

The effects of parental experience on cognition, anxiety-like behaviour, and hippocampal  
plasticity in a biparental species

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## Abstract

Becoming a parent is a transformative event marked by significant changes in behaviour and the brain. In rodents, it is still unclear how parental experience influences behaviours outside of caregiving, such as in spatial cognition and anxiety. Furthermore, studies on the effects of maternal experience have focused on monoparental species, while few studies have investigated the changes associated with fatherhood. To date, a direct comparison on the effects of parental experience on behaviour and the brain in both mothers and fathers of the same species has not been done. The objective of this study was to investigate the effects of parental experience on spatial cognition, anxiety-like behaviour, and hippocampal neuroplasticity-related measures (microglia and perineuronal net expression) in both sexes of the same species, the degu. Degus are biparental rodents allowing us to examine maternal and paternal experiences in addition to maternal experience in single mothers when the male partner is removed (i.e., monoparental maternal experience). Key findings from our study indicated that parental experience differentially affects anxiety-like behaviour and spatial learning and memory in males and females. Biparental females exhibited more anxiogenic behaviour while biparental males showed more anxiolytic behaviour on the elevated plus maze. Furthermore, biparental males exhibited impaired spatial learning, while monoparental females exhibited enhanced spatial learning on the Barnes maze. In the hippocampus, parental experience did not affect the density of microglia and the expression of perineuronal nets in either the dorsal or ventral dentate gyrus. These results demonstrate that parenthood remodels behaviour and affects anxiety and spatial cognition in a differential manner across sexes. However, these alterations in behaviour do not appear to be associated with changes in microglia or perineuronal net expression in the dentate gyrus, suggesting alternative regions and mechanisms are involved.

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### **List of abbreviations**

CA1	Cornu Ammonis 1 (region of the hippocampus)
CA2	Cornu Ammonis 2 (region of the hippocampus)
CA3	Cornu Ammonis 3 (region of the hippocampus)
DAPI	4;6-diamidino-2-phenylindole
DG	Dentate Gyrus
IBA-1	Ionized Calcium Binding Adaptor Molecule 1
IHC	Immunohistochemistry
mPOA	Medial Preoptic Area
MRI	Magnetic Resonance Imaging
NGS	Normal Goat Serum
PBS	Phosphate Buffer Saline
PNN	PNNs
PND	Postnatal Day
PPD	Postpartum Day
ROI	Region Of Interest
SLEAP	Social LEAP Estimates Animal Poses
WFA	Wisteria Floribunda Agglutinin

## **SECTION 1: THESIS GENERAL INTRODUCTION.**

The transition to parenthood is associated with neural, physiological, and behavioural adaptations. Mothers display significant increases in placental and steroid hormones (Duarte-Guterman & Gadea, 2023; Glynn et al., 2016), while at the same time, the brain is remodelling (Hoekzema et al., 2017; Servin-Barthet et al., 2023, 2025). Functionally, these hormones maintain pregnancy, facilitate parturition (Duarte-Guterman et al., 2019; Glynn et al., 2016) and play a role in the onset and development of maternal behaviour (Glynn et al., 2016). Furthermore, when comparing MRI scans of mothers that underwent pregnancy and nulliparous women (i.e., women that have never been pregnant), cortical and subcortical gray matter volume were reduced in the mother's brain (Hoekzema et al., 2017; Martínez-García et al., 2021; Pawluski et al., 2022; Servin-Barthet et al., 2025). The observed reduction in brain volume was found to persist up to 6 years after childbirth and is thought to facilitate postpartum maternal attachment towards the child (Hoekzema et al., 2017; Martínez-García et al., 2021; Pawluski et al., 2022; Servin-Barthet et al., 2025). Although males do not undergo pregnancy or parturition, fathers also exhibit hormonal fluctuations when comparing the period before and after their partners give birth (Bakermans-Kranenburg et al., 2022). In addition, first-time fathers exhibited a decrease in cortical brain volume and thickness when compared to non-fathers comparable to the reductions observed in mothers (Martínez-García et al., 2023). One of the subcortical areas that undergo significant plasticity throughout the peripartum period is the hippocampus, particularly because the hippocampus exhibits partial volume recovery at two years postpartum (Hoekzema et al., 2017). The hippocampus plays a role in regulating spatial learning, memory and anxiety-like behaviour making it a central region of interest (Duarte-Guterman et al., 2023; Knierim, 2015; Servin-Barthet et al., 2023). Despite the presence of parenthood-induced brain

volume changes, the behavioural adaptations accompanied by the transition to parenthood in contexts beyond caregiving behaviour, such as in cognitive and affective behaviours, are not well understood. Additionally, few studies have explored the potential neural mechanisms that underly the observed morphological changes in both sexes.

In addition to the complex changes that accompany parenthood, the experience of being a parent is diverse across mammalian species and ranges from biparental (i.e., cooperative rearing of offspring by both the mother and father) to monoparental maternal care (i.e., rearing of offspring only by the mother) (Abraham & Feldman, 2018; Daryanani et al., 2016). However, the behavioural and neural adaptations associated with different types of parental experiences are not well understood. To further explore the neural mechanisms that potentially underly these behavioural changes, we rely on laboratory rodents. The overarching objective of this study is to examine the behavioural and neuroplastic effects of different types of parental experience (biparental and single motherhood) in a rodent model.

Research on rodents indicate similar transformative changes to humans while also providing insight into the extent of behavioural and neural plasticity that arises due to parenthood. The late postpartum period in primiparous rat mothers (i.e., first-time mothers having undergone one pregnancy) is characterized by enhanced performance in hippocampal-dependent learning and memory tasks when compared to nulliparous controls (Cost et al., 2014; Pawluski, Vanderbyl, et al., 2006). Measures of anxiety-like behaviour during the late postpartum are more ambiguous, however. Various studies indicate a decrease in anxiety-like behaviour in rats during the late postpartum period and up to 8 weeks after weaning (Byrnes & Bridges, 2006; Duarte-Guterman et al., 2019; Wartella et al., 2003). Other studies in both mice and rats, however, have shown an increase in anxiety-like behaviour seven weeks after weaning

(Ladyman et al., 2018; Pawluski et al., 2009). Rodent fathers in biparental species exhibit similar behavioural adaptations to mothers when it comes to spatial learning and memory. Compared to naïve controls and sensitized males (i.e., males exposed to novel pups without previously engaging in sexual experience), California mice fathers exhibit enhanced performance in spatial learning and memory tasks during the early postpartum period (Franssen et al., 2011).

Fatherhood is also associated with changes in affective behaviour, however, the results are inconsistent across biparental rodent species. Studies on California mice have shown that fathers experience a reduction in anxiety-like behaviour (Glasper et al., 2016; Hyer et al., 2016) whereas prairie vole fathers display increased anxiety-like behaviour (Lieberwirth et al., 2013). These findings suggest that the experience of motherhood and fatherhood is accompanied by changes in spatial learning, memory and anxiety-like behaviour, but the effects may be species- and sex-specific. Studies on maternal experience focus almost exclusively on monoparental species, such as rats and mice, and few studies have explored the effects of motherhood in biparental species (Duarte-Guterman & Gadea, 2023). Furthermore, even less is known about the effect of monoparental maternal experience on the brain and behaviour in a biparental species (i.e., when the male partner is removed). To our knowledge, a direct comparison of cognitive and affective behaviours in both mothers and fathers of the same species has not been explored.

Ultimately, these behavioural adaptations in both mothers and fathers are accompanied by changes in neuroplasticity and related neural mechanisms (Duarte-Guterman et al., 2019).

Neuroplasticity associated with parenthood is well documented for particular mechanisms such as hippocampal neurogenesis and cortical brain volume, both of which are reduced throughout gestation and into the postpartum period (Duarte-Guterman et al., 2019, 2023; Eid et al., 2019;

Hyer et al., 2016). However, most of the research was conducted in female rats and little is known about the neural changes when becoming mothers and fathers in biparental species.

Microglia, the resident immune cells of the brain, secrete cytokines and perform phagocytosis during an inflammatory response (van Rossum & Hanisch, 2004). Microglia also regulate neuroplasticity by secreting neurotropic growth factors and pruning synapses, suggesting a role in learning, memory and synapse formation (Parkhurst, Yang, Ninan, Savas, John R. Yates, et al., 2013; van Rossum & Hanisch, 2004). During the late postpartum period, microglia density in the DG of the hippocampus was greater in biparous rat mothers (i.e., mothers having undergone two pregnancies) than in nulliparous females (Duarte-Guterman et al., 2023), but reduced in primiparous mothers (Eid et al., 2019; Haim et al., 2017). However, whether fatherhood induces changes in microglia expression and density have not been explored.

Microglia also interact with PNNs, which further contribute to neuroplasticity (Liu et al., 2021). PNNs are condensed extracellular matrixes that surround neurons, stabilize synapses, and restrict new synaptic connections by balancing excitatory and inhibitory neuronal connections (Fawcett et al., 2019; Härtig et al., 2022; Sanchez et al., 2024; Shaughnessy et al., 2024). Motherhood has also been found to alter the expression and organization of PNNs (Härtig et al., 2022; Lau et al., 2020). In the medial preoptic area, a region pivotal for maternal behaviour in rodents, PNNs become highly organized (i.e., increase in density and become uniformly assembled) in rat mothers, which contribute to stabilizing the neural circuits that mediate maternal behaviour (Uriarte et al., 2020). PNNs in the somatosensory cortex also exhibit an increase in expression after mice exhibit maternal behaviour (Lau et al., 2020). However, the alterations in expression of PNNs due to paternal experience have not been explored.

Furthermore, there is no available research on the expression of PNNs specifically in the hippocampus of either mothers or fathers.

The objective of this study is to uncover how different types of parental experiences influence cognitive and affective behaviour and remodel the brain in both mothers and fathers in a biparental rodent species, the *Octodon degus*. Degus allow us to examine parental experience in both sexes in addition to monoparental maternal experience when the male partner is removed. Therefore, this study aimed to investigate whether biparental experience (in both mothers and fathers) and single motherhood is associated with changes in spatial learning, memory and anxiety-like behaviour in the post-weaning period. From there, we examined neural mechanisms that potentially underly any behavioural changes, focusing on microglia and PNN density in the hippocampus. We predicted that (1) both mothers and fathers will exhibit changes in spatial learning, memory and anxiety-like behaviour compared to naïve degus, (2) both mothers and fathers will exhibit changes in the density of hippocampal microglia and PNNs compared to naïve degus, and (3) monoparental mothers will exhibit behavioural and neural changes compared to biparental mothers.

## **SECTION 2: LITERATURE REVIEW.**

### **2.1. Parental experience in humans**

#### 2.1.1. Different types of parental experience

In many mammalian species, maternal care is the primary form of parental investment due to the necessity of infant nutrition via lactation (Bales, 2017). As a result, the commonly observed parental dynamics in many mammals consist of monoparental, female care. However, for a minority of species (about 3 to 5% of mammalian species), fathers contribute to offspring care and engage in a range of parental behaviours that resemble the care of mothers, except for nursing (Abraham & Feldman, 2018; Leuner et al., 2010). Cooperative systems that exhibit shared care of offspring exist in the form of alloparental or biparental care (Bales, 2017; Kohl et al., 2017; Leuner et al., 2010). Alloparental care refers to systems in which caregiving is provided to the offspring by a group of individuals including the mother, father and other related or unrelated conspecifics (Bales, 2017; Leuner et al., 2010). Biparental care strictly refers to the mother-father dyad that is involved in taking care of their offspring (Leuner et al., 2010). Parenting refers to behaviours that involve nurturing, raising and protecting young and requires intense investment of resources (Kohl et al., 2017). However, research on family dynamics have focused mostly on their effects on offspring, with few studies examining how these different types of parental systems have an effect on mothers and fathers.

In human beings, the experience of being a parent is diverse and family systems are influenced by various external factors. Although typical parental dynamics in humans comprise of both mothers and fathers taking care of their children, parental care is highly variable across societies and cultures (Abraham & Feldman, 2018; Kohl et al., 2017). Socio-cultural and idiosyncratic factors may lead to family systems that are observed in other mammalian species

such as alloparental or single-parent households (Abraham & Feldman, 2018; Daryanani et al., 2016). Across Canada, Australia and the United States, 11 to 33% of families consist of single-parent homes, with 83 to 87% of these being led by single mothers (Abraham & Feldman, 2018; Cheeseman et al., 2011; Daryanani et al., 2016; Gucciardi et al., 2004). According to census data, single-mother households are substantially more likely to live in poverty and experience social discrimination, increasing the likelihood of experiencing depression, anxiety and social isolation (Daryanani et al., 2016; Goodrum et al., 2012). Nonetheless, the cognitive and affective behavioural changes that are associated with different types of parental experiences are not well understood.

#### 2.1.2. Physiological and behavioural effects of parental experience in humans

The transition to parenthood is a transformative event marked by behavioural adaptations that are driven by a range of physiological and endocrinological alterations. Throughout pregnancy, human mothers exhibit cardiovascular and respiratory changes with increases in blood cell volume, coagulation factors, cardiac output, respiratory rate, and oxygen consumption (Heidemann & McClure, 2003; Puri et al., 2023). These changes facilitate the development of the fetus and placenta and although some return to near baseline levels after childbirth, many cardiovascular alterations persist for several weeks into the postpartum period (Chauhan & Tadi, 2025; Heidemann & McClure, 2003). Additionally, these physiological changes are driven by significant increases in placental and steroid hormones (Puri et al., 2023). Peripheral hormones such as estrogens, progestogens, and glucocorticoids increase to maintain pregnancy, facilitate safe delivery of the newborn, and initiate maternal behaviour during the peripartum period (Brunton & Russell, 2010; Duarte-Guterman et al., 2019; Glynn et al., 2016). Changes in the

concentrations of estradiol and progesterone from pregnancy to the postpartum have implications on postpartum attachment to the newborn, which also correlates with the mothers' feeling of well-being (Fleming et al., 1997; Glynn et al., 2016). Mothers exhibiting higher quality maternal caregiving up to one-year postpartum had a slower acceleration of estradiol increase and a lower estradiol to progesterone ratio during gestation (Fleming et al., 1997; Glynn et al., 2016). Furthermore, increases in oxytocin and prolactin levels persist well into the postpartum to initiate lactation and enhance maternal bonding through increasing sensitive mothering, positive affect, and affectionate contact towards the newborn (Brunton & Russell, 2010; Feldman et al., 2007; Servin-Barthet et al., 2023).

Despite being understudied, human fathers also undergo hormonal fluctuations when comparing the period before and after their partners give birth (Bakermans-Kranenburg et al., 2022). Although the functional implications of these changes are not entirely clear, fathers exhibit decreases in testosterone and cortisol levels when compared to individuals that were never fathers (Bakermans-Kranenburg et al., 2022; Berg & Wynne-Edwards, 2001; Gettler et al., 2011). Previous research suggests that lower testosterone salivary concentrations are associated with greater paternal involvement in caretaking and increased emotional sensitivity to the calls of the newborn, allowing fathers to respond more effectively (Bakermans-Kranenburg et al., 2022; Fleming et al., 2002; Gettler et al., 2011). In contrast, increased salivary concentrations of oxytocin and estradiol, which were thought to play a pivotal role in paternal behaviour, are not observed consistently across studies (Bakermans-Kranenburg et al., 2022; Berg & Wynne-Edwards, 2001; Saltzman & Ziegler, 2014).

The literature investigating changes in cognitive functioning and feelings of anxiety experienced by mothers are equivocal. Various studies have indicated cognitive deficits in

pregnant women when compared to never-pregnant controls (reviewed in Anderson & Rutherford, 2012) whereas others found no difference between these groups (Logan et al., 2014; reviewed in Macbeth & Luine, 2010). Although measures of working, recall, and recognition memory were reduced in the postpartum period compared to non-mothers, many studies found the effects to be relatively small and not statistically significant (reviewed in Anderson & Rutherford, 2012; reviewed in Macbeth & Luine, 2010). Studies investigating mothers up to one-year postpartum found that individuals that reported worse subjective memory also reported poorer sleep and higher anxiety- and depressive-like symptoms (Casey et al., 1999; Logan et al., 2014; Orchard et al., 2022). However, no objective measures of cognition (i.e., short-term, working, explicit, and semantic memory tests) were different compared to non-mothers (Casey et al., 1999; Logan et al., 2014; Orchard et al., 2022). In regard to measures of affect, self-reports from mothers indicate a decrease in feelings of anxiety and depression when transitioning from pregnancy to the postpartum period (Heron et al., 2004; Macbeth & Luine, 2010; Pawluski et al., 2017; Pio de Almeida et al., 2012). One hypothesis is that breastfeeding mothers during the postpartum period experience reduced reactivity to stressful events, acting as a protective measure against the deleterious effects of stress (Groer et al., 2002; Tu et al., 2005). Previous research supports this claim, indicating that lactating mothers releasing oxytocin experience a reduction in feelings of anxiety and exhibit an attenuated stress response to physical stressors (Altemus et al., 1995; Tu et al., 2005; Uvnäs-Moberg, 1998). A follow-up study, however, found that there were no differences in physiological measures of the stress response between pregnant, postpartum and never-pregnant women during speech and arithmetic tasks (Altemus et al., 2001). These results suggest that although lactating mothers tend to experience a reduction in feelings of anxiety, attenuation of the stress response is stressor-dependent (Altemus et al., 2001; Tu et al.,

2005). However, a significant portion of healthy, postpartum women may experience postpartum depression which is often comorbid with anxiety (Pawluski et al., 2017). Postpartum depression is characterized by symptoms that resemble major depressive disorder following parturition which includes sadness, restlessness, impaired concentration, and agitation, and affects 10 to 20% of healthy mothers worldwide (Campbell & Cohn, 1991; Patel et al., 2012; Pawluski et al., 2017; Shorey et al., 2018).

Research exploring the effects of fatherhood on cognitive function and anxiety in human beings is limited and scarce. A longitudinal study that compared the working memory of mothers and fathers during pregnancy and in the postpartum period found no differences in their cognition at either time point when compared to each other (Pieters et al., 2021). However, this study lacked a non-parental group with comparisons limited to mothers and fathers. Although no differences were detected between parents, there may be differences between fathers and non-fathers. Similar to mothers, fathers also experience a decrease in feelings of anxiety and stress when comparing the period before and after their partner gives birth (Figueiredo & Conde, 2011; reviewed in Philpott et al., 2017, 2019). The prevalence of paternal perinatal anxiety ranges from 4 to 25% worldwide. Nevertheless, various studies found that a portion of fathers who were highly anxious during their partner's pregnancy had reduced feelings of anxiety in the postpartum, suggesting that fathers experience emotional changes in the transition from their partner's pregnancy to fatherhood (Figueiredo & Conde, 2011; Leiferman et al., 2021; reviewed in Philpott et al., 2019). Endocrinological changes associated with parenthood have been detected in the peripheral circulation. Many of these hormones are capable of entering the brain or are synthesized within it, potentially leading to notable behavioural and neural changes (Brunton & Russell, 2010).

### 2.1.3. Neural effects of pregnancy, postpartum and parental experience in humans

Various studies have described changes in brain volume in mothers during pregnancy and the postpartum period (Hoekzema et al., 2017; Martínez-García et al., 2021; Pritschet et al., 2024; Servin-Barthet et al., 2025). When comparing functional magnetic resonance imaging (MRI) scans of mothers that underwent pregnancy and never-pregnant women, a reduction in cortical gray matter volume was observed as early as the second trimester and persisted for at least six years after parturition (Hoekzema et al., 2017; Martínez-García et al., 2021; Servin-Barthet et al., 2025). The effects of pregnancy on the brain are so robust that women can be classified as mothers or non-mothers with over 90% accuracy based solely on gray matter volume changes (Martínez-García et al., 2021). Several neural regions involved in social processing and cognition show a decrease in gray matter volume associated with pregnancy, including the lateral prefrontal and temporal cortex, fusiform gyrus, cingulate cortex, and precuneus (Hoekzema et al., 2017, 2022; Martínez-García et al., 2021; Pritschet et al., 2024). These higher-order regions are part of the default mode network and play a role in cognitive flexibility, social cognition, social evaluation, and empathy (Andrews-Hanna, 2012; Hoekzema et al., 2022; Mars et al., 2012). As a result, the extent of pregnancy-induced neuroanatomical changes is associated with indices of maternal care and mother-to-infant relationship (Hoekzema et al., 2017; Servin-Barthet et al., 2023, 2025). For instance, gray matter volume changes predicted nesting behaviour during pregnancy, higher quality attachment and absence of hostility towards the newborn in the postpartum (Hoekzema et al., 2017; Martínez-García et al., 2021; Servin-Barthet et al., 2023, 2025). Furthermore, mothers who reported experiencing greater pleasure when interacting with their child exhibited greater decreases in gray matter volume than

mothers who reported lower scores of attachment, suggesting a relationship between attachment quality and gray matter volume loss (Martínez-García et al., 2021). Although the hippocampus also exhibits a substantial reduction in gray matter volume after parturition, there is partial recovery observed at two and six years postpartum, whereas other neural regions exhibit little to no recovery (Hoekzema et al., 2017; Martínez-García et al., 2021). These results further highlight the substantial plasticity of the hippocampus and its potential involvement in parenthood.

