NEURAL AND ENDOCRINE MECHANISMS UNDERLYING ADOLESCENT MATURATION OF SOCIAL REWARD

By

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A DISSERTATION

Submitted to
Michigan State University
In partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Neuroscience

2012

Abstract

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The adolescent transition from childhood to adulthood requires a qualitative shift in the focus on social interactions and reward. The male Syrian hamster provides a unique model for understanding how social information processing matures throughout adolescence, as juvenile males are not attracted to female vaginal secretions (VS) that are essential for sexual behavior in adulthood. I have shown that VS are rewarding in gonad-intact adult but not juvenile hamsters using conditioned place preference (CPP), and that testosterone treatment facilitates a VS-induced CPP in juveniles that is dopamine dependent. Moreover, VS induces Fos expression throughout the mesocorticolimbic system in adult but not juvenile hamsters. This body of work demonstrates that 1) the pubertal rise in testosterone changes the perception of a social stimulus, allowing it to serve as an unconditioned reward; 2) adolescent maturation of social reward is mediated by dopaminergic mechanisms and involves engagement of the mesocorticolimbic reward system.

Acknowledgements

I would like to express my great appreciation to my committee members, Drs. Jim Galligan, Joe Lonstein, Laura Smale, and Cheryl Sisk. Thank you for your time, energy, thoughts, and guidance for the last six years. Cheryl, thank you for your constant support and encouragement. Your enthusiasm and creative thinking are so valued and make your lab a wonderful place to grow as a scientist.

I would also like to express my gratitude to the Neuroscience Program as a whole, including their past and current director, support staff, faculty and students. I am so lucky to have been a part of such an inviting and intelligent community. In particular, Jim and Shari Stockmeyer; thank you for dealing with all of my issues so cheerfully.

I cannot thank the past and present members of the Sisk lab enough for all their help. This dissertation was only possible because of their contributions. Past members include Rayson Figueira, Heather Molinda-Figueira, Sarah Meerts, Kaliris Salas-Ramirez, Kalynn Schulz, Eman Ahmed, and all contributed their thoughts and hands. Undergraduate help was also essential to completing the many hours of tissue analysis and conditioned place preference behavioral work. Many thanks to Dana Gradl, Allison Melkonian, Joshua Olszewicz, Bradley Lawrence, Elise Craig, Shannon O'Conner, Susie Sonnenshine, Nicole Griep, Ashley Pratt, Robyn Weston, Genevieve Trombly, Jennifer La, Christine Azizkhan, and Steven Townsend. Andrew Kneynsberg has also been a terrific help with all things science (and food) related. Jane Venier deserves special thanks for her many (late and weekend) hours in the basement and constant thoughtful help. Thank you, Jane, for keeping the lab going and solving all of our

problems! Kayla De Lorme and Maggie Mohr, you have made the office such a happy, productive, and thoughtful place, and I cannot imagine better lab mates. Thank you for letting me bother you with my questions, helping with experiments, reading my work, and being my friends.

Finally, my utmost thanks to my family and, in particular, my husband, Lance Brinkman, for their unending support and love. My parents have been tireless moral-boosters; thank you for your interest and enthusiasm when I need it most. Lance has shown inexhaustible patience with my unusual work schedule and often long hours. I cannot express how much your support and understanding means to me. Thank you for making this possible.

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Key to Abbreviations

Brain Regions of Interest

Acb	nucleus accumbens
AcbC	nucleus accumbens core
AcbSh	nucleus accumbens shell
AMY	amygdala
AOB	accessory olfactory bulb
BNST	bed nucleus of the stria terminalis
Cg1	anterior cingulate medial prefrontal cortex
DM/PeF	dorsomedial hypothalamus / perifornical area
IF	interfascicular nucleus of the ventral tegmental area
IL	infralimbic medial prefrontal cortex
LH	lateral hypothalamus
Me	medial amygdala
MeP	posterior medial amygdala
MePD	posterdorsal medial amygdala
MePV	posteroventral medial amygdala
MOB	medial olfactory bulb
mPFC	medial prefrontal cortex
MPOA	medial preoptic area
PBP	parabrachial pigmented nucleus of the ventral tegmental area
PeVN	periventricular nucleus of the hypothalamus
PH	posterior hypothalamus
PN	paranigral nucleus of the ventral tegmental area

PrL	prelimbic medial prefrontal cortex
PVN	paraventricular nucleus of the hypothalamus
SuM	supramammillary nucleus
Tail	tail nucleus of the ventral tegmental area
VMH	ventromedial hypothalamus
VMHL	lateral ventromedial hypothalamus
VMHM	medial ventromedial hypothalamus
VTA	ventral tegmental area

Other Acronyms and Abbreviations

ANOVA	analysis of variance
CPP	conditioned place preference
CPA	conditioned place aversion
DOPAC	3,4-dihydroxyphenylacetic acid
GABA	gamma-aminobutyric acid
GnRH	gonadotropin-releasing hormone
ir	immunoreactive
Orx	orexin
SB	sex behavior
TBS	Triton Buffered Saline
TH	tyrosine hydroxylase
VS	vaginal secretions

Chapter 1: Introduction

Adolescent maturation of social information processing: relevance to human mental health

Adolescence, the behavioral transition from child to adult, is a time of dramatic developments in abstract reasoning, cognitive control, and social skills necessary to understand others' emotions and mental state (reviewed in (Spear, 2000, Casey *et al.*, 2005, Blakemore, 2008). Perhaps enabled by this gain in social cognition, adolescents switch their focus from family to peers (Nelson *et al.*, 2005) and are more sensitive to peer influences than are adults (Gardner and Steinberg, 2005). This transition has been described as a time of social reorientation, and, amongst other things, involves changes in the interpretation of social stimuli (Herba and Phillips, 2004). For example, while adults consistently describe a stimulus face as scared, adolescents interpret it as sad, shocked, or confused (Baird *et al.*, 1999). Adolescents also have difficulties shifting their attention from emotional to non-emotional aspects of a face (Monk *et al.*, 2003). These changes in social information processing may affect the performance of normal social behaviors, but also may be associated with mental illness. Anxiety and mood disorders all increase in prevalence during adolescence (Hayward and Sanborn, 2002), and involve distorted interpretation of social stimuli: patients with anxiety disorders show hyper-vigilance or exaggerated attention toward threat (Mogg and Bradley, 1998), and depression is characterized as a bias to interpret information negatively (Gotlib *et al.*, 2004). Thus, it is possible that these disorders may be the result of unsuccessful social reorientation.

Coincident with the general social reorientation is a shift in social and motivational tendencies (Forbes and Dahl, 2010). Adolescence is characterized by increased reward seeking behavior (Galvan, 2010), with frequency of behavior following an inverted U-shaped curve, peaking between10-15 years of age (Steinberg, 2008). This increase in reward seeking may result from reduced responsiveness to potential rewards, as evidenced by reduced activation of reward related brain regions when waiting for an expected reward in adolescents compared to adults, in fMRI studies (Bjork *et al.*, 2004). On the other hand, greater neural activation in response to rewards in adolescence has also been widely documented by fMRI studies (Geier and Luna, 2009). Whether adolescence is a time of partial anhedonia or enhanced hedonic sensitivity is an ongoing debate, but both theories posit immature interpretations of potentially rewarding stimuli in adolescents (Galvan, 2010). The alterations in reward and sensation seeking may affect a range of mental health disorders that increase in prevalence during adolescence. One obvious potential effect of reward dysregulation is that of substance abuse and addiction. Indeed, many suggest that adolescents may be particularly vulnerable to the effects of psychotropic drugs (Chambers *et al.*, 2003).

Pubertal changes in reproductive function

Concurrent with adolescence, puberty specifically is the time of reproductive maturity, and is typically achieved before adolescence is completed (Nussey and Whitehead, 2001). Pubertal onset is determined by a developmentally timed pulsatile release of gonadotropin-releasing hormone from neurons throughout the hypothalamus (as reviewed in (Sisk and Foster, 2004)). This peptide reaches the anterior pituitary gland

via the portal vasculature, and induces the release of luteinizing hormone and follicle stimulating hormone. These gonadotrophins then increase steroidogenesis by acting at Leydig or theca cells in the testes or ovaries, respectively (Nussey and Whitehead, 2001). In the male, Leydig cells produce androgens, including testosterone, androstenedione, and dihydrotestosterone which act at androgen receptors. In addition testosterone can be enzymatically converted by aromatase in the brain to an estrogen, and act at estrogen receptors. Therefore, the effects of testosterone manipulations in many studies (and in this dissertation) may occur through binding either androgen or estrogen receptors. Steroid hormone receptors classically act in the nucleus as transcription factors to regulate gene expression (Spelsberg *et al.*, 1989). While relatively recent findings have revealed non-genomic actions of estrogen and androgen receptors (Boonyaratanakornkit and Edwards, 2007), classical nuclear receptors are likely most relevant to the studies contained within this dissertation.

Human adolescent brain development

The processing of social information has been theorized to include three major processes: 1) detection of the social stimulus, 2) affective and motivational processes and 3) and cognitive and regulatory control (Adolphs, 2001, 2009). In models based on human and non-human primates, the detection node is centered in temporal-parietal cortices important for vision and face detection. Adaptation to rodents would also include olfactory regions in this node. Affective processes are guided by the amygdala (AMY) and accumbens (Acb), while cognitive-regulatory processes are largely controlled by the medial prefrontal cortex (mPFC). In humans, the mPFC and AMY are

involved in the recognition of socially relevant stimuli, judging attractiveness, and social and non-social reward (Hoffman and Haxby, 2000, Aharon *et al.*, 2001, Guyer *et al.*, 2008, Steinberg, 2008). Importantly, these regions all receive extensive dopaminergic and non-dopaminergic projections from the ventral tegmental area (VTA) as part of the complexly and reciprocally connected circuits of the mesocorticolimbic system (Swanson, 1982a, Oades and Halliday, 1987, Thompson and Swanson, 2010).

The "social information processing network" has been adapted to describe adolescent changes in behavior (Nelson *et al.*, 2005). This model suggests that while the detective node is largely functional in childhood, major developmental changes occur within the affective and cognitive nodes that alter emotional attributions to social stimuli and enable complex regulation of social behavior (Nelson *et al.*, 2005). This model of social information processing is strikingly similar to that of motivated behavior, called the "Triadic Model" (Ernst *et al.*, 2006). This "Triadic Model" also proposes a regulatory node that mirrors the above cognitive/regulatory node, and is centered in the mPFC. However, the Triadic Model breaks up the "affective" social information processing node into two nodes to distinguish between "approach" and "avoidance" functions controlled by the Acb and AMY, respectively. The triadic model also describes the adolescent shift in the balance between nodes to control motivated behavior. The increased reward seeking in the face of potentially negative consequences may be due to heightened activity in the approach/reward function of the Acb, a weak harm-avoidant system in the AMY, and/or an inefficient regulatory system in the mPFC (Ernst and Fudge, 2009). Indeed, adolescence is characterized by immature prefrontal and enhanced striatal and amygdalar reactivity to rewards in

comparison to adults (Forbes *et al.*, 2010), as demonstrated with monetary gambling rewards (Ernst *et al.*, 2005, Galvan *et al.*, 2007, Van Leijenhorst *et al.*, 2010a, Van Leijenhorst *et al.*, 2010b), and self-appraisals from peers (Guyer *et al.*, 2009). Thus, a coordinated shift in neural activity may promote social and motivated behaviors during adolescence.

Many of these functional developments may be explained by structural changes in the brain during adolescence. Gross morphological changes have been observed across adolescence: the volume of PFC grey matter and then declines curvilinearly, while white matter increases linearly across adolescence (Giedd *et al.*, 1999, Sowell *et al.*, 2002, Gogtay *et al.*, 2004, Giedd *et al.*, 2006, Lenroot *et al.*, 2007). Also, massive spine and synapse overproduction and pruning occur in the PFC during adolescence (Huttenlocher, 1984, Mrzljak *et al.*, 1990, Casey *et al.*, 2005). Recent work suggests that there are also developmental changes in white matter tracts between the PFC and the AMY and Acb (Eluvathingal *et al.*, 2007), and a general trend throughout the brain to transition from diffuse and short projections to more focused, longer projections across adolescence (Peper *et al.*, 2011b). In the striatum (including the Acb), dopamine receptors are overproduced and then pruned to adult levels (Seeman *et al.*, 1987) and there is an increase in presynaptic dopaminergic markers throughout adolescence (Haycock *et al.*, 2003). Therefore, behavioral changes during adolescence are correlated with neuroanatomical changes in relevant brain regions (Spear, 2000).

It is possible that many of the changes observed during adolescence relate to circulating gonadal hormones (Peper *et al.*, 2011a). In adolescents matched for age, circulating testosterone measures correlate with whole brain white matter in boys but

not in girls; interestingly, the effect is increased in males with a short androgen receptor polymorphism associated with higher basal testosterone (Perrin *et al.*, 2008). This androgen receptor polymorphism also modifies the association between age and white matter volume in a similar fashion (Paus *et al.*, 2010). Acute effects of testosterone have also been observed; a single dose of testosterone in women can cause a decrease in basal activity coupling between the amygdala and orbitofrontal cortex and an increase between the amygdala and thalamus (van Wingen *et al.*, 2010). These effects may also be relevant to socioemotional abilities, as testosterone can cause a decrease in prefrontal-amygdalo connectivity in males performing a task whereby participants were asked to override a natural tendency to approach a happy face and avoid an angry face (Volman *et al.*, 2011). The intersection of age-dependent maturational change with the increase in circulating testosterone likely dictates the effects of both on social behavior and reward, and are a focus of this dissertation.

