Effects of Prenatal Testosterone Exposure and Adult Hormone Manipulation on Social Learning, Social Interactions, and Anxiety-Like Behaviour in Male and Female CD1 Mice

by

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We investigated interactions between organizational and activational effects of gonadal hormones on social behaviour and cognition. Dams were treated with testosterone propionate or sesame oil control. Litters underwent a battery of behavioural assays in adolescence and in adulthood, following sham surgery, gonadectomy, or gonadectomy with estradiol/testosterone replacement. Castration improved social learning in male mice treated prenatally with oil, but blocked learning in mice treated prenatally with testosterone. Social learning was blocked in ovariectomized female mice treated prenatally with testosterone, but was recovered via estradiol replacement. Male mice were less sociable than females, and prenatal testosterone exaggerated this difference. Prenatal testosterone shifted male mice from dominance aggression towards territorial aggression when interacting with a cage-mate, and reduced submissiveness in interactions with an intruder among females. Prenatal testosterone increased anxiety-like behaviour only in male mice. Our results have implications for understanding the role of hormones in mediating social and social cognitive behaviour.
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List of Abbreviations

AR – Androgen receptor
ERα – Estrogen receptor α
ERβ – Estrogen receptor β
GPER1 – G-protein coupled estrogen receptor
ERαKO – Estrogen receptor α knockout
ERβKO – Estrogen receptor β knockout
BDNF – Brain derived neurotrophic factor
T – Testosterone
TP – Testosterone propionate
EB – Estradiol benzoate
GDX – Gonadectomy
OVX – Ovariectomy
GDX+T – Castration with testosterone replacement
GDX+EB – Ovariectomy with estradiol benzoate replacement
STFP – Social transmission of food preferences
**Introduction**

Interesting and robust sex differences in social behaviour can be observed in nearly any social animal. These differences stem from pressure from both natural and sexual selection, and typically serve some adaptive advantage that enhances either reproductive success or survivability (Adkins-Regan, 2013). For example, male ungulates possess dramatic cranial ornaments and engage in ritualized shoving contests in order to win access to a mate. Females, on the other hand, possess smaller cranial structures, typically for defensive purposes or to enhance crypsis, and do not engage in aggressive intrasexual competition (Stankowich & Caro, 2009). Here, while sexual selection has necessitated ritualized aggressive behaviour among males, females of the same species do not engage in intrasexual aggression, and instead possess morphological characteristics that provide an adaptive advantage for avoiding or deterring predators.

It is well established that many aspects of sexually dimorphic behaviour and morphology are driven by gonadal steroid hormones. The actions of gonadal steroids, both during development and adulthood, play an integral role in sexual differentiation, sex-specific social behaviour, and processes related to learning and memory. The present study will endeavor to determine how prenatal hormone environment interacts with gonadal steroid action in adulthood to impact social and social-cognitive behaviours.

**Sexual Differentiation**

Sexual differentiation is driven throughout development and adult life by the actions of gonadal steroid hormones in combination with a number of genomic, epigenetic, and environmental factors (Arnold, 2009; McCarthy & Arnold, 2011; McEwen, 1983). In males, masculinization and defeminization are primarily carried out by both the direct action of
testosterone and its metabolites, including estradiol, following conversion by the aromatase enzyme (Feder & Whalen, 1965; McCarthy & Arnold, 2011). Testosterone is primarily produced in the testes, which are differentiated during prenatal development as a result of genomic action of the Y-chromosome linked SRY gene. Briefly, the SRY gene encodes testes determining factor, which in turn drives differentiation of the testes. The testes then begin to produce testosterone, which acts both directly and through its metabolites, including estradiol, during critical periods of development to alter sexually dimorphic tissues, including various regions in the brain (Arnold, 2009; Berenbaum & Beltz, 2011; McCarthy & Arnold, 2011). The organizing action of testosterone and its metabolites is accompanied by region-specific sexually dimorphic gene expression, which drives sexually differentiated and opposing changes in cell proliferation, cell death, and synapse formation at different brain regions in males and females (McCarthy & Arnold, 2011). Moreover, estradiol acts in development to enact long-term epigenetic changes to DNA and chromatin via sexually dimorphic changes in histone acetylation and steroid receptor promoter gene methylation that vary across brain regions, affecting later life proliferation and sensitivity of the estrogen and progesterone receptors (McCarthy & Arnold, 2011; Nugent, Schwarz, & McCarthy, 2011).

It is important to note that while testosterone has traditionally been thought of as the main gonadal steroid hormone involved in sexual differentiation, with the “default sex” being female, estrogens exert temporally dependent effects in both male and female animals, contributing to feminization and defeminization at different stages in development. Estradiol contributes to defeminization of the male fetus following aromatization, and has been shown to suppress female behaviour in neonatal rats (Feder & Whalen, 1965; Kudwa, Bodo, Gustafsson, & Rissman, 2005). These effects may be due to differences in critical periods for the
developmental effects of steroid hormones. Following the development of the testes until shortly after birth the male fetus is exposed to elevations in testosterone, which readily enters the brain and is aromatized into estradiol, while the female fetus is protected from masculinizing effects of estradiol in the brain as a result of competitive binding of estrogens by alpha-fetoprotein in the periphery (Bakker et al., 2006; Pang, Caggiula, Gay, Goodman, & Pang, 1979). Recent research suggests that, at least in rodents, feminization may be driven through a surge in estradiol during a perinatal critical period after the completion of the masculinization period that occurs in the developing male (which is also mediated by estradiol following aromatization), suggesting that the sex determining actions of gonadal steroids may be temporally dependent (McCarthy, 2010; McCarthy & Arnold, 2011; Wright, Schwarz, Dean, & McCarthy, 2010).

Gonadal steroids continue to drive sexual differentiation throughout adolescent and adult life, inducing further structural changes, as well as more immediate so-called “activational effects” (Arnold, 2009; Vermeersch, T’Sjoen, Kaufman, & Vincke, 2008). Puberty has been put forward as a second critical period for the organizational action of gonadal steroids, during which sexual differentiation is completed through a surge in gonadal steroid production (Elmlinger, Kühnel, Wormstall, & Döller, 2004). In addition, gonadal steroids act on target tissues throughout adult life to promote sexually dimorphic function, mediating behaviours such as aggression, mating, and learning in a sex dependent manner (Arnold, 2009; Mehta & Beer, 2009; Olsen, 1979; Scharfman & MacLusky, 2005).

In summary, adaptive sex-specific function is dependent on both the organizational and activational effects of gonadal steroids. Gonadal steroid action is required throughout early life to guide the development of sexually dimorphic structures, while continued production and activity of these hormones promotes the function of these structures, and produces short term
changes in morphology and behaviour throughout adult life (Arnold, 2009; McCarthy & Arnold, 2011; McEwen, 1983). This combined action of gonadal steroids not only influences classical sex-specific behaviours such as mating and aggression, but also plays an essential role in mediating more nuanced aspects of social behaviour, as well as learning, memory, and other cognitive abilities (Luine, Richards, Wu, & Beck, 1998; Mann & Svare, 1983; Meitzen, Grove, & Mermelstein, 2012; Olsen, 1979; Phoenix, Goy, Gerall, & Young, 1959a).

Gonadal hormone action during development can also alter later life responsiveness to the activational effects of gonadal steroids. Developmental exposure to exogenous testosterone reduces estrogen receptor (ER) expression in both female and male animals, and also reduces sensitivity to inhibitory signaling from luteinizing hormone in female animals (Kühnemann, Brown, Hochberg, & MacLusky, 1995; Sarma et al., 2005). This demonstrates that gonadal hormones act in development to alter gonadal steroid mediated mechanisms underlying cognitive function, and that the role of steroid hormones in mediating cognitive function in adulthood is dependent on the action of gonadal steroids in development.

Though gonadal steroids are primarily produced in the gonads, synthesis of both estradiol and testosterone also occurs in the brain, where testosterone is readily converted to 17β-estradiol and 5α-dihydrotestosterone (DHT) via the aromatase and 5α-reductase and enzymes, respectively (Bidlingmaier, Dörr, Eisenmenger, Kuhnle, & Knorr, 1986; Lephart, 1996). Moreover, testosterone acts independently and via its metabolites at the androgen receptor and estrogen receptors, making it difficult to isolate effects stemming from the action of testosterone from those of estradiol or DHT, which has implications for the interpretation of studies involving steroid hormone manipulation via gonadectomy or administration of testosterone.
Gonadal Steroids in Learning and Memory

Sex differences in aspects of learning, memory, and cognition are well documented. For example, where men, on average, tend to perform better on spatial tasks, such as maze navigation and route learning, women show an advantage in verbal learning tasks (Galea & Kimura, 1993; Kramer, Delis, & Daniel, 1988; Moffat, Hampson, & Hatzipantelis, 1998; Roof, 1993). Gonadal steroids are involved in learning and memory at the cellular level in both males and females, and have been shown in animal models to exert sex specific actions at brain regions essential for learning and memory processes, such as the hippocampus, hypothalamus, amygdala, and cortex (Hajszan, MacLusky, & Leranth, 2008; Meitzen et al., 2012).

Rodent studies have shown that both androgens and estrogens facilitate dendritic spinogenesis and synaptic plasticity, which indicates that these hormones may be a potential mechanism through which learning and memory are mediated (Hajszan, MacLusky, Johansen, Jordan, & Leranth, 2007; Phan, Lancaster, Armstrong, MacLusky, & Choleris, 2011; Woolley, 1998). Studies in rats have demonstrated that estradiol is associated with increased hippocampal NMDA receptor sensitivity and dendritic spine density in female animals, and that elevations in estradiol coinciding with the estrus cycle result in enhanced hippocampal excitability (Scharfman, Mercurio, Goodman, Wilson, & MacLusky, 2003; C. S. Woolley & McEwen, 1992; Catherine S. Woolley, 1998). In addition, female rats show reduced hippocampal dendritic spine density following gonadectomy, which is recovered by exposure to either testosterone, or estradiol. The ameliorative effects of testosterone treatment are blocked by an aromatase inhibitor, indicating that this effect may be dependent on the action of estradiol following aromatization (Leranth, Hajszan, & MacLusky, 2004).
Male rats show similar reductions in hippocampal spine density following gonadectomy, which is reversed after treatment with testosterone propionate, however, current research is conflicting regarding whether or not treatment with estradiol exerts similar effects (Jacome et al., 2016; Leranth, Shanabrough, & Redmond, 2002). Treatment with hydroxyflutamide, an androgen antagonist, and DHT have been shown to increase hippocampal spine density in the CA1 subfield in male rats, while estradiol has no effect, indicating that the effects are likely androgen mediated. Interestingly, these effects are also found in male rats possessing the Tfm (testicular feminization) mutation, which results in synthesis of dysfunctional androgen receptors, implying that these effects are not dependent on the androgen receptor (MacLusky, Hajszan, Johansen, Jordan, & Leranth, 2006). Moreover, both male and female rats show increases in hippocampal spine density in the CA1 subfield following treatment with dehydroepiandrosterone (DHEA) and flutamide, an androgen antagonist. The response to DHEA in males is unaffected by treatment with letrozole, an aromatase inhibitor, suggesting that these changes are not mediated by conversion to estradiol in males (MacLusky, Hajszan, & Leranth, 2004). Furthermore, the reduction in CA3 hippocampal spine density following gonadectomy is accompanied by mossy fiber expansion and increased apical dendritic length in male, but not female rats, which suggests a compensatory response to reductions in gonadal steroids among male, but not female, rats (Mendell et al., 2016). Taken together, these findings demonstrate that, while gonadal hormones are involved in hippocampal plasticity in both male and female rodents, the mechanisms involved are sexually dimorphic. Further research is needed to elucidate the nature of these differences, and to determine the mechanisms through which gonadal steroids effect changes in hippocampal plasticity. In addition, the importance of testosterone for the maintenance of hippocampal spine density has been corroborated in non-
human primates, with male vervet monkeys exhibiting reduced spine density in the CA1 hippocampal subfield following castration (Leranth, Prange-Kiel, Frick, & Horvath, 2004).

Behavioural studies indicate that the effects of gonadal hormones on cellular mechanisms believed to underlie learning and memory are accompanied by changes in performance on learning and memory tasks. Exogenous androgens have been shown to elicit the development of a “male-like” hippocampus, improving performance and eliminating sex differences in spatial learning tasks when administered neonatally to female rats, while castration and treatment with the anti-androgen cyproterone acetate impairs maze performance in male rats. These findings suggest that gonadal steroids facilitate cognition through organizational effects during early development (Joseph, Hess, & Birecree, 1978; Roof, 1993; Roof & Havens, 1992). In later life, elevations in estradiol have been correlated with impaired spatial learning in adult female voles, while treatment with exogenous estradiol improves object recognition memory in female mice (Galea, Kavaliers, Ossenkopp, & Hampson, 1995; Zhao, Fan, Fortress, Boulware, & Frick, 2012). Differences in testosterone among intact adult male rats do not appear to affect performance on spatial learning tasks, though castrated adult male rats exhibit impairments in both object recognition and spatial learning, while performance is recovered following treatment with testosterone (Aubele, Kaufman, Montalmant, & Kritzer, 2008; Galea et al., 1995; Spritzer et al., 2011). Collectively, these results indicate that gonadal steroids affect cognitive performance in spatial learning tasks through both organizational and activational mechanisms. Gonadal sex hormones therefore mediate processes related to spatial learning in a sex dependent manner, both through their actions during development and during adulthood. In addition, these interactive processes may partially underlie sex differences in performance on cognitive tasks.
Neuroendocrinology of Social Behaviour

Much of social behaviour is sexually dimorphic, perhaps due to differences in mate preferences and other evolutionary pressures between male and female animals (Geary, 1998). As discussed above, sexual differentiation is driven in part by the actions of gonadal steroids both in development and throughout later life. The developmental and activational actions of androgens and estrogens have been shown to influence behaviours such as aggression and social interaction, as well as social cognitive behaviours such as social recognition and social learning (reviewed in Ervin et al., 2015).

Developmental Effects of Gonadal Steroids on Social Behaviour

Permanent effects of prenatal hormone manipulations on brain development were first demonstrated as early as the late 1950s. Guinea pigs treated with testosterone prenatally exhibited altered sexual function in adulthood, which not only provided early evidence for organizational effects of testosterone on neural tissue, but also demonstrated that testosterone acts in utero to alter the way in which animals approach and interact with conspecifics (Phoenix et al., 1959). More recent evidence has investigated the effects of prenatal and neonatal androgenic environment on other aspects of social behaviour, such as social interaction, aggression, and maternal behaviour (reviewed below).

In humans, disturbances in prenatal hormone environment have been implicated in developmental disorders, including autism spectrum disorder (ASD). ASD shows a strong male bias and is characterized in part by profound social deficits, which has led researchers to propose the “extreme male brain” hypothesis to explain the perturbation of gonadal steroids observed in ASD (American Psychiatric Association & American Psychiatric Association, 2000; Baron-Cohen et al., 2014; Simon Baron-Cohen et al., 2011; Centers for Disease Control and Prevention,
ASD presents with a number of physiological abnormalities that indicate a potential role of developmental androgens in the etiology of the disorder. These include changes in brain structure that mirror exaggerations of typical testosterone driven development seen in the male fetus (Baron-Cohen et al., 2014). In addition, elevations in placental gonadal steroids are correlated with heightened occurrences of ASD, suggesting that there may be a link between prenatal hormone environment and ASD in humans (Baron-Cohen et al., 2014). Given the role of prenatal testosterone in influencing some aspects of social behaviour, it may be that prenatal testosterone also mediates the social cognitive aspects of ASD.

**Studying Social Behaviour and Social Cognition**

The present study is focused on a set of commonly studied behaviours relevant to the success of social animals, which have been previously shown to depend on gonadal hormones (reviewed in Choleris, Clipperton-Allen, Phan, & Kavaliers, 2009; Choleris, Clipperton-Allen, Phan, Valsecchi, & Kavaliers, 2012; K. Ervin et al., 2015). Social interactions between animals may be understood through investigation of tendencies to approach or avoid social stimuli, interest in investigating a conspecific, or inclination to engage in affiliative or agonistic behaviour in response to a conspecific (reviewed in Choleris, Clipperton-Allen, Phan, & Kavaliers, 2009; Ervin et al., 2015). Moreover, the establishment of stable dominance hierarchies is essential for cohabiting social animals in order to suppress aggressive conflicts between group members, and is of interest in understanding the nature of dominance relationships between animals (Grant & Mackintosh, 1963; Holekamp & Strauss, 2016; Miczek, Maxson, Fish, & Faccidomo, 2001). In addition, cohabiting social animals depend highly on social recognition, which describes the ability of an animal to acquire information about a conspecific, and is essential for mate selection, the avoidance of infected animals, and the
maintenance of dominance hierarchies (reviewed in Choleris, Clipperton-Allen, Phan, & Kavaliers, 2009; Ervin et al., 2015). In addition to learning about conspecifics, animals may also acquire information from conspecifics through social learning in order to avoid the necessity for individual learning (reviewed in Choleris et al., 2009, 2012; Ervin et al., 2015; Heyes & Galef, 1996). These behaviours are relevant to understanding naturalistic social behaviour in rodents, and many of these behaviours mirror human behaviour, making our research relevant to understanding behaviour in human populations.

**Gonadal Steroids and Agonistic Behaviour**

Laboratory studies of aggression typically involve the analysis of an interaction between the experimental animal and a conspecific. In the present study, aggression is taken to encompass multiple facets of agonistic behaviour, which can serve to exclude an intruder from one’s territory, or to establish a dominance hierarchy within a social group (reviewed in Ervin et al., 2015; Grant & Mackintosh, 1963). The task we have adopted here follows the resident-intruder paradigm, which involves the introduction of an unfamiliar conspecific to the home cage of the experimental animal (Clipperton-Allen, 2011; Ogawa et al., 1998). In this task, the resident is more likely to defeat or subdue the intruder animal due to the “prior residency effect”, which describes the tendency for an animal to initiate agonistic or dominant behaviour in familiar territory (Archer, 1998). Intruder animals are typically gonadectomized and group housed in order to reduce aggression and create reliable stimuli through which to test experimental animals. This is necessary because aggressive animals can elicit agonistic behaviour from animals that would not otherwise act aggressively (Denenberg, Gaulin-Kremer, Gandelman, & Zarrow, 1973).
The establishment of dominance hierarchies is important for group living among rodents and other social animals in order to minimize aggression, while determining the nature of social interactions and allocation of resources among group members (reviewed in Holekamp & Strauss, 2016; Miczek, Faccidomo, Fish, & DeBold, 2007). The initial establishment of a dominance hierarchy in rodents typically consists of repetitive agonistic interactions that decline as asymmetrical dominance relationships are established, reducing stress among both dominant and subordinate members and avoiding potentially costly fights between group members, while allowing members to benefit from the advantages of group living, including improved ability to repel predators and defend resources from intruders (Bartolomucci, Palanza, & Parmigiani, 2002; Bartolomucci et al., 2001; Ebensperger, 2001; Uhrich, 1938; Vekovishcheva, Sukhotina, & Zvartau, 2000). Agonistic interactions between high-ranked and low-ranked group members typically consist of aggression from dominant members, and submissive, defensive, and escape behaviour from submissive members, while both male and female mice living in stable hierarchies will attack an unfamiliar intruder introduced to the group (Palanza, Della Seta, Ferrari, & Parmigiani, 2005; Scott & Fredericson, 1951; Uhrich, 1938).

Steroid hormones play an important role in the regulation of dominance behaviour and aggression. Agonistic behaviour is qualitatively different between male and female mice, though female mice engage in as much or more agonistic behaviour than male mice (Clipperton-Allen, Almey, Melichercik, Allen, & Choleris, 2011; Clipperton-Allen, Cragg, Wood, Pfaff, & Choleris, 2010). Agonistic interactions between male mice typically involve more attacks, more boxing and preliminary aggressive behaviours, and more overt aggressive behaviour (Beeman, 1947; Clipperton-Allen et al., 2011, 2010; Ervin et al., 2015; Grant & Mackintosh, 1963). On the other hand, female mice will not typically attack a same-sex intruder, and engage in less
overt aggression than male mice. Rather, female mice tend to engage in agonistic behaviour oriented around the establishment of a dominance hierarchy for longer than male mice (Clipperton-Allen, Almey, Melichercik, Allen, & Choleris, 2011; Clipperton-Allen, Cragg, Wood, Pfaff, & Choleris, 2010; reviewed in Ervin et al., 2015).

Furthermore, gonadectomy reduces agonistic behaviour in both male and female mice (reviewed in Ervin et al., 2015). Following castration, male mice make fewer attacks, and engage in more submissive behaviour than intact male mice (Clipperton-Allen et al., 2011, 2010). In female mice, time spent engaged in agonistic behaviour is reduced following ovariectomy (Clipperton-Allen et al., 2010). Moreover, among castrated male mice, agonistic behaviour is recovered following long-term exposure to testosterone, or DHT, and male-typical agonistic behaviour can be induced in female mice via chronic exposure to testosterone (Brain & Haug, 1992; Brain & Poole, 1976; reviewed in Ervin et al., 2015). Similarly, agonistic behaviour is recovered in castrated male mice following treatment with estradiol, suggesting that the effects of testosterone on aggressive behaviour are at least partly mediated via estrogenic mechanisms (Brain & Poole, 1976; reviewed in Ervin et al., 2015).

Furthermore, while correlational models in humans have shown inconsistent relationships between prenatal steroid hormone exposure and agonistic behaviour, experimental studies in non-human animals have demonstrated a number of hormone effects on adult agonistic behaviour (Book, Starzyk, & Quinsey, 2001). In rodents, adult expression of agonistic behaviour has been correlated with intrauterine position, such that both male and female mice positioned between two male fetuses, consequently receiving greater exposure to intrauterine testosterone, exhibit greater adult agonistic behaviour than those positioned between female fetuses (Gandelman, vom Saal, & Reinisch, 1977; vom Saal, Grant, McMullen, & Laves, 1983).
addition, aggressive behaviour is eliminated in aromatase knockout (ARKO) mice, but can be recovered through administration of 17β-estradiol immediately following birth (Toda, Saibara, Okada, Onishi, & Shizuta, 2001). The presence of estradiol in early development is therefore critical to the development of male-typical aggressive behaviour in adulthood. Furthermore, exposure to exogenous testosterone in early life alters testosterone sensitivity in both male and female mice in adulthood, with gonadectomized mice that received treatment with testosterone early in life exhibiting aggressive behaviour sooner following adult treatment with testosterone (vom Saal, Svare, & Gandelman, 1976). Taken together, these results suggest interplay between organizational and activational effects of steroid hormones in the regulation of aggression.

In addition, evidence implicating estrogen receptor activity in the regulation of aggressive behaviour suggests that the effects of testosterone on aggressive behaviour may be due to actions of estradiol following testosterone aromatization. Male estrogen receptor beta knockout mice show elevated aggression prior to puberty and in adulthood, while estrogen receptor alpha knockout decreases aggression, suggesting that estradiol has opposing effects at different binding sites, though these effects could be the result of either organizational or activational mechanisms (Ogawa, Lubahn, Korach, & Pfaff, 1997; Tsuda, Yamaguchi, Nakata, & Ogawa, 2014). Conversely, targeted ERα knockdown in the ventromedial hypothalamus has been shown to increase aggression, suggesting that ERα may have differing regulatory roles at across brain regions (Spiteri et al., 2010). Taken together, these findings suggest that the organizational effects of testosterone may be the result of the action of estradiol following metabolism by the aromatase enzyme, and that these effects may involve changes in ER function that persist into adulthood.
**Gonadal Steroids and Sociability**

Sociability refers to an animal’s motivation to engage in social interaction, regardless of whether this motivation is agonistic, sexual, or affiliative in nature (Moy et al., 2004). Sociability is often assessed in laboratory rodents through a paradigm that offers the experimental animal a choice between interacting with a conspecific or solitary investigation of a non-social stimulus, relying on the tendency for social rodents to prefer social to non-social stimuli. While some researchers utilize a multi-chambered apparatus that provides the choice between a chamber containing a conspecific or an empty chamber, the sociability test used in our lab involves testing subjects in their home cage in order to reduce any effects of anxiety on social motivation. Mice are given access to an unfamiliar conspecific and a novel object, and sociability is assessed as a ratio of time spent investigating to social stimulus over total investigation of both stimuli (Moy et al., 2004).

The steroid-dependent neuropeptides oxytocin and arginine-vasopressin regulate affiliative behaviour, pair bonding, and aggression across a wide range of species (reviewed in Caldwell, 2012). Gonadal steroids regulate affiliative behaviour through actions exerted throughout development and in adulthood. Administration of an aromatase inhibitor during prenatal development impairs early life ultrasonic vocalizations, social approach behaviour in adolescence, and heterosexual social behaviours in adulthood among female rats (Xu et al., 2014).

In addition, male estrogen receptor α knockout (ERαKO) mice show reduced social interest in both novel and unfamiliar female animals, implicating ERα in the regulation of sexually motivated social approach behaviour in mice (Imwalle, Scordalakes, & Rissman, 2002). Furthermore, neonatal treatment with an aromatase inhibitor reduces the interest of male rats in a
sexually receptive female, while castration abolishes interest in both a sexually receptive female and a male intruder, though these behaviours are restored via treatment with estradiol and DHT (Bakker, Brand, Van Ophemert, & Koos, 1993). Furthermore, among male mice, castration reduces approach behaviour and increases latency to investigate an unfamiliar intruder (Clipperton-Allen et al., 2010). Gonadal steroid hormones therefore appear to be involved both during development and adulthood in the mediation of sexually and competitively motivated sociability among male mice.

**Gonadal Steroids and Social Cognition**

A key aspect of adaptive behaviour in any social animal is the ability of that animal to learn both from and about conspecifics. This information is critical to the success of social structure, and provides social animals with an adaptive advantage in terms of decision-making (Heyes & Galef, 1996). The present study will expand on the literature reviewed below by establishing the nature of the relationship between steroid hormone activity during development, adulthood, and a number of facets of social cognition.

**Social Learning**

Social learning is the ability of an animal to learn from a conspecific. Social learning provides an adaptive advantage by allowing an animal to avoid potentially costly or lengthy individual experimentation by adopting the behaviour of a conspecific (Heyes & Galef, 1996). Social learning has been observed across a wide range of species and taxa. For example, bumble bees adopt novel foraging techniques from conspecifics, while dolphins and chimpanzees are able to acquire and spread tool use strategies via social transmission (Krützen et al., 2005; Leadbeater & Chittka, 2008; Whiten, Horner, & de Waal, 2005). Social learning is often tested in rodents using the social transmission of food preferences (STFP) paradigm, in which an
observer animal acquires a preference for a novel food flavour through interaction with a
demonstrator animal that has recently consumed this flavoured food (Clipperton, Spinato,
Chernets, Pfaff, & Choleris, 2008; Galef & Laland, 2005; Galef & Stein, 1985; Valsecchi &
Galef, 1989).

A number of neuroendocrine factors have been found to influence social learning in
laboratory animals. Female rodents perform better on tasks assessing social learning in
proestrus, when endogenous estradiol levels are highest (Choleris, Clipperton-Allen, Gray, Diaz-
Gonzalez, & Welsman, 2011; Choleris, Clipperton-Allen, Phan, & Kavaliers, 2009). Moreover,
preference for demonstrated food is prolonged in female mice treated with estradiol both 48h
before and immediately prior to testing (reviewed in Ervin et al., 2015; Ervin, Phan, Gabor, &
Choleris, 2013). In addition, estrogen receptor activity is directly involved in the mediation of
social learning behaviour in adult female animals. Estrogen receptor α (ERα) impairs
performance, while estrogen receptor β (ERβ) prolongs the preference for a demonstrated food
(Clipperton et al., 2008; Ervin et al., 2013). In addition, agonism of the G-protein coupled
estrogen receptor (GPER1) enhances social learning in the STFP paradigm when administered
immediately prior to testing (Ervin, Mulvale, Gallagher, Roussel, & Choleris, 2015).

In female rats, neonatal administration of estradiol benzoate does not affect social
learning in later life (Berretti et al., 2014). However, the effects of similar treatments in male
animals, as well as any effects of androgens or treatment in earlier stages of development, have
yet to be examined. Little research has investigated sex differences in social learning directly,
but given the role of steroid hormones in mediating learning, memory, and social behaviour, the
role of sex differences in social learning deserves further attention. The work presented here was
undertaken to further develop our understanding of the role of endocrine function in social
learning behaviour, and has directly examined differences between male and female animals in a number of experimental steroid hormone conditions. In addition, we have investigated the developmental effects of gonadal hormones on social learning earlier in development than previous research.

Social Recognition

Social recognition is the ability of an animal to distinguish between conspecifics. Recognition of social peers is essential for the maintenance of social hierarchies, pair bonds, and maternal behaviour (Choleris et al., 2009). In addition, the ability to distinguish between conspecifics allows animals to base behaviour on past encounters when interacting with a previously encountered conspecific. We have adopted a simple recognition paradigm, which relies on the tendency for mice to preferentially investigate novel social stimuli, in order to investigate social recognition. An experimental mouse is allowed to investigate two same-sex conspecifics over a series of habituation trials. During a test phase, one of these animals is replaced with a novel conspecific, and the extent to which the experimental animal preferentially investigates the novel animal is used to assess recognition (Choleris et al., 2009).

Social recognition is dependent on a number of neuroendocrine factors. As in social learning, performance on social recognition tasks is improved during proestrus in female rats, while research in mice has shown that ovariectomy has an impairing effect on social recognition that is rescued following treatment with estradiol (Hliňáck, 1993; Tang et al., 2005). Furthermore, though female mice exhibit social recognition during all stages of estrus, retention of social recognition memory is improved during proestrus (Sánchez-Andrade & Kendrick, 2011). This suggests a facilitatory role for estrogens in mediating social recognition. Conversely, male rats perform worse on tasks assessing social recognition than females, and
removal of gonadal androgens via castration improves performance (Bluthe, Schoenen, & Dantzer, 1990). This suggests that the female advantage in social recognition is due to differences in steroid hormone function between males and females, though the fact that differences in performance can be abolished through hormone manipulation in adulthood suggests that these differences may be due to activational, rather than organizational, effects of steroid hormones.

Gonadal steroids regulate social recognition via different mechanisms in male and female animals, suggesting that the pathways by which social recognition is mediated by gonadal steroids are, to some extent, qualitatively different. Social recognition in male rodents relies on the activity of both oxytocin and arginine-vasopressin (AVP) receptors, while blockade of AVP receptors in female rats does not extinguish social recognition (Bluthe´ & Dantzer, 1990; Ferguson et al., 2000). Instead, social recognition in female mice is dependent on oxytocin, the G-protein coupled estrogen receptor (GPER1), estrogen receptor α (ERα) and, to a lesser extent, estrogen receptor β (ERβ) (Choleris et al., 2006; Ferguson, Aldag, Insel, & Young, 2001; C. Gabor, Lymer, Phan, & Choleris, 2015; Sánchez-Andrade & Kendrick, 2011; Spiteri et al., 2010). ERαKO female mice show impaired formation and retention of social recognition learning, where ERβKO female mice do not show deficits in social recognition. Existing literature is not conclusive in terms of the role of ERα in male mice. While male ERαKO mice have been shown to exhibit impaired social recognition in trials involving the introduction of a novel ovariectomized female, other work has found intact social recognition when a sedated same-sex conspecific was presented (Imwalle et al., 2002; Sánchez-Andrade & Kendrick, 2011). It is possible that these differences are partly mediated by methodological differences in socially induced anxiety or social interest.
One possible mechanism by which estrogen receptors may influence social recognition is via the regulation of oxytocin, which is also essential for successful social recognition in both male and female mice (Choleris et al., 2006; Ferguson et al., 2001; Gabor, Phan, Clipperton-Allen, Kavaliers, & Choleris, 2012). In male mice, oxytocin knockout abolishes social recognition, while both administration of oxytocin into the lateral ventricle and targeted infusion of oxytocin into the medial amygdala rescue performance on social recognition tasks (Ferguson et al., 2000, 2001). Similarly, in female mice, social recognition is abolished following treatment with oxytocin receptor antisense DNA in the medial amygdala, demonstrating that oxytocin in the medial amygdala is required for social recognition in female mice (Choleris et al., 2007). Interestingly, where androgen-mediated AVP is not necessary for social recognition in female rats, AVP is required for social discrimination in male rats (Bluthe & Dantzer, 1990; Bluthe et al., 1990). This body of evidence indicates that both androgens and estrogens act throughout adulthood to affect social recognition, but that the mechanisms by which social recognition is regulated differ in part between male and female animals. In addition, the differential action of gonadal steroids and their downstream effectors between sexes suggests that sex differences in performance on social recognition tasks is likely mediated, at least in part, by gonadal steroid activity.

While the studies outlined above suggest a role for activational effects of gonadal steroids in regulating of social recognition, the developmental role of gonadal steroids is less clear. Impairments observed in aromatase knockout mice can be rescued in adults by treatment with the androgen metabolites estradiol and dihydrotestosterone, suggesting that these effects are likely activational in nature (Pierman et al., 2008). However, mice treated prenatally with an androgen antagonist, flutamide, do not exhibit deficits in social recognition following treatment with an
AVP antagonist in adulthood, suggesting that androgens act developmentally to organize the male-specific dependency on AVP for social recognition (Axelson, Smith, & Duarte, 1999; Bluthe´ & Dantzer, 1990; Bluthe et al., 1990). Prenatal androgens therefore exert organizational effects during development, which appear to change how social recognition is regulated in male rats. It is still unknown, however, whether elevations in androgens during development affect social recognition. Given the removal of AVP dependency following developmental androgen antagonism, it is possible that heightened exposure to testosterone and its metabolites during development might lead to AVP dependent social recognition in female animals.

**Objectives**

The present study was undertaken in order to elucidate the developmental role of gonadal hormones in the regulation of social and social cognitive behaviour, as well as to assess potential interactions between organizing effects of gonadal steroid hormones in development and activational effects in later life. Using male and female CD1 mice as an animal model, we administered a low dose of testosterone propionate prenatally in order to assess the developmental action of androgens. Furthermore, we manipulated gonadal hormones in adulthood via gonadectomy and hormone replacement in order to assess the activational role of gonadal hormones, as well as interactions between organizational and activational gonadal hormone activity. We administered a set of behaviour assays aimed at assessing social reactivity, social approach behaviour, social learning, and social recognition in adolescence and adulthood, before and after adult hormone manipulation. We hypothesize that early androgen exposure masculinizes behaviour promoting enhanced “male-typical” social behaviour. In addition, early androgen exposure likely affects sensitivity to changes in gonadal steroid hormone activity in adulthood.
Future research will examine the effects of our treatments on socially relevant brain regions, and will assess whether changes in behaviour can be associated with changes in relevant brain systems. We hypothesize that sexually dimorphic expression of steroid receptors is dependent on early androgen exposure, and that early androgen exposure exerts organizational effects on physiological responsiveness to gonadal hormones in adulthood. Furthermore, we predict that changes in steroid receptor expression will be related to behavioural outcomes described in the present study.