Although fathers do not undergo pregnancy, some studies have shown reductions in cortical gray matter volume and cortical thickness when comparing the period before and after their partner gives birth and also when compared to non-fathers (Martínez-García et al., 2023; Orchard et al., 2020; Paternina-Die et al., 2020). The regions most affected in fathers include the orbitofrontal and cingulate cortex, fusiform gyrus, and precuneus, with changes observed up to 16 weeks postpartum (Kim et al., 2014; Martínez-García et al., 2023; Paternina-Die et al., 2020). Furthermore, greater reductions in brain volume were positively correlated with increased neural activation in response to infant cues, including visual and auditory stimuli, in fathers (Horrell et al., 2021; Mascaro et al., 2014; Paternina-Die et al., 2020). However, other studies found no difference in neural volume between fathers and non-fathers at ten weeks postpartum, attributing the neural changes to pregnancy rather than the effect of parenthood (Hoekzema et al., 2017).

In summary, the transition to parenthood is accompanied by an early onset of brain remodeling that extends long after childbirth, especially in mothers (Hoekzema et al., 2017; Martínez-García et al., 2021; Paternina-Die et al., 2020; Servin-Barthet et al., 2023, 2025). However, the functional adaptation of these neuroanatomical changes is unclear since the research on humans investigating the relationship between neural changes and behaviour are

correlational. To further explore the mechanisms underlying these neural adaptations and control for various confounding factors (e.g., timing of pregnancy, number of pregnancies, age of the parents, etc.), we rely on laboratory animal models.

## **2.2. Behavioural effects of parental experience in rodents**

### **2.2.1. Changes in cognition in rodent mothers during the postpartum period**

Although parenthood is accompanied by physiological and behavioural adaptations, little is known about the effects of parental experience in contexts beyond caregiving behaviour (Leuner et al., 2010). Laboratory rats have been used extensively as a model to study maternal experience, with a minority of studies on mice (Duarte-Guterman & Gadea, 2023). Rodent mothers experience changes in cognition throughout pregnancy and in the early and late postpartum period (reviewed in Duarte-Guterman et al., 2019; reviewed in Pawluski et al., 2022). The literature on motherhood typically defines the early postpartum as the first week after birth and the late postpartum as the period around weaning (including the week before and the week after weaning). First-time rat mothers in the early postpartum, between postpartum day (PPD) one to four had impaired spatial memory relative to nulliparous females (Darnaudéry et al., (2007). However, one confound is that mothers are separated from their pups to complete behavioural tasks, which may induce stress and have a significant effect on their behaviour.

In the late postpartum period, around the time of weaning the pups, primiparous rat mothers (i.e., first-time mothers having undergone a single pregnancy) exhibited enhanced spatial and working memory compared to nulliparous females (Cost et al., 2014; Pawluski, Vanderbyl, et al., 2006; Pawluski, Walker, et al., 2006). Primiparous mothers made fewer working and reference memory errors in the baited radial arm maze when compared to

nulliparous controls by making fewer entries into non-baited arms and fewer repeated entries into baited arms (Pawluski, Vanderbyl, et al., 2006; Pawluski, Walker, et al., 2006). However, various studies comparing primiparous mothers to sensitized females (i.e., females exposed to pups that exhibit maternal care without having undergone pregnancy) found no difference in hippocampal-dependent tasks (Kinsley et al., 1999; Pawluski, Vanderbyl, et al., 2006). These results suggest that although mothering alone has an effect on behaviour, the combination of pregnancy and experience of motherhood yields the greatest effects on cognitive abilities in the late postpartum period (Kinsley et al., 1999; Pawluski, Vanderbyl, et al., 2006). Additionally, these studies suggest that first-time mothers exhibit enhanced spatial, reference and working memory even when tested between two to 35 days after weaning (Cost et al., 2014; Pawluski, Vanderbyl, et al., 2006; Pawluski, Walker, et al., 2006). For instance, mothers displayed enhanced performance in the object placement task even when tested at 42 days postpartum (Pawluski, Vanderbyl, et al., 2006). One proposal for an increase in spatial learning and memory of mothers during the late postpartum period is that it allows for more efficient foraging and ensures that mothers spend less time off the nest, away from the pups (Kinsley & Lambert, 2008; Macbeth & Luine, 2010). Overall, rat mothers exhibit changes in cognitive functioning in spatial learning and memory tasks throughout pregnancy and the postpartum period.

Laboratory rats are frequently used as the model organism to study the changes in cognitive function of mothers. However, parental care in rats consist solely of monoparental maternal care because male rats do not engage in caretaking behaviour (Stolzenberg & Mayer, 2019). The cognitive changes exhibited by mothers in biparental rodent species, such as California mice, prairie voles, and degus, have not been explored.

### 2.2.2. Changes in anxiety-like behaviour in rodent mothers during the postpartum period

The results from studies examining anxiety-related behaviours in rodent mothers during the postpartum period are equivocal (Duarte-Guterman et al., 2019; Duarte-Guterman & Gadea, 2023; Macbeth & Luine, 2010). However, the differences observed across studies may be attributed to the timing of the postpartum period when behavioural measures are conducted. Studies utilizing the elevated plus maze as a measure of anxiety-like behaviour suggest that primiparous rat mothers exhibit a reduction in anxiety during the early postpartum (within the first week after giving birth) (Lonstein, 2005; M. Pereira et al., 2005). These studies show that mothers spent more time in the open arms and had more frequent crossings into the open arms of the maze compared to sensitized and nulliparous females (Lonstein, 2005; M. Pereira et al., 2005). Additionally, rat mothers also exhibited more exploratory behaviours in the open field during the early postpartum; mothers crossed the centre of the field and changed quadrants more frequently compared to sensitized and nulliparous controls, further suggesting a reduction in anxiety-related behaviours (Agrati et al., 2008; Fleming & Luebke, 1981). Lastly, a study using the light-dark box as a measure of anxiety found that rat mothers in the first week postpartum spent more time in the light compartment and transitioned between chambers of the box more frequently compared to nulliparous controls, indicating reduced anxiety-like behaviour (Miller et al., 2011). The results from various studies indicated that reproductive and maternal experience in primiparous rats alters the expression of anxiety-related behaviour, particularly when tested during the early postpartum period. However, as noted previously, separation from pups during this period may act as a significant confound that affects the mothers' behaviour. Mothers during the early postpartum exhibit high levels of maternal aggression, which may contribute to reduced anxiety-like behaviour as mothers search for pups (Lonstein, 2005; Lonstein & Gammie, 2002).

This point is made evident in a series of studies that explore the effects of recent pup contact on anxiety-related behaviours of mothers in which it is necessary for rat mothers to receive contact with pups up to four hours before testing to observe a reduction in anxiety-related behaviours (Lonstein, 2005; Miller et al., 2011). Additionally, separation of the pups from mothers beyond four hours induced anxiety-like behaviours in both the elevated plus maze and the light-dark box indicating the dependency on recent pup contact (Lonstein, 2005; Miller et al., 2011).

In the late postpartum period, most findings in the literature exploring anxiety-related behaviours suggest a reduction in anxiety-like behaviour, though a few studies report an increase. Similar to the early postpartum, some studies have found that rat mothers exhibit a decrease in anxiety-like behaviours up to 8 weeks after weaning across various behavioural tests (Byrnes & Bridges, 2006; Love et al., 2005; Wartella et al., 2003). For instance, in the open field, a study found that rat mothers exhibited a decrease in anxiety-related behaviours 2 weeks after weaning (Wartella et al., 2003). In this study, primiparous rats displayed a greater frequency of rearing, total number of centre crosses, changes in the number of quadrants explored, and a reduced frequency of freezing (Wartella et al., 2003). Additionally, primiparous rats tested on the elevated plus maze at six to eight weeks post-weaning spent more time in the open arms compared to nulliparous controls (Byrnes & Bridges, 2006). However, a potential confounding factor is that primiparous mothers also exhibited greater total distance travelled which may inflate the duration in the open arms. Further, one study reported that primiparous rat mothers tested one week after weaning exhibit increased anxiety-like behaviour in both the open field and elevated plus maze (Pawluski et al., 2009). Mothers crossed the centre of the open field less and spent more time in the closed arms of the elevated plus maze compared to naïve females (Pawluski et al., 2009).

In contrast to these results, two studies found that primiparous rat mothers at three weeks postpartum did not exhibit differences in anxiety-related behaviour when compared to nulliparous controls (Lonstein, 2005; Rincón-Cortés & Grace, 2020). Primiparous rats at three weeks postpartum exhibited no differences in the time spent in the open arms and the frequency of crossings between the arms of the elevated plus maze (Lonstein, 2005; Rincón-Cortés & Grace, 2020). These results suggest no change in anxiety of rat mothers in the late postpartum when compared to nulliparous controls. In these studies, the pups were not weaned before behavioural testing and mothers were kept with their litter. This procedure is in contrast to the previous studies that explored the late postpartum period whereby all pups were weaned before behavioural testing (Byrnes & Bridges, 2006; Love et al., 2005; Wartella et al., 2003). An effect of recent pup contact on the behaviour of mothers is suggested in a study in which primiparous mothers displayed increased anxiety-related behaviour in the EPM compared to nulliparous controls and no increase at two and seven weeks post-weaning (five and ten weeks postpartum, respectively) (Furuta et al., 2013). These results also align with a study that indicated primiparous rat mothers tested two weeks after weaning did not differ from nulliparous females in measures of anxiety-like behaviour in either the open field or elevated plus maze (Lemaire et al., 2006). Whether the pups were weaned prior to behavioural testing (and how long after weaning the mothers were tested) may account for some of the differences observed across studies. Additionally, mice mothers exhibited increased anxiety-related behaviours eight weeks after the pups were weaned (Ladyman et al., 2018); mice mothers spent less time in the open arms of the elevated plus maze and exhibited less total distance travelled on the maze compared to nulliparous controls (Ladyman et al., 2018). These results suggest that the expression of

anxiety-related behaviours in rodent mothers may be different across species during the late postpartum period.

In biparental rodent species, two studies have compared anxiety-like behaviour in the early to mid-postpartum between biparental mothers (i.e., both the mother and father take care of pups in the postpartum period), monoparental mothers (i.e., the father is removed a day after mothers give birth, allowing only the mothers to take care of the offspring in the postpartum), and nulliparous females (Bosch et al., 2018; Zhao et al., 2019). In California mice, both biparental and monoparental mothers produced more fecal boli in the open field than did nulliparous female controls, which is typically inferred as an indicator of increased anxiety (Zhao et al., 2019). The California mice were tested at PPD 18, about halfway into the postpartum period as California mice pups are weaned between four to five weeks (Zhao et al., 2019). However, the authors suggested that the increased bowel movements was a condition of parturition and lactation rather than anxiety. Furthermore, there were no differences between maternal groups in any of the behavioural measures (including the open field and tail-suspension test) (Zhao et al., 2019). In prairie voles, primiparous mothers separated from their male partners exhibited increased anxiety-like behaviour (tested at PPD 2) on the elevated plus maze compared to non-separated mothers (Bosch et al., 2018). These results suggest that anxiety-related behaviours in mothers may differ between biparental and monoparental rodent species, with partner separation differentially affecting mothers across biparental species. Ultimately, the current literature on changes in anxiety-related behaviour of primiparous rodent mothers is equivocal and dependent on the timing of the postpartum. Research on the effects of maternal experience in biparental species across different timepoints of the postpartum period is required.

Furthermore, the underlying neural mechanisms driving such behavioural changes are unclear and require investigation.

### 2.2.3. Changes in cognition in rodent fathers during the postnatal period

Few studies have explored the effects of fatherhood on cognitive functioning. In California mice, first-time fathers one week after their pups were born exhibited enhanced spatial learning and memory when tested on a baited dry land maze and compared to both virgin males (i.e., males with no sexual experience and no pup exposure) and pup-exposed males (i.e., males with no sexual experience but exposed to pups and exhibited paternal care) (Franssen et al., 2011). The dry land maze is divided into three phases: a three-day habituation, a single initial acquisition day, and four test days. Over the course of the three habituation days, the number of baited wells decreases by half; all eight wells are baited on the first day, followed by four baited wells on the second day, and two baited wells on day three. On the initial acquisition day, only one out of the eight wells are baited, and this baited well remained the same over the course of four testing days. Fathers did not show behavioural differences from the other groups in the habituation phase and the testing phase of the baited dry land maze, exhibiting similar latencies to approach the baited well (Franssen et al., 2011). On the initial acquisition day however, fathers performed better compared to both virgin and pup-exposed males. Fathers displayed shorter latencies to approach the baited well while also approaching more non-baited wells, suggesting enhanced cognitive flexibility compared to virgin and pup-exposed males (Franssen et al., 2011). Because the initial acquisition phase was the most difficult day of the task, the results suggest that fathers outperform non-fathers on more challenging tasks (Franssen et al., 2011). In another study, there were no differences in recognition memory between fathers and non-fathers in the

object recognition task when tested at 3 weeks after their offspring were born (Glasper et al., 2011). However, the lack of difference may reflect a floor effect because both fathers and non-fathers failed to show a preference for the novel object in their investigation time, suggesting that a 24 hour delay between the stages was too long for retention of the familiar object (Glasper et al., 2011). This suggestion was supported by a recent study of California mice fathers tested two weeks after their pups were born (Colt et al., 2025). In the object recognition task, where the delay between the acquisition and testing phase was 15 minutes, first-time fathers spent more time investigating the novel object compared to virgin males suggesting enhanced recognition memory (Colt et al., 2025). These results confirm that the cognitive adaptations associated with parenthood are not solely driven by pregnancy or lactation because they are present in both fathers and mothers. Yet to this date, a direct behavioural comparison between mothers and fathers of the same species has not been done. Nonetheless, the results suggest that the behavioural modifications that have been observed are likely driven by a combination of sexual experience and pup-exposure in addition to endocrinological and neural changes (Glasper et al., 2011; Horrell et al., 2021; Leuner et al., 2010).

### 2.2.2. Changes in anxiety-like behaviour in rodent fathers during the postnatal period

Similar to mothers, testing fathers on behavioural tasks that measure anxiety-related behaviour is dependent on the timing of the postnatal period. The results from various studies on California mice suggest that a reduction in anxiety-related behaviour of first-time fathers are robustly observed around 16 to 19 days after their pups are born (a time in which paternal care is at its highest in this species) (Colt et al., 2025; Glasper et al., 2011; Hyer et al., 2016). California mice fathers tested one week after their pups were born did not differ from non-fathers on the

novelty-suppressed feeding task, suggesting no differences in anxiety-like behaviour (Glasper et al., 2011). In contrast, another study found that California mice fathers had a reduction in anxiety-related behaviour on the elevated plus maze, spending more time in the open arms, compared to virgin males at postnatal day (PND) 16 but not at PND two or nine (Hyer et al., 2016). A more recent study also reported similar results, whereby California mice fathers exhibited reduced anxiety-like behaviour on the elevated plus maze when tested two weeks after their pups were born compared to both virgin males and pup-sensitized males (Colt et al., 2025). In another biparental species, the prairie vole, fathers exhibited an increase in anxiety-like behaviour when tested one week after their pups were born (Lieberwirth et al., 2013). In this study, fathers exhibited reduced time spent in the open arms and the number of open arm entries compared to both virgin and sexually experienced males (i.e., males placed with ovariectomized females to acquire sexual experience but lacking paternal experience). These results suggest that fatherhood leads to differences in the expression of anxiety-related behaviours across biparental rodent species (Colt et al., 2025; Lieberwirth et al., 2013). Nonetheless, the neural mechanisms driving such behavioural changes in fathers are not entirely known.

## **2.3. Hippocampal neuroplasticity and neuroinflammation in rodents**

### **2.3.1. Hippocampus structure and function**

The hippocampus is responsible for regulating spatial navigation, learning, memory formation, information processing, and emotion regulation (Knierim, 2015; Leuner & Gould, 2010b; Pradip Chauhan et al., 2021; Strange et al., 2014). The trisynaptic pathway of the hippocampus is a memory circuit that is critical for emotional integration and short-term memory formation (Knierim, 2015; Pradip Chauhan et al., 2021). Projections from the dentate gyrus (DG)

send input to the pyramidal cells of the CA3 via the mossy fiber pathway, and CA3 pyramidal cells connect to the apical dendrites of the CA1 via the Schaffer collateral pathway (Knierim, 2015; Pradip Chauhan et al., 2021; H.-J. Shi et al., 2023). Pathways within the CA1 and CA3 regions of the hippocampus play a central role in processing spatial and temporal cues (Kesner et al., 2004). Neural regions beyond the hippocampus are also involved in this circuit and are critical for memory formation. Although the entorhinal cortex provides majority of the cortical inputs to the hippocampus, additional projections come from the cingulate gyrus, perirhinal, and postrhinal cortices, along with projections from various subcortical regions, including the amygdala, medial septum, and raphe nucleus (Knierim, 2015). From there, the CA3 and CA1 regions of the hippocampus will form complex connections and feedback circuits by projecting back to the entorhinal cortex, amygdala, prefrontal cortex, and nucleus accumbens (Knierim, 2015).

In rodents, the hippocampus is divided along the dorsal-ventral axis (which corresponds to the anterior-posterior axis in humans) into three primary regions: the dorsal, intermediate, and ventral hippocampus (Bannerman et al., 2004; Knierim, 2015; Strange et al., 2014). The dorsal hippocampus is primarily involved in learning and memory, specifically spatial memory, and receives most of its input from the medial entorhinal and retrosplenial cortices (Bannerman et al., 2004; Knierim, 2015; Strange et al., 2014). Lesions specific to the dorsal region, leaving about 60% of the remaining hippocampus intact, impairs spatial learning and spatial reference memory (Bannerman et al., 2003; M. B. Moser et al., 1995; M.-B. Moser & Moser, 1998). In contrast, leaving the dorsal hippocampus intact and ablating nearly 75% of the rest of the hippocampus, allows for the acquisition and retrieval of spatial memory (Bannerman et al., 2003; M. B. Moser et al., 1995; M.-B. Moser & Moser, 1998). Such results are observed across various tasks that

measure spatial learning and memory. For instance, rats with lesions in the dorsal region took longer to find the hidden platform on the Morris water maze (Bannerman et al., 2003; M. B. Moser et al., 1995), and entered more incorrect arms on the rewarded T-maze (Bannerman et al., 2002) and radial arm maze (Pothuizen et al., 2004) compared to both sham operated controls and animals with lesions only to the ventral hippocampus (Strange et al., 2014; M. A. Wilson & McNaughton, 1993). Alternatively, the ventral hippocampus is primarily involved in emotional memory and regulates anxiety-related behaviour, receiving majority of inputs from the amygdala, infralimbic, prelimbic, and prefrontal cortices (Bannerman et al., 2004; Knierim, 2015; Strange et al., 2014). Lesions to the ventral hippocampus alter anxiety-like behaviour and reduce neuroendocrine responses without impacting spatial learning and memory (Bannerman et al., 2003; K. G. Kjelstrup et al., 2002). For instance, rats with lesions to the ventral hippocampus exhibited a reduced latency to enter the dark compartment in the light-dark box task (Bannerman et al., 2003) and spent more time on the open arms of the elevated plus maze (K. G. Kjelstrup et al., 2002). Nonetheless, the functions of the dorsal and ventral hippocampus are overlapping. For instance, place cell activity in the ventral hippocampus suggests that it is implicated in large-scale spatial processing and the role of the dorsal hippocampus in conditioned fear suggests that it contributes to emotional memory (K. B. Kjelstrup et al., 2008; Strange et al., 2014). While the hippocampus is established as a central region in spatial memory and anxiety-like behaviour, the mechanisms underlying parenthood-induced hippocampal and behavioural changes in both sexes are not clear.