While great gains are being made in understanding the neurobiology of adolescence, we know very little about the specific neural mechanisms behind adolescent changes in the perception of social stimuli (Kipke, 1999). Moreover, as both social and motivated behaviors change across adolescence, using a social stimulus as the experimental reward might provide a relevant perspective on normative changes in behavior during this developmental period (Galvan, 2010). The overall goal of this proposal is to determine the neural mechanisms important for mature behavioral and physiological responses to female pheromones, a rodent version of social reward, and how these neural systems change across adolescence and hormone treatment.

The Syrian hamster model of social information processing

Animal models offer us a chance to investigate the hormonal and neural mechanisms behind the assessment and interpretation of social stimuli, which is indexed in this dissertation by physiological and behavioral responses to a stimulus in a specific social context. The male Syrian hamster is an excellent model for studying a reproductively-relevant social stimulus as their performance of sociosexual behavior is highly dependent on chemosensory cues, especially female hamster vaginal secretions (VS) (Murphy and Schneider, 1970, Johnston, 1986, Coppola and O'Connell, 1988). Normal adult male copulatory behavior consists of repeated sequences of anogenital investigation, mounts, intromissions and ejaculations (Hull and Dominguez, 2007). In sexually naïve adult males, mating is dependent on the perception of VS, as removal of the olfactory bulb completely blocks odor preferences and copulatory behavior in males (Murphy and Schneider, 1970, Petrulis and Johnston, 1995, Petrulis). In fact, VS are such powerful stimuli that, when applied to a castrated or anesthetized male hamster, they can induce males to mount those same-sex partners (Murphy, 1973, Johnston, 1986). Sexually-naïve adult males show a preference for VS over male odors (Maras and Petrulis, 2006), and an unconditioned attraction to VS, as they spend more time licking VS-containing bottles over clean bottles (Johnston, 1974, Landauer *et al.*, 1977). This salient and socially-relevant cue will be used in this dissertation as a model of a social reward.

VS serve to communicate upcoming sexual receptivity of the female to the male, as females actively apply VS around their burrow entrance by vaginal marking (Petrulis, 2009). While secretions from both non-estrous and ovariectomized females are

attractive to male hamsters, males can detect differences between secretions from different females and prefer secretions from estrous females over non-estrous (Johnston, 1974, Macrides *et al.*, 1984a, Huck *et al.*, 1989, delBarco-Trillo *et al.*, 2009). VS contain a mixture of volatile and nonvolatile compounds, and the relative importance of these fractions in sexual behavior have been well-researched (Clancy *et al.*, 1984, Singer *et al.*, 1984b, Steel and Keverne, 1985). One volatile compound, dimethyl disulfide, peaks in concentration on the day of ovulation, and promotes male investigatory behavior but does not promote sexual behavior (Singer *et al.*, 1984a). However, the importance of this fraction has been questioned recently, as males, females, and castrated males are equally attracted to dimethyl disulfide (Petrulis and Johnston, 1995). Another major component of VS with sexually attractive properties has been identified as "aphrodisin" (Singer *et al.*, 1986). Specifically, it is a high molecular weight, soluble, glycosylated protein in the lipocalin superfamily; therefore, it is possible that aphrodisin is either a carrier protein for some other unidentified molecule, a pheromone in its own right, or both (Henzel *et al.*, 1988, Briand *et al.*, 2004). Regardless of the chemical composition, VS are impressively potent and concentrated; a water dilution experiment showed that one female equivalent (i.e., the amount typically extruded by one estrous female in response to tactile genital stimulation) can be diluted one hundred-fold without significantly reducing male interest (Macrides *et al.*, 1984a). Experiments contained in this dissertation use combined volatile and non-volatile components of VS well-above levels of detection.

Attraction to VS and the performance of sexual behavior are regulated by circulating gonadal steroid hormones in male hamsters (Whalen and Debold, 1974,

Gregory and Bishop, 1975). These effects require activation of both androgen and estrogen receptors, as long-term treatment of neither dihydrotestosterone nor estradiol alone is sufficient to match testosterone effects, but combined treatment brings behavioral levels close to that observed in testosterone-treated animals (DeBold and Clemens, 1978, Steel, 1982, Powers and Bergondy, 1983, Powers *et al.*, 1985, Steel and Hutchison, 1986, 1988). The reduction in attraction to VS after castration is not a result of olfactory deficits, as long-term castrates can still locate diluted VS just as well as gonad-intact males (Peters *et al.*, 2004), and VS activates equivalent numbers of neurons in the olfactory bulb of intact and gonadectomized hamster (Fiber *et al.*, 1993). In addition to long-term modulation of these behaviors, testosterone may also serve to provide short-term feedback in response to chemosensory and sexual cues. In gonad-intact animals, exposure to VS causes a surge in circulating testosterone 30 minutes after exposure (Pfeiffer and Johnston, 1992). Therefore, testosterone may serve as a cue in reinforcement and learning to promote sexual behavior, an effect that will be investigated in this dissertation.

Adolescent developmental of hamster social behavior and evaluation of VS

The process of social reorientation, in its many forms, is observed across species and likely evolved as an adaptive mechanism to leave the nest, achieve independence, try new foods, and gain new territory and mates. Male hamster puberty and adolescence is typically defined as the period between postnatal day (P) 28 and P56, according to when they leave the nest, begin to show adult-like reproductive behaviors, and produce mature concentrations of gonadal hormones (Miller *et al.*, 1977, Vomachka and

Greenwald, 1979, Schoenfeld and Leonard, 1985, Schulz and Sisk, 2006, delBarco-Trillo *et al.*, 2011). The attraction to VS over control odors reaches significance only after P40, suggesting that the pheromones gain hedonic value through adolescent maturation (Johnston and Coplin, 1979). Gonad-intact adults show higher levels of anogenital investigation than gonad-intact juvenile animals, although the differences are not statistically significant (Meek *et al.*, 1997). Gonad-intact juvenile male Syrian hamsters also display very low levels of mounts and intromissions, and absolutely no ejaculations (Meek *et al.*, 1997). In fact, mounting behavior reaches only 50% of adult levels by P42, halfway through adolescence (Miller *et al.*, 1977). Both the latency to perform anogenital investigations and mounts are longer in juvenile animals as compared to adults, suggesting that juvenile animals are less motivated to perform the behaviors (Schulz, 2007). Juvenile hamsters also fail to show an elevation in testosterone in response to VS (Romeo *et al.*, 1998, Romeo *et al.*, 2002). Therefore, male hamsters display developmental changes in behavioral and endocrine response to a social cue.

Given the importance of testosterone in regulating male sexual behaviors, and the dramatic changes in gonadal hormones during adolescence, it follows that the adolescent gain in sociosexual behavior is mediated by gonadal hormones. Indeed, testosterone treatment can induce attractive behaviors to VS in P25 juvenile animals (Johnston and Coplin, 1979), and adult-like levels of anogenital investigation in P28 juvenile males (Meek *et al.*, 1997). However, testosterone treatment cannot induce adult-like levels of mounts, intromissions and ejactulatory behaviors in juvenile males (Meek *et al.*, 1997), even after 17 days of treatment that approximate the normal

adolescent period (Schulz *et al.*, 2009). The immature sex behavior responses to testosterone cannot be explained by immature actions of androgen receptors, estrogen receptors or aromatase, as juveniles and adults have similar expression patterns and levels of activity (Meek *et al.*, 1997, Romeo *et al.*, 1998, Romeo *et al.*, 1999). *Together, these data suggest the juvenile brain shows some immature responses to female social stimuli, and that these behaviors can only partially be activated by testosterone prior to adolescence. As vaginal secretions may act as a reinforcer or promoter of sexual behavior in adulthood, experiments contained in this dissertation will investigate the rewarding properties of VS to gain more insight into the perception of this stimulus across adolescence.*

Chemosensory detection and neural evaluation

As mentioned earlier, chemosensory information is essential to allow mating behavior in Syrian hamsters, as olfactory bulbectomy completely blocks copulatory behavior (Powers *et al.*, 1979). Olfactory cues stimulate two anatomically and functionally distinct systems. The main olfactory system includes the olfactory epithelium, where olfactory receptors quickly detect volatile molecules and project to the main olfactory bulb (MOB). In contrast, the accessory olfactory system generally detects heavier non-volatile cues transported up to the vomeronasal organ, which then projects to the accessory olfactory bulb (Meredith, 1991, Keverne, 2004). Both these systems are likely involved in sexual behavior and attraction to VS (Powers *et al.*, 1979, O'Connell and Meredith, 1984, Petrulis, 2009), however the accessory olfactory system is important in processing the nonvolatile aphrodisin components of VS (Clancy *et al.*,

1984, Singer *et al.*, 1987) and is required for sexual behavior in sexually naïve animals (Meredith, 1986, Pfeiffer and Johnston, 1994).

Both the main and accessory olfactory bulbs project centrally, but by different pathways. The MOB projects to the amygdala mainly via the olfactory cortex, but also with some direct projections to the anterior medial amygdala (Me), whereas the AOB projects directly to the anterior and posterior Me (Davis *et al.*, 1978, Kevetter and Winans, 1981c, b). Therefore, the Me is positioned to integrate social olfactory information for the promotion of social behaviors. Indeed, the Me is essential for mating and chemoinvestigatory behavior (Lehman *et al.*, 1980). The posterodorsal medial amygdala (MePd) is especially important for chemosensory attraction, as lesions there reduce preferences for female over male odors and chemoinvestigatory behavior (Lehman *et al.*, 1980, Maras and Petrulis, 2006, Petrulis).

The Me is part of a well-characterized and tight network of interconnected regions, including the bed nucleus of the stria terminalis (BNST) and the medial preoptic area (MPOA). Lesions in these regions also reduce copulatory and motivated behavior; (Lehman and Winans, 1983, Kondo, 1992, Paredes *et al.*, 1993, Kondo and Arai, 1995, Liu *et al.*, 1997, Sato *et al.*, 1999, Simmons and Yahr, 2002, Melis *et al.*, 2003). Specifically, lesions that disrupt connectivity between the Me and the BNST eliminate preferences for volatile female odors, but not non-volatile compounds or sexual behavior in male hamsters (Been and Petrulis, 2012). On the other hand, functionally disconnecting the Me and the MPOA eliminates copulatory behavior but not olfactory preferences (Been and Petrulis, 2011). Neural activation in response to VS in these brain regions has also been characterized by immunohistochemical identification of the

expression of immediate early genes like Fos. Sexual behavior and VS exposure both induce Fos in Me, BNST, and magnocellular subdivision of the medial preoptic nucleus (MPNmag) above that of handled controls, with sex inducing more Fos than VS exposure alone (Kollack and Newman, 1992, Fiber *et al.*, 1993, Fernandez-Fewell and Meredith, 1994, Kollack-Walker and Newman, 1995, Fiber and Swann, 1996, Kollack-Walker and Newman, 1997). Studying Fos expression is particularly useful when interested in multiple brain regions, it will be used in these dissertation studies.

Just as gonadal hormones regulate the performance of sexual and chemosensory behavior, they also modulate responses of the chemosensory brain to sexual stimuli. The Me, BNST, and MPOA all express large numbers of androgen and estrogen receptors (Simerly *et al.*, 1990, Wood and Newman, 1999) and mediate some effects of testosterone on sexual behavior. Implanting testosterone into the MPOA or MeP of castrated male hamsters restores anogenital investigation and mounts (Wood and Newman, 1995b), while selective blockade of androgen receptors in the MPOA inhibits mating (McGinnis *et al.*, 1996, Harding and McGinnis, 2004). Integration of chemosensory and gonadal steroid information is also essential for the expression of chemoinvestigatory and copulatory behaviors (Wood and Newman, 1995a, Wood and Coolen, 1997, Hull and Dominguez, 2007). In a series of elegant studies in long-term castrated male hamsters, Wood and colleagues gave replacement testosterone unilaterally in the MPOA to enhance sexual behaviors, as described above. This stimulatory effect of testosterone was blocked by unilateral olfactory bulbectomy, but only when ipsilateral to the testosterone treatment (Wood and Newman, 1995a). That is, the MPOA needed to integrate both chemosensory and hormonal information within

the same hemisphere to promote sexual behavior. The MeP serves a similar function, but is more sensitive to disruptions in olfactory information, as removing contralateral olfactory information also affects sexual behavior (Wood and Coolen, 1997). Interestingly, long-term gonadectomy reduces Fos activation to VS in the MPNmag but not MePd or BNST (Fiber and Swann, 1996), demonstrating some site-specific effects of testosterone in the regulation of Fos responses to VS.

The Me, BNST, and MPOA are also connected to several hypothalamic regions that have been implicated in sexual behavior and chemosensory responses (Orsini *et al.*, 1985, Gréco *et al.*, 1996, McGinnis *et al.*, 1996, Kollack-Walker and Newman, 1997, Coolen and Wood, 1998, Choi *et al.*, 2005, Harding and McGinnis, 2005, Wood and Swann, 2005). Namely, the Me projects both directly and indirectly, via the BNST and MPOA, to the paraventricular nucleus (PVN), periventricular nucleus (PeVN), lateral hypothalamus (LH) and the ventromedial hypothalamus (VMH) (Simerly and Swanson, 1986, 1988, Fahrbach *et al.*, 1989, Gomez and Newman, 1992, Coolen and Wood, 1998, Wood and Swann, 2005, Yoshida *et al.*, 2006).

Me and MPOA are also connected to the mesocorticolimbic system, previously described as a complex circuitry important for a range of social and motivated behaviors. The Me sends some sparse projections to the Acb and VTA (Coolen and Wood, 1998, Geisler and Zahm, 2005), while basolateral and central amygdala send more robust projections to the Acb, mPFC, and VTA (Phillips *et al.*, 2003). The MPOA, having received chemosensory information via the MePd, is also a major source of input to the VTA and Acb (Simerly and Swanson, 1988, Groenewegen *et al.*, 1999,

Cunningham *et al.*, 2002, Phillips *et al.*, 2003, Usunoff *et al.*, 2009). The neuroanatomy and function of these different neural circuits are discussed below.

