Devaluation of Sucrose Caused by Social Instability Stress in Adolescent Male Long-Evans Rats in the Presence of an Unfamiliar Peer

Racheal Herlehy

Psychology

Submitted in partial fulfillment

of the requirements of

Master of Arts

Faculty of Social Sciences, Brock University

St. Catharines, Ontario

Abstract

Rats that undergo the Social Instability Stress procedure during adolescence (SS: daily 1-hour isolation + re-pairing with an unfamiliar cage partner for 16 days) display changes in reward-related behaviour. Specifically, SS rats spend less time in social interaction but more time in social approach compared to controls, indicative of an altered social repertoire; SS males also show increased aggression when competing for access to sweet substances. To investigate to what extent SS influences choice behaviour when social and sweet rewards are presented simultaneously, a Social Discounting test was conducted. The SS procedure was administered during either adolescence or adulthood to both male and female rats to investigate sex differences and to determine if SS effects were specific to administration during adolescence. Results showed that increasing concentrations of sucrose (0%, 2%, 5%, 10%) had no influence on time spent near a novel peer during the Social Discounting choice test, but rats drank less of 5% sucrose when in a social condition relative to when drinking alone. The only stress effect to emerge was in adolescent-stressed males tested immediately after the stress procedure; SS adolescent males spent significantly less time drinking sucrose overall compared to controls, indicative of a stress-induced anhedonia. The stress-induced devaluation of sucrose was not long-lasting as it was not found in adolescent males tested after a delay. Thus, Social Instability stress produces short-lasting behavioural changes in reward processing only in adolescent male rats.

Acknowledgments

First and foremost, I want to thank Dr. Cheryl McCormick for all the support and encouragement she's given me. Her expertise has been invaluable and being able to learn from her has been one of the best parts of the last few years. I can't remember ever learning and growing so much in the same amount of time, and I could not have done it without her.

I also want to thank my committee members, Dr. Teena Willoughby and Dr. Cameron Muir for all their support and feedback.

I want to thank all the graduate students in the lab (especially Marina, Akif, and Shealin) for all their help over the years and always being willing to listen as I think out loud. I specifically want to thank Pardis for all her support and for being such a great officemate. I also specifically thank Jennet for always being willing to help, for all the things she's taught me, and for being a friend.

I also want to thank the Brock animal facility's care staff for taking care of all the rats used as subjects in this thesis. I am also grateful to the rats themselves; they're curious and intelligent animals and deserve appreciation.

Lastly, I want to acknowledge the other MA students in the 2018-2020 cohort. The time, understanding, and friendship that was shared in our group made Brock University feel like home. I wouldn't have it any other way.

Table of Contents

List of Tables

List of Figures

Abbreviations

CORT – corticosterone

CPP – conditioned place preference

CTL – control

D1 – dopamine type 1 receptor

D2 – dopamine type 2 receptor

HPA – hypothalamic-pituitary-adrenal

PND – post natal day

SS – Social Instability Stress

VTA – ventral tegmental area

Introduction

General Introduction

Adolescence is a period of neural maturation during which different systems undergo development, including brain regions involved with social behaviour and with reward (Juraska & Markham, 2004; Juraska & Willing, 2017; Teicher, Andersen, & Hostetter, 1995). Adolescents have a prolonged release of adrenal glucocorticoids in response to a stressor compared to adults and glucocorticoids have been shown to have remodelling effects on various brain regions (Fuchs, Flugge, & Czeh, 2006; Romeo, Patel, Pham, & So, 2016). The combination of a prolonged exposure to glucocorticoids and ongoing adolescent neural development has led to the hypothesis that adolescents are particularly vulnerable to stressors (Fuhrmann, Knoll, & Blakemore, 2015). The Social Instability Stress (SS) procedure (16 days of 1 hr isolation + re-pairing with a new cage partner) produces alterations in social behaviour and motivation for sweet rewards under conditions of competition when administered to adolescent male rats (Cumming, Thompson, & McCormick, 2014; Green, Barnes, & McCormick, 2013). A decrease in social interaction was recently observed in adolescent SS female rats as well (Asgari, 2020), but the full extent of how adolescent SS changes the social repertoire of female rats is unknown. This thesis experiment compared sucrose and social rewards against each other to determine how SS affects reward processing when both rewards are presented together. Although a main hypothesis is that adolescents are more vulnerable to stressors than are adults, an adult-stress control group has been infrequently used in previous SS experiments. One study that did use an adult-stress group in addition to adolescent-stress found a stress difference in response to the psychostimulant amphetamine in adolescent-stressed, but not adult-stressed rats (McCormick, Robarts, Kopeikina, & Kelsey, 2005). In the current experiment, the Social Instability Stress

procedure was administered to both adolescent and adult, male and female rats to determine if any SS effects on response to sucrose and social reward are unique to the adolescent period and to explore any sex-differences in how SS affects reward preference.

Adolescent Period

Adolescence is a transitional period from childhood to adulthood. The adolescent period in humans is marked by neural maturation and behavioural changes. Throughout the human brain, white matter (myelination of axons) increases linearly with age during adolescence and grey matter (cell bodies and dendrites) reaches peak volume before adolescence then decreases during the adolescent period (Giedd et al., 1999, 2015). Behaviourally, the social preferences of adolescents shift to spend less time with family and more time with peers, and adolescents rate spending time with peers to be important to them (Palmonari, Pombeni, & Kirchler, 1990). Human adolescents are also typically described as impulsive risk-takers, and health risk behaviours increase during the adolescent period (Hooshmand, Willoughby, & Good, 2012).

Generally, adolescence in rats is said to begin sometime between post-natal day (PND) 21 and 30 and end on post-natal day 59, with adulthood beginning on PND 60 (Eiland & Romeo, 2013; Tirelli, Laviola, & Adriani, 2003; Wulsin, Wick-Carlson, Packard, Morano, & Herman, 2016). This range is sometimes subdivided into three stages: early/pre-pubertal (PND 21-27 to 34), mid/pubertal (PND 34 to 46), and late/post-pubertal (PND 46 to 59), (Tirelli et al., 2003; Wulsin et al., 2016). Puberty occurs during adolescence and is when sexual reproduction becomes possible (Sisk & Zehr, 2005). The onset of puberty as defined by physiological markers occurs in rats during mid adolescence: PND 35 for females and between PND 42 and 45 for males (Drzewiecki, Willing, & Juraska, 2016; McCormick, 2010). Neuroendocrine changes

occur around the onset of puberty, predominately an increase in gonadal hormones (Parker & Mahesh, 1976; Romeo, 2010; Sisk & Zehr, 2005).

Adolescent brain development in rats is similar to that in humans (Juraska & Markham, 2004). Both the decrease in grey matter (Andersen, 2003) as well as the increase in white matter volume (Juraska & Markham, 2004) that is found in human adolescents is also found in rats. This decrease in grey matter in both species is likely due to ongoing synaptic pruning and apoptosis (Eiland & Romeo, 2013). A longitudinal neuroimaging study done in rats found that metabolism increased in the hippocampus and decreased in the striatum and frontal cortex between adolescence and adulthood (Choi et al., 2015), suggesting ongoing functional maturation during the adolescent period.

Adolescent rats share a number of behavioural features with their human counterparts. They show increased impulsivity in comparison to adults. For example, in one study, adolescent rats chose to accept a smaller, immediate reward rather than wait for a larger, delayed reward during most trials whereas adult rats waited for the larger reward in more than half of the trials (Sonntag et al., 2014). They are also more exploratory than are adults and have a larger preference for novelty than adults do (Stansfield & Kirstein, 2006). Adolescent rats also have increased sociability compared with adults and spend more time interacting with peers than do adults (Perkins et al., 2016).

Social Behaviour in Adolescent Rats

The formation and maintenance of complex social relationships is required for social animals to live together successfully (Himmler, Himmler, Pellis, & Pellis, 2016). Individuals must possess an appropriate behavioural repertoire to navigate their social environment. Play fighting allows juveniles to practice and develop social skills with a low risk of the play

escalating to an aggressive fight (Himmler et al., 2016). In domesticated lab rats, adolescents engage in more play fighting than do adults (Douglas, Varlinskaya, & Spear, 2004). The type of play behaviour that rats engage in changes as they age, becoming less spontaneous and more directed (Meaney & Stewart, 1981). Play behaviour is first evident between PND 15 and 16, peaks in mid-adolescence, and then declines into adulthood (Pellis & Pellis, 2007; Thor & Holloway, 1984). Male rats are especially playful; they engage in more play behaviour than do females (Auger & Olesen, 2009; Meaney & Stewart, 1981) and in general spend more time in social interaction than do females (Asgari, 2020). Rats have a preference for social novelty; they prefer to be near and to engage in play behaviour with an unfamiliar peer over a familiar one (Cirulli, Terranova, & Laviola, 1996). This preference is used in social novelty preference test to determine preferences for social investigation (Smith, Wilkins, Mogavero, & Veenema, 2015).

In addition to play, social experience in general during adolescence is required to form the knowledge of appropriate social activities and reactions; disruption of this learning has lasting effects. Social isolation increases motivation for social interaction, and this motivation increases as the time in isolation increases (Niesink & Van Ree, 1982). Rats isolated during their adolescent period and deprived of social play show both greater anticipation for, and time spent in, social interaction compared with non-isolated rats (Panksepp & Beatty, 1980; Van Den Berg et al., 1999). This effect seems to be specific to the adolescent period as rats isolated earlier (PND 15-24) do not differ in their play behaviour compared with rats that were not isolated (Ikemoto & Panksepp, 1992). Although play behaviour starts in rats before the adolescent period, this period after weaning is when these social changes as a result of isolation will occur.

Social Brain Development During Adolescence

The social brain refers to a number of different regions and the connections between them that are responsible for different aspects of play behaviour (Hodges & McCormick, 2019; Pellis & Pellis, 2007). The act of play fighting is a complex behaviour and involves motivation for social interaction, executive functioning to interpret and respond appropriately to the play partner, and the physical motions to execute the play behaviour (Himmler et al., 2016). Processing social information recruits a range of brain regions, including the accessory olfactory bulb for peer identification through olfactory cues, the amygdala and prefrontal cortex to assign salience and meaning to the cues, the ventral tegmental area and nucleus accumbens for social motivation, and the hypothalamus and motor pathways to enact social behaviour (Insel & Fernald, 2004). The neuropeptides vasopressin and oxytocin are important for social recognition (Insel & Fernald, 2004; Veenema & Neumann, 2008).

The prefrontal cortex is an important region for executive functioning and modulating social behaviours (Vanderschuren, Achterberg, & Trezza, 2016). The prefrontal cortex undergoes maturation during the adolescent period, with white matter increasing during adolescence and into adulthood (Juraska & Markham, 2004) and overall volume peaking and then decreasing during adolescence (Juraska & Willing, 2017; van Eden & Uylings, 1985). Dendritic pruning during adolescence is also found in the amygdala, nucleus accumbens, and hypothalamus (Crews, He, & Hodge, 2007).

The Dopaminergic System and Reward Preference

The dopaminergic system is the most well-understood mechanism for reward processing (see Bromberg-Martin, Matsumoto, & Hikosaka, 2010 for review). Dopamine is synthesized from the amino acid tyrosine. Five subtypes of dopamine receptors are known, and these are

divided into two families of receptors: D1 and D2. There are a number of dopaminergic pathways that are implicated in a wide range of functions. The mesocorticolimbic pathway consists of both the mesolimbic pathway and the mesocortical pathway and is comprised of projections originating from the ventral tegmental area (VTA). Both these pathways are involved in predicting future rewards and modulating motivation and reward-seeking behaviours (Bromberg-Martin et al., 2010; Kapur & John Mann, 1992). These pathways have been implicated in substance abuse disorders, schizophrenia, and Parkinson's disease (Kapur & John Mann, 1992).

The mesocortical pathway projects from the VTA to the prefrontal cortex and is implicated broadly in executive functioning. The mesolimbic pathway consists of dopaminergic neurons in the VTA that project to the nucleus accumbens. This pathway is responsible for both reward and aversion motivations (Bromberg-Martin et al., 2010). The mesolimbic pathway is involved in motivation for food, sex, and drug rewards and nucleus accumbens dopamine mediates social play behaviour (Manduca et al., 2016; Salamone & Correa, 2012).

Age and Sex Differences in Reward System

The dopaminergic system undergoes development throughout adolescence and into adulthood. The density of dopaminergic fibers in the prefrontal cortex increases during the adolescent period, between PND 20 and 60, with little change in density found after PND 60 (Kalsbeek, Voorn, Buijs, Pool, & Uylings, 1988). The striatum undergoes pruning of D1 and D2 receptors during late adolescence and into early adulthood, whereas the nucleus accumbens shows a rise in both D1 and D2 receptor density in early- to mid-adolescence, which remain steady into adulthood (Teicher et al., 1995). This effect is sex-dependent; only males show receptor overproduction and subsequent pruning in the striatum. Female receptor density remains

steady in the striatum, but both females and males show similar trajectories in the nucleus accumbens (Andersen, Rutstein, Benzo, Hostetter, & Teicher, 1997). Further investigation has revealed that this sex difference is not driven by pubertal hormones (Andersen, Thompson, Krenzel, & Teicher, 2002), suggesting it may be the organizational effects of gonadal hormones and/or genetics driving this sex difference in dopamine receptor development.

Natural Rewards

Natural rewards (e.g., food, sex) are evolutionarily important for survival, and these activate the mesolimbic dopamine system (Kelley & Berridge, 2002; Olsen, 2011). Drugs of abuse, although not natural rewards in that they are not required for survival, hijack the reward system and act in a similar manner. Food and sweet substances are commonly used as rewards in behavioural testing (Tzschentke, 1998). Sweet and fatty foods are especially rewarding (Olsen, 2011), as sugar provides immediate energy and fat can be stored for long-term metabolic energy, and rats prefer sweet foods over non-sweet foods (Boyer, Cross, & Anderson, 1974). Rats will form a preference for a chamber that has been associated with a sweet reward even when the drink is not present in the chamber (Ågmo, Galvan, & Talamantes, 1995). This task is called Conditioned Place Preference (CPP): one chamber in an apparatus is paired with an unconditioned stimulus and a place preference is formed when the animal later demonstrates an associated preference for that chamber when the stimulus is no longer present. This preference is based on the assumptions that the test animal can remember the stimuli, distinguish between the two chambers (they have different visual and tactile cues), and is motivated to approach the stimulus. Deficits in memory and changes to motivation level can be measured using the CPP test.

The extent to which something is perceived as a reward can depend on developmental stage. Preference for sweetened condensed milk is higher in adolescence compared with adulthood, peaking in late adolescence at PND 50 (Friemel, Spanagel, & Schneider, 2010). There is evidence to suggest that social interactions are more rewarding in adolescence than in adulthood in rats (Foulkes & Blakemore, 2016) as well as in humans (Dreyfuss et al., 2014; Foulkes & Blakemore, 2016).

Males and females have been found to differ in sucrose preference, but this difference emerges with different methodologies (Sclafani, 1987). In a 24 hour two bottle test (rats are allowed to choose between a bottle of sucrose and a bottle of another solution, here water or polycose, a carbohydrate), no difference is found between males and females in intake, but females will consume more sucrose than males when testing is shorter (Sclafani, 1987). Using saccharin, which is more sweet than sucrose, females drank more than males even during the 24 hour bottle test (Valenstein, Cox, & Kakolewski, 1967). Females will consume more saccharin after puberty and this is when the sex difference emerges (Wade & Zucker, 1969). Gonadectomy reduced saccharin preference in females but not males (Zucker, 1969), suggesting the increased preference for sweet substances is driven by gonadal hormones in females, with little influence on male's preference.

Social Interaction as a Reward

Rats have a strong motivation for social contact throughout their life, and this natural preference is used as an incentive in some behavioural lab tasks (Van Den Berg et al., 1999). Because of heightened social play during the adolescent period, there have been investigations to determine whether the value of social reward is heightened during the adolescent period compared to with adulthood. Similar to with sweet reward, adolescent rats will form a place

preference for a chamber that was previously paired with an active play partner (Calcagnetti & Schechter, 1992). To determine whether the presence of a peer is driving the place preference or whether it is specifically the ability to play with a peer that is critical to forming a place preference, a drug was given to the play partner to block them from responding to and initiating play behaviour without impairing locomotor activity. No place preference was formed for the chamber with the non-playing peer, suggesting that it is the play behaviour that is rewarding (Calcagnetti & Schechter, 1992).

The day-to-day social living context determines motivation for social play in adolescents, with social isolation increasing social behaviours relative to group-housed rats (Douglas et al., 2004; Vanderschuren, Stein, Wiegant, & Van Ree, 1995). During conditioning to CPP, Douglas et al. (2004) found that adolescent rats housed alone spent more time play fighting than did adolescent rats that were group housed. Isolated adult rats did not show an increase time in play fighting, but spent more time in social investigation than did group-housed adult rats (Douglas et al., 2004). That isolates of both ages spent more time in social behaviours than did group-housed rats points to social interaction being rewarding during both adolescence and adulthood, with play behaviour particularly rewarding during adolescence. Results from the CPP test showed that adolescent rats formed place preference for the social chamber, and this preference was heightened for isolation-housed male rats compared to both group-housed and female rats (Douglas et al., 2004). Trezza, Damsteegt, & Vanderschuren, (2009) also found a significant preference for social CPP for adolescent isolation-housed rats, as well as a trend towards significance for rats that were isolation-housed for a few hours each day. Even temporary isolation housing appears to enhance motivation for social interaction.

Overlap and Distinctiveness of Reward System and Social Brain

The mesolimbic system is involved in modulating motivation towards both sweet and social rewards. Dopamine activity increases during social interaction (Robinson, Heien, & Wightman, 2002) and dopamine projections from the ventral tegmental area to nucleus accumbens modulate social play (Gunaydin et al., 2014; Manduca et al., 2016). Administering drugs that elevate extracellular levels of dopamine (methylphenidate, amphetamine, and apomorphine) directly into the nucleus accumbens increases play behaviour (Manduca et al., 2016). However, it has been found repeatedly that administering these drugs into the body decreases play behaviours and inhibits formation of social CPP (Trezza et al., 2009; Young, Gobrogge, & Wang, 2011), thus dopamine increasing play behaviour is a local effect specific to the nucleus accumbens. Dopamine has also been found to increase motivation for social play (Achterberg et al., 2016).

Dopamine does not seem to be involved in the rewarding properties of sweet reward but it is required to form associations between the reward and environmental cues (Wise, 1989). Blocking dopamine receptors prevented place preference to be formed with 18% sucrose, but did not alter sucrose consumption (Ågmo et al., 1995). The pleasurable "liking" aspect of sweet rewards appears to be primarily modulated by opioid neurotransmitters in the nucleus accumbens, with dopamine pathways responsible for the "wanting" incentive salience of natural rewards (Kelley & Berridge, 2002).

Similar to with sweet rewards, opioids have been found to increase the hedonic "liking" aspects of social play (Trezza, Baarendse, & Vanderschuren, 2010), and also the motivational "wanting" of social play in adolescent rats (Achterberg, van Swieten, Houwing, Trezza, & Vanderschuren, 2019). Specifically, opioid receptor binding occurs during social interaction

(Panksepp & Bishop, 1981), and opioids in the nucleus accumbens modulate investigation of a novel peer (Smith, Wilkins, Li, Tulimieri, & Veenema, 2018).

Adolescence as a Sensitive Period

A centuries-old question is how a newborn brain becomes a specialized adult brain: is the functionality of the adult brain an innate characteristic from birth or does it form from experiences over the lifespan? It is now known that specialized developmental processes form from a combination of genetic predisposition and environmental experience. The combination of "nature" and "nurture" during childhood and adolescence form what will become adult brain specificity. The theory of neuroconstructivism states that brain regions are biased early in life to become specialized for different functions and emerge over development based on interactions between neural maturation and environmental context (Karmiloff-Smith, 2015).

Given the ongoing development of the reward system and the areas and systems associated with the social brain, the development of these regions are highly malleable in adolescence through environmental experience. Because of this neural malleability and the behavioural changes evident during adolescence, it has been posited that adolescence is a sensitive period of development (Fuhrmann et al., 2015). Individuals encounter a wide range of differences during childhood, such as differences in socioeconomic status, cultural customs, the occurrence of adverse childhood events such as abuse, and access to education. It is possible that a period of sensitivity following childhood provides preparation for future responses while the brain is still maturing based on the individual's environment (Andersen, 2003). Specialized regions can then become "wired" to respond to certain stimuli in a manner that is adaptive in the current environment, but maladaptive in different environments. In recent decades, researchers have advocated for further investigation into the adolescent period to further understand

environmental conditions that may cause long-lasting changes in normal brain development (Hamburg & Takanishi, 1989). One condition that can alter neural development is stress.

What is Stress and When is it Negative

Stress is a state that is entered when homeostasis (a steady state of equilibrium) is perceived to be threatened (Chrousos, 2009). The idea of stress as a reactive measure to an adverse stimulus was first formally conceptualized by Hans Selye in the mid 1930's (Selye, 1936). Stimuli that create stress can be physical or psychological, and once the stress response is triggered, causes both behavioural and physical effects (see next section) that attempt re-establish homeostasis.