### 2.3.2 Effects of parental experience on the hippocampus

The behavioural adaptations associated with parenthood are accompanied by various alterations in the brain. Although hormonal correlates have been explored to account for these behavioural changes in motherhood (Barha et al., 2015; reviewed in Duarte-Guterman et al., 2019; reviewed in Leuner et al., 2010), the neural mechanisms are not completely understood. The hippocampus undergoes substantial neuroplastic changes throughout the peripartum period and is critically involved in cognitive and affective processes (Duarte-Guterman et al., 2019; Eid et al., 2019; Macbeth & Luine, 2010). As discussed previously, parental experience was observed to alter spatial learning, memory, and anxiety-like behaviour, and because the hippocampus is central to these cognitive functions, the hippocampus has become a neural region of focus (Duarte-Guterman et al., 2019; Eid et al., 2019; Macbeth & Luine, 2010). As in human beings, primiparous rats exhibited a reduction in hippocampal volume during the peripartum period (Galea et al., 2000; Hillerer et al., 2014). Between the first and second week of the postpartum, hippocampal volume of primiparous rats was smaller than nulliparous female controls (Hillerer et al., 2014). Furthermore, structural changes in the hippocampus are coupled with neuronal and molecular changes in the postpartum period (Duarte-Guterman et al., 2023; Puri et al., 2023; Strange et al., 2014). For instance, in the early postpartum, primiparous rats exhibited increased dendritic spine density in ventral CA1 pyramidal neurons compared to nulliparous females (Leuner & Gould, 2010). In contrast, in the late postpartum period, primiparous rats exhibited a reduction in neuronal dendritic lengths and fewer branch points of dorsal CA1 and CA3 pyramidal neurons (Pawluski & Galea, 2006). Furthermore, hippocampal neurogenesis in the DG is decreased during pregnancy, starting at gestation day 13 (Eid et al., 2019), and remains lowered in the late postpartum (even after weaning) compared to nulliparous controls

(Darnaudéry et al., 2007; Eid et al., 2019; Leuner et al., 2007; Lieberwirth et al., 2013; Pawluski & Galea, 2007). In biparental rodent species, parental experience induces hippocampal changes in both males and females (Glasper et al., 2011, 2016; Lieberwirth et al., 2013). In California mice, as in primiparous rats mothers, fathers at 19 days in the postnatal period exhibited increased dendritic spine density of DG and CA1 pyramidal neurons relative to non-fathers (Glasper et al., 2016). Furthermore, around the time of weaning, both California mice mothers and fathers had a decrease in hippocampal neurogenesis compared to naïve controls (Glasper et al., 2011). Similarly, prairie vole fathers (when tested within the first week of the postnatal period) displayed decreased neuron cell survival in the DG of the hippocampus, without affecting cell proliferation, relative to non-fathers (Lieberwirth et al., 2013). These results suggest that parental experience in both sexes lead to alterations in hippocampal plasticity. Yet the exact mechanisms that drive such changes in the hippocampus of mothers and fathers are unknown. Although hormonal influences are frequently proposed as a primary driving factor, there is increasing evidence indicating the importance of neuroimmune signaling on hippocampal plasticity that requires further investigation (reviewed in Duarte-Guterman et al., 2019; Eid et al., 2019; reviewed in Galea et al., 2014; Glasper et al., 2016; reviewed in Puri et al., 2023).

### 2.3.3. Function of microglia

Microglia are the resident immune cells of the brain that have neuroprotective functions (Borst et al., 2021; van Rossum & Hanisch, 2004). For instance, microglia are capable of initiating phagocytic responses and recruit peripheral immune cells to mediate either an inflammatory or immunosuppressive response (Borst et al., 2021; van Rossum & Hanisch,

2004). For a healthy brain, it is essential to maintain a balance between pro-inflammatory and anti-inflammatory markers that are released by microglia since imbalances have been observed to have detrimental consequences, such as increasing the risk of neurodegenerative diseases (Cornell et al., 2022; Golia et al., 2019; Nayak et al., 2014). Beyond neuroimmune functions, microglia also play a critical role in neural plasticity by supporting and regulating neuronal and synaptic activity (Borst et al., 2021; Cornell et al., 2022; Green & Rowe, 2024; van Rossum & Hanisch, 2004). For instance, microglia carry out synaptic pruning, which refers to the process of targeting weak or immature synapses to eliminate them via engulfment (Cornell et al., 2022; Nayak et al., 2014). Microglia in the DG have been observed engulfing both pre- and post-synaptic components (including synaptophysin and PSD95<sup>+</sup>), confirming the presence of synaptic pruning in the hippocampus (Wang et al., 2020). The removal of weak synaptic connections are critical for shaping and refining neural circuits, increasing neuronal network efficiency (Cornell et al., 2022; Nayak et al., 2014). Furthermore, microglia are capable of releasing various signaling molecules and trophic factors that are essential in facilitating neuronal health, survival, and maturation, including brain-derived neurotrophic factors, nerve growth factor, and insulin-like growth factor-1 (Cornell et al., 2022; Nayak et al., 2014). Microglia morphology is characterized by the extent of their ramified processes which provide some insight into their function and is remarkably plasticity (Green & Rowe, 2024). Homeostatic microglia possess highly branched processes and play a role in surveilling the neural environment, monitoring synapses and maintaining neural circuits (Augusto-Oliveira et al., 2025; Green & Rowe, 2024; Kettenmann et al., 2011; Paolicelli et al., 2022). The terminal branches of homeostatic microglia are motile and allow them to survey the microenvironment and engage in phagocytosis of apoptotic cells, implicating their role in the support and regulation

of neurogenesis (Augusto-Oliveira et al., 2025; Cornell et al., 2022; Sierra et al., 2010).

Microglia are also able to transition to a morphology with retracted processes and an enlarged cell-body to further regulate and shape neural connectivity (Augusto-Oliveira et al., 2025; Green & Rowe, 2024). Ultimately, microglia play an important role in mediating neuronal and synaptic plasticity to modulate cognition and behaviour.

#### 2.3.4. Function of microglia on hippocampal dependent behaviour

As key regulators of synaptic plasticity, microglia also play a crucial role in regulating hippocampal-dependent learning and memory (Cornell et al., 2022; Parkhurst, Yang, Ninan, Savas, John R. Yates, et al., 2013). For instance, targeted depletion of hippocampal microglia in mice via clodronate injection (a drug used to eliminate microglia populations by inducing apoptosis when phagocytosed by macrophages (Han et al., 2019)) into the CA1 region resulted in impaired spatial memory on the Barnes maze task (Torres et al., 2016). Mice with microglia depletion took longer to approach the target hole and visited more non-target holes than did wild type mice (Torres et al., 2016). In another cohort of mice, when microglia were allowed to recover and repopulate the hippocampus, their performance in the Barnes maze did not differ from that of wild type mice (Torres et al., 2016). Furthermore, another study found that microglia depletion in mice led to deficits in both fear conditioning and novel object recognition tasks (Parkhurst et al., 2013). Microglia knock-out mice also exhibited a decrease in postsynaptic dendritic spines, which are formed in the process learning (Parkhurst, Yang, Ninan, Savas, John R. Yates, et al., 2013). These results further suggest that microglia play a critical role in dendritic spine formation, likely through the secretion of brain-derived neurotrophic factors (Parkhurst, Yang, Ninan, Savas, John R. Yates, et al., 2013). Furthermore, two studies found that

repopulation of microglia (i.e., after acute deletion, surviving microglial populations recover over time) transiently enhanced short-term recognition and object-placement memory in healthy rats while also preserving cognition in older mice that would otherwise show age-induced memory impairments (De Luca et al., 2020; Elmore et al., 2018). In addition to the observed cognitive enhancements, repopulation of microglia increased hippocampal synaptic spine densities when compared to unaffected animals (De Luca et al., 2020; Elmore et al., 2018). Microglia also regulate memory retrieval by mediating forgetting, which occurs when engram cells become dissociated and cannot be reactivated (Cornell et al., 2022; C. Wang et al., 2020). The depletion of microglia in the DG of mice prevented forgetting of contextual fear memories by increasing the time of freezing compared to those without ablated microglia populations (C. Wang et al., 2020). These results demonstrate the role of microglia in modulating learning and memory, synaptic pruning, and neuronal remodeling (Cornell et al., 2022; De Luca et al., 2020; Elmore et al., 2018; C. Wang et al., 2020).

Furthermore, microglia contribute to the regulation of affective behaviour, and overactivation of the neuroimmune system has been identified as a factor in the development of various neuropsychiatric disorders (Stein et al., 2017; Y.-L. Wang et al., 2018). Microglia dysregulation has been implicated in depression and anxiety observed in both humans and animal models (Frick et al., 2013; Stein et al., 2017). For instance, rats exposed to chronic stress had increased hippocampal microglia activation and exhibited increased depressive- and anxiety-like behaviour (Y.-L. Wang et al., 2018). Administering minocycline (an immunosuppressant used to inhibit microglia activity), however, dampened hippocampal microglia activation and the expression of pro-inflammatory markers while also reversing the effects of stress induced depressive- and anxiety-like behaviours (Sun et al., 2016; Y.-L. Wang et al., 2018). These results

suggest that microglia play a role in the pathogenesis of depressive- and anxiety-like behaviour via hippocampal neuroinflammation. Taken together, these results indicate that hippocampal microglia take part in regulating both cognitive and affective behaviours.

### 2.3.5. Effect of parenthood on microglia

In mothers, the peripheral inflammatory response is suppressed throughout gestation to protect the fetus and initiate contractions associated with parturition (Hanson, 2000). However, less is known about the changes that are associated with the neuroimmune response during the peripartum period, especially in the late postpartum. Previous studies indicated that pregnancy and the early postpartum are associated with a decrease in hippocampal microglia density (Duarte-Guterman et al., 2023; Eid et al., 2019; Haim et al., 2017; Posillico & Schwarz, 2016). In particular, primiparous rats throughout pregnancy exhibited fewer dorsal hippocampal Iba-1-expressing cells (a protein uniquely expressed by microglia and widely used as a marker for microglia) that persisted up to 21 days into the postpartum (prior to weaning) (Haim et al., 2017; Posillico & Schwarz, 2016). In contrast, another study reported no difference in microglia density in the DG between primiparous mothers and nulliparous females throughout the peripartum period (Eid et al., 2019). A difference was observed between the early and late postpartum in mothers, however, with PPD 8 showing markedly fewer microglia than at PPD 30 (nine days after weaning) (Eid et al., 2019). Furthermore, at PPD 8, the average length of microglia processes were shorter compared to nulliparous females (Eid et al., 2019). This result reflected primiparous mothers exhibiting fewer ramified microglia and a greater number of cells possessing stout morphology (an intermediate microglial morphology characterized by few, short processes) (Eid et al., 2019). This transition from ramified to stout microglia morphology

potentially suggests a transient increase in microglia activation during the early postpartum period (Eid et al., 2019). Furthermore, biparous mothers (i.e., having experienced two separate pregnancies) in the late postpartum exhibited an increase in microglia density in the DG compared to nulliparous controls; however, this effect was not observed in primiparous mothers (Duarte-Guterman et al., 2023). These results suggest that motherhood is accompanied by changes in microglia expression and plasticity in the neuroimmune environment, with some studies indicating changes persisting into the late postpartum period. The effects of paternal experience on microglia density and function have not been explored. Further research is required to investigate how parental experience in both sexes alter microglia density and function.

#### 2.3.6. Function of perineuronal nets

Perineuronal nets (PNNs) are condensed extracellular matrixes that surround neurons and play an important role in regulating neuroplasticity (Fawcett et al., 2022; Sanchez et al., 2024). The extracellular matrix is composed of extracellular molecules, such as neurocan, tenascin and brevican, that provide support to various neural cell types and are concentrated around perisynaptic regions (Fawcett et al., 2022). PNNs are composed primarily of chondroitin sulfate proteoglycans with a hyaluronic acid backbone that are held together by various link proteins (Fawcett et al., 2022; Hylin et al., 2013; Sanchez et al., 2024). PNNs surround soma and dendrites allowing them to regulate axonal growth and synaptic plasticity (Duncan et al., 2019; Fawcett et al., 2019, 2022; Sanchez et al., 2024). Furthermore, PNNs act as a physical barrier that stabilizes synapses, regulates the extent of synaptic plasticity, and restricts synaptic formation by directing the neuronal connections that are made (Sanchez et al., 2024). In various

cortical and subcortical regions of both mice and rats, including the visual perirhinal cortex and hippocampus, PNNs have been observed surrounding mainly GABAergic parvalbumin (PV)-positive interneurons (Carstens et al., 2016; Fawcett et al., 2022; Lensjø et al., 2017; Sanchez et al., 2024). In the hippocampal CA1 and CA3 regions of mice, PNNs were clearly defined and observed surrounding PV-positive neurons while expression was limited in rats (Lensjø et al., 2017). While in the CA2 region of rats, but not mice, there is localization of PNNs around excitatory pyramidal neurons, suggesting a species difference in PNN expression and regulation of synaptic connections (Carstens et al., 2016; Fawcett et al., 2022; Lensjø et al., 2017; Sanchez et al., 2024). Furthermore, PNNs in different hippocampal regions have been found to induce contrasting effects on synaptic plasticity (Bukalo et al., 2001; Carstens et al., 2016; Sanchez et al., 2024). For instance, degradation of PNNs in the CA1 hippocampal area of mice disrupted and reduced long-term potentiation (LTP; the strengthening of synaptic connections that are essential for memory formation (Sanchez et al., 2024) while enhancing excitatory synaptic transmission (Bukalo et al., 2001). In contrast, PNN degradation in the CA2 region in mice enabled LTP, possibly because PNNs surround and suppress synaptic potentiation of excitatory CA2 pyramidal neurons (Carstens et al., 2016). The behavioural functions of such differences on LTP and synaptic modulation between hippocampal regions are unclear, however. Nonetheless, PNNs balance inhibitory and excitatory synaptic connections to directly modulate neuronal activity and communication between distinct neural regions, which are essential for cognitive processing (Fawcett et al., 2022; Sanchez et al., 2024).

Microglia have also been identified to interact with and regulate PNNs, with molecular components of PNNs found phagocytosed by both mice and human microglia (Crapser et al., 2020). Microglial depletion prevented the loss of PNNs, further contributing to synaptic

remodeling and plasticity (Crapser et al., 2020; Liu et al., 2021; Strackeljan et al., 2021). After microglia depletion, the density and intensity of PNNs increased in the visual cortex (Crapser et al., 2020; Liu et al., 2021) and subiculum (Crapser et al., 2020), also leading to an increase in both excitatory and inhibitory synaptic connections (Liu et al., 2021). In contrast, the absence of microglia in the CA1 region of the hippocampus did not affect the overall PNN density or number of PV-positive neurons (Strackeljan et al., 2021). However, microglia depletion did increase the density of dendritic spines in CA1 pyramidal neurons and elevate the expression of other molecules within the surrounding extracellular matrix that play a role in regulating both pre- and post-synaptic activity, such as brevican (Strackeljan et al., 2021). These results suggest that microglia play a homeostatic role in regulating the expression of PNNs to further remodel synaptic connections in a region-dependent manner.

### 2.3.7. Function of perineuronal nets on hippocampal dependent behaviour

As PNNs are involved in regulating neuroplasticity, PNNs have also been implicated in modulating learning and memory (Duncan et al., 2019). For instance, PNNs have been found to preserve long-term contextual fear memories and are essential for memory consolidation in both mice and rats (Hylín et al., 2013; W. Shi et al., 2019). For instance, degradation of PNNs via chondroitinase ABC and hyaluronidase injection (enzymes that target and degrade the chemical composition of PNNs (Hylín et al., 2013; W. Wang et al., 2017)) into the hippocampus led to impaired long-term contextual fear memory (Hylín et al., 2013). Rats with PNN degradation spent less time freezing when presented with the conditioned stimulus (a tone that was paired with a foot shock) when tested 48 hours after the acquisition phase (Hylín et al., 2013). LTP impairments have also been observed, along with fear memory consolidation impairments in

mice, induced by PNN degradation following chondroitinase ABC administration (Shi et al., 2019). Furthermore, optogenetic inhibition of PV interneurons rescued the memory impairments caused by PNN degradation, highlighting the role of PNNs on PV neurons to regulate GABA release and preserve long-term memories (W. Shi et al., 2019). Inhibiting the expression of neuropeptide Y (a peptide involved in learning and memory processes), led to overexpression of CA1 hippocampal PNNs and resulted in impaired spatial learning and memory on both the Morris water maze and Barnes maze (Bertocchi et al., 2021). Mice with intense expression of PNNs had longer latencies to find the target platform and escape box in both mazes during the acquisition phase but not the probe trial compared to control mice (Bertocchi et al., 2021). These observed deficits were rescued through PNN degradation in the CA1, highlighting the importance of balanced PNN expression to modulate spatial learning and memory (Bertocchi et al., 2021; Sanchez et al., 2024). Beyond the hippocampus, PNN degradation in the medial prefrontal cortex and anterior cingulate cortex have also resulted in impaired contextual fear memory (Fawcett et al., 2022; Hylén et al., 2013; W. Shi et al., 2019). In contrast, degradation of PNNs in the perirhinal cortex and medial prefrontal cortex enhanced recognition memory and spatial memory, respectively (M. D. Anderson et al., 2020; Romberg et al., 2013). Isolating the effects of PNNs on cognition, however, is difficult because the techniques used to deplete PNNs (such as chondroitinase ABC administration) may target other components of the extracellular matrix as well (Sanchez et al., 2024). Nonetheless, the results from previous studies demonstrate the essential yet complex role of PNNs in regulating different types of memory processes, with their influence being dependent on both the memory type and brain region.

Although PNNs are implicated in a range of neuropsychiatric disorders, research is scarce on how hippocampal PNNs modulate affective behaviour (Reichelt et al., 2019). An imbalance

of inhibitory and excitatory synaptic connections due to PNN dysregulation was proposed as a mechanism contributing to anxiety-related behaviour (Fan et al., 2024). Inducing stress (via inescapable foot shock) not only increased anxiety-like behaviour on both the elevated plus maze and open field in mice, it also increased the expression of PNNs surrounding PV-positive interneurons in the CA1 hippocampal region (Fan et al., 2024). PNNs may also play a protective role in modulating anxiety-like behaviour after a stressful event (Colodete et al., 2024).

Degradation of ventral hippocampal PNNs during adulthood followed by stress exposure exhibited long-lasting behavioural deficits and decreases in overall PV-positive interneurons and PNN-PV interactions in rats (Colodete et al., 2024). Rats exposed to stress with PNN degradation exhibited increased anxiety-like behaviour in the light-dark box (spending less time in the light portion of the box), maladaptive social behaviour (indicated by reduced social interaction), and cognitive impairments in the novel object recognition test (lower discrimination index) when compared to rats that were only stressed (Colodete et al., 2024). These results suggest that the relationship between PNNs and PV neurons in the CA1 and ventral hippocampus play a substantial role in regulating anxiety-like behaviour after the experience of a stressful event; highlighting the potential role of PNNs in modulating affective behavioural responses (Colodete et al., 2024; Fan et al., 2024). Beyond the hippocampus, PNN expression in the orbitofrontal cortex was inversely correlated with anxiety-like behaviour in mice, with lower densities of PNN and PV-positive neurons associated with greater basal anxiety-like behaviour in the open field (Lee & Lee, 2021). Furthermore, PNN degradation in the medial prefrontal cortex induced anxiety-like behaviour in both the open field and elevated plus maze (X. Li et al., 2024). Ultimately, these results suggest that PNN expression in various neural regions, including the hippocampus, are critical in modulating anxiety-like behaviour.

### 2.3.8. Effect of parenthood on perineuronal nets

To my knowledge, the role of PNNs in the hippocampus throughout parenthood has yet to be investigated. However, other neural regions that undergo plasticity in response to parental experience have been shown to exhibit changes in the organization and density of PNNs (Lau et al., 2020; Leuner et al., 2023; Uriarte et al., 2020). In the medial preoptic area (mPOA), a region central to the maternal circuit that regulates maternal behaviour, PNN expression fluctuated throughout the peripartum period (Uriarte et al., 2020). The intensity of PNN expression in primiparous rats increased throughout pregnancy, beginning at gestation day 10 and reached maximum intensity just before parturition, on gestation day 21 (Uriarte et al., 2020). The intensity of PNN expression decreased two days after giving birth. This is followed by a transient increase on PPD 7 and then a decrease at PPD 22. Furthermore, PNN intensity is decreased in the mPOA two weeks after weaning, however, their expression was still noticeable compared to nulliparous females that exhibited virtually no PNN expression (Uriarte et al., 2020). These findings suggest that maternal experience generates a molecular signature that enables the sustained expression of PNNs in regions where they are typically absent in rats, such as the mPOA (Uriarte et al., 2020). In addition, PNN intensity is potentially mediated by pregnancy-related hormones (Uriarte et al., 2020). Ovariectomized female rats treated with estradiol and progesterone show increased PNN expression in the mPOA compared to those only treated with one hormone type (or oil-treated controls), suggesting that increases in both gonadal hormones are required to induce an increase in PNN intensity (Uriarte et al., 2020). Furthermore, virgin female mice that showed maternal behaviour toward newborn pups exhibited changes in PNN expression in the somatosensory cortex (SS1) compared to naïve female mice (Lau et al., 2020).

Maternal sensitization appears to modulate PNN expression in a subregion- and hemisphere-dependent manner compared to mice without pup-exposure, through increased expression in the right barrel cortex and decreased expression in the left forelimb regions of the somatosensory cortex (Lau et al., 2020). Overall, these findings suggest that maternal experience (Uriarte et al., 2020) (including pup exposure alone without the experience of pregnancy (Lau et al., 2020)), influence the expression of PNNs to stabilize neural circuits that regulate maternal behaviour, such as the mPOA and somatosensory cortex. However, the cognitive and affective changes associated with these alterations in PNN expression in response to parental experience are unclear. Additionally, no studies to date have explored PNN expression in fathers of a biparental species.