The work presented in this thesis deals with findings related to social learning and social reactivity, as well as the results of a control test investigating anxiety-like behaviour. Results related to social recognition and social approach avoidance, as well as the results of an object recognition control task, are presented in a separate thesis by Cam Wasson (Wasson, 2016).
Methods and Procedures

Table 1: Experimental Timeline

<table>
<thead>
<tr>
<th>Age</th>
<th>Event</th>
<th>Experimental Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-conception</td>
<td>Mating pairs formed</td>
<td>0</td>
</tr>
<tr>
<td>E1</td>
<td>Conception – presence of a vaginal plug detected</td>
<td>1</td>
</tr>
<tr>
<td>E12, E14, E16</td>
<td>Prenatal treatment</td>
<td>12 - 16</td>
</tr>
<tr>
<td>PD 0</td>
<td>Birth and cross-fostering</td>
<td>18 - 21</td>
</tr>
<tr>
<td>PD 20</td>
<td>Weaning</td>
<td>38 - 41</td>
</tr>
<tr>
<td>PD 35 - 42</td>
<td>Behavioural test battery I</td>
<td>53 - 60</td>
</tr>
<tr>
<td>PD 43</td>
<td>Gonadectomy or sham surgery</td>
<td>61 - 64</td>
</tr>
<tr>
<td>PD 58</td>
<td>Hormone replacement</td>
<td>76 - 79</td>
</tr>
<tr>
<td>PD 68 - 76</td>
<td>Behavioural test battery II</td>
<td>86 - 94</td>
</tr>
<tr>
<td>PD 77</td>
<td>Sacrifice and tissue extraction</td>
<td>95 - 98</td>
</tr>
</tbody>
</table>

Note: E – Embryonic day; PD – Postnatal day. E1, E12, E14, and E16 indicate time point of pregnancy in days. PD 0 through PD 77 indicate the age of the subjects in days.

Summary

Mating pairs were formed from 2-3 month old male and female CD1 mice. Following detection of a vaginal plug, designated embryonic day 1 (E1), injections of testosterone propionate were administered subcutaneously to pregnant dams on E12, E14, and E16. Day of birth was designated post-natal day 0 (PD 0). Mice began the first round of behavioural testing on PD 35, and completed testing on PD 42, just prior to the onset of puberty. Animals were gonadectomized or received sham surgery on PD 43. Silastic capsules were filled with either pure crystalline testosterone or cholesterol (males), or 12.5 µg estradiol benzoate dissolved in sesame oil or sesame oil alone (females). Capsules were implanted in gonadectomized animals on PD 58. Animals underwent the same behavioural tests following sexual maturity and adult hormone intervention, between days 68 and 76. Animals were sacrificed for tissue collection following the completion of behavioural testing.
Materials and Methods

Animals and Housing

We used CD1 mice obtained from Charles River Laboratories (St. Constance, QC, Canada) to form breeding pairs and as demonstrator animals. The behavioural assays used in this study have been previously used with CD1 mice to investigate involvement of gonadal steroids in social behaviour. All procedures were approved by the University of Guelph Animal Care and Use Committee, and were in accordance with the guidelines set by the Canadian Council on Animal Care.

Housing

Animals were housed in clear polyethylene cages (26 × 16 × 12 cm) with environmental enrichment (paper cups, paper and cotton nesting material), corncob bedding, plastic housing, and ad libitum access to water and rodent chow (Teklad Global 14% Protein Rodent Maintenance Diet, Harlan Teklad, WI). Dams and litters were kept on a high protein rodent chow diet (Teklad Global 18% Protein Rodent Diet, Harlan Teklad, WI) starting when breeding pairs were created until litters were weaned 20 days after birth. The colony room was kept on a 12-hour reverse light/dark cycle, with lights off between 0800h and 2000h. This allowed us to conduct experiments and procedures during normal working hours while mice were in their active phase (Roedel, Storch, Holsboer, & Ohl, 2006).

Cross-Fostering

Experimental litters were cross-fostered on the day of birth in order to avoid differences in maternal behaviour resulting from treatment with testosterone propionate (Mann & Svare, 1983). An untreated foster CD1 mother with an age-matched litter was removed from the home cage, and her pups were removed and fostered into other non-experimental litters or euthanized.
The experimental litter was rubbed with bedding that had been lightly soiled by the foster mother and placed in her nest. The pups were left alone for 10 minutes to allow them to acquire the odor of the adoptive mother from the nest material. The foster mother was then reintroduced to the cage, and was monitored for 24 hours to ensure adequate maternal care.

**Stimulus and Demonstrator Animals**

Due to the unpredictable nature of breeding, more animals were bred than necessary in order to ensure proper experimental timing. Animals from non-experimental litters were used as age-matched demonstrators and stimulus animals in the behavioural assays described below. Additional age-matched CD1 mice were brought in from Charles River Laboratories (St. Constance, QC, Canada) when there were insufficient non-experimental litters. Stimulus animals were kept triple-housed with same-sex conspecifics. Since these stimulus animals were juveniles at the first round of behavioural testing they were left gonadally intact for tests conducted prior to puberty, but gonadectomized prior to testing in adulthood in order to reduce the effect of variation in stimulus animal behaviour. Care was taken to ensure that an experimental animal did not encounter a stimulus animal more than once throughout the administration of the test batteries.

**Surgeries**

At least one hour prior to surgery, mice received a subcutaneous injection of 50 mg/kg of carprofen at 10 ml/kg (Pfizer Canada Inc, Kirkland, QC, Canada), an analgesic and anti-inflammatory commonly used in veterinary medicine. All surgeries were conducted under anesthesia with isofluorane gas (Benson Medical Industries, Markham, ON). A mixture of 0.67% lidocaine (Alveda Pharmaceuticals, Toronto, ON, Canada) and 0.17% bupivacaine (Hospira Inc., Montreal, QC, Canada) was injected at the incision site as a local anesthetic to
reduce post-surgical discomfort. In addition, animals received a subcutaneous injection of 0.5 ml warm saline in order to maintain hydration. Surgical incisions were closed using EZ Clip surgical staples (Stoelting Co., Illinois, USA). Mice were single-housed and monitored daily for at least 7 days post-surgery.

*Ovariectomy*

Ovaries were removed via a midline dorsal incision in the skin of the lower back. Connecting tissue was separated from the skin using the blunt edges of a pair of surgical scissors, and the ovaries located through the thin dorsal muscles on either side of the midline. Small incisions were made in the muscle above each ovary, and the ovaries were extracted from the pelvic cavity. The oviduct was clamped prior to severing the oviduct and removing the ovary in order to prevent excessive bleeding (Clipperton-Allen, 2011).

*Castration*

Male mice were castrated via a ventral incision in the scrotum. After piercing the tunica, sterile forceps were used to grasp the testicular fat pad and remove the testicle. The blood vessels and spermatic cord were then ligated with heated hemostatic clamps to prevent bleeding, and the testicle was severed with surgical scissors. Remaining tissue was placed back into the tunica, and the process was repeated with the other testicle. Finally, the incision in the scrotum was closed with surgical staples (Matta, Tiessen, & Choleris, 2017).

*Sham Surgery*

Sham surgeries were performed in the same manner as ovariectomies and castrations, except that the incision was closed without manipulating the gonads. In female mice, a midline dorsal incision was made in the skin of the lower back, and was then closed. In male animals, an
incision was made in the scrotum, and was then closed. Mice that underwent sham surgery underwent the same pre- and post-operative procedures as animals that received gonadectomy.

**Hormone Replacement Implants**

All capsules were soaked overnight in 1% bovine albumin solution to avoid a spike in hormone release following insertion. Immediately prior to implantation, capsules were washed in ethanol and saline in order to rinse and sterilize them. Capsules were inserted via a small incision made at the base of the neck. Skin was separated from underlying connecting tissue using the blunt edges of a small pair of surgical scissors, and the capsule was inserted to one the side of the body. The incision was closed using EZ Clip surgical staples. Side of insertion was randomized between subjects. Subjects were left to rest for 10 days to facilitate recovery from the surgery, and to allow circulating hormone levels to reach normal basal levels (Myers, 1971).

**PCR Sex Identification**

Tissue was collected on the day of birth by toe clipping in order to facilitate identification, and was analyzed through polymerase chain reaction (PCR) amplification of the Y chromosome linked SRY gene (317 bp) using beta-actin (220 bp) as a control.

**Drugs**

**Prenatal Testosterone Treatment**

We administered 10 μg testosterone propionate (Galenova, Saint-Hyacinthe, QC, Canada) dissolved in 0.05 ml sesame oil to treatment condition dams subcutaneously three times on alternating days throughout the treatment period (E12, E14, E16). Testosterone propionate is soluble in oil, and must be hydrolyzed by esterases in blood before becoming biologically active, therefore resulting in a more gradual release than if pure testosterone were used. Testosterone propionate was chosen in order to facilitate a stable increase in testosterone in pregnant mice
across the treatment period without the need for hormone implantation surgery (Fujioka, Shinohara, Baba, Irie, & Inoue, 1986; Kintz, 1999). The dosage we used falls at the lower end of a range of doses which have been shown to produce behavioural effects in mice (Mann & Svare, 1983; vom Saal et al., 1976). Our objective was to model heightened intrauterine testosterone exposure without greatly exceeding the bounds of what could occur in the upper physiological range of untreated animals.

**Hormone Replacement**

Testosterone and cholesterol filled capsules were created using 12 mm long pieces of silastic tubing (1.57 mm i.d., 3.18 mm o.d.; Dow Corning) sealed with 1 mm of silicone medical adhesive at either end of the tubing. This created a 10 mm long cavity, which was filled with either pure crystalline testosterone (Galenova, Saint-Hyacinthe, QC, Canada) or cholesterol (Sigma-Aldrich, Oakville, ON, Canada). The functional length, and by extension the releasing surface area, of the capsule directly relates to the rate at which hormone is released. 10 mm of functional capsule length has been shown to produce normal physiological levels of testosterone for approximately 21 days in castrated male mice when prepared in this manner (Myers, 1971).

β-Estradiol 3-benzoate (Sigma-Aldrich, Oakville, ON, Canada) and sesame oil (Sigma-Aldrich, Oakville, ON, Canada; used as a control) filled capsules were created using 30 mm long pieces of silastic tubing (1.98 mm i.d., 3.18 mm o.d.; Dow Corning) sealed with 2.5 mm of silicone medical adhesive at either end of the tubing. This created a 20 mm long cavity with an internal volume of 0.062 ml, which was filled with a solution of 12.5 µg estradiol benzoate dissolved in sesame oil (201.62 µg/ml). This length of capsule and dosage of estradiol has been shown to produce physiological levels of estradiol in ovariectomized female mice (Clipperton-Allen et al., 2010; Ribeiro, Pfaff, & Devidze, 2009).
**Experimental Apparatus**

All testing was conducted under red light between 0900h and 1900h, during the animals’ dark active phase, with the exception of the dark/light task, which was conducted in a lit testing room. Where applicable, behaviour was recorded from above using JVC Everio HD GZ-E300 digital camcorders, and videos were scored by an investigator blind to treatment condition using JWatcher Event Recorder software (Blumstein, Daniel, & Evans, 2006). Perforated clear Plexiglas cage lids were used in place of stainless steel lids in order to allow for unobscured video recording. Materials that were directly interacted with by the animals were washed thoroughly between each use with Sparkleen odorless detergent (Fisher Scientific), and rinsed with baking soda and water in order to eliminate odor.

**Flavoured Diets**

The STFP paradigm requires experimental animals to be naïve to both flavours used in the test. In addition, each flavour needs to be equally as palatable as the flavour it is paired with in order to avoid strong spontaneous preferences. Because we tested animals in the social transmission of food preferences task twice, we used two sets of readily distinguishable and equally palatable flavours.

During testing in adolescence we used two food flavours commonly used in our lab with CD1 mice: 2% premium cocoa powder (Fry & Sons, Ltd.) and 1% ground cinnamon (McCormick & Company, Inc.) mixed with powdered rodent chow. The efficacy of these flavours has been demonstrated in a number of papers published by our lab and by others (Clipperton et al., 2008; Wrenn, Harris, Saavedra, & Crawley, 2003). During testing in adulthood, 1.5% ground turmeric (McCormick & Company, Inc.) and 2% ground thyme (McCormick & Company, Inc.) mixed with powdered rodent chow were used. We conducted
pilot studies and found that these two food flavours are equally palatable in CD1 mice, and that both intact and gonadectomized mice showed robust preferences for demonstrated food when using these flavours in the STFP paradigm (see Appendix A).

Social Transmission of Food Preferences

During the three-day pair housed period prior to the STFP paradigm, a perforated aluminum cage divider was placed in the middle of the cage, separating the animals (25.8 × 0.2 × 10.8 cm; 0.3 cm diameter holes; 0.1 cm between each hole; ~1500 holes per divider). These dividers were designed to allow animals to sniff and interact through the perforations without being able to make direct contact (Matta, 2014).

Demonstrator mice were given access to flavoured chow in clean polyethylene cages (26 × 16 × 12 cm). In order to reduce spillage and facilitate accurate measurement of demonstrator animal food consumption, flavoured food was presented to demonstrator animals in 5 cm tall jars with a diameter of 7.5 cm. Jars were fitted with stainless steel collars with an opening 2.5 cm in diameter, and a perforated steel disk was placed directly on top of the powered food in order to allow feeding while preventing spillage (Dyets Inc., Bethlehem, PA).

Experimental animals were presented with the two novel flavoured foods in polyethylene cages (42.5 × 26.5 × 18.5 cm) with two openings, 2 cm apart, leading to small stainless steel chambers (4.5 × 2.5 × 2.5 cm), which provided access to separate Plexiglas feeders (4.5 × 5 × 3.6 cm; Tecniplast, Verese, Italy). Each feeder contained one of the two novel foods (cinnamon or cocoa during pre-puberty, turmeric or thyme during post-puberty), one of which being the food consumed by the demonstrator. The efficacy of these feeders in accurately measuring food consumption has been demonstrated previously and described in detail (Valsecchi & Galef, 1989). Food consumption was measured by weighing feeders on a digital scale accurate to 0.01g
(Sartorius Analytical Balance, Sartorius Inc., United Kingdom). Observers and demonstrators were given *ad libitum* access to water during feeding.

*Dark/Light Test*

Mice were tested using the VersaMax Animal Activity Monitoring System (AccuScan Instruments, Inc.). The testing box comprised of a 42.5 cm × 42.5 cm Plexiglas box which records vertical and horizontal activity by tracking breaks in beams of infrared light. A dark Plexiglas insert was used to create a 42 cm × 20.5 cm dark half of the cage, and the animal’s activity was monitored using the accompanying VersaMax software. A 60-watt incandescent light bulb was placed directly over the apparatus, illuminating the exposed “light” side of the testing chamber.

*Procedures*

*Subjects and Breeding*

Breeding pairs were formed from sexually and experimentally naïve 2-3 month old male and female CD1 mice (Charles River Laboratories, St. Constance, QC). On arrival, animals were triple-housed with same sex conspecifics for one week prior to breeding in order to allow the animals to adjust to the facility and to the reverse light/dark cycle. Male and female dyads were paired in a fresh cage at the beginning of the dark phase (0800h), and females were monitored daily at 2000h for presence of a vaginal plug, indicating that mating had occurred. The male mouse was removed when a vaginal plug was detected, and the date was designated as embryonic day 1 (E1). Cages were changed on E11 (24 hours before the first injection) and E17 (24 hours following the final injection), and were not changed again until 8 days following delivery of a litter.
**Prenatal Testosterone Treatment**

Dams were injected subcutaneously with either 10 µg of testosterone propionate dissolved in 0.05 ml sesame oil, or with sesame oil alone. Injections took place on E12, E14, and E16. This time period coincides with both sexual differentiation, and with the development of sexually dimorphic brain regions relevant to social and cognitive behaviours including the hippocampus, hypothalamus, and amygdala (Finlay & Darlington, 1995; Ikeda, Tanaka, & Esaki, 2008; Shimogori et al., 2010).

**Cross Fostering and Developmental Data Collection**

On the day of birth, designated post-natal day 0 (PD 0), pups were cross-fostered to foster mothers with age-matched litters in order to control for alterations to maternal behaviour as a result of testosterone treatment. Pups were initially sexed visually on PD 0, but this was abandoned due to poor accuracy and sex was instead determined via *SRY* gene detection on the day of birth (Hotchkiss et al., 2007; Phoenix et al., 1959a; Wolf, Hotchkiss, Ostby, LeBlanc, & Gray, 2002). Where possible, litters were culled to 4 males and 4 females before fostering. When PCR was unsuccessful, litters were culled to 8 pups at random, and sex was determined at weaning. Culled pups were euthanized by lethal exposure to isofluorane and frozen at -80 C to allow for future analysis. Litters were weighed every four days up until PD 20, at which point pups were weaned and housed with same sex littermates in groups of 2-3 mice until the beginning of the first behavioural test battery. In addition, we recorded age of first appearance of fur, eye opening, and ear opening in order to determine whether prenatal treatment with testosterone resulted in overt disruptions in early physiological development. Furthermore, we monitored female mice daily following weaning to determine the day of vaginal opening, and determined the onset of estrus by assessing vaginal cytology collected daily via vaginal lavage.
First estrus was defined as the first appearance of clusters of cornified squamous epithelial cells, which is used as an indicator of estrus in mice (Caligioni, 2009; McLean, Valenzuela, Fai, & Bennett, 2012).

**Gonadectomy and Hormone Replacement**

Mice were gonadectomized or received sham surgery at 42 days of age, immediately following the conclusion of the first behavioural test battery, and just prior to the onset of puberty (Baillie, 1961; Chehab, Mounzih, Lu, & Lim, 1997). Half of gonadectomized animals received hormone replacement with testosterone (males) or estradiol benzoate dissolved in sesame oil (females), while the other half were implanted with control capsules filled with either cholesterol (males) or sesame oil (females) 10 days following gonadectomy surgery. This was intended to control for potential differences in adult production of sex steroids resulting from prenatal testosterone treatment.

**Behavioural Test Battery**

Mice underwent a set of behavioural tasks assessing anxiety, social behaviour, and cognition. The test battery was administered twice during the experiment – once in adolescence, and once following adult gonadectomy/hormone intervention. This design allows us to separate the organizational and activational effects of steroid hormones, as well as to understand how differences in organizational action of gonadal steroids may alter later life responsiveness to these hormones.

Seven behavioural assays were performed: the social transmission of food preferences task, which assesses social learning; the flavour recognition task, which assesses olfaction and flavour discrimination; the dark/light task, which assess anxiety behaviour; an object recognition paradigm; an approach-avoidance task, which assesses social motivation; a test of reactivity to an
intruder animal; and a social recognition task. The social transmission of food preferences paradigm, the social reactivity test, the dark/light test, and the flavour recognition task are presented here. Another graduate student will present the other tests in a separate manuscript. Each mouse underwent a subset of three to four tasks, out of a possible seven, in an order that minimized interference between tests through either practice effects or increased exposure to social stimuli (see Table 2).

Table 2: Order of Behavioural Testing

<table>
<thead>
<tr>
<th>Testing Day</th>
<th>Testing Path A</th>
<th>Testing Path B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>Social Learning</td>
<td>Social Learning or Flavour Recognition</td>
</tr>
<tr>
<td>Day 2</td>
<td>Dark/Light</td>
<td>Dark/Light</td>
</tr>
<tr>
<td>Day 5</td>
<td>Object Recognition</td>
<td>Social Approach/Avoidance</td>
</tr>
<tr>
<td>Day 8</td>
<td>Social Reactivity</td>
<td>Social Recognition</td>
</tr>
</tbody>
</table>

*Note:* Mice were tested in one of two sets of behavioural tests. ¾ of animals were tested in the social transmission of food preferences task, ¼ were tested in flavour recognition, while ½ of animals were tested in each of the other tasks. This table shows the order in which these tasks were performed. Half of animals in each set of behavioural tasks were tested in the light-dark paradigm.

Social Transmission of Food Preferences

Social learning was assessed using the social transmission of food preferences (STFP) paradigm. Briefly, in this behavioural assay an animal is allowed to interact with a demonstrator that has recently consumed a flavour of food that is novel to the observer. The observer is then allowed to make a choice between the demonstrated food and another novel flavour of food. Mice will typically choose the demonstrated food over the non-demonstrated food (Valsecchi & Galef, 1989). The basis of this test is the tendency for mice to use information acquired via interaction with a conspecific when making feeding choices (Galef & Laland, 2005; Valsecchi & Galef, 1989).

Mice were pair housed with a same sex demonstrator separated by a cage divider for 3 days prior to testing. Subjects were food deprived 12 hours before testing in order to encourage
greater food consumption. In addition, demonstrator animals were coloured with black permanent marker 12 hours before testing to facilitate identification when analyzing social interaction videos.

On the day of testing, demonstrator animals were removed from the cage at the beginning of their dark active phase and placed in feeding cages with access to one of two flavoured foods. Following a 60-minute feeding period, the demonstrator animals were returned to their home cage and food was weighed to assess consumption. Enrichment, cage dividers, and housing were removed from the home cage, and the demonstrator animal was reintroduced to the experimental observer for a 30-minute filmed interaction. We assessed a number of behaviours, which were used to generate the following composite behaviour groups: active behaviour; social behaviour, agonistic behaviours delivered and received, dominance behaviour, social investigation, and non-social behaviour (see Appendix B). In addition, oronasal investigation was quantified as the amount of time the observer spent sniffing around the nose and mouth region of the demonstrator mouse. This is of special importance to the understanding of social learning in the STFP given that this is how the observer acquires flavour information from the demonstrator. Only the behaviour of the experimental observer animal was scored.

Following the interaction, observer animals were placed in the test chamber with access to both the demonstrated novel food, and another equally novel flavoured food (food pairs described above in Flavoured Diets). Consumption was measured every two hours over an 8-hour period.

**Flavour Recognition**

Because the STFP is dependent on olfaction, we assessed whether mice were able to distinguish between the food flavours used in the STFP paradigm (1% cinnamon and 2% cocoa
during pre-puberty, and 1.5% turmeric and 2% thyme during post-puberty). Animals were tested in their home cage with enrichment removed during their dark active phase.

The flavour recognition task relies on the tendency for mice to prefer novelty, so no mice that had been tested in the STFP were tested in flavour recognition (Choleris, Clipperton-Allen, Phan, Valsecchi, & Kavaliers, 2012). Mice were habituated to a novel food flavor presented in two glass mason jars (5.5 cm tall × 6.5 cm diameter) with stainless steel mesh lids over three 4-minute habituation sessions, separated by 3-minute rest periods. Following a final 3-minute rest phase, one of the diets was replaced with a novel flavoured food, and olfactory discrimination of flavoured diets was quantified by comparing investigation of the two diets at test phase. Diet pairs used in the flavour recognition task were the same as the pairs used in the social transmission of food preferences paradigm.

**Resident-Intruder Test**

A social reactivity test was used to examine how the subjects interacted with an unfamiliar conspecific presented in the experimental animal’s home cage. This paradigm is similar to the social interaction element of the social transmission of food preferences paradigm, except that experimental animals were socially isolated prior to testing, and the stimulus “intruder” animal was not familiar to the experimental animal.

Experimental animals were socially isolated for 7 days prior to testing. Stimulus animals were kept triple-housed with same-sex conspecifics for at least 10 days prior to testing. Enrichment and housing were removed from the cage, and an age matched, same-sex intruder animal was placed in the home cage of the experimental animal. Interaction videos were scored using the same criteria, set of behaviours, and analysis methods as described above for the social transmission of food preferences interaction videos (see Appendix B).
**Dark/Light**

We assessed anxiety in experimental mice in order to determine whether any observed effects in other behavioural assays may have been related to differences in anxiety-like behaviour between treatment groups. Anxiety-like behaviour was assessed using a dark/light test, in which an animal is placed in an apparatus with two connected chambers, one of which is darkened, and one that is exposed to a bright overhead light.

Animals were tested in a brightly lit testing room during their dark-active phase, but were left undisturbed in the testing room with the lights off for at least one hour prior to testing. Animals were placed in the dark side of the apparatus and allowed to explore freely for a 5-minute test period. Anxiety-like behaviour in the dark/light test is characterized by more time spent in the dark side of the chamber, less frequent light side entries, and greater latency to first enter the light side (Chaouloff, Durand, & Mormède, 1997).

**Tissue and Serum Collection**

Fecal samples were sent to Dr. Gabriella Mastromonaco at the Toronto zoo for analysis in order to assess the efficacy of our prenatal treatment. In addition, dams were sacrificed following the removal of pups at birth (PD 0) and trunk blood was collected in order to assess circulating gonadal steroid levels at the end of pregnancy. Plasma testosterone was assessed via ELISA following hormone extraction using a commercially available kit (Testosterone ELISA Kit, Abcam Inc., Toronto, ON). Following the completion of the final set of behavioural tests, animals were deeply anaesthetized via intraperitoneal injection of at least 340 mg/kg avertin and blood was collected via cardiac puncture. Subjects were then perfused with a solution of 4% paraformaldehyde in phosphate buffered saline, and brains were extracted, post-fixed, cryoprotected, and stored and -80 C. Blood was collected in microvette EDTA coated capillary
tubes (Sarstedt, Montreal, QC, Canada). Blood was centrifuged at 12,000 RPM and plasma was extracted via pipette and stored in 1.5 ml Eppendorf microcentrifuge tubes sealed in small plastic zip bags at -80 C. In addition, animals were weighed prior to perfusion, and prostates (male mice) and gonads (intact mice) were extracted and weighed following perfusion.

**Statistical Analysis and Data Handling**

**General Data Handling Practices**

All data manipulation procedures were performed systematically using scripts written in R Statistical Software (R Foundation) and Python (Python Software Foundation). This automation reduced the chance of error resulting from manual handling of data (source code at https://github.com/chowes).

**Statistical Analysis**

In general, factorial ANOVA was used to compare data between groups, with sex (male, female), prenatal treatment (testosterone propionate, control), and adult treatment condition (post-puberty only; intact, gonadectomy + vehicle, gonadectomy + testosterone/estradiol replacement) as between-subjects factors. Analysis of simple main effects was used to explore interactions where appropriate, and a Sidak correction was used to correct for multiple pairwise comparisons as needed. For measures where we had a specific set of hypotheses, planned comparisons were used to assess differences between mice treated prenatally with testosterone and sesame oil, between male and female mice, between intact and gonadectomized mice, and between gonadectomized mice and mice that received replacement with testosterone or estradiol. We elected to use planned comparisons rather than post-hoc comparisons because correcting for multiple post-hoc comparisons would have resulted in unacceptably high type II error.
Social Transmission of Food Preferences

Consumption data were analyzed by comparing preference for a given food in a pair of flavoured diets between observers whose demonstrator consumed that food flavour, or the other flavour of food in the pair. In adolescence, preference for cinnamon-flavoured diet was compared between cinnamon demonstrated and cocoa demonstrated animals. In adulthood, preference for turmeric-flavoured food was compared between turmeric demonstrated animals and thyme demonstrated animals. Social learning was said to have occurred where demonstrated food flavour had a significant effect on flavour preference. That is, where cinnamon demonstrated animals had a greater preference for cinnamon flavoured diet than cocoa demonstrated animals, or where turmeric demonstrated animals had a greater preference for turmeric than thyme demonstrated animals.

First, a preference ratio was calculated for each animal using the following formula:

\[ \text{Cinnamon Preference Ratio} = \frac{\text{Cinnamon Consumed}}{\text{Total Food Consumed}} \]

The same formula was used in adulthood, with turmeric in place of cinnamon. Animals were only included in the analysis at a given time point if they consumed at least 0.1 grams of food during the two hour time period. In addition, only mice whose demonstrator consumed at least 0.1 grams of food were included in the analysis in order to ensure that animals were able to detect the odor of the demonstrated food through oronasal investigation of the demonstrator.

Preference data were analyzed using a mixed factorial ANOVA with sex, prenatal treatment, and adult treatment condition (post-puberty only) as between-subjects factors, and time (2h, 4h, 6h, 8h) as a within-subjects factor. In addition, factorial ANOVAs were conducted at each time point to assess the effects of prenatal treatment, adult treatment, and sex throughout the test. Social learning within each experimental group was assessed at each time point using a
priori planned comparisons. For each experimental condition, between-samples t-tests were performed at each time point, comparing the cinnamon or turmeric preference ratios of cinnamon and cocoa demonstrated animals, or turmeric and thyme demonstrated animals, respectively.

A demonstrated flavour preference score was calculated using the following formula:

\[
\text{Demonstrated Food Preference Ratio} = \frac{\text{Demonstrated Food Consumed}}{\text{Total Food Consumed}}
\]

As with the preference ratios described above, these scores were square root and arcsine-transformed prior to analysis. Demonstrated food preference ratios were used to test differences in socially acquired food preferences between experimental groups. A mixed factorial ANOVA was conducted in order to assess differences between groups, with sex, prenatal treatment, and adult treatment condition (post-puberty only) as between-subjects factors, and time (2h, 4h, 6h, 8h) as a within-subjects factor. Factorial ANOVAs were conducted at each time point to assess the effects of prenatal treatment, adult treatment condition, and sex on preference for demonstrated food, and a priori planned comparisons were used to assess differences between animals treated prenatally with testosterone and sesame oil within each sex, prenatal treatment, and adult treatment condition.

Total food consumption at each time point was also calculated as follows:

\[
\text{Total Consumption} = \text{Cinnamon Consumed} + \text{Cocoa Consumed}
\]

The same formula was used in adulthood, with turmeric and thyme in place of cinnamon and cocoa. These data were analyzed using a mixed factorial ANOVA with sex, prenatal treatment, and adult treatment condition (adulthood only) as between-subjects factors, and time (2h, 4h, 6h, 8h) as a within-subjects factor. Simple main effects were examined where a significant interaction was found, and a priori planned comparisons were used to assess differences between
animals treated prenatally with testosterone and sesame oil within each sex, prenatal treatment, and adult treatment condition.

**Flavour Recognition**

Flavour recognition was assessed by comparing the ratio of amount of time the animal spent investigating the novel food presented in test phase to the amount of time the animal spent investigating the feeder in the same location during habituation:

\[
\text{Investigation Ratio} = \frac{\text{Time Investigating Target}}{\text{Total Investigation}}
\]

A significant increase in investigation ratio during test compared to habituation, tested using a paired t-test, indicated that animals spent more time investigating the novel food during test than the food in the same location during habituation. This method allows us to control for individual preferences for a certain food flavour. Analysis was only conducted for groups that showed impairment in social learning, and no comparisons were made between experimental groups.

**Social Interactions**

Composite measures of social and non-social behaviours were generated from data of STFP and social reactivity interaction videos (see Appendix B). Data were corrected for normality as required and compared between experimental groups using factorial ANOVAs with sex, prenatal treatment, and adult treatment condition (adulthood only) as factors. Planned comparisons were used to test differences in agonistic behaviour and oronasal investigation (STFP only) between groups. Where data could not be normalized, non-parametric Wilcoxon ranked-sum tests were used in place of t-tests.

**Light-Dark Paradigm**

Anxiety-like behaviour was quantified by calculating total time spent in the light side of the cage, frequency of light side entries, latency to enter the light side of the cage, and total
activity. These measures were analyzed both for the first minute of testing, and over the total 5-minute test period. Factorial ANOVA was conducted in order to assess differences between groups, with sex, prenatal treatment, and adult treatment condition (adulthood only) as between-subjects factors. Planned comparisons were used to assess differences in anxiety-like behaviour and total activity between groups.
Results

Summary of Results

In adolescence, all mice showed robust social learning in the STFP with no effect of prenatal testosterone. In adulthood, social learning was impaired in gonadectomized male and female mice treated prenatally with testosterone, and this was recovered by estradiol replacement in females but not by testosterone replacement in males. Among mice treated prenatally with sesame oil, gonadectomy extended preference for demonstrated food in male mice, but reduced it in female mice. We did not find impairments in flavour discrimination, indicating that deficits in social learning were not due to olfactory impairment.

Adolescent female mice were more active than male mice when interacting with an unfamiliar conspecific and spent more time investigating both familiar and unfamiliar conspecifics. When interacting with an unfamiliar intruder, male mice engaged in more non-social behaviour and spent less time engaged in submissive behaviour than female mice. Additionally, prenatal treatment with testosterone increased time spent engaged in dominance behaviour among adolescent male mice when interacting with an unfamiliar intruder. In social interactions with both familiar cage-mates and unfamiliar intruders in adulthood, female mice were more active than male mice and spent more time investigating the conspecific than male mice, while male mice spent more time engaged in non-social behaviour than female mice.

When interacting with a familiar intruder, prenatal treatment with testosterone shifted adult male mice towards a greater engagement in non-social behaviour. In addition, prenatal treatment with testosterone reduced dominance behaviour and increased ritualized aggression among male mice, and decreased time spent receiving agonistic behaviour among OVX female mice in interactions with a familiar cage-mate. In interactions with an unfamiliar intruder, time spent engaged in
submissive behaviour was reduced by prenatal treatment with testosterone, though this effect was specific to female mice when considering frequency of submissive behaviour. Male mice made more attacks, and engaged in more ritualized and open aggression than female mice when interacting with both familiar and unfamiliar conspecifics in adolescence and adulthood, though these behaviours were reduced in castrated mice.

In adolescence, mice treated prenatally with testosterone exhibited greater anxiety-like behaviour than mice treated prenatally with sesame oil, though male mice appeared to be more sensitive to this effect than female mice. In adulthood, male mice showed greater anxiety-like behaviour than female mice, castration had an anxiolytic effect in male mice and prenatal treatment with testosterone increased anxiety-like behaviour in adult male mice. Conversely, female mice were resilient to these effects.

**Developmental and Physiological Results**

**Summary**

We were unable to detect a difference in either fecal or plasma testosterone between dams treated prenatally with sesame oil and dams treated prenatally with testosterone propionate. We found no differences in litter weight between mice treated prenatally with testosterone and sesame oil during the first 20 days of life, or in adulthood at perfusion. Gonadectomy increased adult body weight in female mice, though this change was reversed in mice that received estradiol benzoate replacement. Treatment with prenatal testosterone resulted in earlier vaginal opening in female animals, but did not change onset of estrus. In male mice, prostate weight was decreased in gonadectomized animals, though this change was reversed in mice that received testosterone replacement.
***Maternal Fecal and Serum Analysis***

We found no difference in the change in fecal testosterone over the course of the prenatal treatment period between dams injected with sesame oil and dams injected with testosterone propionate \([t(24.76) = 0.28, p = 0.78, d = 0.10]\), neither was there a difference in fecal testosterone between dams treated with sesame oil and dams treated with testosterone at the end of the treatment period \([t(24.76) = 0.67, p = 0.51, d = 0.25]\). Moreover, we found no significant difference in plasma testosterone measured at the day of birth between dams treated with sesame oil and dams treated with testosterone propionate \([t(36.98) = -1.51, p = 0.14, d = 0.48]\).