Stress is not always a negative experience; dose response to stress is commonly portrayed as an inverted U-shaped curve. Too low or too high levels of stress are termed distress and are deemed to be negative stress. In the middle of the inverted curve is the optimal level of stress, termed eustress. Although too much stress is generally thought of as the sole cause of distress, too little of a stress response can lead to an individual failing to react appropriately to threatening conditions. For example, an excess of stress when seeing a snake may lead to a panic attack, whereas a lack of stress may lead to the individual approaching the snake and being bitten. An optimal level of stress will lead to the individual moving a safe distance away from the snake.

Stress can be acute (short-lived) or chronic (long-lasting). These terms apply to how long a stressor is present and exerts its primary influence, not how long any changes produced by the stressor last. Both acute and chronic stressors could have relatively short- or long-lived effects. Different types of stressors exist; some are physical, some psychological, some are produced by changes in the environment, and are some produced only by perceived changes. One type of stressor – social stress – is detailed in a later section.

Physiological Response to Stressors

A common mechanism studied is the activation of the hypothalamic-pituitary-adrenal axis (HPA axis) in response to a perceived stressor. When a threat is perceived, corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) are released from the paraventricular nucleus of the hypothalamus. When these reach the anterior pituitary, adrenocorticotropic hormone (ACTH) is released into the bloodstream. The adrenal cortex then produces and secretes glucocorticoids. The control of the HPA axis is partially regulated through negative feedback by glucocorticoid receptors (GRs) and mineralocorticoid receptors (MRs) by glucocorticoid binding which modulates the subsequent release of CRH and ACTH (Lupien, McEwen, Gunnar, & Heim, 2009). These receptors are located throughout the brain, including the hypothalamus, pituitary, frontal cortex, hippocampus, and amygdala, amongst other areas.

Glucocorticoids are implicated in a wide range of neural functions, such as synaptic growth, apoptosis, and glutamate release (McCormick & Hodges, 2017; Popoli, Yan, McEwen, & Sanacora, 2012), and excessive exposure to glucocorticoids can disrupt these processes (Fuchs & Flügge, 1998; Woolley, Gould, & McEwen, 1990). Since stress results in the release of glucocorticoids, stress exposure can have remodeling effects on brain structures (Fuchs, Flugge, & Czeh, 2006). In rats, stress has been found to alter the structure of the prefrontal cortex, hippocampus, and amygdala (Eiland & Romeo, 2013).

Sex and Age Differences in HPA Axis Response

In rats, females generally have higher concentrations of the main glucocorticoid corticosterone both at baseline and in response to a stressor, and take longer to recover to baseline levels than do males (Aloisi, Ceccarelli, & Lupo, 1998; Iwasaki-Sekino, Mano-Otagiri, Ohata, Yamauchi, & Shibasaki, 2009; McCormick, Robarts, Kopeikina, & Kelsey, 2005). This

hyperactivity of the HPA axis could be due to increased reactivity of the axis when a stressor is perceived compared to males, or due to a delay or attenuation in negative feedback to regulate the stress response. That concentrations in corticosterone in females rise more rapidly than males in response to a stressor indicates the former; that females take longer to return to baseline indicates the latter, so perhaps it is a combination of the two.

Adolescents differ in their response to stressors compared to adults. Adolescent female rats show a prolonged HPA response to a stressor (McCormick et al., 2005; Romeo, Lee, & McEwen, 2004), and males show both a delayed and prolonged response (Vázquez & Akil, 1993). Given that various brain regions continue to develop during adolescence in both humans and rats (Giedd, 2004; Juraska & Markham, 2004), the prolonged release of glucocorticoids may influence the development of neural areas. In addition, the onset of puberty during the adolescent period comes with a surge of hormones, and these hormones (testosterone and estradiol predominately), can influence HPA axis functioning (McCormick, Linkroum, Sallinen, & Miller, 2002). Thus, age and sex differences are important considerations in stress research.

Social Instability Stress

The Social Instability Stress (SS) model is a mild chronic stressor developed by Dr. McCormick. It consists of two stressors: a short daily isolation period followed by social instability. Typically administered during the adolescent period from PND 30-45, rats are isolated in ventilated containers for 1 hour daily, then re-paired in a home cage with an unfamiliar age- and sex-matched peer (McCormick, 2010). For the first 15 days of the procedure, each rat is paired with a new peer each day and then repaired with their original cage-mate after isolation on the 16th day. During re-pairing, rats were placed in a different cage than the one they were housed in that day, but in which another SS pair was housed that day. Cages were not

cleaned before rats were placed back into them, so unfamiliar scents and feces from the previous SS inhabitants remained in the cage to produce a novel environment. The age range of PND 30-45 encapsulates the average age of physical markers of puberty for both male and female rats (PND 42 and 35 respectively). This ensures that both sexes are receiving both pre- and post-pubertal stress. Although not designed to be a translational model for humans, but instead a means to investigate developmental stage-specific plasticity of the nervous system, the model can be thought of as similar to the experience of children moving to new schools frequently, or children in the foster care system who are frequently moved to new houses and new families.

It seems to be the combination of daily one hour confinement in a small container (isolation) and re-pairing with a new conspecific that produces a prolonged stress effect in adolescents (Hodges & McCormick, 2015): adolescents re-paired with a new partner each day show a prolonged CORT release after isolation, whereas adolescents paired with a familiar peer and adults paired with either a familiar or unfamiliar peer each day do not. When SS is administered during adolescence, males will typically have reduced weight gain compared to controls (McCormick, 2010). This effect is sometimes also found in males stressed as adults, but is not typically found in females stressed at either developmental stage (Hodges & McCormick, 2015; McCormick, Robarts, Gleason, & Kelsey, 2004; McCormick et al., 2005).

In adolescent male rats, the SS procedure produces alterations in social behaviour, motivation for sweet rewards (both described in more detail below), and intake of ethanol. A male adult-stress group has not been consistently used in all studies, so the extent to which SS in adulthood may influence these factors is not fully known. Because the SS model was designed to study developmental, stage-specific plasticity in the brain, a cohort of adult-stressed rats is an important inclusion for future studies to be able to make stress-based age comparisons and

determine whether SS-induced changes in behaviour and neural development are unique to the adolescent period.

In experiments investigating social behaviour, males are typically used so how the SS procedure (in either adolescence or adulthood) affects social behaviours in female rats is not fully known. This male bias is present in many fields that use animal research, including neuroscience, pharmacology, and physiology (Shansky, 2019), so more research using females is needed on a more global level. Social Instability Stress experiments that have included both adolescent- and adult-stressed males and females have found some stress differences. Adult SS females have higher corticosterone concentrations after a restraint test (an acute stressor) compared with adult female controls (McCormick et al., 2005). This effect is specific to adult-stressed females; neither adolescent SS females, adolescent SS males, or adult SS males differed in CORT response from controls after restraint (McCormick et al., 2005). In response to nicotine, only females show SS-induced effects on locomotor activity (McCormick et al., 2004, 2005), but both males and females show SS-induced effects on locomotor activity in response to amphetamine (Mathews, Mills, & McCormick, 2008). Further details of SS-induced effects are detailed in the subsequent two sections.

Social Instability Stress on Social Interaction and Approach

The SS model produces a decrement in preference for interaction with a novel conspecific, but not for sociability in general (Hodges et al., 2017). When paired with a novel peer, both male and female SS rats spend less time in social interaction than do CTL rats (Asgari, 2020), but when the stimulus rat is separated by mesh, male SS rats (females have yet to be tested) spent either the same amount, or more time, in social approach compared with CTL rats (Green et al., 2013; Hodges et al., 2017, 2019). This decrease in time spent interacting with a

novel peer in SS rats is evident both immediately after the stress procedure as well as weeks after, suggesting a long-lasting change in social repertoire.

In a conditioned place preference (CPP) test, both CTL and SS rats formed a preference for the social chamber when allowed to interact freely with the stimulus rat; neither group formed a preference when the stimulus rat was restrained behind mesh (Hodges et al., 2017). Previous research has found that CPP is formed with less training sessions when physical contact is allowed versus when contact is restricted (Peartree et al., 2012), so perhaps more training sessions would have led to CPP being formed in the restrained condition. Regardless, although SS rats have altered behaviour when physical interaction is possible, they form a place preference just as readily as controls when physical contact is allowed. This points to two possible separate mechanisms of altered social behaviour: social interaction and social motivation, whereby SS alters social interaction but leaves social motivation intact.

Social Instability Stress on Motivation for Sweet Rewards under Social Conditions

Male SS rats also show altered social behaviour in the presence of a rewarding sweet substance. When competing for access to sweetened condensed milk (~30% sucrose + fat and other macro- and micronutrients that not found in sucrose), stressed rats displayed more aggressive behaviours than did controls (Cumming et al., 2014). This increase in aggression may involve an altered social repertoire, an increased motivation for food reward, or a combination of the two. A follow-up study did not find an increase in aggressive behaviour from the SS rats when in competition for 1% sucrose, a somewhat less rewarding substance, but SS rats drank more sucrose compared with controls (Marcolin, Hodges, Baumbach, & McCormick, 2019). When in the presence of a peer but not competing against them for access to 1% sucrose, SS and control rats do not differ in intake of sucrose (Marcolin et al., 2019). These results suggest that

competition heightens the rewarding aspect of sweet rewards for SS rats, but a highly palatable substance is required to elicit aggressive behaviours.

Social Discounting

The thesis experiment also sought to directly compare two rewards against each other. SS rats appear to have an altered social repertoire in that they spend less time in social interaction, but still find social stimuli rewarding. They also show increased aggression when in competition for sweetened condensed milk, but not 1% sucrose. This experiment aims to determine: 1) the degree to which SS rats are sensitive to changes in sucrose concentrations, and 2) if graded changes in sucrose salience will alter corresponding social behaviour in a dose-dependent manner.

To determine this a choice-test was used in which rats had free access to a peer behind mesh that they could approach but had limited physical access, as well as access to drink from a water bottle filled with either water (0%), 2%, 5%, or 10% sucrose concentration by volume. All rats repeated this test over 4 days so that they were given a different concentration of sucrose each day. Time spent drinking from each bottle and time near the peer were measured to determine a preference for each stimulus. The social discounting test was developed and has been used previously by the van Wingerden lab (*unpublished data*) in rodents. The social discounting task used in this thesis was largely based off the van Wingerden model. The overarching hypothesis was that SS, when experienced in adolescence but not in adulthood, would lead to a lasting change in how social context influences sucrose intake relative to control rats.

For this thesis, it is expected that social discounting will be observed, defined here as a shift away from the social stimuli and towards the sweet reward as the concentration increases. The

shift away from the social stimuli and towards the sucrose stimuli is expected to be negatively correlated, so as rats spend more time drinking, they will spend less time with the peer. Two assumptions are needed for this paradigm: first, that rats will naturally drink more sucrose at higher concentrations as higher concentrations will provide greater reward value than lower concentrations. It has been shown in other experiments that rats will freely consume more sucrose as the concentration increases (Sclafani, 1987), until the concentration reaches between 8% and 16%, at which intake plateaus (Sclafani & Ackroff, 2003). The second assumption is that at higher concentrations of sucrose, rats will spend less time near the stimulus peer than they did at lower concentrations; spending less time with a peer as the concentration of sucrose increases would be indicative of social discounting occurring. It is expected that all test rats will have an initial preference for a peer over water, and then for social preference to decline as a function of sucrose concentration. Further specific predictions for this thesis were developed, outlined below.

Specific Predictions.

1. The SS procedure will create differences in behavioural responses to the social discounting task. It is predicted that SS rats will increase their consumption of sucrose (and thereby decrease time spent with a peer) to a greater degree at lower concentrations of sucrose than control rats will. Because adolescent male SS rats spend the same or even more time in social approach as controls (Hodges et al., 2019), it is predicted that the SS rats will spend just as much, or more, time near the novel peer as controls when the other option is to drink 0% sucrose (water).
2. With the theory that adolescents are particularly susceptible to stress, it is hypothesized that the SS procedure will produce differences in social discounting when administered to

adolescent rats compared to when it is administered to adults. That adolescent SS males become more aggressive than controls when in competition for sweetened condensed milk but not 1% sucrose suggests that SS rats may find more palatable substances more rewarding than controls do and may have an increased sensitivity to sweet substances (Cumming et al., 2014; Marcolin et al., 2019) In the social discounting task, the increase in reward value as the concentration of sucrose increases it is predicted to be more salient for the SS rats compared to the controls. Thus, it is predicted that adolescent SS rats will be more sensitive to the changing concentrations of sucrose and to socially discount (more time spent drinking sucrose and less time spent near the novel peer) to a greater degree at lower concentrations than control adolescents. Since adolescence is hypothesized to be a sensitive period of development and a developmental stage that is more susceptible to the effects of stress, it is predicted that rats stressed as adults will not differ from controls during the social discounting task.

3. It is predicted that changes produced by the SS procedure will be evident both immediately after the stress procedure, as well as weeks after, in that it produces a long-lasting change. If prediction 2 is incorrect and rats stressed during adulthood show behavioural differences, it is not expected that this effect will be long-lasting for the adults.
4. Given differences in female HPA axis reactivity in response to stressors and female's higher preference for sucrose and lower preference for social interaction compared to males, it is predicted that males and females will show differences in the social discounting task and that SS females will show greater discounting in favour of sucrose at lower concentrations than will SS males.

Social Context on Drinking 5% Sucrose

A recent study found that adolescent male rats will drink more 1% sucrose when they are alone in an apparatus versus when there is a peer present behind mesh, regardless of whether they were stress or control rats (Marcolin et al., 2019). This study included rats tested either immediately after the termination of the stress procedure (immediate group) or after a delay of 25 days during which rats were allowed to rest in their home cages (delay group) to investigate whether the effects of stress are long-lasting or short-lived (Marcolin et al., 2019). Rats only drank more when alone when they were tested immediately and there were no stress differences in rats tested after a delay (Marcolin et al., 2019). Rats tested both immediately and after a delay were aware of the social conditions and spent more time near the peer when one was present (Marcolin et al., 2019). That social condition influences drinking behaviour is the main hypothesis of the current study. Drinking of 5% sucrose, a more palatable substance than 1% sucrose, when rats were both alone and when there was a peer present were investigated to determine if social condition (the presence of absence of a peer) influenced drinking behaviour. This analysis sought to replicate the findings of Marcolin et al., (2019) with 5% sucrose and extend the results to male rats stressed during adulthood and to females.

Because part of the main hypothesis of the current thesis is that SS will lead to changes in how social context influences sucrose intake relative to control rats, the comparison of drinking alone and drinking when a peer is present also serves as a secondary measure to confirm that social context has an influence on sucrose intake. In the social discounting task, rats were drinking sucrose in a social context, but the full extent to which changes in sucrose intake are influenced by social context (and any ensuring stress differences) cannot be determined without a negative control, hereby measured as time drinking sucrose without a peer present.

Methods

Animals

Two separate cohorts, one of female (n = 120) and one of male (n = 128) Long-Evans rats were sourced from Charles Rivers, Saint Constant, Québec. Table 1 shows the breakdown of all rats into the between-subject groups; these between-subject groups are detailed in subsequent sections. Males arrived on either postnatal day (PND) 23 or 60, whereas females were either PND 23-26 or 60-64 (n = 60/age group). For average age for the younger female rats was 24.5

Table 1. *Number of rats in each between-subjects group. Test rats participated in behavioural tests and their data was collected for analysis. Stimulus rats were control rats that acted as unfamiliar peers during behavioural tests.*

	Test Rats							
	Social Instability Stressed (SS)				Controls			
	Adolescent Arrival		Adult Arrival		Adolescent Arrival		Adult Arrival	
	Male	Female	Male	Female	Male	Female	Male	Female
Tested Immediately	14	12	12	12	14	12	12	12
Tested After a Delay	14	12	12	12	14	12	12	12

+

	Stimulus Rats			
	Adolescent Arrival		Adult Arrival	
Male	12	12	12	12
Female	12	12	12	12

days, this was rounded up and this group was designated an average age of 25. The Social Instability Stress procedure involves placing stressed rats with a new cage partner every day and this requires a minimum number of rats also undergoing the procedure. Rats were given an

average age so that they would all start the stress procedure at the same time to ensure the number of novel cage partners required was met. Because the Social Instability Stress procedure is administered at specific ages (PND 30-45), ages were rounded to a whole number to allow for the stress procedure to begin at the appropriate time. The older females were designated an average age of 63. Rats were housed in age- and sex-matched pairs under a 12-hr light-dark cycle (lights on at 05:00 hr) with food and water available ad libitum. Rats were given a week to acclimate to the animal facility after arrival. Animal use and procedures were approved by the Brock University Institutional Animal Care Committee (ACC) and were carried out in adherence with the Canadian Council on Animal Care guidelines.

Testing Apparatus

Four white acrylic boxes were used in a designated testing room. Boxes comprised of a main test chamber measuring 61 cm x 31 cm x 52 cm, with two side chambers (20 cm x 20 cm x 22 cm) separated from the main chamber by a plastic mesh. A larger hole was drilled into the middle of each mesh screen so that drinking bottles could be placed in the side chambers with their spouts protruding into the main chamber. See Figure 1A for a schematic of the testing apparatus.

Video Tracking

A video camera mounted above the test boxes recorded each behavioural test and provided a live feed to SMART® version 2 tracking software. Using SMART®, each main chamber of the test box was divided into three zones: an upper zone associated with the stimulus in the upper side chamber, a centre zone not associated with any stimulus, and a lower zone associated with the stimulus in the lower side chamber. See Figure 1B for a schematic of the testing apparatus with the upper and lower zones highlighted. Time spent in each zone was then

automatically measured by the software. Videos were later scored by a volunteer blind to the test conditions to determine frequency and duration of contact with the stimuli in the two side chambers.

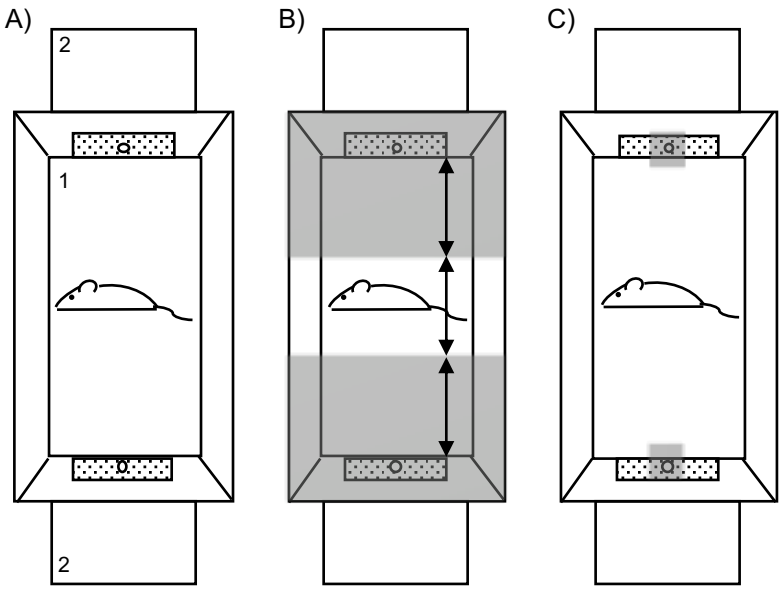

Figure 1. *Schematic of testing apparatus with inclusion zones. A) Schematic of testing apparatus. The box labelled 1 is the main testing chambers and boxes labelled 2 are side chambers in which stimuli were placed. These side chambers were separated from box 1 by a mesh wall. Each mesh wall contains a larger hole drilled into the centre of the mesh. B) Inclusion zones used by the tracking software represented by grey shading. Each zone represents 1/3rd of the testing chamber closest to each side chamber. C) Inclusion zones used in hand scoring represented by grey shading. Each zone represents the area within a 1cm radius from the drilled hole in the mesh.*

Video Scoring

Videos were hand-scored by a rater blind to all experimental conditions (stress group, age, time of testing, and sex of rats, as well as condition of anything in the side chambers, ie. if a peer was familiar or unfamiliar to the test rats and any concentration of sucrose in the bottles). Scorers used the program PlusMZ, a free computer software designed for behavioural data scoring. For all behavioural tests a score was counted when a rat was on one side of the main chamber and touching or within 1cm of the hole in the centre of the mesh (for the stimulus side) or of the spout of the bottle (for the sucrose side). See Figure 1C for a schematic of the testing apparatus with the area scored highlighted. Duration of each instance was recorded for as long as

the rat remained within a 1cm radius of either the hole or spout.

Social Instability Stress Procedure

The Social Instability Stress (SS) procedure was as described in McCormick (2010) and consisted of 16 days of 1-hour isolation in ventilated containers (13 cm x 8 cm or 17 cm x 9 cm), followed by return to a novel cage-partner in a new cage for the first 15 days. After isolation on the 16th day, rats were placed with their original cage partner and then left undisturbed until behavioural testing began. Isolation occurred at variable times of the light cycle to avoid habituation to when the procedure occurred, excluding the first hour after lights on or the hour prior to lights off. Male (n = 52) and female (n = 48) rats were randomly assigned to undergo the SS procedure. Adolescent rats of both sexes (n = 52) underwent the procedure from PND 30-45, while adult female rats (n = 24) underwent the procedure from PND 68-83, and adult male rats (n = 24) from PND 67-82. Separate male (n = 26 per age) and female (n = 24 per age) rats were designated as control rats and left undisturbed for the duration of the stress procedure except for weighing and regular cage maintenance.