Mesocorticolimbic circuitry and dopaminergic function

The literature describing the mesocorticolimbic system, the VTA, Acb, and mPFC, is extensive, and cannot be reviewed exhaustively here. Instead, I will provide a general description of the circuitry and synaptic effects of dopamine as a foundation for understanding adolescent changes in mesocorticolimbic structure and function. In addition, I will describe structural and functional heterogeneity within components of the mesocorticolimbic circuit that forms the basis for evaluating particular subregions of these components in certain experiments in this dissertation. Overall the VTA, mPFC, AMY, and Acb form a network of looping circuits (Thompson and Swanson, 2010), functioning in synchrony to regulate motivated behaviors.

The mesocorticolimbic circuitry is known for its robust projections of dopaminergic cells from the A10 population in the VTA to corticolimbic structures (Oades and Halliday, 1987, Koob and Nestler, 1997, Tzschentke, 2000). The released dopamine can act on two families of dopamine receptors, D1 and D2 with different functional properties, imparting to dopamine a wide range of actions in the CNS (Missale *et al.*, 1998). D1-like receptors (D1 and D5) exist primarily in a low-affinity state for dopamine and require high levels of dopamine to be activated, while D2-like receptors have a higher dopamine affinity and are more likely to be activated at lower concentrations of dopamine (Richfield *et al.*, 1989, Goto and Grace, 2005). Additionally, D1 receptors are linked to stimulatory G proteins which increase cyclic adenosine

monophosphate (cAMP) activity, while the D2-like receptors (D2, D3, and D4) are linked to inhibitory G proteins and reduce cAMP (Kebabian and Calne, 1979, Surmeier *et al.*, 2007, Carlezon and Thomas, 2009). Both receptors can also modulate a range of ion channels, including AMPA and NMDA glutamate receptors. D2 receptors can also at as autoreceptors on presynaptic dopaminergic terminals and on dopamine cell bodies in the VTA, regulating dopamine release and uptake (Missale *et al.*, 1998).

The ventral tegmental area (VTA). The VTA is known for its population of dopaminergic cells. These dopamine neurons typically fire at some spontaneous tonic rate until glutamatergic stimulation induces bursts of firing (Grace and Bunney, 1984b, a, Goto *et al.*, 2007). This burst firing raises extracellular DA from tonic baseline concentrations to phasic transients (Goto et al 2007). Thus, tonic DA cell activity may induce basal levels of the high-affinity D2 receptor activation, while phasic release can activate D1 receptors (Creese *et al.*, 1983, Richfield *et al.*, 1989, Grace, 1991). Only 60% of the cells in the VTA are actually dopaminergic; 30-35% are gamma-aminobutyric acid (GABA)-ergic, and 2-3% glutamatergic (Swanson, 1982b, Nair-Roberts *et al.*, 2008, Dobi *et al.*, 2010). While distinct populations of cells have been identified based on either their location, co-expression of peptides such as cholecystokinin and neurotensin, whether they are autoinhibited, and their firing patterns, recent evidence suggests that a combination of techniques are required for accurate phenotyping (Swanson, 1982a, Hokfelt *et al.*, 1984, Seroogy *et al.*, 1987, Rayport *et al.*, 1992, Lammel *et al.*, 2008, Margolis *et al.*, 2008).

VTA gets input from a range of brain regions characterized as the “isodendritic core”, similar to that observed in the reticular formation (Geisler and Zahm, 2005). In

addition, mesopontine tegmental area neurons provide cholinergic modulation (Maskos, 2008). However, the major excitatory and inhibitory inputs to the VTA are from the mPFC and striatum, respectively, and are discussed below. These connections are bidirectional, as the VTA sends distinct projections to the striatum (including the Acb), PFC, amygdala, and hypothalamus (Margolis *et al.*, 2008). Both dopaminergic and GABAergic cells can either synapse locally or project to corticolimbic sites (Groenewegen *et al.*, 1999, Tzschentke, 2000, Brenhouse *et al.*, 2008, Margolis *et al.*, 2008, Carlezon and Thomas, 2009, Ernst *et al.*, 2009). Projections from the VTA to the striatum follow a roughly topographic continuum, with medial regions, including the interfascicular (IF) and paranigral (PN) subregions, projecting to medial accumbens (including the medial shell), and more lateral subdivisions, including the parabrachial pigmented nucleus (PBP), projecting to lateral accumbens (including the core) (Fallon and Moore, 1978, Ikemoto, 2007). There are also many interconnections between these subdivisions of the midbrain (Ikemoto, 2007, Ferreira *et al.*, 2008). In addition, distinct responses to local drug manipulations and genetic manipulations have also been reported along the rostro-caudal extent of the VTA (Ikemoto and Kohl, 1997, Carlezon *et al.*, 2000, Olson and Nestler, 2007) and the posterior hypothalamus (PH) / supramammilary nucleus (SuM) and “Tail” have been suggested as rostral and caudal extensions of the VTA, respectively (Ikemoto, 2010).

Medial prefrontal cortex (mPFC). The mPFC contributes both affective and regulatory processes for the performance of motivated behavior (Seitz *et al.*, 2006). Within the mPFC, both GABAergic interneurons and glutamatergic pyramidal neurons receive dopaminergic input from the VTA (Tzschentke, 2000). D1 receptors are

expressed in a greater proportion of GABAergic interneurons than pyramidal cells, however, both D1 and D2 receptors are expressed in both cell types. Also, D2 receptors are expressed mainly in cortical layer V (known for its subcortical projections), while D1 receptors are expressed relatively evenly in all layers (Santana *et al.*, 2009). In addition to afferents from the VTA, the mPFC also receives major inputs from the mediodorsal thalamus (a source of striatal and olfactory information), the insula (a source of emotional information), and the SuM nucleus (an extension of the VTA, described above) (Heimer, 1972, Hoover and Vertes, 2007, Ikemoto, 2010, Damasio *et al.*, 2012). The mPFC sends glutamatergic projections to the accumbens, where it modulates accumbens activity by binding AMPA and NMDA postsynaptically, but also on presynaptic dopaminergic terminals in complex three-way synapses (David *et al.*, 2005). The mPFC also sends glutamatergic projections to the VTA; these glutamatergic cells preferentially target GABAergic VTA cells that project to the Acb, and dopaminergic VTA cells that project back to the mPFC (Carr and Sesack, 2000b).

Like the rest of the mesocorticolimbic circuitry, the mPFC can be segregated into subregions with distinct functional and anatomical characteristics (Phillipson, 1979, Heidbreder and Groenewegen, 2003, Geisler and Zahm, 2005). The most dorsal subregion is the anterior cingulate (Cg), which is involved in motor control and is less interconnected with limbic and VTA regions (Vertes, 2006). The ventral mPFC includes the prelimbic (PrL) and infralimbic (IL) subregions, which, on the basis of connectivity and behavioral relevance, have been associated with the executive functions of the dorsolateral PFC and affective functions of the orbitomedial PFC in primates, respectively (Uylings *et al.*, 2003, Vertes, 2006). While both the PrL and IL are tightly

connected with the Acb, amygdala, and VTA, the IL projects more to the BNST and hypothalamus than PrL (Vertes, 2004).

Amygdala (AMY). In addition to the chemosensory role the Me plays in rodent social behavior, the basolateral and central amygdala is also important in fear and avoidance conditioning (LeDoux, 2007). The VTA sends dopaminergic projections to the central and basolateral amygdala (Inglis and Moghaddam, 1999), where it releases dopamine in response to stressors to enhance amygdalar dependent fear-conditioning and aversive responses. This likely occurs via dopamine dependent inhibition of a specific group of GABAergic neurons (Marowsky *et al.*, 2005), which then disinhibit amygdalar glutamatergic cells that project to the Acb (Charara and Grace, 2003) and mPFC (Hoover and Vertes, 2007).

Accumbens (Acb). The Acb is generally thought of as the link between limbic processes and motor output, and receives projections from the VTA, mPFC, and AMY (Sesack and Grace, 2009). The Acb primarily consists of GABAergic medium spiny neurons that express D1 and D2 receptors, cholinergic interneurons that express D2 but not D1 receptors, and GABAergic interneurons that express several peptides but not dopamine receptors (Meredith, 1999, Bertran-Gonzalez *et al.*, 2008). The Acb is interconnected with the dorsal striatum, and may display similar cellular organization. The medium spiny neurons in the dorsal striatum have been organized into two populations that exert balanced but antagonistic influences on their midbrain target and motor output (Albin *et al.*, 1989, Surmeier *et al.*, 2007). The “direct” population of cells typically expresses D1-type dopamine receptors on their cell bodies and dendrites and co-expresses dynorphin. This cell group feed backs to the substantia nigra to inhibit

non-dopaminergic cells via GABA receptor activation, and is generally thought to facilitate motor output. The “indirect” population expresses D2 family receptors and co-express enkephalin. It projects mainly to the ventral pallidum and subthalamic nuclei to transynaptically reduce motor output.

A parallel organization of direct and indirect cell populations is present in the Acb, but with considerably less exclusive projection patterns (Sesack and Grace, 2009, Lobo *et al.*, 2010). Indeed, recent evidence suggests that a small percentage (6-17%) of Acb neurons even express both D1 and D2 receptors (Bertran-Gonzalez *et al.*, 2008). Generally, though, the direct and indirect pathway disinhibits and inhibits, respectively, motor pathways for reward acquisition and motivated behavior (Sesack and Grace, 2009). The Acb is divided anatomically into two subdivisions, called the core and shell, which vary in their neurochemistry and projection patterns (Groenewegen *et al.*, 1999). The core projects more to substantia nigra while shell projects more to VTA (Heimer *et al.*, 1991). The shell projections synapse on GABAergic, and not dopaminergic, cells in the VTA, including local interneurons and GABAergic projections back to the Acb (Xia *et al.*, 2011). The shell is often associated with functions in drug reward, while core has been implicated more in cue-conditioning; however these are likely oversimplifications of their complex functions (Sesack and Grace, 2009).

While D1 and D2 receptors have typically been described as excitatory or inhibitory of cellular membrane potential and activity, more recent data demonstrate that this is an oversimplification (reviewed in (Seamans and Yang, 2004). Instead, the effect of activating either receptor can depend on whether or not the post-synaptic neuron has been recently activated (Arbuthnott and Wickens, 2007). The membrane potentials of

medium spiny neurons in the accumbens and pyramidal cells in the cortex fluctuate from periods of hyperpolarization to plateaus of relative depolarization, creating baseline states of "down" or "up" (O'Donnell and Grace, 1995). In "up" states, lower amplitude excitatory post-synaptic potentials are required to induce an action potential. Thus, multiple coordinated synaptic inputs are needed to most readily induce cell firing; in the Acb, this is often means glutamatergic input from the amygdala and mPFC (Seamans and Yang, 2004). Importantly, dopamine modulates these up states to amplify the signal:noise ratio: D1 receptors tend to holding the membrane potential at the "up" state longer, while D2 receptors serve to reducing the number of spontaneously evoked action potentials (O'Donnell, 2003). Generally, dopaminergic action is thought to be more modulatory than other typical neurotransmitters. Thus, the Acb integrates glutamate release from the mPFC and amygdala with dopamine modulation from the VTA and, often in complicated three-way synapses, encodes motivational salience and potentiates goal-directed behavior.

This circuitry is widely studied for its ability to sustain site-directed self-administration of drugs and electrical stimulation (as reviewed in (Ikemoto, 2007). For example, animals self-stimulate in the mPFC, supporting its role in reward circuits, and this activity induces Fos in the Acb, MPOA and VTA (Avanitogiannis *et al.*, 2000). However, this circuitry evolved in response to natural, not artificial, rewards. As such, the VTA, Acb, and mPFC are activated by chemosensory sexual stimuli and copulatory behaviors (Greco *et al.*, 1998, Balfour *et al.*, 2004, Balfour *et al.*, 2006), and are important in sexual motivation and chemosensory attraction. Specifically, lesions of the mPFC and Acb inhibit anogenital investigation, sexual behavior and motivation

(Hendricks and Scheetz, 1973, Pfaus and Phillips, 1991, Fernandez-Gausti *et al.*, 1994, Agmo and Villalpando, 1995, Liu *et al.*, 1998, Kippin *et al.*, 2004, Afonso *et al.*, 2007).

Behavioral paradigms of reward and motivation

Before a more nuanced discussion of the mesocorticolimbic function in sociosexual behaviors, concepts of reward must be discussed. Reward has three different components: hedonic value, motivational salience, and learning (Berridge and Robinson, 2003). Hedonic value, or how pleasurable or "liked" a stimulus is, can be determined without previous learning and is primarily expressed as an affective reaction (e.g. lateral tongue protrusions upon sucrose administration). In contrast, motivational salience is the process whereby a stimulus is deemed attention-grabbing and worthy of goal directed behavior, or "wanted". While stimuli that are "wanted" are often initially "liked", these qualities can be dissociated from one another, e.g., drug addicts who no longer enjoy the drug but still have cravings. This theory suggests that mesocorticolimbic dopamine is important for imbuing rewarding stimuli with motivational salience, or wanting (Berridge and Robinson, 1998, Ikemoto and Panksepp, 1999, Salamone *et al.*, 2005, Berridge, 2007, Ikemoto, 2007). While I acknowledge these different facets of reward, almost all behavioral tasks fail to discriminate between the components; therefore I will use "reward" throughout the dissertation to refer to all three.

