CANNABINOID RECEPTOR TYPE 1 (CB1) DENSITIES IN MONOGAMOUS AND NON-MONOGAMOUS NEW WORLD MONKEYS

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ABSTRACT

The endocannabinoid system plays an important role in social behaviors across a variety of species. Recently, the distribution of cannabinoid receptor 1 (CB1) was linked to the mating patterns of voles (i.e., monogamy versus non-monogamy) (Simmons et al., 2021). This raises the question of whether a similar mechanism exists in primates. The goal of the present study was to address that question by examining the relationship between CB1 density and mating patterns in monogamous (owl monkey, marmoset) and non-monogamous (capuchin) New World monkeys. Our study found that CB1 axon length density was higher in the monogamous species than the promiscuous in layer III of the anterior cingulate cortex. Our study suggests a potential correlation between monogamy and CB1 in New World monkeys. More research is needed to determine the extent of this trend in other primate species.

CHAPTER 1

INTRODUCTION

The endocannabinoid system

The endocannabinoid system (ECS) uses endocannabinoids to regulate the central nervous system via neuromodulation (Lu & Mackie, 2016). Endocannabinoids are lipid-derived neurotransmitters produced in an on-demand process and released from the postsynaptic membrane in response to an action potential that triggers calcium to flood into the cell (Moreira et al., 2009). Endocannabinoids bind to cannabinoid receptors to affect the neurotransmitter release by surrounding neurons (Moreira et al., 2009). More broadly, the ECS functions by processing both environmental and internal inputs to produce adaptable behaviors (Lu & Mackie, 2016; Lutz et al., 2015) and dysregulation within the ECS has been linked to a myriad of mental disorders, including anxiety, depression, bipolar disorder, and schizophrenia (Koethe et al., 2007; Lutz et al., 2015; Moreira et al., 2009).

Cannabinoid receptor type 1 (CB1) is the primary endocannabinoid receptor of the central nervous system, located mostly on GABAergic and glutamatergic neurons (Fagundo et al., 2013; Howlett & Abood, 2017; Lu & Mackie, 2016). CB1 receptors are the most abundant G-protein coupled receptors in the brain (Chiarlone et al., 2014) and are found on 53% of human sensory neurons (Ford et al., 2021). Although CB1 receptors are present throughout the brain, they are particularly dense within the forebrain's limbic, frontal, and temporal lobes that deal with cognition and the areas of the brain that control movement (Glass et al., 1997; Herkenham et al., 1990). Regions in the limbic and frontal association cortices that are linked to emotional processing and learning respectively were found to have the highest CB1 receptor density (Glass et al., 1997). The localization of CB1 receptors in regions of the brain responsible for behavior demonstrates the importance CB1 plays in behavioral regulation.

Much of the research on CB1 receptors have included cannabinoids such as Δ9-tetrahydrocannabinol (THC), including their effects on aggression, sociality, and appetite (Howlett & Abood, 2017; Shahbazi et al., 2020; Zou & Kumar, 2018). Cannabinoids directly bind to CB1 receptors in the central nervous system and can result in a variety of harmful symptoms with chronic use (Bloomfield et al., 2019). Research into cannabinoids have also helped researchers better understand CB1's role in the development of psychosis (D'Souza et al., 2004; Nestoros et al., 2017). In addition to exogenous drugs such as THC that bind to cannabinoid receptors, anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are two major endocannabinoids commonly studied (Lu & Mackie, 2016).

The role of CB1 in behavior

CB1 plays a major role in behaviors through social reward, motivation, and withdrawal (Panagis et al., 2014; Wei et al., 2017). A study conducted in mice found that

CB1 activation via the CB1 ligand anandamide was required to activate the rewarding feelings associated with social behavior in the nucleus accumbens (Wei et al., 2015). Furthermore, this study found that activation of CB1 led to increased motivation to socialize in mice (Wei et al., 2015). The same mechanisms that link CB1 with rewarding feelings also play a role in addiction when they become maladaptive (Serrano & Parsons, 2011).

