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Temporal Changes in *N*-acylethanolamine Content and Metabolism Throughout the Peri-Adolescent Period

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Abstract

Fatty acid amide hydrolase (FAAH) regulates tissue concentrations of *N*-acylethanolamines (NAE), including the endocannabinoid, *N*-arachidonylethanolamide (anandamide; AEA). FAAH activity and NAEs are widely distributed throughout the brain and FAAH activity regulates an array of processes including emotion, cognition, inflammation, and feeding. However, there is relatively little research describing how this system develops throughout adolescence, particularly within limbic circuits regulating stress and reward processing. Thus, this study characterized temporal changes in NAE content (AEA, oleoylethanolamine [OEA] and palmitoylethanolamide [PEA]) and FAAH activity across the peri-adolescent period, in four corticolimbic structures (amygdala, hippocampus, prefrontal cortex and hypothalamus). Brain tissue of male Sprague-Dawley rats were collected on post-natal days (PND) 25, 35, 45 and 70, representing pre-adolescence, early-mid adolescence, late adolescence and adulthood, respectively. Tissue was analyzed for AEA, OEA and PEA content as well as FAAH activity at each time point. AEA, OEA and PEA exhibited a similar temporal pattern in all four brain regions; NAE concentrations were lowest at PND 25 and highest at PND 35. NAE concentrations decreased between PNDs 35 and 45 and increased between PNDs 45 and 70. FAAH activity changed in the opposite direction to NAE content; activity decreased between PNDs 25 and 35; increased between PNDs 35 and 45 and decreased between PNDs 45 and 70. These age-dependent patterns of NAE content and FAAH activity demonstrate temporal specificity to the development of this system, and could contribute to alterations in stress sensitivity, emotionality and executive function which also fluctuate during this developmental period.

Keywords

Adolescence; adolescent development; endocannabinoid; anandamide; oleoylethanolamine; palmitoylethanolamide; fatty acid amide hydrolase; corticolimbic circuit

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INTRODUCTION

N-Acylethanolamines (NAE) are a class of fatty acid-derived signalling lipids that are generated by the transfer of a sn-1 fatty acid from a donor phospholipid to phosphatidylethanolamine (PE) to form *N*-acyl PE (NAPE) by a currently undefined calcium-activated *N*-acyltransferase (Ahn, McKinney et al. 2008; Ueda, Tsuboi et al. 2010). NAPE is then converted to the active NAE through several potential mechanisms, including NAPE-specific phospholipase D (NAPE-PLD), α/β -hydrolase-4 or phospholipase C/tyrosine phosphatase pathways (Ahn, McKinney et al. 2008; Ueda, Tsuboi et al. 2010). With respect to the central nervous system, however, it is unknown which of these mechanisms (or an alternative) is the primary pathway mediating NAE biosynthesis. In fact, the possibility exists that different NAE molecules may utilize distinct biosynthetic pathways (Ueda et al., 2010). While there are many species of NAEs, the most studied of the NAE molecules is the endocannabinoid, *N*-arachidonylethanolamine (also known as anandamide; AEA), which is an endogenous agonist of the cannabinoid type 1 (CB₁) receptor (De Petrocellis and Di Marzo 2009). AEA also possesses non-CB₁ receptor signaling actions to L-type Ca²⁺ channels and transient changes in intracellular Ca²⁺, and disruption to gap function. Lastly, the most well studied of AEA non-endocannabinoid actions is the activation of vanilloid receptors (see review, Howlett and Mukhopadhyay 2000), which has been reported to enhance fear and anxiety-related responses (Moreira, Aguiar et al. 2012). In addition to AEA, two other prominent NAEs are oleoylethanolamine (OEA) and palmitoylethanolamine (PEA). In contrast to AEA, OEA and PEA do not act as agonists at the cannabinoid receptors (Hansen 2010) and it appears that their synthesis is significantly driven by NAPE-PLD (Leung, Saghatelian et al. 2006). OEA and PEA have been found to activate peroxisome proliferator-activated receptor- α (PPAR α ; Fu, Gaetani et al. 2003; Lo Verme, Fu et al. 2005) and OEA is also an agonist at the orphan G-protein coupled receptor 119 (GPR119; Overton, Babbs et al. 2006). Notwithstanding differences in their biosynthetic pathways and functional targets, all three molecules are hydrolyzed by fatty acid amide hydrolase (FAAH), resulting in the formation of ethanolamine and the parent fatty acid (i.e., AEA is metabolized into ethanolamine and arachidonic acid; Ueda, Tsuboi et al. 2010).