A rewarding stimulus may be used to condition an operant behavior. In operant behavior tasks, such as bar pressing or nose pokes, an animal's behavior is modified by receiving a reinforcement or punishment. This form of leaning requires action on the part of the subject, and is contrasted with Pavlovian conditioning. In Pavlovian

conditioning, an initially rewarding (unconditioned) stimulus is repeatedly paired with a neutral stimulus; the animal then learns that the neutral stimulus is associated with the unconditioned reward, such that it is also “wanted.” Both forms of conditioning require leaning about a stimulus and can indicate how motivated an animal is to receive the reward. One behavioral paradigm of Pavlovian conditioning useful for the rewarding value of a stimulus is conditioned place preference (CPP, (Perks and Clifton, 1997). In this paradigm, an unconditioned reward (that is liked) is repeatedly paired with a neutral stimulus (a conditioning compartment), which then acquires rewarding properties of its own. These rewarding properties are determined in a test whereby the subject “wants” that conditioned environment, and spends more time in the stimulus-paired compartment over an unconditioned compartment.

CPP has long been used in determining the rewarding aspects of drugs, copulatory and feeding behaviors (Schechter and Calcagnetti, 1993), as well as which neurotransmitters, hormones, and brain regions are important for reward (Schechter and Calcagnetti, 1993, Tzschentke, 1998). Male and female rodents show a CPP to sexual interactions (Miller and Baum, 1987, Agmo and Berenfeld, 1990, Hughes *et al.*, 1990, Mehrara and Baum, 1990, Oldenburger *et al.*, 1992, Agmo and Gomez, 1993, Meisel and Joppa, 1994, Meisel *et al.*, 1996, Paredes and Alonso, 1997, Pankevich *et al.*, 2006, Agustin-Pavon *et al.*, 2007, Martínez-Ricós *et al.*, 2007). Dopamine has been widely implicated in CPP, as both systemic and intra-Acb delivered D1 and D2 agonists and antagonists induce a CPP or conditioned place aversion, respectively (Hoffman and Beninger, 1988, Papp, 1988, Hoffman and Beninger, 1989, Shippenberg *et al.*, 1991, White *et al.*, 1991, Papp *et al.*, 1993, Acquas and Di Chiara, 1994, Tzschentke, 1998).

More recently, optogenetic techniques have demonstrated that activation of phasic firing of VTA dopaminergic neurons is sufficient to induce a CPP (Tsai *et al.*, 2009).

Qualities of sexual reward and motivation have been assessed with a range of behavioral tasks and parameters. The classic appetitive / consummatory dichotomy (Everitt, 1990) has been used to parse out motivational aspects of sexual interactions. Simply stated, stereotyped acts of sexual behavior including mounts, intromissions, and ejaculations, are deemed consummatory. Behaviors prior to the first direct copulatory act are described as appetitive. For example, attraction or preferences for stimuli, anticipatory behavior or locomotor excitement in advance of access to a receptive mate and investigatory behavior have all be used as indicators of sexual arousal and motivation (Everitt, 1990). However, many of these behavioral measures are dependent on locomotor ability, which may be affected by age and dopaminergic manipulations. Indeed, Everitt proposed using instrumental conditioning and CPP to better assess sexual reward and motivation. Drug and age confounds are avoided in CPP, as animals are tested for their place preference in a drug-free state, and are compared with age-matched controls that did not received conditioning (Schechter and Calcagnetti, 1993). In addition, a conditioned place aversion (CPA, (Acquas *et al.*, 1989) can be used to determine the aversive qualities of stimuli. Multiple indicators of reward provide the most complete understanding of the motivational properties of a stimulus; CPP will be used in this dissertation to complement previous work on attractive responses to VS, in our attempt to measure rewarding properties of the important social cue.

Dopamine and sociosexual reward

In general, dopamine appears important for male sexual behavior, as systemic apomorphine (a D1/D2 agonist) increases performance of copulatory behavior in male rats and hamsters (Malmnas, 1976, Agmo and Berenfeld, 1990, Scaletta and Hull, 1990, Mas *et al.*, 1995, Rodríguez-Manzo, 1999, Lopez and Ettenberg, 2000, 2001, Arteaga *et al.*, 2002, Lopez and Ettenberg, 2002, Niikura *et al.*, 2002, Hull and Dominguez, 2007). Several of the behavioral paradigms described above have been used to parse sexual motivation and reward from the consummatory aspects of sexual behavior. Systemic flupenthixol (a D1/D2 antagonist) decreases the number of operant conditioning responses for access to a receptive female at a dose that did not affect copulatory behavior (Everitt, 1990). Similarly, systemic haloperidol (a D2 antagonist) reduces anticipatory level changes for a female in a two-level chamber, while only affecting sexual behavior at high doses that likely affect general motor behavior (Pfaus and Phillips, 1989, Pfaus and Phillips, 1991). D2 receptors also appear important in motivation for female cues, as systemic haloperidol reduced the unconditioned incentive value of estrous female cues in sexually-naïve male rats and sex-associated cues after conditioning (Lopez and Ettenberg, 2001, 2002). The effects of systemic dopamine manipulations in CPP have been mixed. Pimozide, a D2 antagonist, fails to block ejaculation-induced CPP in male rats (Agmo and Berenfeld, 1990), and systemic D1 D2 antagonists fail to block a CPP for male bedding in female mice (Martínez-Hernández *et al.*, 2006, Agustin-Pavon *et al.*, 2007). However, in hamsters, sulpiride, but not raclopride, both D2 antagonists, blocked the sexual behavior-induced CPP (Meisel *et*

al., 1996). Thus, systemic manipulations, often with D2 receptors, can selectively affect some species-specific, appetitive, motivational aspects of sociosexual behavior.

Dopamine in the mesocorticolimbic system has also been implicated in sexual performance and motivation (Asmus, 1994, Hull *et al.*, 1995, van Furth *et al.*, 1995, Berridge and Robinson, 1998, Balfour *et al.*, 2004, Balfour *et al.*, 2006). Dopamine activity increases in the male rat Acb in response to female chemosensory and auditory cues and during copulation in male and female rats and hamsters (Mas *et al.*, 1990, Damsma *et al.*, 1992, Mitchell and Gratton, 1992, Wenkstern *et al.*, 1993). Intra-Acb delivery of D1 and D2 antagonists decreases anticipatory behavior for access to a receptive female and increases latencies to mount the female, while agonists increase sex behavior (Pfaus *et al.*, 1990, Pfaus and Phillips, 1991, Meisel *et al.*, 1993). However, one study of unconditioned chemosensory reward with site-specific manipulations found that dopamine depletion in the shell did not affect female mouse attraction to male bedding (Martínez-Hernández *et al.*, 2012). Thus, dopamine release in the accumbens may be important for appetitive preparatory sexual activity and copulation, however its role in sexual reward requires further study. Effects of dopamine in the mPFC on the performance of sexual behavior or reward have not been well-characterized.

In the MPOA, dopamine appears important for mediating male sexual behavior, as MPOA dopamine is released in response to female cues and during copulation (Hull *et al.*, 1995, Sato *et al.*, 1995). These dopamine responses to females are dependent on chemosensory stimuli in male hamsters (Wood, 2004, Triemstra *et al.*, 2005). Moreover, microinjections of nonspecific dopamine agonists (apomorphine) or

antagonist (flupenthixol) into the MPOA promotes or attenuates sexual behavior in male rats, respectively (Hull *et al.*, 1986, Warner *et al.*, 1991, Arteaga *et al.*, 2002). MPOA dopamine is also important in penile responses: D1 receptors appear important for erections (which, when spontaneous, may indicate motivation), while D2 receptors appear important for seminal emission associated with ejaculation (Hull *et al.*, 1992). While some have proposed that MPOA dopamine effects are mainly consummatory, as opposed to appetitive (Everitt, 1990), there is some evidence to the contrary. Namely, the D2 antagonist raclopride into the MPOA reduces the number of animals choosing a female box in a 4 choice X-maze, and also reduces the number of animals copulating once they reached her, without affecting movement (Moses *et al.*, 1995). Thus, MPOA dopamine appears important in both motivational and consummatory aspects of copulatory behavior.

Lesion and knife cut studies suggest that almost all of the dopamine input to the MPOA is from dopaminergic incerto-hypothalamic projections from the A14 cell group in the PeVN (Bjorklund *et al.*, 1975, Palkovits *et al.*, 1977, Day *et al.*, 1980, Horvath *et al.*, 1993). However a recent anatomical tracer study suggests that the MPOA may receive dopamine innervation from a wide range of brain regions, including the MPOA itself, PVN, SuM, PH, and VTA (Oades and Halliday, 1987, Miller and Lonstein, 2009). Through an extensive series of studies, Hull and colleagues have demonstrated that basal and female-induced release of dopamine from MPOA terminals is the result of local glutamate to release nitric oxide (Lorrain and Hull, 1993, Lorrain *et al.*, 1996, Sato *et al.*, 1998, Dominguez *et al.*, 2004). Moreover, increasing and decreasing both glutamate and nitric oxide in the MPOA increase and decrease sexual behavior, ex

copula erections, and anticipatory ultrasonic vocalizations (Giuliano *et al.*, 1997, Moses and Hull, 1999, Brudzynski and Pniak, 2002, Lagoda *et al.*, 2004, Dominguez *et al.*, 2006). An important source of glutamate to the MPOA relevant to sexual behavior is the MeP. Lesioning the MeP reduces both sexual behavior and the release of dopamine in the MPOA in response to a stimulus female, and supplementing this MPOA dopamine release with a D1/D2 agonist (apomorphine) restores sexual behavior to pre-lesion levels (Dominguez *et al.*, 2001, Dominguez and Hull, 2001). These studies emphasize the importance of MeP input to the MPOA to relay chemosensory information important for sociosexual behaviors.

Dopamine is also released in response to female cues and during copulation in the nearby PVN (Melis *et al.*, 2003), are shares many similarities with MPOA dopamine, described above. The PVN is also innervated by dopaminergic neurons from the A14 cell group in the PeVN and A13 cell group in the medial zona incerta (Bjorklund *et al.*, 1975, Swanson and Sawchenko, 1980, Buijs *et al.*, 1984, Lindvall *et al.*, 1984, Cheung *et al.*, 1998), and is the site of oxytocin neurons that project to the spinal cord to promote erections (Melis and Argiolas, 2003). Dopamine release in the PVN acts on D2 receptors to increase nitric oxide, stimulate these oxytocin neurons, and increase non-contact erections in response to female cues (Melis *et al.*, 1987, Eaton *et al.*, 1991, Melis *et al.*, 1997, Melis *et al.*, 1998, Melis *et al.*, 2003). Non-contact erections in response to female cues may be an indicator of sexual motivation (Melis and Argiolas, 2003). Interestingly, the dopamine stimulated PVN oxytocin neurons also project to the VTA, where they increase nitric oxide to stimulate VTA dopaminergic neurons and increase the release dopamine in the Acb (Melis *et al.*, 2007, Succu *et al.*, 2007, Succu

et al., 2008). The role of PVN DA in these erections, and its connectivity with mesocorticolimbic dopamine, suggests that the PVN is also involved in both consummatory and motivational aspects of sexual behavior

Orexin and sociosexual reward

Another neural mediator of reward is orexin (also known as hypocretin), a neuropeptide expressed exclusively in the lateral hypothalamic area (LHA) and the perifornical–dorsomedial hypothalamus (DM-PeF) in two forms, orexin-A and orexin-B (de Lecea *et al.*, 1998, Sakurai *et al.*, 1998). While initially implicated in regulating food intake and sleep and arousal, recent studies have demonstrated its involvement in food, drug, and sexual reward. Specifically, orexin cells express Fos in response to copulation (Muschamp *et al.*, 2007), food, cocaine, and morphine (Harris *et al.*, 2005), and orexin agonists and antagonists increase and decrease male rat sexual behavior, respectively (Gulia *et al.*, 2003, Muschamp *et al.*, 2007, Di Sebastiano *et al.*, 2010). Moreover, orexin neurons appear essential for sex and morphine- induced CPP (Harris *et al.*, 2007, Di Sebastiano *et al.*, 2011). Although drug reward effects seem specific to cells lateral of the fornix, copulation increases Fos throughout the cell group. There are dense orexinergic projections to, and Orx 1 and 2 receptor expression in, the accumbens, BNST, MPOA, PVN, Me, VMH, SuM and VTA (Peyron *et al.*, 1998, Trivedi *et al.*, 1998, Marcus *et al.*, 2001, Fadel and Deutch, 2002, Schmitt *et al.*, 2012), suggesting that some of these reward-related functions may be via interactions with the mesocorticolimbic system. Indeed, orexin excites VTA dopaminergic and GABAergic

neurons, and causes dopamine release in the Acb and PFC (Korotkova *et al.*, 2003, Narita *et al.*, 2006, Muschamp *et al.*, 2007, Vittoz *et al.*, 2008).

Testosterone and sociosexual reward

As noted above, many chemosensory and sociosexual behaviors are dependent on testosterone. Therefore, it follows that testosterone may also play a role in the rewarding and motivational properties of these stimuli. Accordingly, gonadectomy blocks male rats' ability to form a CPP to an estrous female and to emit ultrasonic vocalizations in response to female bedding, another indicator of sexual motivation (Harding and McGinnis, 2004). Testosterone-induced restoration of this CPP and vocalization is blocked by either infusion of the antiandrogen flutamide into or lesion of the VMH (Harding and McGinnis, 2004). While these studies suggest some local action of testosterone within VMH, VMH could also relay hormonal information via projections to the BNST, Me, MPOA, and PVN. However, the VMH is not well-connected with mesocorticolimbic structures (Saper *et al.*, 1976, Canteras *et al.*, 1994). One study also reports rapid effects of testosterone, after aromatization to estradiol, on cellular firing in the male rat lateral hypothalamus (Orsini, 1982). Effects of testosterone on the orexin system have not been well-studied, except for one report of gonadecomy reducing orexin expression in the anterior hypothalamus, but not prefrontal cortex (Silveyra *et al.*, 2009).