Vaginal Opening

There is evidence that stressors during childhood influence pubertal timing (Belsky, Steinberg, & Draper, 1991). Studies in humans have found that family stressors such as parental divorce and family conflict result in an earlier age of first menarche in women (Moffitt, Caspi, Belsky, & Silva, 1992; Wierson, Long, & Forehand, 1993). For female rats, a physical marker of puberty is vaginal opening. Earlier vaginal opening is found in female rats who experienced early-life stressors compared with rats who did not experience a stressor (Cowan & Richardson, 2019). It is not known if prepubertal adolescent stress has similar effects on pubertal timing in

females as childhood stress does. Vaginal opening in female SS rats was recorded to investigate if the SS procedure resulted in an earlier average age of pubertal onset compared to controls.

SS rats were monitored for both partial (pinhole) and full vaginal opening daily while they were already being handled during the stress procedure. Excess handling was kept to a minimum, and vaginal inspection was done in as short a time as possible for each rat. Vaginal opening data were not collected from female control rats to avoid disturbing them and creating any confounding factors due to excess handling.

Behavioural Tests

Rats began behavioural testing either the day after the SS procedure (Immediate testing, n = 100) or after a delay of 25 days for females and 26 days for the males (Delay testing, n = 100). Rats tested at both time points underwent behavioural tests in the same order: a habituation test, two social novelty tests, four social discounting tests, and a sucrose preference test, schematics of test are shown in Figure 2. All tests were 10 minutes in duration and were conducted one hour after the onset of the dark cycle under red light.

Weight Gain

SS (n = 100) and CTL (n = 100) rats were weighed on both the first (Ado: PND 30; Adult male: PND 67; Adult female: PND 68) and last day of the SS procedure (Ado: PND 45; Adult male: PND 82; Adult female: PND 83).

Habituation Test

The first behavioural test for both immediate and delay groups involved habituation to the test apparatus and drinking bottles. Each testing box had a bottle of water in one side chamber

and a bottle of 5% sucrose in the other chamber, counterbalanced between boxes. After the 10-minute test, all rats tested were given 75 ml of 5% sucrose in their home cages overnight.

Social Novelty Tests

The current experiment uses the Social Novelty Preference Test developed in the Veenema lab which has found that rats will show a preference for an unfamiliar peer over their cage-mate (Smith et al., 2015; Smith, Mogavero, Tulimieri, & Veenema, 2017).
Over two test days, test rats were placed in the test chamber for 10 minutes, with their cage-partner placed in one side chamber and a novel stimulus rat in the other side chamber. Stimulus rats were control rats (age- and sex-matched) that only acted as unfamiliar peers during

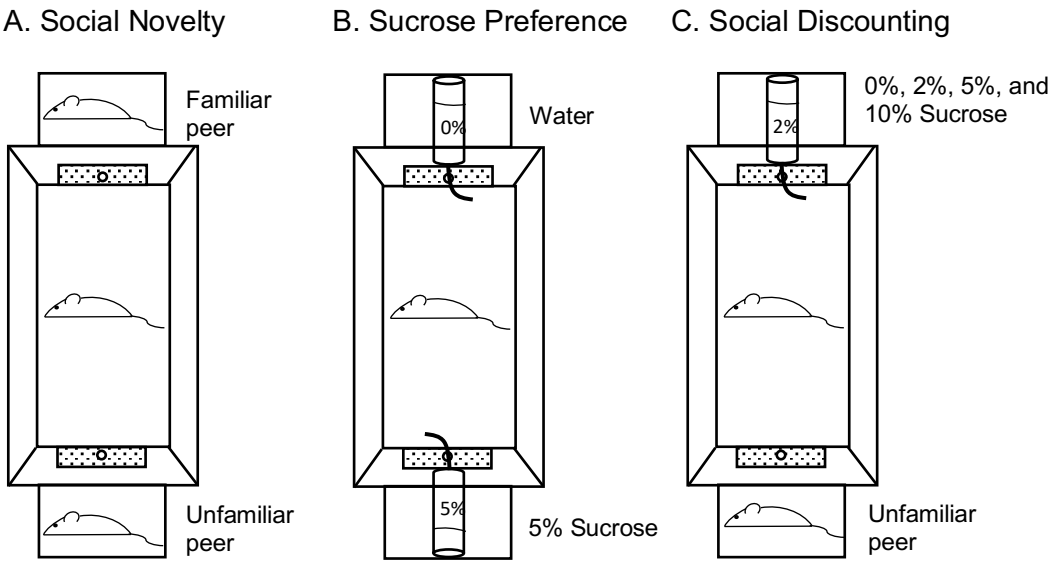

Figure 2. *Schematic drawings of behavioural tests, all tests were 10 minutes in duration. Placement of stimuli in each side chamber was counterbalanced between apparatuses and between test rats. A) Social Novelty test. The test rat was placed in the middle chamber. A familiar peer (test rat's cage-mate) was placed in one side chamber and an unfamiliar peer (stimulus rat) was placed in the other side chamber. B) Sucrose Preference test. The test rat was placed in the middle chamber. A bottle of water (0% sucrose) was placed in on side chamber and a bottle of 5% sucrose was placed in the other side chamber. Spouts of both bottles protruded through the mesh so that the test rat could drink from the spouts. The habituation test was conducted the same as the sucrose preference test. C) Social Discounting test. The test rat was placed in the middle chamber on four separate days. A bottle of sucrose was placed in one side chamber on each of the four days (0%, 2%, 5%, and 10% in counterbalanced order) and an unfamiliar peer (stimulus rat, new rat each day) was placed in the other side chamber. Placement of stimuli in the side chambers was counterbalanced across days.*

behavioural tests. Placement of cage-partners and stimulus rats were counterbalanced between boxes. Time spent in both zones closest to each peer was scored by SMART® version 2 tracking software, as shown in Figure 1B, and these times were used for statistical analyses.

Social Discounting Tests

Over four test days, rats were placed in the test chamber for 10 minutes and allowed a choice between one of four concentrations of sucrose (0, 2, 5, and 10%) and a novel stimulus rat. A new rat was used each test day, and rats were exposed to a different concentration of sucrose each day. Placement of the stimuli in the side chambers was counterbalanced across boxes and between days, and order to which rats were exposed to the concentrations of sucrose was randomized between rats and across days so that each rat underwent a different order. Each test was manually scored for the frequency and duration of time that the test rat had its snout touching, poking through, or within 1cm of the hole in the mesh on the stimulus rat side and for time spent drinking from the bottle on the sucrose side.

Sucrose Preference Test

A 10-minute sucrose preference test was conducted to investigate any differences in sucrose preference between groups that could interfere with the interpretation of the social discounting results. Rats were placed in the test chamber and allowed access to a bottle of water and 5% sucrose. Placement of the bottles were counterbalanced between boxes. Each test was manually scored for the frequency and duration of time spent drinking from either bottle.

The current experiment measured sucrose consumed in units of time spent drinking, but studies that measure fluid intake frequently report their results as ingestion in milligrams, grams or millilitres relative to body weight rather than total consumption to adjust for possible weight differences in intake (Bekris, Antoniou, Daskas, & Papadopoulou-Daifoti, 2005; Hong et al.,

2012; Wilmouth & Spear, 2009). It has been reported that body weight is not a confounding factor in reporting sucrose consumption (Bekris et al., 2005). Time spent drinking has also been found to be highly correlated with volume consumed in an ethanol consumption study (Varlinskaya, Truxell, & Spear, 2015), and so time drinking can be used as a reliable measure of solution intake.

Social Context on Drinking 5% Sucrose

Results from the Sucrose Preference Test and Social Discounting Test from Experiment 1 were used. The Sucrose Preference Test represented time drinking 5% sucrose alone, and the Social Discounting data when rats had 5% sucrose represented time drinking 5% in the presence of a peer.

Statistical Analyses

All data analysis was done with SPSS version 25 and graphs were made with Prism GraphPad version 8. Unless otherwise noted, analyses were run with the following independent variables: stress group (control or stress group), sex (male or female), initial developmental stage (adolescent or adult), and time of testing (immediate or delay). Univariate and repeated measures ANOVAs were used except for the analysis of vaginal opening which consisted solely of an independent samples t-test. For repeated measures ANOVAs, stimuli presented in either side chamber was used as the repeated measure. For the social discounting task, the overall shape of the curve across sucrose concentrations was a main interest, so polynomial contrasts were run to determine polynomial patterns. Post-hoc analyses consisted of paired-samples t-tests or the Fisher LSD test for pairwise comparisons. Partial eta squares ($\eta_p 2$) are reported as a measure of effect size for main effects and interactions of ANOVAs. The alpha level of $p < 0.05$ was used for all analyses. Non-significant main effects and interactions were not reported.

Results

Weight Gain

Control rats gained an average of 8.76 grams more than the stressed rats over the duration of the stress procedure, $F(1,184) = 17.18$, $p < 0.001$, $\eta p2 = 0.085$ (see Figure 3A). Adolescents gained more weight than adults ($F(1,184) = 288.74$, $p < 0.001$, $\eta p2 = 0.611$), and males gained more weight than females ($F(1,184) = 319.68$, $p < 0.001$, $\eta p2 = 0.635$). Main effects of both age

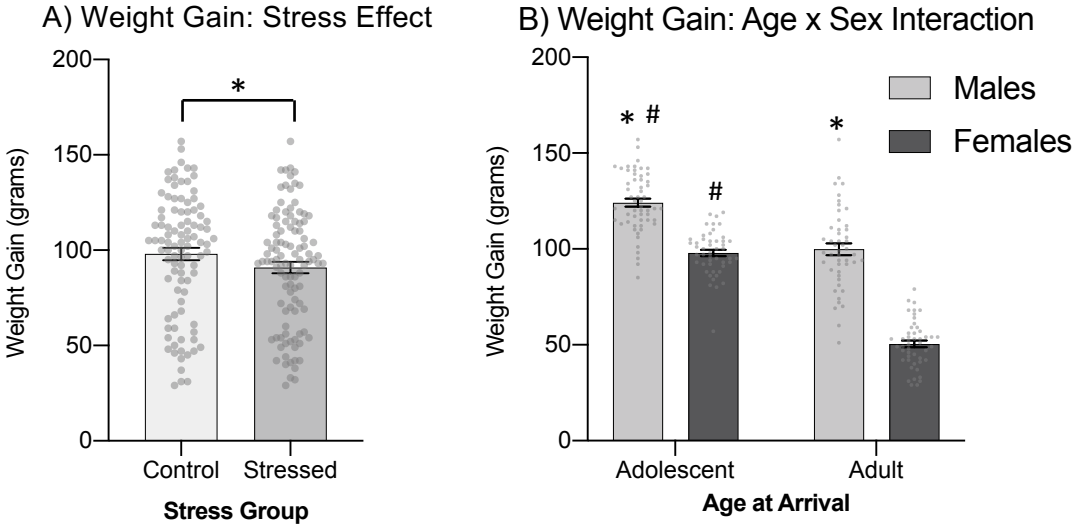

Figure 3. *Weight gain during the stress procedure for all groups of rats. Bars represent group averages (+ SEM) and dots represent individual data points. A) Stressed rats gained less weight than controls. B) Interaction between age and sex; * = sex difference in which males gained more weight than females; # = age difference in which adolescents gained more weight than adults.*

at stress and sex were obviated by an interaction between the two, $F(1,184) = 30.15$, $p < 0.001$, $\eta p2 = 0.141$. Post-hocs showed that adolescent females and adult males gained about the same amount of weight, whereas adolescent males gained the most and adult females gained the least (see Figure 3B).

Vaginal Opening

Previous data from the McCormick lab has observed the average and most common day of vaginal opening for control Long-Evans rats at PND 33 ($n = 28$, range = PND 30-38, SD = 1.86), (unpublished observations by Simone, J.J.).

Mean day of vaginal opening in the current experiment for SS rats was PND 33.5 (n = 24), with a range between PND 30 and PND 37, SD = 2.02, (see Figure 4). An independent samples t-test between average day of vaginal opening for SS rats and the data for control rats was not significant, $t(50) = 0.741$, $p = 0.462$.

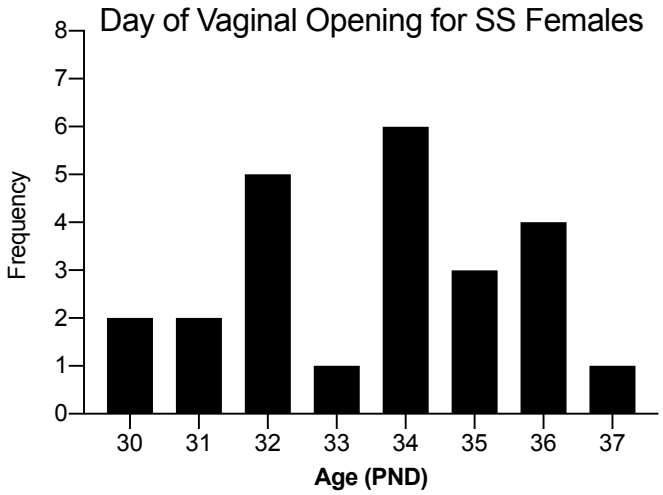

Figure 4. *Frequency distribution for day of vaginal opening of SS female rats. Mean day was 33.5, mode was 34.*

Social Novelty. There was a main effect showing a preference to spend time near the unfamiliar rat over the familiar rat, $F(1, 184) = 63.64)$, $p < 0.001$, $\eta_p^2 = 0.257$ (Figure 5a). In Figure 5, each line represents an individual rat with bars representing group means. Results also indicated an interaction between partner novelty, time of testing, and age at stress, $F(1,183) = 5.46$, $p = 0.021$, $\eta_p^2 = 0.029$ (Figure 5b), in which all groups spent more time near the unfamiliar rat with the exception of the adult group tested after a delay. This group spent an average of 23.10 seconds more with the unfamiliar rat over the familiar rat, but this difference failed to reach statistical significance, ($p = 0.141$).

A main effect of sex was observed, $F(1, 184) = 4.49$, $p = 0.036$, $\eta_p^2 = 0.024$, in which males spent on average 4.79 seconds more than females in total time near either peer.

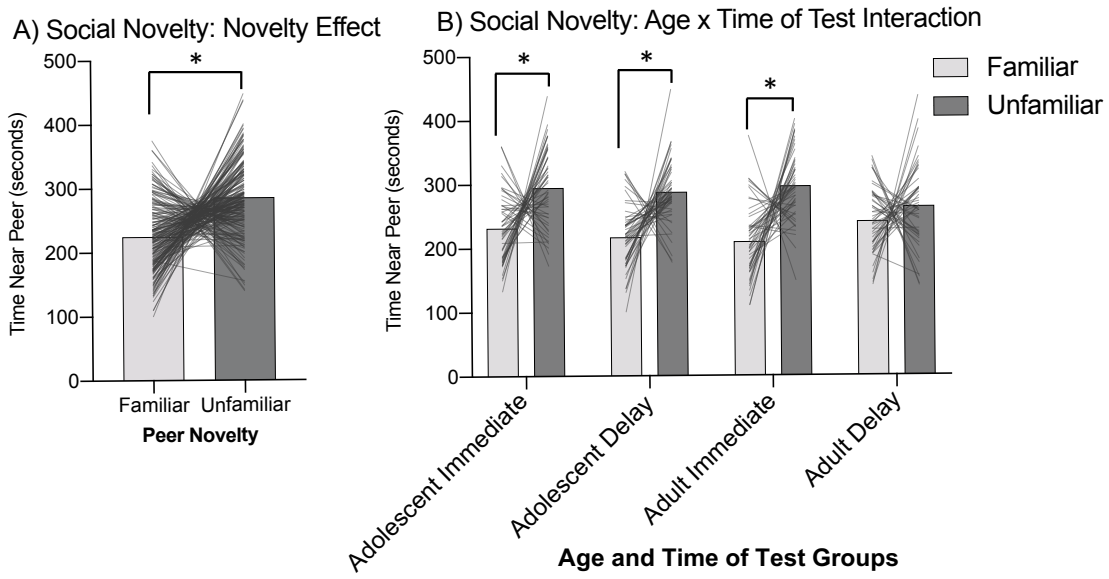

Figure 5. *Social novelty preference test – time spent with both a familiar and unfamiliar peer (Mean + SEM) with individual data points represented as lines. A) Main effect of novelty in which rats significantly spent more time near the unfamiliar peer. B) Adult rats tested after a delay spent more time near the unfamiliar peer, but this difference failed to reach significance. All other groups spend significantly more time near the unfamiliar peer.*

Preliminary Results: Delay Females

A preliminary analysis of the data showed that the female group tested after a delay spent significantly less time drinking than did the other sex and time of testing groups in both the social discounting and sucrose preference tests, see Figure 6 for drinking results from the social discounting task, (sucrose preference: main effect of time of test by sex, $F(1, 184) = 4.31$, $p =$ 0.039l social discounting: main effect of time of test by sex, $F(1, 182) = 11.30$, $p = 0.001$). When looked at separately, this group still responded to the increasing concentrations of sucrose (sucrose preference: $F(1,44) = 7.53$, $p = 0.009$, $\eta_p^2 = 0.146$; social discounting, 4 concentrations of sucrose: $F(3,132) = 4.30$, $p = 0.006$, $\eta_p^2 = 0.089$). Further, that within the delay female group, both control and SS, adolescent and adult, rats showed this decrease in drinking, leads to the conclusion that this was a difference caused within this particular cohort and did not rely on any manipulated factors. For this reason, all subsequent analyses that involve sucrose consumption were analyzed with the delay females separately to prevent any effects being detected that would

be driven by the delay female's lower overall consumption of sucrose. Further discussion of the possible reasons for this difference in drinking behaviour are detailed later in the discussion section.

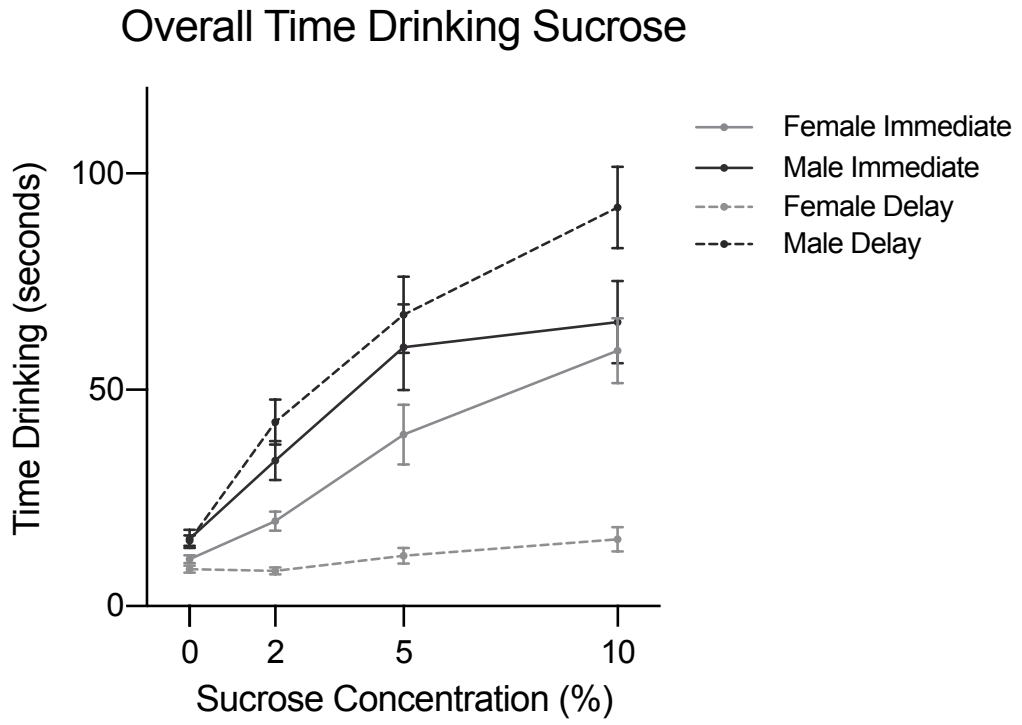

Figure 6. *Time drinking different concentrations of sucrose by sex and time of testing (Mean + SEM). Females tested after a delay spent significantly less time drinking at all concentrations compared to the other groups.*

The delay females being analyzed separately created a problem with the remaining dataset in that both the variables sex and time of testing are no longer complete: the dataset is missing half the female subjects and half the delay subjects and so time of testing by sex interactions cannot be analyzed. To remedy this, three options are available: 1) also analyze the immediate females separately and analyze males tested at both time points together, 2) also analyze delay males separately and analyze both males and females tested immediately together, or 3) analyze all time of test and sex groups separately. Some of main questions of this thesis were: 1) to determine if males and females will differ after the stress procedure, and 2) if there

are any stress effects, to determine if they are long-lasting. Based on these questions, option 2 was used for analysis, so both males and females tested immediately were analysed together to determine any sex differences. Females tested after a delay and males tested after a delay were both analyzed separately. Analyzing delay males and delay females separately from the immediate groups will not allow for a direct statistical analysis of time of testing effects, but will still allow some determination of how long-lasting any stress effects found are.

Sucrose Preference

Repeated measures ANOVAs (dependent variables: time spent drinking water, and time spent drinking 5% sucrose) were conducted for each of the three groups: males and females tested immediately, delay females, and delay males. One rat was excluded from the control, adult, immediate female group for failure to complete the test. All groups spent more time drinking sucrose than water (**Immediate Males and Females**: $F(1, 91) = 103.81$, $p < 0.001$, $\eta_p^2 = 0.533$; **Delay Females**: $F(1, 44) = 7.53$, $p = 0.009$, $\eta_p^2 = 0.146$; **Delay Males**: $F(1, 48) = 61.54$, $p < 0.001$, $\eta_p^2 = 0.562$), see Figure 7.