While the determination of the functional targets of NAE signaling is progressing, relatively little is known about the ontogeny of this system. With respect to synthesis, it has been demonstrated that the enzymatic activity of the *N*-acyl transferase responsible for NAPE synthesis dramatically declines from birth to the onset of adolescence and also remains low in adulthood (Moesgaard, Petersen et al. 2000). Similarly, FAAH mRNA expression and activity are found to be very high at birth and decline into adulthood (Thomas, Cravatt et al. 1997). These data suggest that the capacity for both synthesis and metabolism of NAEs is high in early life, but declines with age. Whether NAE synthesis, contents or hydrolysis change during the peri-adolescent period remains unknown. This question may be of particular relevance for the regulation of AEA signaling, as CB₁ receptor activation is known to be important for many neurodevelopmental processes (Harkany, Keimpema et al. 2008), some of which continue in adolescence (e.g. Gogtay, Giedd et al. 2004; Gogtay, Nugent et al. 2006). Furthermore, profound changes in behavioural repertoire and physiological status occur during the adolescent period; it is during this stage that mammals achieve sexual maturation, leave their primary caregivers and establish a sense of independence (Spear 2000; McCormick, Mathews et al. 2010; Romeo 2010; Pattwell, Bath et al. 2011). This process of development involves a fine balance of several behavioural processes, including stress perception, reward sensitivity and higher order cognition, all of which are processes that are influenced by NAE signaling, particularly AEA (Solinas, Goldberg et al. 2008; Zanettini, Panlilio et al. 2011; Hill and Tasker 2012).

The aim of the current experiment was to characterize developmental patterns of AEA, PEA and OEA, and their metabolism by FAAH, in forebrain structures primarily involved in emotion, cognition and decision-making, structures that are also known to undergo functional and architectural changes during the peri-adolescent period. To achieve this, we measured both NAE content and FAAH activity at time points representing pre-adolescence (post natal day [PND] 25), early to mid-adolescence (PND 35), late adolescence (PND 45) and adulthood (PND 70). Our data indicate time-specific changes in NAE content and metabolism throughout these forebrain structures, the functional significance of which remains to be determined.

MATERIALS AND METHODS

Subjects

Male Sprague Dawley rats (Charles River, QC, Canada) were received at the University of British Columbia on post-natal (PND) 21 regardless of the age at which they were studied. Rats were pair housed by age in clear polyurethane cages (48×27×20 cm) filled with cedar bedding and paper towels for enrichment, and maintained on a 12h/12h light/dark cycle (lights on at 9 am). Food (Purina rat chow) and water were provided *ad libitum*. All protocols were carried out in accordance with the Canadian Council for Animal Care guidelines and were approved by the Animal Care Committee at the University of British Columbia. Four ages were compared: PND 25 (pre- adolescence; n=8), PND 35 (early/mid-adolescence; n=8), PND 45 (late adolescence; n=8), and PND 70 (adulthood, n=8).

Procedure

All animals were sacrificed by rapid decapitation during the first third of the light cycle and brain tissue was collected for eCB content analysis. Prefrontal cortex, hippocampus, amygdala, and hypothalamus were dissected within 5 min, as previously described (Hill, Karatsoreos et al. 2010), frozen on dry ice, and stored at −80° C until analysis.