The dopaminergic dynamics in the MPOA are also highly sensitive to testosterone. Several studies have demonstrated that long-term (2-8 weeks) gonadectomy results in an increase in dopamine in MPOA tissue punches (Engel *et al.*,

1979, Simpkins *et al.*, 1983, Mitchell and Stewart, 1989, Du *et al.*, 1998). Further study demonstrated that this increase in intracellular dopamine was accompanied by a decrease in basal extracellular dopamine (and associated low dopamine turnover), and an increase in amphetamine-induced extracellular dopamine (Gunnet *et al.*, 1986, Du *et al.*, 1998), without a change in dopaminergic cell number in the MPOA or PeVN (Du and Hull, 1999). This suggests that testosterone is necessary for MPOA dopamine release, but not synthesis. The MPOA dopaminergic release in response to sexual stimuli in the MPOA is also dependent on testosterone in male rats (Hull *et al.*, 1995, Putnam *et al.*, 2003). As discussed above, nitric oxide is important in the release of dopamine, both at basal levels and in response to a sexual stimulus. Importantly, nitric oxide is also sensitive to testosterone, as long-term gonadectomy reduces expression of nitric oxide synthase in the MPOA both hamsters and rats (Hadeishi and Wood, 1996, Du and Hull, 1999)). This effect of testosterone is due to its metabolism to estradiol, which, after gonadectomy, maintains nitric oxide synthase and intracellular dopamine levels at that of testosterone treated male rats (Putnam *et al.*, 2005). Thus, dopamine release in the MPOA, and presumably the PVN, is sensitive to gonadal hormones.

In addition to hypothalamic sites of action, testosterone may exert effects on sociosexual reward via modulation of dopaminergic action in the mesocorticolimbic circuit. Long-term castration (30, but not 14, days) causes an increase in the number of TH-ir cells in the VTA of adult male rats (McArthur *et al.*, 2007, Johnson *et al.*, 2010). This parallels effects observed in the mPFC, where 28 day gonadectormy causes an increase in TH innervation and extracellular dopamine (Aubele and Kritzer, 2011b). Moreover, long-term gonadectomy blocks or reverses the effects of AMPA and NMDA

glutamate receptor administration on dopaminergic tone in the mPFC, respectively (Aubele and Kritzer, 2011a). Testosterone could affect dopamine in the mPFC via androgen receptors in dopaminergic cells in the VTA (Kritzer, 1997, Kritzer and Creutz, 2008), or alternatively, via androgen sensitive MPOA projections to the VTA (Sato *et al.*, 2008). The functional relevance of these studies is unclear: gonadectomy reduces performance on PFC dependent operant tasks (Kritzer *et al.*, 2007). However, these tasks are also known to be dependent on dopamine innervation, making the gonadectomy induced *increase* in PFC dopamine perplexing. In addition, short-term (4 day) gonadectomy causes a decrease in TH innervation and extracellular dopamine, effects that are opposite those observed with 28 day gonadectomy (Aubele and Kritzer, 2011a). Thus, the typical effect of testosterone on prefrontal dopamine is still undetermined.

Effects of short-term (>14days) castration in the ventral striatum have not been observed (Engel *et al.*, 1979, Mitchell and Stewart, 1989) however 28 day gonadectomy generally reduces dopamine and DOPAC concentrations in Acb tissue (Alderson and Baum, 1981, Mitchell and Stewart, 1989) but see (Baum *et al.*, 1986). While further study is needed to determine the effects of testosterone treatment, as opposed to withdrawal, these studies demonstrate that testosterone may be needed to maintain dopamine function in the Acb.

Testosterone itself also has intrinsically reinforcing properties (Wood, 2004). Animals form a CPP to peripheral injections of testosterone (De Beun *et al.*, 1992, Alexander *et al.*, 1994, Wood, 2004); the CPP can be disrupted by flupenthixol (D1/D2 antagonist) infusion into the Acb on the test day (Packard *et al.*, 1998). Moreover, CPP

is also formed to intra-Acb (Frye *et al.*, 2002) and intra-MPOA (King *et al.*, 1999) testosterone injections. Therefore, the endogenous testosterone surge in response to copulatory behavior or chemosensory stimuli may imbue them with rewarding properties. However, gonadectomized adult hamsters with replacement testosterone are still attracted to VS (Gregory and Bishop, 1975), suggesting that tonic levels of circulating testosterone, without elevations in testosterone specifically in response to pheromones, are sufficient for attraction to female pheromones. This dissertation will test whether exogenous testosterone is also sufficient for a conditioned place preference for VS.

Overall, it is clear that there are many interactions between mesocorticolimbic and hypothalamic circuitry, including the actions of dopamine, orexin, and testosterone, that may mediate sociosexual reward. A simplified presentation of these brain regions and neurochemicals is shown below in Figure 1.1. Importantly, many of these regions and neurochemicals mature in structure and function across adolescence, and these developmental changes may underlie the behavioral shifts in sociosexual responses at that same time.

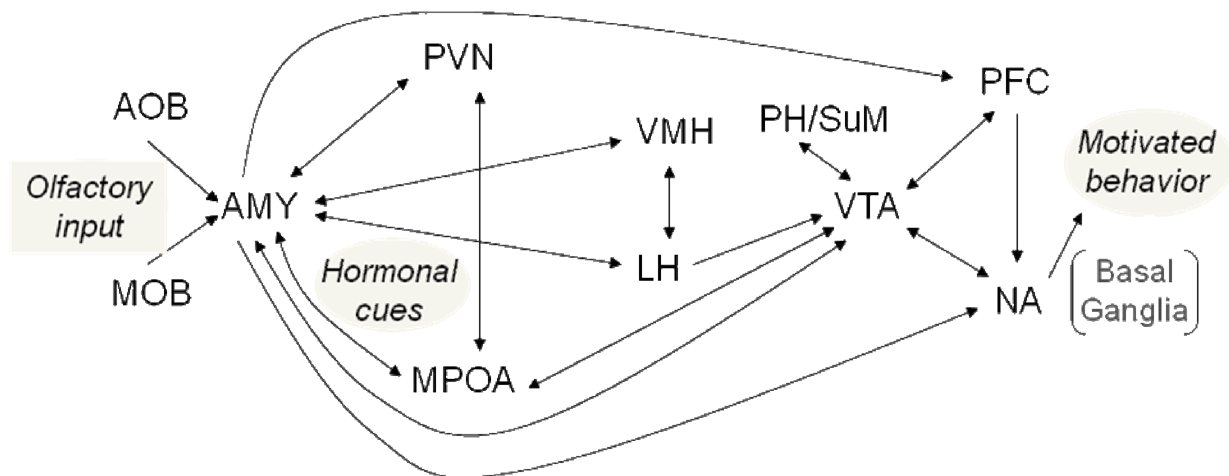


Figure 1.1: Diagram of brain regions involved in social behavior and motivation. Olfactory information is integrated with hormonal cues in the amygdala, sent to the preoptic area and hypothalamus, and then relayed to the mesocorticolimbic system for motivated behavioral output.

Developmental changes in motivational neural circuitry

As sexual behavior and attractive responses to VS mature across adolescence, neural responses to VS (as indexed by Fos expression) in brain regions important in the initial olfactory processing were investigated in juvenile and adult male hamsters. Gonad-intact juvenile and adult hamsters expressed increases in Fos expression in response to VS in the MeP, BNST, MPN, and MPNmag (Romeo *et al.*, 1998, Parfitt *et al.*, 1999). Therefore, although this study suggests juveniles can detect VS so as to induce neural activation in olfactory-related brain regions, it failed to identify the neural mechanism behind the behavioral changes in response to VS. While Fos expression was similar between juvenile and adult hamsters, juvenile did hamsters fail to show the adult-like MPOA dopamine release (indexed via dihydroxyphenylacetic acid concentrations in

tissue punches) in response to VS (Schulz *et al.*, 2003), highlighting the MPOA as one possible site of maturation. However, further research is needed to identify the neural maturation behind changes in chemoinvestigatory and sexual behaviors during adolescence.

As dopamine has been strongly implicated in reward, motivational salience and sexual motivation pathways (Berridge and Robinson, 1998, Andersen *et al.*, 2002, Bjork *et al.*, 2004, Hull and Dominguez, 2007), changes in dopamine circuitry that occur during adolescence may be important for the maturation of social stimulus interpretation and assessment (Bjork *et al.*, 2004). Indeed, there is good evidence for mesocorticolimbic and dopaminergic maturation over adolescence (Spear, 2000). However, it should be mentioned that the great majority of these studies were performed in rats and mice. While the overarching processes are likely similar to that in hamsters, exact timing could differ between the species. These studies are given to demonstrate possibilities, and future studies are needed in hamsters.

Multiple aspects of the mesocorticolimbic dopaminergic circuit mature during adolescence. In the midbrain, the firing rates of dopaminergic neurons peak mid-adolescence (McCutcheon and Marinelli, 2009). In male rats, striatal tissue content of dopamine reaches adult levels by P60 (Giorgi *et al.*, 1987, Broaddus and Bennett, 1990), and microdialysis studies show extracellular dopamine peaks at mid adolescence in the Acb (Philpot and Kirstein, 2004, Badanich *et al.*, 2006, Philpot *et al.*, 2009). Dopamine transporter density, another marker of innervation, also peaks in late adolescence in the accumbens (Coulter *et al.*, 1996). In the mPFC, there is an relatively linear adolescent increase in dopamine innervation and therefore dopamine

tissue concentrations in the mPFC in male monkeys (Rosenberg and Lewis, 1995) and rats (Kalsbeek *et al.*, 1988), and an increase in amygdalar input in male rats (Cunningham *et al.*, 2002). At the same time, the number of neurons in the PrL and IL decrease (Markham *et al.*, 2007), and glutamatergic projections from the PFC to the accumbens increase (Brenhouse *et al.*, 2008) during adolescence in male rats. Together, these data suggest gains in dopaminergic and glutamatergic activity and projections throughout adolescence.

In addition to changes in production and innervation, dopamine receptor expression matures during adolescence in both humans (Seeman *et al.*, 1987) and rats. Specifically, there is an overproduction and then pruning of D1 and D2 dopamine receptors in the dorsal striatum and Acb in males (and less so in females), as evidenced by binding assays (Teicher *et al.*, 1995, Andersen *et al.*, 1997b). In the mPFC, D1 and D2 density also peak mid-adolescence, particularly on pyramidal cells that project to the AcbC (Andersen *et al.*, 2000). Studies also suggest that autoreceptor function in the mPFC is only present prior to adolescence (Teicher *et al.*, 1991, Andersen *et al.*, 1997a). Thus, even if dopamine is released in a similar fashion between adults and juveniles, its postsynaptic consequences could be very different.

Lastly, not only does density of receptor expression shift, but the functional outcome of receptor activation changes during adolescence. In both the mPFC and Acb, D2 receptor activation excites GABAergic interneurons through some unknown mechanism, but only after P50 (Tseng and O'Donnell, 2007, Benoit-Marand and O'Donnell, 2008). These GABAergic interneurons then attenuate pyramidal and medium spiny neuron responses to excitatory input, potentially enhancing the

signal:noise ratio in adulthood (Tseng and O'Donnell, 2007, Benoit-Marand and O'Donnell, 2008). In the mPFC, interactions between dopamine and glutamate receptors also mature during adolescence (O'Donnell, 2003). As discussed previously, the membrane potential of mPFC pyramidal neurons can be brought into a more excitable "up" state by coordinated activation of glutamatergic NMDA and dopaminergic D1 receptor activation. Importantly, the amplitude of these up states is lower in juvenile animals compared to adults (O'Donnell *et al.*, 2002), and the D1 facilitation is only present after P48 (Tseng and O'Donnell, 2005). In the striatum, excitatory post synaptic potentials in response to cortical input reach adult levels between P30 and P40, while spontaneous burst firing patterns do not mature until after P40 (Tepper *et al.*, 1998). Together, all these changes suggest that any dopamine dependent behavioral responses to a social cue may change dramatically during adolescence, due to the extensive developmental changes in the relevant circuitry and electrophysiological properties.

There have been many attempts to relate these developmental changes in dopamine circuits to a range of reward-related behaviors. For example, the mid-adolescent peak in D1 expression on mPFC pyramidal cells that project to the Acb have been associated with a peak in cocaine reward at P40 in male rats (Brenhouse *et al.*, 2008). Similarly, Acb dopamine transients to a conspecific fail to show adult-like habituation to the social stimulus in a subsequent social interaction in juveniles, and have been linked to enhanced levels of peer-directed social interactions at this same age (Varlinskaya and Spear, 2008, Robinson *et al.*, 2011). Enhanced reward sensitivity to palatable food, cocaine, and amphetamine in juveniles compared to adults are

detected both behaviorally and with Fos expression in the Acb and mPFC (Andersen *et al.*, 2001, Cao *et al.*, 2007, Friemel *et al.*, 2010). As Fos expression is a useful way to monitor cellular activity in many brain regions at once, it will be used in this dissertation to provide a systems-level perspective for adolescent changes in responses to VS.

There are also reports of orexinergic maturation during adolescence in male rats. Dramatic increases in prepro-orexin mRNA (the uncleaved precursor to orexin A and B) are seen from P1 – P25, and reached adult levels at P40 (Yamamoto *et al.*, 2000). However, orexin-A and orexin-B immunoreacitve cells peak at P70, and then decline to adult levels over the next 30 days (Sawai *et al.*, 2010). Given the known interactions between orexinergic and dopaminergic systems in motivated behaviors, development in both systems may interact to affect reward-seeking behavior.