## **2.4. Biparental model: *Octodon degus***

### 2.4.1. The degu as an animal model

*Octodon degus*, commonly referred to as degus, are a diurnal moderate-sized rodent species dispersed across northern and central Chile (Ardiles et al., 2013; Colby et al., 2012; Colonnello et al., 2011). In the wild, degus exist in social colonies composed of about four to six females and one to two males, in addition to the offspring (Colonnello et al., 2011; S. C. Wilson, 1982). The gestation period of a degu mother is around 90 days with litters ranging from four to ten pups (Colby et al., 2012). Degu pups are born precocial and readily mobile and weaning typically occurs between five to six weeks after birth (Colby et al., 2012). In both laboratory and wild settings, males contribute to pup care and rear their offspring (Colonnello et al., 2011; S. C. Wilson, 1982). Furthermore, degus have been observed to exhibit strong social bonds and produce a wide range of vocalizations, including courtship calls, predator alarm calls, and

mother-offspring specific calls (Colby et al., 2012; Colonnello et al., 2011). In contrast to biparental rodents that have been previously discussed, such as California mice and prairie voles, degus are not a monogamous species (Colby et al., 2012). Their complex social organization and behaviour have made them a species of interest to study parental separation, social deprivation, and mother-infant attachment (Colby et al., 2012; Colonnello et al., 2011).

#### 2.4.2. Parental care in degus

In contrast to laboratory rats that have been used extensively to study monoparental maternal experience, degus exhibit alloparental and biparental care; whereby conspecifics and both mothers and fathers engage in pup caretaking behaviours, respectively (Ardiles et al., 2013; Colby et al., 2012; Colonnello et al., 2011). The total time of parental care in degus is comprised of 37% paternal care and 63% maternal care (Pinkernelle et al., 2009). Degu mothers spend a significant proportion of time nursing the pups (51% of the total maternal behaviour), along with huddling, licking and grooming, and engaging in play behaviour with the pups (Pinkernelle et al., 2009). Degu fathers also engage in huddling, licking and grooming, and playing with the pups (Pinkernelle et al., 2009). Monoparental degu mothers, however, have been observed to spend more time engaging with pups compared to biparental degu mothers in certain studies (S. C. Wilson, 1982); in contrast with Pinkernelle et al., 2009. Furthermore, there is an inverse relationship between the quantity of maternal and paternal care in degus, indicating that reduced duration of maternal interaction with pups is associated with increased paternal involvement (Aspillaga-Cid et al., 2021). In contrast, a positive relationship was observed in the quality of maternal and paternal care, such that an increase in the frequency of affiliative interaction with pups in mothers is associated with an increase in the quality of paternal care (Aspillaga-Cid et

al., 2021). These findings suggest that paternal caretaking behaviour is dependent on the expression of maternal behaviour and additionally, partner presence influences the expression of maternal behaviour.

Previous studies in degus have focused extensively on parental behaviours and the effects of parental-infant bonds on neural development of pups (De Schultz et al., 2020, 2023; Ebensperger et al., 2010). However, the biparental nature of degus make them a suitable model to study the effects of motherhood (including both biparental and monoparental maternal experience) and fatherhood on cognitive and affective behaviour and neuroplasticity.

## **2.5. Study objectives and predictions**

The overarching goal of this study aims to explore the effects of different types of parental experience on cognition, anxiety-like behaviour, and the expression of hippocampal microglia and PNNs in both sexes of a biparental species. Based on the current literature, I predicted: (1) both mothers and fathers will exhibit behavioural differences in both measures of cognition and anxiety-like behaviour compared to naïve controls, (2) both mothers and fathers will exhibit differences in the density of hippocampal microglia and PNNs compared to naïve controls, and (3) monoparental mothers will exhibit behavioural and neural differences compared to biparental mothers and naïve controls.

## **SECTION 3: METHODS**

### **3.1. Animals**

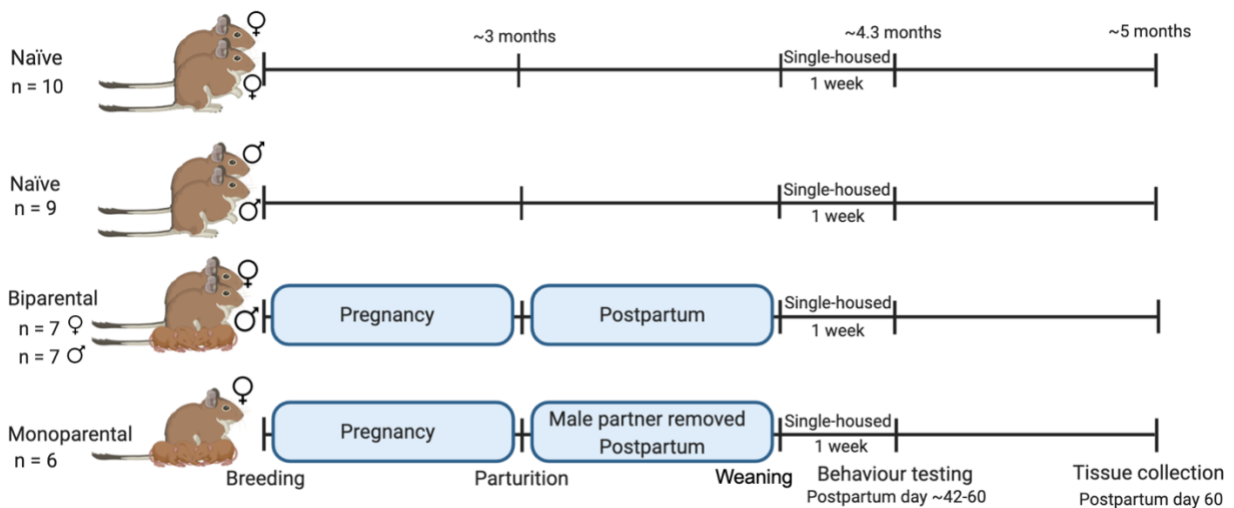
Captive-born degus were pair-housed at the animal facility at Brock University under standard laboratory conditions ( $21 \pm 1$  °C,  $54 \pm 3\%$  humidity, and 12:12 light-dark cycle) inside techniplast cages (L 48 cm, W 37.5 cm, H 21 cm). The breeder pairs received a breeder diet (Lab Diet #5P06 – Prolab RMH 2000) whereas the naïve pairs received a standard pellet diet (Teklad #2041). Home cages had *ad libitum* access to their respective diet and water with bedding (Inotiv (Envigo) – 7099P Tek-Fresh Laboratory Animal Bedding) and a variety of enrichment materials (including a paper hut, paper straw, paper tubes and either wooden sticks or blocks – supplied by Teklad). Alfalfa hay and oats were provided three times a week and home cages were changed weekly. Animal care protocols and experimental procedures were approved by and conducted in strict compliance with the Institutional Animal Care Committee of Brock University.

### **3.2. Breeding and experimental design**

Adult degus were randomly assigned to one of three conditions, outlined in Figure 1: (1) naïve controls, (2) biparental care, or (3) monoparental care. The degus had no previous mating or parental experience such that for the ones breeding, this would be their first litter. The age of the degus ranged from 9.5 to 13.2 months old at the time of pairing and breeding groups were age-matched with naïve controls. From the initial date of pairing, breeder females were weighed biweekly to confirm that the females were pregnant. During this period, breeder males and naïve pairs were also weighed biweekly to ensure that all experimental degus received equal handling. After giving birth, the mothers and pups were weighed weekly to ensure the mothers' weights returned to near baseline levels and the pups were healthy. During this period, fathers and the

naïve pairs were also weighed weekly to ensure equal handling of all individuals. Litter sizes ranged from four pups to eight pups, with the exception of one pair having only two pups.

In the biparental condition ( $n = 7$  mothers,  $n = 7$  fathers), unrelated males and females were paired for breeding for the first time. After the offspring were born, both the mother and the father were allowed to take care of the pups in the postpartum period until weaning. In the monoparental condition ( $n = 6$  mothers), unrelated males and females were paired for breeding, however, after parturition, only the mothers took care of the pups. One day after the pups were born, the fathers were removed and housed in a separate home cage allowing only the mothers to take care of the pups in the postpartum period until weaning. The naïve condition ( $n = 10$  males,  $n = 9$  females) comprised of related and unrelated pair-housed degus of the same sex. The naïve degus were never previously bred or had any parental experience in taking care of pups.



**Figure 1: Schematic representation of the experimental timeline.** All degus were between 9.5 to 13.2 months old at the time of pairing and were randomly assigned to one of three groups. Naïve: the naïve controls comprised of same-sex pair housed degus ( $n = 10$  for males,  $n = 9$  for females). Biparental: the biparental group consisted of both the mothers and fathers taking care of the pups during the postpartum period ( $n = 7$  for mothers,  $n = 7$  for fathers). Monoparental: in the monoparental group, only the mothers took care of the pups during the postpartum period as

the males were removed one day after the females gave birth ( $n = 6$ ). For both parental conditions, mothers were pregnant for about 3 months before giving birth and at 5 weeks  $\pm 2$  into the postpartum period, the pups were weaned. All the degus were single housed for 1 week before behavioural testing was conducted, which occurred at about 42 days postpartum or 4.3 months after the date of pairing. On the final day of behavioural testing, the degus were euthanized and their tissue were collected, which occurred at PPD 60 or about 5 months after the initial date of pairing.

Pups were weaned at 5 weeks  $\pm 2$  days postpartum (PPD~35). At the time of weaning, adult degu breeding pairs were single housed in a separate home cage to avoid pregnancies during behavioural testing. Simultaneously, same-sex naïve pairs were also single housed to control for effects of single housing degus throughout the duration of behavioural testing. A series of behavioural tests began one week after the pups were weaned (PPD~42) to allow the degus to acclimatize to being single housed and separated from the pups. All behavioural tests were conducted between 9:00 am and 2:00 pm. The degus were habituated in the behavioural testing room for 30 minutes before the start of the task, starting at 8:30 am. A Logitech camera was placed on the ceiling of the testing room and connected to a computer to record the degus' behaviour on each task. The tests were conducted on the adult degus to analyze the effects of different forms of parental experience on cognition and anxiety-like behaviour. The behavioural measures were conducted with two-day intervals between each, in the following order: the open-field test (PPD~42), elevated plus maze (PPD~45), and Barnes maze (PPD~48 to PPD~60). Tissue collection occurred 90 minutes after the Barnes maze retention task (PPD~60).

### **3.3. Open field**

#### **3.3.1. Open field apparatus.**

The open field test was the first behavioural measure that was assessed and began 1 week after weaning the pups. The open field test was used to measure anxiety-like behaviour and

overall locomotor activity in the degus, including the total distanced travelled and the average velocity. The apparatus consisted of a white box with no lid (L 60 cm x W 60 cm x H 60 cm). Two degus were placed in the testing room at a time, each in their individual open field box that were located side by side, placed directly in centre view of the camera. In between testing the degus, the bottom platform and the inner walls of the apparatus were cleaned using a Peroxigard solution (1:40 dilution) and fecal pellets were counted. After ensuring the apparatus was completely dry, the next degu was placed in the open field box.

### 3.3.2. Exploration.

Degus were placed in the centre of the open field box and allowed to explore uninterrupted for 10 minutes. After 10 minutes, degus were removed from the box and placed back in their home cage.

### 3.3.3. Open field scoring.

The open field videos were scored using the SLEAP (Social LEAP Estimates Animal Poses) program (T. D. Pereira et al., 2022). SLEAP is an open-source program that utilizes a deep-learning framework to track animal behaviour. We used training data from the video recordings to fine tune and improve the tracking of SLEAP to accurately analyze a variety of the degus' behaviour. The centre of the open field was defined based on a distance from the walls and the centre of the box. The amount of time that each degu spent within or beyond this range was then quantified as a measure of anxiety-like behaviour. The total distance travelled throughout the open field was also analyzed.

### **3.5. Elevated plus maze.**

#### 3.5.1. Elevated plus maze apparatus.

The elevated plus maze was conducted 48 hours after the open field test as an additional measure of anxiety-like behaviour. The elevated plus maze consisted of a plus-shaped platform that is elevated 51 cm off the ground. The platform has two open arms (L 114 cm in total) with no surrounding walls and two closed arms (L 112 cm in total) that are enclosed with walls 40 cm high. Because the open arms were uncovered, when the degu fell or jumped off the platform, they were gently picked up and placed back on the centre of the elevated plus maze. The apparatus was placed directly in centre view of the camera and a single degu was tested on the elevated plus maze at a time. In between testing the degus, the arms of the platform and the inner walls of the apparatus were cleaned using a Peroxigard solution (1:40 dilution). Fecal pellets were counted and surfaces were sprayed and wiped thoroughly. After ensuring the apparatus was completely dry, the next degu was placed on the elevated plus maze.

#### 3.5.2. Exploration.

Degus were placed in the centre of the elevated plus maze and allowed to explore uninterrupted for 10 minutes. After 10 minutes, the degus were removed from the platform and placed back in their home cage.

#### 3.5.3. Elevated plus maze scoring.

The elevated plus maze videos were also scored using the SLEAP (Social LEAP Estimates Animal Poses) program (T. D. Pereira et al., 2022). Similar to the open field, training data from the video recordings were used to fine tune and improve the tracking of SLEAP to

analyze the degus' behaviour. The amount of time that each degu spent in the open and closed arms of the apparatus was quantified as a measure of anxiety-like behaviour. The total distance travelled on the elevated plus maze was also analyzed.

### **3.6. Barnes maze.**

#### **3.6.1. Barnes maze apparatus.**

The Barnes maze was conducted 48 hours after the elevated plus maze as a measure of spatial learning and memory. The Barnes maze consisted of a circular platform (with a diameter of 122 cm) that was elevated 60 cm off the ground. On the periphery of the platform, there were 20 open holes, each with a diameter of 9 cm that were 7.5 cm apart. Underneath one of the holes contained an escape box (H: 11 cm, L: 32 cm, W: 23cm) whereas the remaining 19 holes were empty and uncovered. The hole with the escape box, defined as the “target hole”, was randomized across degus and were placed in one of four locations which were separated by 90 degrees from each other. The apparatus was placed directly in centre view of the camera and each individual degu had a different target hole that remained the same across the trials. In the testing room, there were visual cues that were taped on each of the walls for the degus to utilize as spatial cues (including the door, a triangle, an “X” and vertical lines). The cues on the walls remained consistent throughout the testing for all degus. The Barnes maze task occurred over a 13-day period and comprised of four distinct phases: habituation, learning, probe and retention. Each phase of the task aimed at assessing a distinct aspect of spatial learning and memory. The order in which the degus were tested was randomized across the various days to account for the time of testing. In between testing the degus, the Barnes maze platform and the inside of the escape box were cleaned using a Peroxigard solution (1:40 dilution). Areas with fecal pellets and

urine were sprayed and wiped thoroughly. After ensuring the apparatus was completely dry, the next degu was placed on the Barnes maze platform.

### 3.6.2. Habituation.

The habituation phase (day 1 of the Barnes maze testing period) was conducted to allow the degus to familiarize themselves with the novel environment and learn the task by exploring the Barnes maze platform and escape box. First, each degu was directly placed in the escape box placed underneath the target hole for 2 minutes. If the degu left the escape box, it was gently nudged and guided back in to allow it to habituate to the box. After those 2 minutes, the degu was removed from the box and placed directly on the Barnes maze platform near the target hole where they were allowed to approach and enter the escape box for 1 minute. If the degu did not enter after the allotted time, they were gently nudged and guided into the escape box where they were then left to habituate for 2 minutes. Finally, the degu was removed from the box again and placed directly in the centre of the Barnes maze platform facing the target hole. They were allowed 4 minutes to freely explore, approach and enter the escape box. Similarly, if the degu did not enter after the allotted time, they were gently nudged and guided into the escape box and left to habituate for another 2 minutes. After each degu underwent the habituation phase, they were removed from the escape box and placed back in their home cage.

### 3.6.3. Learning.

The learning phase of the Barnes maze began 24 hours after the habituation phase and occurred over the course of 4 days (day 2 to 5 of the Barnes maze testing period). Each degu completed 4 trials per day (for a total of 16 trials) in which each trial was spaced by 15 minutes.

At the start of the trial, each degu was placed under an empty bowl on the centre of the maze to randomize the starting orientation and direction that the degu was facing. After a 10 second delay, the bowl was lifted and the trial officially started when the testing room door closed. The degus were given 4 minutes to explore the maze, approach the target hole and enter the escape box. If the degu did not approach the escape box during the allotted time, they were gently nudged and guided to the target hole. After each trial, the degu was allowed to stay in the box for 1 minute, after which they were removed from the escape box and placed back in their home cage.

#### 3.6.4. Probe.

The probe phase (day 6 of the Barnes maze testing period) began 24 hours after the last day of the learning phase. The probe task consisted of a single trial where the escape box was removed from the platform and the degu were allowed to explore the maze freely for 4 minutes. The start of the probe phase was the same as the learning phase where the degus were placed under an empty bowl and the trial started when the bowl was lifted and the door closed. After the allotted time, the degus were removed from the platform and placed back in their home cage.

#### 3.6.5. Retention.

The retention phase (day 13 of the Barnes maze testing period) took place 7 days after the probe phase to assess long-term memory of the degus. The retention task consisted of a single trial where the escape box returned and was placed underneath the target hole. As in the previous phases, the start of the retention phase consisted of placing the degu under an empty bowl where the trial started when the bowl was lifted, and the door closed. The degu was allowed to freely

explore the maze for 4 minutes to locate and enter the escape hole. If the degu was unsuccessful at locating the escape hole after the allotted time, they were gently removed from the platform and placed back into their home cage.

#### 3.6.6. Barnes maze scoring.

The Barnes maze trials were scored manually by a blinded observer to quantify the total number of approaches for each degu. An approach was defined as the total number of visits each degu made to a non-target hole before approaching the target hole contained the escape box.

### **3.7. Tissue collection and processing.**

#### 3.7.1. Tissue collection.

On the final day of behavioural testing, 90 mins after the retention phase of the Barnes maze task, degus were deeply anesthetized using isoflurane, which was confirmed by the absence of a pedal reflex, and euthanized by exsanguination via cardiac puncture. For each degu, body weights were recorded and brains were collected. After extracting the brains, the hemispheres were separated and either fixed in 4% paraformaldehyde (PFA) solution or frozen on dry ice and later stored at  $-80^{\circ}\text{C}$  for future analyses. The brains were placed in the 4% PFA solution at  $4^{\circ}\text{C}$  for 24 hours and then transferred to a 30% sucrose solution in phosphate buffer (PB) for cryoprotection. The brains were confirmed to be cryoprotected when they sank to the bottom of the solution. Following this, brains were coronally sliced using a sliding freezing microtome at a thickness of  $40\ \mu\text{m}$  and were separated into series of 4 for the prefrontal cortex and series of 7 for the hippocampal area. The brain slices were carefully collected in an

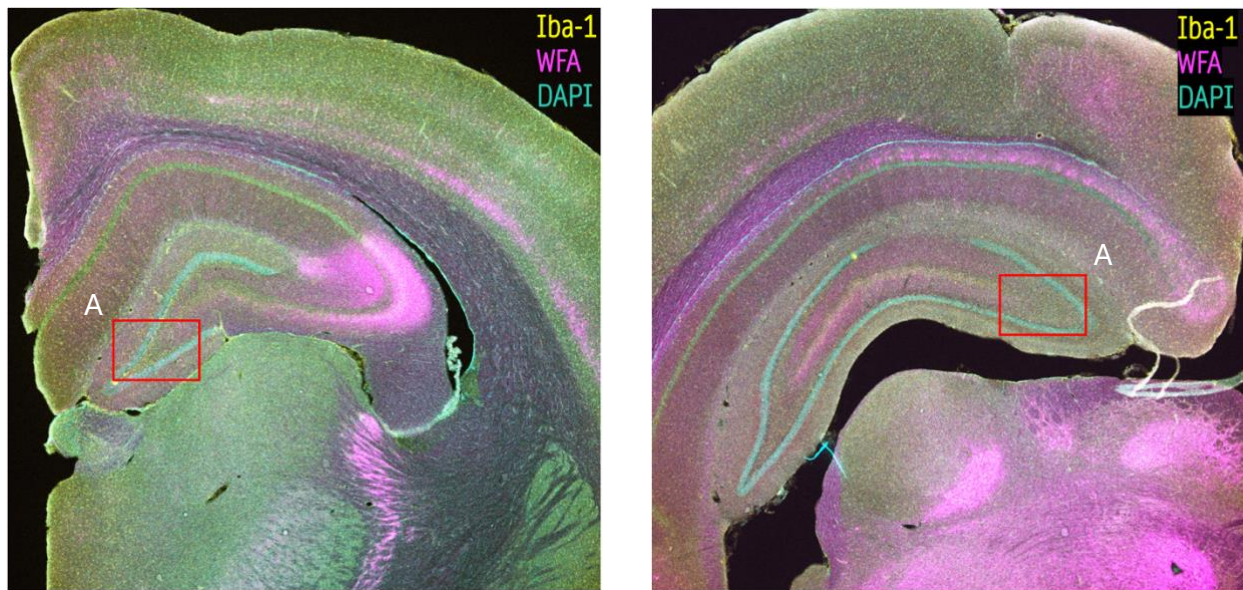
antifreeze solution that comprised of 30% ethylene glycol and 20% glycerol and stored at -20°C freezer until they were analyzed using immunohistochemistry techniques.