Endocannabinoid Extraction and Analysis

Brain regions underwent a lipid extraction process as previously described (Patel, Rademacher et al. 2003). The tissue samples were weighed then placed in borosilicate glass culture tubes containing 2 mL of acetonitrile with 84 pmol of [²H₈] anandamide. These samples were homogenized with a glass rod and sonicated for 30 min, at which point they were incubated overnight at −20°C to precipitate proteins. The samples were centrifuged at 1500 g to remove particulates. Supernatants were removed to a new glass culture tube and evaporated to dryness under N₂ gas, then re-suspended in 300 µL of methanol to recapture any lipids adhering to the tube and re-dried again under N₂ gas. The final lipid extracts were suspended in 20 µL of methanol and stored at −80°C until analysis. NAE contents (AEA, PEA and OEA) within lipid extracts were determined using isotope-dilution, liquid chromatography-mass spectrometry as described earlier (Patel, Carrier et al. 2005).

Membrane Preparation

Frozen brain tissue samples (i.e. amygdala, hippocampus, hypothalamus, prefrontal cortex) were homogenized in 10 volumes of TME buffer (50 mM Tris HCl, pH = 7.4; 1 mM EDTA, and 3 mM MgCl₂). Homogenates were then centrifuged at 18, 000×g for 20 min and the resulting crude membrane fraction-containing pellet was re-suspended in 10 volumes of TME buffer. Protein concentrations were determined using the Bradford method (Bio-Rad, Hercules, CA, USA).

FAAH Activity Assay

FAAH activity was measured by conversion of AEA labelled with [^3H] in the ethanolamine portion of the molecule ([^3H]AEA; Omeir, Chin et al. 1995) to [^3H] ethanolamine preparations as reported earlier (Hillard, Wilkison et al. 1995). Membranes were incubated in a final volume of 0.5 mL TME buffer (50 mM Tris-HCl, 3.0 mM MgCl_2 , and 1.0 mM EDTA, pH 7.4) that contained 1.0 mg/ml fatty acid-free bovine serum albumin and 0.2 nM [^3H]AEA. Isotherms were constructed using eight concentrations of AEA at concentrations between 10 nM and 10 mM. Incubation was carried out at 37 °C and the enzymatic reaction was stopped by the addition of 2 ml of chloroform/methanol (1 : 2). After remaining in room temperature for 30 min with frequent mixing, 0.67 ml of chloroform and 0.6 ml of water were added and aqueous and organic phases were separated by centrifugation at 1000 rpm for 10 min. The amount of [^3H] in 1 ml each of the aqueous and organic phases was determined by liquid scintillation counting and conversion of [^3H]AEA to [^3H]ethanolamine was calculated. The K_i and V_{max} values for this conversion were determined by fitting the data to a single site Michaelis-Menten equation using Prism.

Statistics

AEA, OEA, and PEA contents and FAAH activities were analyzed using one-way ANOVAs, as a function of age for each brain region. Bonferroni post-hoc tests were used to determine whether there were any significant differences between age groups in each brain structure.

RESULTS

Age-dependent changes in AEA content throughout structures of the corticolimbic circuit

One way ANOVA revealed significant effects of age on AEA content in the amygdala ($F_{(3,15)} = 15.54$, $p=0.0003$; Fig. 1), hypothalamus ($F_{(3,15)} = 10.01$, $p=0.001$; Fig. 1), prefrontal cortex ($F_{(3,15)} = 11.43$, $p=0.0008$; Fig. 1) and hippocampus ($F_{(3,15)} = 5.63$, $p=0.01$; Fig. 1). In the amygdala, post-hoc analyses revealed that AEA content was significantly lower on PND 25 relative to PND 35 ($p < 0.05$) and 70 ($p < 0.005$). PND 70 AEA content was also significantly higher than on PND 45 ($p < 0.005$). Post-hoc comparisons of AEA content in the hypothalamus also demonstrated significantly higher AEA content on PND 70 relative to PND 25 ($p < 0.05$) and 45 ($p < 0.005$), whereas AEA content on PND 35 was significantly higher than on PND 45 ($p < 0.05$). Examination of AEA content in the prefrontal cortex also revealed that on PND 70, content was significantly higher than on PND 25 ($p < 0.005$) and 45 ($p < 0.005$). Furthermore, AEA content on PND 35 was significantly higher than on PND 25 ($p < 0.005$) and PND 45 ($p < 0.05$). Post-hoc analyses revealed that AEA content in the hippocampus was significantly greater on PND 70 than on PND 25 ($p < 0.05$) and 45 ($p < 0.05$).