Studies show that CB1 plays a role in aggression (Haller et al., 2004; Rodriguez-Arias et al., 2013). Research using global gene knockouts of CB1 found that deletion of CB1 receptors led to increased aggressiveness in mice (Haller et al., 2004; Rodriguez-Arias et al., 2013). However, some research shows variability in the effects of CB1 deletion with CB1 deletion increasing aggression in the home cage scenario, and social avoidance in an unfamiliar cage scenario (Haller et al., 2004). These results show the importance of the interaction with the environment on CB1 expression as it relates to social behavior.

In addition to CB1's role in social behavior and aggression, CB1 modulation of glutamatergic and GABAergic neurons regulates novelty seeking and investigatory behaviors (Häring et al., 2011; Lafenêtre et al., 2009; Van Laere et al., 2009). However, there is seemingly conflicting research on CB1's role in the drive to investigate due to differing experimental designs. Specifically, the way in which behavior is modulated through CB1 is dependent on factors such as drug dose and environmental conditions (Häring et al., 2011; Terzian et al., 2014). For instance, Terzian et al. (2014) used mice gene knockouts in CB1 receptors of glutamatergic and GABAergic neurons in the cortex and found that there was a sex and social context difference. They found that males showed no difference in behavior in the presence of another male, while male mice that had their CB1 receptors on cortical glutamatergic neurons deleted interacted less with females (Terzian et al. 2014).

In a recent study conducted on mice, the effects of CB1 deletion changed depending on what type of neurons were targeted for downregulation of the CB1 receptor (Häring et al., 2011). Removal of CB1 receptors in GABAergic neurons of the cortex and striatum increased exploratory behavior while the same removal of CB1 receptors from glutamatergic neurons of the cortex led to decreased exploration (Häring et al., 2011). In another study, mice whose CB1 receptors were blocked and were shown a novel type of food did not eat as much as the control group (Lafenêtre et al., 2009). Lafenêtre et al. (2009) found that activation of CB1 receptors located on glutamatergic neurons of the rodent cortex promoted investigatory behavior while activation of the same receptors of inhibitory GABAergic neurons led to decreased investigation. The researchers concluded that the role of CB1 was as a modulator between investigatory and withdrawal behavior, the improper balance of which could lead to disorders like ADHD that are characterized by heightened novelty seeking (Lafenêtre et al., 2009).

Recent studies suggest that CB1 might have a role in even more complex behaviors such as temperament and mating strategy (Simmons et al., 2021; Van Laere et al., 2009). A study conducted on humans demonstrated CB1's role in the novelty seeking element of Cloninger's personality theory on temperaments (Van Laere et al., 2009). This study indicated that the availability of CB1 receptors is linked to personality (Van Laere et al., 2009). Specifically, Van Laere et al. (2009) found that as CB1 availability increased in the cerebrum, novelty seeking behaviors decreased in humans and vice versa, with the largest effect found in the amygdala. This means that novelty-seeking personalities had lower CB1 overall. Simmons et al. (2021) investigated how CB1 is linked to mating patterns in different Microtus species. They found that CB1 receptors within limbic regions were more dense in the monogamous prairie vole than in the promiscuous meadow vole (Simmons et al., 2021). Our current understanding of the role of CB1 in primate social behavior is not as well researched. The current project aims to increase this understanding by examining CB1 densities in a brain region associated with social behavior in monogamous and non-monogamous nonhuman primate species. Based on the results from Microtus species (Simmons et al., 2021), we hypothesized that CB1 axon length density will be higher in monogamous species than non-monogamous species.

Anterior cingulate cortex

The anterior cingulate cortex (ACC) is located anterior to the corpus callosum and integrates both cognitive and emotional information (Bhattacharyya & Sendt, 2012; Stevens et al., 2011). The ACC contributes to such processes as: decision making, learning, motivation, theory of mind, emotion, pain, and attention (Botvinick, 2007; Braem et al., 2017; Etkin et al., 2011; Gallagher & Frith, 2003; Holroyd & Yeung, 2012; Okine et al., 2016; Zhong et al., 2017). Importantly, the ACC serves as an integration point where information coming into the brain from the limbic system and prefrontal cortex is interpreted as rewarding or harmful (Rolls, 2019; Stevens et al., 2011).