Summary of Dissertation Experiments

The differences between gonad-intact juvenile and adult male hamsters in their unconditioned behavioral and physiological responses to social stimuli suggest that female cues are being interpreted differently at the two ages. ***This dissertation tested the hypothesis that adult but not juvenile animals find VS rewarding and that this shift in stimulus interpretation is mediated by changes in dopaminergic mesocorticolimbic circuitry due to pubertal increases in testosterone.*** To do this, behavioral, immunohistochemical and pharmacological methods were used to test predictions of this hypothesis.

Chapter 2 tested the hypothesis that adult male hamsters find VS rewarding. Conditioned place preference was used by training males to associate one

of two chambers with female pheromones and then determining in which chamber the males choose to spend more time. Additionally, CPP was used to compare the reinforcing properties of VS to that of sexual behavior.

Chapter 3 tested the hypothesis that both rewarding and mesocorticolimbic responses to VS are gained during adolescence. Conditioned place preference (CPP) paradigm was used to compare the rewarding properties of VS between juvenile and adult hamsters. In parallel, immunohistochemistry for Fos and tyrosine hydroxylase was used to compare mesocorticolimbic activation patterns in response to VS between juveniles and adults. Hamsters were gonad intact in order to determine normal developmental changes in the evaluation of female pheromones.

Chapter 4 tested the hypothesis that testosterone treatment is sufficient in juveniles, and necessary in adults, for mesocorticolimbic responses to VS. Immunohistochemistry for Fos and tyrosine hydroxylase was used to compare mesocorticolimbic activation patterns in response to VS between gonadectomized juvenile and adult males with or without testosterone replacement.

Chapter 5 tested the hypothesis that dopamine receptor activation mediates the testosterone-dependent CPP formation for VS. Adult hamsters were gonadectomized and juvenile hamsters were given testosterone prior to testing for a CPP to VS. Additionally, testosterone-treated juveniles were given a dopamine antagonist to block a CPP to VS.

Although other brain regions, including the bed nucleus of the stria terminalis and dorsal striatum, and other neurotransmitters, including oxytocin and opioids, are involved in social information processing, social behaviors, and social reward, the

dramatic adolescent changes in mesocorticolimbic dopamine circuitry make it a good candidate for focused study in this dissertation. Overall, this work furthers our understanding of the neurobiological mechanisms underlying the adolescent maturation of social information processing and social reward.

Chapter 2: Male Syrian hamsters demonstrate a conditioned place preference for sexual behavior and female chemosensory stimuli

Introduction

Sexual activity is an effective natural reward in rodents; male rodents approach and prefer opposite sex conspecifics and perform operant behavior tasks for sexual stimuli (Murphy, 1973, Everitt, 1990, Crawford *et al.*, 1993, Agmo, 2003, Paredes, 2009). Another paradigm used extensively to assess the rewarding properties of a range of stimuli is conditioned place preference (CPP) (Schechter and Calcagnetti, 1993, Tzschentke, 1998, Tzschentke, 2007). CPP is a form of classical conditioning in which animals develop a preference for a distinctive environment associated with a rewarding stimulus such as an addictive drug, food, water, copulation, and other social behaviors (Pfaus and Phillips, 1991, Robbins and Everitt, 1996, Kelley and Berridge, 2002). CPP procedures have demonstrated the rewarding value of sexual behavior in male and female rats and mice, and in female hamsters (Miller and Baum, 1987, Agmo and Berenfeld, 1990, Agmo and Picker, 1990, Hughes *et al.*, 1990, Mehrara and Baum, 1990, Kippin and van der Kooy, 2003, Popik *et al.*, 2003, Harding and McGinnis, 2004). Specific components of male sexual behavior, including somatosensory stimuli resulting from intromission and ejaculation, are rewarding in and of themselves (Agmo and Gomez, 1993, Kudwa *et al.*, 2005, Tenk *et al.*, 2009). Chemosensory stimuli encountered during sexual behavior may also be rewarding, but this component of sexual interactions has not been as thoroughly investigated. Of the several rodents species for which sexual behavior induces a CPP, only mice are known to show a CPP for volatile (but not non-volatile) chemosensory stimuli derived from the opposite sex

(Pankevich *et al.*, 2006, Pierman *et al.*, 2006, Agustin-Pavon *et al.*, 2007, Martínez-Ricós *et al.*, 2007).

Male Syrian hamsters are ideal for investigating the rewarding properties of chemosensory stimuli, because hamsters are unique from rats and mice in their utter reliance on female chemosensory stimuli for the initiation of sexual behavior (Murphy and Schneider, 1970, Johnston, 1986, Coppola and O'Connell, 1988). For example, olfactory bulb removal eliminates odor preferences and copulatory behavior in sexually experienced male hamsters (Murphy and Schneider, 1970, Powers and Winans, 1975, Petrulis and Johnston, 1995, Petrulis, 2009). Vaginal secretions (VS) are the primary source of female chemosensory stimuli (Murphy and Schneider, 1970). Sexually-naïve male hamsters show an unconditioned attraction to VS (Johnston, 1974, Landauer *et al.*, 1977), and a preference for VS over male odors (Maras and Petrulis, 2006). In fact, VS are such powerful stimuli that male hamsters will mount another castrated or anesthetized male scented with VS (Murphy, 1973, Johnston, 1986). The data showing that female chemosensory stimuli are strongly attractive to male hamsters suggest that neural processing of female chemosensory stimuli encountered during anogenital investigation may be a specific component of sexual behavior that is rewarding for male hamsters (Wood, 2004). Surprisingly, however, male hamsters fail to bar press for female hamster VS, but operant conditioning may not have been well-suited to detect responses for VS (Coppola and O'Connell, 1988). Whether sexual behavior or its components can induce a CPP in male Syrian hamsters is unknown.

Syrian hamsters are an animal model that has yielded important insights into the mechanisms underlying the integration of sensory stimuli and hormones that are crucial

to the expression of appropriate behaviors during sexual and social interactions (Wood, 2004, Sato *et al.*, 2008, Petrulis, 2009). As we continue to investigate the neurobiology underlying sexual behavior using this model, we must determine whether sexual behavior and related chemosensory stimuli are rewarding to better direct research on brain regions and neurotransmitters involved in these behaviors. To assess the rewarding components of sexual behavior in the male Syrian hamster model, Experiment 1 tested the prediction that male hamsters demonstrate a CPP for sexual behavior, and Experiment 2 tested the prediction that male hamsters demonstrate a CPP for VS. The present study is the first to report that both sexual behavior and VS alone induce a CPP in male hamsters.

Methods

Animals

Forty sexually naïve adult male Syrian hamsters (*Mesocricetus auratus*) obtained from Harlan Sprague-Dawley laboratories (Madison, WI) were experimental subjects. All testing began when hamsters were 61-70 days old. Twenty-eight ovariectomized adult female hamsters, approximately 12 months old, were primed with estradiol benzoate and progesterone, 52 h and 4 h respectively, prior to use as stimulus females. Before use in Experiment 1, the females were tested for receptivity by placing a non-experimental, sexually experienced male from our colony in the females' home cage until the female showed lordosis but before the male achieved an intromission. All hamsters were singly housed in clear polycarbonate cages (30.5 x 10.2 x 20.3 cm) with ad libitum access to food (Teklad Rodent diet no. 8640, Harlan, Madison, WI) and

water. Male and female hamsters were housed in separate temperature- and humidity-controlled vivaria with a reversed light:dark cycle (14:10; lights off at 1400 h). Hamsters were treated in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animals, and protocols were approved by the Michigan State University Institutional Animal Care and Use Committee.

Experimental Design

Place preference conditioning occurred in an apparatus with three distinct compartments (Med Associates, St. Albans, VT). The middle compartment (12 x 21 x 21 cm) was gray with a smooth Plexiglas floor and was connected to the two outer compartments (28 x 21 x 21 cm) by manually controlled sliding guillotine doors. One outer compartment was white, with metal grid flooring. Fresh pine pellets were placed in the waste pan beneath the floor before each conditioning session. The other outer compartment was black, with black scalloped solid Plexiglas flooring, and scented with a 2% acetic acid solution swabbed along the top of the walls and ceiling before each conditioning session. These contextual cues were used in both Experiment 1 and 2, as we felt that that an olfactory contextual cue was important for this olfactory-oriented species and would not interfere with conditioning for VS. Pilot experiments determined that these conditions reduced initial preferences for one or the other compartment. Time spent in each compartment was recorded using MED-PC software connected to infrared photobeams spaced at five cm intervals along the bottom of the apparatus. All conditioning and tests were conducted under dim red light at least one hour into the dark phase.

An initial place preference test, here called the pretest, was used to determine each hamster's initial compartment preference. Following a 5-min habituation period in the middle gray compartment, the doors were raised and the hamster could move freely throughout the apparatus for 15 min. The outer compartment in which the hamster spent the most time was defined as the initially preferred compartment. Experimental and control groups, n = 10/group, were matched such that average initial preferences were similar across the groups. Following the pretest, the hamsters received a series of 30 min conditioning sessions, one session per day on consecutive days. Stimulus-paired (sex behavior, SB or vaginal secretions, VS) or no-stimulus conditioning sessions occurred on alternating days, beginning with the no-stimulus conditioning session. Although three stimulus-paired sessions were sufficient to induce a CPP for SB (Experiment 1), in a pilot study using similar procedures, three stimulus-paired sessions were insufficient to induce a CPP for VS. Therefore, in Experiment 2 we increased the number of stimulus-paired sessions to five with the idea that more exposure to VS would lead to a CPP. During the no-stimulus conditioning sessions, hamsters in both the experimental and control groups were taken directly from their homecages and placed into their initially preferred compartments, where they remained alone for 30 min. During stimulus-paired conditioning sessions, hamsters in the experimental group were taken from their homecages, placed in the initially non-preferred compartments and given access to SB or VS. The hamsters in the control group were also placed in their initially non-preferred compartments, but were not given the stimulus so that their experiences in the two compartments were comparable. The CPP apparatus was cleaned thoroughly with 25% ethanol following each conditioning session and test.

Twenty-four hours after the last conditioning session, hamsters were tested for their place preference following the same procedure used for the pretest.

Experiment 1: CPP for Sexual Behavior. In Experiment 1, the stimulus tested for its rewarding value was sexual behavior with a receptive female hamster. In the SB-paired conditioning sessions, a female was placed into the compartment immediately before the male. Hamsters were observed for the duration of these sessions to verify that each male performed sexual behavior. They engaged in bouts of sexual behavior, including ejaculations, throughout the 30-min session, although behavior was not quantified. Hamsters in the control group were alone in compartments for both conditioning sessions, while hamsters in the SB group were alone in the compartment during the no-stimulus sessions. Six total conditioning sessions occurred, including three no-stimulus and three stimulus-paired sessions. The experiment took place over eight consecutive days including the pretest and test days.

Experiment 2: CPP for Vaginal Secretions. Experiment 2 was similar to Experiment 1, except the experimental stimuli tested for rewarding value were VS. VS were collected by vaginally palpating 14 hormone-primed female hamsters approximately two hours before conditioning sessions began. Because both volatile and non-volatile components of VS have important, and potentially different, roles in male hamster sexual behavior, the paradigm was designed to ensure that both were present throughout the conditioning session. Several pilot studies were performed to determine the optimal method for VS delivery, including cotton swabs and Eppendorf tubes. The latter proved to be the most effective at keeping VS moist and allowing for continued exposure to volatile cues throughout the conditioning session. Approximately

20 µl of VS were applied to water-moistened cotton gauze packed into a 2 ml Eppendorf tube. The tube was out of reach of the male, taped to the top of the back wall of the initially non-preferred compartment in VS-paired conditioning sessions for the VS group. Empty Eppendorf tubes were used for the control group in all conditioning sessions and for the VS group in the no-stimulus conditioning sessions. To ensure exposure to non-volatile components of VS, the remaining VS were mixed with 1.5 ml of mineral oil (see (Woodley and Baum, 2004)), and approximately 50 µl of this mixture was applied with a metal spatula directly onto the nose of the hamsters in the VS group immediately before being placed in the VS-paired compartment. The concentration of VS used in this experiment far exceeds that which is attractive to male hamsters, but mimics the amount available to a male hamster upon anogenital investigation of an estrous female (Macrides *et al.*, 1984b). Clean oil was applied to the nose of hamsters in the control group for all conditioning sessions and in the VS group for no-stimulus conditioning sessions. Ten total conditioning sessions occurred, including five no-stimulus and five stimulus-paired sessions. The experiment took place over 12 consecutive days including the pretest and test days.

Data Analysis

To assess whether the stimuli (Experiment 1 SB; Experiment 2 VS) induced a CPP, data from the pretests and final tests were used to calculate a preference score, defined as time in the stimulus-paired compartment / (time in stimulus-paired compartment + time in no-stimulus compartment), and a difference score, defined as the time in the no-stimulus compartment – time in the stimulus-paired compartment

(Meisel and Joppa, 1994, Paredes and Alonso, 1997, Martínez and Paredes, 2001, Meerts and Clark, 2007, 2009c, b, a, Tenk *et al.*, 2009, Parada *et al.*, 2010). Paired *t*-tests were used to evaluate the change in preference score and difference score pre- and post-conditioning, and the alpha level was set at $p < 0.05$ (Meisel and Joppa, 1994, Paredes and Alonso, 1997, Martínez and Paredes, 2001, Meerts and Clark, 2007, 2009c, b, a, Tenk *et al.*, 2009, Parada *et al.*, 2010). One methodological concern when using CPP is that the change in time spent in the compartments may be due to habituation across the conditioning sessions. This concern was addressed in these experiments by the use of the control group that received the same experience in the testing apparatus without any stimulus pairings (Meisel and Joppa, 1994).