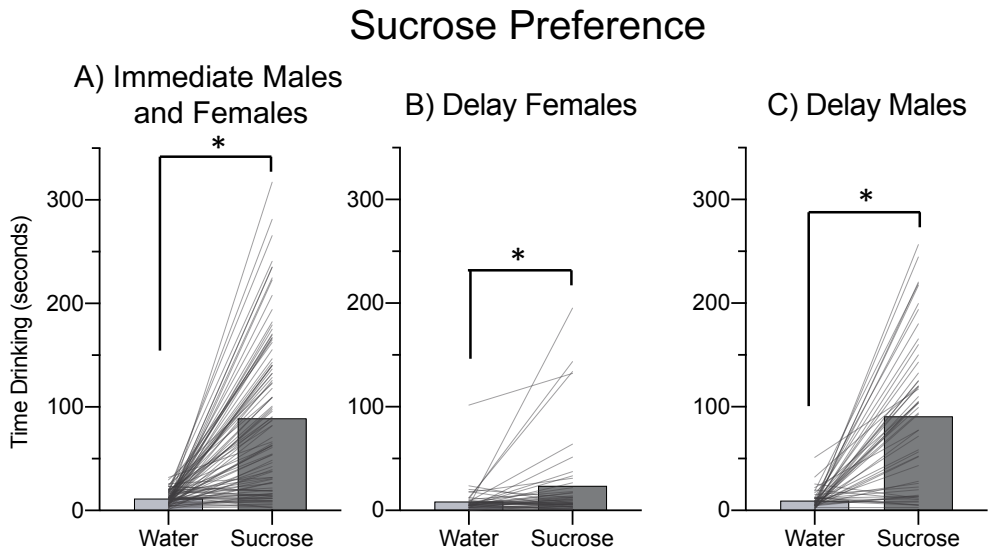

Figure 7. *Sucrose preference test. Average time drinking water and sucrose as bars (Mean + SEM) with individual data points are represented by each line. A) Immediate Males and Females, B) Delay Females, and C) Delay Males all spent more time drinking sucrose than water.*

For the Immediate Males and Females group, a within-subjects effect of sex ($F(1,91) = 4.43$, $p = 0.038$, $\eta_p^2 = 0.046$) showed that females spent on average 5.95 seconds more than males drinking water ($p < 0.001$), but males and females did not differ in time drinking sucrose ($p = 0.083$). There were no other interactions or main effects (all p's > 0.153).

Social Discounting

The initial plan was to calculate the percentage of time rats explored the unfamiliar peer out of their total time spent in both the social and sucrose the inclusion zones for each concentration of sucrose with the following equation:

$$Social\ \% = \frac{time\ near\ peer}{time\ near\ peer + time\ drinking} \times 100$$

From the calculation of social percentage at each concentration, a rat would be considered to be social discounting if their social percentage decreased as the concentration of sucrose increased (example: social discounting would have occurred is a rat's social percentage decreased from 70% at 0% sucrose to 50% at 2% sucrose).

Before the social percentage was calculated, an initial analysis was conducted for the time spent near a peer and time spent drinking sucrose separately and as measured in seconds. From initial analysis, it was observed that rats increased time spent drinking as the concentration increased, but time spent near the peer remained as a flat line across the concentrations (see Figure 8). From this, calculating the social percentage to measure social discounting would be driven by the time spent drinking and would not accurately reflect changes in social behaviour. Because of this, time spent near a peer and time spent drinking sucrose were left as variables measured in time and analyzed separately.

Response to Sucrose Concentrations

The main interest in this analysis was to determine a growth curve for time spent drinking as concentration increased. The question was then whether the shape of this curve would differ based on the between-subject factors. Given the small sample size per group and the number of between-subjects variables, a repeated measures ANOVA was used as quadratic and polynomial contrasts will allow for the determination of the shape of the sucrose curve.

To determine and then manage outliers, the main concern was whether an outlier at any given concentration would impact the overall shape of the curve to make the curve for any given rat differ from the mean shape for that group. To do this, the standardized residuals followed up with Cook's Distance for time drinking at the four different concentrations were calculated. Given that 99% of the sample should lie between -2.6 and 2.6 standard deviations around the mean, a standardized residual value less then -2.6 or greater than 2.6 was deemed a possible outlier and further investigated. Any subject that had 2 outliers over the 4 days of testing was removed from analysis for this test, given that having outliers on two out of four factors for the dependent variable was likely having too great of an influence on the shape of that subject's curve. This decision was followed up by manual inspection of the shape of the overall curve for these cases to confirm that it differed from the mean. Because it is expected that time drinking sucrose at the different concentrations and time spent near the novel peer will be negatively correlated, subjects whose data were excluded from analysis for time drinking sucrose also had their data for corresponding time near a peer excluded.

Outlier removal resulted in the following numbers of subjects being removed from analysis by analysis group: Immediate Males and Females = 3 removed, Delay Females = 1 removed, Delay Males = 0 removed. In addition, two females were removed from analysis for

failure to complete the test, leaving the total group numbers as follows. Immediate adolescent females: CTL = 10, SS = 12; Immediate adult females: CTL = 11, SS = 12; Immediate adolescent males: CTL = 13, SS = 13; Immediate adult males: CTL = 12, SS = 12; Delay adolescent females: CTL = 11, SS = 12; Delay adult females: CTL = 12, SS = 12; Delay adolescent males: CTL = 14, SS = 14; Delay adult males: CTL = 12, SS =12.

A repeated measures ANOVA was conducted for time spent drinking sucrose at the four concentrations (0, 2, 5, and 10%) for the three groups.

Immediate Males and Females. All rats increased time spent drinking as sucrose concentration increased, $F(3, 261) = 30.66$, $p < 0.001$, $\eta_p^2 = 0.261$. An interaction between concentration, stress group, and sex ($F(3, 261) = 3.01$, $p = 0.031$, $\eta_p^2 = 0.033$) revealed that male controls spent more time drinking 0% and 5% than did SS rats ($p = 0.003$ and $p = 0.004$ respectively). Contrasts revealed a subsequent cubic interaction between sucrose concentration, stress group, and sex, $F(1, 87) = 8.66$, $p = 0.004$, $\eta_p^2 = 0.091$.

An interaction between concentration, stress group, and age at stress failed to reach significance ($F(3, 261) = 2.00$, $p = 0.114$, $\eta_p^2 = 0.022$), but a cubic interaction did reach significance ($F(1, 87) = 3.94$, $p = 0.05$, $\eta_p^2 = 0.043$) and was investigated further.

Repeated measures ANOVA's were carried out to investigate stress effects for each age at stress and sex group.

Adolescent Females. For adolescent females, there was no effect of stress either as a main effect or interaction ($p = 0.474$ and $p = 0.982$ respectively), see Figure 8a.
All rats responded to changes in sucrose concentration, $F(3, 60) = 8.17$, $p < 0.001$, $\eta_p^2 = 0.290$. Specifically, time spent drinking increased from 0% to 2% ($p = 0.047$), did not differ from 2% to 5% ($p = 0.353$), but increased between 5% and 10% ($p = 0.021$). As Figure 8 shows separate

lines for both control and SS rats, values for each stress group are as follows, **CTL**: 0%-2%: p = 0.276; 2%-5%: p = 0.557; 5%-10%: p = 0.167; **SS**: 0%-2%: p = 0.100; 2%-5%: p = 0.479; 5%-10%: p = 0.061. All rats increased time spent drinking between 0% and 10% (CTL: p = 0.032; SS: p = 0.012).

Adult Females. For adult females, there was no effect of stress as a main effect or interaction (p = 0.527 and p = 0.164 respectively), but a cubic contract revealed a difference in the shape of the curve for control and stressed rats ($F(1,21) = 4.91$, p = 0.038, $\eta_p^2 = 0.190$), see Figure 8C.

Post-hocs revealed that neither control nor SS rats increased time spent drinking between 0% and 2% sucrose (CTL: p = 0.092; SS: p = 0.055). Control rats did not increase in time spent drinking between 2% and 5% (p = 0.498), but SS rats did (p = 0.021). Neither group increased significantly in time drinking between 5% and 10% (CTL: p = 0.150; SS: p = 0.465).

Adolescent Males. Adolescent males revealed a significant main effect of stress group ($F(1,24) = 6.73$, p = 0.016, $\eta_p^2 = 0.219$) and interaction between stress group and concentration ($F(3,72) = 3.86$, p = 0.013, $\eta_p^2 = 0.139$). A cubic contrast revealed differences in the shape of the curves for stressed and control rats ($F(1,24) = 7.01$, p = 0.014, $\eta_p^2 = 0.226$), see Figure 8B.

Post-hocs revealed that control rats spent on average 41.87 seconds more drinking overall than did SS rats (p = 0.016). Specifically, CTL rats spent more time drinking than SS rats did at both 0% ($t(24) = 2.44$, p = 0.023) and 5% ($t(24) = 2.90$, p = 0.008, with the difference approaching, but failing to reach significance at 10% ($t(24) = 1.87$, p = 0.074) and non-significant at 2% ($t(24) = 1.17$, p = 0.254). Neither control nor SS rats increased in time spent drinking from 0% to 2% (CTL: p = 0.078; SS: p = .103). Control rats increased in time drinking between 2% and 5% (p = 0.015), while SS rats did not (p = 0.418). Neither control nor SS rats

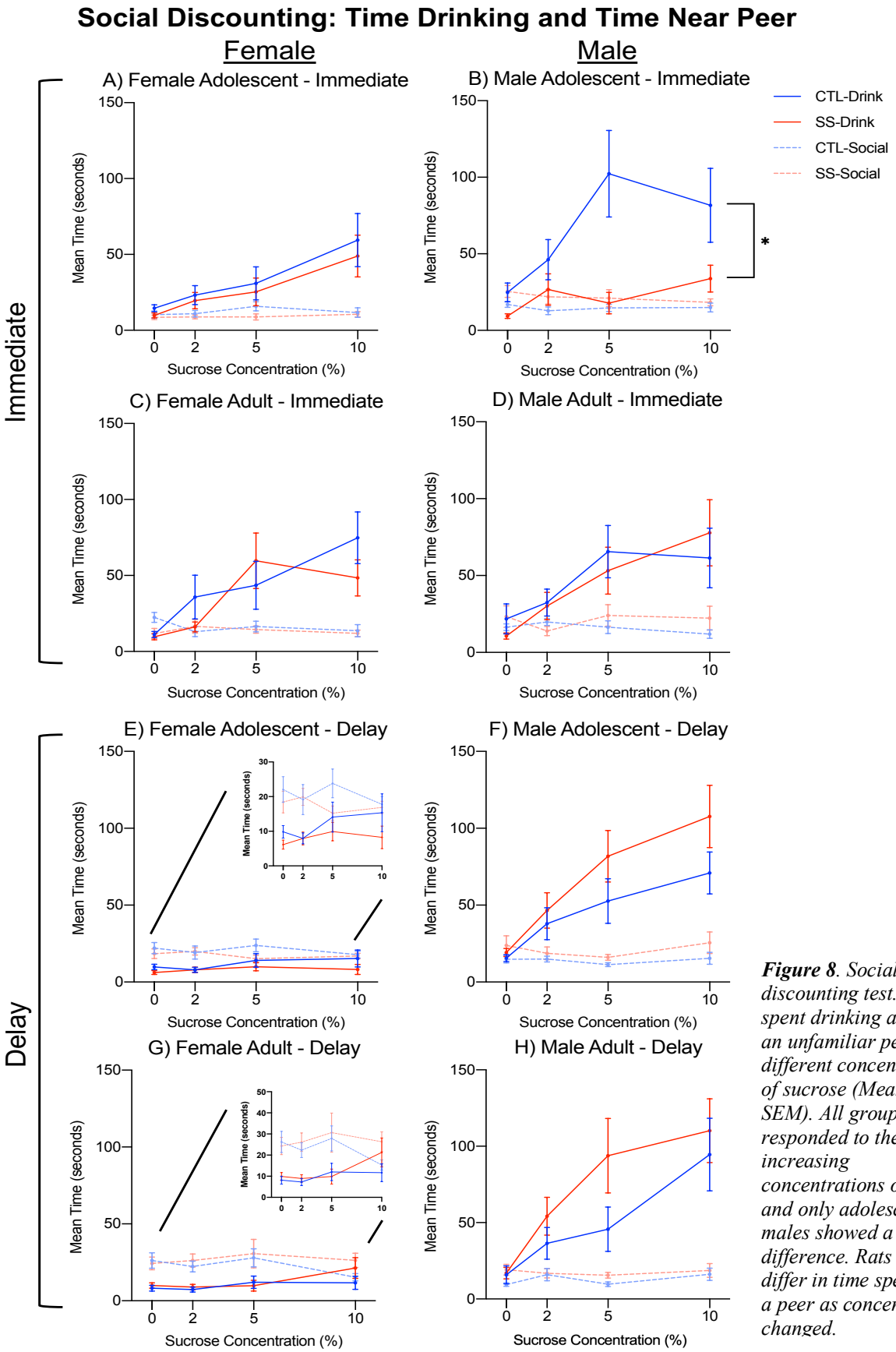

Figure 8. *Social discounting test. Time spent drinking and near an unfamiliar peer at different concentrations of sucrose (Mean + SEM). All groups responded to the increasing concentrations of sucrose and only adolescent males showed a stress difference. Rats did not differ in time spent near a peer as concentration changed.*

increased time spent drinking between 5% and 10% (CTL: $p = 0.393$; SS: $p = 0.077$). Both control and SS rats increased significantly in time spent drinking between 0% and 10% sucrose (CTL: $p = 0.024$; SS: $p = 012$).

Adult Males. For adult males, there was no main effect or interaction of stress group ($p = 0.873$ and $p = 0.659$ respectively). All rats responded to changes in sucrose concentration, $F(3, 66) = 9.34$, $p < 0.001$, $\eta_p^2 = 0.298$, see Figure 8D. Specifically, rats increased time spent drinking from 0% to 2% ($p = 0.039$), from 2% to 5% ($p = 0.009$), but did not increase between 5% and 10% ($p = 0.474$). As figure 8 shows separate lines for both control and SS rats, values for each stress group are as follows, **CTL**: 0%-2%: $p = 0.304$; 2%-5%: $p = 0.041$; 5%-10%: $p = 0.851$; **SS**: 0%-2%: $p = 0.073$; 2%-5%: $p = 0.127$; 5%-10%: $p = 0.191$. All rats increased time spent drinking between 0% and 5% (CTL: $p = 0.010$; SS: $p = 0.021$). The increased between 0% and 10% for controls failed to reach significance ($p = 0.062$) but was significant for SS rats ($p = 0.012$).

Delay Females. Rats responded to the changes in sucrose concentration, $F(3, 129) = 03.48$, $p = 0.018$, $\eta_p^2 = 0.075$. Specifically, rats did not increase time spent drinking between 0% and 2% ($p = 0.634$) but increased between 2% and 5% ($p = 0.047$), and then did not increase between 5% and 10% ($p = 0.351$). Rats spent more time drinking at 10% than they did at 0% ($p = 0.036$) and 2% ($p = 0.002$).

There were no other main effects or interactions (all p's > 0.134). For consistency, figure 8E and 7G shows the curve for control and SS rats by each age group.

Delay Males. Rats responded to the changing concentrations of sucrose, $F(3, 144) = 37.12$, $p < 0.001$, $\eta_p^2 = 0.436$. Specifically, rats increased time spent drinking at each sucrose concentration increase (0%-2%: $p < 0.001$; 2%-5%: $p = 0.001$; 5%-10%: $p = 0.002$). There were

no other main effects or interactions (all p's > 0.072). For consistency, figure 8F and 8H shows the curve for control and SS rats by each age group.

Time Spent Near Peer at Different Sucrose Concentrations

Because the original hypothesis was that the concentration of sucrose available would influence time spent with a peer, even though that did not seem to be the case based on preliminary observation of the data, any rat that was omitted from the response to sucrose concentration task as an outlier was also omitted from this analysis. Standardized residuals and Cook's Distance were used to investigate potential outliers that may influence on the shape of the curve for time spent near the novel peer, as described previously for the response to sucrose concentrations analysis. Only one rat had two out of four standardized residuals greater than 2.6 but was not removed from analysis as the residuals were not greatly above 2.6 and the shape of the curve for this rat upon manual inspection did not appear to differ greatly from the mean shape of the curve. Leaving this rat in the dataset also prevented unnecessarily reducing the sample size.

Immediate Males and Females. Rats tested immediately did not alter time near the novel peer as the concentration of sucrose changed, $F(3, 261) = 1.10$, $p = 0.350$, $\eta_p^2 = 0.012$ (see Figure 8A, B, C, and D). There was a main effect of males spending on average 5.51 seconds more with any peer than did females ($F(1, 87) = 11.21$, $p = 0.001$, $\eta_p^2 = 0.114$), obviated by an interaction between sex and stress ($F(1, 87) = 6.95$, $p = 0.010$, $\eta_p^2 = 0.074$). Male SS rats spent on average 5.81 seconds more near any novel peer than did control males ($p = 0.012$), while SS and control females did not differ in time spent near the novel peer ($p = 0.233$), see Figure 9. SS males also spent on average 9.86 seconds more near a novel peer compared to SS females ($p < 0.001$), with

no difference between male and female controls ($p = 0.622$). There were no other main effects or interactions (all p's > 0.123).

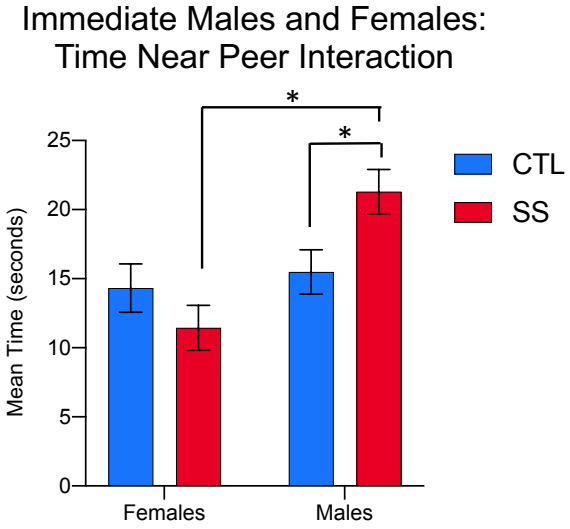

Figure 9. *Interaction of stress group and sex on time spent near novel peer (Mean + SEM). Females did not differ in time near the peer, but SS males spent more time neat the peer than did CTL males.*

Delay Females. Delay females did not alter time spent near the novel peer as the concentration of sucrose changed, $F(3, 129) = 1.25$, $p = 0.296$, $\eta_p^2 = 0.028$ (see Figure 8E and G). The only significant effect that was observed was a main effect of age at stress ($F(1, 43) = 4.65$, $p = 0.037$, $\eta_p^2 = 0.098$), in which adults spent an average of 5.75 seconds more near any novel peer than did the adolescents.

Delay Males. Delay males did not alter time spent near the novel peer as the concentration of sucrose changed, $F(3, 144) = 1.80$, $p = 0.149$, $\eta_p^2 = 0.036$ (see Figure 8F and H). There was a significant main effect of stress ($F(1, 48) = 7.44$, $p = 0.009$, $\eta_p^2 = 0.134$), in which SS rats spent an average of 5.82 seconds more with any novel peer than did the controls. There were no other main effects of interactions (all p's > 0.149).

Social Context on Drinking 5% Sucrose

A repeated measures ANOVA (dependant variables: time spent drinking 5% in sucrose preference test – alone, and time spent drinking 5% sucrose in social discounting test – with peer) was conducted for the three groups. Rats that were excluded from the responding to sucrose concentrations analysis were also excluded from this analysis.