OEA content in corticolimbic structures follow similar age-dependent changes to AEA content

One way ANOVA revealed an age effect on OEA content in the amygdala ($F_{(3,15)} = 20.67$, $p=0.0001$; Fig. 2), hypothalamus ($F_{(3,15)} = 5.08$, $p=0.02$; Fig. 2), prefrontal cortex ($F_{(3,15)} = 13.41$, $p=0.0004$; Fig. 2) and hippocampus ($F_{(3,15)} = 12.13$, $p=0.0006$; Fig. 2). Adult (i.e., PND 70) OEA content in the amygdala was significantly higher relative to content on PND 25 ($p < 0.0005$), 35 ($p < 0.05$) and 45 ($p < 0.0005$). Furthermore, PND 35 OEA content was significantly higher than on PND 25 ($p < 0.05$). In the hypothalamus, OEA content at PND 35 was significantly greater than on PND 25 ($p < 0.05$). Post-hoc analyses of OEA content in the prefrontal cortex revealed that relative to animals on PND 70, animals that were 25 and 45 days old had significantly lower OEA content ($p < 0.005$ for both comparisons). OEA

content on PND 35 was also significantly greater than on PND 25 ($p < 0.005$) and 45 ($p < 0.005$) in the prefrontal cortex. OEA content in the hippocampus on PND 70 was significantly higher than on PNDs 25 ($p < 0.005$) and 45 ($p < 0.005$). OEA content within the hippocampus on PND 25 was significantly lower relative to content on PND 35 ($p < 0.05$).

Age-dependent changes observed in corticolimbic PEA content

One way ANOVA revealed a significant effect of age on PEA content in the amygdala ($F_{(3,15)} = 6.26$, $p=0.009$; Fig. 3), prefrontal cortex ($F_{(3,15)} = 6.92$, $p=0.006$; Fig. 3) and hippocampus ($F_{(3,15)} = 9.26$, $p=0.002$; Fig. 3); however, no significant age dependent effects were observed in PEA content of the hypothalamus ($F_{(3,15)} = 1.852$, $p=0.19$; Fig. 3). Amygdalar PEA content at PND 70 was significantly higher than on PND 25 ($p < 0.05$) and 45 ($p < 0.05$). Furthermore, PEA content in the prefrontal cortex was significantly greater on PND 70 relative to PND 25 ($p < 0.05$) and 45 ($p < 0.05$), and PEA content on PND 35 was higher relative to PND 25 ($p < 0.05$). In the hippocampus, PEA content was higher on PND 70, relative to content on PND 25 ($p < 0.005$) and PND 45 ($p < 0.005$).

Age-dependent changes in FAAH activity throughout the corticolimbic stress circuit