### 3.7.2. Immunohistochemistry.

For each degu, the first series of the hippocampal area was selected for immunohistochemistry staining. Each series was double stained for Iba-1 (as a marker for microglia) and a lectin from *Wisteria floribunda* (WFA, as a marker for PNNs). The hippocampal sections underwent three quick washes followed by three 10-minute washes in 0.1M phosphate buffer saline (PBS) to ensure the antifreeze solution was washed off the tissue. The sections were then submerged and incubated into a blocking solution that comprised of 3% normal goat serum (NGS) and 0.3% Triton-X for 1 hour at room temperature in order to prevent non-specific binding of the tissue. The sections were incubated in a rabbit anti-Iba-1 primary antibody (dilution 1:1000, Wako Chemicals, Catalogue No. 016-26721) to detect microglia, and a lectin from WFA (dilution 1:500, Sigma-Aldrich, Catalogue No. 00166187) to detect PNNs. The primary antibody solution contained 4% NGS and 0.3% Triton-X and the sections were incubated for 24 hours at 4°C. The sections were then rinsed with PBS and incubated in secondary antibody of goat anti-rabbit Alexa 488 (dilution 1:500, ThermoFisher, Catalogue No. A-11008) and a streptavidin Alexa 568 (dilution 1:500, ThermoFisher, Product No. S-11226) for 3 hours at room temperature. Following this, the sections were washed using PBS and counter stained with DAPI (dilution 1:1000, ThermoFisher, Product No. D-1306) for 5 minutes. The stained sections were mounted onto Superfrost/Plus microscope slides and coverslipped using polyvinyl alcohol with DABCO (PVA-DABCO, Sigma-Aldrich). The slides were left to dry completely at 4°C before they were imaged for analysis.

### 3.7.3. Iba-1 and WFA imaging.

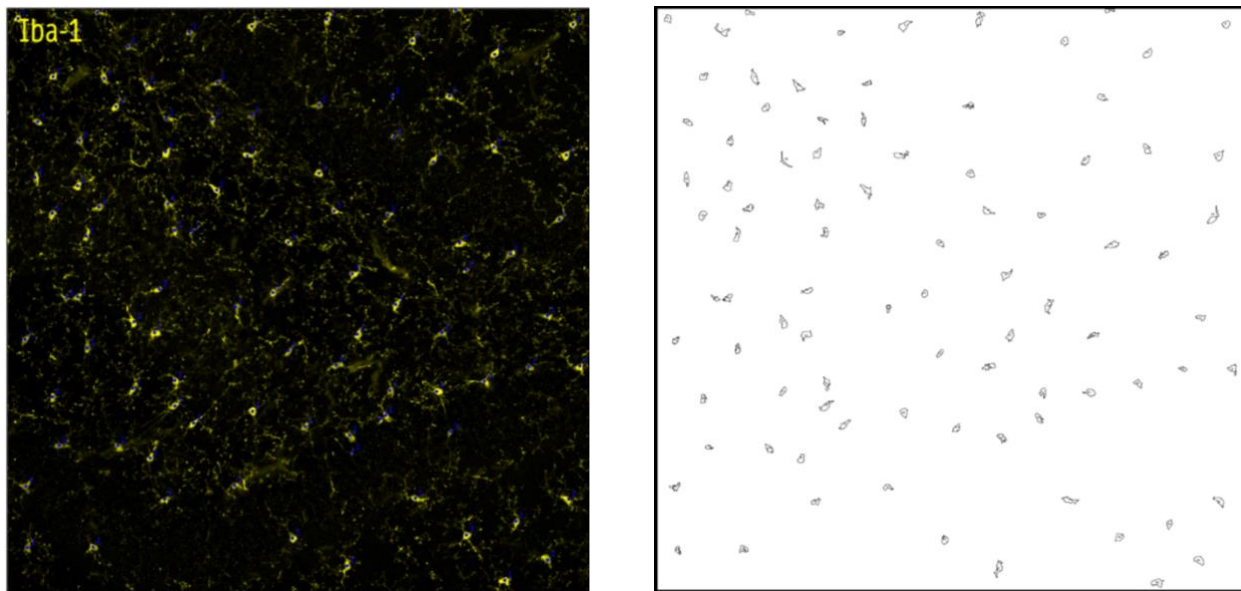
Microscope slides containing the stained tissue were observed and imaged using a laser confocal microscope (Olympus FV3000) using a 20x objective. Similar laser power and high voltage (HV) settings were used across images for each of the channels to ensure consistent contrast and exposure settings. Additionally, the Z-stack settings were adjusted and optimized to ensure that the complete depth of the tissue (which were sliced at a thickness of 40  $\mu\text{m}$ ) was captured for each image with a step size of around 17 per image. Each slice was scanned to produce images at a resolution of 2048x2048 pixels. The DG of the dorsal and ventral hippocampus were imaged.



**Figure 2: Co-labelled immunofluorescence of Iba-1 (yellow), WFA (magenta), and DAPI (cyan).** A representation of the dorsal hippocampus (left) and the ventral hippocampus right (right) taken at 4x. (A) The DG of the hippocampus.

#### 3.7.4. Iba-1 analysis for microglia.

On ImageJ, each image was Z-projected with maximum intensity and converted to an 8-bit image type before analyzing the images. For Iba-1 quantification, the threshold was adjusted in order to minimize the background while keeping the Iba-1-positive particles visible. The area of various Iba-1 cells were measured to acquire a range of their cell body size, which ranged from 25 to 150  $\mu\text{m}^2$ . Particle analysis was then performed and the total number of Iba-1-positive particles were recorded. Two images were taken of the DG and particle analysis counts were averaged across the images.

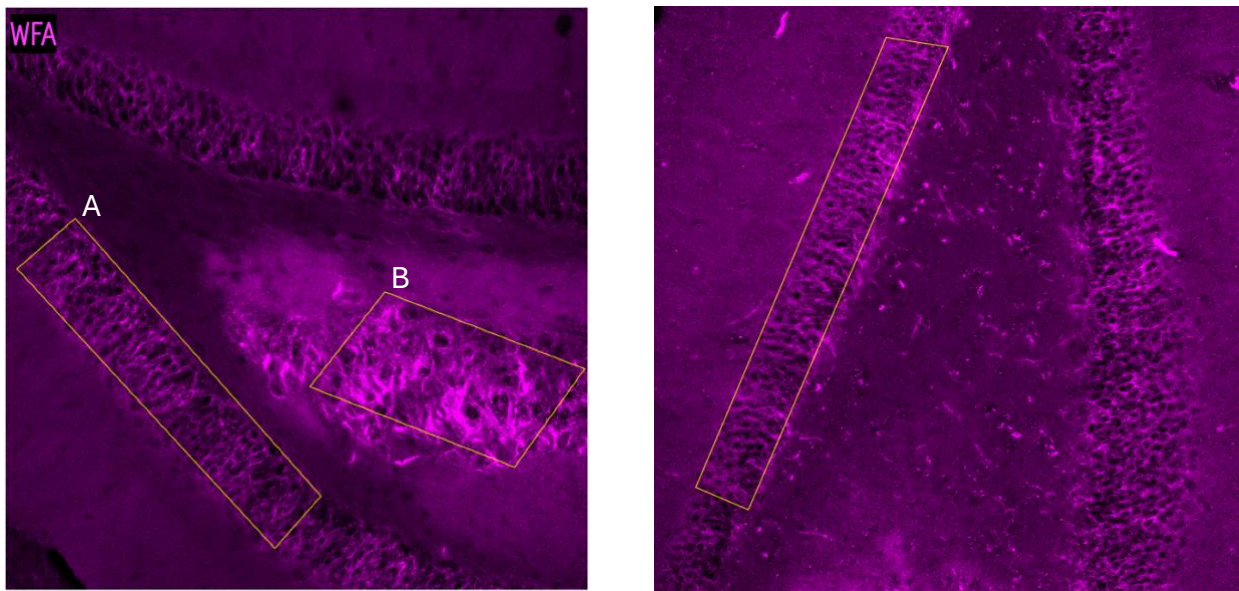


**Figure 3: Particle analysis of Iba-1.** A representation of the expression of Iba-1 cells (yellow, left) and the particles that were captured using particle analysis (right).

#### 3.7.5. WFA analysis for PNNs.

For WFA quantification, an ROI was drawn around the hilus and infrapyramidal blade of the granule cell layer for the dorsal DG (Figure 4). For the ventral region, an ROI was drawn only around the infrapyramidal blade of the DG because the expression of WFA in the hilus was

limited (Figure 4). For each image, circles were placed in areas that did not contain WFA expression to acquire the average background gray value. A calibrated rodbard equation was used to quantify the optical density for the ROIs that were drawn and the background. The average optical density of the background was then subtracted by the optical density of the infrapyramidal blade and hilus. Lastly, the optical density difference was divided by the area of the ROIs that were drawn (in  $\mu\text{m}^2$ ) to normalize the values and control for differences in ROI size across images. Two images were taken of the dorsal and ventral DG and optical density values were averaged across the images.



**Figure 4: ROIs of the DG to calculate optical density of WFA expression.** A representation of the expression of WFA with an ROI drawn around the infrapyramidal blade (A) and the hilus (B) of the dorsal DG (left) and the infrapyramidal blade of the ventral DG (right).

### 3.8. Statistical analyses.

Statistical analyses were performed using R (R version 4.4.1; R Core Team) with RStudio as the integrated development environment (RStudio version 2024.09.0+375; Posit Software,

PBC). A linear mixed-effects model was conducted to analyze the effect of parental condition, sex, and their interaction as fixed effects on all behavioural measures and markers of neural plasticity. Additionally, the cohort was included in the model as a random effect to account for the variability in the timing of behavioural testing. The data were analyzed using the lmerTest package (version 3.1-3; Kuznetsova et al., 2017). The DHARma package (version 0.4.7; Hartig, 2024) was used to assess the normality and variance of the residuals and Q-Q residual plots were analyzed to confirm no significant deviations in the normality and homoscedasticity of the residuals. Furthermore, an arcsine square root transformation was applied to normalize the distribution of the data and stabilize the variances for the proportion of time spent in the centre of the open field and the proportion of time spent in the centre, closed and open arms of the elevated plus maze. Due to an imbalanced design, a-priori contrasts were established based on theoretical hypotheses and a limited number of meaningful comparisons that could be made. Estimated marginal means were computed using the emmeans package (version 1.11.1; Lenth, 2025) to evaluate contrasts. The planned contrasts were as follows: (1) naïve females minus naïve males, (2) biparental females minus naïve females, (3) monoparental females minus naïve females, (4) biparental females minus monoparental females, (5) biparental males minus naïve males, (6) and the difference between biparental females and naïve females, minus the difference between biparental males and naïve males. The final contrast examines the interaction effect, comparing the impact of biparental experience in males relative to females. To analyze the influence of outliers, I conducted an analysis using winsorized values at the 5<sup>th</sup> and 95<sup>th</sup> percentiles within each combination of parental group and sex, which allowed us to cap extreme values while preserving the sample size. Outliers were detected using the winsorize function from the DescTools package (version 0.99.59; Signorell, 2025). However, the results presented

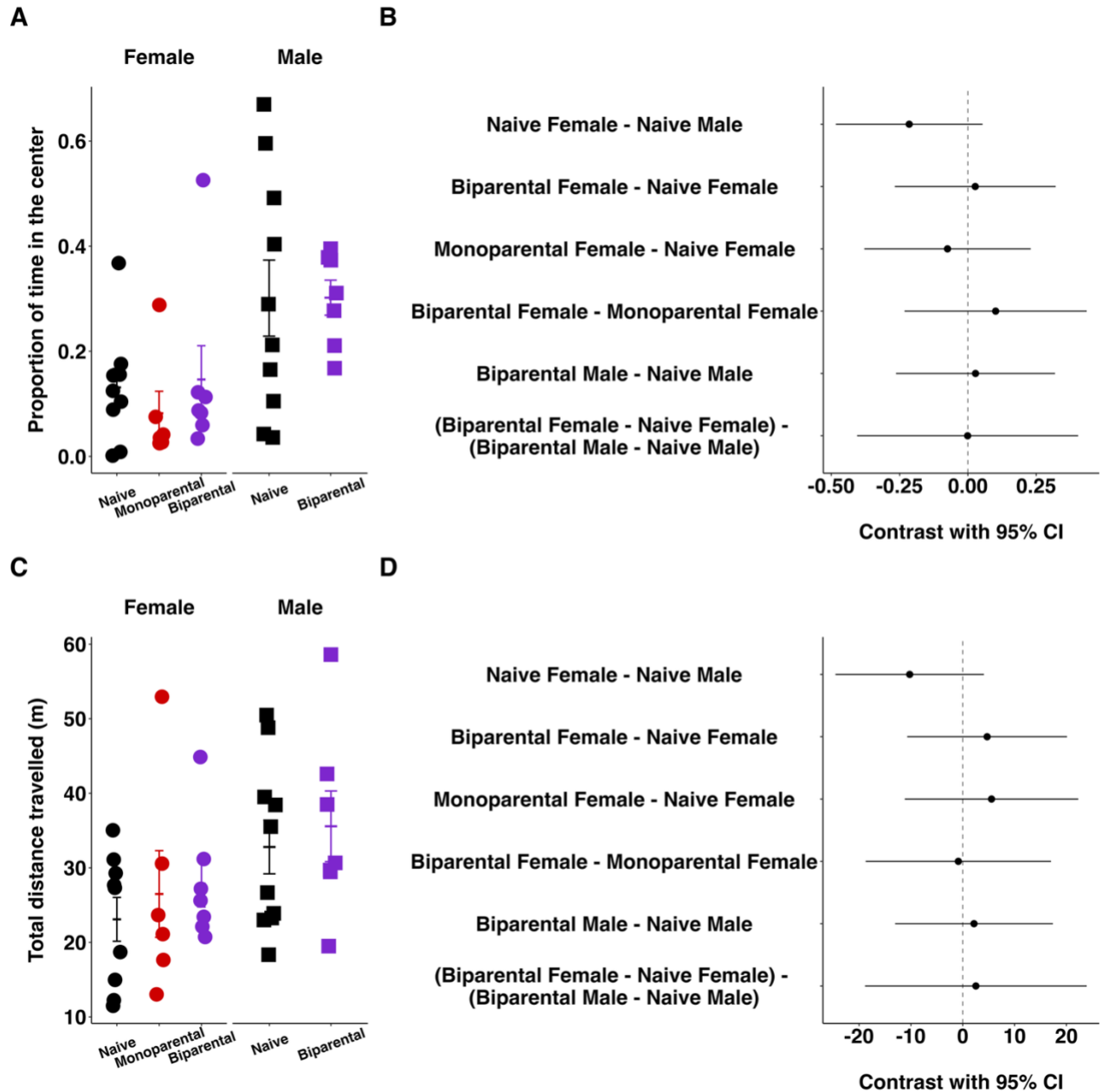
are unadjusted values because controlling for outliers did not alter the interpretation of the analysis. The figures in the results section with individual data points are untransformed, however, the contrast plots represent the contrasts that were performed on transformed data. Significance thresholds were set at  $p < 0.05$ , trends were set at  $0.05 < p < 0.08$ , and multiple comparisons were corrected using false discovery rate (Benjamini & Hochberg, 1995). Furthermore, in the results section, contrast estimates are presented in the figures with p-values reported for significant contrasts.

The video files for the first cohort of degus on training day 4 and the probe day of the Barnes maze are missing. The data for this cohort are missing completely at random and included a total of 10 degus: three biparental mothers, three biparental fathers, two naïve males, and two naïve females. For these two days, we used data that were collected in the behavioural testing room as the degus performed the task. Because the in-room manual scores were consistent with the video-recorded scores across individuals in the other cohorts, we were able to use them to fill in the missing data.

## **SECTION 4: RESULTS.**

### **4.1. Open field**

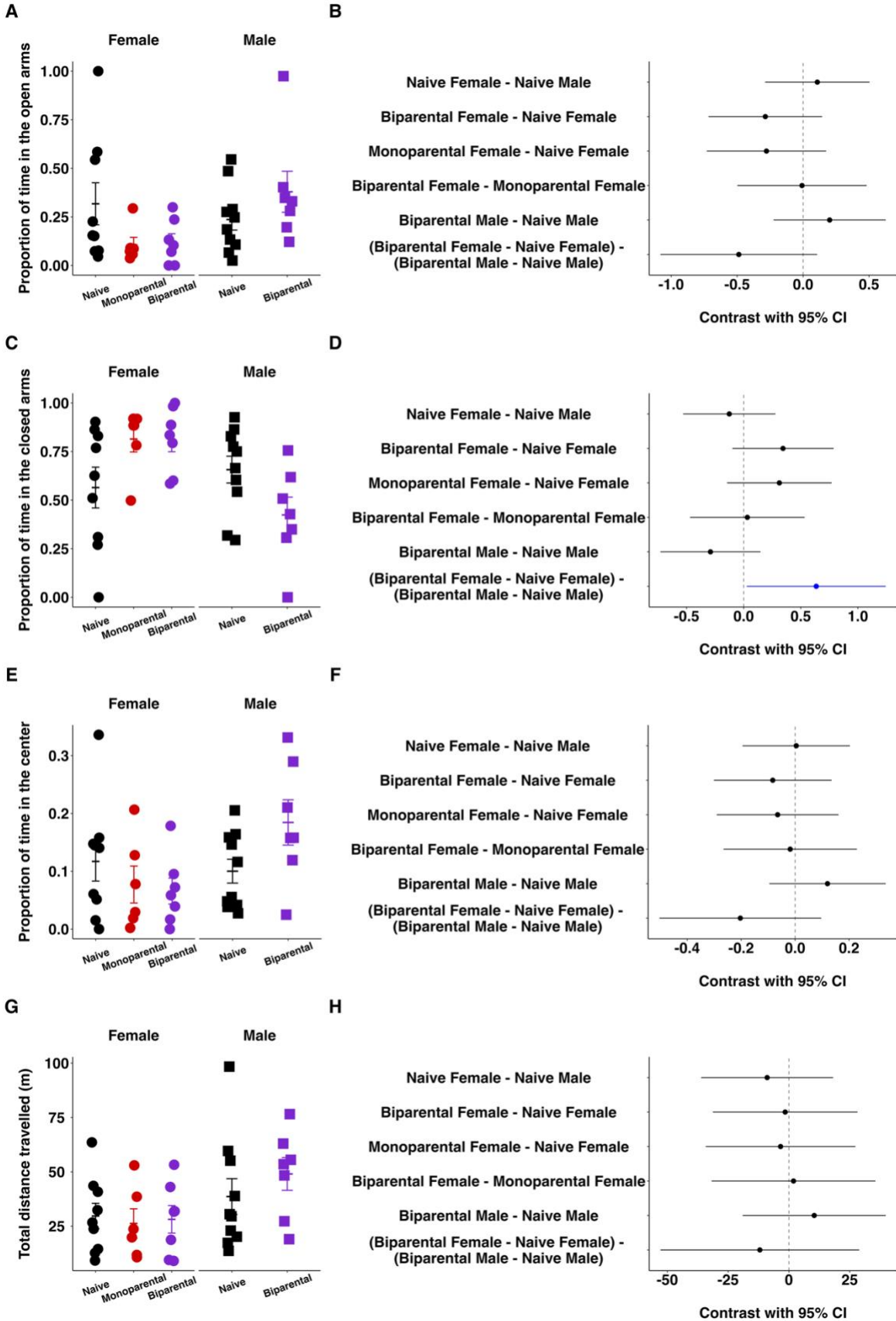
Planned contrasts detected no differences across parental groups or sexes in the proportion of time spent in the centre of the open field (Figure 5A and 5B). Furthermore, there were no differences across parental groups or sexes in the total distance travelled in the open field (Figure 5C and 5D).



**Figure 5: Measures of anxiety-like behaviour in the open field.** (A) Proportion of time spent in the centre of the open field for each individual degu. Data points are unadjusted for interpretability, and the means are indicated by the line  $\pm$  SEM ( $n = 6$  to  $10$  per group). (B) Contrasts for the proportion of time spent in the centre of the open field with 95% confidence interval. Contrasts and significance testing were performed on arcsine square root transformed data. There were no differences across parental conditions or sexes in the proportion of time spent in the centre of the open field. (C) Total distance travelled (in meters) in the open field for each individual degu. (D) Contrasts for the total distance travelled in the open field with 95% confidence interval. There were no differences across parental conditions or sexes in the total distance travelled in the open field.