Immediate Males and Females. All rats spent on average 38.6 seconds more drinking alone than they did with a peer, $F(1,87) = 38.76$, $p < 0.001$, $\eta_p^2 = 0.308$ (see Figure 10A). A between-subjects effect of sex approached but failed to reach significance, $F(1,87) = 3.62$, $p = 0.060$, $\eta_p^2 = 0.040$, which showed that males drank for an average of 23.24 seconds more than females regardless of social condition. Follow-up revealed an effect of social context for females rats in which they spent an average of 35.12 seconds more drinking when alone than when there was a peer present, $F(1,41) = 17.09$, $p < 0.001$, $\eta_p^2 = 0.294$. There were no other main effects of interactions for females (all p's > 0.063). Male rats spent an average of 42.08 seconds more

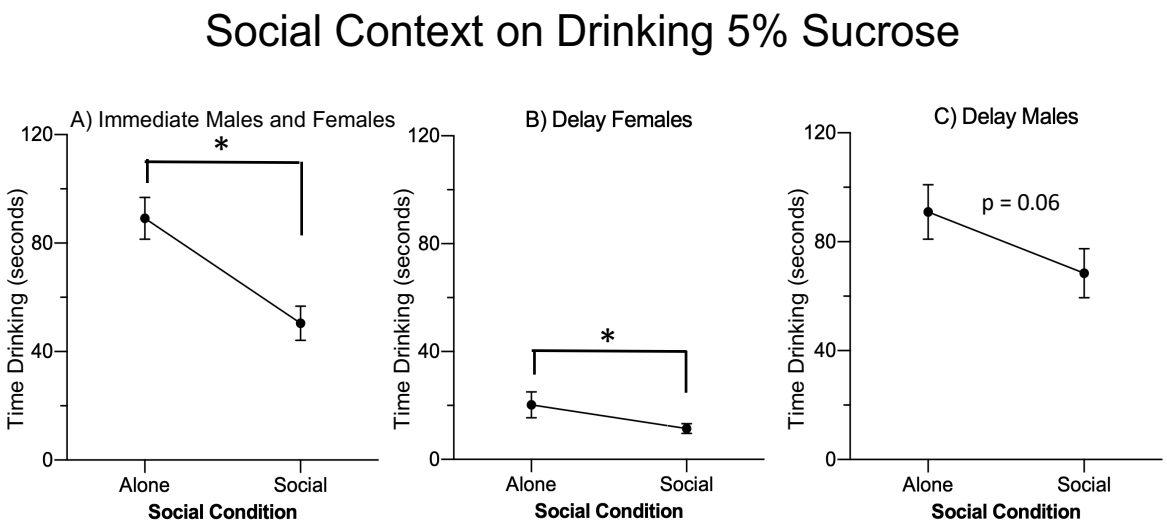

Figure 10. *Influence of social condition on drinking 5% sucrose (Mean + SEM). A) Immediates spent more time drinking when alone. B) Delay females spent more time drinking when alone. C) Delay males spent more time drinking when alone, but this trended towards significance.*

drinking when alone compared to when a peer was present, $F(1,46) = 22.19$, $p < 0.001$, $\eta_p^2 = 0.325$. An interaction between social condition and stress group ($F(1,46) = 4.11$, $p = 0.048$, $\eta_p^2 = 0.082$), revealed that there was no difference between stressed and control males in average time spent drinking sucrose when alone ($p = 0.577$), but when a peer was present stressed rats spent less time drinking than controls did ($p = 0.013$). A between-subjects effect of stress condition and age at stress that failed to reach significance ($F(1,46) = 3.68$, $p = 0.061$, $\eta_p^2 = 0.074$) indicated that adolescent controls spent more time drinking overall compared to stress rats ($p = 0.013$) whereas adult stressed and control did not differ ($p = 0.862$). There were no other significant interactions or main effects (all p's > 0.061).

Delay Females. All rats spent on average 10.93 seconds more drinking alone than they did with a peer, $F(1, 44) = 4.88$, $p = 0.032$, $\eta_p^2 = 0.100$ (see Figure 10B). There were no other interactions or main effects (all p's > 0.070).

Delay Males. Males spent on average 22.36 seconds more drinking alone than they did with a peer, but the difference was not significant $F(1, 48) = 3.65$, $p = 0.062$, $\eta_p^2 = 0.071$ (see Figure 10C). There were no other interactions or main effects (all p's > 0.079).

Discussion

Response to Sucrose

Although the specific predictions for the current experiment were in regard to social discounting, the general predictions they were derived from are still relevant to the separate measures of time spent drinking sucrose and time spent near the novel peer. The general predictions were: 1) that SS rats would increase time drinking sucrose and decrease time near the peer to a greater degree than would CTL rats as the concentrations of sucrose increased during the social discounting task, 2) an effect of stress would only be found in the adolescent-stressed

and not the adult-stressed rats, 3) SS effects in adolescent-stressed rats would be long-lasting, and if the previous prediction was incorrect and SS effects were evident in adult-stressed rats, than this effect would not be long-lasting, and 4) males and females would differ in their time spent drinking and with the peer, with SS females spending more time drinking sucrose than SS males.

The original predictions that stress effects would differ based on sex and age at stress were met in so far as the only stress effect evident was in the adolescent-stressed males. Male rats stressed during adolescence and tested immediately after the stress procedure spent less time drinking sucrose compared to controls, whereas no stress effect for sucrose consumption was found in males stressed during adulthood or in females stressed during either adolescence or adulthood. No stress effects were observed in any age or sex group tested 25 days after the stress procedure. Separate discussion sections below address each of these sex, age, and time of testing differences specifically.

Overall, during the social discounting task, rats increased the time spent drinking as the concentration of sucrose increased. That rats were given 0%, 2%, 5%, and 10% sucrose in a counter-balanced order and spent more time drinking at the higher concentrations indicates that rats were able to distinguish between the different concentrations despite encountering only one concentration per day and in a random order. In past experiments, when allowed to freely drink different concentrations of sucrose, adult male rats were found to increase consumption as the concentration rose, with a peak between 8% and 16%, and then decrease their intake at concentrations higher than 16% due to satiation (Sclafani, 1987; Sclafani & Ackroff, 2003). Thus, it is possible that a ceiling effect for 10% sucrose consumption was reached in the present experiment.

The current experiment is focused on between-group differences in sucrose consumption, yet it is important to note that individual differences exist as well. Rats naturally differ in baseline sucrose consumption and several investigations have split rats into groups based on low and high sucrose consumption (Brennan, Roberts, Anisman, & Merali, 2001; Desousa, Wunderlich, De Cabo, & Vaccarino, 1998; Sills & Crawley, 1996). Baseline differences in sucrose consumption may be a more stable trait that is also related to anxiety, because rats that had low consumption of sucrose also had higher levels of generalized anxiety compared to high consumption rats (Desousa et al., 1998). However, one study that determined the extent that rats were willing to work for access to different concentrations of sucrose (0%, 10%, 20%, and 30%) found similar response patterns in both low and high sucrose consumers, with both groups increasing responses as the concentration of sucrose increased (Brennan et al., 2001). While individual differences could have been evaluated in the current experiment, the main interest was how the overall response to increasing concentrations of sucrose is affected by the SS procedure. Because both low and high consumers respond in the same pattern to sucrose concentration changes (Brennan et al., 2001), only between-group differences were analysed for this thesis.

The SS adolescent male rats had a decrease in time drinking sucrose compared to controls during the social discounting task but not during the sucrose preference test. It is important to note the difference between sucrose intake and sucrose preference tasks. Sucrose preference often involves a two-bottle test where one bottle is filled with sucrose or saccharin and the other with water (Hoffman, 2016). Sucrose intake can be reported as the intake of sucrose during a sucrose preference test or can be a different test altogether that only utilizes one bottle containing sucrose or saccharin. Whereas sucrose intake is reported in amount consumed (in millilitres or grams), sucrose preference is usually reported as the percent sucrose consumed out of total fluid

consumed and so adjusts for total drinking behaviour. For the same two-bottle test, both sucrose intake and preference can be reported. From the same test, between-subjects differences in sucrose intake do not always produce significant between-subjects differences in sucrose preference when reported as a percent score (Wilmouth & Spear, 2009). However, the different results based on reporting sucrose intake versus preference may be at least partially due to methodology, as Wilmouth & Spear, (2009) used a 24 hour access test and D'Aquila, Newton, & Willner, (1997) reported that stress differences caused by chronic mild stress in both sucrose intake and preference are observed only when rats are tested during their dark phase and not during the light phase. With the consideration that sucrose intake and sucrose preference may not result in the same findings, some insight can still be gained from studies that report sucrose preference rather than sucrose intake.

It was originally predicted that SS rats would spend more time than controls would drinking sucrose as the concentration increased and thus be more sensitive to the changing concentrations (even though concentrations were presented in counter-balanced order). The opposite was found; instead of being more sensitive to the changing concentrations of sucrose, SS rats reacted less to the changing concentrations than controls did. The SS adolescent male rat's devaluation of sucrose could be described as anhedonia. Anhedonia is defined as an inability to feel pleasure; reduced consumption of and motivation for sucrose has been used previously as a measure of anhedonia (Muscat et al., 1990; Papp et al., 1991; Willner, 2017). Both anhedonia and a reduced motivation for reward are trademark symptoms of major depressive disorder. Adolescence appears to be a period that is vulnerable to the onset of affective disorders, with diagnoses of clinical depression in humans increasing during adolescence (Hankin et al., 1998; Lewinsohn, Clarke, Seeley, & Rohde, 1994; Zisook et al.,

2007). Based on the mesocortical pathway's involvement in reward motivation and reactivity, an often-proposed theory is that this circuit is implicated in depressive disorders (Dunlop & Nemeroff, 2007; Kapur & John Mann, 1992; Nestler & Carlezon, 2006; Stein, 2008). A decrease in sucrose or saccharine consumption is used as an indicator of anhedonia (Willner, Towell, Sampson, Sophokleous, & Muscat, 1987; Wilmouth & Spear, 2009) and that adolescent male SS rats spent less time drinking sucrose compared to controls when in a social context could be classified as a sex- and age-specific anhedonia in social conditions. Further discussion of how the adolescent male's decrease in sucrose consumption relates to depression (as measured by anhedonia) and also drug self-administration are outlined in subsequent sections.

The question arises of whether the adolescent male's decrease in sucrose consumption could have been due to an increase in anxiety as Desousa et al., (1998) found that male rats who consumed low amounts of sucrose also displayed increased generalized anxiety. Generalized anxiety, as measured by time spent on the open arm of an elevated plus maze, did not differ between SS and control adolescent males when tested immediately after the stress procedure (Hodges, Baumbach, & McCormick, 2018; McCormick, Smith, & Mathews, 2008). Conversely, a decrease in anxiety in adolescent SS males relative to controls emerged after controlling for novelty seeking, as measured by locomotor behaviour in an unfamiliar arena (Hodges et al., 2018). Adolescent SS females also displayed a decrease in generalized anxiety as measured by increased time in the open arm compared to controls (McCormick et al., 2008), but did not differ in time drinking sucrose in the current experiment. Taken together, general anxiety-related changes are an unlikely explanation for the SS adolescent male's drinking behaviour during the social discounting task. That adolescent SS males only spent less time drinking sucrose in a social context could indicate an effect of social anxiety specifically, considering the reduced time

SS adolescent males spend in social interaction relative to controls (Green et al., 2013; Hodges et al., 2019). However, that rats were only allowed social approach and not social interaction in the current experiment and that SS and CTL adolescent males did not differ in social approach does not support the idea that devaluation of sucrose in SS rats was caused by an increase in social anxiety.

Stress Effect Specific to Males. Results for sucrose intake and preference after isolation stress is not uniform, but some studies have found similar results to the current study, in that isolated males show a devaluation of sucrose compared to control males. Isolated males show less hyperreactivity in anticipation for 5% sucrose compared to group-housed males (Van Den Berg et al., 1999), indicating reduced motivation for sucrose. Sex differences in how adolescent isolation affects sucrose intake has been infrequently studied but when they have been, results are mixed. Hong et al., (2012) reported that in adulthood, after isolation during adolescence (PND 30-50), isolated females increased preference for 1% sucrose relative to non-isolated controls, with no difference between isolated and non-isolated males. A recent study that isolated both males and females between PND 21 and 49 found a decrease in sucrose preference in both sexes, but the authors note that the decrease appears greater for males than for females (Begni, Zampar, Longo, & Riva, 2020). Another study that isolated both male and female rats during their adolescent period (PND 30-60) found that isolated males drank less of a 32% sucrose solution than did group-housed males, with no differences in consumption based on housing conditions in females rats (Pisu et al., 2016). The sex difference found in Pisu et al., (2016) supports other studies that have suggested that females may be more resistant than males to stress-produced behavioural changes (Gomes & Grace, 2017; Klinger, Gomes, Rincón-Cortés, &

Grace, 2019). Females may be more resistant than males to Social Instability Stress-induced changes in sucrose consumption.

Whereas isolation studies employ the removal of social relationships, social instability stress manipulates social experiences to change the quality of social relationships without removing the relationships completely. The combination of social isolation and disruption of cage-partners in the McCormick Social Instability Stress procedure decreases time drinking sucrose only in a social context. Although there was no decrease in time drinking 1% sucrose in SS males in a social context in a previous study, SS males drank more sucrose when in competition against a cage partner (Marcolin et al., 2019) and displayed more aggressive behaviours when in competition for sweetened condensed milk (Cumming et al., 2014). Other models of social instability stress often observe a decrease in sucrose preference in stressed rats and mice (reviewed in Goñi-Balentziaga, Perez-Tejada, Renteria-Dominguez, Lebeña, & Labaka, 2018). Thus, changing the quality of social relationships, rather than removing them, is sufficient to influence sucrose intake and preference, but only in a social context and with sucrose concentrations greater than 1% in the McCormick SS procedure.

Sex differences in sucrose intake are not found in all stress models, in particular chronic mild stress and different versions of social instability stress. The chronic mild stress procedure produces a decrease in sucrose intake in both male (Grønli et al., 2004; Nielsen, Arnt, & Sánchez, 2000; Willner et al., 1987) and female rats (Baker, Kentner, Konkle, Santa-Maria Barbagallo, & Bielajew, 2006), suggesting that in tests of sucrose consumption, females may not be resistant to chronic mild stress. A review of different versions of social instability stress in female rats and mice found mixed results for sucrose preference, but most studies found a decrease in sucrose preference in stressed females relative to controls (Goñi-Balentziaga et al.,

2018), thus even different versions of social instability stress produced different results in the sucrose preference test. It is possible that the lack of stress difference in females in the current experiment is specific to the McCormick Social Instability Stress procedure. It is also possible that females of the Long-Evans strain specifically do not show a decrease in sucrose consumption; of the 17 articles included in Goñi-Balentziaga et al., (2018)'s systematic review, no other version of social instability stress outside the McCormick lab involved Long-Evans rats. Overall, although sex differences in sucrose consumption varies by stress procedure, the McCormick Social Instability Stress procedure produces a decrease in time drinking sucrose only in adolescent-stressed males, with no difference in females.

Males may be more likely than females to display anhedonia as measured by laboratory tests since lower consumption of sucrose only in males is also found in two rat strains that are used as models of depression. The Wistar-Kyoto strain is hyperresponsive to stress and spends less time in social interaction compared to the Sprague-Dawley strain (Pardon et al., 2002). Compared to Sprague-Dawley rats, Wistar-Kyoto males had a lower sucrose preference whereas females did not (Burke et al., 2016). Two separate lines of Sprague-Dawley rats were bred to be either susceptible or resistant to behavioural despair after an acute stressor as measured by activity in the forced swim test (Harrell, Hardy, Boss-Williams, Weiss, & Neigh, 2013). After exposure to chronic stressors during adolescence, stressed males from the susceptible line drank less sucrose than non-stressed susceptible males, with no stress difference in sucrose consumption in susceptible females (Harrell et al., 2013). In the resistant line, both male and female stressed rats consumed less sucrose than non-stressed resistant rats (Harrell et al., 2013). Thus, in strains susceptible to the effects of stress, only males display anhedonia in terms of

sucrose consumption and preference and results from these strains are consistent with the decreased time drinking sucrose specific to adolescent male SS rats in the current experiment.

Stress Effect Specific to Adolescents. The effects of SS were age-specific with adolescents being more vulnerable, consistent with the original prediction that stress effects would only be found in adolescent-stress rats. During the social discounting task, decreased consumption of sucrose was only evident in adolescent-stressed rats, with no differences in sucrose consumption caused by adult stress. Non-stressed adolescent males have been found to be more sensitive to changes in sucrose concentration than adults (Wilmouth & Spear, 2009), and the SS procedure may have dampened this sensitivity. A study that measured taste reactivity to different concentrations of sucrose (0%, 0.34%, 3.4%, and 34% in random order) by a 45 second oral infusion found that both adolescent and adult male rats had more positive responses to 34% sucrose compared to water, but only adolescents showed more positive responses to 3.4% sucrose than they did to water (Wilmouth & Spear, 2009). Adolescents are more sensitive than adults to the hedonic properties of both low and high value sweet rewards as adolescents drink more 1% sucrose adjusted to body weight (Wilmouth & Spear, 2009), spend more time drinking 1% sucrose than adults do (Marcolin et al., 2019), and consumption of sweetened condensed milk peaks during adolescence (Friemel et al., 2010). That stress in the current experiment attenuated sucrose consumption only for the adolescent males and not for adults would suggest that even though adolescents are more sensitive to the rewarding properties of sucrose than are adults, this sensitivity is more susceptible to alteration by stressors.

Although the Social Instability Stress procedure consists partly of an isolation stressor and a stress-induced change in sucrose consumption in adolescent males was found in the present experiment, changes in sucrose intake after isolation housing are mixed. Isolation-housing in

adolescence decreases motivation for sweet substances (Van Den Berg et al., 1999), but in terms of sucrose consumption across multiple studies, isolation has been found to decrease (Carrier & Kabbaj, 2012; Pisu et al., 2016), not change (Hall, Humby, Wilkinson, & Robbins, 1997), or increase consumption of sucrose (Brenes & Fornaguera, 2008) compared to group-housed rats. Although adolescent SS rats do not differ from controls in intake for 1% sucrose (Marcolin et al., 2019), adolescent SS males in the current experiment spent less time drinking sucrose than controls did. A previous study found evidence that re-pairing with an unfamiliar partner following isolation more so drives adolescent-susceptibility in the Social Instability Stress procedure rather than the isolation component alone. Given that isolation stress is not uniform in its effects on sucrose intake, it is possible that the changes in adolescent SS rat's consumption of sucrose solutions in the current experiment was driven more so by social instability, or its interaction with isolation stress, rather than by isolation stress alone.

Stress Effect Specific to Adolescent Rats Tested Immediately after SS. Adolescent SS male rats that were tested immediately after the stress procedure drank less sucrose than did controls, but no effect of stress was observed in the adolescent males tested after a delay of 25 days. A previous study that administered a chronic stress paradigm to male rats for 5 weeks found that stressed rats drank less sucrose relative to baseline for the first two weeks, and then this decrease in consumption attenuated after two weeks although still remained significant (Grønli et al., 2004). The attenuation of stressed-induced anhedonia, even while the stressor is still present, as found in Grønli et al., (2004), indicates that changes in reward-related behaviours due to stress may be temporary. Hong et al., (2012) found that after adolescent isolation, males did not differ from controls in sucrose preference later in adulthood, although females did, so the temporary anhedonia may be sex-specific depending on the type of stressor. It is possible that

anhedonia as measured by sucrose intake due to stress is a relatively short-lived effect that disappears after a few weeks, but continuous stress can prolong the stress effects.

For the Social Instability Stress procedure specifically, whether SS differences are long-lasting depends on the measure. Some stress effects only emerge with time. A decrease in spatial location memory is only found in adolescent SS rats when they are tested weeks after the SS procedure and not when they are tested immediately after (McCormick et al., 2012). When examined weeks after the stress procedure, males who were stressed during adolescence had decreased protein expression of two markers of synaptic plasticity (PSD-95 and αCaMKII) in the prefrontal cortex compared to controls, while rats examined immediately after stress procedure did not differ from controls (Marcolin, Baumbach, Hodges, & McCormick, 2020). For other measures, only immediate effects of stress are found. After a swim test conducted the day after the SS procedure ends, adolescent-stressed males have a potentiated corticosterone response and females have a prolonged response compared to controls, but no stress differences are found when the swim test is conducted 25 days after the last day of the SS procedure (Mathews, Wilton, Styles, & McCormick, 2008). During a 30 minute testing session, males tested immediately will consume more 10% ethanol than controls, with no stress difference found when tested weeks later (Marcolin et al., 2019). The SS adolescent male's reduction in time drinking sucrose in the current experiment is a stress effect that is only evident when tested immediately after the stress procedure and is thus not long-lasting.

Effect of Social Context on Drinking Sucrose

A comparison on time spent drinking sucrose alone and when a peer was present revealed that SS rats spent less time drinking sucrose when a peer was present. A previous study found that adolescent male rats, regardless of whether stressed or not, drank more of 1% sucrose when

they were alone compared to when a peer was present (Marcolin et al., 2019). The current study replicated these results and extended them to females and to rats stressed as adults, as well as to a new concentration of sucrose, 5%. No stress effects were found in the sucrose preference test when rats were drinking alone but were observed in the social discounting task when a peer was present. Although adolescent male SS rats did not alter their time near a novel peer as the concentrations of sucrose changed in the social discounting task, a peer being present was necessary for a stress effect in sucrose drinking to be observed.

Some insight into the influence of social context on sucrose reward can be gained by looking at how social context affects responses towards drugs of abuse. Both sucrose and drugs of abuse activate the mesolimbic system, and the dopaminergic response to both substances are thought to be analogous (De Jong, Vanderschuren, & Adan, 2016). The full extent of the neural overlap between social reward and drug reward is not fully known, but the two rewards have interacting effects and, under different contexts, can either be protective against, or have a synergistic effect with, self-administration of drugs of abuse (reviewed in Beloate & Coolen, 2017). In adolescent male rats, pairing social context and nicotine together facilitates CPP formation (Thiel, Sanabria, & Neisewander, 2009); however, in choice tests between a social stimuli and drug administration, the two stimuli have competing reward values. When rats trained to self-administer either methamphetamine or heroin are allowed to choose between the drug reward and a social reward, both male and female rats chose the social reward more frequently than they did the drug reward, preventing drug self-administration (Venniro et al., 2018). Competing reward values between sucrose and social stimuli could explain why rats in the current experiment drank less sucrose when a peer was present. That rats spent more time drinking 5% sucrose when alone may indicate that social reward competed against sucrose

reward to such a degree that it created a statistically significant effect. Age and internal motivational states influence the choice between social and drug reward. In a CPP test in which one chamber is associated with social interaction and the other with amphetamine administration, adolescent male rats prefer the social side if they have been housed individually and the amphetamine side if they have been pair-housed (Yates, Beckmann, Meyer, & Bardo, 2013). Housing conditions only affected choice preference in adolescent rats, adults did not have a preference for either compartment, regardless of housing condition (Yates et al., 2013). The decreased time adolescent SS males spent drinking in the social discounting test is consistent with Yates et al., (2013) as the altered housing environment (SS procedure) altered the choice preference between two natural rewards only in adolescents.