Recent studies have examined the contributions of the ACC to social behavior (Apps et al., 2016; Zhong et al., 2017). Research investigated how rats decide on foraging strategies in different social scenarios. The ACC could provide insight into how decision-making changes based on different social settings and competition because the ACC is involved in both decision making and interpretation of social cues (Zhong et al., 2017). Additionally, one study found that the ACC played an important role in social information processing (Apps et al., 2016). This was linked to the ability to evaluate and detect faults during social encounters (Apps et al., 2016). In addition, Okine et al. (2016) administered an endocannabinoid antinociceptive drug (N-palmitoylethanolamide (PEA) in the ACC of rats and found that the drug activated CB1 receptors via anandamide to lessen pain.

Several studies have addressed the role that CB1 receptors play in the ACC in relation to behavior, including mental disorders like schizophrenia and depression (Koethe et al., 2007; Zavitsanou et al., 2004). For instance, Koethe et al. (2007) looked at CB1 receptors in ACC of postmortem humans and found there was no change in CB1 density in schizophrenia despite the fact that previous research (Zavitsanou et al., 2004) found the opposite to be true. Zavitsanou et al. (2004) used a CB1 antagonist in the anterior cingulate cortex (ACC) and found that there was a 64% increase in binding in humans with schizophrenia. Zavitsanou et al. (2004) concluded that malfunctioning of CB1 is partly responsible for some aspects of schizophrenia. This variation in study results could be because of different experimental approaches. In addition, Koethe et al. (2007) found that there were far less CB1 receptors found on glial cells in people with major depression.

Another social behavior that the ACC is involved in is pair-bonding of monogamous species (Jiang & Platt, 2018; Li et al., 2021; López-Gutiérrez et al., 2021). Most of the research conducted thus far has used rodents as their subjects (Li et al., 2021; López-Gutiérrez et al., 2021). Li et al. (2021) found that affiliative behaviors like allogrooming in a monogamous vole species increased serotonin release in the ACC. Activation of serotonin receptors in the ACC also helped voles with social and consolation issues (Li et al., 2021). Another study determined that affiliative behaviors were triggered by connectivity within functional networks of a monogamous vole's brain (López-Gutiérrez et al., 2021). In humans, activation of the ACC was associated with material attachment and long-term romantic relationships (Acevedo et al., 2012). For example, human ACCs were found to activate when they were shown either an image of a loved one or an arousing visual (Esch & Stefano, 2005).

Mating patterns

Primates are known for their diverse social behavior, including mating patterns (Clutton-Brock & Janson, 2012; Kappeler & van Schaik, 2002). Primate mating patterns can be divided into monogamous and non-monogamous. Monogamy is more common in primates relative to other mammalian species and is generally associated with biparental care, pair-bonding of mates, and little to no sexual dimorphism (French et al., 2018; Schacht & Kramer, 2019). Non-monogamous patterns are generally more widespread among primates (French et al., 2018). Additionally, many biological mechanisms underlie primate mating patterns and can help distinguish between monogamy and non-monogamy. For example, neuropeptides like oxytocin and vasopressin have been linked to mating pattern expression in primates (French et al., 2018; Lieberwirth & Wang, 2014).

Owl monkeys (genus *Aotus*) are monogamous primates native to South America (Fernandez-Duque, 2015). Owl monkeys exhibit strong pair bonding, living in small familial groups of a mating pair and their offspring (Fernandez-Duque, 2015; Huck et al., 2014). Characteristic of monogamous primates, owl monkeys display little sexual dimorphism (Fernandez-Duque, 2011). However, intra-sexual competition between owl monkeys leads to mating pairs frequently being replaced by an intruding owl monkey (Fernandez-Duque & Huck, 2013). These regular intrusions have been shown to have a negative impact on the reproductive success of the broken pair (Fernandez-Duque & Huck, 2013). In the instances where owl monkeys were never replaced by an intruder, Fernandez-Duque & Huck (2013) found that there was a 25% increase in offspring produced every decade compared to mates that were replaced.