## 4.2. Elevated plus maze

There were no differences detected across parental groups or sexes in the proportion of time spent in the open arms of the elevated plus maze (Figure 6A and 6B). However, there was a significant interaction effect in the proportion of time spent in the closed arms ( $p > 0.0366$ ) (Figure 6C and 6D). The interaction contrast explores whether the effect of parental experience on the time spent in arms of the elevated plus maze differs between sexes. Furthermore, there were no differences detected in the centre of the elevated plus maze across parental groups or between sexes (Figure 6E and 6F). Lastly, there were no differences detected in the total distance travelled on the elevated plus maze (Figure 6G and 6H).

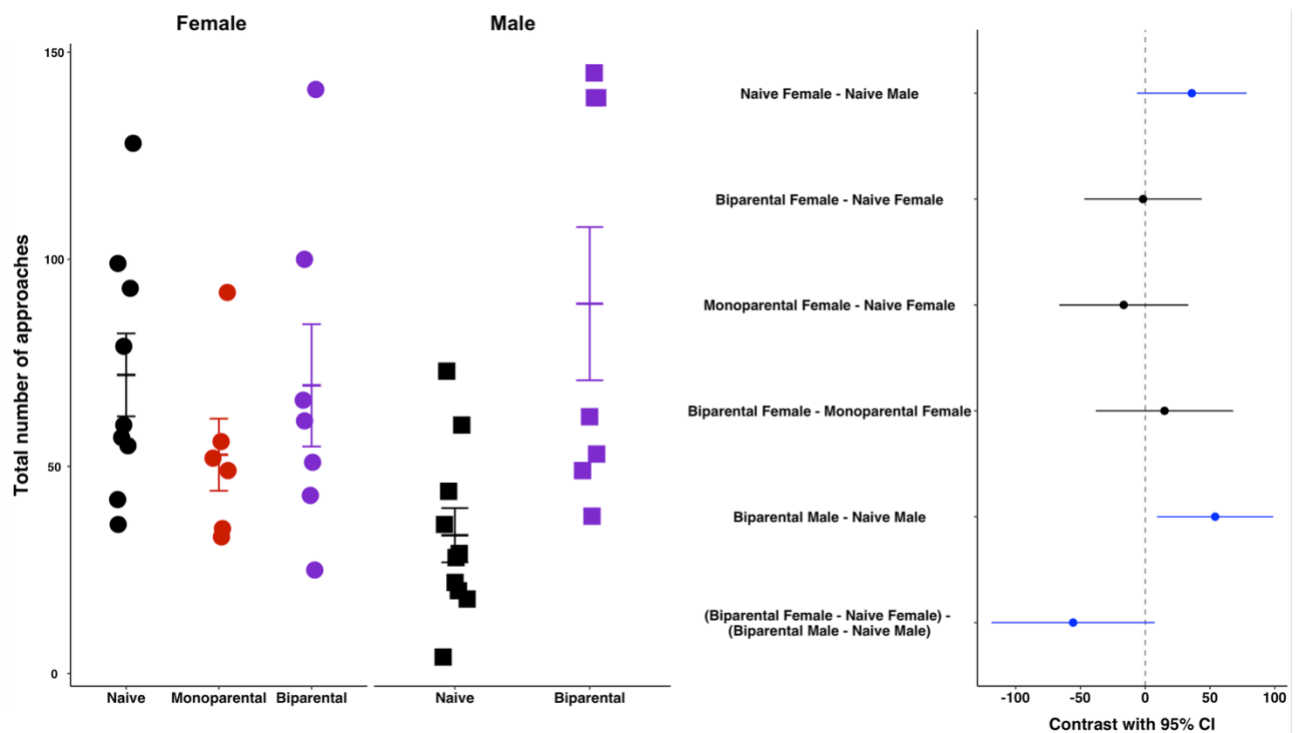


**Figure 6: Measures of anxiety-like behaviour on the elevated plus maze.** (A) Proportion of time spent in the open arms of the elevated plus maze for each individual degu. Data points are unadjusted for interpretability and means are indicated by the line  $\pm$  SEM (n = 6 to 10 per group). (B) Contrasts for the proportion of time spent in the open arms of the elevated plus maze with 95% confidence interval. Contrasts and significance testing were performed on arcsine square root transformed data. There were no differences across parental conditions or sexes in the proportion of time spent in the open arms of the elevated plus maze. (C) Proportion of time spent in the closed arms of the elevated plus maze for each individual degu. (D) Contrasts indicated that the difference in the proportion of time spent in the closed arms between biparental females and naïve females was greater compared to the difference between biparental males and naïve males. (E) Proportion of time spent in the centre of the elevated plus maze for each individual degu. (F) Contrasts indicated no differences across parental conditions or between sexes in the proportion of time spent in the centre of the maze. (G) Total distance travelled (in meters) in the elevated plus maze for each individual degu. (H) There were no differences across parental conditions or between sexes in the total distance travelled. Contrasts that are significantly different from zero ( $p < 0.05$ ) are indicated by a blue line.

### 4.3. Barnes maze

#### 4.3.1. Total number of approaches to non-target holes on the Barnes maze during learning

The total number of approaches to non-target holes before locating the target hole were summed across all four trials over all four training days. Contrasts revealed a difference in naïve degus, whereby naïve females had more total approaches to non-target holes compared to naïve males ( $p = 0.046$ ) (Figure 7). Furthermore, biparental males approached more non-target holes compared to naïve males ( $p = 0.0114$ ) (Figure 7). Lastly, the total number of approaches to non-target holes between biparental females and naïve females was lower compared to the difference between biparental males and naïve males ( $p = 0.046$ ) (Figure 7).



**Figure 7: Total number of approaches on the Barnes maze across all four training days. (A)** The total number of approaches to non-target holes before locating the hole was summed across all the trials, over all four training for each individual degu. Means are indicated by the line  $\pm$  SEM ( $n = 6$  to  $10$  per group). **(B)** Contrasts for the total number of approaches on the Barnes maze with 95% confidence interval. Contrasts indicated both naïve females and biparental males approached more non-target holes compared to naïve males. Furthermore, the difference between the total number of approaches to non-target holes was lower between biparental females and naïve females compared to the difference between biparental males and naïve males. Contrasts that are significantly different from zero ( $p < 0.05$ ) are indicated by a blue line.

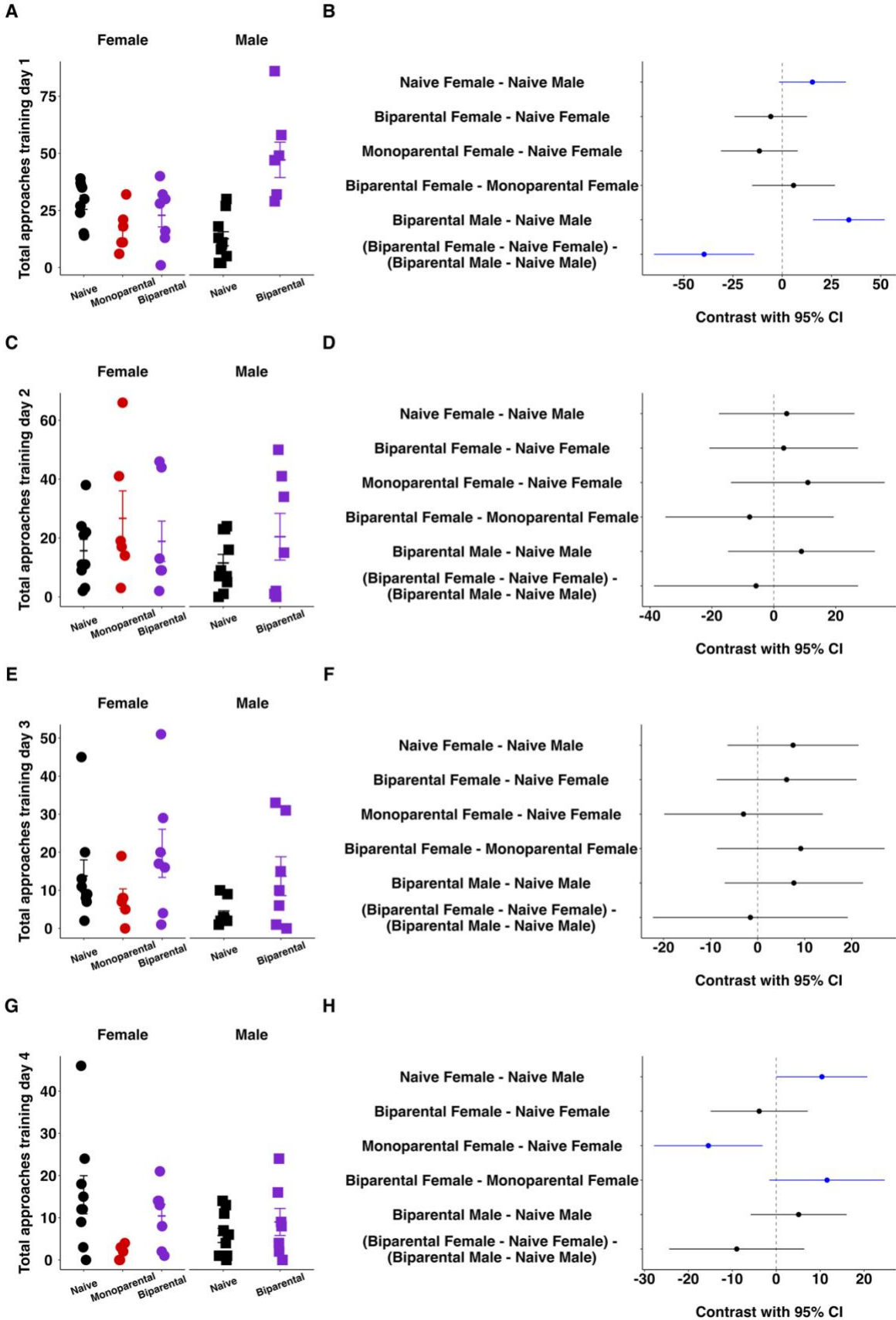
#### 4.3.2. Total number of approaches to non-target holes for each training day on the Barnes maze

The total number of approaches to non-target holes before locating the target hole was summed across all four trials for each of the training days of the Barnes maze. For training day 1, there was a difference between naïve degus, where naïve females approached more non-target holes compared to naïve males ( $p = 0.032$ ) (Figure 8A and 8B). Furthermore, there was a difference between males, with biparental males approaching more non-target holes compared to

naïve males ( $p = 0.0001$ ) (Figure 8A and 8B). Lastly, the difference in the number of approaches to non-target holes between biparental females and naïve females was lower compared to the difference between biparental males and naïve males ( $p = 0.0004$ ) (Figure 8A and 8B).

On training day 2 (Figure 8C and 8D) and training day 3 (Figure 8E and 8F) of the Barnes maze, contrasts detected no differences across parental groups or sexes in the number of approaches to non-target holes.

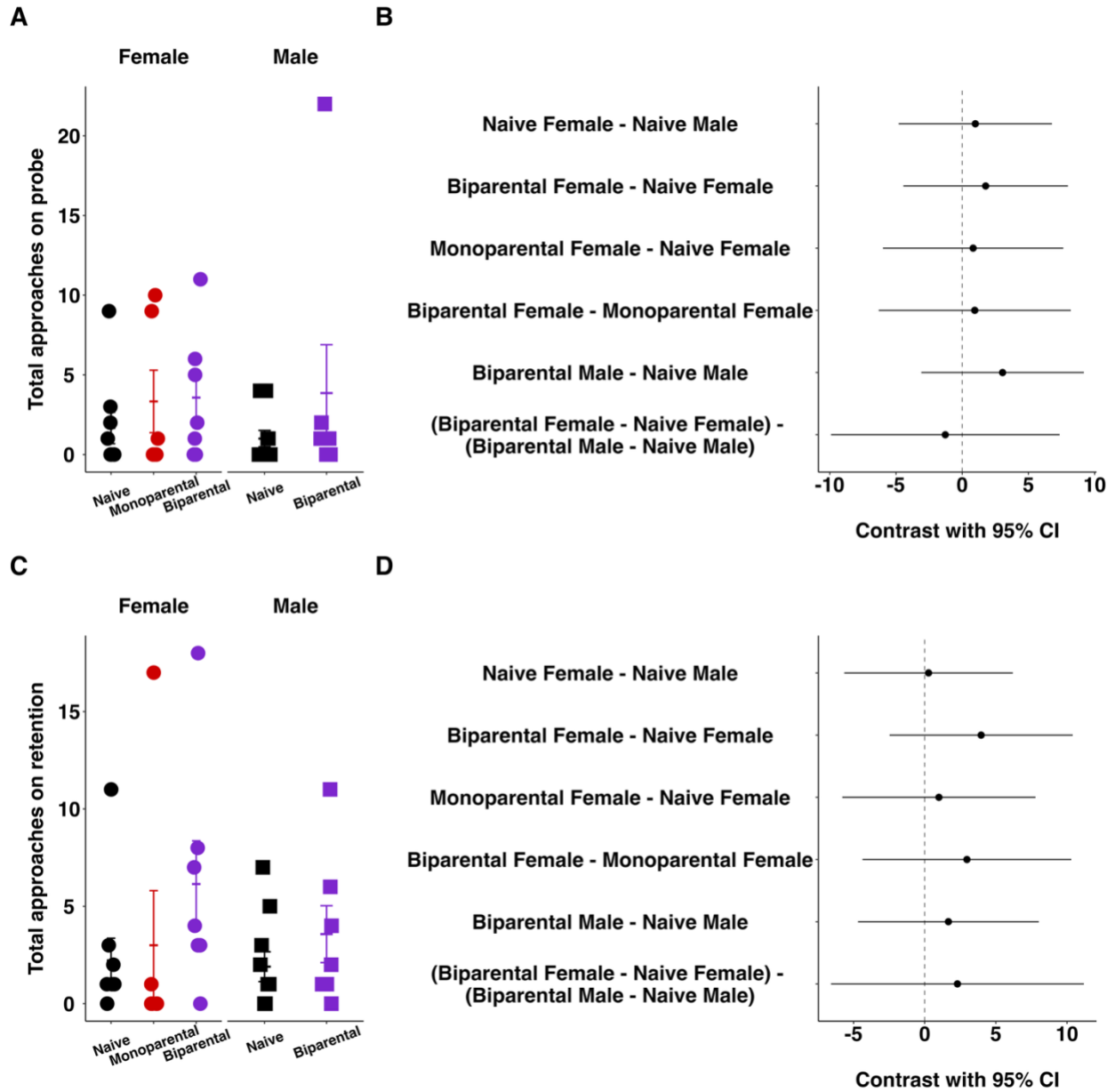
On training day 4 of the Barnes maze, contrasts revealed a difference between naïve degus, where naïve females approached more non-target holes compared to naïve males ( $p = 0.024$ ) (Figure 8G and 8H). Furthermore, monoparental females approached fewer non-target holes compared to both naïve females ( $p = 0.037$ ) and biparental females ( $p = 0.008$ ) (Figure 8G and 8H).



**Figure 8: Number of approaches on the Barnes maze by training day.** (A) The total number of approaches to non-target holes prior to locating the hole on training day 1 for each individual degu. Means are indicated by the line  $\pm$  SEM ( $n = 6$  to  $10$  per group). (B) Contrasts for the total number of approaches on the Barnes maze with 95% confidence interval. Contrasts revealed both naïve females and biparental males approached more non-target holes compared to naïve males on training day 1. Furthermore, the difference in the total number of approaches was lower between biparental females and naïve females compared to the difference between biparental males and naïve males on training day 1. (C) The total number of approaches to non-targets holes on training day 2 for each degu. (D) Contrasts detected no differences across parental groups or sexes on training day 2. (E) The total number of approaches to non-targets holes on training day 3 for each degu. (F) Contrasts detected no differences across parental groups or sexes on training day 3. (G) The total number of approaches to non-targets holes on training day 4 for each degu. (H) Contrasts indicated naïve females approached more non-target holes compared to naïve males on training day 4. Additionally, monoparental females approached less non-target holes compared to both naïve and biparental females on training day 4. Contrasts that are significantly different from zero ( $p < 0.05$ ) are indicated by a blue line.

#### 4.3.3. Total number of approaches to non-target holes on the probe and retention days of the Barnes maze

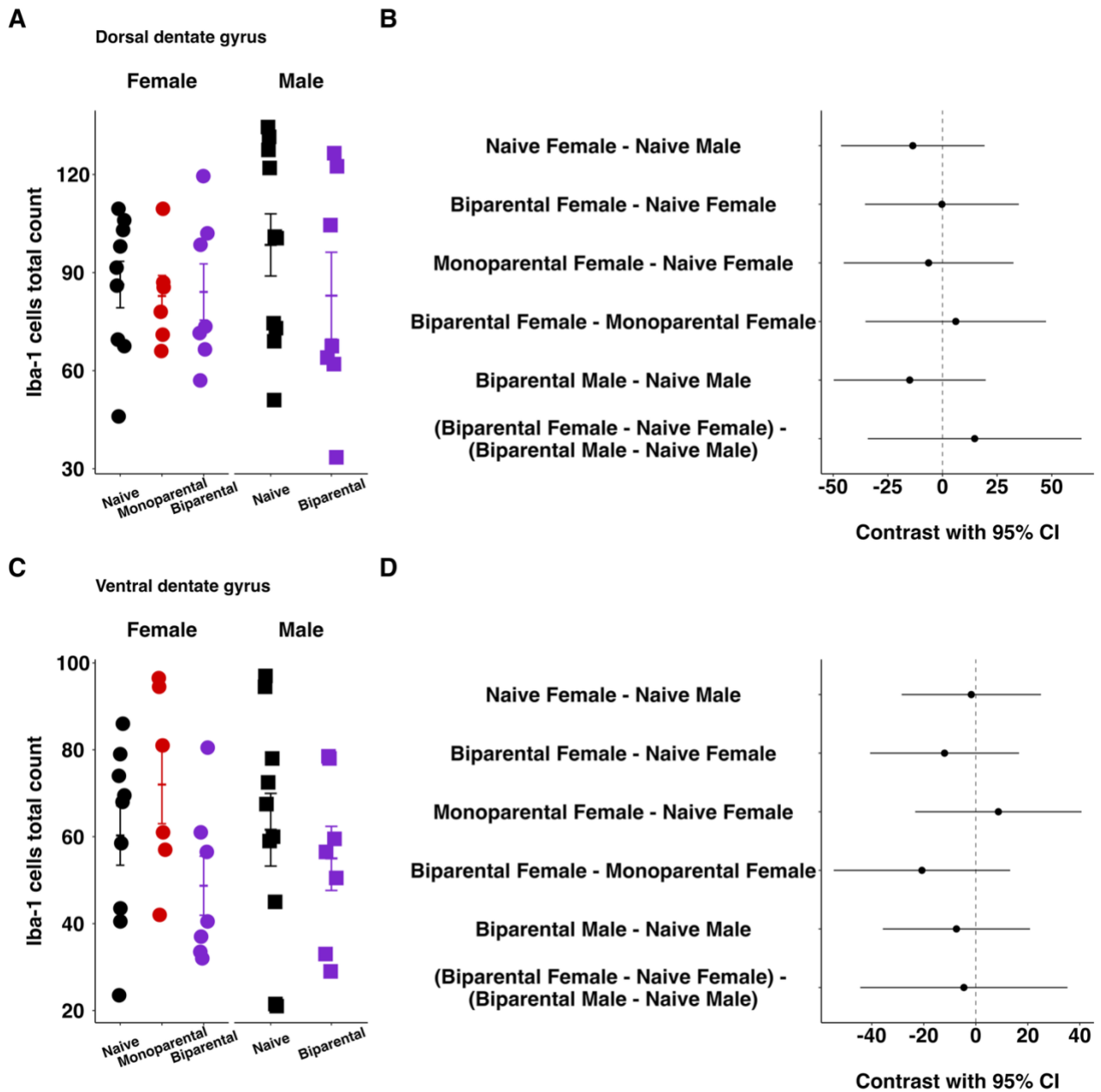
On the probe day of the Barnes maze task, contrasts indicated no differences across parental groups or sexes in the total number of approaches to non-target holes (Figure 9A and 9B). Furthermore, on the retention day of the Barnes maze task, conducted one week after the probe, there were no differences across parental groups or sexes in the total number of approaches to non-target holes (Figure 9C and 9D).



**Figure 9: Total approaches on probe and retention day of Barnes maze.** (A) The total number of approaches to non-target holes prior to locating the hole on the probe day for each individual degu. Means are indicated by the line  $\pm$  SEM ( $n = 6$  to  $10$  per group). (B) Contrasts for the total number of approaches on the probe day with 95% confidence interval. There were no differences across parental conditions or sexes. (C) The total number of approaches to non-target holes prior to locating the hole on the retention day for each individual degu. (D) Contrasts for the total number of approaches on the retention day with 95% confidence interval. There were no differences across parental conditions or sexes.