Although social context has also been found to protect against drug self-administration in a choice test, social context facilitates drug administration when the two are presented together. Although the Social Discounting task was designed to be a choice task between social and sucrose rewards, the two stimuli were presented together, and so synergistic effects of both rewards may have been present during the task. Varlinskaya et al., (2015) found that adolescent and adult males consumed more ethanol when in a social condition. Gipson et al., (2011) found that the presence of a peer increased self-administration of amphetamine, although the presence of a peer decreased intake of sucrose pellets. The decrease in time drinking sucrose when in the presence of a peer in the current study is consistent with the decreased sucrose intake in Gipson et al., (2011). Whereas social context facilitated drug administration, social context decreased sucrose intake and so social context affects both drug and sucrose administration, but in opposite directions. That no stress difference was found in the social novelty and sucrose preference tests, but a stress effect was found when social and sucrose rewards were presented together suggests

that the interaction between the two rewards is displayed differently, or altered, in stressed adolescent male rats.

With the exception of the delay females, rats spent more time drinking sucrose than they did approaching a novel peer in the social discounting task, suggesting that sucrose contained a higher reward value than the social stimuli. When administered to adolescent males, the Social Instability Stress procedure has been previously found to alter social behaviour (Green et al., 2013; Hodges et al., 2019) and it is possible that the SS adolescent male's altered social repertoire is competing against the sucrose reward and that the subsequent devaluation of sucrose is the manifestation of this motivational competition. Other laboratory tests, such as conditioned place preference or progressive ratio self-administration, could aid in determining the motivational properties of both stimuli and if they differ in SS rats. If the devaluation of sucrose under social conditions is a result of an altered social repertoire, this stress effect is then specific to adolescent males. This adolescent vulnerability would be consistent with findings from another lab that has shown that adolescent male rats will change their preference for either social interaction or amphetamine reward depending on their housing (isolation or group-housing), but adult males have no preference, even when their housing environment is manipulated (Yates et al., 2013). Most animal studies in various fields, including neuroscience, use male subjects which has led to little known about sex differences (Shansky, 2019). However, that females did not show a stress difference in the current experiment is consistent with a previous finding that isolation stress enhances the reward value of social stimuli in adolescent male, but not female, rats (Douglas et al., 2004). Future studies that include both males and females would be largely beneficial to determine what stress effects are sex specific.

Social Approach During Social Novelty and Social Discounting Tasks

In the social novelty test, a main effect of partner familiarity emerged whereby rats spent more time near the novel peer than they did with the familiar one, consistent with previous reports using the same novelty paradigm [e.g., Smith et al., (2015); Smith et al., (2017)]. From the data in Figure 5, some rats spent more time with the familiar over the unfamiliar rat. The individual variability in time near either peer was likely a result of using the larger zone for tracking (1/3rd of the main chamber, see Figure 1B). Whereas the smaller zones that were used during the social discounting test (Figure 1C) only included time spent directly approaching the stimulus rats, the larger zones likely also included time when the rats were not approaching either peer but still within the zone. Hand scoring the social novelty test would determine whether a smaller zone results in less individual variability in novelty preference. However, hand scoring for the current experiment would be unnecessary as a main effect of novelty preference emerged and the zones on either side of the apparatus were the same size so that neither the familiar nor unfamiliar side was biased. Thus, using a larger tracking zone to measure social novelty preference is a suitable alternative to hand scoring a smaller inclusion zone.

The amount of time that rats spent near the novel peer during the social discounting task is likely not representative of their full engagement with the peer. The design of the testing apparatus allowed rats to receive audio and olfactory cues from the stimulus rat even when they were drinking sucrose and not directed towards the stimulus rat. Rats communicate using ultrasonic vocalizations (Brudzynski, 2009) and it was more likely than not that auditory communication was occurring between the test and stimulus rats during the social discounting test that could not be detected by human ears. However, the communication happening because of these calls is a normal occurrence when utilizing rats in research. Further, these calls have

been found to elicit approach behaviour (Wöhr & Schwarting, 2007) and so may have helped to draw the test rat towards the stimulus rat rather than have acted as a deterrent. Preliminary results not included in this thesis analyzed the time spent near the novel peer as a measure of time within 1 cm of the whole mesh wall (whereas this thesis only includes time spent within 1 cm of the hole in the centre of the mesh) and time spent in the larger zones covering 1/3rd of the central test chamber nearest to the peer (see Figure 1B for depiction of the latter) to determine if a larger inclusion zone would capture more social approach. Use of these larger inclusion zones for social approach produced results largely similar to the ones reported here, and thus using the smaller zone and only including time spent at the hole did not affect the results of the test. The ultrasonic vocalizations between the test and stimulus rat were likely either not having a significant effect on approach behaviour during the social discounting test or were having an effect that was uniformly captured by different sized inclusion tracking zones. The time spent near the hole in the mesh was used in the final analyses reported in this thesis to include only directed social approach and to not bias a larger zone of social approach over the smaller zone around the sucrose bottle spout.

Rats spent less time near the novel peer during the social discounting task than was expected, but these results are not believed to be abnormal as there was an overall preference for partner novelty during the social novelty test, which was expected. Although the increased time that the adult delay group spent with the unfamiliar peer over the familiar peer did not reach significance, given that this was the oldest group, this is likely the result of a well-reported, age-related decrease in social interest (Baenninger, 1967; Panksepp, 1981; Thor & Holloway, 1984). The results from the time spent near the novel peer during the social discounting task are consistent with previous findings using the Social Instability Stress paradigm. That SS males

spent more time near the novel peer than CTL males did during the social discounting task replicates previous findings that SS males will often spend more time near a novel peer in social approach measurements than controls will (Green et al., 2013; Hodges et al., 2017). SS females have not been previously tested on social approach, but that there was not a stress difference in females in the current experiment extends the results previously found in males to females (Green et al., 2013; Hodges et al., 2017). Together with recent evidence from the McCormick lab that found that SS females spend less time in social interaction than do controls (Asgari, 2020), the Social Instability Stress procedure produces alterations in time spent in social interaction in both male and female rats but does not alter social approach behaviour.

Social Discounting

For the current experiment, social discounting was defined as a reduction in preference for a novel peer in favour of increasing concentrations of sucrose. An increase in time drinking sucrose as the concentration increased was expected to be observed simultaneously with a decrease in time near the novel peer. Under this definition, social discounting was not observed in the current experiment, as the increase in time spent drinking sucrose as the concentrations increased did not result in less time spent near the novel peer.

A possible reason for the lack of adjustment of time spent near the peer as the concentrations of sucrose changed could be the design of the testing apparatus used. As displayed in Figure 1, the apparatus consisted of one main chamber for the test rat and two attached side chambers in which stimuli could be placed. An apparatus that is commonly used in social behavioural tasks is the three-chambered apparatus, which consists of three chambers separated by walls that contain a door to allow access into each chamber. A diagram of an example three-chamber apparatus is shown in Figure 11. The two walls separate the box into

three main chambers, the shaded two of which the test rat must enter into to be considered in the inclusion zone. When the test rat is in either of the two chambers that have stimuli present, they are then primarily exposed to that stimuli. Although they are likely receiving auditory and

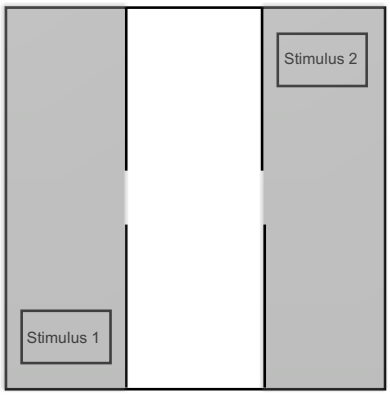

Figure 11. *Schematic of a three-chambered testing apparatus. Shaded areas represent zones used for tracking.*

olfactory cues from the stimulus in the far chamber, these are reduced by the two walls. The testing apparatuses used in the current experiment did not have separate chambers within the main testing chamber. One continuous chamber allowed rats to still receive a large amount of auditory and olfactory cues from the unfamiliar peer when they were drinking from the sucrose bottle at the other end of the main chamber. Because rats were still receiving cues from the unfamiliar stimulus peer while drinking in the apparatus, this could have diminished some of the choice aspect of this test as rats were not having to choose between drinking sucrose and visiting the peer to the same degree as they would have had to in a three-chambered apparatus.

The opposite of social discounting was found when analyzing social context on time spent drinking of 5% sucrose. Rather than adjusting time near the peer as the concentration of sucrose changed as was expected with social discounting, rats adjusted their time drinking based on the social context. That rats drink more of 5% sucrose when alone than they did when there was a peer present is consistent with previous findings that rats will drink more of a 1% sucrose solution when alone than they will with a peer (Marcolin et al., 2019). Whereas Marcolin et al.

(2019) only found an effect of social context on drinking in rats tested immediately and not after a delay, this study found that both rats tested immediately and those tested after a delay drank more when alone than they did when a peer was present. As the rewarding value of sweet substances decreases as rats age (Friemel et al., 2010), perhaps a higher concentration of sucrose (5% over 1%) provided a higher reward value that was less diminished with age. In the current experiment, the results that adolescent male rats drank more sucrose alone than they did with a peer was extended to female rats and adult rats of both sexes.

The Veenema lab recently developed the Social versus Food Preference Test which involves a choice between social approach and food consumption (Reppucci, Brown, Chambers, & Veenema, 2020). The Social versus Food Preference Test is similar to the Social Discounting test but rather uses food pellets instead of sucrose. Both adolescent and adult, male and female Wistar rats preferred to approach the novel peer over consuming food and 24 hour isolation did not change rat's preference for the novel peer (Reppucci et al., 2020). Food deprivation for 24 hours increased time spent eating for both adolescents and adults, and also decreased time investigating the novel peer, but only for adolescents (Reppucci et al., 2020), similar to what was expected in the current Social Discounting test. Although the Social Instability Stress procedure decreased time spent drinking for adolescent male rats, it appears that food deprivation is required to also observe a reduction in time spent near a novel peer in favour of consummatory behaviour in both male and female adolescents, and isolation alone is not sufficient to induce any changes in choice behaviour.

Although the current experiment did not measure neural correlates during the social discounting task, theories can still be generated as to the mechanisms involved, with the predominant transmitters likely being dopamine and oxytocin regulation. Drinking sucrose

increased dopamine in the nucleus accumbens and administering a dopamine reuptake inhibitor to the nucleus accumbens increased sucrose intake (Hajnal & Norgren, 2001). Decreases in both sucrose consumption and preference in male Wistar and Sprague-Dawley rats was reversed by chronic administration of an antidepressant which blocked dopamine D2 receptors (Bekris et al., 2005). Administering a dopamine antagonist did not decrease sucrose intake, suggesting other mechanisms besides dopamine in the nucleus accumbens are also involved in sucrose consumption (Hajnal & Norgren, 2001). Thus, administration of dopamine directly into the nucleus accumbens increases sucrose consumption, but this is not the sole mechanism for sucrose intake.

Oxytocin in the VTA, hypothalamus, and nucleus accumbens has been found to promote social behaviours and interact with dopamine to regulate social behaviours (Song, Kalyani, & Becker, 2018). Oxytocin enhanced motivation for social rewards but diminished sucrose intake, but to what extent this is due to shared or distinct mechanisms or to a mediator is not fully known (Song et al., 2018). In both males and females, oxytocin decreased lever pressing for sucrose, but females were more sensitive to lower doses than males (Zhou, Ghee, See, & Reichel, 2015). This may reflect sex differences in oxytocin receptor binding as females had lower receptor binding densities in the nucleus accumbens, hippocampus CA1, and medial amygdala compared to males (Dumais, Bredewold, Mayer, & Veenema, 2013).

In mice, social stress decreased oxytocin receptor expression in females (Williams et al., 2020). Oxytocin in the nucleus accumbens differentially regulates two social behaviours: approach and vigilance (Williams et al., 2020). In male rats and mice, oxytocin has been found to reverse stress-induced decreases in social approach (Lukas et al., 2011). In SS and CTL rats, oxytocin antagonist administered as an injection had no effect on social approach, but increased

social avoidance in adolescent SS males relative to controls and to vehicle-injected SS males (Hodges et al., 2019). As adults, females who had SS administered during adolescence had lower oxytocin receptor gene expression in their hippocampus compared to controls (Asgari*, in preparation*). In adolescent SS males, it is expected that differences in dopamine and oxytocin expression will be observed after the Social Discounting task, perhaps with diminished expression of both neurotransmitters relative to non-stressed adolescent controls.

Low Sucrose Consumption of Delay Females

The finding that the females tested after a delay drank less sucrose than the other time of testing and sex groups was not expected. Because of the delay before testing, the decrease in time drinking sucrose may have been partially driven by age. A previous study in male rats found that adolescents drank more of a 1% sucrose solution relative to body weight than did adults (Wilmouth & Spear, 2009). Adolescent males also displayed more positive reactions to 10% sucrose than did adults (Wilmouth & Spear, 2009), suggesting that sucrose provides a greater hedonic "liking" value to adolescents than it does to adults. Further, the rewarding value of sweetened condensed milk peaks during late adolescence at PND 50 and then declines into adulthood (Friemel et al., 2010), making an age-related decline in reward value for sweet substances a possible explanation for the delay female's low consumption. Whether an age-related decrease in sucrose intake is evidence for a heightened reactivity of adolescents or a decline in reactivity with age is not known. In Figure 8B and 8D, it appears as though the adolescent males (Figure 8B) spent more time drinking than did the adult males (Figure 8D), but this difference was non-significant as an appropriate interaction did not emerge. However, age differences in female drinking behaviour have not been investigated previously and the delay female's lowered time drinking relative to females tested immediately may have been an age-

related anhedonia of sucrose analogous to the age difference found in males by Wilmouth & Spear (2009) and Friemel et al., (2010).

An issue with the age hypothesis is present. In the current experiment, the females tested after a delay but stressed as adolescents that had lowered sucrose consumption were actually younger (PND 72-75) than the females tested immediately but stressed as adults (PND 87-90) during the four days of testing. The rats used in Wilmouth & Spear (2009) were bred from a colony in the lab whereas the rats in the current experiment were ordered from an external supplier at their appropriate ages, thus the adolescent-stress group arrived as adolescents and the adult-stress group arrived as adults. A similarity between the current experiment and Wilmouth & Spear (2009) is that their adult male group was housed in the colony room longer than the adolescent male group and in the current experiment the delay-testing group was housed in the colony room longer than the immediate-testing group. Thus, the difference in time drinking sucrose may be an effect of delay before testing. Rather than a sole effect of time, this may be an effect of time spent in a particular housing environment. Female rats appear to be sensitive to their housing environment, and have a reduction in sucrose preference after four weeks in isolation housing relative to before isolation (Baker & Bielajew, 2007). Female rats housed in an enriched environment for four weeks displayed a greater sucrose preference than rats housed in standard laboratory conditions (Baker & Bielajew, 2007). In males, after roughly five weeks in an enriched environment, sucrose preference does not differ from males housed in standard environments (Brenes & Fornaguera, 2008). Enriched environments typically will house a group of rats in a large cage with multiple toys that are changed at intervals to maintain novelty. In comparison, standard housing consists of two or three rats housed together in a standard-sized cage with one toy. In recent years, the question has arisen of whether enriched environments are

providing a benefit over standard housing or are actually reversing a deficit caused by standard housing (Kempermann, 2019). Thus, if females are more sensitive to their housing environment than are males, the extended period of time in standard housing may have had an effect on sucrose preference for the female rats. Replication and further investigation into how age and time spent in different housing conditions affects sucrose preference is needed.

Although the factors that produced anhedonia in the delay females are not known, the factors that likely did not contribute to this effect are more established. That both control and SS delay females spent less time drinking sucrose indicates that this was not an effect of stress. That the immediate adult-stressed rats did not show anhedonia, but the delay adolescent-stressed females did despite these two groups being similar in age at the time of testing (immediate adults: PND 87-90, delay ado: PND 72-75) indicates that this is likely not solely an age effect. The possibility remains that age was a contributing, but not the primary, factor. The difference in time drinking is likely not solely a cohort difference caused by breeding or shipping as females that were tested both immediately and after a delay arrived at the facility from the same supplier at the same time and only the delay group displayed the attenuated drinking behaviour. The delay female group received the same handling, manipulations, and order of testing that the other time of test and sex groups received, making it unlikely that the difference was caused by an unintentional procedural or experimental manipulation. The delay female's decrease in sucrose consumption requires replication to determine if this is a valid effect or a result of an environmental factor not accounted for.

Changes in Physical Development

SS rats gained less weight during the stress procedure than did controls who remained in their home cages, a finding that has been consistently observed in males stressed during

adolescence (McCormick, 2010). Both age groups and both sexes of SS rats in the current experiment had reduced weight gain. Previous studies have found mixed results for weight gain in adult-stressed males: one study found reduced weight after stress in adolescent-stressed males but not adult-stressed (Hodges & McCormick, 2015), and another study found reduced weight gain in both adolescent- and adult-stressed males relative to controls (McCormick et al., 2005). Although reduced weight gain has not typically been found in either adolescent- or adult-stressed SS females (McCormick et al., 2004, 2005), SS females in the current experiment stressed at both ages also had reduced weight gain relative to controls. Regardless of stress group, a main effect of sex and a main effect of age on weight gain were also found. That adolescent rats had greater weight gain than did adults, and that males had greater weight gain than females, were expected based on normal developmental trajectories (Charles River Laboratories, 2020).

Vaginal opening for adolescent SS females was monitored daily to determine if the SS procedure had an effect on physical markers of puberty. No difference was found in mean day of vaginal opening between the SS females and previously gathered data for control females. A previous study found that early-life stressors resulted in an earlier average age of vaginal opening (Cowan & Richardson, 2019). The amount of time that females were exposed to the SS procedure before vaginal opening was observed was likely not sufficient for the stress procedure to have an effect on pubertal timing, in fact, some of the SS females already had vaginal openings on PND 30 when the stress procedure began. Since males have a later age of pubertal onset (~PND 42) than females do, stress differences in pubertal onset in males are more likely to be found since they would have been exposed to the stress procedure for longer prepubertally. A previous report has found that early-life stress has the opposite effect for males as it does for females, with males having delayed pubertal onset relative to controls (Cowan & Richardson,

2019). However, it is possible that only early-life stress has an effect on pubertal onset, and that pubertal stress occurs too close to when pubertal markers appear to have an effect. It has also been found previously in adult female rats that chronic stress disrupts the estrous cycle by causing cycles to become more irregular (Baker et al., 2006; Konkle et al., 2003). Although no stress differences were found in vaginal opening, future studies could determine if SS disrupts the estrous cycle of adult females.

Conclusion

Overall, contrary to initial predictions, rats did not adjust time spent with a novel peer as they were presented with increasing concentrations of sucrose. Adolescent male rats that underwent the Social Instability Stress procedure devalued sucrose when a peer was present in relation to non-stressed controls, but this result was not long-lasting. No effects of stress were observed in adult-stressed rats, supporting the hypothesis that adolescents are more susceptible to the effects of Social Instability Stress than adults are. Thus, the Social Instability Stress procedure produces changes in reward processing for sweet rewards that is specific to the adolescent period of male rats, and from which rats recover.

References

Achterberg, E. J.M., van Swieten, M. M. H., Houwing, D. J., Trezza, V., & Vanderschuren, L. J. M. J. (2019). Opioid modulation of social play reward in juvenile rats. *Neuropharmacology*, *159*, 107332. https://doi.org/10.1016/j.neuropharm.2018.09.007

Achterberg, E. J.M., Van Kerkhof, L. W. M., Servadio, M., Van Swieten, M. M. H., Houwing, D. J., Aalderink, M., … Vanderschuren, L. J. M. J. (2016). Contrasting Roles of Dopamine and Noradrenaline in the Motivational Properties of Social Play Behavior in Rats. *Neuropsychopharmacology*, *41*(3), 858–868. https://doi.org/10.1038/npp.2015.212

Ågmo, A., Galvan, A., & Talamantes, B. (1995). Reward and reinforcement produced by drinking sucrose: Two processes that may depend on different neurotransmitters. *Pharmacology, Biochemistry and Behavior*, *52*(2), 403–414. https://doi.org/10.1016/0091-3057(95)00128-J

Aloisi, A. M., Ceccarelli, I., & Lupo, C. (1998). Behavioural and hormonal effects of restraint stress and formalin test in male and female rats. *Brain Research Bulletin*, *47*(1), 57–62. https://doi.org/10.1016/S0361-9230(98)00063-X

Andersen, S. L. (2003). Trajectories of brain development: Point of vulnerability or window of opportunity? *Neuroscience and Biobehavioral Reviews*, *27*(1–2), 3–18. https://doi.org/10.1016/S0149-7634(03)00005-8

Andersen, S. L., Rutstein, M., Benzo, J. M., Hostetter, J. C., & Teicher, M. H. (1997). Sex differences in dopamine receptor overproduction and elimination. *NeuroReport*, *8*(6), 1495–1498. https://doi.org/10.1097/00001756-199704140-00034

Andersen, S. L., Thompson, A. P., Krenzel, E., & Teicher, M. H. (2002). Pubertal changes in gonadal hormones do not underlie adolescent dopamine receptor overproduction.