Results

Experiment 1: CPP for Sexual Behavior. Male hamsters in the sexual behavior group showed a CPP for the SB-paired compartment, whereas control hamsters did not (Figure 2.1). Paired *t*-tests showed that the preference score increased significantly for the SB group, $t(9)=-4.26$, $p<0.01$, but not the controls, $t(9)=.49$, $p=0.63$. Likewise, the difference score decreased significantly for the SB group, $t(9)=4.11$, $p<0.01$, but not the controls, $t(9)=-.54$, $p=0.60$.

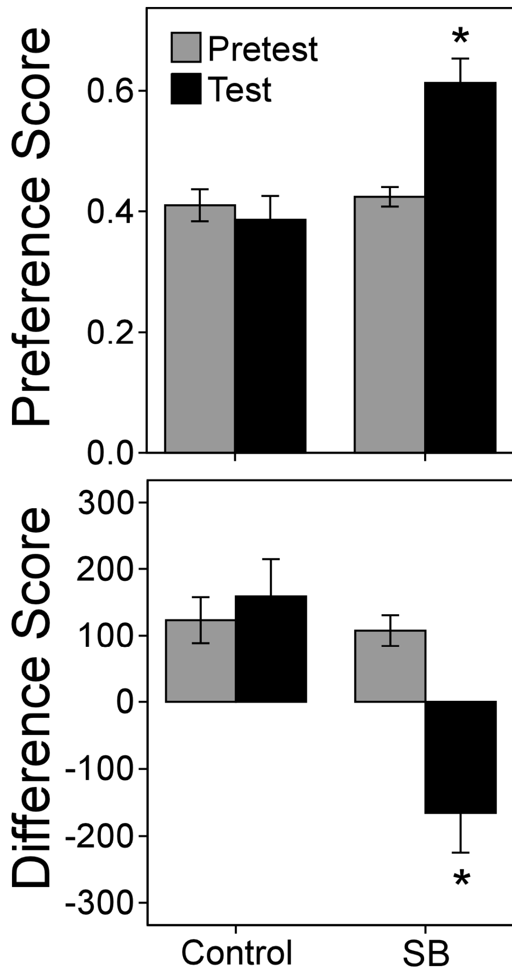


Figure 2.1. CPP to sexual interaction in adult hamsters. Mean±SEM preference score (top) and difference score (bottom) on pretest (gray bars) and test (black bars) for control and SB groups, n=10 per group. Asterisks indicate that the SB group showed a CPP for the compartment associated with sexual behavior; there was a significant change in preference score and difference score for the SB group but not control group, $p<0.05$.

Experiment 2: CPP for Vaginal Secretions. Male hamsters that were given VS showed a CPP for the VS-paired compartment, whereas control hamsters did not (Figure 2.2). Paired *t*-tests showed that the preference score increased significantly for

the VS group, $t(9)=-2.772$, $p<0.05$, but not the controls, $t(9)=-0.89$, $p=0.40$. Likewise, the difference score decreased significantly for the VS group, $t(9)=0.35$, $p<0.01$, but not the controls, $t(9)=2.07$, $p=0.07$.

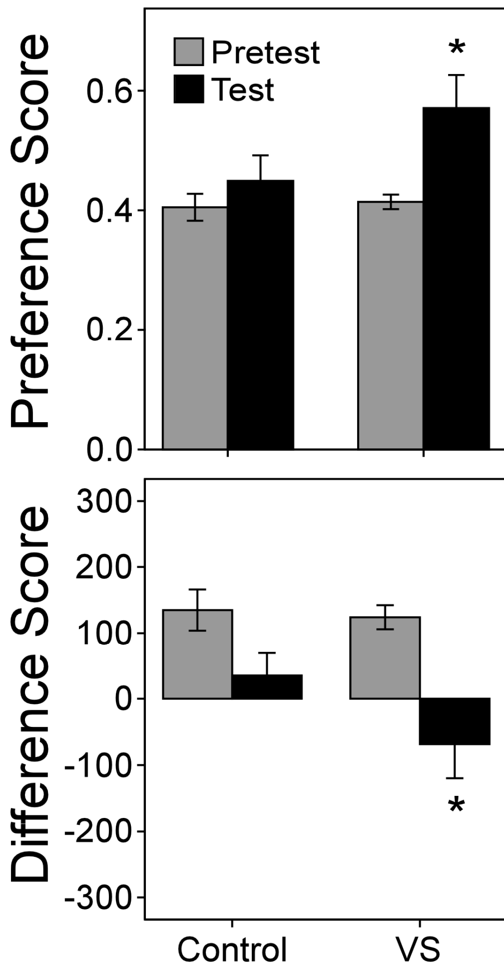


Figure 2.2. CPP to VS in adult hamsters. Mean±SEM preference score (top) and difference score (bottom) on pretest (gray bars) and test (black bars) for control and VS groups, n=10 per group. Asterisks indicate that the VS group showed a CPP for the compartment associated with VS; there was a significant change in preference score and difference score for the VS group but not control group, $p<0.05$.

Discussion

The present study is the first to demonstrate that male Syrian hamsters show a CPP for both sexual behavior with a receptive female hamster and VS, reinforcing the concept that sexual activity is a strong natural reward for male rodents (Pfaus and Phillips, 1991). Moreover, the CPP for VS indicates that female chemosensory stimuli are an unconditioned reward to male hamsters because the males were sexually naïve and VS had not been previously associated with sexual activity. Fewer conditioning sessions may be needed to induce a CPP for sexual behavior than for VS because the additional somatosensory, auditory and visual aspects likely contribute to the rewarding properties of sexual behavior. However, differences in VS stimulus intensity when presented by an experimenter versus by a female hamster during a sexual encounter may also be a factor. The present data agree with those from mice showing that chemosensory stimuli are rewarding (Pankevich *et al.*, 2006, Agustin-Pavon *et al.*, 2007, Martínez-Ricós *et al.*, 2007). Mice are also sensitive to chemosensory stimuli in certain reproductive contexts (e.g., pregnancy block and puberty acceleration (Bruce, 1959, Vandenbergh, 1973). Together, the findings from hamsters and mice suggest that the behavioral salience and rewarding value of a chemosensory stimulus may be positively correlated.

Rewarding Components of VS

The specific compounds in VS that are rewarding remain to be identified. The chemosensory stimulus used in the present study contained both volatile and non-volatile components. The volatile component of VS, dimethyl disulfide, has been proposed to promote anogenital investigation of the female (Singer *et al.*, 1976,

Macrides *et al.*, 1977, Pfeiffer and Johnston, 1994), whereas the non-volatile component, aphrodisin, is thought to stimulate sexual arousal and copulatory behavior (Macrides et al., 1977; (Singer *et al.*, 1984a, Singer *et al.*, 1987). Generally speaking, the different components bind different receptors; volatile olfactants bind receptors located in the main olfactory epithelium, whereas non-volatile molecules bind those in the vomeronasal organ (Rowe and Edwards, 1972, Meredith, 1991, Keverne, 2004, Keller *et al.*, 2009). In male hamsters, the vomeronasal organ is important for the display of sexual behavior and plasma testosterone responses to VS (Meredith, 1986). Main olfactory epithelium lesions have little effect on sexual behavior whereas vomeronasal organ lesions alone reduce sexual behavior in sexually naïve male hamsters; combined lesions completely eliminate male courtship and sexual activity (Powers and Winans, 1975). The accessory olfactory system also appears to be critical for chemosensory reward in mice (Pankevich *et al.*, 2006, Martínez-Ricós *et al.*, 2007). Although additional studies are needed to pinpoint the specific components of VS and their site of action that lead to a CPP in male hamsters, the literature suggests that the accessory olfactory system is important to motivate sexual behavior in male hamsters.

Neural Mechanisms of Sexual Reward

Dopamine and opioids have both been implicated in the rewarding aspects of rodent sexual behavior. Dopamine is released in the medial preoptic area (MPOA) and Acb following copulation and exposure to chemosensory stimuli in male and female rodents (Mas *et al.*, 1990, Pfaus *et al.*, 1990, Damsma *et al.*, 1992, Mitchell and Gratton, 1992, Meisel *et al.*, 1993, Wenkstern *et al.*, 1993, Asmus, 1994, Sato *et al.*, 1995, Schulz *et*

al., 2003). In fact, the MPOA dopamine release in hamsters requires exposure to female chemosensory stimuli (Triemstra *et al.*, 2005), further reinforcing the obligatory nature of chemosensory stimuli for sexual responses in this species. Dopamine is implicated in CPP for sexual behavior in hamsters, as CPP induced by sexual behavior in female hamsters is blocked by administration of a D2 receptor antagonist (Meisel *et al.*, 1996) but see (Agmo and Berenfeld, 1990, Agustin-Pavon *et al.*, 2007) for different findings in other rodent species. Opioid receptor activation has also been observed after male rat copulation (Balfour *et al.*, 2004). Studies on the role of opioids in sexual reward have consistently shown that opiate receptor antagonists block CPP for sexual behavior in male rats (Miller and Baum, 1987, Agmo and Berenfeld, 1990, Mehrara and Baum, 1990, Agmo and Gomez, 1993). Although opioids may modulate dopamine activity in reward-related responses in male rats (Balfour *et al.*, 2004), more research is needed to determine the role of dopamine and opioids in mediating the rewarding value of VS in male hamsters.

Testosterone is another potential mediator of chemosensory reward because sexual stimuli elicit an acute increase in plasma testosterone (Pfeiffer and Johnston, 1994, Romeo *et al.*, 1998), and testosterone is intrinsically rewarding. For example, hamsters self-administer intracerebroventricularly-delivered testosterone (Wood, 2004) and rats and mice show a CPP for systemic testosterone injections (De Beun *et al.*, 1992, Alexander *et al.*, 1994). In addition, castration prevents formation of CPP for an estrous female rat (Harding and McGinnis, 2004). Thus, the sex- and VS-induced increase in testosterone in hamsters (Pfeiffer and Johnston, 1994, Romeo *et al.*, 1998)

may be a neuroendocrine response that contributes to the rewarding aspects of these stimuli.

In summary, adult male hamsters show a CPP for sexual behavior and VS, indicating that the chemosensory stimuli encountered during anogenital investigation is one specific component of sexual behavior in male hamsters that is rewarding. It is noteworthy that the chemosensory stimuli are rewarding in both hamsters and mice, as the animals had never before associated the chemosensory stimuli with sexual behavior. Both these species are heavily influenced by chemosensory stimuli in different aspects of social behavior, suggesting that there may be an evolutionary connection between the dependence on these stimuli for social behaviors and their intrinsically rewarding properties.

Chapter 3: Adolescent gain in positive valence of a socially relevant stimulus: engagement of the mesocorticolimbic reward circuitry

Introduction

A universal feature of mammalian adolescence is the restructuring of social spheres as interactions with peers become more salient than those with family (Nelson *et al.*, 2005). This reallocation of interest involves maturation of social information processing, i.e., the perception of and responses to social stimuli. Social stimuli evoke different neural responses in human adolescents as compared with adults, particularly in the prefrontal cortex (Blakemore, 2008), but the neuronal mechanisms underlying this developmental change remain unclear. Given the importance of appropriately interpreting social stimuli in successful adult social interactions, the overall goal of this study is to determine which brain regions and neurotransmitter systems are associated with adolescent changes in the perception of social stimuli.

The male Syrian hamster is an ideal animal model for investigating the neural substrates of adolescent maturation of social information processing. Information about female reproductive status is conveyed via pheromone-containing vaginal secretions (VS). Appropriate neural processing of VS is required for the performance of male sexual behavior and is sufficient to induce a conditioned place preference (CPP) in sexually naïve adult male hamsters, indicating that VS are inherently rewarding to adults (Murphy and Schneider, 1970, Petrulis, 2009, Bell *et al.*, 2010). In contrast, juvenile hamsters are not attracted to VS (Johnston and Coplin, 1979), nor do they mate with a receptive female when primed with exogenous testosterone (Meek *et al.*, 1997, Schulz *et al.*, 2009). Studies using the immediate early gene Fos as a proxy for neural

activation reveal that juvenile hamsters do detect VS, as it elicits an increase in Fos expression in brain regions typically associated with processing of chemosensory social stimuli, e.g. the medial amygdala (Romeo *et al.*, 1998). Thus, the social salience of VS changes across adolescent development, and this naturally occurring maturation of social information processing is critical for successful reproduction.

The neural underpinnings of these age-related changes in responses to VS are unknown. The ability of adults to form a CPP for VS suggests involvement of reward-related neural systems in the processing of this social stimulus. In particular, the rodent mesocorticolimbic dopamine and hypothalamic orexin systems are implicated in sexual, food and psychotropic drug reward (Meisel *et al.*, 1996, Becker *et al.*, 2001b, Harris *et al.*, 2005, Muschamp *et al.*, 2007, Ikemoto, 2010, Lajtha and Sershen, 2010, Di Sebastiano *et al.*, 2011), and these systems often operate in concert (Fadel and Deutch, 2002, Korotkova *et al.*, 2003, Narita *et al.*, 2006). Both dopaminergic and orexinergic circuitries undergo functional and structural changes during adolescence (Kuhn *et al.*, 2010, Sawai *et al.*, 2010), however developmental changes in response to social stimuli, including VS, have not been examined within them. The present study seeks to determine if juveniles differ from adults in their proclivity to 1) show CPP for VS, and 2) express Fos in response to VS in mesocorticolimbic and hypothalamic reward circuits.