***Development***

Mixed ANOVA revealed a significant main effect of time on total litter weight \([F(1.71, 54.80) = 900.18, p < 0.001]\). Planned comparisons revealed no differences in total litter weight between litters treated prenatally with sesame oil and testosterone at any time point. Moreover, there were no differences between litters treated prenatally with sesame oil and testosterone in terms of age of ear opening \([t(18.73) = 0.17, p = 0.87, d = 0.07]\), eye opening \([t(16.97) = 0.16, p = 0.87, d = 0.07]\), or first appearance of fur \([t(20.18) = 0.46, p = 0.65, d = 0.19]\).

***Adult Weights***

Overall, male mice had significantly greater body weights than female mice \([F(1, 343) = 424.38, p < 0.001, \eta^2 = 0.55]\). ANOVA also revealed a significant main effect of adult treatment condition \([F(2, 343) = 7.23. p = 0.001, \eta^2 = 0.04]\) on adult body weight, as well as a significant interaction between sex and adult treatment condition \([F(2, 343) = 13.86. p < 0.001, \eta^2 = 0.08]\). Analysis of simple main effects revealed an effect of adult treatment condition on body weight among female mice \([F(2, 167) = 24.70. p < 0.001, \eta^2 = 0.23]\). Pairwise comparisons revealed that intact female mice had lower body weights than both ovariectomized female mice \([p < 0.001]\), and
ovariectomized female mice that received estradiol replacement \( p < .001 \), while ovariectomized mice that received replacement with estradiol had lower body weights than ovariectomized mice that did not receive replacement \( p = .006 \) (see Figure 1).

Planned comparisons revealed that ovariectomized mice were heavier than intact mice among female mice treated prenatally with sesame oil \( t(48.59) = 6.51, \ p < .001, \ d = 1.78 \) and testosterone \( t(51.97) = 3.37, \ p = .001, \ d = 0.90 \). Furthermore, among mice treated prenatally with sesame oil, body weight was reduced in ovariectomized mice that received estradiol replacement compared to ovariectomized mice that did not receive replacement \( t(46.13) = 3.66, \ p < .001, \ d = 1.00 \).

Planned comparisons showed that male mice had significantly greater body weights than female mice across all treatment conditions. Among mice treated prenatally with sesame oil, intact male mice had greater body weights than intact female mice \( t(56.46) = 13.86, \ p < .001, \ d = 3.52 \), castrated male mice had greater body weights than ovariectomized female mice \( t(46.76) = 6.10, \ p < .001, \ d = 1.74 \), and castrated mice that received testosterone replacement had greater body weights than ovariectomized mice that received replacement with estradiol benzoate \( t(44.69) = 10.42, \ p < .001, \ d = 2.77 \). Similarly, among mice treated prenatally with testosterone, intact male mice had greater body weights than intact female mice \( t(62.29) = 10.24, \ p < .001, \ d = 2.52 \), castrated male mice had greater body weights than ovariectomized female mice \( t(49.10) = 5.31, \ p < .001, \ d = 1.41 \), and castrated mice that received testosterone replacement had greater body weights than ovariectomized mice that received replacement with estradiol benzoate \( t(51.81) = 6.87, \ p < .001, \ d = 1.69 \).
Figure 1: Adult body weight ± SEM. # indicates a significant difference between male and female within the same prenatal and adult treatment group; & indicates a significant difference between adult treatment groups within the same sex and prenatal treatment group, either between intact and GDX mice, or between GDX and GDX+EB/T replacement mice.

Vaginal Opening and Onset of Estrus

Vaginal opening occurred significantly earlier in female mice treated prenatally with testosterone (M = 29.56 days) compared to those treated with sesame oil (M = 30.30 days) \( [t(76.73) = 2.16, p = .03, d = 0.49] \). We found no significant difference in onset of estrus cycling between female mice that received prenatal treatment with sesame oil (M = 36.53 days) and testosterone (M = 36.11 days).

Gonad and Prostate Weights

Planned comparisons revealed no significant differences in gonad weight between adult mice treated prenatally with sesame oil and testosterone among either male or female animals. However, ANOVA revealed a main effect of adult treatment condition on prostate weight among male mice \( [F(2, 175) = 23.95, p < .001, \eta^2 = .22] \). Pairwise comparisons showed that prostate weights were significantly greater among intact male mice than both castrated mice \( [p < .001] \) and castrated mice that received testosterone replacement \( [p = .004] \). Furthermore, prostate...
weights were higher among castrated mice that received replacement with testosterone compared to castrated mice that did not receive testosterone replacement \( p < .001 \). Planned comparisons revealed that castration reduced prostate weight relative to intact mice among mice treated prenatally with sesame oil \( t(48.49) = 6.53, \ p < .001, \ d = 1.69 \) and testosterone \( t(46.28) = 3.71, \ p < .001, \ d = 0.98 \). Furthermore, testosterone replacement in adulthood increased prostate weight among castrated males treated prenatally with sesame oil \( t(45.35) = 3.14, \ p < .001, \ d = 0.84 \) and testosterone \( t(50.54) = 2.56, \ p = .01, \ d = 0.67 \) (see Figure 2).

![Prostate weight ± SEM](image)

**Figure 2**: Prostate weight ± SEM. & indicates a significant difference between adult treatment groups within the same sex and prenatal treatment group, either between intact and GDX mice, or between GDX and GDX+T replacement mice.

**Social Transmission of Food Preferences – Feeding Behaviour**

**Summary**

In adolescence, mice showed preference for demonstrated food at all time points throughout the 8-hour test regardless of sex or prenatal treatment. Furthermore, there were no differences in preference for demonstrated food flavour due to sex or prenatal treatment. In adulthood, social learning was impaired in gonadectomized male and female mice treated prenatally with testosterone and this was recovered by estradiol replacement in female mice but


not by testosterone replacement in male mice. Among mice treated prenatally with testosterone, castration extended preference for demonstrated food in male mice, while ovariectomy reduced preference for demonstrated food in female mice. None of the groups that showed impairments in STFP exhibited impairments in the flavour discrimination task.

**Adolescence**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Prenatal Treatment</th>
<th>Sample Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Sesame Oil</td>
<td><em>Cinnamon: 33; Cocoa: 40</em></td>
</tr>
<tr>
<td></td>
<td>Testosterone</td>
<td><em>Cinnamon: 38; Cocoa: 34</em></td>
</tr>
<tr>
<td>Female</td>
<td>Sesame Oil</td>
<td><em>Cinnamon: 38; Cocoa: 35</em></td>
</tr>
<tr>
<td></td>
<td>Testosterone</td>
<td><em>Cinnamon: 31; Cocoa: 34</em></td>
</tr>
</tbody>
</table>

**Social Learning – Cinnamon Preference**

Mixed analysis of variance revealed a main effect of demonstrated food on preference for cinnamon flavoured diet \(F(1, 228) = 153.01, p < .001, \eta^2 = .40\), as well as an interaction between demonstrated food flavour and sex \(F(1, 228) = 3.96, p = .05, \eta^2 = .02\). In addition, we found a trend towards significance for a main effect of time \(F(2.61, 594.19) = 2.59, p = .06, \eta^2 = .01\), as well as a significant interaction between time and demonstrated food flavour \(F(2.61, 594.19) = 11.05, p < .001, \eta^2 = .05\). Separate ANOVAs conducted at each time point revealed a significant main effect of demonstrated food flavour at 2 hours \(F(1, 252) = 282.38, p < .001, \eta^2 = .82\), 4 hours \(F(1, 258) = 119.09, p < .001, \eta^2 = .32\), 6 hours \(F(1, 255) = 67.49, p < .001, \eta^2 = .21\), and 8 hours \(F(1, 242) = 48.95, p < .001, \eta^2 = .17\), as well as a trend towards significance for an interaction between sex and demonstrated food at the 8-hour time point \(F(1, 242) = 3.64, p = .06, \eta^2 = .02\).

Planned comparisons revealed a significant difference in preference for cinnamon flavoured diet between cinnamon and cocoa demonstrated mice at all time points, across all
Male mice treated prenatally with sesame oil whose demonstrator was given cinnamon flavoured diet showed a greater preference for cinnamon flavoured diet than those whose demonstrator was given a cocoa flavoured diet at all time points [2h: $t(57.27) = 7.21, p < .001$, $d = 1.80$; 4h: $t(63.92) = 5.54, p < .001$, $d = 1.34$; 6h: $t(60.79) = 3.60, p < .001$, $d = 0.89$; 8h: $t(63.55) = 2.54, p = .01$, $d = 0.63$].

Male mice treated prenatally with testosterone whose demonstrator consumed cinnamon flavoured chow showed a significantly greater preference for cinnamon flavoured diet than those whose demonstrator was given cocoa flavoured diet at all time points [2h: $t(60.90) = 6.57, p < .001$, $d = 1.59$; 4h: $t(61.66) = 3.44, p = .001$, $d = 0.83$; 6h: $t(63.93) = 2.99, p = .003$, $d = 0.73$; 8h: $t(58.00) = 2.34, p = .02$, $d = 0.59$].

Female mice treated prenatally with sesame oil whose demonstrator was fed cinnamon flavoured diet showed a greater preference for cinnamon flavoured diet than those whose demonstrator consumed cocoa flavoured diet at all time points [2h: $t(65.34) = 12.15, p < .001$, $d = 2.93$; 4h: $t(63.09) = 6.98, p < .001$, $d = 1.65$; 6h: $t(61.50) = 5.47, p < .001$, $d = 1.29$; 8h: $t(64.12) = 5.61, p < .001$, $d = 1.34$].

Female mice treated prenatally with testosterone whose demonstrator animal consumed cinnamon flavoured chow showed a significantly greater preference for cinnamon flavoured diet than those whose demonstrator was given cocoa flavoured diet at all time points [2h: $t(50.01) = 9.44, p < .001$, $d = 2.47$; 4h: $t(47.93) = 6.54, p < .001$, $d = 1.71$; 6h: $t(52.31) = 4.68, p < .001$, $d = 1.23$; 8h: $t(50.56) = 4.08, p < .001$, $d = 1.09$].
Figure 3: Ratio of cinnamon consumed to total consumption ± SEM. * indicates a significant difference in cinnamon preference between cinnamon and cocoa demonstrated mice.

Social Learning – Demonstrated Food Preference

Mixed analysis of variance revealed a main effect of time on preference for demonstrated food \( F(2.61, 605.42) = 11.46, p < .001, \eta^2 = .05 \), as well as a trend towards significance for a main effect of sex \( F(1, 232) = 3.67, p = .06, \eta^2 = .02 \). In addition, separate ANOVAs conducted at each time point revealed a trend towards significance for a main effect of sex the 8-hour time point \( F(1, 250) = 3.66, p = .06, \eta^2 = .02 \). Planned comparisons revealed no significant differences in demonstrated flavour preference due to prenatal treatment group or sex at any time point.
**Total Consumption**

Mixed analysis of variance revealed main effects of time \([F(2.48, 691.02) = 102.32, p < .001, \eta^2 = .27]\) and prenatal treatment \([F(1, 279) = 5.06, p = .02, \eta^2 = .02]\) on total food consumption, as well as a trend towards significance for a main effect of sex \([F(1,279) = 2.77, p = .09, \eta^2 = .01]\). Furthermore, there were significant interactions between time and sex \([F(2.48, 691.02) = 2.81, p = .05, \eta^2 = .01]\) and time and prenatal treatment \([F(2.48, 691.02) = 3.83, p = .02, \eta^2 = .01]\). Separate ANOVAs conducted at each time point revealed that mice treated prenatally with testosterone consumed more chow than mice treated prenatally with sesame oil at the 2-hour time point \([F(1, 279) = 3.09, p = .007, \eta^2 = .02]\), and a trend towards a significance suggested that male mice consumed more chow than female mice at the 2-hour time point \([F(1, 279) = 2.89, p = .09, \eta^2 = .01]\). At the 4-hour time point, there was a trend towards significance for an interaction between sex and prenatal treatment \([F(1, 282) = 3.15, p = .08, \eta^2 = .01]\). At the 6-hour time point ANOVA revealed that male mice consumed more chow than female mice \([F(1, 282) = 7.18, p = .008, \eta^2 = .03]\) (see Figure 4).

Planned comparisons revealed a significant difference between female mice treated prenatally with sesame oil and testosterone at the 2-hour and 4-hour time points, such that mice treated prenatally with testosterone consumed more food than mice treated prenatally with sesame oil at the 2-hour time point, but less at the 4-hour time point [2-hour: \(t(131.14) = 2.79, p = .006, d = .48\); 4-hour: \(t(135.99) = 2.33, p = .02, d = .40\)]. In addition, sesame oil treated male mice consumed significantly more chow than sesame oil treated female mice at the 2-hour time point \([t(143.79) = 2.14 p = .03, d = .35]\).
Figure 4: Total consumption ± SEM. * indicates a significant difference between prenatal treatments; # indicates a significant sex difference.

**Adulthood**

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**Social Learning – Turmeric Preference**

In adulthood, mixed analysis of variance showed a significant interaction effect between time and demonstrated food on preference for turmeric \( F(2.73, 478.98) = 7.57, p < .001, \eta^2 = .04 \), as well as a significant interaction between time, sex, prenatal treatment, adult treatment condition, and demonstrated food \( F(5.47, 478.98) = 2.72, p = .02, \eta^2 = .03 \). In addition, we found significant main effects of prenatal treatment \( F(1, 175) = 5.25, p = .02, \eta^2 = .03 \) and
demonstrated food flavour \( F(1, 175) = 23.36, p < .001, \eta^2 = .12 \) on preference for turmeric-flavoured diet, as well as a trends towards significance for interactions between prenatal treatment and demonstrated food flavour \( F(1, 175) = 2.92, p = .09, \eta^2 = .02 \) and adult treatment condition, prenatal treatment, and demonstrated food flavour \( F(1, 175) = 2.45, p = .09, \eta^2 = .03 \).

Further ANOVAs were conducted at each time point. At 2 hours, turmeric-demonstrated mice had a greater preference for turmeric than thyme-demonstrated mice \( F(1, 227) = 82.35, p < .001, \eta^2 = .27 \), and a trend towards significance suggested that mice treated prenatally with testosterone may have had an increased preference for turmeric relative to mice treated prenatally with sesame oil \( F(1, 227) = 2.97, p = .09, \eta^2 = .01 \). At 4 hours, mice whose demonstrated consumed turmeric flavoured diet continued to demonstrate a greater preference for turmeric than mice whose demonstrated consumed thyme flavoured diet \( F(1, 227) = 24.10, p < .001, \eta^2 = .10 \). At 6 hours, turmeric-demonstrated mice had a greater preference for turmeric than thyme-demonstrated mice \( F(1, 238) = 12.74, p < .01, \eta^2 = .06 \), and mice treated prenatally with testosterone had a greater preference for turmeric than mice treated prenatally with sesame oil \( F(1, 238) = 9.41, p = .002, \eta^2 = .04 \). In addition, ANOVA revealed a significant interaction between demonstrated food and adult treatment condition \( F(2, 238) = 4.26, p = .02, \eta^2 = .02 \), a significant interaction between demonstrated food and prenatal treatment \( F(1, 238) = 4.09, p = .04, \eta^2 = .02 \), a significant interaction between demonstrated food, adult treatment condition, and prenatal treatment \( F(2, 238) = 4.52, p = .01, \eta^2 = .03 \), and a significant interaction between adult treatment condition, sex, and prenatal treatment \( F(2, 338) = 3.26, p = .03, \eta^2 = .03 \). At the 8-hour time point, ANOVA revealed that mice treated prenatally with testosterone had a greater preference for turmeric than mice treated prenatally with sesame oil \( F(1, 207) = 4.18, p \)
=.04, $\eta^2 = .02$], as well as a trend towards an interaction between demonstrated food, adult treatment condition, and prenatal treatment [$F(2, 207) = 2.23, p = .07, \eta^2 = .03$] (see Figure 5).

Among intact male mice treated prenatally with sesame oil, planned comparisons showed a significant difference in preference for turmeric flavoured diet between turmeric and thyme demonstrated mice at the 2-hour time point, and a trend towards significance at 6 hours [2h: $t(14.12) = 5.89, p < .001, d = 2.76$; 4h: $t(16.74) = 1.27, p = .22, d = 0.59$; 6h: $t(13.70) = 1.84, p = .09, d = 0.91$; 8h: $t(7.19) = 0.44, p = .68, d = 0.27$]. Furthermore, in castrated male mice treated prenatally with sesame oil, planned comparisons showed a significant difference in preference for turmeric flavoured diet between mice with turmeric and thyme fed demonstrators at the 2-hour, 6-hour, and 8-hour time points, and a trend towards significance at the 4-hour time point [2h: $t(17.80) = 3.44, p = .003, d = 1.47$; 4h: $t(17.78) = 2.09, p = .05, d = 0.91$; 6h: $t(17.66) = 3.07, p = .006, d = 1.37$; 8h: $t(12.21) = 2.99, p = .01, d = 1.39$]. Furthermore, among castrated male mice treated prenatally with sesame oil that received testosterone replacement, there was a significant difference in preference for turmeric flavoured diet between mice with turmeric and thyme demonstrated mice at the 2-hour and 4-hour time points, and a trend towards significance at the 6-hour time point [2h: $t(13.78) = 3.10, p = .04, d = 0.85$; 4h: $t(15.02) = 2.20, p = .04, d = 0.99$; 6h: $t(14.49) = 1.85, p = .09, d = 0.85$; 8h: $t(9.49) = 1.40, p = .19, d = 0.70$].

Among intact male mice treated prenatally with testosterone, there was a significant difference in turmeric preference between mice with turmeric and thyme fed demonstrators at the 2-hour, 4-hour, and 6-hour time points [2h: $t(16.33) = 3.47, p = .03, d = 1.53$; 4h: $t(16.84) = 2.15, p = .05, d = 0.94$; 6h: $t(19.17) = 2.69, p = .01, d = 1.13$; 8h: $t(11.58) = 0.51, p = .61, d = 0.25$]. Conversely, among castrated male mice treated prenatally with testosterone, thyme demonstrated mice exhibited a greater preference for turmeric than turmeric demonstrated mice, which is the
opposite of what we expected and what we observed in other groups. However, we observed a
trend towards significance at the 2-hour time point, suggesting that turmeric demonstrated mice
had a greater preference for turmeric than thyme demonstrated mice [2h: \( t(14.11) = 1.95, p = .07, \\
d = 0.92; 4h: t(16.63) = 1.08, p = .29, d = 0.49; 6h: t(17.00) = 2.96, p = .008, d = 1.30; 8h: \\
t(12.92) = 1.47, p = .17, d = 0.71\)]. Furthermore, there were no differences in preference for
turmeric flavoured diet at any time point between turmeric and thyme demonstrated castrated
male mice treated prenatally with testosterone that received testosterone replacement in
adulthood [2h: \( t(16.73) = 0.71, p = .49, d = 0.30; 4h: t(17.00) = 0.57, p = .58, d = 0.23; 6h: \\
t(13.05) = 0.03, p = .97, d = 0.02; 8h: t(13.61) = 1.25, p = .23, d = 0.55\]. Among intact female mice treated prenatally with sesame oil, planned comparisons
showed significant differences in preference for turmeric flavoured diet between turmeric and
thyme demonstrated mice at the 2-hour and 6-hour time points, and trends towards significance
at 4 hours and 8 hours [2h: \( t(19.86) = 2.88, p = .009, d = 1.23; 4h: t(19.48) = 1.74, p = .10, d = \\
0.74; 6h: t(17.75) = 2.53, p = .02, d = 1.13; 8h: t(16.70) = 1.82, p = .09, d = 0.83\]]. Moreover,
among ovariectomized female mice treated prenatally with sesame oil, mice whose demonstrator
consumed turmeric flavoured diet had a greater preference for turmeric at the 2-hour time point
[2h: \( t(10.51) = 2.24, p = .05, d = 1.11; 4h: t(7.96) = 1.61, p = .15, d = 0.90; 6h: t(13.73) = 1.11, p \\
= .29, d = 0.51; 8h: t(4.43) = 0.14, p = .90, d = 0.09\]. Among ovariectomized female mice
treated prenatally with sesame oil that received estradiol replacement, mice demonstrated with
turmeric flavoured diet showed a greater preference for turmeric flavoured chow than those
demonstrated with thyme at the 2-hour time point [2h: \( t(20.05) = 2.53, p = .02, d = 1.04; 4h: \\
t(16.89) = 1.39, p = .18, d = 0.60; 6h: t(14.96) = 0.15, p = .88, d = 0.06; 8h: t(20.30) = 0.36, p = \\
.72, d = 0.15\].
Among intact female mice treated prenatally with testosterone, planned comparisons showed significant differences in preference for turmeric flavoured diet between turmeric and thyme demonstrated mice at the 2-hour and 6-hour time points [2h: \( t(18.99) = 3.26, p = .004, d = 1.42; 4h: t(17.59) = 1.22, p = .24, d = 0.54; 6h: t(17.90) = 2.33, p = .03, d = 1.04; 8h: t(11.61) = 1.43, p = .18, d = 0.71\]. However, among gonadectomized female mice treated prenatally with testosterone, planned comparisons showed no differences in preference for turmeric flavoured diet between turmeric and thyme demonstrated mice at any time point [2h: \( t(21.66) = 0.63, p = .54, d = 0.25; 4h: t(21.96) = 0.28, p = .79, d = 0.11; 6h: t(22.00) = 0.73, p = .48, d = 0.29; 8h: t(11.63) = 0.56, p = .58, d = 0.28\]. Conversely, among ovariectomized female mice treated prenatally with testosterone that received estradiol replacement, turmeric demonstrated mice showed a greater preference for turmeric than thyme demonstrated mice at the 2-hour and 4-hour time points [2h: \( t(9.68) = 2.59, p = .02, d = 1.37; 4h: t(11.61) = 2.27, p = .04, d = 1.13; 6h: t(9.16) = 0.64, p = .54, d = 0.35; 8h: t(11.42) = 0.30, p = .77, d = 0.15\].
Figure 5: Ratio of turmeric consumed to total consumption ± SEM. * indicates a significant difference between turmeric and thyme demonstrated mice.

Social Learning – Demonstrated Food Preference

Mixed analysis of variance revealed a main effect of time on preference for demonstrated food flavour \([F(2.44, 509.27) = 7.01, p < .001, \eta^2 = .04]\), as well as a significant interaction between time, sex, prenatal treatment, and adult treatment condition \([F(5.45, 509.27) = 2.24, p = \ldots]\).
.04, \eta^2 = .03]. In addition, we found a trend towards a main effect of prenatal treatment on preference for demonstrated food \([F(1, 187) = 3.16, p = .06, \eta^2 = .02]\) and a trend towards an interaction between prenatal treatment and adult treatment condition \([F(2, 175) = 2.44, p = .09, \eta^2 = .03]\). Separate ANOVAs conducted at each time point revealed a trend towards a main effect of prenatal treatment at the 2-hour time point \([F(2, 251) = 2.99, p = .09, \eta^2 = .02]\); main effects of adult treatment condition \([F(2, 238) = 3.18, p = .04, \eta^2 = .03]\) and prenatal treatment \([F(2, 238) = 4.88, p = .03, \eta^2 = .03]\), as well as an interaction between adult treatment condition and prenatal treatment \([F(2, 238) = 3.56, p = .02, \eta^2 = .03]\), at the 6-hour time point; and a trend towards significance for an interaction between prenatal treatment and adult treatment condition at the 8-hour time point \([F(2, 207) = 2.94, p = .06, \eta^2 = .03]\) (see Figure 6).

Among mice treated prenatally with testosterone, planned comparisons revealed that ovariectomized mice that received estradiol replacement exhibited a greater preference for demonstrated food at the 4-hour time point than castrated mice that received testosterone replacement \([t(33.23) = 2.17, p = .04, d = 0.72]\). Furthermore, among castrated mice, prenatal testosterone treatment reduced preference for demonstrated diet relative to mice treated prenatally with sesame oil at the 6-hour \([t(36.85) = 3.74, p < .001, d = 1.19]\) and 8-hour \([t(30.26) = 2.56, p = .02, d = 0.89]\) time points. There was also a trend among castrated mice that received testosterone replacement suggesting that prenatal testosterone treatment reduced demonstrated flavour preference at the 4-hour time point \([t(38.81) = 1.93, p = .06, d = 0.60]\). In addition, among male mice treated prenatally with sesame oil, castrated mice showed a greater preference for demonstrated food at the 8-hour time point than castrated mice that received replacement with testosterone \([t(27.92) = 2.70, p = .01, d = 0.71]\). In addition, preference for demonstrated food at the 6-hour time point was reduced among castrated male mice that had been treated
prenatally with testosterone compared to intact male mice \( t(38.83) = 2.94, p = .005, d = 0.91 \).

Similarly, among female mice treated prenatally with testosterone, demonstrator preference at the 6-hour time point was reduced in ovariectomized mice when compared to intact female mice \( t(40.53) = 2.29, p = .03, d = 0.69 \), with a similar trend at the 2-hour time point \( t(43.00) = 1.90, p = .06, d = 0.56 \). A trend towards significance suggested that hormone replacement with testosterone may have increased demonstrated food preference at the 6-hour time point among castrated male mice treated prenatally with testosterone \( t(25.95) = 1.69, p = .10, d = 0.55 \).

Additional trends towards significance suggested a marginal increase in preference for demonstrated food due to replacement with estradiol among ovariectomized mice treated prenatally with testosterone at the 2-hour \( t(37.40) = 1.79, p = .08, d = 0.55 \) and 4-hour \( t(38.05) = 1.72, p = .09, d = 0.53 \) time points.

In addition, among intact female mice, ANOVA revealed an effect of estrus on preference for demonstrated food at the 2-hour and 4-hour time points a \( 2h: F(2, 26) = 4.52, p = .02, \eta^2 = .36; 4h: F(2, 26) = 4.52, p = .02, \eta^2 = .36 \), such that mice in proestrus had a greater preference for demonstrated diet than those in estrus at the 2-hour \( p = .02 \) and 4-hour \( p = .04 \) time points.
Figure 6: Ratio of demonstrated diet consumed over total intake ± SEM. * indicates a significant difference between prenatal treatments; # indicates a significant difference between males and females; & indicates a significant difference between adult treatment groups, either between intact and GDX mice, or between GDX and GDX+EB/T replacement mice.

Total Consumption

Mixed analysis of variance revealed a main effect of time on total consumption \([F(2.79, 711.78) = 155.09, p < .001, \eta^2 = .38]\), as well as a significant interaction between time and adult treatment condition \([F(5.58, 711.78) = 2.53, p = .02, \eta^2 = .02]\), and a trend towards significance for an interaction between time and sex \([F(2.79, 715.03) = 2.53, p = .06, \eta^2 = .01]\). In addition, we found a trend towards a main effect of sex \([F(1, 255) = 3.74, p = .05, \eta^2 = .01]\), and significant interactions between sex and adult treatment condition \([F(2, 255) = 3.52, p = .03, \eta^2 = .03]\), and sex and prenatal treatment \([F(1, 255) = 5.22, p = .02, \eta^2 = .02]\).

Separate ANOVAs conducted at each time point revealed a significant interaction effect between sex and adult treatment condition on consumption at the 2-hour time point \([F(2, 267) = 4.06, p = .02, \eta^2 = .03]\); main effects of sex \([F(1, 267) = 9.09, p = .003, \eta^2 = .03]\) and prenatal
treatment \( F(1, 267) = 5.77, p = .02, \eta^2 = .02 \) on total consumption at the 4-hour time point; a main effect of adult treatment condition on total consumption \( F(2, 267) = 3.54, p = .03, \eta^2 = .03 \) at the 6-hour time point; and a main effect of adult treatment condition \( F(2, 267) = 3.53, p = .03, \eta^2 = .03 \) along with a significant interaction effect between sex and prenatal treatment \( F(1, 267) = 8.12, p = .005, \eta^2 = .03 \) on consumption at the 8-hour time point (see Figure 7).

Among intact mice treated prenatally with sesame oil, planned comparisons showed that female mice consumed more than male mice at the 4-hour \( t(39.45) = 2.77, p = .009, d = 0.83 \) and 8-hour \( t(39.88) = 2.33, p = .02, d = 0.71 \) time points. Additionally, among sesame oil treated mice that underwent gonadectomy, female mice consumed significantly more total food at the 8-hour time point than male mice \( t(36.21) = 2.15, p = .04, d = 0.66 \). Similarly, among gonadectomized mice treated prenatally with testosterone, female mice consumed significantly more chow at the 4-hour time point than male mice \( t(40.13) = 2.06, p = .05, d = 0.62 \), though we found a trend at the 2-hour time point in the opposite direction \( t(41.99) = 1.94, p = .06, d = 0.58 \). Furthermore, among castrated mice, mice treated prenatally with testosterone consumed more food than mice treated prenatally with sesame oil at the 8-hour time point \( t(36.06) = 3.20, p = .003, d = 0.99 \). In addition, there were trends towards significance among intact female mice at the 4-hour \( t(42.90) = 1.84, p = .07, d = 0.55 \), and 8-hour \( t(41.11) = 1.77, p = .08, d = 0.52 \) time points, suggesting that consumption was lower among mice treated prenatally with testosterone compared to mice treated prenatally with sesame oil. Moreover, among female mice treated prenatally with testosterone, intact mice consumed significantly more food than ovariectomized mice at the 6-hour time point \( t(40.44) = 2.37, p = .02, d = 0.71 \). Similarly, among female mice treated prenatally with sesame oil, intact mice consumed more food than ovariectomized mice at the 8-hour time point \( t(38.53) = 2.08, p = .04, d = 0.62 \). In addition,
testosterone replacement significantly increased consumption among castrated male mice treated prenatally with sesame oil at the 8-hour time point \([t(35.55) = 2.41, p = .02, d = 0.68]\).

Figure 7: Total consumption ± SEM. * indicates a significant different between prenatal treatment groups; # indicates a significant sex difference; & indicates a significant difference between adult treatment groups, between either intact and GDX+VEH mice, or between GDX+VEH and GDX+EB/T replacement mice.

**Flavour Recognition Control Task**

All groups that showed impairment in social learning in the social transmission of food preferences paradigm were able to discriminate between the turmeric and thyme flavoured diets in a flavour recognition task. Among castrated mice treated prenatally with testosterone \((n = 8)\), there was a significant increase in investigation of the target food jar after switching the habituated food flavour for a novel food flavour during the test phase \([t(11.96) = 2.62, p = .02, d = 1.31]\). Similarly, among castrated mice treated prenatally with testosterone that received testosterone replacement \((n = 9)\), there was a significant increase in investigation of the novel food jar in the test phase \([t(2.49) = 15.96, p = .02, d = 1.17]\). Lastly, among ovariectomized mice treated prenatally with testosterone \((n = 12)\), there was a significant increase in investigation of
the target food jar in the test phase after switching the habituated food flavour for a novel food flavour \[ t(21.85) = 2.24, p = .04, d = 0.92 \] (see Figure 8).

![Flavour Recognition](image)

**Figure 8:** Investigation ratio ± SEM. * indicates a significant different between habituation and test.

**Social Transmission of Food Preference – Social Interaction**

**Summary**

In adolescence, we saw no differences in measures of non-social behaviour based on sex or prenatal treatment condition. Male mice engaged in more frequent overall agonistic behaviour than female mice, likely driven by differences in reciprocal attacks. Moreover, female mice spent more time receiving agonistic behaviour than male mice, likely due to more time spent engaged in submissive behaviour. Female mice also engaged in social investigation for longer than male mice, spent longer investigating the body of the demonstrator, and made more frequent investigations of the demonstrator’s anogenital region. However, we did not find any differences in oronasal investigation, indicating that there were not differences between groups in terms of availability of information about the flavoured diet consumed by the demonstrator.

In adulthood, female mice spent more time engaged in active behaviour than male mice, while male mice engaged in non-social behaviour for longer and more frequently than female mice. Prenatal treatment with testosterone increased behavioural switching in general in female
mice, but only increased the frequency of engagement in non-social behaviour in male mice, and shifted male mice further towards a greater performance of for non-social activity. Female mice investigated the demonstrator for longer than male mice, while gonadectomy reduced investigation of the demonstrator. Furthermore, female mice spent more time engaged in agonistic behaviour than male mice, and we did not see a significant sex difference in dominance scores. Prenatal treatment with testosterone reduced dominance scores among male mice as well as intact female mice, while ovariectomized female mice treated prenatally with testosterone were markedly dominant over the demonstrator, likely due to a reduction in agonistic behaviours received. Furthermore, we found that only male mice delivered attacks and engaged in ritualized and open aggression, and that these behaviours were greatly reduced in castrated mice. Lastly, among male mice, prenatal treatment with testosterone reduced dominance behaviour, but increased ritualized aggression.

**Adolescence**

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**Social Interaction**

**Social Investigation**

Adolescent female mice spent longer engaged in overall social investigation than male mice \(F(1, 212) = 4.76, p = .03, \eta^2 = .02\). Specifically, female mice spent more time investigating the body of the demonstrator than male mice \(F(1, 212) = 10.75, p = .001, \eta^2 = .05\), and made more frequent investigations of the demonstrator’s anogenital region \(F(1, 212) = 4.06, p = .05, \eta^2 = .02\). However, ANOVA and planned comparisons revealed no differences in
either frequency or duration of oronasal investigation based on either sex or prenatal treatment, indicating that there were no differences in opportunity to acquire information about demonstrated diet between groups (see Figure 9).

![Image of Figure 9: Measures of social investigation ± SEM.]

**Figure 9**: Measures of social investigation ± SEM.

**Agonistic Behaviour**

Overall, dyads of male mice engaged in agonistic behaviour more frequently than dyads of female mice \(F(1, 212) = 13.78, p < .001, \eta^2 = .06\). Planned comparisons showed that social interactions between male mice consisted of more instances of agonistic behaviour among than interactions between female mice in mice treated prenatally with both sesame oil \([W = 875, Z = -2.71, p = .006, r = -.27]\) and testosterone \([W = 1191.5, Z = -2.51, p = .01, r = -.24]\). Moreover, male mice delivered agonistic behaviours more frequently than female mice \(F(1, 212) = 8.17, p = .005, \eta^2 = .04\), and spent less time receiving agonistic behaviour from the demonstrator than female mice \(F(1, 212) = 5.03, p = .02, \eta^2 = .02\). Among mice treated prenatally with sesame oil, planned comparisons revealed that male mice delivered agonistic behaviours more frequently than female mice \([W = 905.5, Z = -2.51, p = .01, r = -.25]\), while female mice treated prenatally
with testosterone spent significantly longer as the recipient of agonistic behaviour than male mice treated prenatally with testosterone \( [W = 2067, Z = 2.36, p = .02, r = .22] \). Furthermore, among mice treated prenatally with testosterone, male mice had greater duration-based dominance scores than female mice \( [W = 1253, Z = -2.21, p = .03, r = -.21] \), while male mice had greater frequency-based dominance scores than female mice among mice treated prenatally with sesame oil \( [W = 960, Z = -2.13, p = .03, r = -.21] \). In addition, male mice treated prenatally with sesame oil were dominant over the demonstrator [Duration: \( V = 984, p = .007 \); Frequency: \( V = 1050, p < .001 \)], as were male mice treated prenatally with testosterone [Duration: \( V = 1426, p < .001 \); Frequency: \( V = 1223, p = .002 \)]. Similarly, female mice treated prenatally with sesame oil were dominant over the demonstrator [Duration: \( V = 829, p = .03 \); Frequency: \( V = 852, p < .001 \)], though female mice treated prenatally with testosterone were only dominant over the demonstrator when considering frequency-based dominance score \( [V = 1226, p < .001] \) (see Figure 10).