One way ANOVAs of maximal hydrolytic activity (V_{max}) of FAAH revealed a significant age-dependent effect that was the inverse of that seen in NAE content in the amygdala ($F_{(3,15)} = 6.50$, $p=0.007$; Fig. 4), hypothalamus ($F_{(3,15)} = 4.48$, $p=0.03$; Fig. 4), prefrontal cortex ($F_{(3,14)} = 5.00$, $p=0.02$; Fig. 4) and hippocampus ($F_{(3,14)} = 4.26$, $p=0.03$; Fig. 4). Post-hoc comparisons of FAAH activity in the amygdala revealed that FAAH activity on PND 25 was significantly higher than on PND 35 ($p < 0.05$) and 70 ($p < 0.05$). In the hypothalamus, FAAH activity was greater on PND 45 than on PND 70 ($p < 0.05$). Post-hoc analyses of FAAH activity in the prefrontal cortex revealed significantly higher levels of FAAH activity on PND 45 relative to activity on PND 70 ($p < 0.05$). In the hippocampus, FAAH activity was significantly greater on PND 25 than on PND 70 ($p < 0.05$).

A one-way ANOVA indicated that there were no age effects on the K_m values of FAAH activity (see Table I) in the amygdala ($F_{(3,15)} = 0.72$, $p = 0.56$), hypothalamus ($F_{(3,15)} = 0.36$, $p = 0.79$) and hippocampus ($F_{(3,14)} = 0.41$, $p = 0.75$). However, there was a significant effect of age on K_m values in the prefrontal cortex ($F_{(3,14)} = 4.25$, $p = 0.03$) such that K_m values were significantly lower on PND 35 relative to PND 25 ($p < 0.05$; see Table I).

DISCUSSION

The results of the current study reveal significant age-dependent changes in NAE content (i.e., AEA, OEA and PEA) as well as FAAH activity throughout the peri-adolescent period into adulthood. Generally, the contents of all of the NAEs increased from PND 25 to 35, and then decreased between PND 35 and 45. By PND 70, these ligands had increased to adult concentrations (Hill et al., 2010). These temporal changes in tissue NAE content were accompanied by opposite changes in FAAH activity. FAAH was generally high on PND 25, declined by PND 35, and then increased again by PND 45. By PND 70, FAAH activity was significantly reduced relative to the earlier time points, especially relative to PND 25 and 45. Collectively, these data would indicate that there is a peak in NAE content in the brain during the early phases of adolescence, which is likely mediated by a transient reduction in FAAH-mediated metabolism. Furthermore, considering the similarity amongst the NAE content throughout adolescence, it seems likely that these age-dependent changes in NAEs are influenced by changes in FAAH activity. However, we have not explored age-related changes in rates of NAE biosynthesis.

While there is much debate over a specific age range for the adolescent period, both in the human and animal model literature, most agree that pubertal onset and development occur during this phase (Spear 2000; Morishita, Okamoto et al. 2005). This raises the possibility that the observed changes in AEA, PEA and OEA contents may also be related to shifts in gonadal hormone concentrations which facilitate pubertal maturation. In support of this, Wenger and colleagues (2002) reported peak AEA contents immediately prior to pubertal onset (PND 30–31) in the hypothalamus of female rats which declined with vaginal opening. Furthermore, pre-pubertal administration of an exogenous CB1 receptor agonist was shown to delay pubertal onset relative to that of vehicle-injected female rats (Wenger, Croix et al. 1988). Evidence that estrogens reduce the expression of FAAH and elevate AEA content in the brain and periphery (Maccarrone, De Felici et al. 2000; Scorticati, Mohn et al. 2003; Hill, Karacabeyli et al. 2007; De Petrocellis and Di Marzo 2009), suggests the possibility that an increase in estrogens during the onset of puberty can modulate FAAH activity and AEA content in females. Our current understanding of male pubertal onset and testosterone in relation to endocannabinoids and related NAEs is far less developed. A frequently accepted estimate of the adolescent period in males is PND 28–42, with pubertal onset at about PND 30 (Spear 2000). We observed peak content of AEA throughout the corticolimbic circuit at PND 35. Since measurements of AEA, PEA and OEA were not performed between PND25 and 35, the timing of the changes in NAEs in relationship to the onset of puberty is not known. Similarly, the relationship of these changes to circulating levels of testosterone are unknown; although previous studies have shown that male rodents have little to no testosterone on PND 25 but exhibit circulating testosterone on PND 35 (Romeo, Lee et al. 2004; Romeo, Bellani et al. 2006). Future studies should include concise temporal measures during this period of pubertal onset to determine whether changes in FAAH activity or AEA, PEA and OEA content correlate with the emergence of circulating gonadal androgens and maturation of the testes.