Psychoneuroendocrinology, *27*(6), 683–691. https://doi.org/10.1016/S0306-4530(01)00069-5

Asgari, P. (2020). *Stress and the Development of Social Behaviour in Adolescent Female Rats*. Brock University. https://doi.org/10.1155/2010/706872

Auger, A. P., & Olesen, K. M. (2009). Brain sex differences and the organisation of juvenile social play behaviour. *Journal of Neuroendocrinology*, *21*(6), 519–525. https://doi.org/10.1111/j.1365-2826.2009.01871.x

Baenninger, L. P. (1967). Comparison of behavioural development in socially isolated and grouped rats. *Animal Behaviour*, *15*(2–3), 312–323. https://doi.org/10.1016/0003-3472(67)90018-8

Baker, S., & Bielajew, C. (2007). Influence of housing on the consequences of chronic mild stress in female rats. *Stress*, *10*(3), 283–293. https://doi.org/10.1080/10253890701265362

Baker, S. L., Kentner, A. C., Konkle, A. T. M., Santa-Maria Barbagallo, L., & Bielajew, C. (2006). Behavioral and physiological effects of chronic mild stress in female rats. *Physiology and Behavior*, *87*(2), 314–322. https://doi.org/10.1016/j.physbeh.2005.10.019

Begni, V., Zampar, S., Longo, L., & Riva, M. A. (2020). Sex differences in the enduring effects of social deprivation during adolescence in rats: Implications for psychiatric disorders. *Neuroscience*, *437*, 11–22. https://doi.org/10.1016/j.neuroscience.2020.04.018

Bekris, S., Antoniou, K., Daskas, S., & Papadopoulou-Daifoti, Z. (2005). Behavioural and neurochemical effects induced by chronic mild stress applied to two different rat strains. *Behavioural Brain Research*, *161*(1), 45–59. https://doi.org/10.1016/j.bbr.2005.01.005

Beloate, L. N., & Coolen, L. M. (2017). Influences of social reward experience on behavioral responses to drugs of abuse: Review of shared and divergent neural plasticity mechanisms

for sexual reward and drugs of abuse. *Neuroscience and Biobehavioral Reviews*, *83*(October), 356–372. https://doi.org/10.1016/j.neubiorev.2017.10.024

Belsky, J., Steinberg, L., & Draper, P. (1991). Childhood experience, interpersonal development, and reproductive strategy: An evolutionary theory of socialization. *Child Development*, *62*(4), 647–670. https://doi.org/10.1111/j.1467-8624.1991.tb01558.x

Boyer, W. N., Cross, H. A., & Anderson, C. (1974). Quality reward preference in the rat. *Bulletin of the Psychonomic Society*, *3*(5), 332–334. https://doi.org/10.3758/BF03333486

Brenes, J. C., & Fornaguera, J. (2008). Effects of environmental enrichment and social isolation on sucrose consumption and preference: Associations with depressive-like behavior and ventral striatum dopamine. *Neuroscience Letters*, *436*(2), 278–282. https://doi.org/10.1016/j.neulet.2008.03.045

Brennan, K., Roberts, D. C. S., Anisman, H., & Merali, Z. (2001). Individual differences in sucrose consumption in the rat: Motivational and neurochemical correlates of hedonia. *Psychopharmacology*, *157*(3), 269–276. https://doi.org/10.1007/s002130100805

Bromberg-Martin, E. S., Matsumoto, M., & Hikosaka, O. (2010). Dopamine in motivational control: Rewarding, aversive, and alerting. *Neuron*, *68*(5), 815–834. https://doi.org/10.1016/j.neuron.2010.11.022

Brudzynski, S. M. (2009). Communication of adult rats by ultrasonic vocalization: Biological, sociobiological, and neuroscience approaches. *ILAR Journal*, *50*(1), 43–50. https://doi.org/10.1093/ilar.50.1.43

Burke, N. N., Coppinger, J., Deaver, D. R., Roche, M., Finn, D. P., & Kelly, J. (2016). Sex differences and similarities in depressive- and anxiety-like behaviour in the Wistar-Kyoto rat. *Physiology and Behavior*, *167*, 28–34. https://doi.org/10.1016/j.physbeh.2016.08.031

Calcagnetti, D. J., & Schechter, M. D. (1992). Place conditioning reveals the rewarding aspect of social interaction in juvenile rats. *Physiology and Behavior*, *51*(4), 667–672. https://doi.org/10.1016/0031-9384(92)90101-7

Carrier, N., & Kabbaj, M. (2012). Testosterone and imipramine have antidepressant effects in socially isolated male but not female rats. *Hormones and Behavior*, *61*(5), 678–685. https://doi.org/10.1016/j.yhbeh.2012.03.001

Charles River Laboratories (2020). Long-Evans Rat. https://www.criver.com/products-services/find-model/long-evans-rat?region=24

Choi, H., Choi, Y., Kim, K. W., Kang, H., Hwang, D. W., Kim, E. E., … Lee, D. S. (2015). Maturation of metabolic connectivity of the adolescent rat brain. *ELife*, *4*(NOVEMBER2015), 1–12. https://doi.org/10.7554/eLife.11571

Chrousos, G. P. (2009). Stress and disorders of the stress system. *Nature Reviews Endocrinology*, *5*(7), 374–381. https://doi.org/10.1038/nrendo.2009.106

Cirulli, F., Terranova, M. L., & Laviola, G. (1996). Affiliation in periadolescent rats: Behavioral and corticosterone response to social reunion with familiar or unfamiliar partners. *Pharmacology Biochemistry and Behavior*, *54*(1), 99–105. https://doi.org/10.1016/0091-3057(95)02169-8

Cowan, C. S. M., & Richardson, R. (2019). Early-life stress leads to sex-dependent changes in pubertal timing in rats that are reversed by a probiotic formulation. *Developmental Psychobiology*, *61*(5), 679–687. https://doi.org/10.1002/dev.21765

Crews, F., He, J., & Hodge, C. (2007). Adolescent cortical development: A critical period of vulnerability for addiction. *Pharmacology Biochemistry and Behavior*, *86*(2), 189–199. https://doi.org/10.1016/j.pbb.2006.12.001

Cumming, M. J., Thompson, M. A., & McCormick, C. M. (2014). Adolescent social instability stress increases aggression in a food competition task in adult male Long-Evans rats. *Developmental Psychobiology*, *56*(7), 1575–1588. https://doi.org/10.1002/dev.21252

D'Aquila, P. S., Newton, J., & Willner, P. (1997). Diurnal variation in the effect of chronic mild stress on sucrose intake and preference. *Physiology and Behavior*, *62*(2), 421–426. https://doi.org/10.1016/S0031-9384(97)00042-5

De Jong, J. W., Vanderschuren, L. J. M. J., & Adan, R. A. H. (2016). The mesolimbic system and eating addiction: What sugar does and does not do. *Current Opinion in Behavioral Sciences*, *9*(March), 118–125. https://doi.org/10.1016/j.cobeha.2016.03.004

Desousa, N. J., Wunderlich, G. R., De Cabo, C., & Vaccarino, F. J. (1998). Individual differences in sucrose intake predict behavioral reactivity in rodent models of anxiety. *Pharmacology Biochemistry and Behavior*, *60*(4), 841–846. https://doi.org/10.1016/S0091-3057(98)00069-0

Douglas, L. A., Varlinskaya, E. I., & Spear, L. P. (2004). Rewarding properties of social interactions in adolescent and adult male and female rats: Impact of social versus isolate housing of subjects and partners. *Developmental Psychobiology*, *45*(3), 153–162. https://doi.org/10.1002/dev.20025

Dreyfuss, M., Caudle, K., Drysdale, A. T., Johnston, N. E., Cohen, A. O., Somerville, L. H., … Casey, B. J. (2014). Teens impulsively react rather than retreat from threat. *Developmental Neuroscience*, *36*(3–4), 220–227. https://doi.org/10.1159/000357755

Drzewiecki, C. M., Willing, J., & Juraska, J. M. (2016). Synaptic number changes in the medial prefrontal cortex across adolescence in male and female rats: A role for pubertal onset. *Synapse*, *70*(9), 361–368. https://doi.org/10.1002/syn.21909

Dumais, K. M., Bredewold, R., Mayer, T. E., & Veenema, A. H. (2013). Sex differences in oxytocin receptor binding in forebrain regions: Correlations with social interest in brain region- and sex- specific ways. *Hormones and Behavior*, *64*(4), 693–701. https://doi.org/10.1016/j.yhbeh.2013.08.012

Dunlop, B. W., & Nemeroff, C. B. (2007). The role of dopamine in the pathophysiology of depression. *Archives of General Psychiatry*, *64*(3), 327–337. https://doi.org/10.1001/archpsyc.64.3.327

Eiland, L., & Romeo, R. D. (2013). Stress and the developing adolescent brain. *Neuroscience*, *249*(212), 162–171. https://doi.org/10.1016/j.neuroscience.2012.10.048

Foulkes, L., & Blakemore, S. J. (2016). Is there heightened sensitivity to social reward in adolescence? *Current Opinion in Neurobiology*, *40*, 81–85. https://doi.org/10.1016/j.conb.2016.06.016

Friemel, C. M., Spanagel, R., & Schneider, M. (2010). Reward sensitivity for a palatable food reward peaks during pubertal developmental in rats. *Frontiers in Behavioral Neuroscience*, *4*(JUL), 1–10. https://doi.org/10.3389/fnbeh.2010.00039

Fuchs, E., & Flügge, G. (1998). Stress, glucocorticoids and structural plasticity of the hippocampus. *Neuroscience and Biobehavioral Reviews*, *23*(2), 295–300. https://doi.org/10.1016/S0149-7634(98)00031-1

Fuchs, E., Flugge, G., & Czeh, B. (2006). Remodeling of neuronal networks by stress. *Frontiers in Bioscience*, *11*(SUPPL. 2), 2746–2758. https://doi.org/10.2741/2004

Fuhrmann, D., Knoll, L. J., & Blakemore, S. J. (2015). Adolescence as a sensitive period of brain development. *Trends in Cognitive Sciences*. https://doi.org/10.1016/j.tics.2015.07.008

Giedd, J. N. (2004). Structural magnetic resonance imaging of the adolescent brain. *Annals of the New York Academy of Sciences*, *1021*, 77–85. https://doi.org/10.1196/annals.1308.009

Giedd, J. N., Blumenthal, J., Jeffries, N. O., Castellanos, F. X., Liu, H., Zijdenbos, A., … Rapoport, J. L. (1999). Brain development during childhood and adolescence: A longitudinal MRI study. *Nature Neuroscience*, *2*(10), 861–863. https://doi.org/10.1063/1.1652711

Giedd, J. N., Raznahan, A., Alexander-Bloch, A., Schmitt, E., Gogtay, N., & Rapoport, J. L. (2015). Child psychiatry branch of the national institute of mental health longitudinal structural magnetic resonance imaging study of human brain development. *Neuropsychopharmacology*, *40*(1), 43–49. https://doi.org/10.1038/npp.2014.236

Gipson, C. D., Yates, J. R., Beckmann, J. S., Marusich, J. A., Zentall, T. R., & Bardo, M. T. (2011). Social facilitation of d-amphetamine self-administration in rats. *Experimental and Clinical Psychopharmacology*, *19*(6), 409–419. https://doi.org/10.1037/a0024682

Gomes, F. V., & Grace, A. A. (2017). Adolescent stress as a driving factor for schizophrenia development - A basic science perspective. *Schizophrenia Bulletin*, *43*(3), 486–489. https://doi.org/10.1093/schbul/sbx033

Goñi-Balentziaga, O., Perez-Tejada, J., Renteria-Dominguez, A., Lebeña, A., & Labaka, A. (2018). Social instability in female rodents as a model of stress related disorders: A systematic review. *Physiology and Behavior*, *196*(July), 190–199. https://doi.org/10.1016/j.physbeh.2018.09.001

Green, M. R., Barnes, B., & McCormick, C. M. (2013). Social instability stress in adolescence increases anxiety and reduces social interactions in adulthood in male long-evans rats. *Developmental Psychobiology*, *55*(8), 849–859. https://doi.org/10.1002/dev.21077

Grønli, J., Murison, R., Bjorvatn, B., Sørensen, E., Portas, C. M., & Ursin, R. (2004). Chronic mild stress affects sucrose intake and sleep in rats. *Behavioural Brain Research*, *150*(1–2), 139–147. https://doi.org/10.1016/S0166-4328(03)00252-3

Gunaydin, L. A., Grosenick, L., Finkelstein, J. C., Kauvar, I. V., Fenno, L. E., Adhikari, A., … Deisseroth, K. (2014). Natural neural projection dynamics underlying social behavior. *Cell*, *157*(7), 1535–1551. https://doi.org/10.1016/j.cell.2014.05.017

Hajnal, A., & Norgren, R. (2001). Accumbens dopamine mechanisms in sucrose intake. *Brain Research*, *904*(1), 76–84. https://doi.org/10.1016/S0006-8993(01)02451-9

Hall, F. S., Humby, T., Wilkinson, L. S., & Robbins, T. W. (1997). The effects of isolation-rearing on sucrose consumption in rats. *Physiology and Behavior*, *62*(2), 291–297. https://doi.org/10.1016/S0031-9384(97)00116-9

Hamburg, D. A., & Takanishi, R. (1989). Preparing for life: The critical transition of adolescence. *American Psychologist*, *44*(5), 825–827. Retrieved from https://0-search-proquest-com.opac.unicatt.it/docview/614321906/fulltextPDF/522A86DC226445D3PQ/6?accountid=9941

Hankin, B. L., Abramson, L. Y., Moffitt, T. E., Angell, K. E., Silva, P. A., & McGee, R. (1998). Development of depression from preadolescence to young adulthood: Emerging gender differences in a 10-year longitudinal study. *Journal of Abnormal Psychology*, *107*(1), 128–140. https://doi.org/10.1037/0021-843X.107.1.128

Harrell, C. S., Hardy, E., Boss-Williams, K., Weiss, J. M., & Neigh, G. N. (2013). Sex and lineage interact to predict behavioral effects of chronic adolescent stress in rats. *Behavioural Brain Research*, *24*(3), 57–61. https://doi.org/10.1016/j.physbeh.2017.03.040

Himmler, S. M., Himmler, B. T., Pellis, V. C., & Pellis, S. M. (2016). Play, variation in play and the development of socially competent rats. *Behaviour*, *153*(9–11), 1103–1137. https://doi.org/10.1163/1568539X-00003307

Hodges, T. E., Baumbach, J. L., Marcolin, M. L., Bredewold, R., Veenema, A. H., & McCormick, C. M. (2017). Social instability stress in adolescent male rats reduces social interaction and social recognition performance and increases oxytocin receptor binding. *Neuroscience*, *359*, 172–182. https://doi.org/10.1016/j.neuroscience.2017.07.032

Hodges, T. E., Baumbach, J. L., & McCormick, C. M. (2018). Predictors of social instability stress effects on social interaction and anxiety in adolescent male rats. *Developmental Psychobiology*, *60*(6), 651–663. https://doi.org/10.1002/dev.21626

Hodges, T. E., Eltahir, A. M., Patel, S., Bredewold, R., Veenema, A. H., & McCormick, C. M. (2019). Effects of oxytocin receptor antagonism on social function and corticosterone release after adolescent social instability in male rats. *Hormones and Behavior*, *116*(July), 104579. https://doi.org/10.1016/j.yhbeh.2019.104579

Hodges, T. E., & McCormick, C. M. (2015). Adolescent and adult male rats habituate to repeated isolation, but only adolescents sensitize to partner unfamiliarity. *Hormones and Behavior*, *69*, 16–30. https://doi.org/10.1016/j.yhbeh.2014.12.003

Hodges, T. E., & McCormick, C. M. (2019). Stress and social development in adolescence in a rodent model. *Routledge International Handbook of Social Neuroendocrinology*, *59*, 479–503. https://doi.org/10.4324/9781315200439-28

Hoffman, K. L. (2016). What can animal models tell us about depressive disorders? *Modeling Neuropsychiatric Disorders in Laboratory Animals*. https://doi.org/10.1016/b978-0-08-100099-1.00002-9

Hong, S., Flashner, B., Chiu, M., ver Hoeve, E., Luz, S., & Bhatnagar, S. (2012). Social isolation in adolescence alters behaviors in the forced swim and sucrose preference tests in female but not in male rats. *Physiology and Behavior*, *105*(2), 269–275. https://doi.org/10.1016/j.physbeh.2011.08.036

Hooshmand, S., Willoughby, T., & Good, M. (2012). Does the direction of effects in the association between depressive symptoms and health-risk behaviors differ by behavior? A longitudinal study across the high school years. *Journal of Adolescent Health*, *50*(2), 140–147. https://doi.org/10.1016/j.jadohealth.2011.05.016

Ikemoto, S., & Panksepp, J. (1992). The effects of early social isolation on the motivation for social play in juvenile rats. *Developmental Psychobiology*, *25*(4), 261–274. https://doi.org/10.1002/dev.420250404

Insel, T. R., & Fernald, R. D. (2004). How the brain processes social information: Searching for the social brain. *Annual Review of Neuroscience*, *27*(1), 697–722. https://doi.org/10.1146/annurev.neuro.27.070203.144148

Iwasaki-Sekino, A., Mano-Otagiri, A., Ohata, H., Yamauchi, N., & Shibasaki, T. (2009). Gender differences in corticotropin and corticosterone secretion and corticotropin-releasing factor mRNA expression in the paraventricular nucleus of the hypothalamus and the central nucleus of the amygdala in response to footshock stress or psychological stress in rats. *Psychoneuroendocrinology*, *34*(2), 226–237. https://doi.org/10.1016/j.psyneuen.2008.09.003

Juraska, J. M., & Markham, J. A. (2004). The cellular basis for volume changes in the rat cortex during puberty: White and gray matter. *Annals of the New York Academy of Sciences*, *1021*, 431–435. https://doi.org/10.1196/annals.1308.058

Juraska, J. M., & Willing, J. (2017). Pubertal onset as a critical transition for neural development and cognition. *Physiology & Behavior*, *1654*(Pt B), 87–94. https://doi.org/10.1016/j.physbeh.2017.03.040

Kalsbeek, A., Voorn, P., Buijs, R. M., Pool, C. W., & Uylings, H. B. M. (1988). Development of the dopaminergic innervation in the prefrontal cortex of the rat. *Journal of Comparative Neurology*, *269*(1), 58–72. https://doi.org/10.1002/cne.902690105

Kapur, S., & John Mann, J. (1992). Role of the dopaminergic system in depression. *Biological Psychiatry*, *32*(1), 1–17. https://doi.org/10.1016/0006-3223(92)90137-O

Karmiloff-Smith, A. (2015). An alternative to domain-general or domain-specific frameworks for theorizing about human evolution and ontogenesis. *AIMS Neuroscience*, *2*(2), 91–104. https://doi.org/10.3934/Neuroscience.2015.2.91

Kelley, A. E., & Berridge, K. C. (2002). The neuroscience of natural rewards: Relevance to addictive drugs. *Journal of Neuroscience*, *22*(9), 3306–3311. https://doi.org/10.1523/jneurosci.22-09-03306.2002

Kempermann, G. (2019). Environmental enrichment, new neurons and the neurobiology of individuality. *Nature Reviews Neuroscience*, *20*(4), 235–245. https://doi.org/10.1038/s41583-019-0120-x

Klinger, K., Gomes, F. V., Rincón-Cortés, M., & Grace, A. A. (2019). Female rats are resistant to the long-lasting neurobehavioral changes induced by adolescent stress exposure. *European Neuropsychopharmacology*, *29*(10), 1127–1137. https://doi.org/10.1016/j.euroneuro.2019.07.134

Konkle, A. T. M., Baker, S. L., Kentner, A. C., Barbagallo, L. S. M., Merali, Z., & Bielajew, C. (2003). Evaluation of the effects of chronic mild stressors on hedonic and physiological

responses: Sex and strain compared. *Brain Research*, *992*(2), 227–238. https://doi.org/10.1016/j.brainres.2003.08.047

Lewinsohn, P. M., Clarke, G. N., Seeley, J. R., & Rohde, P. (1994). Major depression in community adolescents: Age at onset, episode duration, and time to recurrence. *Journal of the American Academy of Child and Adolescent Psychiatry*, *33*(6), 809–818. https://doi.org/10.1097/00004583-199407000-00006

Lukas, M., Toth, I., Reber, S. O., Slattery, D. A., Veenema, A. H., & Neumann, I. D. (2011). The neuropeptide oxytocin facilitates pro-social behavior and prevents social avoidance in rats and mice. *Neuropsychopharmacology*, *36*(11), 2159–2168. https://doi.org/10.1038/npp.2011.95

Lupien, S. J., McEwen, B. S., Gunnar, M. R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature Reviews Neuroscience*, *10*(6), 434–445. https://doi.org/10.1038/nrn2639

Manduca, A., Servadio, M., Damsteegt, R., Campolongo, P., Vanderschuren, L. J. M. J., & Trezza, V. (2016). Dopaminergic neurotransmission in the nucleus accumbens modulates social play behavior in rats. *Neuropsychopharmacology*, *41*(9), 2215–2223. https://doi.org/10.1038/npp.2016.22

Marcolin, M. L., Baumbach, J. L., Hodges, T. E., & McCormick, C. M. (2020). The effects of social instability stress and subsequent ethanol consumption in adolescence on brain and behavioral development in male rats. *Alcohol*, *82*, 29–45. https://doi.org/10.1016/j.alcohol.2019.08.003