ANOVA revealed that male mice engaged in ritualized aggression for longer than female mice \( [F(1, 212) = 31.81, p < .001, \eta^2 = .01] \), as well as a trend suggesting that mice treated prenatally with testosterone engaged in ritualized aggression for longer than mice treated prenatally with sesame oil \( [F(1, 212) = 3.06, p = .08, \eta^2 = .01] \). Planned comparisons showed that male mice spent more time engaged in ritualized aggression than female mice across both prenatal treatment groups [Prenatal Sesame Oil: \( W = 949, Z = -2.68, p < .001, r = -.27 \); Prenatal Testosterone: \( W = 878.5, Z = -4.78, p < .001, r = -.45 \)]. Furthermore, male mice engaged in open aggression for significantly longer than female mice \( [F(1, 212) = 10.64, p = .001, \eta^2 = .05] \), and planned comparisons showed that male mice engaged in open aggression for longer than female mice across both prenatal treatment groups [Prenatal Sesame Oil: \( W = 882, Z = -4.19, p < .001, r \)
Additionally, ANOVA revealed that female mice spent more time engaged in submissive behaviour than male mice \([F(1, 212) = 8.69, p = .004, \eta^2 = .04]\). Specifically, among mice treated prenatally with testosterone, planned comparisons indicated that female mice spent significantly longer engaged in submissive behaviour than male mice \([W = 2183, Z = 3.01, p = .002, r = .28]\) (see Figure 10).

Moreover, male mice both delivered \([F(1, 212) = 40.38, p < .001, \eta^2 = .16]\) and received \([F(1, 212) = 22.31, p < .001, \eta^2 = .10]\) more attacks than female mice. Planned comparisons showed that male mice delivered more attacks than female mice across both prenatal treatment groups \([\text{Prenatal Sesame Oil}: W = 870, Z = -3.53, p < .001, r = -.35; \text{Prenatal Testosterone}: W = 951.5, Z = -4.43, p < .001, r = -.41]\), and received more attacks than female mice across both prenatal treatment groups \([\text{Prenatal Sesame Oil}: W = 939, Z = -3.15, p = .002, r = -.13; \text{Prenatal Testosterone}: W = 1081, Z = -3.94, p < .001, r = -.37]\) (see Figure 10).
Figure 10: A: Duration of agonistic behaviour; B: Duration-based dominance score; C: Frequency-based dominance score; D: Frequency of agonistic behaviour delivered; E: Duration of agonistic behaviour received; F: Submissive behaviour; G: Duration of open aggression; H: Duration of ritualized aggression; I: Attacks delivered; J: Attacks received. Frequency based dominance score presented as a median because mean was not representative of the results of our non-parametric test. All other measures ± SEM. # indicates a significant sex difference; % indicates dominance scores significantly > 0.
Adulthood

<table>
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</table>

Overall Activity

Female mice spent more time engaged in active behaviour than male mice \(F(1, 238) = 4.40, p = .04, \eta^2 = .02\], while animals treated prenatally with testosterone engaged in active behaviour more frequently than those treated with sesame oil \(F(1, 238) = 6.34, p = .01, \eta^2 = .03\]. There was also a main effect of adult treatment condition on frequency of active behaviour \(F(2, 238) = 11.04, p < .001, \eta^2 = .09\], and further investigation via pairwise comparisons showed that intact mice engaged in more frequent active behaviour than both gonadectomized mice \(p < .001\] and gonadectomized mice that received hormone replacement with either estradiol benzoate (females) or testosterone (males) \(p = .002\] (see Figure 11).
ANOVA also showed main effects of prenatal treatment \([F(1, 238) = 4.21, p = .04, \eta^2 = .02]\) and sex \([F(1, 238) = 8.09, p = .005, \eta^2 = .03]\) on total duration of non-social behaviour, as well as interactions between sex and adult treatment condition \([F(2, 238) = 3.44, p = .03, \eta^2 = .03]\), and sex and prenatal treatment \([F(1, 238) = 6.52, p = .01, \eta^2 = .03]\). Analysis of simple main effects showed that prenatal treatment with testosterone was associated with increased duration of non-social behaviour among male mice \([F(1, 124) = 11.60, p = .001, \eta^2 = .09]\). Furthermore, frequency of non-social behaviour was higher among mice treated prenatally with testosterone compared to mice treated prenatally with sesame oil \([F(1, 238) = 9.66, p = .002, \eta^2 = .04]\), and male mice engaged in a greater number of non-social behaviours overall than female mice.
mice \( [F(1, 238) = 17.82, p < .001, \eta^2 = .07] \). In addition, ANOVA revealed a main effect of adult treatment condition on frequency of non-social behaviour \( [F(2, 238) = 5.87, p = .003, \eta^2 = .05] \), such that intact mice engaged in non-social behaviours more frequently than both gonadectomized mice \( [p = .005] \), and gonadectomized mice that received testosterone (male) or estradiol (female) replacement \( [p = .02] \) (see Figure 12).

![Non-Social Behaviour - Duration](image1)

![Non-Social Behaviour - Frequency](image2)

*Figure 12: Duration and frequency of non-social behaviour ± SEM.*

ANOVA revealed a main effect of sex on duration of non-social locomotor behaviour \( [F(1, 238) = 7.89, p = .005, \eta^2 = .03] \), as well as significant interactions between sex and prenatal treatment \( [F(1, 238) = 5.34, p = .02, \eta^2 = .02] \), and sex and adult treatment condition \( [F(2, 238) = 4.03, p = .02, \eta^2 = .03] \). Simple main effects indicated that prenatal treatment with testosterone
increased the duration of non-social locomotor behaviour among male mice \( F(1, 124) = 8.19, p = .005, \eta^2 = .06 \), and there were trends towards significance for an effect of adult treatment condition among both male \( F(2, 124) = 2.74, p = .07, \eta^2 = .04 \) and female mice \( F(2, 114) = 2.43, p = .09, \eta^2 = .04 \). Moreover, mice treated prenatally with testosterone engaged in more frequent non-social locomotor behaviour than mice treated prenatally with sesame oil \( F(1, 238) = 9.04, p = .003, \eta^2 = .04 \), and male mice engaged in more frequent instances of non-social locomotive behaviour than female mice \( F(1, 238) = 15.60, p < .001, \eta^2 = .06 \). ANOVA also revealed a main effect of adult treatment condition on frequency of non-social locomotor behaviour \( F(2, 238) = 7.12, p = .001, \eta^2 = .06 \), such that intact mice engaged in non-social locomotor behaviour more frequently than both gonadectomized mice \( p < .001 \) and gonadectomized mice that received hormone replacement \( p = .01 \). Male mice also engaged in more instances of inactive non-social behaviour than female mice \( F(1, 238) = 9.65, p = .002, \eta^2 = .04 \), and a trend towards significance suggested that mice that received prenatal treatment with testosterone engaged in inactive non-social behaviour more often than those treated prenatally with sesame oil \( F(1, 238) = 3.51, p = .06, \eta^2 = .02 \) (see Figure 13).
ANOVA revealed significant interaction effects between sex and prenatal treatment [$F(1, 238) = 4.86, p = .02, \eta^2 = .02$] and sex and adult treatment condition [$F(2, 238) = 3.31, p = .04, \eta^2 = .03$] on time spent engaged in horizontal activity. Analysis of simple main effects showed that prenatal treatment with testosterone increased the duration of active horizontal behaviour among male mice, [$F(1, 124) = 6.02, p = .02, \eta^2 = .05$], while adult treatment condition affected active horizontal behaviour in female mice [$F(2, 114) = 3.12, p = .05, \eta^2 = .05$], such that ovariectomized mice that received estradiol replacement spent less time engaged in active horizontal behaviour than ovariectomized mice that did not receive estradiol replacement [$p = .04$]. Furthermore, mice treated prenatally with testosterone engaged in horizontal activity more
frequently than mice treated prenatally with sesame oil \(F(1, 238) = 8.93, p = .003, \eta^2 = .04\],
while male mice engaged in horizontal activity more frequently than female mice \(F(1, 238) = 7.60, p = .006, \eta^2 = .03\]. In addition, ANOVA revealed a significant effect of adult treatment condition on frequency of horizontal activity \(F(2, 238) = 5.35, p = .005, \eta^2 = .04\], such that frequency of horizontal activity was higher among intact mice than gonadectomized mice \(p = .01\] and gonadectomized mice that received replacement with either testosterone (males) or estradiol (females) \(p = .02\] (see Figure 14).

![Figure 14: Duration and frequency of horizontal activity ± SEM.](image)

Male mice engaged in active vertical activity for longer than female mice \(F(1, 238) = 11.53, p = .001, \eta^2 = .05\], and more frequently than female mice \(F(1, 238) = 25.41, p < .001, \eta^2\]
In addition, mice treated prenatally with testosterone engaged in vertical activity more frequently than mice treated prenatally with sesame oil \(F(1, 238) = 6.91, p = .009, \eta^2 = .03\), while a trend towards significance indicated that mice treated prenatally with testosterone engaged in vertical activity for marginally longer than mice treated prenatally with sesame oil \(F(1, 238) = 3.69, p = .06, \eta^2 = .02\). Furthermore, ANOVA revealed a main effect of adult treatment condition on duration \(F(2, 238) = 3.78, p = .02, \eta^2 = .03\) and frequency \(F(2, 238) = 8.11, p < .001, \eta^2 = .06\) of vertical activity. Pairwise comparisons showed that intact mice spent significantly more time engaged in vertical behaviour than gonadectomized mice \(p = .02\), and engaged in vertical activity more frequently than gonadectomized mice \(p < .001\) and gonadectomized mice that received replacement with testosterone (males) or estradiol (females) \(p = .02\). There was also a trend toward significance suggesting a possible interaction effect between sex and adult treatment condition on both frequency \(F(2, 238) = 2.60, p = .08, \eta^2 = .02\) and duration \(F(2, 238) = 2.73, p = .07, \eta^2 = .02\) of vertical activity, and a trend towards significance suggesting an interaction effect between sex and prenatal treatment on frequency of vertical activity \(F(1, 238) = 2.99, p = .09, \eta^2 = .01\) (see Figure 15).
Figure 15: Duration and frequency of active vertical behaviour ± SEM.

ANOVA revealed a significant interaction effect between sex and prenatal treatment on duration of social inactivity \( [F(1, 238) = 8.34, p = .004, \eta^2 = .03] \), such that male mice treated prenatally with testosterone spent less time engaged in social inactivity with the demonstrator than male mice treated prenatally with sesame oil \( [F(1, 124) = 4.89, p = .03, \eta^2 = .04] \). In addition, male mice engaged in solitary inactivity for longer than female mice \( [F(1, 238) = 4.98, p = .03, \eta^2 = .02] \), and ANOVA showed a trend towards a main effect of adult treatment condition on duration of solitary inactivity \( [F(2, 238) = 2.86, p = .06, \eta^2 = .02] \) (see Figure 16). Furthermore, mice treated prenatally with testosterone spent more time engaged in grooming than mice treated prenatally with sesame oil \( [F(1, 238) = 5.12, p = .03, \eta^2 = .02] \).
Figure 16: Solitary and social inactivity ± SEM.

Social Behaviour

ANOVA revealed main effects of sex \( [F(1, 238) = 8.08, p = .005, \eta^2 = .03] \) and prenatal treatment \( [F(1, 238) = 4.34, p = .04, \eta^2 = .02] \) on total duration of social behaviour, as well as significant interactions between sex and prenatal treatment \( [F(1, 238) = 6.53, p = .01, \eta^2 = .03] \), and sex and adult treatment condition \( [F(2, 238) = 3.46, p = .03, \eta^2 = .03] \). Analysis of simple main effects showed that prenatal testosterone treatment decreased the duration of engagement in social behaviours among male mice \( [F(1, 124) = 11.63, p = .001, \eta^2 = .09] \), but not female mice. ANOVA also revealed a main effect of adult treatment condition on frequency of social behaviour \( [F(2, 238) = 7.63, p = .001, \eta^2 = .06] \), such that both gonadectomized mice \( [p = .001] \)
and gonadectomized mice that received hormone replacement with either testosterone (males) or EB (females) \( [p = .02] \) engaged in fewer instances of social behaviour than intact mice. In addition, there was a significant interaction between sex and prenatal treatment \( [F(1, 238) = 4.46, p = .04, \eta^2 = .02] \). Further analysis revealed that testosterone treated female mice engaged in social behaviour more frequently than those treated prenatally with sesame oil \( [F(1, 114) = 4.05 \ p = .04, \eta^2 = .03] \) (see Figure 17). Differences in findings between the frequency and duration of overall social behaviour may stem from the inclusion of attacks delivered and received in the composite measure for frequency (discussed below).

*Figure 17: Duration and frequency of social behaviour ± SEM.*
Social Investigation

Overall, female mice spent more time engaged in social investigation than male mice $[F(1, 238) = 12.65, p < .001, \eta^2 = .05]$. Moreover, ANOVA revealed main effects of both sex $[F(1, 238) = 6.02, p = .02, \eta^2 = .03]$ and adult treatment condition on frequency of social investigations $[F(2, 238) = 4.70, p = .01, \eta^2 = .04]$, as well as a significant interaction between sex and prenatal treatment $[F(1, 238) = 4.59, p = .03, \eta^2 = .02]$. Overall, intact mice made more investigations of the demonstrator animal than gonadectomized mice $[p = .01]$, while a trend towards significance suggests that intact mice may have also made more social investigations than gonadectomized mice that received hormone replacement $[p = .05]$. Additionally, prenatal treatment with testosterone increased the frequency of social investigation only among female animals $[F(1, 114) = 5.28, p = .02, \eta^2 = .04]$ (see Figure 18).
Figure 18: Duration and frequency of social investigation ± SEM.

ANOVA revealed a main effect of adult treatment condition on duration of oronasal investigation \([F(2, 238) = 5.44, p = .005, \eta^2 = .04]\), as well as a trend towards an interaction between sex, prenatal treatment, and adult hormone condition \([F(2, 238) = 2.74, p = .07, \eta^2 = .02]\). Pairwise comparisons showed that intact animals spent significantly more time investigating the oronasal region of the demonstrator than both gonadectomized animals \([p < .001]\) and gonadectomized animals that received hormone replacement with either testosterone (males) or EB (females). Planned comparisons showed that intact female mice treated prenatally with testosterone spent more time engaged in oronasal investigation than gonadectomized female
mice treated prenatally with testosterone \( W = 296, Z = 2.61, p = .009, r = .41 \) and intact female mice treated prenatally with sesame oil \( W = 131, Z = -2.04, p = .04, r = -.32 \) (see Figure 19).

**Figure 19:** Duration of oronasal investigation ± SEM. * indicates a significant different between prenatal treatment groups; & indicates a significant difference between intact and GDX+VEH mice.

Female mice spent significantly longer investigating the demonstrator’s body than male mice \( F(1, 238) = 29.35, p < .001, \eta^2 = .11 \), and ANOVA revealed main effects of sex \( F(1, 238) = 9.12, p = .003, \eta^2 = .04 \) and adult treatment condition \( F(2, 238) = 5.09, p = .007, \eta^2 = .04 \) on the frequency of investigations of the demonstrator’s body, as well as an interaction between sex and prenatal treatment \( F(1, 238) = 4.15, p = .04, \eta^2 = .02 \). Overall, intact mice made more frequent investigations of the body of the demonstrator animal than both gonadectomized mice \( p = .02 \) and mice that were gonadectomized and received hormone replacement with either testosterone (males) or estradiol (females) \( p = .02 \). Among female mice, there was a trend towards significance suggesting that mice treated prenatally with testosterone made marginally more frequent investigations of the demonstrator’s body than those treated prenatally with sesame oil \( F(1, 114) = 2.82, p = .10, \eta^2 = .02 \) (see Figure 20).
Figure 20: Duration and frequency of investigation of the demonstrator’s body ± SEM.

ANOVA revealed a significant interaction effect between sex and prenatal treatment on duration of anogenital investigation \(F(1, 238) = 4.57, \ p = .04, \ \eta^2 = .02\), and analysis of simple main effects revealed that prenatal treatment with testosterone increased frequency of anogenital investigation compared to mice treated prenatally with sesame oil among female mice \(F(1, 238) = 4.20, \ p = .04, \ \eta^2 = .04\) (see Figure 21).
Overall, dyads of female mice engaged in agonistic behaviour for longer than dyads of male mice \([F(1, 238) = 14.08, p < .001, \eta^2 = .06]\), while prenatal treatment with testosterone reduced the total duration of agonistic behaviour between demonstrators and observers compared to mice treated prenatally with sesame oil \([F(1, 238) = 14.12, p < .001, \eta^2 = .06]\). In addition ANOVA revealed a main effect of adult treatment condition \([F(2, 238) = 3.29, p = .04, \eta^2 = .27]\) on the duration of total agonistic behaviour that occurred between dyads during the interaction, and trends towards significance suggested that dyads containing a gonadectomized observer engaged in agonistic behaviour for longer than both intact mice \([p = .07]\), and gonadectomized mice that received hormone replacement \([p = .09]\) (see Table 3, Figure 22).

Furthermore, ANOVA revealed main effects of both sex \([F(1, 238) = 12.38, p = .001, \eta^2 = .05]\) and adult treatment condition \([F(2, 238) = 7.88, p < .001, \eta^2 = .06]\) on the frequency of agonistic behaviour that occurred between dyads, as well as a significant interaction between sex and adult treatment condition \([F(2, 238) = 6.57, p = .002, \eta^2 = .05]\). Analysis of simple main effects revealed an effect of adult treatment condition among male mice \([F(2, 124) = 12.16, p < .001, \eta^2 = .16]\), with a trend towards significance among female mice \([F(2, 114) = 2.46, p = .09, \eta^2 = .05]\).
$\eta^2 = .04$. Pairwise comparisons showed that among male mice, castrated reduced the frequency of agonistic behaviour relative to intact mice [$p < .001$], while testosterone replacement recovered it [$p = .001$]. The differences in these findings from those dealing with duration likely stem from the inclusion of attacks delivered and received, which are only scored in terms of frequency (see Table 4, Figure 22).

Table 3: Planned Comparisons: Agonistic Behaviour - Duration

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<th>Sex</th>
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<td>F-T-GDX+EB &gt; M-T-GDX+T</td>
<td>$W = 317, Z = 2.11, p = .03, r = .32$</td>
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Prenatal Treatment

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Adult Treatment

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<td>$W = 120, Z = -2.15, p = .03, r = -.34$</td>
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Table 4: Planned Comparisons: Agonistic Behaviour - Frequency

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<table>
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Legend:
Sex:
M: Male; F: Female
Prenatal Treatment:
T: Prenatal Testosterone; O: Prenatal Sesame Oil
Adult Treatment:
SHAM: Intact; GDX: GDX + Vehicle; GDX+T/EB: GDX + Hormone Replacement
Figure 22: Duration and frequency of total agonistic behaviour ± SEM. * indicates a significant difference between prenatal treatment groups; # indicates a significant sex difference; & indicates a significant difference between adult treatment groups, between either intact and GDX+VEH mice, or between GDX+VEH and GDX+EB/T replacement mice.

Mice treated prenatally with testosterone spent less time delivering agonistic behaviour than mice treated prenatally with sesame oil \([F(1, 238) = 4.53, p = .03, \eta^2 = .02]\). In addition, there was a significant interaction effect between sex and adult treatment condition on duration of agonistic behaviour delivered \([F(2, 238) = 7.88, p = .02, \eta^2 = .03]\), and simple main effects revealed that adult treatment condition affected duration of agonistic behaviour in female mice, such that replacement with estradiol benzoate significantly decreased the duration of agonistic behaviour delivered among ovariectomized mice \([p = .04]\) (see Table 5, Figure 23).
Additionally, ANOVA showed significant main effects of sex \(F(1, 238) = 11.23, p = .001, \eta^2 = .05\) and adult treatment condition \(F(2, 238) = 15.72, p < .001, \eta^2 = .12\) on the frequency of agonistic behaviours delivered, as well as interactions between gonad and sex \(F(2, 238) = 14.35, p < .001, \eta^2 = .11\), and gonad, sex, and prenatal treatment condition \(F(2, 238) = 3.23, p = .04, \eta^2 = .03\). Analysis of simple main effects revealed a significant effect of adult treatment condition among male animals \(F(2, 130) = 28.51, p < .001, \eta^2 = .32\), such that castration reduced the frequency of agonistic behaviours delivered compared to intact male mice \([p < .001]\), while replacement with testosterone increased frequency compared to castrated mice \([p < .001]\). There was also a trend towards significance for an interaction between adult treatment condition and prenatal treatment condition among both male mice \(F(2, 124) = 2.56, p = .08, \eta^2 = .04\) and female mice \(F(2, 114) = 2.86, p = .06, \eta^2 = .05\) (see Table 6, Figure 23).
Table 5: Planned Comparisons: Agonistic Behaviour Delivered - Duration

<table>
<thead>
<tr>
<th>Sex</th>
<th>Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prenatal Treatment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F-O-SHAM &gt; F-T-SHAM</td>
<td>( W = 290, Z = 2.12, p = .02, r = .33 )</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>M-O-SHAM &gt; M-T-SHAM</td>
<td>( W = 345, Z = 2.43, p = .02, r = .37 )</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>M-O-GDX &gt; M-T-GDX</td>
<td>( W = 323, Z = 2.64, p = .008, r = .41 )</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>F-T-GDX &gt; M-T-GDX</td>
<td>( W = 352, Z = 4.13, p &lt; .001, r = .65 )</td>
<td>***</td>
</tr>
</tbody>
</table>

| **Adult Treatment**      |                         |                 |      |
|                          | F-O-SHAM > F-O-SHAM     | \( W = 96, Z = -2.80, p = .005, r = .44 \) | **   |
|                          | M-O-GDX+T > F-O-GDX+EB | \( W = 113.5, Z = -2.13, p = .03, r = -.34 \) | *    |
|                          | M-O-GDX > M-T-GDX       | \( W = 194, Z = 1.94, p = .05, r = .30 \) | T    |
|                          | M-T-GDX+T > F-T-GDX+EB | \( W = 344, Z = 3.92, p < .001, r = .62 \) | ***  |
|                          | F-T-GDX > M-T-GDX       | \( W = 164.5, Z = -1.61, p = .07, r = -.27 \) | T    |

Table 6: Planned Comparisons: Agonistic Behaviour Delivered - Frequency

<table>
<thead>
<tr>
<th>Sex</th>
<th>Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prenatal Treatment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M-O-SHAM &gt; F-O-SHAM</td>
<td>( W = 129, Z = -2.48, p = .01, r = -.38 )</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>M-O-GDX+T &gt; F-O-GDX+EB</td>
<td>( W = 397.5, Z = 3.67, p &lt; .001, r = .55 )</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>M-O-GDX &gt; M-T-GDX</td>
<td>( W = 401, Z = 4.61, p &lt; .001, r = .71 )</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>M-O-GDX+T &gt; M-O-GDX</td>
<td>( W = 155, Z = -2.03, p = .04, r = -.30 )</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>M-T-GDX+T &gt; M-O-GDX</td>
<td>( W = 27, Z = -4.84, p &lt; .001, r = -.75 )</td>
<td>***</td>
</tr>
</tbody>
</table>

Legend:
Sex:
M: Male; F: Female
**Prenatal Treatment:**
T: Prenatal Testosterone; O: Prenatal Sesame Oil
**Adult Treatment:**
SHAM: Intact; GDX: GDX + Vehicle; GDX+T/EB: GDX + Hormone Replacement
Figure 23: Duration and frequency of agonistic behaviours delivered ± SEM. * indicates a significant different between prenatal treatment groups; # indicates a significant sex difference; & indicates a significant difference between adult treatment groups, between either intact and GDX+VEH mice, or between GDX+VEH and GDX+EB/T replacement mice.

ANOVA revealed significant main effects of sex \(F(1, 238) = 12.91, p < .001, \eta^2 = .05\] and adult treatment condition \(F(2, 238) = 33.78, p = .002, \eta^2 = .05\] on the duration of agonistic behaviour received, as well as a significant interaction effect between sex and adult treatment condition \(F(2, 238) = 5.34, p = .005, \eta^2 = .04\]. Simple main effects revealed an effect of adult treatment condition among male animals \(F(2, 124) = 8.10, p < .001, \eta^2 = .12\], such that,
castrated male mice received agonistic behaviour for longer than both intact male mice \( p = .002 \), and castrated mice that received testosterone replacement \( p = .002 \) (see Table 7, Figure 24).

**Table 7: Planned Comparisons: Agonistic Behaviour Received - Duration**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-O-SHAM &gt; M-O-SHAM</td>
<td>( W = 326 ), ( Z = 2.31 ), ( p = .02 ), ( r = .35 )</td>
<td>*</td>
</tr>
<tr>
<td>F-O-GDX+EB &gt; M-O-GDX+T</td>
<td>( W = 302 ), ( Z = 2.41 ), ( p = .02 ), ( r = .38 )</td>
<td>*</td>
</tr>
<tr>
<td>F-T-GDX+EB &gt; M-T-GDX+T</td>
<td>( W = 352 ), ( Z = 2.97 ), ( p = .003 ), ( r = .45 )</td>
<td>**</td>
</tr>
<tr>
<td>M-T-GDX &gt; F-T-GDX</td>
<td>( W = 134 ), ( Z = -1.77 ), ( p = .08 ), ( r = -.28 )</td>
<td>T</td>
</tr>
</tbody>
</table>

**Prenatal Treatment**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-O-GDX &gt; F-T-GDX</td>
<td>( W = 264 ), ( Z = 2.11 ), ( p = .03 ), ( r = .34 )</td>
<td>*</td>
</tr>
</tbody>
</table>

**Adult Treatment**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-O-GDX &gt; F-O-SHAM</td>
<td>( W = 123 ), ( Z = -2.04 ), ( p = .04 ), ( r = -.32 )</td>
<td>*</td>
</tr>
<tr>
<td>M-O-GDX &gt; M-O-SHAM</td>
<td>( W = 70 ), ( Z = -4.03 ), ( p &lt; .001 ), ( r = -.61 )</td>
<td>***</td>
</tr>
<tr>
<td>M-O-GDX &gt; M-O-GDX+T</td>
<td>( W = 359 ), ( Z = 2.76 ), ( p = .006 ), ( r = .42 )</td>
<td>**</td>
</tr>
<tr>
<td>M-T-GDX &gt; M-T-SHAM</td>
<td>( W = 101 ), ( Z = -2.97 ), ( p = .003 ), ( r = -.46 )</td>
<td>**</td>
</tr>
<tr>
<td>M-T-GDX &gt; M-T-GDX+T</td>
<td>( W = 349 ), ( Z = 3.30 ), ( p &lt; .001 ), ( r = .51 )</td>
<td>***</td>
</tr>
</tbody>
</table>

**Legend:**
- **Sex:** M: Male; F: Female
- **Prenatal Treatment:** T: Prenatal Testosterone; O: Prenatal Sesame Oil
- **Adult Treatment:** SHAM: Intact; GDX: GDX + Vehicle; GDX+T/EB: GDX + Hormone Replacement
In the duration-based dominance scores, ANOVA showed a trend towards significance for a main effect of adult treatment condition \[ F(2, 238) = 2.98, \ p = .05, \ \eta^2 = .02 \], as well as significant interactions between adult treatment and sex \[ F(2, 238) = 9.61, \ p < .001, \ \eta^2 = .08 \], and adult treatment condition and prenatal treatment \[ F(2, 238) = 2.86, \ p = .02, \ \eta^2 = .02 \].

Simple main effects revealed an effect of adult treatment condition in male mice \[ F(2, 124) = 12.58, \ p < .001, \ \eta^2 = .17 \], but only a trend towards significance in female mice \[ F(2, 114) = 2.92, \ p = .06, \ \eta^2 = .05 \]. Analysis of simple main effects showed that male mice treated prenatally with testosterone had lower duration-based dominance scores than those treated prenatally with sesame oil \[ p < .001 \]. Furthermore, pairwise comparisons revealed that castrated mice had significantly lower duration-based dominance scores than both intact male mice \[ p < .001 \] and castrated mice that received replacement with testosterone \[ p < .001 \] (see Table 8, Figure 25).
Table 8: Planned Comparisons: Dominance Score - Duration

<table>
<thead>
<tr>
<th>Sex</th>
<th>Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-O-GDX+T &gt; F-O-GDX+EB</td>
<td>$W = 91, Z = -3.10, p = .002, r = -.58$</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>F-O-GDX &gt; M-T-GDX</td>
<td>$W = 332, Z = 3.59, p &lt; .001, r = .57$</td>
<td>***</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Prenatal Treatment</th>
<th>Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-O-SHAM &gt; M-T-SHAM</td>
<td>$W = 340, Z = 2.31, p = .02, r = .35$</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>M-O-GDX &gt; M-T-GDX</td>
<td>$W = 308, Z = 2.26, p = .02, r = .35$</td>
<td>*</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Adult Treatment</th>
<th>Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-O-SHAM &gt; M-O-GDX</td>
<td>$W = 356, Z = 2.69, p = .007, r = .41$</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>M-O-GDX+T &gt; M-O-GDX</td>
<td>$W = 152, Z = -2.10, p = .04, r = -.32$</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>M-T-SHAM &gt; M-T-GDX</td>
<td>$W = 372, Z = 3.88, p &lt; .001, r = .60$</td>
<td>***</td>
<td></td>
</tr>
<tr>
<td>M-T-GDX+T &gt; M-T-GDX</td>
<td>$W = 71, Z = -3.72, p &lt; .001, r = -.58$</td>
<td>***</td>
<td></td>
</tr>
<tr>
<td>F-T-GDX &gt; F-T-GDX+EB</td>
<td>$W = 286, Z = 1.98, p = .05, r = .51$</td>
<td>*</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dominance over Demonstrator</th>
<th>Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-O-SHAM &gt; 0</td>
<td>$V = 236, p = .01$</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>F-T-GDX &gt; 0</td>
<td>$V = 104, p = .02$</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>M-O-SHAM &gt; 0</td>
<td>$V = 209, p &lt; .001$</td>
<td>***</td>
<td></td>
</tr>
<tr>
<td>M-O-GDX+T &gt; 0</td>
<td>$V = 211, p &lt; .001$</td>
<td>***</td>
<td></td>
</tr>
<tr>
<td>M-T-SHAM &gt; 0</td>
<td>$V = 226, p = .006$</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>M-T-GDX &lt; 0</td>
<td>$V = 33, p = .01$</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>M-T-GDX+T &gt; 0</td>
<td>$V = 236, p = .002$</td>
<td>**</td>
<td></td>
</tr>
</tbody>
</table>

Legend:

Sex:
M: Male; F: Female

Prenatal Treatment:
T: Prenatal Testosterone; O: Prenatal Sesame Oil

Adult Treatment:
SHAM: Intact; GDX: GDX + Vehicle; GDX+T/EB: GDX + Hormone Replacement
Figure 25: Duration based dominance score ± SEM. * indicates a significant different between prenatal treatment groups; # indicates a significant sex difference; & indicates a significant difference between adult treatment groups, between either intact and GDX+VEH mice, or between GDX+VEH and GDX+EB/T replacement mice; % indicates dominance score significantly > or < 0.

ANOVA revealed main effects of sex \( [F(1, 238) = 36.98, p < .001, \eta^2 = .13] \) and adult treatment condition \( [F(2, 238) = 3.69, p = .03, \eta^2 = .03] \) on duration of ritualized aggression, as well as interactions between sex and prenatal treatment \( [F(1, 238) = 4.45, p = .04, \eta^2 = .02] \), and sex and adult treatment condition \( [F(2, 238) = 6.21, p = .002, \eta^2 = .05] \). Planned comparisons revealed that male mice treated prenatally with testosterone spent more time engaged in ritualized aggression than male mice treated prenatally with sesame oil \( [F(1, 124) = 4.34, p = .04, \eta^2 = .03] \). Furthermore, adult treatment condition affected duration of ritualized aggression among male mice \( [F(1, 124) = 5.71, p = .004, \eta^2 = .08] \), such that castrated mice spent less time engaged in ritualized aggression than both intact male mice \( [p = .01] \), and castrated mice that received testosterone replacement \( [p = .01] \). In addition, male mice engaged in open aggression for significantly longer than female mice \( [F(1, 238) = 12.41, p = .03, \eta^2 = .02] \) (see Table 9, Table 10, Figure 26).
Table 9: Planned Comparisons: Ritualized Aggression - Duration

<table>
<thead>
<tr>
<th>Sex</th>
<th>Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M-O-SHAM &gt; F-O-SHAM</td>
<td>W = 129, Z = -2.66, p = .008, r = -.41</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>M-O-GDX+T &gt; F-O-GDX+EB</td>
<td>W = 122, Z = -2.47, p = .01, r = -.39</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>M-T-SHAM &gt; F-T-SHAM</td>
<td>W = 65, Z = -4.24, p &lt; .001, r = -.65</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>M-T-GDX+T &gt; F-T-GDX+EB</td>
<td>W = 72, Z = -4.09, p &lt; .001, r = -.62</td>
<td>***</td>
</tr>
</tbody>
</table>

**Adult Treatment**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M-O-SHAM &gt; M-O-GDX</td>
<td>W = 350, Z = 2.79, p = .005, r = .42</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>M-O-GDX+T &gt; M-O-GDX</td>
<td>W = 140, Z = -2.60, p = .009, r = -.39</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>M-T-SHAM &gt; M-T-GDX</td>
<td>W = 305, Z = 2.28, p = .02, r = .35</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>M-T-GDX+T &gt; M-T-GDX</td>
<td>W = 122, Z = -2.52, p = .01, r = -.39</td>
<td>*</td>
</tr>
</tbody>
</table>

Table 10: Planned Comparisons: Open Aggression - Duration

<table>
<thead>
<tr>
<th>Sex</th>
<th>Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M-O-SHAM &gt; F-O-SHAM</td>
<td>W = 187, Z = -2.12, p = .03, r = -.32</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>M-O-GDX+T &gt; F-O-GDX+EB</td>
<td>W = 140, Z = -2.78, p = .005, r = -.43</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>M-T-SHAM &gt; F-T-SHAM</td>
<td>W = 114, Z = -3.14, p &lt; .001, r = -.53</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>M-T-GDX+T &gt; F-T-GDX+EB</td>
<td>W = 138.5, Z = -2.91, p = .004, r = -.44</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>M-T-GDX &gt; F-T-GDX</td>
<td>W = 147, Z = -2.47, p = .01, r = -.39</td>
<td>**</td>
</tr>
</tbody>
</table>

**Prenatal Treatment**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M-T-GDX &gt; M-O-GDX</td>
<td>W = 168, Z = -2.01, p = .04, r = -.32</td>
<td>*</td>
</tr>
</tbody>
</table>

**Adult Treatment**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M-O-GDX+T &gt; M-O-GDX</td>
<td>W = 168 Z = -2.57, p = .01, r = -.39</td>
<td>*</td>
</tr>
</tbody>
</table>

Legend:

Sex:
M: Male; F: Female

**Prenatal Treatment**:
T: Prenatal Testosterone; O: Prenatal Sesame Oil

**Adult Treatment**:
SHAM: Intact; GDX: GDX + Vehicle; GDX+T/EB: GDX + Hormone Replacement
Figure 26: Duration of open aggression and ritualized aggression ± SEM. * indicates a significant different between prenatal treatment groups; # indicates a significant sex difference; & indicates a significant difference between adult treatment groups, between either intact and GDX+VEH mice, or between GDX+VEH and GDX+EB/T replacement mice.