#### 4.4. Iba-1 expression for microglia

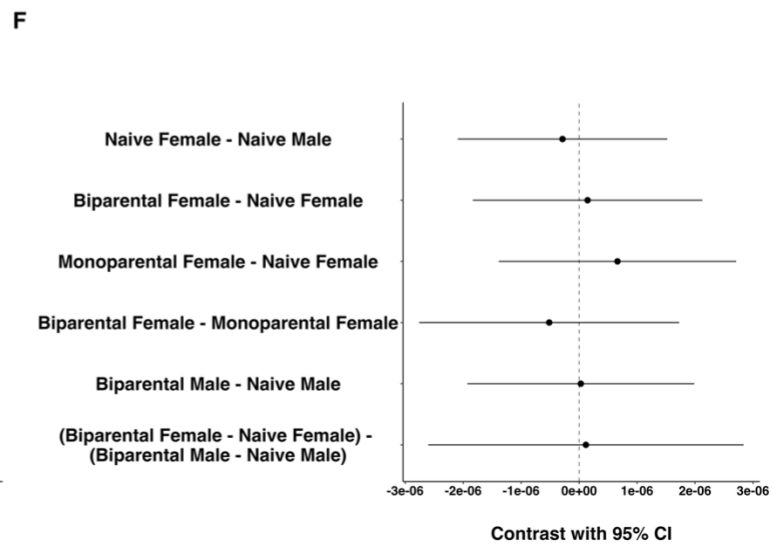
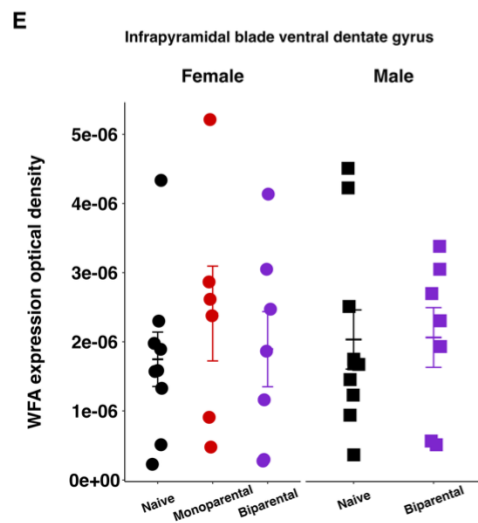
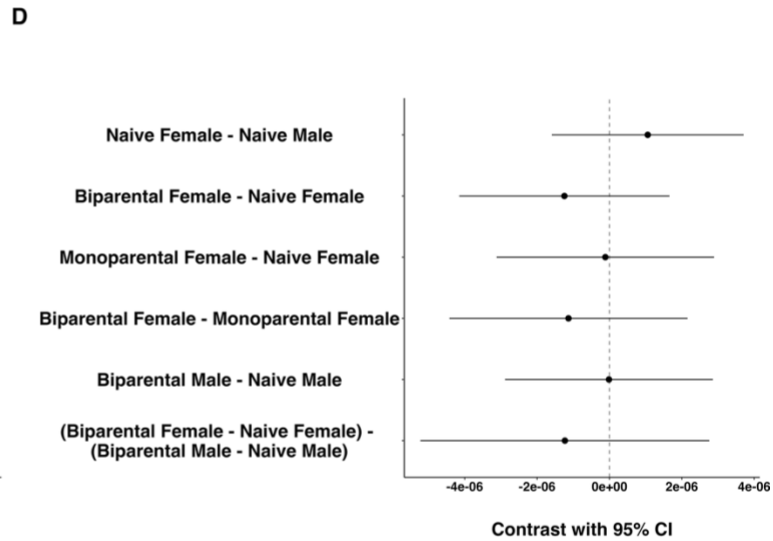
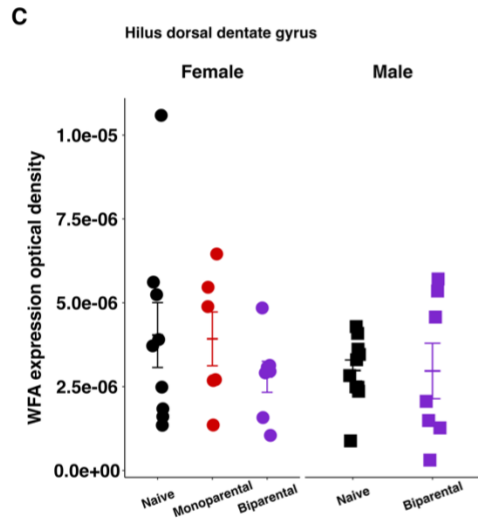
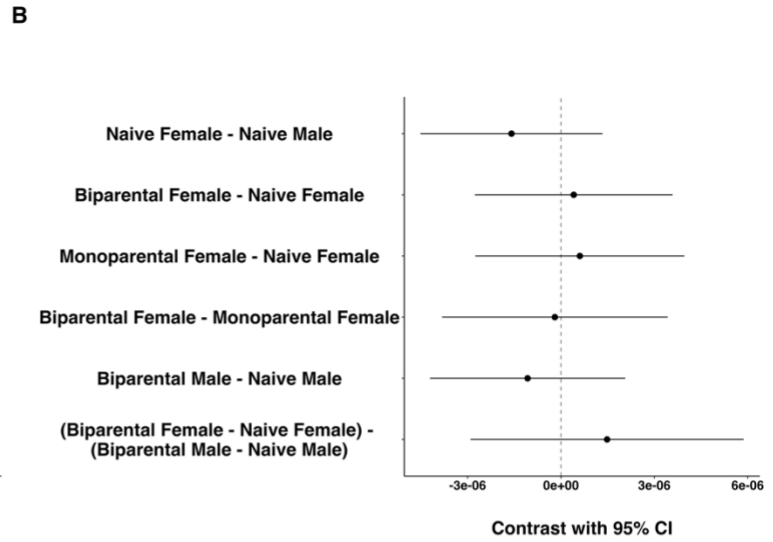
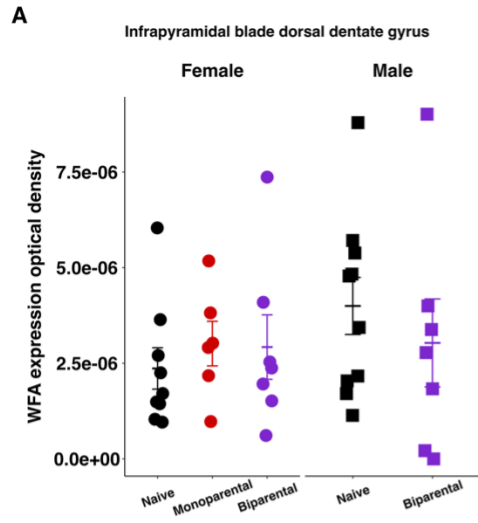
In the dorsal DG, contrasts revealed no differences across parental groups or sexes in the particle analysis of Iba-1-expressing cells (Figure 10A and 10B). Furthermore, there were no differences detected across parental groups or sexes in the ventral DG for the particle analysis of Iba-1-expressing cells (Figure 10C and 10D).



**Figure 10: Number of Iba-1 expressing cells in the DG.** (A) The total count of Iba-1-expressing cells (microglia) in the dorsal DG. Means are indicated by the line  $\pm$  SEM (n = 6 to 10 per group). (B) Contrasts for the total number of Iba-1 cells in the dorsal DG with 95% confidence interval. There were no differences across parental conditions or sexes. (C) The total count of Iba-1-expressing cells in the ventral DG. (D) Contrasts for the total number of Iba-1 cells in the ventral DG with 95% confidence interval. There were no differences across parental conditions or sexes.

#### **4.5. WFA expression for PNNs**

In the dorsal region, there were no differences across parental groups or sexes in the optical density of WFA expression in the infrapyramidal blade (Figure 11A – 11B) and the hilus (Figure 11C – 11D). Furthermore, there were no differences in the optical density of PNN expression in the infrapyramidal blade of the ventral DG across parental groups or sexes (Figure 11E – 11F).



**Figure 11: Optical density of WFA expression in the DG.** (A) The average optical density of WFA in the infrapyramidal blade of the dorsal DG. Means are indicated by the line  $\pm$  SEM (n = 6 to 10 per group). Values were normalized by dividing the optical density by the area of the ROIs (in  $\mu\text{m}^2$ ) that were drawn for each image. (B) Contrasts for the optical density of WFA expression in the infrapyramidal blade of the dorsal DG with 95% confidence interval. There were no differences across parental conditions or sexes. (C) The average optical density of WFA in the hilus of the dorsal DG. (D) Contrasts for the optical density of WFA expression in the hilus of the dorsal DG. There were no differences across parental conditions or sexes. (E) The average optical density of WFA in the infrapyramidal blade of the ventral DG. (F) Contrasts for the optical density of WFA expression in the infrapyramidal blade of the dorsal DG. There were no differences across parental conditions or sexes.

## **SECTION 5: DISCUSSION.**

### **5.1. General discussion**

The objective of this study was to investigate the effects of different types of parental experience on spatial cognition, anxiety-like behaviour, and hippocampal plasticity in female and male degus. Degus are a biparental rodent species in which both males and females exhibit pup-caretaking behaviour during the postpartum period. This model allows us to examine the effects of parenthood in both mothers and fathers while also exploring the effects of biparental and monoparental maternal experience (i.e., when the male partner is absent during the postpartum period).

Our first prediction was that both mothers and fathers will exhibit changes in anxiety-like behaviour and spatial cognition compared to naïve controls. Contrary to our prediction, we did not detect an effect of parental experience on measures of anxiety-like behaviour in the open field or the open arms of the elevated plus maze. However, we observed biparental experience differentially modulating the proportion of time spent in the closed arms between sexes. Biparental females exhibited more anxiogenic behaviours (spending more time in the closed arms of the elevated plus maze) while biparental males exhibited more anxiolytic behaviour (spending less time in the closed arms). Furthermore, measures of spatial learning and memory on the Barnes maze were also altered in a sex-dependent manner across parental groups when analyzing the total number of approaches to non-target holes. Most notably, biparental males exhibited impaired spatial cognition compared to naïve males and the difference observed in spatial abilities were more pronounced in biparental males than biparental females.

Secondly, we predicted that parental experience would alter the expression of hippocampal microglia and PNNs in both sexes compared to naïve controls. However, there were

no differences detected across parental groups in microglia cell density or PNN density in the dorsal or ventral DG of the hippocampus in either males or females.

Lastly, we predicted that monoparental mothers will display differences in behaviour and neuroplasticity compared to biparental mothers. No differences were observed in measures of anxiety-like behaviour between monoparental and biparental mothers. However, on the last day of Barnes maze training, we observed monoparental mothers exhibiting enhanced spatial cognition (suggested by a lower number of approaches to non-target holes) compared to both biparental and naïve females. Finally, measures of hippocampal microglia and PNN density were not different between monoparental and biparental mothers.

Overall, our findings indicate that parental experience remodels behaviour and differentially affects anxiety-like behaviour and spatial cognition across mothers and fathers. However, these behavioural changes are not accompanied by changes in hippocampal microglia or PNN expression.

## **5.2. Anxiety-like behaviour.**

### **5.2.1. Biparental experience differentially modulates anxiety-like behaviour in mothers.**

In our study, maternal experience did not affect measures of anxiety-like behaviour in the open field. There were no differences in the proportion of time spent in the centre of the open field in either biparental or monoparental mothers. Additionally, there were no differences in the total distance travelled indicating that all degus exhibited similar locomotor activity. On the elevated plus maze, we observed no differences in the time spent in the open arms, the centre, and the total distance travelled across maternal groups. However, biparental experience affects females differently compared to males when analyzing the proportion of time spent in the closed

arms of the elevated plus maze. Although both naïve females and males exhibit a lot of variability across individuals, the results from the interaction contrast suggest that parental experience modulates anxiety-like behaviour differently between females and males, leading to differences in behavioural phenotype. On average, biparental females exhibit an increase in anxiety-like behaviour as all of them spent more than half of the time on the closed arms. Majority of biparental males, however, spent less than half of the time on the closed arms, suggesting a decrease in anxiety-like behaviour. However, because no differences were detected between biparental females and naïve females, or between biparental males and naïve males, it is difficult to draw definitive conclusions about the effect of parental experience on anxiety-like behaviour in degus.

Research examining the effects of maternal experience on anxiety-like behaviour in rats has been equivocal (reviewed in Duarte-Guterman & Gadea, 2023; Macbeth & Luine, 2010). Some studies with postpartum timepoints similar to our own, in which behavioural measures were conducted after weaning, also reported no differences in anxiety-like behaviour between rat mothers and naïve controls (Furuta et al., 2013; Lemaire et al., 2006). However, in contrast to our results, other studies have found that the late postpartum period (specifically after weaning the pups) in rat mothers is characterized by a reduction in anxiety (Byrnes & Bridges, 2006; Love et al., 2005; Wartella et al., 2003) whereas another study indicated that rat mothers exhibit increased anxiety-like behaviour (Pawluski et al., 2009, 2009). Differences in measures of anxiety-like behaviour observed across studies may be attributed to the effect of estrous cycle on affective behaviour in mothers. Rat mothers exhibit a reduction in anxiety-like behaviour on the elevated plus maze (even when tested six to eight weeks post-weaning) compared to naïve females only during the proestrous phase (Byrnes & Bridges, 2006). In rats, proestrous is

characterized by elevated levels of gonadal hormones including estradiol and prolactin (Ajayi & Akhigbe, 2020). These results indicate that changes in hormonal concentration interact with maternal experience to influence anxiety-like behaviour into the postpartum period, emphasizing the importance of accounting for estrous cycle (Byrnes & Bridges, 2006). The duration of the estrous cycle in degus are different from other rodent species as they range from 10 to 22 days (Mahoney et al., 2011). In contrast, the estrous cycle of rats are typically 5 days long while mice exhibit more variability, ranging from 2 to 8 days (Lovick & Zangrossi, 2021). In our study, we visually observed vaginal openings of female degus to characterize their estrous cycle on each day that behavioural testing was conducted. Degus have closed vaginal membranes which inhibits our ability to perform vaginal swabs and cell inspections (Mahoney et al., 2011). The estrous cycle of degus were classified into three categories: open (when degus are in estrous), partially open, and closed (Mahoney et al., 2011). Future steps involve analyzing whether behavioural differences exist across the estrous cycle of degus which may account for the observed variability in behavioural measures. Overall, in our current study, although we detected no behavioural differences between biparental females and naïve females, the results of our interaction (for the closed arms of the elevated plus maze) suggest that biparental experience differentially modulates anxiety-like behaviour in mothers compared to fathers.

Previous research on the effects of single motherhood (i.e., monoparental experience when their male partner is removed during the postpartum period) in biparental rodent species are scarce. However, our findings are consistent with a previous study that reported no differences in measures of anxiety-like behaviour across biparental mothers, monoparental mothers, and naïve females (Zhao et al., 2019). The absence of behavioural differences across different maternal groups reflects our own results where we also observed no differences across

biparental, monoparental, and naïve females in anxiety-like behaviour. Contrary to our results, prairie vole mothers that were isolated from their male partners exhibited increased anxiety-like behaviour, when tested a day after giving birth, compared to non-separated mothers (Bosch et al., 2018). One potential explanation for the observed difference across biparental species is the timing of partner separation, in which removing partners during pregnancy (Bosch et al., 2018) may induce different effects on mothers compared to removing partners after giving birth (such as in our study). Another potential explanation is that prairie voles are socially monogamous (i.e., a pair-bonding species (Bosch et al., 2018)) while degus are socially polygamous (Aspillaga-Cid et al., 2021). Degus have much more flexible family structures compared to other biparental species because in the wild, degus are communal breeders that exhibit alloparental care (Aspillaga-Cid et al., 2021). As a result, partner separation and bond disruption may have a greater effect on prairie vole mothers and lead to more pronounced depressive- and anxiety-like behaviours (Bosch et al., 2018). Nonetheless, our study on degus expands the literature on the effects of parenthood to include a species with a different social and mating system. Lastly, all the studies on behavioural measures of anxiety in single mothers of a biparental species have been conducted in the early postpartum period, within a week of giving birth (Bosch et al., 2018; Zhao et al., 2019). During this timepoint, separating mothers from their pups can significantly impact behaviour and previous studies have highlighted the effects of pup-contact on anxiety-like behaviour (Lonstein, 2005; Miller et al., 2011). In our current study, we conducted behavioural measures in the post-weaning period to reduce the effect of pup-contact and separation on behaviour. Ultimately, behavioural differences observed between our results and previous studies may be attributed to differences in anxiety-like behavioural profiles between species and the exact timing of the postpartum period (Lonstein, 2005; Miller et al., 2011).

Overall, our results in connection to the established literature suggest that anxiety-like behaviour is influenced by maternal experience and dynamically remodelled throughout the postpartum period. Our current study extends the literature on the effects of parental experience on anxiety-like behaviour and offers a novel contrast that analyzes how parenthood might affect biparental and monoparental mothers in distinct ways.

#### 5.2.2. Biparental experience differentially modulates anxiety-like behaviour in fathers.

The results of our study indicate that biparental experience leads fathers to exhibit decreased anxiety-like behaviour compared to monoparental mothers, by spending less time in the closed arms of the elevated plus maze. Previous research in fathers in prairie voles and California mice has also been equivocal. For instance, prairie vole fathers early in the postnatal period exhibited increased anxiety-like behaviour compared to naïve males (Lieberwirth et al., (2013)). In California mice, fathers exhibit a reduction in anxiety-like behaviour only when tested during the mid-postnatal period (Colt et al., 2025; Hyer et al., 2016). A potential explanation for the observed reduction on the elevated plus maze is because pup retrieval is at its highest during this timepoint in California mice (Colt et al., 2025; Hyer et al., 2016). As a result, fathers may experience increased emotional resilience and exhibit reduced anxiety-like behaviour compared to earlier or later in the postnatal period (Colt et al., 2025; Hyer et al., 2016). However, in our current study, we did not detect a difference between biparental males and naïve males in behavioural measures of anxiety on the elevated plus maze when tested during the post-weaning period. In addition to there being little research on the effects of paternal experience on anxiety-like behaviour, no studies have analyzed the effect of fatherhood in the late postnatal period as the literature has exclusively focused on the early to mid-postnatal period (Colt et al., 2025; Hyer

et al., 2016). Our study was conducted in the post-weaning period which limits the effect of pup contact and pup separation on the fathers. Nonetheless, our results in addition to the extant literature suggest that the expression of anxiety-related behaviour varies across biparental species and is heavily dependent on the timing of the postnatal period.

An additional factor to consider when investigating anxiety-like behaviour in the open field and elevated plus maze is the duration of the task. Previous studies vary in the lengths of exploration time, ranging from eight to thirty minutes (Fleming & Luebke, 1981; Wartella et al., 2003; Zhao et al., 2019), which can further contribute to differences across studies. Differences in exploration time may influence the degu's behaviour as they become accustomed to the apparatus and explore more when the duration of the task is longer (Pawluski et al., 2009). Furthermore, previous studies have reported differences in open field behaviour across rat strains which further emphasize the importance of the duration of the task (Schmitt & Hiemke, 1998; Stöhr et al., 1998). For instance, Lewis rats have been observed to exhibit greater locomotor activity early in the open field compared to Fischer rats that become more active towards the end of the task (Stöhr et al., 1998). These results emphasize the importance of considering strain and species differences and the duration of the open field task when assessing behavioural measures of anxiety. Although our current study detected no behavioural differences across parental groups throughout the entire ten minutes of the open field, it is worth investigating whether differences exist within specific time increments of the task. For instance, analyzing potential differences in exploratory behaviour of the degus between the first and second five-minute intervals of the open field. In our current study, administering the open field for ten minutes may have potentially drowned out our ability to detect any behavioural differences. Analyzing the progression of the

degus' behaviour across different timepoints in the open field will allow us to further investigate whether parental experience affects anxiety-like behaviour in degus.

Overall, although we detected no differences in measures of anxiety-like behaviour between biparental fathers and naïve males, our results provide novel contrasts to suggest the differential effects of parental experience between mothers and fathers.

### **5.3. Spatial learning and memory.**

5.3.1. Monoparental maternal experience alters spatial cognition on the final day of the training phase.

In the Barnes maze task, spatial learning and memory were altered across maternal groups towards the end of the training phase. When analyzing the total number of approaches to non-target holes across all four training days, neither biparental nor monoparental mothers were different from naïve females. On training day 4, however, monoparental females made fewer approaches to non-target holes, suggesting enhanced spatial learning compared to both biparental and naïve females. Although there is considerable variability in the behaviour of naïve and biparental females, monoparental mothers consistently displayed better performance on the maze. Our results suggest that the experience of partner absence differentially effects spatial cognition in mothers.