Marmosets (genus *Callithrix*) are also considered to be a monogamous primate (Kleiman, 1977). Marmosets are typically organized in small tight-knit groups wherein only the dominant female reproduces (Yamamoto et al., 2009). Like many monogamous primates, marmosets provide biparental care for their young (Carp et al., 2018). Unlike many other primates who give birth to a single infant at a time, marmosets often give birth to twins which requires much support from the members of the group (Yamamoto et al., 2009). Overall, monogamy in marmosets is characterized by pair-bonding, affiliative behaviors, and hostility toward outsiders (Ågmo et al., 2012; Carp et al., 2018; French et al., 2018).

Capuchin monkeys (genus *Cebus*) are polygamous and live in multi-male multi-female groups (Alfaro, 2005; Carosi et al., 2005). Characteristic of a multi-male multi-female groups, capuchin monkeys display sexual dimorphism with the males having a larger body and canine size (Carosi et al., 2005). Female capuchins choose partners to mate with, focusing their efforts on the alpha male of the group (Alfaro, 2005; Carosi et al., 2005; Janson, 1984; Tiddi et al., 2018). For this reason, capuchins experience high degrees of female competition for the alpha male’s attention; the dominant females restrict subordinate females' access to males (Carosi et al., 2005).

CHAPTER 2

METHODS

The study sample included 15 individuals and included capuchins (n=6), marmosets (n=6) and owl monkeys (n=3) (Table 1). All individuals were adult and free of gross neuropathology. Sexes were balanced as equally as possible.

Table 1: Specimen sex and age

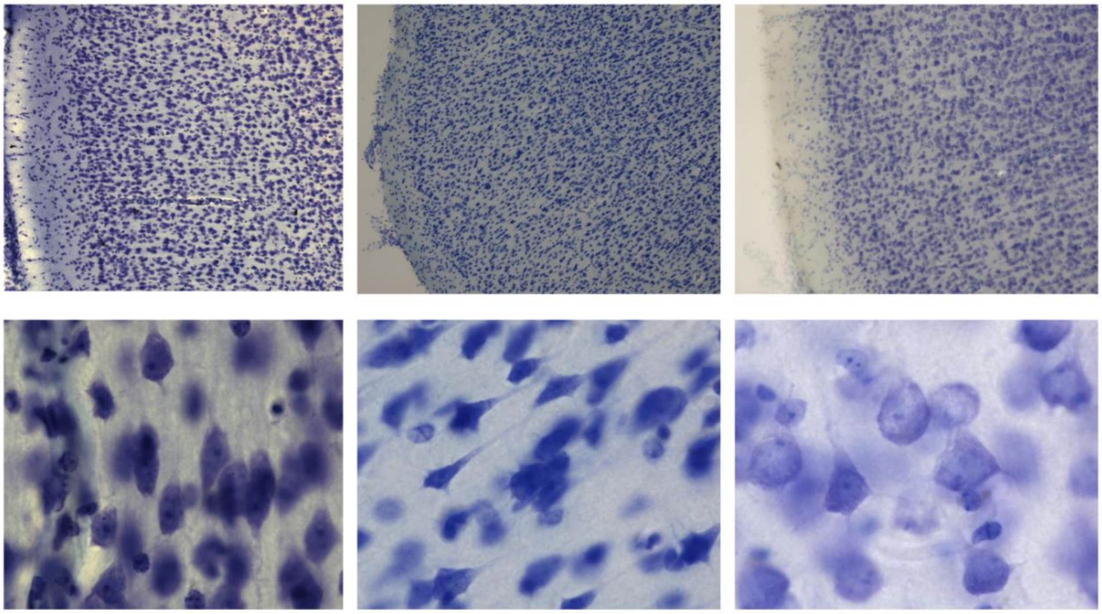
Specimens

Species	**Sex**	**Age**
Owl monkey	M	18
Owl monkey	F	5
Owl monkey	F	3
Marmoset	F	6.11
Marmoset	F	6.2
Marmoset	F	5.1
Marmoset	M	4.1
Marmoset	M	4.5
Marmoset	M	6
Capuchin	F	12.6
Capuchin	F	18.3
Capuchin	F	17.5
Capuchin	M	16.6
Capuchin	M	15.9
Capuchin	M	3.9

Note: Individual sex and age for each specimen of owl monkey, marmoset, and capuchin.