ANOVA revealed a main effect of prenatal treatment on duration of dominance behaviour [$F(1, 238) = 7.98, p = .005, \eta^2 = .03$], as well as interactions between prenatal treatment and sex [$F(1, 238) = 4.60, p = .03, \eta^2 = .02$], and adult treatment condition and sex [$F(2, 238) = 6.28, p = .002, \eta^2 = .05$]. There was an effect of adult treatment condition in female mice [$F(2,114) = 3.15, p = .04, \eta^2 = .05$], such that ovariectomized mice engaged in dominance
behaviour for longer than ovariectomized mice that received estradiol replacement \( p = .04 \).

Moreover, in male mice, mice treated prenatally with testosterone spent less time engaged in
dominance behaviour than mice treated prenatally with sesame oil \( F(1, 124) = 15.78, p < .001, \eta^2 = .11 \) (see Table 11, Figure 27).

Furthermore, ANOVA indicated significant main effects of sex \( F(1, 238) = 10.54, p = .001, \eta^2 = .04 \) and adult treatment condition \( F(2, 238) = 5.49, p = .005, \eta^2 = .04 \) on duration of
submissive behaviour, as well as an interaction between sex and adult treatment condition \( F(2, 238) = 5.91, p = .003, \eta^2 = .05 \). There was a significant effect of adult treatment condition
among male mice \( F(2,124) = 14.66, p < .001, \eta^2 = .19 \), such that duration of submissive
behaviour was higher in castrated mice than in intact male mice \( p < .001 \) and castrated mice
that received testosterone replacement \( p < .001 \) (see Table 12, Figure 27).
Table 11: Planned Comparisons: Dominance Behaviour - Duration

<table>
<thead>
<tr>
<th>Sex</th>
<th>Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-T-GDX &gt; M-T-GDX</td>
<td>$W = 352$, $Z = 4.13$, $p &lt; .001$, $r = .65$</td>
<td>***</td>
<td></td>
</tr>
</tbody>
</table>

**Prenatal Treatment**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-O-SHAM &gt; M-T-SHAM</td>
<td>$W = 337$, $Z = 2.24$, $p = .02$, $r = .34$</td>
<td>*</td>
</tr>
<tr>
<td>M-O-GDX &gt; M-T-GDX</td>
<td>$W = 327$, $Z = 2.74$, $p = .006$, $r = .42$</td>
<td>**</td>
</tr>
<tr>
<td>F-O-SHAM &gt; F-T-SHAM</td>
<td>$W = 289$, $Z = 2.09$, $p = .04$, $r = .33$</td>
<td>*</td>
</tr>
</tbody>
</table>

**Adult Treatment**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-T-GDX &gt; F-T-SHAM</td>
<td>$W = 90$, $Z = -.97$, $p = .002$, $r = -.47$</td>
<td>**</td>
</tr>
<tr>
<td>F-T-GDX &gt; F-T-GDX+T</td>
<td>$W = 296$, $Z = 2.24$, $p = .02$, $r = .35$</td>
<td>*</td>
</tr>
<tr>
<td>M-T-SHAM &gt; M-T-GDX</td>
<td>$W = 316$, $Z = 2.46$, $p = .01$, $r = .38$</td>
<td>*</td>
</tr>
<tr>
<td>M-T-GDX+T &gt; M-T-GDX</td>
<td>$W = 168$, $Z = -1.68$, $p = .09$, $r = -.26$</td>
<td>T</td>
</tr>
</tbody>
</table>

Table 12: Planned Comparisons: Submissive Behaviour - Duration

<table>
<thead>
<tr>
<th>Sex</th>
<th>Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-O-SHAM &gt; M-O-SHAM</td>
<td>$W = 330$, $Z = 2.41$, $p = .02$, $r = .37$</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>F-O-GDX+T &gt; M-O-GDX+EB</td>
<td>$W = 311$, $Z = 2.65$, $p = .008$, $r = .41$</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>F-T-SHAM &gt; M-T-SHAM</td>
<td>$W = 289$, $Z = 1.78$, $p = .07$, $r = .27$</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>F-T-GDX+T &gt; M-T-GDX+EB</td>
<td>$W = 354$, $Z = 3.02$, $p = .003$, $r = .46$</td>
<td>**</td>
<td></td>
</tr>
</tbody>
</table>

**Adult Treatment**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-O-GDX &gt; M-O-SHAM</td>
<td>$W = 69$, $Z = -4.05$, $p &lt; .001$, $r = -.61$</td>
<td>***</td>
</tr>
<tr>
<td>M-O-GDX &gt; M-O-GDX+T</td>
<td>$W = 371$, $Z = 3.04$, $p = .002$, $r = -.46$</td>
<td>**</td>
</tr>
<tr>
<td>M-T-GDX &gt; M-T-SHAM</td>
<td>$W = 100.5$, $Z = -2.98$, $p = .003$, $r = -.46$</td>
<td>**</td>
</tr>
<tr>
<td>M-T-GDX &gt; M-T-GDX+T</td>
<td>$W = 341$, $Z = 3.10$, $p = .002$, $r = .48$</td>
<td>**</td>
</tr>
</tbody>
</table>

**Legend:**

**Sex:**
M: Male; F: Female

**Prenatal Treatment:**
T: Prenatal Testosterone; O: Prenatal Sesame Oil

**Adult Treatment:**
SHAM: Intact; GDX: GDX + Vehicle; GDX+T/EB: GDX + Hormone Replacement
Figure 27: Duration of dominant and submissive behaviour ± SEM. * indicates a significant different between prenatal treatment groups; # indicates a significant sex difference; & indicates a significant difference between adult treatment groups, between either intact and GDX+VEH mice, or between GDX+VEH and GDX+EB/T replacement mice.

ANOVA revealed significant main effects of both sex \( F(1, 238) = 113.83, p < .001, \eta^2 = .32 \) and adult treatment condition \( F(2, 238) = 23.32, p < .001, \eta^2 = .16 \) on frequency of attacks delivered, as well as a significant interaction between sex and adult treatment condition \( F(1, 238) = 17.50, p < .001, \eta^2 = 13 \). There was a significant effect of adult treatment condition among male mice \( F(2, 124) = 24.37, p < .001, \eta^2 = .28 \), such that gonadectomized mice made
significantly fewer attacks than intact mice \( p < .001 \) and gonadectomized mice that received testosterone replacement \( p < .001 \) (see Table 13, Figure 28).

*Table 13: Planned Comparisons: Attacks Delivered*

<table>
<thead>
<tr>
<th>Sex</th>
<th>Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>O-Sham &gt; F-Sham</td>
<td>( W = 84, Z = -4.05, p &lt; .001, r = -.63 ) ***</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>O-GDX+T &gt; F-O-GDX+EB</td>
<td>( W = 71, Z = -4.07, p &lt; .001, r = -.63 ) ***</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>O-GDX &gt; F-O-GDX</td>
<td>( W = 171, Z = -1.84, p = .06, r = -.29 ) T</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>T-Sham &gt; F-T-Sham</td>
<td>( W = 45.5, Z = -4.63, p &lt; .001, r = -.71 ) ***</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>T-GDX+T &gt; F-T-GDX+EB</td>
<td>( W = 66, Z = -4.45, p &lt; .001, r = -.68 ) ***</td>
<td></td>
</tr>
</tbody>
</table>

**Adult Treatment**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>O-Sham &gt; O-GDX</td>
<td>( W = 391, Z = 3.89, p &lt; .001, r = .59 ) ***</td>
<td></td>
</tr>
<tr>
<td>O-GDX+T &gt; O-GDX</td>
<td>( W = 107.5, Z = -3.49, p &lt; .001, r = -.53 ) ***</td>
<td></td>
</tr>
<tr>
<td>T-Sham &gt; T-GDX</td>
<td>( W = 379.5, Z = 4.24, p &lt; .001, r = .65 ) ***</td>
<td></td>
</tr>
<tr>
<td>T-GDX+T &gt; T-GDX</td>
<td>( W = 84.5, Z = -4.05, p &lt; .001, r = -.55 ) ***</td>
<td></td>
</tr>
</tbody>
</table>

**Legend:**

Sex: M: Male; F: Female

Prenatal Treatment:

T: Prenatal Testosterone; O: Prenatal Sesame Oil

Adult Treatment:

SHAM: Intact; GDX: GDX + Vehicle; GDX+T/EB: GDX + Hormone Replacement

**Figure 28:** Attacks delivered ± SEM. # indicates a significant sex difference; & indicates a significant difference between adult treatment groups, between either intact and GDX+VEH mice, or between GDX+VEH and GDX+EB/T replacement mice.
ANOVA also revealed a significant interaction between prenatal treatment and estrus phase on total duration of agonistic behaviour $[F(2, 23) = 6.35, p = .006, \eta^2 = .36]$. Analysis of simple main effects indicated a significant effect of estrus phase on duration of agonistic behaviour among intact female mice treated prenatally with sesame oil $[F(2, 12) = 8.01, p = .006, \eta^2 = .57]$, and pairwise comparisons showed that, among intact female mice treated prenatally with sesame oil, mice in diestrus engaged in agonistic behaviour with the intruder for less time than those in proestrus $[p = .008]$, and marginally less than those in estrus $[p = .07]$. Furthermore, ANOVA revealed a main effect of estrus phase on duration of submissive behaviour $[F(2, 23) = 5.76, p = .009, \eta^2 = .33]$, as well as a significant interaction between prenatal treatment and estrus phase on duration of submissive behaviour $[F(2, 23) = 4.26, p = .03, \eta^2 = .27]$. Analysis of simple main effects indicated an effect of estrus phase on duration of submissive behaviour only among mice treated prenatally with sesame oil $[F(2, 12) = 7.54, p = .008, \eta^2 = .56]$. Pairwise comparisons revealed that among mice treated prenatally with sesame oil, mice in estrus spent more time engaged in submissive behaviour than mice in diestrus $[p = .007]$ and proestrus $[p = .03]$.

**Resident-Intruder Test**

**Summary**

In adolescence, female mice spent more time engaged in active behaviour, social behaviour and social investigation than male mice, while male mice engaged in both active and inactive non-social behaviours for longer than female mice. We did not find a significant sex difference in time spent engaged in agonistic behaviour. However, prenatal treatment with testosterone resulted in an increase in dominance behaviour only in male mice. Furthermore,
male mice engaged in reciprocal attacks, ritualized aggression and open aggression more than female mice.

In adulthood, prenatal treatment with testosterone reduced time spent engaged in submissive behaviour, though this effect was specific to female mice when considering the frequency of submissive behaviour. Furthermore, female mice spent more time engaged in active behaviour than male mice, while male mice shifted between behaviours more often, than female mice. Male mice spent more time engaged in non-social behaviour than female mice, while castration reduced locomotor behaviour and increased social inactivity in male mice. Female mice spent more time engaged in social behaviour than male mice, and spent more time investigating the intruder than male mice. In addition, dyads of female mice spent more time engaged in agonistic behaviour than male mice, though male mice had higher dominance scores than female mice. Male mice delivered agonistic behaviour more frequently and for longer than female mice, while female mice received agonistic behaviour more frequently and for longer than male mice. Specifically, male mice delivered more attacks than female mice, and engaged in open and ritualized aggression for longer than female mice, while female mice engaged in submissive behaviour more frequently and for longer than male mice. In addition, castration reduced agonistic behaviour in male mice, though this effect was reversed in mice that received testosterone replacement among mice treated prenatally with sesame oil.

**Adolescence**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Prenatal Treatment</th>
<th>Sample Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Sesame Oil</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>Testosterone</td>
<td>47</td>
</tr>
<tr>
<td>Female</td>
<td>Sesame Oil</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>Testosterone</td>
<td>39</td>
</tr>
</tbody>
</table>
Total Activity

Overall, female mice engaged in active behaviours for longer than male mice \([F(1, 167) = 5.52, p = .02, \eta^2 = .03]\), while male mice engaged in non-social behaviours for longer than female mice \([F(1, 167) = 28.75, p < .001, \eta^2 = .15]\). Moreover, male mice engaged in both active and inactive non-social behaviour for significantly longer than female mice [Active Non-Social: \(F(1, 167) = 31.06, p < .001, \eta^2 = .16\); Inactive Non-Social: \(F(1, 167) = 6.35, p = .01, \eta^2 = .04\)]. Furthermore, ANOVA revealed that male mice spent significantly longer engaged in horizontal activity \([F(1, 167) = 46.12, p < .001, \eta^2 = .22]\) and vertical activity \([F(1, 167) = 7.04, p = .009, \eta^2 = .04]\) than female mice. In addition, male mice engaged in digging behaviour for longer than female mice \([F(1, 167) = 5.02, p = .02, \eta^2 = .03]\), and engaged in grooming behaviour more frequently than female mice \([F(1, 167) = 7.94, p = .005, \eta^2 = .05]\). Male mice also spent more time engaged in solitary inactivity than female mice \([F(1, 167) = 18.75, p < .001, \eta^2 = .10]\), and a trend towards significance indicated that mice treated prenatally with testosterone spent marginally longer engaged in solitary inactivity than mice treated prenatally with sesame oil \([F(1, 167) = 3.39, p = .07, \eta^2 = .02]\) (see Figure 29).
Figure 29: A: Duration of total activity; B: Duration of non-social behaviour; C: Duration of non-social locomotive behaviour; D: Duration of inactive non-social behaviour; E: Duration of horizontal exploration; F: Duration of vertical exploration; G: Duration of digging behaviour; H: Frequency of grooming behaviour; I: Duration of solitary inactivity. All measures ± SEM.

Social Interaction

ANOVA revealed that female mice spent significantly more time than male mice engaged in social behaviour [$F(1, 167) = 31.46, p < .001, \eta^2 = .16$] (see Figure 30).
Social Investigation

Overall, female mice spent more time investigating the intruder than male mice \([F(1, 167) = 84.92, p < .001, \eta^2 = .34]\). Moreover, female mice spent more time than male mice investigating the body of the intruder mouse \([F(1, 167) = 25.91, p < .001, \eta^2 = .14]\), and investigated the anogenital area of the intruder mouse for longer than male mice \([F(1, 167) = 98.13, p < .001, \eta^2 = .37]\) (see Figure 30).

![Figure 30: A: Duration of social behaviour; B: Duration of social investigation; C: Duration spent investigating the intruder’s body; D: Duration of anogenital investigation. All measures ± SEM.](image)

Agonistic Behaviour

Dyads of male mice engaged in agonistic behaviour for significantly longer than female mice \([F(1, 167) = 5.02, p = .02, \eta^2 = .03]\). Moreover, male mice delivered agonistic behaviours for significantly longer than female mice \([F(1, 167) = 4.02, p = .05, \eta^2 = .02]\), while a trend
towards significance suggested that mice treated prenatally with sesame oil received agonistic behaviour more frequently than mice treated prenatally with testosterone \(F(1, 167) = 3.71, p = .06, \eta^2 = .02\]. Planned comparisons revealed that male mice treated prenatally with testosterone engaged in agonistic behaviour for significantly longer than female mice treated prenatally with testosterone \(W = 619, Z = -2.59, p = .009, r = -.52\] (see Figure 31).

Male mice engaged in ritualized aggression for significantly longer than female mice \(F(1, 167) = 20.18, p < .001, \eta^2 = .11\], and spent more time engaged in open aggression than female mice \(F(1, 167) = 9.79, p = .002, \eta^2 = .06\]. Furthermore, male mice delivered more attacks than female mice \(F(1, 167) = 13.96, p < .001, \eta^2 = .08\]. In addition, planned comparisons indicated that male mice spent more time engaged in ritualized aggression than female mice among mice treated prenatally with both sesame oil and testosterone \[Prenatal Sesame Oil: W = 614, Z = -3.01, p = .003, r = -.60; Prenatal Testosterone: W = 591, Z = -3.17, p = .002, r = -.63\]. Similarly, among mice treated with sesame oil and testosterone, male mice spent more time engaged in open aggression than female mice \[Prenatal Sesame Oil: W = 688, Z = -3.37, p < .001, r = -.68; Prenatal Testosterone: W = 682, Z = -3.37, p < .001, r = -.67\]. Moreover, among mice from both prenatal treatment groups, male mice delivered significantly more attacks than female mice \[Prenatal Sesame Oil: W = 640.5, Z = -3.97, p < .001, r = -.80\] (see Figure 31).

ANOVA revealed a main effect of sex on duration of dominance behaviour \(F(1, 167) = 9.01, p = .003, \eta^2 = .05\], as well as a significant interaction between sex and prenatal treatment \(F(1, 167) = 4.73, p = .03, \eta^2 = .03\]. Analysis of simple main effects revealed that among male mice, prenatal treatment with testosterone increased dominance behaviour compared to mice treated prenatally with sesame oil \(F(1, 87) = 5.37, p = .02, \eta^2 = .06\]. In addition, planned
comparisons revealed that male mice treated prenatally with testosterone engaged in dominant
behaviour than female mice treated prenatally with testosterone \([W = 599, Z = -2.75, p = .006, r
= -.55]\), and a trend towards significance suggested that male mice treated prenatally with sesame
oil spent more time engaged in dominance behaviour than female mice treated prenatally with
sesame oil \([W = 701, Z = -1.78, p = .07, r = -.36]\) (see Figure 31).

Furthermore, ANOVA showed a significant main effect of sex on time spent following
the intruder \([F(1, 167) = 11.30, p = .001, \eta^2 = .06]\), as well as a significant interaction between
sex and prenatal treatment on time spent following the intruder \([F(1, 167) = 12.72, p < .001, \eta^2 = .07]\). Among female mice, mice treated prenatally with testosterone spent significantly longer
following the intruder than mice treated prenatally with sesame oil \([F(1, 167) = 4.75, p = .03, \eta^2
= .06]\). Conversely, male mice treated prenatally with testosterone spent less time following the
intruder mouse than male mice treated prenatally with sesame oil \([F(1, 167) = 7.33, p = .008, \eta^2
= .08]\). Planned comparisons also revealed that female mice treated prenatally with testosterone
spent more time following the intruder than male mice treated prenatally with testosterone \([W = 1329, Z = 4.01, p < .001, r = -.63]\) (see Figure 31).
Figure 31: A: Total duration of agonistic behaviour; B: Duration of agonistic behaviour delivered; C: Duration of ritualized aggression; D: Duration of open aggression; E: Duration of dominance behaviour; F: Duration of following behaviour; G: Frequency of attacks delivered. All measures ± SEM.
Adulthood

<table>
<thead>
<tr>
<th>Sex</th>
<th>Prenatal Treatment</th>
<th>Adult Treatment</th>
<th>Sample Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sesame Oil</td>
<td>Intact</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GDX</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GDX+T</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Testosterone</td>
<td>Intact</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GDX</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GDX+T</td>
<td>15</td>
<td></td>
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<tr>
<td>Female</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Sesame Oil</td>
<td>Intact</td>
<td>13</td>
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<tr>
<td></td>
<td>GDX</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GDX+EB</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Testosterone</td>
<td>Intact</td>
<td>16</td>
<td></td>
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<tr>
<td></td>
<td>GDX</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GDX+EB</td>
<td>15</td>
<td></td>
</tr>
</tbody>
</table>

Total Activity

Female mice engaged in active behaviour for longer than male mice \(F(1, 152) = 12.07, p = .001, \eta^2 = .07\) and ANOVA revealed a main effect of adult treatment condition \(F(2, 152) = 4.27, p = .02, \eta^2 = .05\] on duration of total active behaviour, as well as interactions between prenatal treatment and adult treatment condition \(F(2, 152) = 4.23, p = .02, \eta^2 = .05\], and sex and adult treatment condition \(F(2, 152) = 3.14, p = .05, \eta^2 = .04\]. Simple main effects revealed a significant effect of adult treatment condition on duration of total active behaviour among male mice \(F(2, 72) = 4.64, p = .01, \eta^2 = .11\], such that castrated mice spent less time engaged in active behaviour than castrated mice that received testosterone replacement \(p = .01\]. In addition, there was a significant effect of adult treatment condition among mice treated prenatally with sesame oil \(F(2, 72) = 6.57, p = .02, \eta^2 = .15\], such that gonadectomized mice treated prenatally with sesame oil spent less time engaged in active behaviours than intact mice treated prenatally with sesame oil \(p = .02\] and gonadectomized mice treated prenatally with sesame oil that received replacement with testosterone (males) or estradiol (females) \(p = .004\]. Moreover, male
mice engaged in active behaviours more frequently than female mice \([F(1, 152) = 6.75, p = .01, \eta^2 = .04]\), and ANOVA revealed a trend towards a significant main effect of adult treatment condition on frequency of active behaviour \([F(1, 152) = 2.70, p = .07, \eta^2 = .03]\) (see Figure 32).

Figure 32: Duration and frequency of active behaviour ± SEM.

There was a main effect of adult treatment condition on duration of non-social behaviour \([F(2, 152) = 3.80, p = .02, \eta^2 = .05]\), such that intact mice spent more time engaged in non-social behaviour than gonadectomized mice that received replacement with estradiol (females) or testosterone (males) \([p = .02]\). In addition, male mice spent longer engaged in inactive behaviour than female mice \([F(1, 152) = 96.42, p < .001, \eta^2 = .39]\), and ANOVA indicated a trend towards
significance for an interaction between prenatal treatment and adult treatment condition on duration of non-social behaviour \([F(2, 152) = 2.47, p = .09, \eta^2 = .03]\) (see Figure 33).

![Figure 33: Duration of non-social behaviour ± SEM.](image)

Male mice engaged in non-social locomotor behaviour for longer than female mice \([F(1, 152) = 95.40, p < .001, \eta^2 = .39]\), and ANOVA indicated a main effect of adult treatment condition on duration of non-social locomotor behaviour \([F(2, 152) = 4.63, p = .01, \eta^2 = .06]\), as well as an interaction effect between sex and adult treatment condition \([F(2, 152) = 3.89, p = .02, \eta^2 = .05]\). Analysis of simple main effects revealed an effect of adult treatment condition among male mice \([F(2, 72) = 5.47, p = .006, \eta^2 = .13]\), such that intact male mice engaged in non-social locomotor behaviour for longer than castrated mice \([p = .008]\) and castrated mice that received testosterone replacement \([p = .05]\). There was also a significant effect of adult treatment condition among female mice \([F(2, 80) = 3.75, p = .03, \eta^2 = .09]\), such that ovariectomized mice that received replacement with estradiol engaged in active non-social behaviour for less time than ovariectomized mice that did not receive replacement \([p = .03]\) (see Figure 34).
Overall, male mice spent more time engaged in horizontal activity than female mice \(F(1, 152) = 122.05, p < .001, \eta^2 = .45\). In addition, ANOVA revealed a significant interaction effect between sex and adult treatment condition on duration of horizontal activity \(F(1, 152) = 3.20, p = .04, \eta^2 = .04\), and analysis of simple main effects revealed a trend towards a significant effect of adult treatment condition among female mice \(F(1, 152) = 2.95, p = .06, \eta^2 = .07\). Similarly, male mice engaged in vertical activity for longer than female mice \(F(1, 152) = 46.03, p < .001, \eta^2 = .23\), and ANOVA revealed an interaction effect between sex and adult treatment condition on time spent engaged in vertical activity. Analysis of simple main effects revealed an effect of adult treatment condition among male mice \(F(1, 72) = 6.65, p = .002, \eta^2 = .16\), such that intact mice spent longer engaged in vertical activity than castrated mice \(p = .002\). In addition, a trend towards significance suggested that intact male mice engaged in vertical activity for longer than castrated mice that received testosterone replacement \(p = .05\). There was also a trend towards significance for an effect of adult treatment condition on duration of vertical activity among female mice \(F(1, 80) = 2.60, p = .08, \eta^2 = .06\) (see Figure 39). Additionally, male mice spent more time self-grooming than female mice \(F(1, 152) = 16.67, p < .001, \eta^2 = .10\) (see Figure 35, 36).
There was a main effect of adult treatment condition on time spent engaged in social inactivity \( F(2, 152) = 4.42, p = .01, \eta^2 = .06 \), as well as a significant interaction effect between
sex and adult treatment condition on duration of social inactivity \([F(2, 152) = 5.65, p = .02, \eta^2 = .04]\). Analysis of simple main effects indicated a significant effect of adult treatment condition on social inactivity among male mice \([F(2, 72) = 3.70, p = .03, \eta^2 = .09]\), and planned comparisons revealed that castrated mice spent more time engaged in social inactivity with the intruder than intact male mice \([p = .03]\). There was also a trend towards an effect of adult treatment condition on duration of social inactivity in female mice, though it did not reach significance \([F(1, 80) = 3.09, p = .05, \eta^2 = .07]\) (see Figure 37).

![Figure 37: Duration of social inactivity ± SEM.](image)

**Social Interaction**

Female mice engaged in social behaviour for longer than male mice \([F(1, 152) = 95.71, p < .001, \eta^2 = .39]\), and ANOVA indicated a main effect of adult treatment condition on duration of social behaviour \([F(2, 152) = 4.69, p = .01, \eta^2 = .06]\). Pairwise comparisons indicated that mice that received replacement with testosterone (males) or estradiol (females) spent more time engaged in social behaviour than intact mice \([p = .008]\). There were also trends towards significance for interaction effects between sex and adult treatment condition \([F(2, 152) = 2.80, p = .06, \eta^2 = .04]\) and prenatal treatment and adult treatment condition \([F(2, 152) = 2.44, p = .09, \eta^2 = .03]\) on time spent engaged in social behaviour (see Figure 38).
**Social Investigation**

Female mice spent more time investigating the intruder than male mice $[F(1, 152) = 83.40, p < .001, \eta^2 = .36]$. In addition, there was a main effect of adult treatment condition on duration of social investigation $[F(2, 152) = 4.66, p = .01, \eta^2 = .06]$, and pairwise comparisons showed that gonadectomized mice that received hormone replacement spent more time investigating the intruder mouse than both intact mice $[p = .02]$ and gonadectomized mice $[p = .03]$. ANOVA also indicated a trend towards significance for an interaction effect between sex and adult treatment condition $[F(2, 152) = 2.60, p = .07, \eta^2 = .03]$ (see Figure 39).
ANOVA revealed a significant interaction effect between sex and adult treatment condition on time spent investigating the intruder’s oronasal area \([F(1, 152) = 3.98, p = .02, \eta^2 = .05]\). Analysis of simple main effects revealed that, among male mice, there was a significant effect of adult treatment condition on duration of oronasal investigation \([F(1, 72) = 9.14, p < .001, \eta^2 = .20]\), and pairwise comparisons revealed that gonadectomized male mice that received testosterone replacement spent more time investigating the oronasal region of the intruder than intact male mice \([p = .006]\) and gonadectomized mice \([p < .001]\). In addition, there were trends towards significance for a main effect of adult treatment condition \([F(2, 152) = 2.88, p = .06, \eta^2 = .04]\) and an interaction effect between adult treatment condition and prenatal treatment on duration of oronasal investigation \([F(2, 152) = 2.74, p = .07, \eta^2 = .04]\) (see Figure 40).

**Figure 40**: Duration of oronasal investigation ± SEM.

Female mice spent made more frequent investigations of the intruder’s body \([F(1, 152) = 16.34, p < .001, \eta^2 = .10]\), and spent more time investigating the intruder’s body than male mice \([F(1, 152) = 52.48, p < .001, \eta^2 = .26]\). In addition, there was a main effect of adult treatment condition of frequency of investigations of the intruder’s body \([F(2, 152) = 3.62, p = .03, \eta^2 = .05]\), and pairwise comparisons showed that gonadectomized mice that received testosterone (males) or estradiol (females) replacement made more frequent investigations of the body of the
intruder mouse than gonadectomized mice that did not receive replacement \([p = .03]\). There were also trends towards significance for interaction effects of sex and prenatal treatment \([F(1, 152) = 3.07, p = .08, \eta^2 = .02]\) and adult treatment condition and prenatal treatment \([F(2, 152) = 2.69, p = .07, \eta^2 = .03]\) on frequency of investigations of the intruder’s body (see Figure 41).

![Investigation of Intruder's Body – Duration](image)

![Investigation of Intruder's Body – Frequency](image)

**Figure 41**: Duration and frequency of investigation of the intruder’s body ± SEM.

Overall, female mice spent more time investigating the intruder’s anogenital region than male mice \([F(1, 152) = 51.90, p < .001, \eta^2 = .26]\). In addition, there was a significant interaction between sex and adult treatment condition \([F(2, 152) = 2.68, p = .03, \eta^2 = .05]\), and analysis of simple main effects revealed an effect of adult treatment condition among male mice \([F(2, 72) = 3.14, p = .05, \eta^2 = .08]\). Pairwise comparisons showed a trend towards significance suggesting
that intact male mice spent less time investigating the anogenital region of the intruder mouse than castrated mice \[p = .06\] (see Figure 42).

![Figure 42: Duration of anogenital investigation \( \pm \) SEM.](image)

**Agonistic Behaviour**

Overall, dyads of female mice engaged in agonistic behaviour for longer than male mice \[F(1, 152) = 5.53, p = .02, \eta^2 = .04\], and mice treated prenatally with sesame oil engaged in agonistic behaviour for longer than those treated prenatally with testosterone \[F(2, 152) = 6.76, p = .01, \eta^2 = .04\]. Furthermore, there was a significant interaction effect between sex and prenatal treatment on frequency of agnostic behaviour \[F(1, 152) = 13.32, p < .001, \eta^2 = .08\]. Among female mice, prenatal treatment with testosterone reduced frequency of agonistic behaviour between the resident and intruder compared to mice treated prenatally with sesame oil \[F(1, 80) = 2.74, p < .001, \eta^2 = .15\]. Conversely, a trend towards significance among male mice that suggested that frequency of agonistic behaviour was higher among male mice treated prenatally with testosterone than among those treated with sesame oil \[F(1, 72) = 2.74, p = .08, \eta^2 = .07\]. There was also a trend towards significance for a main effect of adult treatment condition on frequency of agonistic behaviour \[F(2, 152) = 2.78, p = .07, \eta^2 = .04\] (see Table 14, Table 15, Figure 43).
Table 14: Planned Comparisons: Agonistic Behaviour - Duration

<table>
<thead>
<tr>
<th>Sex Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-O-SHAM &gt; M-O-SHAM</td>
<td>$W = 114$, $Z = 1.96$, $p = .05$, $r = .39$</td>
<td>T</td>
</tr>
<tr>
<td>F-O-GDX &gt; M-O-GDX</td>
<td>$W = 124$, $Z = 2.03$, $p = .04$, $r = .40$</td>
<td>*</td>
</tr>
</tbody>
</table>

**Prenatal Treatment**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-O-GDX+T &gt; M-T-GDX+T</td>
<td>$W = 134$, $Z = 2.67$, $p = .008$, $r = .52$</td>
<td>**</td>
</tr>
<tr>
<td>F-O-SHAM &gt; F-T-SHAM</td>
<td>$W = 154$, $Z = 2.19$, $p = .02$, $r = .41$</td>
<td>*</td>
</tr>
<tr>
<td>F-O-GDX &gt; F-T-GDX</td>
<td>$W = 118$, $Z = 1.72$, $p = .09$, $r = .34$</td>
<td>T</td>
</tr>
</tbody>
</table>

Table 15: Planned Comparisons: Agonistic Behaviour - Frequency

<table>
<thead>
<tr>
<th>Sex Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-O-SHAM &gt; M-O-SHAM</td>
<td>$W = 120$, $Z = 2.28$, $p = .02$, $r = .46$</td>
<td>*</td>
</tr>
<tr>
<td>F-O-GDX &gt; M-O-GDX</td>
<td>$W = 123$, $Z = 1.98$, $p = .05$, $r = .39$</td>
<td>*</td>
</tr>
<tr>
<td>M-T-SHAM &gt; F-T-SHAM</td>
<td>$W = 65.5$, $Z = -2.16$, $p = .03$, $r = .39$</td>
<td>*</td>
</tr>
</tbody>
</table>

**Prenatal Treatment**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-T-SHAM &gt; M-O-SHAM</td>
<td>$W = 47.5$, $Z = -2.08$, $p = .04$, $r = -.40$</td>
<td>*</td>
</tr>
<tr>
<td>F-O-SHAM &gt; F-T-SHAM</td>
<td>$W = 157.5$, $Z = 2.35$, $p = .02$, $r = .41$</td>
<td>*</td>
</tr>
<tr>
<td>F-O-GDX &gt; F-T-GDX</td>
<td>$W = 123.5$, $Z = 2.00$, $p = .05$, $r = .39$</td>
<td>*</td>
</tr>
<tr>
<td>F-O-GDX+EB &gt; F-T-GDX+EB</td>
<td>$W = 178.5$, $Z = 2.31$, $p = .02$, $r = .42$</td>
<td>*</td>
</tr>
</tbody>
</table>

**Adult Treatment**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-O-GDX+T &gt; M-O-GDX</td>
<td>$W = 25$, $Z = -.70$, $p = .007$, $r = -.52$</td>
<td>**</td>
</tr>
</tbody>
</table>

Legend:

*Sex:*
M: Male; F: Female

*Prenatal Treatment:*
T: Prenatal Testosterone; O: Prenatal Sesame Oil

*Adult Treatment:*
SHAM: Intact; GDX: GDX + Vehicle; GDX+T/EB: GDX + Hormone Replacement
Figure 43: Duration and frequency of agonistic behaviour ± SEM. * indicates a significant different between prenatal treatment groups; # indicates a significant sex difference; & indicates a significant difference between adult treatment groups, between either intact and GDX+VEH mice, or between GDX+VEH and GDX+EB/T replacement mice.