The pattern of FAAH activity observed throughout adolescent development is in agreement with that previously reported in rat whole brain samples where FAAH activity increased from birth to PND 21, decreased moderately by PND 42 then increased again by PND 56 (Morishita, Okamoto et al. 2005). In that same study, NAPE-PLD exhibited a sharp increase in specific activity from birth to PND 21 and a slower increase to PND 56, which was also consistent with NAPE-PLD mRNA expression in the rat brain during development (Morishita, Okamoto et al. 2005). The modest change in NAPE-PLD activity and expression during peri-adolescence suggests AEA biosynthesis is not completely linked, or only provides a partial contribution to the clear pattern of age-dependent NAE changes reported here. It should be noted that there are at least 3 other AEA biosynthetic pathways (Di Marzo 2011) that do not include NAPE-PLD which may exhibit other age-dependent changes that contribute to the observed peak NAE levels at PND 35; however, methods of assessing these mechanisms do not yet exist. Furthermore, Thomas and colleagues (1997) reported a progressive increase in FAAH mRNA contents between embryonic day 14 to PND 10, which remained relatively high until PND 30 and was subsequently decreased by adulthood (although no measures were taken between PND 30 and 60). This study also demonstrated that the enzymatic activity of FAAH was strongly correlated with the time-course of mRNA in the same regions (Thomas, Cravatt et al. 1997). Taken together with the current data, there are age-dependent changes in FAAH activity, which are likely mediated by transcriptional regulation of FAAH during development.

In this study, OEA and PEA increased from PND 25, peaking at PND 35 and then decreased to adult levels (PND 70). The temporal changes in OEA and PEA during adolescence parallel previous reports of age-dependent effects on AEA content throughout the different structures of the brain (e.g. Berrendero, Sepe et al. 1999; Wenger, Gerendai et al. 2002; Ellgren, Artmann et al. 2008). In particular, a spike in AEA content has been observed

during mid-adolescence within both the hypothalamus of females (first day of proestrus [PND 30–31]; Wenger, Gerendai et al. 2002) and the nucleus accumbens of males (PND 38; Ellgren, Artmann et al. 2008). Temporal changes in CB1 receptor density are also seen during early development and into adolescence (see Lee and Gorzalka 2012). However, while AEA content exhibits a general trend of increasing from early life into adulthood, CB1 receptor density declines between adolescence and adulthood (see Lee and Gorzalka 2012). As such, early adolescence provides a unique temporal window in which, despite opposite trajectories, there is a combined peak of AEA/CB1 receptor activity.