Formalin-fixed postmortem brain samples were first saturated in a series of 10%, 20%, and 30% sucrose solutions. The brains were then sectioned in the sagittal plane at 40 micrometers with a Leica SM2000R freezing microtome, placed in individual centrifuge tubes, and numbered sequentially. A 1-in-10 series of sections was histologically processed for Nissl substance to show cell somata. Nissl substance was stained through immersion in solutions of chloroform and ethanol, followed by a cresyl violet stain of 0.05%. Finally, the slides were coverslipped using DPX to be used for stereological processes (Figure 1). Species and individual information was blinded during data collection to avoid bias.

Figure 1: Nissl stain for New World primates



Note: Owl monkey Nissl stain in leftmost panels, marmoset in middle, and capuchin on the right. The top row of pictures were taken at 4x magnification in the ACC where layer III and layers V/VI are visible.

This project used existing sections that had been immunohistochemically processed for CB1. The antibody used was an anti-rabbit CB1 primary antibody (abcam ab23703) at a dilution of 1:6,000. The data were collected using advanced stereology to measure CB1 axon length density in the ACC between the monogamous and promiscuous primates in layers III and layers V/VI. The StereoInvestigator software (MBF Bioscience) on an Olympus BX 51 Optical Fluorescent Microscope was used for quantification.

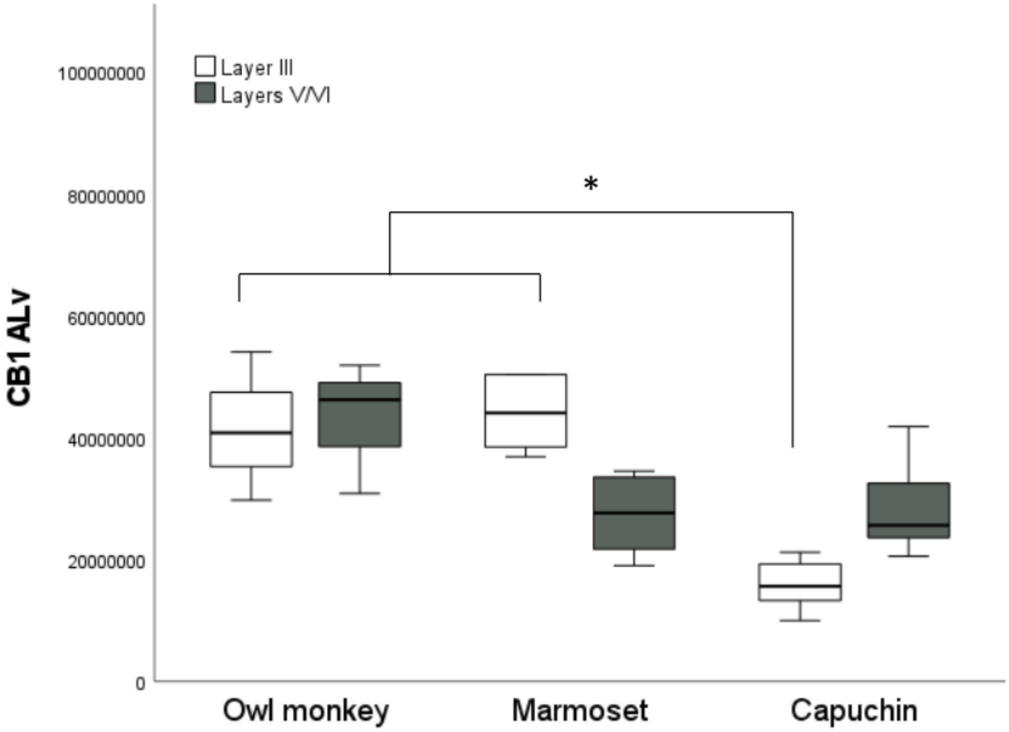
The data were analyzed using ANOVA and post hoc analyses (Tukey HSD). The variable for comparison between species was CB1-ir axon length density. The between-subjects measure was species (owl monkey, capuchin, marmoset) and the cortical layers (III and V/VI) served as the repeated measure. Mixed model ANOVA tests were used to evaluate among-species differences in axon length density. T-tests were used to determine the difference between sexes of the same species.