Male mice had higher duration-based \([F(1, 152) = 33.04, p < .001, \eta^2 = .18]\) and frequency based \([F(1, 152) = 59.52, p < .001, \eta^2 = .28]\) dominance scores than female mice. In addition, ANOVA revealed main effects of prenatal treatment \([F(1, 152) = 5.96, p = .02, \eta^2 = .04]\) and adult treatment condition \([F(2, 152) = 3.87, p = .02, \eta^2 = .05]\) on frequency-based dominance scores, as well as interaction effects between sex and adult treatment condition \([F(2, \ldots)]\).
152) = 5.65, \( p = .004, \eta^2 = .07 \), and sex, prenatal treatment, and adult treatment condition \( [F(2, 152) = 4.56, \ p = .01, \eta^2 = .06 \] on frequency-based dominance scores. Analysis of simple main effects revealed that, among female mice, frequency-based dominance scores were higher among mice treated prenatally with testosterone than among those treated prenatally with sesame oil \( [F(1, 78) = 5.60, \ p = .02, \eta^2 = .07] \). In addition, there was an effect of adult treatment condition among male mice \( [F(2, 72) = 6.81, \ p = .002, \eta^2 = .16] \), and pairwise comparisons indicated that frequency-based dominance scores were lower among castrated mice than both intact male mice \( [p = .002] \) and castrated mice that received testosterone replacement \( [p = .04] \). There was also a trend towards significance for an interaction effect between prenatal treatment and adult treatment condition on frequency-based dominance scores among male mice \( [F(2, 72) = 2.76, \ p = .07, \eta^2 = .07] \). In addition, there were trends towards significance for a main effect of prenatal treatment on duration-based dominance scores \( [F(1, 152) = 2.78, \ p = .10, \eta^2 = .02] \), and an interaction effect between sex and adult treatment condition on duration-based dominance scores \( [F(2, 152) = 2.54, \ p = .08, \eta^2 = .03] \) (see Table 16, Table 17, Figure 44).
Table 16: Planned Comparisons: Dominance Score - Duration

<table>
<thead>
<tr>
<th>Sex Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-O-SHAM &gt; F-O-SHAM</td>
<td>$W = 31, Z = -2.56, p = .01, r = .51$</td>
<td>**</td>
</tr>
<tr>
<td>M-O-GDX + T &gt; F-O-GDX + EB</td>
<td>$W = 17, Z = -3.50, p &lt; .001, r = -.67$</td>
<td>***</td>
</tr>
<tr>
<td>M-T-SHAM &gt; F-T-SHAM</td>
<td>$W = 49, Z = -2.62, p = .009, r = -.47$</td>
<td>**</td>
</tr>
<tr>
<td>M-T-GDX + T &gt; F-T-GDX + EB</td>
<td>$W = 51, Z = -2.36, p = .02, r = -.43$</td>
<td>*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Prenatal Treatment Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-T-GDX + EB &gt; F-O-GDX + EB</td>
<td>$W = 64, Z = -2.00, p = .05, r = -.36$</td>
<td>*</td>
</tr>
</tbody>
</table>

Table 17: Planned Comparisons: Dominance Score - Frequency

<table>
<thead>
<tr>
<th>Sex Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-O-SHAM &gt; F-O-SHAM</td>
<td>$W = 33, Z = -2.45, p = .01, r = -.49$</td>
<td>*</td>
</tr>
<tr>
<td>M-O-GDX + T &gt; F-O-GDX + EB</td>
<td>$W = 1, Z = -4.30, p &lt; .001, r = -.83$</td>
<td>***</td>
</tr>
<tr>
<td>M-T-SHAM &gt; F-T-SHAM</td>
<td>$W = 33, Z = -3.44, p &lt; .001, r = -.37$</td>
<td>***</td>
</tr>
<tr>
<td>M-T-GDX + T &gt; F-T-GDX + EB</td>
<td>$W = 33, Z = -2.03, p = .04, r = -.37$</td>
<td>*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Prenatal Treatment Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-T-GDX &gt; F-O-GDX</td>
<td>$W = 28.5, Z = -3.62, p = .01, r = -.65$</td>
<td>*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Adult Treatment Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-O-SHAM &gt; M-O-GDX</td>
<td>$W = 115, Z = 2.01, p = .04, r = .40$</td>
<td>*</td>
</tr>
<tr>
<td>M-O-GDX + T &gt; M-O-GDX</td>
<td>$W = 18.5, Z = -3.07, p = .002, r = -.63$</td>
<td>**</td>
</tr>
<tr>
<td>M-T-SHAM &gt; M-T-GDX</td>
<td>$W = 136.5, Z = 2.27, p = .02, r = .44$</td>
<td>*</td>
</tr>
<tr>
<td>F-O-GDX &gt; F-O-GDX + EB</td>
<td>$W = 163, Z = 2.59, p = .01, r = .48$</td>
<td>**</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dominance over Demonstrator Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-O-GDX + EB &lt; 0</td>
<td>$V = 0, p &lt; .001$</td>
<td>***</td>
</tr>
<tr>
<td>M-O-SHAM &gt; 0</td>
<td>$V = 69, p = .02$</td>
<td>*</td>
</tr>
<tr>
<td>M-O-GDX + T &gt; 0</td>
<td>$V = 63, p = .04$</td>
<td>*</td>
</tr>
<tr>
<td>M-T-SHAM &gt; 0</td>
<td>$V = 117, p = .001$</td>
<td>***</td>
</tr>
</tbody>
</table>

Legend:
Sex:
M: Male; F: Female
Prenatal Treatment:
T: Prenatal Testosterone; O: Prenatal Sesame Oil
Adult Treatment:
SHAM: Intact; GDX: GDX + Vehicle; GDX + T/EB: GDX + Hormone Replacement
Figure 44: Duration and frequency based dominance scores ± SEM. * indicates a significant different between prenatal treatment groups; # indicates a significant sex difference; & indicates a significant difference between adult treatment groups, between either intact and GDX+VEH mice, or between GDX+VEH and GDX+EB/T replacement mice; % indicates dominance score significantly > or < 0.

ANOVA revealed that frequency and duration of duration of agonistic behaviour delivered were higher among male mice than among female mice [Frequency: $F(1, 152) = 28.77, p < .001$, $\eta^2 = .15$; Duration: $F(1, 152) = 22.84, p < .001$, $\eta^2 = .13$], and indicated significant interactions effect between sex, prenatal treatment, and adult treatment condition on both frequency and duration of agonistic behaviour delivered [Frequency: $F(2, 152) = 6.68, p = .002$,
\[ \eta^2 = .08; \text{Duration: } F(2, 152) = 5.37, p = .006, \eta^2 = .07 \]. Analysis of simple main effects revealed a significant interaction between prenatal treatment and adult treatment condition on both frequency and duration of agonistic behaviour delivered among male mice [Frequency: \( F(2, 72) = 4.20, p = .02, \eta^2 = .10 \); Duration: \( F(2, 72) = 3.32, p = .04, \eta^2 = .09 \)]. Further analysis indicated that adult treatment condition only affected frequency and duration of agonistic behaviour among male mice treated prenatally with sesame oil [Frequency: \( F(2, 33) = 7.39, p = .002, \eta^2 = .31 \); Duration: \( F(2, 33) = 4.41, p = .02, \eta^2 = .21 \)], and pairwise comparisons revealed that castrated mice treated prenatally with sesame oil that received testosterone replacement delivered agonistic behaviours more frequently \([p = .001]\), and for longer \([p = .02]\) than castrated mice treated prenatally with sesame oil that did not receive replacement (see Table 18, Table 19, Figure 45).
### Table 18: Planned Comparisons: Agonistic Behaviour Delivered - Duration

<table>
<thead>
<tr>
<th>Sex</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-O-GDX+T &gt; F-O-GDX+EB</td>
<td>$W = 17, Z = -3.50, p &lt; .001, r = -.67$</td>
<td>***</td>
</tr>
<tr>
<td>M-T-SHAM &gt; F-T-SHAM</td>
<td>$W = 52, Z = -2.69, p = .007, r = -.48$</td>
<td>**</td>
</tr>
<tr>
<td>M-T-GDX &gt; F-T-GDX</td>
<td>$W = 42, Z = -1.95, p = .05, r = -.39$</td>
<td>T</td>
</tr>
<tr>
<td>M-T-GDX+T &gt; F-T-GDX+EB</td>
<td>$W = 61, Z = -2.14, p = .03, r = -.39$</td>
<td>*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Prenatal Treatment</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-T-GDX &gt; M-O-GDX</td>
<td>$W = 42, Z = -1.96, p = .05, r = -.39$</td>
<td>T</td>
</tr>
<tr>
<td>M-O-GDX+T &gt; M-T-GDX+T</td>
<td>$W = 120, Z = 1.95, p = .05, r = .38$</td>
<td>T</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Adult Treatment</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-O-GDX+T &gt; M-O-GDX</td>
<td>$W = 27, Z = -2.58, p = .01, r = -.53$</td>
<td>**</td>
</tr>
<tr>
<td>F-O-GDX &gt; F-O-GDX+EB</td>
<td>$W = 164, Z = 2.63, p = .008, r = .49$</td>
<td>**</td>
</tr>
</tbody>
</table>

### Table 19: Planned Comparisons: Agonistic Behaviour Delivered - Frequency

<table>
<thead>
<tr>
<th>Sex</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-O-GDX+T &gt; F-O-GDX+EB</td>
<td>$W = 13, Z = -3.71, p &lt; .001, r = -.71$</td>
<td>***</td>
</tr>
<tr>
<td>M-T-SHAM &gt; F-T-SHAM</td>
<td>$W = 34, Z = -3.40, p &lt; .001, r = -.61$</td>
<td>***</td>
</tr>
<tr>
<td>M-T-GDX &gt; F-T-GDX</td>
<td>$W = 33, Z = -2.45, p = .01, r = -.49$</td>
<td>**</td>
</tr>
<tr>
<td>M-T-GDX+T &gt; F-T-GDX+EB</td>
<td>$W = 47.5, Z = -2.70, p = .007, r = -.49$</td>
<td>**</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Prenatal Treatment</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-T-GDX &gt; M-O-GDX</td>
<td>$W = 38, Z = -2.18, p = .03, r = -.44$</td>
<td>*</td>
</tr>
<tr>
<td>M-T-SHAM &gt; M-O-SHAM</td>
<td>$W = 51, Z = -1.90, p = .06, r = -.37$</td>
<td>T</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Adult Treatment</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-O-GDX+T &gt; M-O-GDX</td>
<td>$W = 9, Z = -3.63, p &lt; .001, r = -.74$</td>
<td>***</td>
</tr>
<tr>
<td>M-T-SHAM &gt; M-T-GDX</td>
<td>$W = 128.5, Z = 1.88, p = .06, r = .36$</td>
<td>T</td>
</tr>
</tbody>
</table>

Legend:

- **Sex:**
  - M: Male; F: Female

- **Prenatal Treatment:**
  - T: Prenatal Testosterone; O: Prenatal Sesame Oil

- **Adult Treatment:**
  - SHAM: Intact; GDX: GDX + Vehicle; GDX+T/EB: GDX + Hormone Replacement
Figure 45: Duration and frequency of agonistic behaviour delivered ± SEM. * indicates a significant difference between prenatal treatment groups; # indicates a significant sex difference; & indicates a significant difference between adult treatment groups, between either intact and GDX+VEH mice, or between GDX+VEH and GDX+EB/T replacement mice.

Furthermore, female mice received agonistic behaviour from the intruder for longer than male mice \([F(1, 152) = 31.60, p < .001, \eta^2 = .17]\), and mice treated prenatally with sesame oil received agonistic behaviour for longer than mice treated prenatally with testosterone \([F(1, 152) = 7.58, p = .007, \eta^2 = .05]\). Similarly, there were main effects of both sex \([F(1, 152) = 33.66, p < .001, \eta^2 = .18]\) and prenatal treatment \([F(1, 152) = 9.15, p = .003, \eta^2 = .06]\) on frequency of agonistic behaviour received, though ANOVA also revealed a significant interaction between sex
and prenatal treatment \([F(1, 152) = 7.90, p = .006, \eta^2 = .05]\). Analysis of simple main effects showed an effect of prenatal treatment in female mice, such that prenatal treatment with testosterone significantly reduced the frequency of agonistic behaviours received relative to mice treated prenatally with sesame oil \([F(1, 78) = 16.05, p < .001, \eta^2 = .17]\). In addition, there were trends towards significance for main effects of adult treatment condition on frequency \([F(2, 152) = 2.95, p = .06, \eta^2 = .04]\) and duration \([F(2, 152) = 2.65, p = .07, \eta^2 = .03]\) of agonistic behaviour received, as well as towards an interaction effect between sex and adult treatment condition on frequency of agonistic behaviour received \([F(2, 152) = 2.58, p = .08, \eta^2 = .03]\) (see Table 20, Table 21, Figure 46).

**Table 20**: Planned Comparisons: Agonistic Behaviour Received - Duration

<table>
<thead>
<tr>
<th>Sex</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-O-SHAM &gt; M-O-SHAM</td>
<td>(W = 134, Z = 2.05, p = .002, r = .61)</td>
<td>**</td>
</tr>
<tr>
<td>F-O-GDX+EB &gt; M-O-GDX+T</td>
<td>(W = 146, Z = 2.86, p = .004, r = .55)</td>
<td>**</td>
</tr>
<tr>
<td>F-T-GDX+EB &gt; M-T-GDX+T</td>
<td>(W = 168, Z = 2.30, p = .02, r = .42)</td>
<td>*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Prenatal Treatment</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-O-GDX+EB &gt; F-T-GDX+EB</td>
<td>(W = 165, Z = 1.93, p = .05, r = .36)</td>
<td>T</td>
</tr>
<tr>
<td>F-O-SHAM &gt; F-T-SHAM</td>
<td>(W = 148, Z = 1.94, p = .05, r = .36)</td>
<td>T</td>
</tr>
</tbody>
</table>

**Table 21**: Planned Comparisons: Agonistic Behaviour Received - Frequency

<table>
<thead>
<tr>
<th>Sex</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-O-SHAM &gt; M-O-SHAM</td>
<td>(W = 145.5, Z = 3.68, p &lt; .001, r = .74)</td>
<td>***</td>
</tr>
<tr>
<td>F-O-GDX+EB &gt; M-O-GDX+T</td>
<td>(W = 169, Z = 4.00, p &lt; .001, r = .77)</td>
<td>***</td>
</tr>
<tr>
<td>F-T-SHAM &gt; M-T-SHAM</td>
<td>(W = 169.5, Z = 1.96, p = .05, r = .35)</td>
<td>***</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Prenatal Treatment</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-O-GDX+EB &gt; F-T-GDX+EB</td>
<td>(W = 200, Z = 3.17, p = .002, r = .57)</td>
<td>**</td>
</tr>
<tr>
<td>F-O-SHAM &gt; F-T-SHAM</td>
<td>(W = 147.5, Z = 1.91, p = .06, r = .35)</td>
<td>T</td>
</tr>
</tbody>
</table>

Legend:
- **Sex**: M: Male; F: Female
- **Prenatal Treatment**: T: Prenatal Testosterone; O: Prenatal Sesame Oil
- **Adult Treatment**: 
SHAM: Intact; GDX: GDX + Vehicle; GDX+T/EB: GDX + Hormone Replacement

**Figure 46:** Duration and frequency of agonistic behaviour received ± SEM. * indicates a significant different between prenatal treatment groups; # indicates a significant sex difference.

Male mice spent more time engaged in dominance behaviour than female mice [$F(1, 152) = 21.23, p < .001, \eta^2 = .12$]. In addition, ANOVA revealed interaction effects between sex and adult treatment condition on duration of dominance behaviour [$F(2, 152) = 3.27, p = .04, \eta^2 = .04$] as well as between sex, prenatal treatment, and adult treatment condition [$F(2, 152) = 3.11, p = .05, \eta^2 = .04$]. Analysis of simple main effects revealed a significant interaction effect
between prenatal treatment and adult treatment condition on duration of dominance behaviour in male mice \[ F(2, 72) = 3.15, p = .05, \eta^2 = .08 \], and further analysis revealed an effect of adult treatment condition only in male mice treated prenatally with sesame oil \[ F(2, 33) = 4.98, p = .01, \eta^2 = .23 \]. Pairwise comparisons revealed that, among mice treated prenatally with sesame oil, castrated mice that received testosterone replacement spent more time engaged in dominance behaviour than castrated mice that did not receive replacement \([p = .01]\) (see Table 22, Figure 47).

**Table 22: Planned Comparisons: Dominance Behaviour - Duration**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-O-SHAM &gt; F-O-SHAM</td>
<td>[ W = 45.5, Z = -1.86, p = .06, r = -.34 ]</td>
<td>T</td>
</tr>
<tr>
<td>M-O-GDX+T &gt; F-O-GDX+EB</td>
<td>[ W = 16, Z = -3.56, p &lt; .001, r = -.68 ]</td>
<td>***</td>
</tr>
<tr>
<td>M-T-SHAM &gt; F-T-SHAM</td>
<td>[ W = 45.5, Z = -2.96, p = .003, r = -.53 ]</td>
<td>**</td>
</tr>
<tr>
<td>M-T-GDX+T &gt; F-T-GDX+T</td>
<td>[ W = 64, Z = -2.05, p = .04, r = -.37 ]</td>
<td>*</td>
</tr>
</tbody>
</table>

### Prenatal Treatment

<table>
<thead>
<tr>
<th>Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-O-GDX+T &gt; M-T-GDX+T</td>
<td>[ W = 118, Z = 1.84, p = .07, r = .36 ]</td>
<td>T</td>
</tr>
</tbody>
</table>

### Adult Treatment

<table>
<thead>
<tr>
<th>Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-O-GDX+T &gt; M-O-GDX</td>
<td>[ W = 9, Z = -3.63, p &lt; .001, r = -.74 ]</td>
<td>***</td>
</tr>
<tr>
<td>M-O-SHAM &gt; M-O-GDX</td>
<td>[ W = 112, Z = 1.86, p = .06, r = .34 ]</td>
<td>T</td>
</tr>
<tr>
<td>F-O-GDX &gt; F-O-GDX+EB</td>
<td>[ W = 146, Z = 1.85, p = .06, r = .34 ]</td>
<td>T</td>
</tr>
</tbody>
</table>

**Legend:**

- **Sex:** M: Male; F: Female
- **Prenatal Treatment:** T: Prenatal Testosterone; O: Prenatal Sesame Oil
- **Adult Treatment:** SHAM: Intact; GDX: GDX + Vehicle; GDX+T/EB: GDX + Hormone Replacement
Figure 47: Duration of dominance behaviour ± SEM. * indicates a significant different between prenatal treatment groups; # indicates a significant sex difference; & indicates a significant difference between adult treatment groups, between either intact and GDX+VEH mice, or between GDX+VEH and GDX+EB/T replacement mice.

Female mice engaged in submissive behaviours for longer than male mice \( [F(1, 152) = 29.90, p < .001, \eta^2 = .16] \), and more frequently than male mice \( [F(1, 152) = 27.81, p < .001, \eta^2 = .16] \). In addition, mice treated prenatally with testosterone spent less time engaged in submissive behaviour than those treated prenatally with sesame oil \( [F(1, 152) = 6.89, p = .01, \eta^2 = .04] \), and engaged in submissive behaviour less frequently than mice that received prenatal treatment with sesame oil \( [F(1, 152) = 21.30, p = .004, \eta^2 = .05] \). There was also a significant interaction between prenatal treatment and sex in terms of frequency of submissive behaviour \( [F(1, 152) = 5.88, p = .02, \eta^2 = .04] \). Further investigation revealed a significant effect of prenatal treatment only in female mice, such that female mice treated prenatally with testosterone engaged in significantly fewer instances of submissive behaviour than those treated prenatally with sesame oil \( [F(1, 78) = 13.39, p < .001, \eta^2 = .14] \) (see Table 23, Table 24, Figure 48).
Table 23: Planned Comparisons: Submissive Behaviour - Duration

<table>
<thead>
<tr>
<th>Sex</th>
<th>Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F-O-SHAM &gt; M-O-SHAM</td>
<td>W = 134, Z = 3.05, p = .002, r = .61</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>F-O-GDX+EB &gt; M-O-GDX+T</td>
<td>W = 137, Z = 2.42, p = .02, r = .47</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>F-T-SHAM &gt; M-T-SHAM</td>
<td>W = 178, Z = 2.29, p = .02, r = .41</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>F-T-GDX+EB &gt; M-T-GDX+T</td>
<td>W = 169, Z = 2.34, p = .02, r = .43</td>
<td>*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Prenatal Treatment</th>
<th>Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F-O-SHAM &gt; F-T-SHAM</td>
<td>W = 148, Z = 1.93, p = .05, r = .36</td>
<td>T</td>
</tr>
</tbody>
</table>

Table 24: Planned Comparisons: Submissive Behaviour - Frequency

<table>
<thead>
<tr>
<th>Sex</th>
<th>Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F-O-SHAM &gt; M-O-SHAM</td>
<td>W = 143.5, Z = 3.57, p &lt; .001, r = .71</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>F-O-GDX+EB &gt; M-O-GDX+T</td>
<td>W = 157, Z = 3.41, p &lt; .001, r = .66</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>F-T-SHAM &gt; M-T-SHAM</td>
<td>W = 171, Z = 2.02, p = .04, r = .36</td>
<td>*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Prenatal Treatment</th>
<th>Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F-O-SHAM &gt; F-T-SHAM</td>
<td>W = 146, Z = 1.84, p = .07, r = .34</td>
<td>T</td>
</tr>
<tr>
<td></td>
<td>F-O-GDX+EB &gt; F-T-GDX+EB</td>
<td>W = 189, Z = 2.73, p = .006, r = .49</td>
<td>**</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Adult Treatment</th>
<th>Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M-O-GDX &gt; M-O-SHAM</td>
<td>W = 47.5, Z = 1.66, p = .10, r = .33</td>
<td>T</td>
</tr>
<tr>
<td></td>
<td>M-T-GDX &gt; M-T-SHAM</td>
<td>W = 53, Z = 1.81, p = .07, r = .35</td>
<td>T</td>
</tr>
</tbody>
</table>

Legend:

Sex:
M: Male; F: Female

Prenatal Treatment:
T: Prenatal Testosterone; O: Prenatal Sesame Oil

Adult Treatment:
SHAM: Intact; GDX: GDX + Vehicle; GDX+T/EB: GDX + Hormone Replacement
Figure 48: Duration and frequency of submissive behaviour ± SEM. * indicates a significant
difference between prenatal treatment groups; # indicates a significant sex difference; & indicates
a significant difference between adult treatment groups, between either intact and GDX+VEH
mice, or between GDX+VEH and GDX+EB/T replacement mice.

Overall, male mice spent significantly more time engaged in ritualized aggression than
female mice than female mice \( [F(1, 152) = 16.47, p < .001, \eta^2 = .10] \). In addition, there was a
main effect of adult treatment condition on duration of ritualized aggression \( [F(2, 152) = 4.93, p
= .08, \eta^2 = .06] \), such that gonadectomized mice engaged in ritualized aggression for less time
than intact mice \( [p = .03] \) and gonadectomized mice that received hormone replacement
(Duration: \( p = .02 \)). ANOVA also revealed a significant interaction between sex and adult
treatment condition on duration of ritualized aggression \([F(2, 152) = 8.02, p = .005, \eta^2 = .05]\), and simple main effects revealed a significant effect of adult treatment condition on duration of ritualized aggression only in male mice \([Duration: F(1, 72) = 5.67, p = .005, \eta^2 = .14]\). Pairwise comparisons revealed that, among male mice, castrated mice spent significantly less time engaged in ritualized aggression than both intact mice \([p = .01]\) and castrated mice that received testosterone replacement \([p = .01]\) (see Table 25, Figure 49).

**Table 25: Planned Comparisons: Ritualized Aggression - Duration**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-T-SHAM &gt; F-T-SHAM</td>
<td>(W = 45, Z = -3.38, p &lt; .001, r = -.61)</td>
<td>***</td>
</tr>
<tr>
<td>M-T-GDX+T &gt; F-T-GDX+T</td>
<td>(W = 53, Z = -2.86, p = .004, r = -.52)</td>
<td>**</td>
</tr>
</tbody>
</table>

**Prenatal Treatment**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-T-SHAM &gt; M-O-SHAM</td>
<td>(W = 57, Z = -1.71, p = .09, r = -.33)</td>
<td>T</td>
</tr>
<tr>
<td>M-T-GDX &gt; M-O-GDX</td>
<td>(W = 58.5, Z = -1.88, p = .06, r = -.38)</td>
<td>T</td>
</tr>
</tbody>
</table>

**Adult Treatment**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-O-SHAM &gt; M-O-GDX</td>
<td>(W = 104, Z = 2.21, p = .03, r = .44)</td>
<td>*</td>
</tr>
<tr>
<td>M-O-GDX+T &gt; M-O-GDX</td>
<td>(W = 39, Z = -2.65, p = .008, r = -.54)</td>
<td>**</td>
</tr>
<tr>
<td>M-T-SHAM &gt; M-T-GDX</td>
<td>(W = 129.5, Z = -2.08, p = .04, r = .40)</td>
<td>*</td>
</tr>
</tbody>
</table>

**Legend:**

- **Sex:** M: Male; F: Female
- **Prenatal Treatment:** T: Prenatal Testosterone; O: Prenatal Sesame Oil
- **Adult Treatment:** SHAM: Intact; GDX: GDX + Vehicle; GDX+T/EB: GDX + Hormone Replacement
Figure 49: Duration of ritualized aggression ± SEM. * indicates a significant difference between prenatal treatment groups; # indicates a significant sex difference; & indicates a significant difference between adult treatment groups, between either intact and GDX+VEH mice, or between GDX+VEH and GDX+EB/T replacement mice.

Overall, male mice delivered significantly more attacks than female mice [$F(1, 152) = 29.94, p < .001, \eta^2 = .17$]. ANOVA also revealed a significant main effect of adult treatment condition [$F(2, 152) = 6.16, p = .003, \eta^2 = .08$], as well as a significant interaction between sex and adult treatment condition [$F(2, 152) = 6.30, p = .002, \eta^2 = .08$]. Simple main effects revealed an effect of adult treatment condition only among male mice [$F(1, 72) = 5.75, p = .005, \eta^2 = .14$], such that castrated male mice delivered significantly fewer attacks than intact mice [$p = .004$] (see Table 26, Figure 50).
Table 26: Planned Comparisons: Attacks Delivered

<table>
<thead>
<tr>
<th>Sex</th>
<th>Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M-O-SHAM &gt; F-O-SHAM</td>
<td>( W = 39, Z = -2.83, p = .005, r = -.57 )</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>M-O-GDX+T &gt; F-O-GDX+T</td>
<td>( W = 58.5, Z = -1.81, p = .07, r = -.35 )</td>
<td>T</td>
</tr>
<tr>
<td></td>
<td>M-T-SHAM &gt; F-T-SHAM</td>
<td>( W = 32, Z = -4.07, p &lt; .001, r = -.73 )</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>M-T-GDX+T &gt; F-T-GDX+T</td>
<td>( W = 48.5, Z = -3.16, p = .002, r = -.58 )</td>
<td>**</td>
</tr>
</tbody>
</table>

**Adult Treatment**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Test Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-O-SHAM &gt; M-O-GDX</td>
<td>( W = 114, Z = 2.47, p = .01, r = .49 )</td>
<td>*</td>
</tr>
<tr>
<td>M-O-GDX+T &gt; M-O-GDX</td>
<td>( W = 42, Z = -2.21, p = .03, r = -.45 )</td>
<td>*</td>
</tr>
<tr>
<td>M-T-SHAM &gt; M-T-GDX</td>
<td>( W = 143, Z = 2.79, p = .005, r = .54 )</td>
<td>**</td>
</tr>
<tr>
<td>M-T-GDX+T &gt; M-T-GDX</td>
<td>( W = 52, Z = -2.08, p = .04, r = -.40 )</td>
<td>*</td>
</tr>
</tbody>
</table>

Legend:

Sex: M: Male; F: Female

_Prenatal Treatment:_

T: Prenatal Testosterone; O: Prenatal Sesame Oil

_Adult Treatment:_

SHAM: Intact; GDX: GDX + Vehicle; GDX+T/EB: GDX + Hormone Replacement

**Figure 50:** Attacks delivered ± SEM. # indicates a significant sex difference; & indicates a significant difference between adult treatment groups, between either intact and GDX+VEH mice, or between GDX+VEH and GDX+EB/T replacement mice.

ANOVA also revealed a significant interaction effect between estrus phase and prenatal treatment on frequency-based dominance scores in intact female mice \([F(2, 22) = 7.04, p = .004, \eta^2 = .39]\). There was an effect of estrus phase on frequency-based dominance score among intact
Female mice treated prenatally with testosterone \([F(2, 13) = 3.96, p = .05, \eta^2 = .38]\), such that mice in proestrus had significantly lower frequency-based dominance scores than those in diestrus \([p = .05]\). Furthermore, ANOVA revealed a significant interaction effect of estrus phase and prenatal treatment on agonistic behaviours received among intact female mice \([F(2, 22) = 9.68, p = .001, \eta^2 = .47]\). Specifically, estrus phase had a significant affect on frequency of agonistic behaviours received among intact female mice treated prenatally with testosterone \([F(2, 13) = 5.47, p = .02, \eta^2 = .46]\), such that those in proestrus received agonistic behaviour from the intruder mouse more frequently than those in estrus \([p = .05]\) and diestrus \([p = .02]\).

Moreover, ANOVA revealed a significant interaction effect between prenatal treatment and estrus phase on frequency of submissive behaviour. Analysis of simple main effects revealed an effect of estrus phase among intact female mice treated prenatally with testosterone \([F(2, 13) = 5.38, p = .02, \eta^2 = .45]\). Pairwise comparisons indicated that intact female mice treated prenatally with testosterone engaged in more frequent instances of submissive behaviour when in proestrus than diestrus \([p = .02]\) or estrus \([p = .05]\).

**Dark/Light**

**Summary**

In adolescence, mice treated prenatally with testosterone exhibited greater anxiety like behaviour than mice treated prenatally with sesame oil, particularly during the first minute of testing. Male mice appeared to be more sensitive to this effect than female mice. In addition, female mice were more active overall than male mice. In adulthood, anxiety-like behaviour in male mice was affected by both prenatal treatment and adult treatment condition, while female mice were resilient to these effects. Furthermore, female mice exhibited less anxiety-like
behaviour overall than male mice. However, female mice engaged in more horizontal activity overall, and exhibited differences in activity between prenatal and adult treatment groups.

**Adolescence**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Prenatal Treatment</th>
<th>Sample Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Sesame Oil</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Testosterone</td>
<td>47</td>
</tr>
<tr>
<td>Female</td>
<td>Sesame Oil</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>Testosterone</td>
<td>38</td>
</tr>
</tbody>
</table>

Prior to puberty, ANOVA showed that mice treated prenatally with testosterone made fewer light side entries during the first minute of the test than mice treated prenatally with sesame oil [$F(1, 154) = 17.04, p < .001, \eta^2 = .10$]. In addition, there was a significant interaction between sex and prenatal treatment [$F(1, 154) = 4.89, p = .03, \eta^2 = .03$] on light-side entries made in the first minute of testing. Planned comparisons showed that prenatal treatment with testosterone reduced the number of first minute light-side entries among male mice [$t(57.46) = 3.96, p < .001, d = 0.93$], but not female mice. In addition, among mice treated prenatally with sesame oil, male mice made significantly more light side entries during the first minute than female mice [$t(59.60) = 2.34, p = .02, d = .56$]. Furthermore, mice treated prenatally with testosterone made fewer light-side entries than mice treated prenatally with sesame oil over the duration of the test [$F(1, 154) = 15.74, p < .001, \eta^2 = .10$]. Planned comparisons revealed that prenatal testosterone treatment reduced total light side entries among both male mice [$t(59.63) = 2.76, p = .008, d = 0.65$] and female mice [$t(70.83) = 2.83, p = 0.006, d = 0.65$] (see Figure 51).

Moreover, mice treated prenatally with testosterone spent significantly less time in the light side of the chamber than mice treated prenatally with sesame oil during the first minute of testing [$F(1, 154) = 7.65, p = .006, \eta^2 = .05$]. Planned comparisons showed that prenatal treatment with testosterone reduced time spent in the light side of the chamber during the first
minute of testing among male mice \( t(67.53) = 2.13, p = 0.04, d = 0.49 \), and a similar trend was observed in female mice \( t(73.99) = 1.75, p = 0.09, d = 0.40 \), though this effect did not reach statistical significance. No significant differences were found between groups in terms of total time spent in the light side of the chamber throughout the duration of the test (see Figure 51).

In addition, mice treated prenatally exhibited a greater latency to enter the light side of the test chamber than mice treated prenatally with sesame oil \( F(1, 148) = 6.67, p = .01, \eta^2 = .04 \). Planned comparisons showed that male mice treated prenatally with testosterone took longer to enter the light side of the chamber than male mice treated with sesame oil \( W = 490, Z = -2.79, p = .005, r = .31 \) (see Figure 51).

Furthermore, ANOVA revealed that female mice engaged in more horizontal activity than male mice \( F(1, 154) = 6.42, p = .01, \eta^2 = .04 \) on total horizontal activity throughout the duration of the test. Among mice treated prenatally with testosterone, planned comparisons showed that female mice were significantly more active than males throughout the duration of the test and males \( t(82.78) = 2.71, p = .008, d = 0.58 \) (see Figure 51).
Figure 51: A: Number of 1st minute light-side entries; B: Number of total light-side entries; C: Latency to enter the light side of the chamber; D: Time spent in the light side of the chamber in the first minute of testing; E: Total time spent in the light side of the test chamber; F: Horizontal activity (number of beam breaks); All measures ± SEM. * indicates a significant difference between prenatal treatment conditions; # indicates a significant sex difference.

### Adulthood

<table>
<thead>
<tr>
<th>Sex</th>
<th>Prenatal Treatment</th>
<th>Adult Treatment</th>
<th>Sample Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Sesame Oil</td>
<td>Intact</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GDX</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GDX+T</td>
<td>17</td>
</tr>
<tr>
<td>Testosterone</td>
<td></td>
<td>Intact</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GDX</td>
<td>18</td>
</tr>
<tr>
<td></td>
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<td>GDX+T</td>
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<td>Female</td>
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Female mice made significantly more light-side entries than male mice during the first minute of testing [$F(1, 137) = 6.20, p = .01, \eta^2 = .04$]. Furthermore, planned comparisons
revealed that among mice treated prenatally with testosterone, intact male mice made significantly fewer light-side entries during the first minute of testing than intact female mice \([t(23.00) = 3.03, p = .006, d = 1.21]\), and castrated mice that received testosterone replacement made significantly fewer first minute light-side entries than ovariectomized mice that received estradiol replacement \([t(21.65) = 2.08, p = .05, d = 0.83]\) (see Figure 52).