Marcolin, M. L., Hodges, T. E., Baumbach, J. L., & McCormick, C. M. (2019). Adolescent social stress and social context influence the intake of ethanol and sucrose in male rats soon

and long after the stress exposures. *Developmental Psychobiology*, *61*(1), 81–95. https://doi.org/10.1002/dev.21800

Mathews, I. Z., Mills, R. G., & McCormick, C. M. (2008). Chronic social stress in adolescence influenced both amphetamine conditioned place preference and locomotor sensitization. *Developmental Psychobiology*, *50*(5), 451–459. https://doi.org/10.1002/dev.20299

Mathews, Iva Z., Wilton, A., Styles, A., & McCormick, C. M. (2008). Increased depressive behaviour in females and heightened corticosterone release in males to swim stress after adolescent social stress in rats. *Behavioural Brain Research*, *190*(1), 33–40. https://doi.org/10.1016/j.bbr.2008.02.004

McCormick, C. M., & Hodges, T. E. (2017). Stress, glucocorticoids, and brain development in rodent models. In *Stress: Neuroendocrinology and Neurobiology* (Vol. 2, pp. 197–206). Elsevier Inc. https://doi.org/10.1016/B978-0-12-802175-0.00019-X

McCormick, C. M., Smith, C., & Mathews, I. Z. (2008). Effects of chronic social stress in adolescence on anxiety and neuroendocrine response to mild stress in male and female rats. *Behavioural Brain Research*, *187*(2), 228–238. https://doi.org/10.1016/j.bbr.2007.09.005

McCormick, C. M. (2010). An animal model of social instability stress in adolescence and risk for drugs of abuse. *Physiology and Behavior*, *99*(2), 194–203. https://doi.org/10.1016/j.physbeh.2009.01.014

McCormick, C. M., Linkroum, W., Sallinen, B. J., & Miller, N. W. (2002). Peripheral and central sex steroids have differential effects on the HPA axis of male and female rats. *Stress*, *5*(4), 235–247. https://doi.org/10.1080/1025389021000061165

McCormick, C. M., Robarts, D., Gleason, E., & Kelsey, J. E. (2004). Stress during adolescence enhances locomotor sensitization to nicotine in adulthood in female, but not male, rats. *Hormones and Behavior*, *46*(4), 458–466. https://doi.org/10.1016/j.yhbeh.2004.05.004

McCormick, C. M., Robarts, D., Kopeikina, K., & Kelsey, J. E. (2005). Long-lasting, sex- and age-specific effects of social stressors on corticosterone responses to restraint and on locomotor responses to psychostimulants in rats. *Hormones and Behavior*, *48*(1 SPEC. ISS.), 64–74. https://doi.org/10.1016/j.yhbeh.2005.01.008

McCormick, C. M., Thomas, C. M., Sheridan, C. S., Nixon, F., Flynn, J. A., & Mathews, I. Z. (2012). Social instability stress in adolescent male rats alters hippocampal neurogenesis and produces deficits in spatial location memory in adulthood. *Hippocampus*, *22*(6), 1300–1312. https://doi.org/10.1002/hipo.20966

Meaney, M. J., & Stewart, J. (1981). A descriptive study of social development in the rat (Rattus norvegicus). *Animal Behaviour*, *29*(1), 34–45. https://doi.org/10.1016/S0003-3472(81)80149-2

Moffitt, T. E., Caspi, A., Belsky, J., & Silva, P. A. (1992). Childhood experience and the onset of menarche: A test of a sociobiological model. *Child Development*, *63*(1), 47–58. https://doi.org/10.1111/j.1467-8624.1992.tb03594.x

Muscat, R., Sampson, D., & Willner, P. (1990). Dopaminergic mechanism of imipramine action in an animal model of depression. *Biological Psychiatry*, *28*(3), 223–230. https://doi.org/10.1016/0006-3223(90)90577-O

Nestler, E. J., & Carlezon, W. A. (2006). The mesolimbic dopamine reward circuit in depression. *Biological Psychiatry*, *59*(12), 1151–1159. https://doi.org/10.1016/j.biopsych.2005.09.018

Nielsen, C. K., Arnt, J., & Sánchez, C. (2000). Intracranial self-stimulation and sucrose intake differ as hedonic measures following chronic mild stress: interstrain and interindividual differences. *Behavioural Brain Research*, *107*, 21–33.

Niesink, R. J. M., & Van Ree, J. M. (1982). Short-term isolation increases social interactions of male rats: A parametric analysis. *Physiology and Behavior*, *29*(5), 819–825. https://doi.org/10.1016/0031-9384(82)90331-6

Olsen, C. M. (2011). Natural rewards, neuroplasticity, and non-drug addictions. *Neuropharmacology*, *61*(7), 1109–1122. https://doi.org/10.1016/j.neuropharm.2011.03.010

Palmonari, A., Pombeni, M., & Kirchler, E. (1990). Adolescents and their peer groups: a study on the significance of peers social categorization processes and coping with developmental tasks. *Social Behaviour*, *5*(1), 33–48.

Panksepp, J. (1981). The ontogeny of play in rats. *Developmental Psychobiology*, *14*(4), 327–332. https://doi.org/10.1002/dev.420140405

Panksepp, J., & Beatty, W. W. (1980). Social deprivation and play in rats. *Behavioral and Neural Biology*, *30*(2), 197–206. https://doi.org/10.1016/S0163-1047(80)91077-8

Panksepp, J., & Bishop, P. (1981). An autoradiographic map of (3H) diprenorphine binding in rat brain: Effects of social interaction. *Brain Research Bulletin*, *7*(4), 405–410. https://doi.org/10.1016/0361-9230(81)90038-1

Papp, M., Willner, P., & Muscat, R. (1991). An animal model of anhedonia: attenuation of sucrose consumption and place preference conditioning by chronic unpredictable mild stress. *Psychopharmacology*, *104*(2), 255–259. https://doi.org/10.1007/BF02244188

Pardon, M. C., Gould, G. G., Garcia, A., Phillips, L., Cook, M. C., Miller, S. A., … Morilak, D. A. (2002). Stress reactivity of the brain noradrenergic system in three rat strains differing in

their neuroendocrine and behavioral responses to stress: Implications for susceptibility to stress-related neuropsychiatric disorders. *Neuroscience*, *115*(1), 229–242. https://doi.org/10.1016/S0306-4522(02)00364-0

Parker, C. R., & Mahesh, V. B. (1976). Hormonal events surrounding the natural onset of puberty in female rats. *Biology of Reproduction*, *14*(3), 347–353. https://doi.org/10.1095/biolreprod14.3.347

Peartree, N. A., Hood, L. E., Thiel, K. J., Sanabria, F., Pentkowski, N. S., Chandler, K. N., & Neisewander, J. L. (2012). Limited physical contact through a mesh barrier is sufficient for social reward-conditioned place preference in adolescent male rats. *Physiology and Behavior*, *105*(3), 749–756. https://doi.org/10.1016/j.physbeh.2011.10.001

Pellis, S. M., & Pellis, V. C. (2007). Rough-and-tumble play and the development of the social brain. *Current Directions in Psychological Science*, *16*(2), 95–98. https://doi.org/10.1111/j.1467-8721.2007.00483.x

Perkins, A. E., Doremus-Fitzwater, T. L., Spencer, R. L., Varlinskaya, E. I., Conti, M. M., Bishop, C., & Deak, T. (2016). A working model for the assessment of disruptions in social behavior among aged rats: The role of sex differences, social recognition, and sensorimotor processes. *Experimental Gerontology*, *76*, 46–57. https://doi.org/10.1016/j.exger.2016.01.012

Pisu, M. G., Garau, A., Boero, G., Biggio, F., Pibiri, V., Dore, R., … Serra, M. (2016). Sex differences in the outcome of juvenile social isolation on HPA axis function in rats. *Neuroscience*, *320*, 172–182. https://doi.org/10.1016/j.neuroscience.2016.02.009

Popoli, M., Yan, Z., McEwen, B. S., & Sanacora, G. (2012). The stressed synapse: The impact of stress and glucocorticoids on glutamate transmission. *Nature Reviews Neuroscience*, *13*(1), 22–37. https://doi.org/10.1038/nrn3138

Reppucci, C. J., Brown, L. A., Chambers, A. Q., & Veenema, A. H. (2020). Wistar rats and C57BL/6J mice differ in their motivation to seek social interaction versus food in the Social versus Food Preference Test. *BioRxiv*. https://doi.org/10.1101/2020.06.04.134437

Robinson, D. L., Heien, M. L. A. V., & Wightman, R. M. (2002). Frequency of dopamine concentration transients increases in dorsal and ventral striatum of male rats during introduction of conspecifics. *Journal of Neuroscience*, *22*(23), 10477–10486. https://doi.org/10.1523/jneurosci.22-23-10477.2002

Romeo, R. D. (2010). Pubertal maturation and programming of hypothalamic-pituitary-adrenal reactivity. *Frontiers in Neuroendocrinology*, *31*(2), 232–240. https://doi.org/10.1016/j.yfrne.2010.02.004

Romeo, R. D., Lee, S. J., & McEwen, B. S. (2004). Differential stress reactivity in intact and ovariectomized prepubertal and adult female rats. *Neuroendocrinology*, *80*(6), 387–393. https://doi.org/10.1159/000084203

Romeo, R. D., Patel, R., Pham, L., & So, V. M. (2016). Adolescence and the ontogeny of the hormonal stress response in male and female rats and mice. *Neuroscience and Biobehavioral Reviews*, *70*, 206–216. https://doi.org/10.1016/j.neubiorev.2016.05.020

Salamone, J. D., & Correa, M. (2012). The mysterious motivational functions of mesolimbic dopamine. *Neuron*, *76*(3), 470–485. https://doi.org/10.1016/j.neuron.2012.10.021

Sclafani, A. (1987). Carbohydrate taste, appetite, and obesity: An overview. *Neuroscience and Biobehavioral Reviews*, *11*(2), 131–153. https://doi.org/10.1016/S0149-7634(87)80019-2

Sclafani, A., & Ackroff, K. (2003). Reinforcement value of sucrose measured by progressive ratio operant licking in the rat. *Physiology and Behavior*, *79*(4–5), 663–670. https://doi.org/10.1016/S0031-9384(03)00143-4

Selye, H. (1936). A syndrome produced by diverse nocuous agents. *Nature*.

Shansky, R. M. (2019). Are hormones a "female problem" for animal research? *Science*, *364*(6443), 825–826.

Sills, T. L., & Crawley, J. N. (1996). Individual differences in sugar consumption predict amphetamine-induced dopamine overflow in nucleus accumbens. *European Journal of Pharmacology*, *303*(3), 177–181. https://doi.org/10.1016/0014-2999(96)00161-6

Sisk, C. L., & Zehr, J. L. (2005). Pubertal hormones organize the adolescent brain and behavior. *Frontiers in Neuroendocrinology*, *26*(3–4), 163–174. https://doi.org/10.1016/j.yfrne.2005.10.003

Smith, C. J.W., Wilkins, K. B., Mogavero, J. N., & Veenema, A. H. (2015). Social novelty investigation in the juvenile rat: Modulation by the μ-opioid system. *Journal of Neuroendocrinology*, *27*(10), 752–764. https://doi.org/10.1111/jne.12301

Smith, C. J.W., Mogavero, J. N., Tulimieri, M. T., & Veenema, A. H. (2017). Involvement of the oxytocin system in the nucleus accumbens in the regulation of juvenile social novelty-seeking behavior. *Hormones and Behavior*, *93*, 94–98. https://doi.org/10.1016/j.yhbeh.2017.05.005

Smith, C. J.W., Wilkins, K. B., Li, S., Tulimieri, M. T., & Veenema, A. H. (2018). Nucleus accumbens mu opioid receptors regulate context-specific social preferences in the juvenile rat. *Psychoneuroendocrinology*, *89*(December 2017), 59–68. https://doi.org/10.1016/j.psyneuen.2017.12.017

Song, Z., Kalyani, M., & Becker, J. B. (2018). Sex differences in motivated behaviors in animal models. *Current Opinion in Behavioral Sciences*, *23*, 98–102. https://doi.org/10.1016/j.physbeh.2017.03.040

Sonntag, K. C., Brenhouse, H. C., Freund, N., Thompson, B. S., Puhl, M., & Andersen, S. L. (2014). Viral over-expression of D1 dopamine receptors in the prefrontal cortex increase high-risk behaviors in adults: Comparison with adolescents. *Psychopharmacology*, *231*(8), 1615–1626. https://doi.org/10.1007/s00213-013-3399-8

Stansfield, K. H., & Kirstein, C. L. (2006). Effects of novelty on behavior in the adolescent and adult rat. *Developmental Psychobiology*, *48*(1), 10–15. https://doi.org/10.1002/dev.20127

Stein, B. D. J. (2008). Psychomotor symptoms : The role of dopaminergic neurocircuitry. *CNS Spectrums*, *13*(July), 561–565.

Teicher, M. H., Andersen, S. L., & Hostetter, J. C. (1995). Evidence for dopamine receptor pruning between adolescence and adulthood in striatum but not nucleus accumbens. *Developmental Brain Research*, *89*(2), 167–172. https://doi.org/10.1016/0165-3806(95)00109-Q

Thiel, K. J., Sanabria, F., & Neisewander, J. L. (2009). Synergistic interaction between nicotine and social rewards in adolescent male rats. *Psychopharmacology*, *204*(3), 391–402. https://doi.org/10.1007/s00213-009-1470-2

Thor, D. H., & Holloway, W. R. (1984). Developmental analyses of social play behavior in juvenile rats. *Bulletin of the Psychonomic Society*, *22*(6), 587–590. https://doi.org/10.3758/BF03333916

Tirelli, E., Laviola, G., & Adriani, W. (2003). Ontogenesis of behavioral sensitization and conditioned place preference induced by psychostimulants in laboratory rodents.

Neuroscience and Biobehavioral Reviews, *27*(1–2), 163–178. https://doi.org/10.1016/S0149-7634(03)00018-6

Trezza, V., Baarendse, P. J. J., & Vanderschuren, L. J. M. J. (2010). The pleasures of play: Pharmacological insights into social reward mechanisms. *Trends in Pharmacological Sciences*, *31*(10), 463–469. https://doi.org/10.1016/j.tips.2010.06.008

Trezza, V., Damsteegt, R., & Vanderschuren, L. J. M. J. (2009). Conditioned place preference induced by social play behavior: parametrics, extinction, reinstatement and disruption by methylphenidate. *European Neuropsychopharmacology*, *19*(9), 659–669. https://doi.org/10.1038/jid.2014.371

Tzschentke, T. M. (1998). Measuring reward with the conditioned place preference paradigm: A comprehensive review of drug effects, recent progress and new issues. *Progress in Neurobiology*, *56*(6), 613–672. https://doi.org/10.1016/S0301-0082(98)00060-4

Valenstein, E. S., Cox, V. C., & Kakolewski, J. W. (1967). Further studies of sex differences in taste preferences with sweet solutions. *Psychological Reports*, *20*(3_suppl), 1231–1234. https://doi.org/10.2466/pr0.1967.20.3c.1231

Van Den Berg, C. L., Pijlman, F. T. A., Koning, H. A. M., Diergaarde, L., Van Ree, J. M., & Spruijt, B. M. (1999). Isolation changes the incentive value of sucrose and social behaviour in juvenile and adult rats. *Behavioural Brain Research*, *106*(1–2), 133–142. https://doi.org/10.1016/S0166-4328(99)00099-6

van Eden, C. G., & Uylings, H. B. M. (1985). Postnatal volumetric development of the prefrontal cortex in the rat. *Journal of Comparative Neurology*, *241*(3), 268–274. https://doi.org/10.1002/cne.902410303

Vanderschuren, L. J. M. J., Achterberg, E. J. M., & Trezza, V. (2016). The neurobiology of social play and its rewarding value in rats. *Neuroscience and Biobehavioral Reviews*, *70*, 86–105. https://doi.org/10.1016/j.neubiorev.2016.07.025

Vanderschuren, L. J. M. J., Stein, E. A., Wiegant, V. M., & Van Ree, J. M. (1995). Social isolation and social interaction alter regional brain opioid receptor binding in rats. *European Neuropsychopharmacology*, *5*(2), 119–127. https://doi.org/10.1016/0924-977X(95)00010-M

Varlinskaya, E. I., Truxell, E. M., & Spear, L. P. (2015). Sex differences in sensitivity to the social consequences of acute ethanol and social drinking during adolescence. *Behavioural Brain Research*, *282*, 6–13. https://doi.org/10.1016/j.bbr.2014.12.054

Vázquez, D. M., & Akil, H. (1993). Pituitary-adrenal response to ether vapor in the weanling animal: Characterization of the inhibitory effect of glucocorticoids on adrenocorticotropin secretion. *Pediatric Research*, *34*(5), 646–653. https://doi.org/10.1203/00006450-199311000-00017

Veenema, A. H., & Neumann, I. D. (2008). Central vasopressin and oxytocin release: regulation of complex social behaviours. *Progress in Brain Research*, *170*(08), 261–276. https://doi.org/10.1016/S0079-6123(08)00422-6

Venniro, M., Zhang, M., Caprioli, D., Hoots, J. K., Golden, S. A., Heins, C., … Shaham, Y. (2018). Volitional social interaction prevents drug addiction in rat models. *Nature Neuroscience*, *21*(November), 1520–1529. https://doi.org/10.1038/s41593-018-0246-6

Wade, G. N., & Zucker, I. (1969). Hormonal and developmental influences on rat saccharin preferences. *Journal of Comparative and Physiological Psychology*, *69*(2), 291–300. https://doi.org/10.1037/h0028208

Wierson, M., Long, P. J., & Forehand, R. L. (1993). Toward a new understanding of early menarche: the role of environmental stress in pubertal timing. *Adolescence*, *28*(112), 913–924.

Williams, A. V., Duque-Wilckens, N., Ramos-Maciel, S., Campi, K. L., Bhela, S. K., Xu, C. K., … Trainor, B. C. (2020). Social approach and social vigilance are differentially regulated by oxytocin receptors in the nucleus accumbens. *Neuropsychopharmacology*, (December 2019), 1–8. https://doi.org/10.1038/s41386-020-0657-4

Willner, P., Towell, A., Sampson, D., Sophokleous, S., & Muscat, R. (1987). Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. *Psychopharmacology*, *93*(3), 358–364. https://doi.org/10.1007/BF00187257

Willner, P. (2017). The chronic mild stress (CMS) model of depression: History, evaluation and usage. *Neurobiology of Stress*. https://doi.org/10.1016/j.ynstr.2016.08.002

Wilmouth, C. E., & Spear, L. P. (2009). Hedonic sensitivity in adolescent and adult rats: Taste reactivity and voluntary sucrose consumption. *Pharmacology Biochemistry and Behavior*, *92*(4), 566–573. https://doi.org/10.1016/j.pbb.2009.02.009

Wise, R. (1989). Brain dopamine and reward. *Annual Review of Psychology*, *40*(1), 191–225. https://doi.org/10.1146/annurev.psych.40.1.191

Wöhr, M., & Schwarting, R. K. W. (2007). Ultrasonic communication in rats: Can playback of 50-kHz calls induce approach behavior? *PLoS ONE*, *2*(12). https://doi.org/10.1371/journal.pone.0001365

Woolley, C. S., Gould, E., & McEwen, B. S. (1990). Exposure to excess glucocorticoids alters dendritic morphology of adult hippocampal pyramidal neurons. *Brain Research*, *531*(1–2), 225–231. https://doi.org/10.1016/0006-8993(90)90778-A

Wulsin, A. C., Wick-Carlson, D., Packard, B. A., Morano, R., & Herman, J. P. (2016). Adolescent chronic stress causes hypothalamo-pituitary-adrenocortical hypo-responsiveness and depression-like behavior in adult female rats. *Psychoneuroendocrinology*, *65*, 109–117. https://doi.org/10.1016/j.psyneuen.2015.12.004

Yates, J. R., Beckmann, J. S., Meyer, A. C., & Bardo, M. T. (2013). Concurrent choice for social interaction and amphetamine using conditioned place preference in rats: Effects of age and housing condition. *Drug and Alcohol Dependence*, *129*(3), 240–246. https://doi.org/10.1016/j.drugalcdep.2013.02.024

Young, K. A., Gobrogge, K. L., & Wang, Z. (2011). The role of mesocorticolimbic dopamine in regulating interactions between drugs of abuse and social behavior. *Neuroscience and Biobehavioral Reviews*, *35*(3), 498–515. https://doi.org/10.1016/j.neubiorev.2010.06.004.THE

Zhou, L., Ghee, S. M., See, R. E., & Reichel, C. M. (2015). Oxytocin differentially affects sucrose taking and seeking in male and female rats. *Behavioural Brain Research*, *283*, 184–190. https://doi.org/10.1016/j.bbr.2015.01.050

Zisook, S., Rush, A. J., Lesser, I., Wisniewski, S. R., Trivedi, M., Husain, M. M., … Fava, M. (2007). Preadult onset vs. adult onset of major depressive disorder: A replication study. *Acta Psychiatrica Scandinavica*, *115*(3), 196–205. https://doi.org/10.1111/j.1600-0447.2006.00868.x

Zucker, I. (1969). Hormonal determinants of sex differences in saccharin preference, food intake and body weight. *Physiology and Behavior*, *4*(4), 595–602. https://doi.org/10.1016/0031-9384(69)90160-7