Given that AEA/CB1 receptor signalling in corticolimbic circuits is known to regulate reward sensitivity (Mahler, Smith et al. 2007) and stress responsivity (Hill and Tasker 2012), these changes may be key to some of the neurobehavioural changes which occur during the adolescent period. For example, adolescence is a period of sensation seeking, impulsivity and reduced contextual fear (Spear and Brake 1983; Casey, Jones et al. 2011; Pattwell, Bath et al. 2011), in which greater exploration and risk taking occurs, presumably in an effort to establish independence (Spear 2000). Inhibition of endocannabinoid signalling has been reported to improve inhibitory control and reduce impulsivity (Pattij, Janssen et al. 2007; Wiskerke, Stoop et al. 2011), suggesting that tonic endocannabinoid signalling may facilitate impulsive behaviours. Similarly, inhibition of FAAH has been shown to reduce anxiety in an array of animal models (Kathuria, Gaetani et al. 2003; Rossi, De Chiara et al. 2010; Kinsey, O'Neal et al. 2011; Hill, Kumar et al. in press), although one caveat of these findings is that the anxiolytic effects of FAAH inhibition are dependent on factors such as dose, route of administration, timing of exposure and context (see review Zanettini, Panlilio et al. 2011). Of interest, human carriers of the C385A polymorphism of FAAH (which compromises cellular FAAH expression and increases circulating AEA levels; Chiang, Gerber et al. 2004; Sipe, Scott et al. 2010) exhibit blunted activation of the amygdala in response to threat and an increase in ventral striatal activation in response to reward (Hariri, Gorka et al. 2009). Collectively, these data suggest that lower FAAH activity and increased AEA/CB1 receptor signaling subserve a shift in corticolimbic circuits promoting a state of low anxiety, high responsivity to rewards and reduced inhibitory control. Given that the emergence to adolescence represents a period when this behavioural profile is maximal, which is coincident with a peak in AEA/CB1 receptor activity and reduced FAAH activity, it is tempting to speculate that these temporal changes in FAAH activity and AEA content represent a neural substrate of the adolescent phenotype. Further research is required to delineate the contributions these changes in FAAH activity and AEA content play with respect to this phenotype.

It is only relatively recently that researchers have demonstrated adolescence as a distinct maturational period. The fact that the functionality of corticolimbic circuits during early adolescence is unique compared to that during younger or older age intervals further highlights the idea that this maturational stage has its own unique demands including behavioural and neural adaptations to facilitate establishing independence. The data presented herein, demonstrate that the hydrolytic activity of FAAH and the tissue content of its NAE substrates: AEA, PEA and OEA, exhibit discrete, temporal-specific changes throughout the peri-adolescent period. Future research will endeavour to determine the functional role of these changes in the onset of puberty, development of the adolescent phenotype and maturation into adulthood.

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Figure 1.

Mean (± SEM) anandamide (AEA) concentrations throughout structures of the corticolimbic circuit in adolescent and adult male rats. AEA generally increased from PND 25 to 35, then decreased by PND 45. By adulthood, AEA concentrations were significantly higher than all adolescent age points. * denotes $p < 0.05$; ** denotes $p < 0.005$; *** denotes $p < 0.0005$.

Figure 2.

Mean (± SEM) oleoylethanolamine (OEA) concentrations throughout structures of the corticolimbic circuit in adolescent and adult male rats. OEA generally increased from PND 25 to 35, then decreased by PND 45. By adulthood, OEA concentrations were significantly higher than all adolescent age points. * denotes $p < 0.05$; ** denotes $p < 0.005$; *** denotes $p < 0.0005$.

Figure 3.

Mean ($\pm$ SEM) palmitoylethanolamide (PEA) concentrations throughout structures of the corticolimbic circuit in adolescent and adult male rats. PEA generally increased from PND 25 to 35, then decreased by PND 45. By adulthood, PEA concentrations were significantly higher than all adolescent age points. * denotes $p < 0.05$; ** denotes $p < 0.005$; *** denotes $p < 0.0005$.

Figure 4.

Mean ($\pm$ SEM) fatty acid amide hydrolase (FAAH) activity ($V_{\max}$ values) throughout structures of the corticolimbic circuit in adolescent and adult male rats. FAAH activity generally decreased from PND 25 to 35, then increased by PND 45. By adulthood, FAAH activity was generally reduced relative to activity during adolescence. * denotes $p < 0.05$.

Table I

Binding affinity (Km) of anandamide for fatty acid amide hydrolase (FAAH) throughout structures of the corticolimbic circuit during adolescence.^I

	PND 25	PND 35	PND 45	PND 70
Amygdala	0.5421	0.448925	0.46175	0.445825
Hypothalamus	0.413025	0.4782	0.537375	0.3877
Prefrontal Cortex	0.466667	0.2946 *	0.43565	0.39765
Hippocampus	670.2	470.25	463.025	362.6

^I All values represented are in nM and

* indicate significantly different from PND 25, $p < 0.05$.