CHAPTER 3

RESULTS

No sex differences were detected in marmosets or capuchins (there was an insufficient sample for owl monkeys). Sexes were collapsed for among-species analyses. Mixed model ANOVA was used to evaluate species differences in CB1-ir axon length density (Figure 2). Cortical layer (III and V/VI) was the repeated measure and species was the between-subjects measure.

Figure 2: CB1 axon length density



Note: Species and layer specific CB1 axon length density.

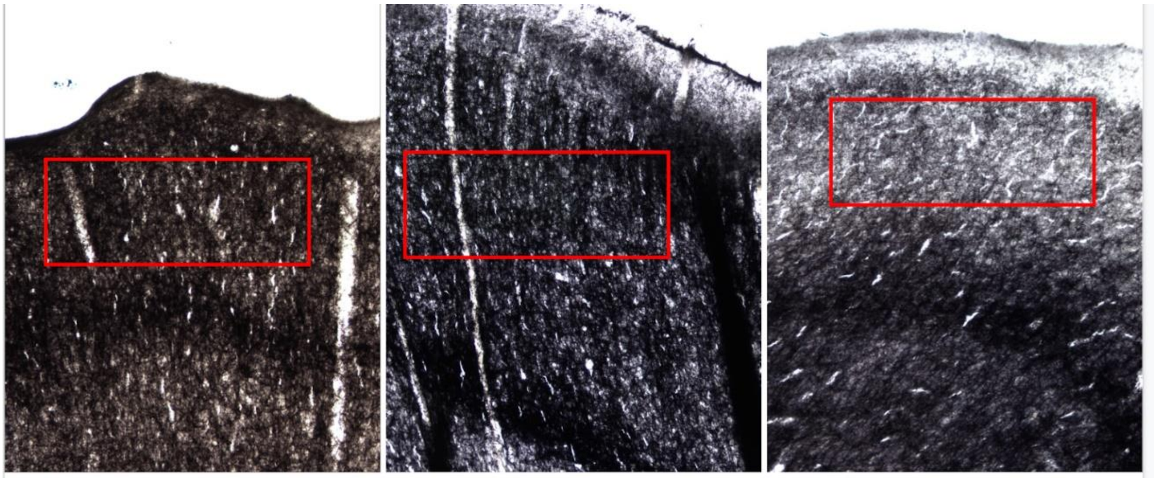
Table 2: Values for CB1 axon length density (ALv)

Summary data

Species	**ALv, layer III**	**ALv, layers V/VI**
Owl monkey	41,514,548 ± 12,179,731	637 ± 90
Marmoset	49,120,979 ± 16,537,527	528 ± 177
Capuchin	15,816,696 ± 4,203,565	389 ± 68

The interaction between species and layer was significant as shown in Figure 2 (F 2,12 = 17.06, $p < 0.001$). The main effect of the layer was not significant (F 1,12 = 0.89, $p = 0.37$) while the main effect of species was significant (F 2,12 = 7.2, $p = 0.009$). Post hoc analyses revealed that marmosets and owl monkeys possessed higher CB1-ir ALv in layer III relative to that of capuchin layer III (all p's < 0.05).

Figure 3: CB1 stain for New World primates



Note: Owl monkey CB1 stain in leftmost panels, marmoset in middle, and capuchin on the right. Layer III is highlighted in red for each species.