**Figure 52**: Light-side entries (frequency) made in the first minute of test ± SEM. # indicates a significant sex difference;

Overall, female mice made significantly more light side entries throughout the duration of the test than male mice \([F(1, 180) = 14.17, p < .001, \eta^2 = .07]\). In addition, ANOVA revealed a significant interaction between sex and prenatal treatment on total light-side entries \([F(1, 180) = 5.30, p = .02, \eta^2 = .03]\). Analysis of simple main effects indicated that, among male mice, those treated prenatally with testosterone made significantly fewer light-side entries than male mice treated prenatally with sesame oil \([F(1, 89) = 7.18, p = .009, \eta^2 = .08]\). Furthermore, planned comparisons showed that gonadectomized male mice that received testosterone replacement differed by prenatal treatment, such that mice that were treated prenatally with testosterone made significantly fewer light-side entries than male mice treated prenatally with sesame oil \([t(30.73) = 2.10, p = .04, d = 0.72]\). In addition, among mice treated prenatally with
testosterone, intact female mice made significantly more light-side entries throughout the duration of the test than intact males \([t(30.96) = 3.73, p < .001, d = 1.29]\). Similarly, gonadectomized female mice that received hormone replacement with estradiol benzoate and were treated prenatally with testosterone made significantly more light-side entries than gonadectomized male mice treated prenatally with testosterone that received testosterone replacement \([t(29.91) = 3.19, p = .003, d = 1.06]\) (see Figure 53).

![Figure 53](image)

**Figure 53**: Light-side entries (frequency) made throughout the duration of the test ± SEM. * indicates a significant different between prenatal treatment groups; # indicates a significant sex difference

ANOVA revealed a significant interaction effect between sex and prenatal treatment on time spent in the light side of the chamber during the first minute of testing \([F(1, 137) = 3.14, p = .04, \eta^2 = .03]\). Analysis of simple main effects indicated that male mice treated prenatally with testosterone spent significantly less time in the light side of the chamber during the first minute of testing than male mice treated prenatally with sesame oil \([F(1, 70) = 4.99, p = .03, \eta^2 = .07]\). Among male mice treated prenatally with sesame oil, planned comparisons showed that gonadectomized mice spent more time in the light side compared to intact mice \([t(14.66) = 2.46, \ldots]\).
Among mice treated prenatally with testosterone, intact female mice spent significantly more time in the light side of the test chamber than intact male mice \([t(23.00) = 3.32, p = .003, d = 1.32]\). There was a similar trend among gonadectomized male and female mice treated prenatally with testosterone \([t(21.68) = 1.94, p = .07, d = 0.73]\), though it did not reach significance. Among gonadectomized male mice, planned comparisons showed that mice treated prenatally with testosterone spent less time in the light side during the first minute of testing than those treated with sesame oil \([t(11.55) = 2.69, p = .02, d = 1.28]\) (see Figure 54).

**Figure 54:** Duration spent in the light side (seconds) during the first minute of testing ± SEM. # indicates a significant sex difference; & indicates a significant difference between adult treatment groups, between either intact and GDX+VEH mice, or between GDX+VEH and GDX+EB/T replacement mice.

Female mice spent significantly longer in the light side of the test chamber than male mice \(F(1, 180) = 3.89, p = .05, \eta^2 = .02\). Furthermore, planned comparisons revealed that gonadectomized male mice treated prenatally with sesame oil spent significantly longer in the light side of the chamber than intact males treated prenatally with sesame oil \([t(25.94) = 2.38, p = .02, d = 0.89]\). In addition, gonadectomized male mice treated prenatally with testosterone spent significantly less time in the light side of the chamber than gonadectomized female mice.
treated prenatally with testosterone \[t(29.92) = 2.42, p = .02, d = 0.81\]. Furthermore, among gonadectomized male mice, prenatal treatment with testosterone reduced time spent in the light side \[t(28.29) = 2.57, p = .02, d = 0.91\] (see Figure 55).

**Figure 55**: Total duration spent in the light side (seconds) ± SEM. * indicates a significant difference between prenatal treatment groups; # indicates a significant sex difference; & indicates a significant difference between adult treatment groups, between either intact and GDX+VEH mice, or between GDX+VEH and GDX+EB/T replacement mice.

Male mice took significantly longer to enter the light side of the test chamber than female mice \[F(1, 177) = 10.54, p = .001, \eta^2 = .06\]. Furthermore, ANOVA revealed a significant main effect of adult treatment condition \[F(2, 177) = 4.77, p = .01, \eta^2 = .05\], such that intact mice took significantly longer to enter the light side of the chamber than gonadectomized mice \[p = .01\], while a trend towards significance suggested that intact mice took longer to enter the light side than gonadectomized mice that received replacement with estradiol (females) or testosterone (males) \[p = .08\]. Among male mice treated prenatally with sesame oil, planned comparisons showed that gonadectomy reduced latency to enter the light side of the chamber \[W = 151, Z = 2.46, p = .01, r = .47\]. In addition, intact male mice treated prenatally with sesame oil took longer to enter the light side of the chamber than intact female mice \[W = 71, Z = -2.13, p = .03, r\]
Similarly, among mice treated prenatally with testosterone, intact male mice showed a
greater latency to enter the light side of the chamber than intact female mice \([W = 80, Z = -1.99, p = .05, r = .35]\), and gonadectomized male mice that received testosterone replacement took
longer to enter the light side of the chamber than female mice that received replacement with
estradiol \([W = 115, Z = -2.30, p = .02, r = .41]\) (see Figure 56).

![Figure 56: Latency to Enter Light Side](image)

**Figure 56:** Latency to enter the light side (seconds) ± SEM. # indicates a significant sex
difference; & indicates a significant difference between adult treatment groups, between either
intact and GDX+VEH mice, or between GDX+VEH and GDX+EB/T replacement mice.

Overall, female mice engaged in more horizontal activity than male mice \([F(1, 180) =
8.12, p = .005, \eta^2 = .04]\), though ANOVA also revealed a significant interaction effect between
sex and adult treatment condition on horizontal activity \([F(2, 180) = 3.59, p = .03, \eta^2 = .04]\).
Analysis of simple main effects revealed a significant effect of adult treatment condition in
female mice \([F(1, 91 = 4.88, p = .01, \eta^2 = .10]\), such that gonadectomized female mice that
received estradiol replacement exhibited reduced horizontal activity compared to both intact
female mice \([p = .02]\) and gonadectomized female mice \([p = .02]\). Furthermore, planned
comparisons revealed a significant increase in overall horizontal activity in gonadectomized
female mice treated with testosterone prenatally compared with intact female mice treated
prenatally with testosterone \([t(31.13) = 2.47, p = .02, d = 0.82]\), and gonadectomized female
mice treated prenatally with testosterone that received estradiol replacement \([t(18.21) = 3.28, p =
.004, d = 1.22]\). Moreover, among mice treated prenatally with sesame oil, intact female mice
exhibited significantly greater horizontal activity than intact male mice \([t(26.32) = 2.28, p = .03,
d = 0.82]\). Furthermore, among mice treated prenatally with testosterone, gonadectomized
females showed significantly greater horizontal activity compared with gonadectomized male
mice \([t(25.44) = 4.24, p < .001, d = 1.41]\). In addition, among gonadectomized female mice,
those treated prenatally with testosterone exhibited greater horizontal activity than mice treated
with sesame oil \([t(16.4) = 2.55, p = .02, d = 1.03]\) (see Figure 57).

**Figure 57:** Total horizontal activity – number of beam breaks ± SEM. * indicates a significant
different between prenatal treatment groups; # indicates a significant sex difference; & indicates
a significant difference between adult treatment groups, between either intact and GDX+VEH
mice, or between GDX+VEH and GDX+EB/T replacement mice.
Discussion

We found that prenatal treatment with a low dose of testosterone propionate interacted with activational hormone effects to influence social learning in adult mice. Moreover, prenatal testosterone treatment and adult treatment condition exerted sex dependent effects on engagement in social behaviour, social investigation, and agonistic behaviour in response to both familiar and unfamiliar conspecifics. In addition, prenatal treatment with testosterone increased anxiety-like behaviour in male, but not female mice, while male mice showed more anxiety-like behaviour overall.

Developmental and Physiological Measures

We were unable to detect a difference in either fecal or plasma testosterone between dams treated prenatally with sesame oil and dams treated prenatally with testosterone, and were therefore not able to confirm that our treatment was successful in increasing exposure to testosterone among treated litters. However, given that on the day of pup birth plasma testosterone was higher among dams treated with testosterone propionate, but not significantly so, we appear to have been successful in designing our treatment to slightly, but not drastically, increase levels of testosterone. It is also possible that heightened testosterone levels had decreased by the time samples were collected and that any difference in testosterone that may have been present during the treatment period was no longer significant.

Prenatal treatment with testosterone did not affect growth of litters or other markers of development in neonatal and juvenile pups, though growth retardation has been previously reported as a result of prenatal treatment with high doses of testosterone propionate in rats and sheep (Manikkam et al., 2004; Wolf et al., 2002). However, prenatal treatment with testosterone caused vaginal opening to occur earlier in female mice, which is suggestive of early onset of
puberty. This is consistent with studies indicating that neonatal exposure to both testosterone and DHT advances the time of vaginal opening in female rats (Harris & Levine, 1965; McDonald & Doughty, 1972; Wagner, Erwin, & Critchlow, 1966), though our findings contradict prior work showing that elevated testosterone in utero due to positioning between male mice delays vaginal opening (McDermott, Gandelman, & Reinisch, 1978; vom Saal, 1989).

Castration reduced prostate weight in male mice (see Figure 2) and testosterone replacement restored it, but not to levels observed in intact mice. Rodent and human literature indicates that prostate weight fluctuates with testosterone levels, suggesting that testosterone replacement was successful in partially restoring testosterone levels in castrated mice (Holmäng, Mårin, Lindstedt, & Hedelin, 1993; Shao, Kong, & Cunningham, 1994). It is therefore possible that our testosterone implants were not successful in fully restoring testosterone to normal physiological levels, though given the importance of puberty as a second critical period for sexual differentiation, including development of the prostate, prostate development may have been disrupted by removal of the testes during this period (De Klerk & Lombard, 1986). In addition, ovariectomy caused an increase in body weight in adulthood, which was reversed by estradiol replacement (see Figure 1). These findings are consistent with, and add to an existing body of research that suggests a role for estradiol in regulation of feeding behaviour and maintenance of body weight (Blaustein, Gentry, Roy, & Wade, 1976; Dubuc, 1985; Tarttelin & Gorski, 1973). Neonatal treatment with high doses of testosterone has been previously shown to increase adult body weight and decrease ovary weight in female rats, while both body weight and testes weight are decreased in male rats (Beatty, Powley, & Keesey, 1970; Harris & Levine, 1965; Swanson & Bosch, 1963). We did not find effects of prenatal treatment with testosterone
on gonad weight, prostate weight, or body weight in adult mice, indicating that our low dose of testosterone propionate was not sufficient to elicit gross physiological changes in adulthood.

**Social Learning**

**Adolescence**

Adolescent mice exhibited a robust preference for demonstrated food over the entire 8-hour test period, regardless of sex and prenatal treatment condition (see Figure 3). While this may indicate a longer preference in juvenile than in adult mice, it may also be due to differences in statistical power resulting from the larger sample size in adolescence (mean n ≈ 35) compared to adulthood (mean n ≈ 11). However, even at the 8-hour time point, observed effect sizes of differences in cinnamon preference ratio between cocoa and cinnamon demonstrated adolescent mice ranged from medium to very large based on Cohen’s guidelines (Cohen, 1988). Previous literature has shown that social influence has a stronger impact on feeding behaviour among juvenile rats than adult rats, and juvenile chimpanzees show an advantage in the acquisition of socially learned tool use strategies (Biro et al., 2003; Bruner, 1972; Galef, 1977). Interestingly, adult demonstrators produce a longer lasting preference for demonstrated food than younger demonstrators in adult observers, which may indicate that demonstrator age in relation to observer age is an important factor in the STFP (Choleris, Guo, Liu, Mainardi, & Valsecchi, 1997). Our findings confirm that social transmission of food preferences occurs in adolescent mice, and suggest that adolescent mice show an advantage in social learning compared to adult mice, which is consistent with literature in both rats and non-human primates. Notably, prenatal testosterone treatment did not affect social learning in adolescent male or female mice.

Among mice treated prenatally with sesame oil, male mice consumed more chow than female mice over the first two hours of testing (see Figure 4). Moreover, among female mice,
prenatal treatment with testosterone significantly increased feeding behaviour over the first two hours of testing, and abolished the sex difference in feeding behaviour. However, over the second 2-hour time period, feeding behaviour was lower among female mice treated prenatally with testosterone than among those treated prenatally with sesame oil. This could indicate a difference in response to hunger or stress induced by food deprivation, and shows parallels with previous work showing that prenatal stress increases initial feeding behaviour in rats following a period of food deprivation, and adds to a body of literature suggesting that prenatal stress affects feeding behaviour in later life (Lesage et al., 2004; Pankevich, Mueller, Brockel, & Bale, 2009; Vallée, Mayo, Maccari, Le Moal, & Simon, 1996). The greater consumption of the females prenatally treated with testosterone may also be indicative of faster body development, though we did not take measurements of body weight at this time point and no differences were found between mice treated prenatally with testosterone and sesame oil at 20 days of age or in adulthood.

**Adulthood**

Among control male mice treated prenatally with sesame oil, castration resulted in a robust extended preference for demonstrated food, while testosterone replacement reduced duration of preference to levels of intact males (see Figure 5). Given that castrated mice treated prenatally with sesame oil showed a shift away from territorial aggression compared to intact mice in the STFP social interactions, but did not exhibit changes in dominance aggression, prolonged preference for demonstrated food in these mice might be related to an increase in willingness to cohabitate and share food resources with a familiar conspecific. Additionally, this improvement may be the result of a decrease in anxiety-like behaviour (discussed below), which is inversely related to recognition memory, associative memory, and spatial learning ability in
rodents (Darcet et al., 2014; Harrison, Hosseini, & McDonald, 2009). Accordingly, treatment with an anxiolytic facilitates social learning in gerbils, suggesting a direct relationship between reduced anxiety and social learning in rodents (Choleris et al., 1998). Additionally, this improvement following gonadectomy may indicate an impairing effect of testosterone or its metabolites on social learning among adult male mice. Given that the majority of hormone effects on social learning have been explored in female mice, it is difficult to put this explanation in context with existing literature.

Interestingly, prenatal treatment with testosterone extended preference for demonstrated food in gonadally intact male mice, while castration blocked social learning in mice treated prenatally with testosterone, and testosterone replacement was not sufficient to recover it. This suggests that the gonadal steroid effects in adolescence and adulthood depend on organizing action of testosterone and its metabolites among male mice, and our findings appear to reflect an improving effect of increased exposure to testosterone on social learning in gonadally intact males. Moreover, increased exposure to testosterone and its metabolites in early development also appears to foster a dependency on testosterone or its metabolites, such that the improving effect of castration observed in untreated mice is reversed, and social learning is blocked entirely in mice treated prenatally with testosterone. Furthermore, given that testosterone replacement was not sufficient to recover social learning in castrated mice, testosterone or its metabolites may exert further organizational actions during adolescence or early adulthood, such that improving effects of increased exposure to testosterone in early development depend on continuous exposure to testosterone into adult life. This is consistent with the notion that puberty is a second critical period for the organizational effects of gonadal steroid hormones, though previous
research has focused mainly on organizational effects of gonadal steroid hormones in puberty on reproductive and agonistic behaviours (reviewed in Romeo, 2003).

Ovariectomy reduced the duration of preference for demonstrated food in female mice treated prenatally with sesame oil, and preference for demonstrated food was not extended by estradiol replacement. This contradicts previous research in our lab which showed that preference for demonstrated food was prolonged among ovariectomized mice that received this dosage of estradiol benzoate delivered through silastic capsules made to the same specifications as the ones used in this study (Clipperton-Allen, 2011). However, ovariectomy blocked the STFP in female mice treated prenatally with testosterone, though social learning was recovered by estradiol replacement. This suggests that heightened exposure to prenatal androgens increases dependency on estradiol for social learning in female mice. Given that heightened exposure to testosterone during development has been shown to reduce estrogen receptor density, it is possible that local synthesis of estradiol in the brain is not sufficient to maintain social learning in ovariectomized mice (Kühnemann et al., 1995). Overall, these results are consistent with previous work that has shown the importance of estradiol for social learning in female mice (reviewed in Ervin et al., 2015).

Together, these findings indicate that prenatal treatment with testosterone results in greater dependency on gonadal hormones for social learning among both female and male mice, and that prenatal treatment with testosterone affects sensitivity to changes in steroid hormones in adulthood. Given that we did not find impairments in olfactory flavour discrimination among groups that did not show social learning, it is unlikely that these impairments are due to effects of prenatal or adult treatment on olfaction. It is also unlikely that our findings are the result of deficits in learning, given that all groups were able to distinguish between a novel and familiar
object in an object recognition task, which also indicates that this deficit is specific to social learning (Wasson, 2016).

We found differences in time spent investigating the demonstrator’s oronasal area, which is of particular interest due to the importance of transfer of food flavour information between the observer and demonstrator to the STFP (see Figure 19). Prenatal testosterone treatment increased time spent engaged in oronasal investigation in intact female mice, though this difference was eliminated among ovariectomized mice. It is possible that this increase in oronasal investigation among intact female mice treated prenatally with testosterone was a compensatory strategy that may have masked impairment in social learning.

We did not observe consistent effects of prenatal or adult treatment on feeding behaviour in female mice, which is inconsistent with the observed increase in body weight in adulthood. It is possible that this is due to our use of overnight food deprivation to promote feeding behaviour, and that previously reported effects of ovariectomy and estradiol replacement on feeding behaviour in rodents may relate to satiety (Tarttelin & Gorski, 1973). Alternatively, the weight gain resulting from ovariectomy may be due to changes in metabolic function, rather than a direct consequence of changes in feeding behaviour. It has been previously demonstrated that food intake returns to baseline levels over time in ovariectomized female rats, while body mass remains higher than in control animals (McElroy & Wade, 1987). In addition, previous work has suggested that changes in both body weight and feeding behaviour in female rats are consequences of changes in fat metabolism following ovariectomy (Wade & Gray, 1979).

Overall, we have shown that the role of gonadal hormones in mediating social learning behaviour in adulthood is dependent on organizational effects of gonadal hormones in early development. The increased dependency on estradiol for social learning observed in female
mice fits well with past research demonstrating the importance of estradiol and its receptors for social learning, and with literature showing that treatment with prenatal androgens decreases expression of estrogen receptors in female mice (Kühnemann et al., 1995). However, the role of gonadal steroids in mediating social learning in male mice has not been extensively studied. Our findings show that activational effects of gonadal steroids are affected by organizational effects of heightened exposure to testosterone or its metabolites in development. It has been previously demonstrated that androgens are involved in mediating spine density, mossy fiber expansion, and apical dendritic length in male rats (MacLusky, Hajsan, Prange-Kiel, & Leranth, 2006; Neil J. MacLusky et al., 2006, 2004; Mendell et al., 2016), and this region has been shown to be important for the effects of estrogentic facilitation of social cognitive behaviour in female mice (Gabor et al., 2015; Phan et al., 2015, 2011). It is possible that similar hippocampal androgenic and/or estrogentic mechanisms affect social learning or other social cognitive behaviours in male mice, making this region a promising target for future investigation.

**Social Interactions with a cage-mate in the Social Transmission of Food Preferences Test**

**Adolescence**

In adolescence, we observed neither sex differences, nor effects of prenatal testosterone administration on overall activity or non-social active or inactive behaviours during interactions with a familiar cage-mate during the STFP test. Given the effects seen in the resident-intruder task (discussed below), the lack of effects on activity in the STFP interactions might be due to reduced arousal in observer mice, possibly caused by the use of overnight food deprivation the night prior to testing. Short term food deprivation has been shown to increase general activity and activity in response to environmental stimuli in rats, suggesting that differences in activity between groups may have been masked by an overall increase in behaviour due to overnight
food deprivation (Campbell & Sheffield, 1953; Finger, 1951; Teghtsoonian & Campbell, 1960). In addition, interactions between familiar rodents tend to involve less aggression than interactions between unfamiliar animals, while rodents typically show greater interest in investigating unfamiliar conspecifics, suggesting that interactions with an unfamiliar conspecific may be more arousing than interactions with familiar conspecifics (Choleris et al., 2006; Engelmann, Wotjak, & Landgraf, 1995; Ferkin, 1988; Kareem & Barnard, 1982; Stefano Parmigiani & Brain, 1983).

Female mice engaged in more social investigation than male mice, which is consistent with what we observed in the resident-intruder test (discussed below), suggesting that adolescent female mice show greater interest in investigating conspecifics than male mice regardless of familiarity. Moreover, while sex or prenatal treatment did not affect duration of agonistic behaviour, frequency of agonistic behaviour was higher among male mice, likely due to the inclusion of attacks delivered and received, which are only scored in terms of frequency. Moreover, male mice exhibited more territorial aggression than female mice. Specifically, male mice spent more time engaged in open aggression and ritualized aggression, and both delivered and received more attacks than female mice. This is consistent with previous research showing that adolescent male rats engage in more rough-and-tumble play with familiar cage-mates than female rats (Meaney & Stewart, 1981; reviewed in Vanderschuren, Niesink, & Van Pee, 1997). However, where previous studies have shown that neonatal treatment with high doses of testosterone or DHT elicits male-typical play behaviour in female rats (Meaney & Stewart, 1981; Olioff & Stewart, 1978), the low-dose prenatal treatment regimen used in this study was not sufficient to elicit this behaviour in adolescent female mice. This pattern of aggressive behaviour has been previously observed in adult mice, and is consistent with what we observed in the
resident-intruder test, as well as with existing literature suggesting that overt aggressive behaviour emerges in adolescent male mice between 30 and 35 days of age (Clipperton-Allen, Cragg, Wood, Pfaff, & Choleris, 2010; McKinney & Desjardins, 1973; Svare & Gandelman, 1975).

Taken together, these findings demonstrate that sex differences in agonistic behaviour and social investigation in response to a familiar conspecific emerge during or prior to adolescence, and prenatal treatment with testosterone did not alter this behaviour in either male or female mice.

**Adulthood**

In interactions with familiar cage-mates, female mice spent more time engaged in active behaviour and social behaviours than males, while male mice engaged in non-social behaviours for longer than females, which is similar to what we observed in the resident-intruder test (discussed below) (see Figure 11, 12). Male mice also switched between non-social behaviours (“behavioural shifting”) more frequently than female mice, and gonadectomy reduced behavioural shifting in both sexes. These findings are indicative of greater arousal in male mice and reduced arousal in gonadectomized mice, and are consistent with what we, and others, have observed in the resident-intruder task with an unfamiliar conspecific (discussed below) (Clipperton-Allen et al., 2011, 2010).

Prenatal treatment with testosterone increased behavioural shifting among both male and female mice, suggesting an overall increase in arousal (see Figure 11). This increase in behavioural shifting was driven by higher frequency of engagement in non-social behaviour, and in particular, non-social locomotor activity and grooming behaviour among both male and female mice (see Figures 12 – 15). Interestingly, prenatal treatment with testosterone also
increased frequency of social behaviour among female, but not male mice (see Figure 17). Therefore, while increased exposure to prenatal androgens in early development increases behavioural shifting in general among adult female mice, this effect is specific to non-social behaviour in male mice. Moreover, prenatal treatment with testosterone resulted in a sex-specific increase in duration of non-social behaviour, and in particular, non-social locomotor activity among male mice. This was accompanied by a decrease in time spent engaged in social behaviour among male mice treated prenatally with testosterone compared to those treated prenatally with sesame oil, indicating that heightened exposure to androgens in utero shifts male mice towards a greater performance of non-social activity over social behaviour.

Furthermore, we found that female mice spent more time investigating the familiar demonstrator than males (see Figure 18), and, prenatal treatment with testosterone increased the frequency of social investigation among female mice, which indicates that heightened exposure to gonadal steroid hormones in early development may affect motivation to investigate a conspecific in female mice. Furthermore, gonadectomy reduced frequency of investigation of the demonstrator in both sexes, indicating that motivation to investigate a familiar conspecific is dependent on the activational effects of gonadal hormones in both sexes. This is consistent with research indicating that social investigatory behaviour is mediated by estradiol in female rodents and testosterone in male rodents (Tang et al., 2005; Thor, 1980).

In addition, prenatal treatment with testosterone reduced duration of agonistic behaviour, though the nature of this effect was dependent on both sex and adult treatment condition (see Figure 22). Interestingly, prenatal treatment with testosterone reduced dominance over the demonstrator among male mice, and castrated mice treated prenatally with testosterone in particular were strikingly submissive to the gonadectomized demonstrator (see Figure 25). This
reduction in dominance score appears to stem from a decrease in time spent engaged in dominance behaviour among male mice treated prenatally with testosterone.

Moreover, prenatal treatment with testosterone also reduced dominance behaviour in intact female mice, but not ovariectomized mice or mice that received estradiol replacement (see Figure 27). This suggests a possible interaction between organizational effects of heightened exposure to testosterone and its metabolites and the activational effects of progesterone, which was not replaced in mice that received estradiol replacement, and has been shown to attenuate aggression in female rats (Albert, Jonik, & Walsh, 1992; Erpino & Chappelle, 1971). Moreover, in contrast to the effects observed in castrated male mice, ovariectomized mice treated prenatally with testosterone were markedly dominant over the demonstrator due to a decrease in duration of agonistic behaviours received and an increase in frequency of agonistic behaviours delivered relative to ovariectomized mice treated prenatally with sesame oil. These findings suggest that testosterone or its metabolites exert sex-dependent effects during prenatal development, reducing hierarchical dominance behaviour among male mice, but reducing tolerance for hierarchical aggression from a conspecific among female mice. Interestingly, these effects were reversed (or partly reversed) in both intact female mice and ovariectomized mice that received estradiol replacement in the STFP interactions, but not in the resident-intruder task (discussed below).

Overall, female mice spent more time engaged in agonistic behaviour than males, and we did not find a difference in duration-based dominance scores between intact male and female mice (see Figure 22, 25). This is similar to what has been previously observed in interactions with unfamiliar intruders, but not with what we observed in the resident-intruder test (discussed below) (Clipperton-Allen et al., 2011, 2010). Male mice engaged in agonistic behaviour more frequently than female mice, likely due to the inclusion of reciprocal attacks, which are only
scored in terms of frequency, and typically occur almost exclusively in male rodents, though these differences were eliminated by gonadectomy (see Figure 23). Furthermore, only male mice engaged in ritualized and open aggression, and delivered attacks against the demonstrator (see Figure 26, 28), which is consistent with previous research, and with what we observed in the resident-intruder test (discussed below) (Beeman, 1947). Conversely, female mice exhibited agonistic behaviour primarily through dominance aggression, which is consistent with what has been previously observed in interactions in the STFP paradigm (Clipperton et al., 2008).

Ritualized aggression and delivery of attacks were reduced or eliminated among castrated mice but recovered by testosterone replacement. Furthermore, duration of ritualized aggression was higher among male mice treated prenatally with testosterone, which contrasts with the reduction in dominance behaviour. This suggests a shift from hierarchical aggression towards territorial aggression in interactions with a familiar conspecific as a result of heightened exposure to testosterone in early development.

Overall, these results are consistent with previous research that examined interactions with unfamiliar intruders, and suggest that female mice engage in as much or more agonistic behaviour than male mice overall, but their agonistic behaviour is more the dominant type, while male mice exhibit more territorial aggression than female mice, even in response to a familiar cage-mate. In male mice, increased exposure to testosterone in utero resulted in a shift away from hierarchical aggression and towards territorial aggression in response to a familiar conspecific. We did not see an increase in territorial aggression among female mice treated prenatally with testosterone, though ovariectomized female mice treated prenatally with testosterone were markedly dominant over the demonstrator, were less submissive, and delivered agonistic behaviour more frequently than ovariectomized mice treated prenatally with sesame
oil. This suggests that heightened exposure to prenatal androgens exerts sexually differentiated effects on agonistic behaviour in male and female mice. While male mice shift away from hierarchical aggression towards territorial aggression in response to a familiar cage-mate, exposure to elevated testosterone *in utero* causes female mice to be less tolerant of dominance aggression, though estradiol appears to be protective against this effect. These changes may indicative of less willingness to build and maintain hierarchical relationships, which facilitate cohabitation and are essential for social function in gregarious mammals (Bartolomucci et al., 2001; Miczek et al., 2007; Scott & Fredericson, 1951).

These effects on social and agonistic behaviour may have implications for interpretation of our findings on social learning (discussed above). OVX mice treated prenatally with testosterone showed blocked social learning, as well as reduced tolerance for dominance aggression from the demonstrator, suggesting that the observed impairment in the STFP may be related to reduced willingness to cohabitate with a familiar conspecific. In addition, it has been previously shown that subordinate mice are more likely to learn from conspecifics than dominant mice, suggesting that the marked dominance of OVX mice treated prenatally with testosterone over the demonstrator may have inhibited learning, though it should be noted that castrated male mice treated prenatally with testosterone were submissive to the demonstrator and also showed blocked social learning (Kavaliers, Colwell, & Choleris, 2005). Similarly, male mice treated prenatally with testosterone showed a shift away from dominance aggression towards territorial aggression, which indicates reduced willingness to share territory, and may explain the impairment in social learning observed in castrated mice and mice that received testosterone replacement that were treated prenatally with testosterone. However, given that prenatal treatment with testosterone improved social learning in intact male mice, continuous exposure to
testosterone appears to mitigate any potential negative relationship between impairments in social learning and dysfunctional establishment of dominance hierarchies.

**Resident-Intruder Test**

**Adolescence**

When interacting with an unfamiliar intruder, adolescent female mice were more active than male mice and spent more time engaged in social behaviour, though male mice spent more time engaged in both active and inactive non-social behaviours than females, which was manifested through more time spent engaged in horizontal and vertical activity, digging behaviour, solitary grooming behaviour, and solitary inactivity (see Figure 29, 30). This suggests that adolescent male mice show a greater inclination for non-social activity in the presence of an unfamiliar intruder, while female mice are more active overall, and spend more time engaged in social behaviour. Moreover, adolescent female mice spent more time engaged in social investigation, body and anogenital, than male mice (see Figure 30). This is consistent with the results of the STFP interactions, and suggests that female mice are more interested in investigating both familiar and unfamiliar conspecifics in adolescence.

There was no sex difference in the duration of agonistic behaviour in adolescent mice treated prenatally with sesame oil, which is consistent with previous research showing that adult female mice respond to a same-sex intruder with as much or more agonistic behaviour than male (see Figure 31) (Clipperton-Allen et al., 2011, 2010). However, a sex difference emerged in mice that received prenatal treatment with testosterone, such that male mice treated prenatally with testosterone exhibited increased agonistic and dominance behaviour. We did not observe a similar effect when mice interacted with a familiar demonstrator, suggesting that the organizational effects of testosterone and its metabolites drive the agonistic response to an
unfamiliar intruder in adolescent male mice, but do not affect interactions with a familiar conspecific. Alternatively, the differences between these findings may be due to age, given that the STFP paradigm was administered at 35 days of age, and the resident-intruder test was conducted later in adolescence, at 42 days of age (see Methods, Table 2).

Furthermore, consistent with the STFP interactions in adolescence, male mice engaged in more ritualized and open aggression than female mice, and delivered more attacks than female mice, regardless of prenatal treatment. These results are consistent with previous research, which has shown that territorial aggression in male mice emerges in early adolescence, between 30 and 35 days of age, coinciding with a spike in plasma androgens and the development of the testes (McKinney & Desjardins, 1973; Svare & Gandelman, 1975).

Taken together, these findings indicate that sex differences in non-social behaviour, agonistic behaviour, and social investigation in response to an unfamiliar conspecific emerge during or prior to adolescence. Furthermore, heightened exposure to testosterone or its metabolites exerts a sex-specific organizational effect on agonistic response, increasing dominance behaviour over an unfamiliar intruder in adolescent male mice. Interestingly, neonatal treatment with a high dose of testosterone propionate has been shown to masculinize rough and tumble play behaviour in adolescent female rats in response to an unfamiliar conspecific, but does not affect play behaviour in male rats, though rough-and-tumble play with familiar cage-mates has been shown to depend on the presence of neonatal androgens in male rats (Meaney & Stewart, 1981; Olioff & Stewart, 1978). Given that rough-and-tumble play in rats is thought to be pro-social in nature and is more highly expressed than in mice, the pattern of agonistic behaviour described here may not be indicative of play behaviour, and may instead be representative of emerging territorial aggression as suggested by previous work with adolescent
mice (McKinney & Desjardins, 1973; Power, 1999; Svare & Gandelman, 1975; Vanderschuren et al., 1997).

**Adulthood**

As in the STFP interactions, adult female mice spent more time engaged in active behaviour than male mice during interactions with an unfamiliar conspecific (see Figure 32). Male mice also exhibited more behavioural shifting than female mice, which is consistent with previous research, and with what we observed in the STFP interactions, and suggests greater arousal among male mice (Clipperton-Allen et al., 2011, 2010).

Similar to what we observed in the STFP interactions, male mice engaged in more non-social behaviour than female mice, and in particular, spent more time engaged in self-grooming and horizontal and vertical locomotor behaviour. In male mice, castration reduced active non-social behaviour and vertical locomotor behaviour, and increased social inactivity, which is consistent with previous research (see Figures 33 – 37) (Clipperton-Allen et al., 2011). Conversely, female mice that received estradiol replacement engaged in active non-social behaviours for less time than ovariectomized mice, though this is due to an increase in time spent engaged in social behaviour, and is not indicative of decreased activity. Moreover, female mice engaged in social behaviour for longer than male mice, and investigated the intruder for longer overall than male mice, which has been previously shown in our lab and is consistent with what we observed in the STFP interactions, and suggests that female mice show greater interest in investigating both a familiar or an unfamiliar conspecific than male mice (see Figure 38, 39, 40, 42) (Clipperton-Allen et al., 2011).

Dyads of female mice spent more time engaged in agonistic behaviour than male mice, which is consistent with the results of the STFP interactions, and with previous research showing
that female mice engage in as much or more agonistic behaviour than male mice (see Figures 43 – 50). However, we also found that male mice had higher dominance scores than female mice, which conflicts with both the results of the STFP interactions, and with previous research from our lab (Clipperton-Allen et al., 2011, 2010). Though overall duration of agonistic behaviour that occurred between dyads was higher among female mice, male mice delivered agonistic behaviour for longer and more frequently than female mice, while female mice received agonistic behaviour for longer and more frequently than male mice. Specifically, males spent more time engaged in dominance behaviour and ritualized aggression, and delivered more attacks than female mice, while in females, duration and frequency of submissive behaviour were higher than in males. However, negative dominance scores indicated that female mice were submissive to the OVX intruder across all prenatal and adult treatment conditions, indicating that differences between male and female mice may have been the result of behaviour of the intruder, and inconsistencies with previous research conducted in our lab using the same protocols make these results difficult to interpret (discussed below) (Clipperton-Allen, 2011; Clipperton-Allen et al., 2011, 2010).