Previous studies have shown rat mothers exhibit enhanced spatial memory compared to naïve females even when tested two to 35 days post-weaning (Cost et al., 2014; Pawluski, Vanderbyl, et al., 2006; Pawluski, Walker, et al., 2006). In contrast, we observed no differences between both groups of mothers and naïve females, except for on the final training day where monoparental females outperformed both biparental and monoparental females. However, it is

important to note that our results may be attributed to differences in levels of motivation and anxiety in biparental and naïve females rather than changes in spatial cognition. A previous study investigating degus' performance on the Barnes maze indicated changes in levels of motivation and learning ceiling effects over the course of training (Popović et al., 2010). Degus exhibit a decrease in the number of approaches to non-target holes over the first few days which is indicative of learning (Popović et al., 2010). However, around training day five and six, the number of approaches began to increase thereafter, likely due to reduced motivation and levels of anxiety which prompted exploratory behaviours (Popović et al., 2010). The results from our study suggest that levels of motivation between monoparental, and naïve and biparental females may be different and play a significant role in exploratory behaviour, since enhanced performance of monoparental females was only observed on the final training day. Future directions involve analyzing the learning curve for each individual degu across the four training days using a repeated measures design to acquire more insight into differences in learning across groups.

To my knowledge, no study has analyzed behavioural measures of spatial learning and memory in mothers of a biparental species, including spatial abilities in single mothers. With our study being the first to do so, our results provide a novel analysis on how different types of maternal experiences may modulate spatial cognition. Enhanced spatial performance observed in monoparental mothers, whether due to improved spatial cognition or differences in motivation levels, is a novel finding that extends the literature on the differential effects of maternal experience.

### 5.3.2. Biparental paternal experience alters spatial cognition in fathers.

Across all four training days and training day 1, our results indicate that biparental males exhibit reduced spatial abilities compared to naïve males. Furthermore, the difference between biparental males and naïve males was greater than the difference between biparental females and naïve females.

Previous research have observed fathers exhibiting enhanced cognitive functioning compared to naïve males, including on spatial learning and recognition memory tasks during the early postpartum period (Colt et al., 2025; Franssen et al., 2011). There are several plausible explanations for the discrepancy between our results and the established literature. As mentioned previously, the timing of the postnatal period and pup contact may significantly contribute to differences observed across studies (Lonstein, 2005). Studies investigating the effects of fatherhood conduct spatial tasks exclusively during the early to mid-postnatal period which may result in behavioural changes due to pup-separation (Colt et al., 2025; Franssen et al., 2011). Our current study offers a novel timepoint by investigating the effects of paternal experience on spatial learning and memory in the post-weaning period. Another potential explanation is that fatherhood-induced spatial memory enhancements may become more pronounced only when the complexity of the task increases (Franssen et al., 2011). As mentioned previously, California mice fathers exhibited no differences in spatial abilities across any training days except when the task was the most challenging (Franssen et al., 2011). Furthermore, studies exploring the effects of parental experience often use baited mazes with food rewards to measure spatial cognition (Franssen et al., 2011; Pawluski, Vanderbyl, et al., 2006). In our study, the Barnes maze uses a mild aversive stimulus (being in open space which induces anxiety in rodent species) to motivate the degus to locate the target hole and find the escape box. However, having the ability to

efficiently and effectively forage as a parent is incredibly important for various rodent species as it increases the rate of survival of their young (Kinsley & Lambert, 2008; Lambert et al., 2005). As a result, both mothers and fathers may perform better on tasks that simulate foraging due to increased motivation levels to search for resources. This may be especially relevant in degus since males become highly territorial, increase in foraging and exhibit more exploratory behaviours around the breeding season (Quispe et al., 2009; Soto-Gamboa et al., 2005). Although various factors may contribute to differences observed across studies, our research offers novel results that biparental degu fathers exhibit impaired spatial learning and memory compared to naïve males on the Barnes maze.

### 5.3.3. Naïve male degus exhibit enhanced performance compared to naïve females.

In the Barnes maze task, we also observed sex differences in spatial abilities across naïve degus. In particular, naïve males showed enhanced performance compared to naïve females across all four days and on training day 1. Our results align with previous studies that have reported enhanced spatial learning and memory in male compared to female rodents (Gresack & Frick, 2003; Harrison et al., 2006; Hawley et al., 2012; Safari et al., 2021). This difference has been reported in mice and rats across various behavioural tests that have been used to measure spatial cognition (Gresack & Frick, 2003; Harrison et al., 2006; Hawley et al., 2012; reviewed in Jonasson, 2005; Safari et al., 2021). However, in contrast to our results, previous studies in mice and degus have shown opposing effects where females outperform males on spatial tasks (Maheu et al., 2025; Popović et al., 2010). For instance, female degus made less reference memory errors (i.e., approaches to non-target holes) compared to males over the course of training; suggesting that female degus exhibit enhanced spatial abilities compared to males (Popović et al., 2010).

However, performance differences on these spatial tasks may be attributed to strategy selection rather than underlying differences in spatial abilities between males and females (Hawley et al., 2012). Female degus have been found to employ a spatial strategy (i.e., using spatial cues to locate the target hole) while male degus use a random (i.e., approaching non-adjacent holes and crossing over the centre of the maze to locate the target hole) or serial strategy (i.e., locating the target hole by systematically approaching consecutive holes) (Popović et al., 2010). Future work could investigate search strategies across individuals to further analyze performance on the Barnes maze.

#### 5.3.4. Parental experience does not alter long-term spatial memory.

Our study observed no differences across parental groups on the probe (one day after the last training day) or retention day (one week after probe), suggesting that parental experience does not alter long-term memory. Contrary to our results, rat mothers (tested two weeks post-weaning) exhibited enhanced long-term measures of spatial memory compared to naïve females (Love et al., 2005). Using a baited dry land maze, a probe test was conducted one week following the habituation phase where rats with maternal experience displayed improved spatial cognition (Love et al., 2005). However, long-term spatial memory in fathers or monoparental mothers in a biparental species have not yet been explored. As our study observed no differences in the retention task (conducted one week after the final training day), there may be species differences in the effect of parental experience on long-term spatial memory.

#### **5.4. Parental experience does not alter microglia expression in the DG.**

In our current study, we analyzed microglia expression in the DG of the hippocampus to investigate possible neural mechanisms that are regulating changes in spatial cognition and anxiety-like behaviour. However, in both the dorsal and ventral DG, there were no differences in microglia cell density across parental groups. Microglia are often implicated in neuroinflammatory responses but also play a critical role in synaptic and neuronal plasticity to regulate hippocampal-dependent learning and memory, and anxiety-like behaviour (Cornell et al., 2022; Parkhurst, Yang, Ninan, Savas, John R. Yates, et al., 2013).

Our results align with two previous studies that observed no differences in microglia density in the DG between primiparous rat mothers and naïve females (Duarte-Guterman et al., 2023; Eid et al., 2019). However, an increase in microglia count was observed in biparous mothers (mothers that underwent two separate pregnancies) compared to naïve females (Duarte-Guterman et al., 2023). These results suggest that the amount of parity remodels the hippocampus and influences the expression of microglia in the DG, with multiple pregnancies potentially inducing more pronounced effects.

Furthermore, previous studies suggest that changes in microglia density begin during pregnancy and persist into the postpartum period, although changes become less pronounced. Microglia density in the dorsal hippocampus was reduced in rat mothers compared to naïve females during late pregnancy and persisted into the late postpartum period (up to PPD 21) (Haim et al., 2017). However, in our study, brains were collected and analyzed at the end of behavioural testing (around PPD 60) which is significantly later into the postpartum period. Taken together, the results suggest that maternal experience influences the density of microglia during the postpartum, but the effects may not be as pronounced after weaning. Nonetheless, the

timing across studies is worth considering to further identify how hippocampal microglia density is remodelled over the course of the postpartum period and into the long-term. However, differences between studies may also exist due to the hippocampal subregions that were analyzed. Previous studies averaged microglia cell counts across the DG, CA1 and CA3 dorsal region (Haim et al., 2017) while our current study focuses exclusively on the DG. Future directions in our study involve analyzing microglia density in the dorsal and ventral CA1 and CA3 hippocampal regions. This will allow for a more complete understanding of any potential region-specific changes within the hippocampus.

In addition to analyzing the density of microglia in the brain, studies quantify subpopulations of microglia based on their morphology. Studies analyzing the morphology of microglia have identified differences between mothers and naïve females specifically during the early postpartum period (Eid et al., 2019; Haim et al., 2017), while others have observed no differences in the late postpartum period (Duarte-Guterman et al., 2023). At PPD 8, rat mothers have fewer ramified microglia and greater intermediate microglia than naïve females, which are characterized by a larger cell body and shorter processes (Eid et al., 2019). A transition to microglia morphology with reduced processes have been observed during pro-inflammatory responses in disease models (including PTSD, brain injury, and Alzheimer's (Green & Rowe, 2024)) and suggest a transient change in the dynamic remodeling of microglia morphology during the early postpartum (Eid et al., 2019). However, it is important to note that the exact function of microglia cannot be deduced by analyzing morphology alone since microglia also carry out various functions that play a role in neuroplasticity (including synaptic pruning and secreting neurotrophic and growth factors) (Borst et al., 2021; Cornell et al., 2022; Eid et al., 2019; Green & Rowe, 2024; van Rossum & Hanisch, 2004). Future directions involve analyzing

microglia morphology in addition to other markers of neuroinflammation and neuroplasticity in the hippocampus to acquire insight into their function.

Furthermore, our study observed no differences in hippocampal microglia density between monoparental females and both biparental and naïve females. Previous studies on biparental species that include a monoparental group often focus on the effects of paternal deprivation on the pups (De Schultz et al., 2020, 2023; Helmeke et al., 2009). Few studies focus on the experience of partner removal on mothers themselves and no study to date has analyzed microglia density in monoparental mothers. Overall, our results further contribute to the current literature by investigating the effects of single motherhood, suggesting no differences across monoparental, biparental, and naïve females on microglia density well after the postpartum period.

In biparental fathers, our results indicate that microglia density in the DG were not different from naïve males. Research investigating the effects of fatherhood on markers of neuroinflammation has not been conducted. In regard to other markers of hippocampal plasticity, previous studies have shown evidence of changes in the expression of hippocampal neurogenesis in fathers that reflect the changes observed in mothers (Eid et al., 2019; Glasper et al., 2011, 2011; Hyer et al., 2016; Lieberwirth et al., 2013). These results suggest that similar mechanisms in the hippocampus may be altered in both mothers and fathers to regulate behavioural changes. However, it is important to note that studies investigating neural changes in fathers have been done exclusively during the early to mid-postpartum period, with our study being the first to investigate the long-term effects of paternal experience on hippocampal neuroplasticity (at PND 60). Although we observed no differences across groups, the results from our study are novel and

add to the literature by exploring the effects of parental experience on hippocampal microglia density in biparental mothers, monoparental mothers, and fathers.

### **5.5. Parental experience does not alter PNN expression in the DG.**

Lastly, we analyzed the density of PNN expression in the DG due to its role in regulating hippocampal-dependent cognitive and affective behaviour (Duncan et al., 2019; Reichelt et al., 2019). In our current study, we observed no differences in the optical density of WFA (a marker of PNNs) in either the dorsal or ventral region of the DG. PNNs are extracellular matrixes that play a pivotal role in synaptic plasticity and neuroplasticity (Fawcett et al., 2022; Sanchez et al., 2024). PNNs surround neurons, regulate axonal growth, and direct dendritic projections to stabilize synapses and modulate the extent of synaptic plasticity (Fawcett et al., 2022; Sanchez et al., 2024).

In regard to the effects of maternal experience on PNN expression, previous studies have focused on neural regions that are critical in regulating maternal behaviour (Lau et al., 2020; Leuner et al., 2023; Uriarte et al., 2020). For instance, increased expression of PNNs in the mPOA was observed at gestation day 10, peaked at gestation day 21, and decreased thereafter (Uriarte et al., 2020). An increase in PNN density was also observed in the somatosensory cortex of virgin female mice that exhibited maternal behaviour toward conspecific pups (Lau et al., 2020). These results suggest that parturition and the postpartum period remodel the expression and density of PNNs, however, it may be dependent on the timing (i.e., more pronounced effects during gestation and early in the postpartum) and neural region (i.e., more pronounced in regions that are directly associated with regulating maternal behaviours). It is worth mentioning that there are methodological differences in the techniques that are used to quantify PNN density.

Across studies, different techniques are used due to variation in the intensity of PNN expression across rodent species which may account for contrasting results between studies. For instance, research on mice often rely on manual counts to calculate PNN density in the DG (Lau et al., 2020; Maheu et al., 2025; W. Shi et al., 2019). In the DG of degus, however, the dense expression of PNNs make manual counting challenging and inaccurate. As a result, studies analyzing PNN expression in degus, including our own, rely on optical density or particle analysis measurements (Garduño et al., 2024; Tan et al., 2022). Furthermore, despite WFA being a commonly used marker for PNNs, a study by Härtig et al., (2022) reported that alternative markers may be used to label PNNs depending on the neural region. For instance, in the CA3 hippocampal region of mice, there are differences in the expression of PNNs between WFA and ACAN (which labels PNNs by binding specifically to aggrecan) (Härtig et al., 2022). These results suggest that markers used to label PNNs are region-specific and may contribute to differences across studies.

Our results indicate no differences between biparental fathers and naïve males in the density of PNNs in the DG. To my knowledge, no studies to date have investigated PNNs in fathers of a biparental species in any brain region, including the hippocampus. Our results also indicate no differences between monoparental mothers and naïve females. No studies have explored the effects of monoparental maternal experience in PNN expression either. Overall, our study offers a novel contrast to investigate the effects of parental experience on PNN density in the hippocampus and is the first to explore potential changes in mothers, fathers, and monoparental mothers.

## **5.6. Strengths and limitations.**

A major strength of our current study is the inclusion of both males and females undergoing parental experience. In the established literature, there is currently no published study that investigates the effects of parental experience on behaviour and the brain in both mothers and fathers of the same species. To my knowledge, a single study observed reductions in hippocampal neurogenesis in both mother and fathers of California mice (Glasper et al., 2011), however, a comparison on behaviour between sexes has not been conducted. In our study, we include both sexes and provide a novel contrast to explore how parental experience might differentially affect males and females. Furthermore, our study includes an understudied group in monoparental mothers providing a contrast to biparental and naïve females. Previous studies analyzing monoparental maternal experience in biparental species are scarce. There have been two previous studies that analyze the effects of partner separation on anxiety-like behaviour in mothers (Bosch et al., 2018; Zhao et al., 2019), however, no studies to date have analyzed measures of spatial learning and memory.

Another strength of our study is conducting behavioural testing one-week post-weaning. As discussed previously, studies analyzing the effect of fatherhood and partner separation on mothers in biparental species have focused exclusively on the early to mid-postpartum period (Bosch et al., 2018; Franssen et al., 2011; Glasper et al., 2016). Pup separation is a significant confound and may impact the behaviour of both mothers and fathers, especially in regard to anxiety-like behaviour (Lonstein, 2005; Miller et al., 2011). In our study, we considered the effects of pup separation and conducted all behavioural tasks one-week post-weaning. However, conducting behavioural tests during the late postpartum period may also serve as a limitation since the brains were not analyzed until about three-weeks after weaning. At this point, the

influence of parental experience on neuroplasticity may be reduced compared to earlier in the postpartum period.

Another limitation of this study is the single housing of all degu pairs a week prior to and throughout the duration of behavioural testing. This was done to ensure that biparental females did not become pregnant after weaning, since previous research has shown substantial effects of pregnancy on cognition and anxiety-like behaviour (Cost et al., 2014; Galea et al., 2000; Macbeth, Gautreaux, et al., 2008; Pawluski, Vanderbyl, et al., 2006). To control for the effects of single housing the biparental degus, we separated naïve pairs at the same time. Although this was done out of necessity to prevent the effects of pregnancy on behaviour, previous research has shown the effects of single housing on cognition and anxiety-like behaviour across various rodent species (Cavieres et al., 2023; Hebda-Bauer et al., 2019; Nádeníček et al., 2022; Popović et al., 2023; Starkey et al., 2007). Female degus that were single housed exhibited changes in spatial cognition compared to group housed degus (Cavieres et al., 2023). On the Barnes maze, single housed degus exhibited improvements in spatial learning on the final training day but had impaired long-term memory when tested one week after, on the retention trial (Cavieres et al., 2023). In a follow-up study, socially isolated female degus exhibited increased anxiety-like behaviour in the open field, and impairments in both social and working memory (Cavieres et al., 2023). Although we controlled for the effects of individual housing by separating all degu pairs, the maladaptive effects of social isolation on both cognitive and affective behaviour are worth mentioning.

Nonetheless, our study significantly contributes to the current literature by offering a comprehensive overview on the effects of parental experience on cognitive and anxiety-like behaviour and markers of neuroplasticity in both sexes of the same species. In doing so, our

study provides a novel contrast in analyzing the differential effects of the transition to parenthood in males and females. To my knowledge, our current study is the first to provide a direct comparison on how parental experience alters behaviour and remodels the brain in both mothers and fathers. Furthermore, the inclusion of monoparental females (in addition to biparental females) extends the literature on the effects of partner separation and bond disruption on behaviour and the brain. This allows for a novel analysis on the behavioural and neural effects of different types of parental experiences.

### **5.7. Future directions.**

As discussed previously, a next step in our study regarding the analysis of anxiety-like measures include analyzing specific timepoints within the ten minutes of both the open field and elevated plus maze. This will allow us to investigate motivation levels across individuals and provide further insight into behavioural changes that potentially occur over the course of testing. For the Barnes maze, analyzing the learning curve for individuals across each individual training day will give us insight into individual differences in spatial learning, including levels of motivation and ceiling effects. Furthermore, investigating the strategies that are used on the Barnes maze will provide context into the spatial strategies that are employed across groups and sexes. We also conducted the novel object recognition task during behavioural testing which will be analyzed to observe any potential effects of parental experience on recognition memory. Furthermore, both microglia and PNN density will be explored for the dorsal and ventral CA1 and CA3 regions. Additional markers of neuroinflammation will be analyzed including microglia morphology and cytokine levels (including both pro- and anti-inflammatory cytokines). This will

provide insight and a more complete understanding of any potential neuroinflammation changes across various subregions of the hippocampus.

A future direction to take that would further our current study is to isolate the effects of pregnancy and parental experience on behaviour and the brain through the addition of other types of parental groups. For instance, the inclusion of pup-sensitized females (i.e., naïve females that are exposed to pups and exhibit in pup-caretaking behaviour) and experienced females without maternal experience (i.e., females that undergo pregnancy and parturition but the pups are removed after birth) would provide further insight into the differential effects of reproductive experience and maternal experience. Previous research has indicated that naïve female rats exposed to pups exhibit increased neuron proliferation and cell survival compared to primiparous and biparous females (Pawluski & Galea, 2007) without affecting spatial cognition (Pawluski, Vanderbyl, et al., 2006;) and anxiety-like behaviour (Pawluski et al., 2009). These results suggest that the experience of mothering alone alters the extent of neuroplasticity but not behaviour in rats. However, the inclusion of these groups and their effect on behaviour and the brain has not been explored in biparental rodent species. Degus exhibit alloparental pup-care which will allow for a paradigm where naïve female degus exhibit maternal behaviour toward pups without undergoing pregnancy. This will allow us to isolate the effects of pregnancy and pup-caretaking behaviour on spatial cognition, anxiety-like behaviour, and hippocampal plasticity. Furthermore, the inclusion of a male group with sexual experience (i.e., preventing fathers from taking care of pups by removing males after mating with females) will allow for further analysis into the effects of sexual experience on behaviour and the brain in the absence of fathering.

## **5.8 Conclusion.**

This current study provides a comprehensive overview into the effects of parenthood on behaviour and the brain in both males and females of the same species. Previous studies have used rats almost exclusively to study the effects of maternal experience. Additionally, there are few studies on the effects of fatherhood which must rely on biparental species such as California mice and prairie voles. Even more scarce are the effects of different types of maternal experience, including both biparental and monoparental maternal experience, in a biparental species. To date, a direct comparison on the effects of parenthood on behaviour and the brain between males and females of the same species has not been done. Our study comprehensively addresses these gaps in the literature by investigating spatial cognition, anxiety-like behaviour, and neural changes that potentially accompany parental experience in both sexes of a biparental species. Our results provide novel contrasts which suggest that parental experience differentially affects anxiety-like behaviour and spatial abilities between females and males. In particular, biparental experience differentially modulates anxiety-like behaviour in mothers compared to fathers. Furthermore, biparental fathers exhibit impaired spatial learning compared to naïve males, while monoparental females exhibit enhanced spatial learning compared to both biparental and naïve females. Lastly, in the dorsal and ventral DG, parental experience did not alter the expression of microglia and PNNs. This study contributes to further our understanding of how such an important life experience, namely the transition to parenthood, influences behaviour and remodels the brain.

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