CHAPTER 4

DISCUSSION AND CONCLUSION

The endocannabinoid system is important for regulating complex social behaviors and has been shown to vary between species, sexes, and even individuals of a species (Silver, 2019; Simmons et al., 2021). Simmons et al. (2021) mapped the distribution of CB1 receptors in two vole species throughout their limbic regions and found that monogamous voles showed higher CB1 density in regions of the brain that are involved in spatial memory and social bonding such as the periaqueductal gray and hippocampus. To build on those findings, we examined CB1 receptor density in the ACC of monogamous and non-monogamous New World monkeys and found a significant correlation between monogamy and CB1 density. Our data suggest that CB1 receptors may be involved in monogamous behaviors that are critical to pair bonding success.

The ACC plays a role in social behavior through mechanisms of decision making and processing of social cues (Apps et al. 2016; López-Gutiérrez et al., 2021; Zhong et al., 2017). The neurological mechanisms that involve the ACC are integral to maintaining the pair-bond of monogamous species (Jiang & Platt, 2018; Li et al., 2021; López-Gutiérrez et al., 2021). These behaviors include partner guarding, distress during separation, biparental care, and affiliative behaviors like allogrooming (López-Gutiérrez et al., 2022). Studies have found that affiliative behaviors in monogamous vole species are connected with increased serotonin release and connectivity within the ACC (Li et al., 2021; López-Gutiérrez et al., 2021). While another study found that oxytocin release within the ACC of rats played a role in helping behaviors (Yamagishi et al., 2020).

At a basic level the ACC plays a role in the bonding of monogamous pairs by increasing the reward value of having a single sexual partner (López-Gutiérrez et al., 2022). Jealousy is another mechanism used to maintain pair-bonds. Maninger et al. (2017) found that fluorodeoxyglucose uptake in the left ACC of monogamous titi monkeys showed higher levels when they were in a jealousy evoking situation. The ACC's involvement in social behaviors, in particular those that relate to mating behavior of monogamous species, made this brain region relevant for our study (Li et al., 2021; López-Gutiérrez et al., 2021). However, future research could address other areas of the brain in the reward circuit that are involved in monogamous behaviors.

The ECS regulates social behaviors via CB1 receptor activation (Wei et al., 2017). As previously discussed CB1 plays a major role in social behaviors such as those associated with social reward (Panagis et al., 2014; Wei et al. 2017), aggression (Haller et al., 2004; Rodriguez-Arias et al., 2013), and investigation (Häring et al., 2011; Lafenêtre et al., 2009; Van Laere et al., 2009). In addition, CB1 dysregulation has been shown to lead to a variety of mental disorders like schizophrenia and depression (Koethe et al., 2007; Zavitsanou et al., 2004). In particular, the rewarding function of CB1 is of importance to the current study as it relates to mating patterns. Wei et al. (2015) found that social contact triggered activation of CB1 receptors in the mouse nucleus accumbens which reinforced the associated reward of contact.

Our results revealed that CB1 axon length density was higher in the monogamous owl monkey and marmoset than the promiscuous capuchin in layer III of the ACC. It is possible that layer-specific functions played a role. Layer III of the ACC consists of many pyramidal neurons that project into layers V/VI (Wu et al., 2009). Layers V/VI, in turn, serve as the cerebral cortex's primary output for memory recall, cognition, and motor control (Miyashita, 2022; Prasad et al., 2020; Wu et al., 2009). Furthermore, the function of ACC pyramidal neurons is dependent on the layer and target pathway (Medalla et al., 2022). Medalla et al. (2022) found that amygdala-targeting neurons within layer V of a rhesus macaque's ACC were more excitable than those of layer III.

This study was the first to investigate the relationship between CB1 axon length density and mating patterns of primates. Our results support the findings in vole species (Simmons et al., 2021) by extending this trend to New World primates. The results of our study suggest a potential correlation between monogamy and CB1 in New World monkeys. However, more primate species comparisons are required to determine the extent of these findings. Further studies could investigate the role of CB1 in Old World primates and humans, in addition to continuing to investigate other regions of the brain implicated in mating behavior. In conclusion, we hope that the results of our study will permit a better understanding of how the endocannabinoid functions in relation to mating behavior.

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