Prenatal treatment with testosterone reduced the duration of agonistic behaviours, driven by a reduction in the duration of agonistic behaviour received by the resident, and specifically, in time spent engaged in submissive behaviour. However, these effects were specific to female mice when considering the frequency of these behaviours, suggesting that this effect was stronger in female mice. Among female mice, prenatal treatment with testosterone increased frequency-based dominance scores, decreased the frequency of agonistic behaviours received, and of submissive behaviour. We observed a similar effect on submissive behaviour among female mice in the STFP interactions, but it was specific to ovariectomized mice. This suggests
that the organizational effects of gonadal steroids interact with activational effects of estradiol in mediating the agonistic response to both familiar and unfamiliar conspecifics in female mice. While exposure to exogenous testosterone in early development reduced submissive behaviour in response to an intruder in female mice, estradiol masked this effect when the conspecific was familiar.

Furthermore, we observed effects of adult treatment condition on measures of agonistic behaviour only among male mice. Castrated mice had lower frequency-based dominance scores than intact mice and mice that received testosterone replacement. However, an increase in dominance scores in adult mice that received testosterone replacement was found only in control males treated prenatally with sesame oil. Furthermore, we found a reducing effect of castration on frequency and duration of agonistic behaviours delivered only in mice treated prenatally with sesame oil. In addition, only in males treated prenatally with sesame oil, we observed that testosterone replacement increased time spent engaged in dominance behaviour and ritualized aggression, though duration of ritualized aggression was decreased by castration regardless of prenatal treatment. We also found that castration decreased the number of attacks made against the intruder and this effect was reversed by testosterone replacement regardless of prenatal treatment. Previous studies have indicated that castration reduces aggression in male mice, while treatment with testosterone restores it (Beeman, 1947; Clipperton-Allen et al., 2011, 2010). The results presented here suggest that changes in measures of agonistic behaviour in response to changes in gonadal hormones in adulthood were blunted among male mice treated prenatally with testosterone.

Notably, female mice and castrated male mice had negative duration-based dominance scores, suggesting that they were submissive to the intruder. This contradicts previous research
conducted in our lab using the same protocol, and runs contrary to what we expected, given that resident mice typically win contests over intruders in their home territory, and that we used gonadectomized intruders which typically show minimal aggressive behaviour (Archer, 1998; Clipperton-Allen et al., 2011, 2010). Furthermore, these results conflict with what we observed in the STFP interactions with familiar demonstrators, which followed a similar protocol. It is unlikely that this discrepancy is due to inconsistencies in video scoring, given that a single observer blind to treatment condition scored all social interaction videos from testing in adulthood. Intruders were age matched to within 2 - 3 days of age to resident mice during testing in both adolescence and adulthood, making it unlikely that our results were due to differences in age between the resident and intruder. While we used as many unique intruders as possible to avoid repeated exposure to potential aggressive resident mice, the resident-intruder test was the final test performed in the behaviour test battery, and it is possible that these results occurred as a result of stress experienced by the experimental mice due to testing in other behavioural assays, or stress experienced by the intruders as a result of their use as stimulus mice in other behavioural assays. (see Methods - Table 2).

Overall, these results suggest that female mice are more motivated to engage in social investigation than male mice. Moreover, while total agonistic behaviour is similar between male and female mice presented with an unfamiliar intruder, female mice are more submissive to an unfamiliar intruder than male mice, though there may have been confounding effects of repeated testing in either experimental mice or stimulus mice used as intruders. Furthermore, exposure to exogenous testosterone in early development reduces submissiveness to an unfamiliar intruder, though this effect appears to be stronger in female mice. Among male mice, agonistic behaviour is dependent on testosterone, though exposure to exogenous testosterone in early development
appears to blunt changes in agonistic behaviour resulting from manipulation of gonadal hormones in adulthood. Finally, heightened exposure to testosterone in early development is not sufficient to elicit male-typical territorial aggression among female mice in response to an unfamiliar intruder.

Female mice did not deliver attacks or engage in open or ritualized aggression in response to a familiar conspecific or an unfamiliar intruder, regardless of prenatal treatment and adult treatment condition. Previous studies have shown that treatment with testosterone in adulthood elicits territorial aggression more readily from female mice when they have been treated prenatally with a high dose of testosterone, suggesting that prenatal treatment with testosterone interacts with testosterone, but not estradiol, in adulthood to promote territorial aggression in female mice (vom Saal et al., 1976). In addition, prenatal treatment with testosterone has been shown to elevate postpartum aggression in mice, though postpartum aggression is fundamentally different from the territorial aggression typical of male mice, in that it is less ritualized and is focused around inflicting damage to the intruder (Mann & Svare, 1983; S. Parmigiani, Brain, Mainardi, & Brunoni, 1988; S. Parmigiani, Ferrari, & Palanza, 1998; Svare & Gandelman, 1973). Collectively, these findings suggest that while heightened exposure to testosterone and its metabolites in early development sensitizes female mice to activational effects of testosterone in adulthood and elevates female-typical territorial aggression in lactating mice, prenatal treatment alone is not sufficient to elicit territorial aggression in female mice in response to either familiar or unfamiliar conspecifics.

In summary, the effects of prenatal and adult treatment condition on agonistic behaviour were dependent on both sex and familiarity of the conspecific. Exposure to exogenous testosterone causes male mice to shift away from hierarchical aggression towards territorial
aggression when interacting with a familiar conspecific, while OVX female mice treated prenatally with testosterone are less submissive to familiar conspecifics. This suggests that heightened exposure to testosterone in early development alters the agonistic response to a familiar conspecific, though estradiol seems to protect against this effect in female mice. Interestingly, in interactions with unfamiliar intruders, this reduction in submissive behaviour was observed in female mice regardless of adult treatment suggesting that estradiol may facilitate maintenance of dominance hierarchies with familiar mice, but not unfamiliar intruders.

**Dark/Light Test**

The dark-light test was intended as a control test of anxiety-like behaviour meant to facilitate interpretation of other behavioural measures. However, the results presented here have implications for our understanding of the role of organizational and activational effects of gonadal steroid hormones in mediating anxiety-like behaviour.

Gonadal hormones have been previously shown to exert activational effects on anxiety-like behaviour in both male and female rodents. In adult male mice, testosterone and DHT have been shown to rapidly reduce anxiety-like behaviour (Aikey, Nyby, Anmuth, & James, 2002). In female rodents, estrogens have been shown to exert anxiogenic effects at ERα and anxiolytic effects at ERβ, while systemic treatment with estradiol or progesterone produces anxiolytic effects (Lund, Rovis, Chung, & Handa, 2005; Picazo & Fernandez-Guasti, 1995; Spiteri et al., 2010; Walf & Frye, 2009). Furthermore, gonadal hormones have been shown to modulate activity of the hypothalamic-pituitary-adrenal (HPA) axis, which interacts reciprocally with the hypothalamic-pituitary-gonadal (HPG) axis, is involved in regulating the stress response, and is closely associated with anxiety and depressive disorders in humans (reviewed in Abelson, Khan, Liberzon, & Young, 2007; Pariente & Lightman, 2008). Specifically, estradiol has a stimulatory
effect on the HPA system in adult female rats, while testosterone has been shown to inhibit the
HPA response to stress in male rodents (Burgess & Handa, 1992; Handa et al., 1994; Viau &
Meaney, 1991, 1996). Moreover, the inhibitory effects of testosterone on the HPA response to
stress in male rats have been shown to depend on organizational effects of gonadal steroids in
both prenatal and neonatal development, which suggests that gonadal steroids exert
organizational effects in development that have implications for later life stress reactivity (C. M.
McCormick, Furey, Child, Sawyer, & Donohue, 1998; Cheryl M. McCormick & Mahoney,
1999). In addition, developmental exposure to high doses of testosterone propionate, synthetic
estrogenic compounds, or the anti-estrogen tamoxifen have been shown to increase in later life
anxiety-like behaviour in female rodents, demonstrating that gonadal steroids exert long lasting
effects on anxiety behaviour through organizational effects in early development (Min Hu et al.,
2015; Ryan & Vandenberghe, 2006; Zimmerberg & Farley, 1993).

**Adolescence**

In adolescence, prenatal treatment with testosterone resulted in greater anxiety-like
behaviour as indexed by reduced total light-side entries, greater latency to enter the light side of
the test chamber, and reduced time spent in the light side of the test chamber during the first
minute of testing (see Figure 51). Moreover, the effects of prenatal exposure to testosterone on
anxiety-like behaviour were more profound among male mice. Prenatal treatment with
testosterone reduced latency to enter the light side and light-side entries and during the first
minute of testing only in male mice, though female mice treated prenatally with testosterone did
show an overall reduction in light-side entries. It therefore appears that prenatal treatment with
testosterone has an enhancing effect on anxiety-like behaviour in adolescence, which affects
male mice more strongly than female mice.
Among mice treated prenatally with sesame oil, males made more light-side entries during the first minute of testing than female mice, though this difference was extinguished in mice treated prenatally with testosterone. While this may indicate that male mice are less anxious when first introduced to a new environment, the increase in light-side entries in the absence of an increase in time spent in the light side indicates that male mice made more rapid transitions between the light and dark sides of the chamber early in the test period, and could indicate reticence to remain in the light side. Female mice also exhibited more locomotor activity than male mice, which could be indicative of increased exploratory behaviour, and is consistent with the greater activity observed in female than male mice at adolescence in the resident-intruder test.

Therefore, although we did not find strong evidence for a sex difference in anxiety behaviour in adolescence, heightened exposure to testosterone or its metabolites seems to increase anxiety-like behaviour through organizational actions in early development, though these actions appear to exert a stronger effect in male mice. Interestingly, research investigating the effects of social stress in adolescent rats has indicated that isolation and introduction of new cage-mates increases basal levels of corticosterone and CRH mRNA, and reduces social interaction with familiar cage-mates (McCormick, Merrick, Secen, & Helmreich, 2007). Furthermore, social instability stress in adolescent male rats has been shown to increase anxiety-like behaviour in adulthood, and reduces time spent engaged in social interaction (Green, Barnes, & McCormick, 2013). Similarities between the social stress protocol and the experimental procedures implemented throughout the behaviour test battery in the present study indicate that we may have unintentionally introduced a stress component, which may explain the inconsistent results we observed in the resident-intruder test in adulthood. In addition, interactions between
the HPA and HPG axes may have affected steroid mediated agonistic behaviour, and in fact, previous research has associated aggression with anxiety-like behaviour in the light/dark test in male mice (Armario & Castellanos, 1984; Guillot & Chapouthier, 1996; Mastorakos, Pavlatou, & Mizamtsidi, 2006).

**Adulthood**

Overall, prenatal treatment with testosterone increased anxiety-like behaviour in adult male mice, but had no effect on females (see Figures 52 – 57). Among male mice, prenatal treatment reduced time spent in the light side of the test chamber and number of light-side entries during the first minute of testing, as well as overall light side entries. Conversely, female mice were resilient to these effects regardless of adult treatment condition, perhaps due to lower levels of aromatase activity, and consequently, reduced conversion of testosterone to estradiol, during early development compared to male mice. This suggests that greater exposure to testosterone or its metabolites exerts developmental actions in male mice that have implications for later-life anxiety behaviour, while female mice are protected. This contradicts previous research showing that exposure to high doses of testosterone propionate, anti-estrogens, and synthetic estrogenic compounds in early development affect later-life anxiety-like behaviour in female rodents (Min Hu et al., 2015; Ryan & Vandenbergh, 2006; Zimmerberg & Farley, 1993). Interestingly, male rats were previously shown to be protected against anxiogenic effects of prenatal treatment with high doses of testosterone propionate, indicating that sex-dependent vulnerability to prenatal testosterone exposure may be dose dependent (Zimmerberg & Farley, 1993).

Previous research has indicated sexually differentiated changes in anxiety behaviour and HPA disruption as a result of prenatal manipulations in male and female rodents. Some studies indicate that female rats exhibit greater elevations in anxiety-like behaviour and greater HPA
sensitization as a result of prenatal stress and prenatal exposure to ethanol than male rats (Bowman et al., 2004; Hellemans, Verma, Yoon, Yu, & Weinberg, 2008; Weinberg, 1988)

However, other research has found that males are more profoundly affected by prenatal stress in early gestation, and that CRF is increased in both male and female prenatally stressed rats, suggesting later life HPA dysregulation in both male and female rodents (Mueller & Bale, 2008; Weinstock, 2002). In addition, male rodents show impairments in social memory and enhanced aggression as a result of prenatal stress, while prenatal treatment with ethanol produces greater deficits in social interaction among male rats than in females (Hellemans et al., 2010; Kinsley & Svare, 1987; Souza et al., 2013; Wilson & Terry, 2013). Our findings suggest that female mice are more resilient to perturbations in steroid hormone milieu in prenatal development, while impairments in social learning and changes in social interactions show parallels with what has been observed in prenatally stressed rodents and rodents treated prenatally with alcohol.

Among male mice treated prenatally with sesame oil, castrated mice spent more time in the light side of the chamber, and entered the light side of the chamber sooner than intact mice, suggesting an anxiolytic effect of castration. Interestingly, previous research has indicated that acute treatment with testosterone is anxiolytic, suggesting that the role of testosterone in mediating anxiety behaviour depends on length or timing of exposure (Aikey et al., 2002). These findings suggest that testosterone or its metabolites may be involved in the regulation of anxiety-like behaviour, but that heightened exposure to testosterone in development blunts responsiveness to hormonal changes in adulthood, which is interesting given that the anxiolytic effects of testosterone in adulthood have been previously shown to depend on the presence of testosterone during the neonatal period in rats (McCormick et al., 1998). Our findings are consistent with findings suggesting that castration prior to puberty decreases anxiety-like
behaviour in male mice, though castration has been shown to enhance anxiety when performed after puberty in adult mice, suggesting that testosterone exerts further organizational actions in puberty that affect anxiety behaviour in adulthood (McDermott, Liu, & Schrader, 2012; Zuloaga, Jordan, & Breedlove, 2011). Furthermore, anxiety-like behaviour among female mice was unaffected by adult treatment condition, which contradicts previous results suggesting a role for estradiol in the regulation of anxiety behaviour in adult female rodents (Lund et al., 2005; Morgan & Pfaff, 2001; Spiteri et al., 2010).

Overall, female mice made more light-side entries than male mice both during the first minute of testing and over the entire course of the test, spent more time overall in the light side of the test chamber than male mice, and took less time to enter the light side of the test chamber than male mice. However, sex differences in anxiety were dependent on both prenatal treatment and adult treatment condition. Among intact mice treated prenatally with testosterone, male mice took longer to enter the light side of the chamber, made fewer light side entries and spent less time in the light side of the chamber than female mice, while intact male mice only exhibited greater latency to enter the light side of the chamber among control mice treated prenatally with sesame oil. This suggests that, while male mice exhibit more anxiety-like behaviour in the dark/light test, prenatal treatment with testosterone intensifies this difference. Though anxiety-like behaviour is generally accepted to be sexually dimorphic, differences between male and female rodents depend on the test used. Male mice typically show greater anxiety-like behaviour than female mice in the similar light/dark test – in which the animal is initially placed in the open, light side of the chamber – suggesting that the sex differences observed in our study are in line with existing literature (reviewed in Kokras & Dalla, 2014).
Overall, female mice seem to be resilient to the anxiogenic developmental action of heightened exposure to testosterone and its metabolites, and do not show a change in anxiety-like behaviour as a result of hormone manipulation in adulthood. Conversely, in male mice, greater exposure to testosterone or its metabolites acts in development to enhance anxiety behaviour in adulthood. In addition, castration in adolescence has an anxiolytic effect among male mice, which is not reversed by testosterone replacement, though this effect appears to be blunted among male mice treated prenatally with testosterone. Collectively, these results show that heightened exposure to testosterone exerts organizational effects on anxiety-like behaviour both in adolescence and adulthood, and that female mice are resilient to these effects.

**Estrous Cycle Effects**

Intact female mice in proestrus, when circulating estradiol is highest (Caligioni, 2009; McLean et al., 2012), exhibited a greater preference for demonstrated food flavour than mice in estrus during the first half of the STFP test. This adds to previous research indicating that preference for demonstrated food is prolonged in female mice in proestrus compared to those in estrus, and further highlighting the involvement of female sex steroids in the STFP (Choleris et al., 2011). Similarly, female mice show an advantage in social recognition in proestrus suggesting that other facets of social cognition may be enhanced in proestrus, while our findings suggest that prenatal treatment with testosterone may diminish this effect (Sánchez-Andrade & Kendrick, 2011).

Effects of estrous phase on agonistic behaviour in the social interaction tests were dependent on prenatal treatment and familiarity of the conspecific. When interacting with a familiar conspecific, female mice treated prenatally with sesame oil spent more time engaged in agonistic behaviour when in proestrus than when in diestrus, while mice in estrus were more
submissive than mice in proestrus and diestrus. This is consistent with previous research in female hamsters, which showed that aggression towards conspecifics was drastically reduced in estrus, when animals were more submissive and spent more time in lordosis (Floody & Pfaff, 1977). Conversely, among female mice treated prenatally with testosterone, mice in proestrus were less dominant over an unfamiliar intruder than mice in diestrus, received agonistic behaviour more frequently than mice in estrus and diestrus, and engaged in submissive behaviours more frequently than mice in estrus and diestrus. This is consistent with early research showing that prenatal treatment with high doses of testosterone suppresses estrus and reduces lordosis in guinea pigs (Goy, Bridson, & Young, 1964; Phoenix, Goy, Gerall, & Young, 1959). In addition, given that these effects were dependent on familiarity of the conspecific, these results may be related to the involvement of estrus cycle phase in affecting performance in social recognition (Sánchez-Andrade & Kendrick, 2011).

However, given the low number of mice in each phase of estrus, these results should be interpreted with caution.

Conclusions and Implications

We found that prenatal treatment with a low dose of testosterone propionate interacted with steroid hormone manipulations in adulthood to produce sexually differentiated effects on social learning, social interaction, agonistic behaviour, and anxiety-like behaviour. The results presented here have implications for our understanding of ASD, which is characterized in part by deficits in communication skills, difficulty engaging in appropriate social interactions, increased aggressive behaviour, and heightened anxiety (American Psychiatric Association, 2000; Loveland, Pearson, Tunali-Kotoski, Ortegon, & Gibbs, 2001; Moore & Calvert, 2000; White, Oswald, Ollendick, & Scahill, 2009). Elevated exposure to gonadal hormones in early
development has been associated with increased incidence of ASD, and a number of our findings mirror symptoms typical of the disorder (Auyeung et al., 2009; S. Baron-Cohen et al., 2014). Furthermore, our results have implications for our understanding of the underpinnings of anxiety disorders, and in particular, add to existing literature demonstrating the importance of maternal health as a determining factor for future susceptibility to mental illness (Bowman et al., 2004; Hellemans et al., 2008; McCormick et al., 2007; Weinberg, 1988). In addition, the present study adds to an existing body of research examining the role of gonadal steroids in mediating social learning behaviour and social reactivity to familiar and novel conspecifics (reviewed in Ervin et al., 2015).

We have shown that heightened exposure to testosterone in prenatal development sensitizes mice to impairments in social learning, which has implications for our understanding of learning impairments in ASD. Individuals diagnosed with ASD show difficulty learning from peers and teachers, which represents a significant problem for students diagnosed with ASD learning in a traditional classroom environment (Hobson & Lee, 1999; M. Moore & Calvert, 2000; Williams, Whiten, & Singh, 2004). Further investigation into interactions between organizational and activational effects of gonadal steroids in mediating social learning behaviour may lead to treatment targets that could help individuals with ASD function more effectively in the classroom and in other social environments.

Furthermore, our results indicate that male mice are less sociable than females, do not pay as much attention to social stimuli, and allocate more time to non-social activity than females. Interestingly, exposure to heightened prenatal androgens appears to shift male mice further towards a non-social behavioural phenotype, suggesting that these behaviours are further masculinized in male mice, but not in females. Additionally, prenatal treatment with testosterone
caused male mice to shift away from dominance aggression towards territorial aggression in interactions with familiar cage-mates, and prenatal treatment with testosterone reduced tolerance for dominance aggression from an unfamiliar intruder in females, though estradiol was protective against this effect when the conspecific was familiar. These changes in agonistic behaviour are suggestive of reduced willingness or ability to build and maintain a dominance hierarchy, which is an important aspect of adaptive cohabitation in social rodents, though estradiol may be protective against these effects (Miczek et al., 2007, 2001; Scott & Fredericson, 1951). Our findings show parallels with social deficits and inappropriate social behaviour typically associated with ASD, which lends credence to the theory that *in utero* elevations in gonadal steroids are a risk factor for ASD, and that autism represents a shift towards an extreme male behavioural pattern (Simon Baron-Cohen, Knickmeyer, & Belmonte, 2005; Loveland et al., 2001).

We found that male mice showed greater arousal when interacting with a conspecific than female mice, while heightened exposure to testosterone in development appears to increase arousal and reduce sociability in male mice. Moreover, we found that heightened exposure to testosterone in early development elevates anxiety-like behaviour in male mice, while female mice are resilient to this effect. This adds to a body of literature showing that effects of prenatal manipulations on later life anxiety behaviour tend to be sexually dimorphic (Bowman et al., 2004; Hellemans et al., 2010, 2008; Kinsley & Svare, 1987; Mueller & Bale, 2008; Souza et al., 2013; Weinberg, 1988; Weinstock, 2002; Wilson & Terry, 2013). Taken in context with previous work, our findings suggest that male mice are more sensitive than female mice to changes in gonadal steroid milieu during critical periods for masculinization and defeminization. Furthermore, anxiety disorders are often comorbid with ASD, which shows a strong male bias.
Therefore, it is possible that the suspected role of heightened intrauterine gonadal steroids in increasing vulnerability to ASD may also extend to increased susceptibility to comorbid anxiety disorders, especially among males. These findings also have implications for our interpretation of the results of our tests of social reactivity and social learning. Anxiety has been associated with decreased sociability in rats, while fear has been shown to potentiate aggressive behaviour in both rats and humans, suggesting that some of the changes in sociability and agonistic behaviour we observed in male mice treated prenatally with testosterone may have been related to increased anxiety (Marsee, Weems, & Taylor, 2007; Neumann et al., 2010). In addition, anxiety has been shown to impair recognition memory, associative memory, and spatial learning in rodents, while anxiolytic treatment has been shown to enhance social learning, suggesting that heightened anxiety may have contributed to impairments in social learning among male mice treated prenatally with testosterone (Choleris et al., 1998; Darcet et al., 2014; Harrison et al., 2009).

In summary, social learning, social interaction, and anxiety like behaviour are affected by interactions between organizational and activational effects of gonadal steroids. Male mice, in general, tend to be more profoundly affected by prenatal treatment with testosterone than female mice. In addition, we found responsiveness to activational effects of gonadal steroids depend on organizational effects in early development, suggesting that heightened exposure to gonadal steroids in development affects sensitivity to hormonal changes in adulthood. We observed a number of parallels between the effects of our prenatal and adult treatments and symptoms seen in ASD, which is consistent with the hypothesis that heightened intrauterine exposure to gonadal steroids increases vulnerability to ASD in humans (Baron-Cohen et al., 2014; Baron-Cohen et al., 2005). Furthermore, recent research has suggested that early inflammation and immune
response may be related to incidence of ASD. Given the anti-inflammatory and immune-modulating properties of sex steroids, it is possible that heightened exposure to testosterone and its metabolites in early development may affect vulnerability to ASD via related mechanisms (Angele et al., 1999; Angelidou et al., 2012; Depino, 2013; Malkin et al., 2004; Theoharides, Asadi, & Patel, 2013). In conclusion, our research has implications for the understanding of the role of gonadal hormones in mediating social behaviour and anxiety, and as risk factors for ASD and comorbid anxiety disorders.

**Limitations**

A major limitation of this study is that we were not able to verify that our low dose of testosterone propionate successfully increased exposure to androgens in our experimental litters. The results of the fecal analysis were highly variable, though analysis of plasma collected on the day of birth showed a slight, non-significant increase in testosterone among dams given injections with testosterone compared to those treated with sesame oil. It is unlikely that sufficient levels of testosterone persisted in pregnant mice up until the day of birth, though plasma testosterone may be useful in assessing the validity of prenatal treatment in the future. However, blood collection is inherently stressful, and the introduction of further stressors to experimental dams would introduce a prenatal stress component, which may interact with hormone manipulations and confound the results of behavioural and biological assays in offspring (Moore & Power, 1986; Sachser & Kaiser, 1996). A promising and non-invasive alternative approach to the assessment of gonadal steroid hormones in plasma is the use of ELISA to quantify these hormones in urine rather than plasma, which has been used and validated in rodent species at a range of developmental stages (deCatanzaro, Muir, Beaton, Jetha, & Nadella, 2003). In order to validate our prenatal treatment, further breeding pairs will be
created and subject to the same treatment regimen described here (see Methods). Dams will be sacrificed 6 hours after the final injection with testosterone propionate, and blood will be collected from both the dam and fetuses in order to determine whether our treatment was successful in increasing maternal testosterone, and whether any increase in maternal testosterone affected fetal testosterone.

Another limitation in our study was the use of intact mice as demonstrators and intruders during adolescence. Based on vaginal opening and estrus data, female mice approached sexual maturity earlier than expected, and it is therefore likely that age-matched stimulus mice had higher circulating levels of gonadal hormones during testing in adolescence than intended. Male mice in particular exhibited higher than expected territorial aggression in adolescence, which is consistent with literature suggesting that these behaviours emerge in male mice as early as 30 days of age (McKinney & Desjardins, 1973; Svare & Gandelman, 1975). Therefore, demonstrator and intruder mice were potentially more variable in adolescence than in testing in adulthood following gonadectomy, making it more difficult to draw direct comparisons between results from adolescence and adulthood (Archer, 1998).

Furthermore, given that female mice typically went through estrus prior to gonadectomy, and male mice showed overt aggressive behaviours typically thought to coincide with a spike in androgens during testing in adolescence, it is likely that we performed the gonadectomy surgeries after the onset of puberty (Ahima, Dushay, Flier, Prabakaran, & Flier, 1997; McKinney & Desjardins, 1973). The time delay between gonadectomy and hormone replacement surgery meant that gonadal hormones were likely absent among gonadectomized mice for at least part the proposed second organizational period, during which gonadal steroid hormones are thought to carry out further structural changes affecting behaviour in adulthood (reviewed in Romeo,
Therefore, although animals that received replacement with either estradiol or testosterone likely had normal circulating levels of these hormones in adulthood, their absence during this critical period may have resulted in non-typical adult behaviour in these mice due to removal of the gonads during puberty.

**Future Research**

Tissue collected during this experiment will be analyzed in order to assess the effects of our developmental and adult treatments on brain regions relevant to social learning, social recognition, aggression, and sociability in mice. This will not only allow us to examine the organizational and activational roles of gonadal steroids in mediating structural changes in the brain, but will also allow us to correlate these biological outcomes with the behavioural results reported here. Specifically, we are interested in quantifying steroid receptors in the ventral medial hypothalamus (VMH), medial amygdala, hippocampus, and medial prefrontal cortex (mPFC). The VMH has been shown to regulate aggression in mice, and gonadal steroid receptors are highly expressed in this region, suggesting that gonadal steroid activity in the VMH may be important for regulation of agonistic behaviour (Fuxjager et al., 2010; Lin et al., 2011; Merchenthaler, Lane, Numan, & Dellovade, 2004). Similarly, the medial amygdala affects aggression in both male and female mice via local aromatization of testosterone to estradiol, and is involved in social cognitive processes and anxiety behaviour (Ferguson et al., 2001; Spiteri et al., 2010; Unger et al., 2015). In addition, estrogenic mechanisms in the hippocampus are involved in social recognition in female mice, and administration of gonadal steroids in both developmental and adulthood has been previously shown to exert structural changes in this region (Gabor et al., 2015; MacLusky et al., 2006; MacLusky et al., 2006; Mendell et al., 2016; Phan et al., 2015, 2011; Roof & Havens, 1992; Scharfman et al., 2007). Moreover, the mPFC is
influenced by gonadal steroids and stress hormones, is involved in regulating both social and anxiety behaviour, and is under-responsive in individuals diagnosed with ASD (Barak & Feng, 2016; Kolb et al., 2012; Lee et al., 2016; Li, Nakajima, Ibañez-Tallon, & Heintz, 2016; Loveland, Bachevalier, Pearson, & Lane, 2008; Nakajima, Görlich, & Heintz, 2014).

Specifically, oxytocin receptors in the mPFC mediate sociosexual behaviour in female mice, and anxiety behaviour in male mice, suggesting that oxytocin receptors in this region may be particularly relevant to understanding the sexually different behavioural effects described here (Li et al., 2016; Nakajima et al., 2014).

Finally, while our lab has extensively investigated the role of estrogens and the estrogen receptors in mediating social learning in female mice, the role of the androgen receptor is unknown (reviewed in Ervin et al., 2015). Furthermore, the importance of gonadal hormones in facilitating social learning in male mice has not been investigated previously. Our findings suggest that testosterone is involved in mediating social learning, but that this effect is highly dependent on actions of gonadal hormones in early development. Future research should investigate if previous findings demonstrating the importance of estrogenic mechanisms in mediating social learning in female mice extend to male mice, and whether or not the androgen receptor is involved in the mediation of social learning behaviour in male or female mice.
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Appendix A

**Figure 58:** Turmeric preference ratio ± SEM. * Indicates a significant difference between turmeric demonstrated mice and thyme demonstrated mice. All mice showed social learning until at least 4 hours.

**Figure 59:** Consumption of turmeric and thyme ± SEM. There was no difference in consumption of turmeric and thyme, indicating that mice did not show a preference for either flavour (p = .56)
## Appendix B

### Behaviours Scored

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chasing the Intruder</td>
<td>The resident mouse actively follows, or pursues and chases the intruder.</td>
</tr>
<tr>
<td>Dominant Behaviour</td>
<td>The resident mouse is in control; includes pinning of the intruder, aggressive grooming, crawling over or on top, and mounting attempt.</td>
</tr>
<tr>
<td>Attacks Delivered</td>
<td>Physical attacks, including dorsal/ventral bites. Only the frequency of attacks was measured.</td>
</tr>
<tr>
<td>Ritualized Aggression</td>
<td>Physical attacks which include box/wrestle, offensive and defensive postures, lateral sideways threats and tail rattle.</td>
</tr>
<tr>
<td>Open Aggression</td>
<td>Physical attacks with a locked fight including tumbling, kick-away and counterattack where the attacker cannot be identified.</td>
</tr>
<tr>
<td>Avoidance of the Intruder</td>
<td>The resident withdraws and runs away from the intruder while the intruder is chasing.</td>
</tr>
<tr>
<td>Submissive Behaviour</td>
<td>The intruder mouse is in control; includes crawl under, supine posture (ventral side exposed), prolonged crouch, and any other behaviour in which the intruder is dominant (e.g., the intruder pins, aggressively grooms, etc., the resident).</td>
</tr>
<tr>
<td>Attacks Received</td>
<td>Physical attacks including bites to dorsal/ventral regions.</td>
</tr>
<tr>
<td>Defensive Upright Posturing</td>
<td>Species-typical defensive behaviour; upright with the head tucked and the arms ready to push away.</td>
</tr>
<tr>
<td>Social Inactivity</td>
<td>Includes sit/lie/sleep together.</td>
</tr>
<tr>
<td>Oronasal Investigation</td>
<td>Active sniffing of the intruder’s oronasal area.</td>
</tr>
<tr>
<td>Body Investigation</td>
<td>Active sniffing of the intruder’s body.</td>
</tr>
<tr>
<td>Anogenital Investigation</td>
<td>Active sniffing of the intruder’s anogenital region.</td>
</tr>
<tr>
<td>Stretched Approaches</td>
<td>Risk assessment behaviour; back feet do not move and front feet approach the intruder. Only the frequency of stretched approaches was measured.</td>
</tr>
<tr>
<td>Approach/Attend</td>
<td>Often from across the cage; the resident’s attention is focused on the intruder, head tilted toward the intruder and movements toward the intruder; this becomes “Chasing the Intruder” once along the tail or sniff if within 1.5 cm of the intruder.</td>
</tr>
<tr>
<td>Horizontal Activity</td>
<td>Movement around the cage; includes active sniffing of air and ground.</td>
</tr>
<tr>
<td>Vertical Activity</td>
<td>Movement to investigate upwards, both front feet off the ground; includes sniffing, wall leans and lid chews (less than 3).</td>
</tr>
<tr>
<td>Digging</td>
<td>Rapid stereotypical movement of forepaws in the bedding.</td>
</tr>
<tr>
<td>Abnormal Stereotypies</td>
<td>“Strange” behaviours, including spin-turns, repeated jumps/lid chews/head shakes (more than 3).</td>
</tr>
<tr>
<td>Solitary Inactivity</td>
<td>No movement; includes sit, lie down and sleep.</td>
</tr>
<tr>
<td>Self-Grooming</td>
<td>Rapid movement of forepaws over facial area and along body.</td>
</tr>
</tbody>
</table>
**Composite and Grouped Behaviours**

<table>
<thead>
<tr>
<th>Description</th>
<th>Behaviours Included</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Activity</td>
<td>All behaviours involving activity.</td>
</tr>
<tr>
<td>Total Social Behaviour</td>
<td>All social behaviours.</td>
</tr>
<tr>
<td>Agonistic Behaviour Delivered</td>
<td>Follow Intruder, Dominant Behaviour, and Attack Delivered.</td>
</tr>
<tr>
<td>Agonistic Behaviour Received</td>
<td>Avoid Intruder, Submissive Behaviour, Attack Received, and Defensive Upright Posturing.</td>
</tr>
<tr>
<td>Total Agonistic Behaviour</td>
<td>This composite behaviour represents the overall levels of agonism present in the resident-intruder interactions and does not indicate the direction of the agonistic behaviour (i.e., whether agonistic behaviour is directed toward the resident or toward the intruder).</td>
</tr>
<tr>
<td>Dominance Score</td>
<td>Total agonistic behaviour delivered minus total agonistic behaviour received. A negative score indicates that the resident was the submissive animal in the pair, while a positive score signifies that the resident was the dominant animal.</td>
</tr>
<tr>
<td>Social Investigation</td>
<td>Oronasal Investigation, Body Investigation, Anogenital Investigation, Stretched Approaches, and Attend To/Approach Intruder.</td>
</tr>
<tr>
<td>Non-Social Behaviour</td>
<td>Horizontal Exploration, Vertical Exploration, Dig, Stereotypies, Inactive Alone, and Self-Groom.</td>
</tr>
</tbody>
</table>

Modified from Ervin et al., 2014 and Clipperton-Allen et al., 2011