Methods

Animals

Syrian hamsters (*Mesocricetus auratus*) were obtained from Harlan Laboratories (Madison, WI) and were housed in temperature- and humidity-controlled vivaria with a shifted light:dark cycle (14:10, dark phase began at 1:00pm) and *ad libitum* access to food (Teklad Rodent diet 8640, Harlan, Madison, WI) and water. Juveniles in Exp 1 arrived at postnatal day 13 (P13) and were housed with their littermates and biological mother until weaning at P18. Adults in Exp 1 arrived at ages ranging from P56-62, juveniles in Exp 2 at P20, and adults in Exp 2 at P54. Weanlings and adult males were singly housed in clear polycarbonate cages (30.5 x 10.2 x 20.3 cm) and were all sexually naïve. Sixty adult female hamsters, approximately 12 months old, were housed under similar conditions in separate vivaria and used as the source of VS. Female hamsters were ovariectomized several weeks before hormone administration and collection of VS. They were injected subcutaneously with 10 μg estradiol benzoate and 500 μg progesterone in sesame oil, 52 and 4 hours respectively, prior to collection of VS by gentle vaginal palpation. All experiments were conducted under <4 lux red light 1-5 hours into the dark phase. Hamsters were treated in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animals, and protocols were approved by the Michigan State University Institutional Animal Care and Use Committee.

Experiment 1: Conditioned Place Preference for potential rewards

Experimental Design: Place preference conditioning occurred as described previously (Bell *et al.*, 2010) in an apparatus with one middle compartment and two outer compartments distinct in their visual, tactile and olfactory cues (Med Associates, St. Albans, VT). To acclimate subjects to handling and novel chambers, male hamsters were placed in glass aquaria in the behavioral testing room for 10 minutes every day, for three days prior to the start of the CPP regimen. A 17-minute pretest (2 minutes in the middle compartment followed by 15 minutes access to all compartments) was used to determine each hamster's initial compartment preference and to create groups with similar initial preferences, when possible. The outer compartment in which the hamster spent more time was defined as the initially preferred compartment. Hamsters that did not enter each compartment at least 5 times were excluded from further training. Following the pretest, the hamsters received a series of 30-minute conditioning sessions in the side compartments, one session per day on consecutive days, alternating no-stimulus or stimulus-paired sessions. During the no-stimulus conditioning sessions, hamsters in both the experimental and the control groups were placed in their initially preferred compartments, where they remained alone. During stimulus-paired conditioning sessions, hamsters in the experimental group were placed in the initially nonpreferred compartments with the stimulus. The hamsters in the control groups were also placed in their initially nonpreferred compartments but were not given the stimulus. This group served to demonstrate that any change in preference or difference score across tests seen in the experimental group was not due to chance or habituation during conditioning. Twenty-four hours after the last conditioning session, hamsters

were tested for their place preference following the same procedure used for the pretest. Pretest, conditioning sessions, and test all occurred at the same time of day (+/- an hour) for each hamster. VS were used as a stimulus in the first experiment, and palatable food and cocaine were used as stimuli in two other experiments.

Experiment 1a: CPP for VS. To test for a CPP for VS, 22 sexually naïve adult and 18 juvenile hamsters were assigned to control and experimental groups, n=9-11. In order to reduce the number of cohorts required and prevent exposing control animals to the smell of the stimuli, control animals were housed in a separate but similar room in which the dark phase began at 8:00am, and testing at 9:00am. Ten total conditioning sessions occurred, including 5 no-stimulus and 5 stimulus-paired sessions. Including the pretest and test, the experiment took place over 12 consecutive days, from P20 to 31 for juvenile animals and P63-69 to 74-80 for adult animals. An hour before use, VS were collected from 30 females and mixed together to total approximately 500µl. Approximately 15 µl of VS were applied to water-moistened cotton gauze packed into a 2-ml Eppendorf tube, one tube for each male. Immediately before testing, the tube was placed out of reach from the male at the top of the back wall in the initially nonpreferred compartment in VS-paired conditioning sessions for the VS group. Empty Eppendorf tubes were used for the control group in all conditioning sessions and for the VS group in the no-stimulus conditioning sessions. To ensure exposure to nonvolatile components of VS, the remaining ~200 µl of VS were mixed with 1.5 ml of mineral oil, and approximately 20 µl of this mixture was applied with a metal spatula directly onto the nose of hamsters in the VS group immediately before being placed in the VS-paired compartment. Clean oil was applied to the nose of hamsters in the control group for all

conditioning sessions and in the VS group for no-stimulus conditioning sessions. One hour after completion of the CPP test, hamsters were euthanized with an overdose of sodium pentobarbital (150 mg/kg, ip) and a terminal blood sample was collected via cardiac puncture for radioimmunoassay of circulating plasma testosterone.

Experiment 1b: CPP for palatable food. To test for a CPP for palatable food, 22 juvenile hamsters were assigned to control and experimental groups, n=11. Ten total conditioning sessions occurred, including 5 no-stimulus and 5 stimulus-paired sessions, and the experiment lasted 12 days from P20-31. Approximately 10 pieces of Hoola Fruits cereal (~2.5 g, Our Family Foods, Minneapolis, MN) were scattered on the floor of the initially non-preferred compartment, while none were present in the initially preferred compartment for the experimental group. There was no palatable food placed in either compartment for the control group. Quantity of normal chow and palatable food consumed and body weights were recorded each day.

Experiment 1c: CPP for cocaine. To test for a CPP for cocaine, 16 juvenile hamsters were assigned to control and experimental groups, n=8. Six total conditioning sessions occurred, including 3 no-stimulus and 3 stimulus-paired sessions, and the experiment lasted 8 days from P23-30. Hamsters in the experimental group were injected intraperitoneally with cocaine (20mg/kg, Lannett Company, Inc. Philadelphia, PA) immediately before being placed in the initially nonpreferred compartment for stimulus-paired sessions, whereas they received a 0.9% saline vehicle injection before being placed in the initially preferred compartment for no-stimulus sessions. The control group received saline injections before being placed in either compartment in conditioning sessions.

Statistical analysis. To assess whether the stimuli (VS, palatable food, or cocaine) induced a CPP, data from the pretests and final tests were used to calculate a preference score, defined as [time in the stimulus-paired compartment/(time in stimulus-paired compartment+time in no-stimulus compartment)], and a difference score, defined as [time in the no-stimulus compartment–time in the stimulus-paired compartment] (Bell *et al.*, 2010). Paired t-tests within each group were used to evaluate the change in preference score and difference score pre- and post-conditioning (Bell *et al.*, 2010). Here and with all other reported analyses, $p < 0.05$ was considered significant, and all statistical analyses were done with SPSS software (PASW Statistics 18; SPSS: An IBM Company, Chicago, IL).

Experiment 2: Neural Responses to VS

Experimental Design. 16 juvenile (P28) and 16 adult (P64) hamsters were weighed and randomly assigned to either the VS or control group, n = 8. VS were collected less than two hours before use and delivered via cotton applicator swabs to minimize effects of handling the animals on Fos expression. VS-containing or clean blank swabs were dropped into VS or control hamsters' home cages, respectively, and behavior was monitored while the hamsters interacted with the swab for one hour. Hamsters were often observed to pick up the swab, chew on it, and place it in their cheek pouches for several minutes at a time. To prevent control hamsters from smelling volatile components of VS, they were given access to blank swabs and then removed from the room for tissue collection prior to giving the VS group the VS-containing swabs. Blank and VS-containing swabs were delivered 1-2 and 3-4 hours

after lights off, respectively. One hour after introduction of swab into the cage, hamsters were euthanized with an overdose of sodium pentobarbital (150 mg/kg, ip) and a terminal blood sample was collected via cardiac puncture for radioimmunoassay of circulating plasma testosterone. Hamsters were perfused transcardially with heparinized buffered saline rinse followed by 4% paraformaldehyde. Brains were post-fixed in 4% paraformaldehyde for 24 hours and stored in 20% sucrose/phosphate buffered saline solution until sectioning.

Histological procedures. Brains were sectioned with a cryostat into 4 series of 40 μm thick sections and stored in cryoprotectant at -20°C until histological processing. The first series of sections was mounted onto glass slides, dehydrated with a series of ethanols, and stained with cresyl violet before coverslipping for identification of regions of interest. A second and third series of sections were used to double-label cFos with tyrosine hydroxylase (TH) and Orexin-A (Orx) immunoreactivity, respectively, with free-floating immunohistochemistry. cFos is an immediate early gene used to indicate transcriptional activation (Sheng and Greenberg, 1990, Hughes and Dragunow, 1995), in many studies on sex and VS-induced neural activity. Thus, the use of Fos allows for more facile inter-study comparisons and provides an excellent way to survey general activity patters in many brain regions at once. TH is the rate-limiting enzyme for catecholamine production. Dopamine-β-hydroxylase, the enzyme that converts dopamine to norepinephrine, is absent in the ventral tegmental area in hamsters (Vincent, 1988), thus TH immunoreactivity in the ventral tegmental area was used here to identify dopaminergic cells. Orexin-A is one of two active orexinergic peptides (de Lecea *et al.*, 1998, Sakurai *et al.*, 1998), and, in particular, has been implicated in

sexual reward (Muschamp *et al.*, 2007, Di Sebastiano *et al.*, 2011). While both TH and Orx have AP-1 binding sites in their promoters (Hughes and Dragunow, 1995, Chen and Randeva, 2010), conclusions about DA or orexinergic activity upon observations of double-labeling must be made cautiously (Hoffman and Lyo, 2002).

Immunohistochemistry work occurred at room temperature unless otherwise noted. Rinses with Trizma buffered saline (TBS, 0.05M, pH = 7.6) occurred initially and between steps, and all antibodies were diluted in 2% of the appropriate serum and 0.3% Triton-X TBS (see Table 3.1). To visualize Fos and TH, residual aldehydes were removed with 0.1% sodium borohydride after the first series of TBS rinses, and endogenous peroxidase activity was quenched with 1% hydrogen peroxide. Tissue was blocked and made permeable with 20% goat serum and 0.3% Triton-X TBS, followed by incubation in the cFos primary antibody for 48 hours at 4°C. Tissue was then incubated consecutively in the Fos secondary antibody and avidin-biotin complex for one hour each. Lastly, sections were reacted for ~2 minutes with 10 mg 3,3'-Diaminobenzideine tetrahydrochloride in 50 ml TBS and 45 µl of 30% hydrogen peroxide to produce a dark brown reaction product in the nucleus of Fos-immunoreactive (ir) cells. After rinsing, tissue was again blocked and made permeable and then incubated overnight in TH primary antibody. TH secondary antibody and avidin-biotin complex were then each applied consecutively for 1 hour. Finally, sections were reacted for ~2 minutes with 1 drop of Vector SG enzyme substrate in 7 ml TBS and 50 µl 30% hydrogen peroxide to produce a cytoplasmic blue reaction product in TH-ir cells. To visualize Fos and Orx, a similar immunohistochemistry protocol was used, but with the appropriate reagents (see Table 3.1).

Table 3.1. Immunohistochemical reagents

Fos/TH Immunohistochemistry		
Fos	Serum	Normal goat serum. Pel-Freez Biologicals, Rogers, AR
	Primary	c-Fos (4): rabbit, sc-52. 1:10,000, 0.02μg IgG/ml solution, Santa Cruz Biotech, Santa Cruz, CA
	Secondary	Biotinylated goat anti-rabbit IgG (H+L). 1:500, 3μg IgG/ml solution, Vector Laboratories, Burlingame, CA
TH	Serum	Normal goat serum. Pel-Freez Biologicals, Rogers, AR
	Primary	Mouse anti-TH monoclonal antibody. 1:2,000, Millipore-Chemicon, Billerica, MA
	Secondary	Biotinylated goat anti-mouse IgG (H+L). 1:500, 3μg IgG/ml solution, Vector Laboratories, Burlingame, CA
Fos/Orx Immunohistochemistry		
Fos	Serum	Normal donkey serum. Jackson ImmunoResearch Laboratories, West Grove, PA
	Primary	c-Fos (4): rabbit, sc-52. 1:10,000, 0.02μg IgG/ml solution, Santa Cruz Biotech, Santa Cruz, CA
	Secondary	Biotinylated donkey anti-rabbit IgG (H+L). 1:500, 2.4μg IgG/ml solution, Jackson ImmunoResearch, West Grove, PA
Orx	Serum	Normal horse serum, S-2000. Vector Laboratories, Burlingame, CA
	Primary	Orexin-A (C-19): goat, sc-8070, 1:5,000, 0.04μg IgG/ml solution, Santa Cruz Biotech, Santa Cruz, CA
	Secondary	Biotinylated horse anti-goat IgG (H+L), 1:500, 3μg IgG/ml solution, Vector Laboratories, Burlingame, CA
Other reagents		
Avid :Biotin Complex		Peroxidase- Vectastain ABC Kit PK-6100, Vector Laboratories, Burlingame, CA
Enzyme Substrate		Peroxidase- Vector SG Substrate Kit SK-4700, Vector Laboratories, Burlingame, CA
Enzyme Substrate		Peroxidase- 3,3' Diaminobenzidine tetrahydrochloride, Sigma-Aldrich, St. Louis, MO

Primary and secondary antibody deletion control studies were run on separate sections. Non-specific background staining was low or absent in these sections.

Tissue sections were mounted onto glass slides and dehydrated with a series of ethanols before coverslipping.

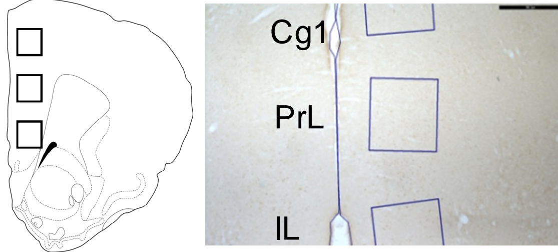
Microscopic analysis. Regions of interest included the nucleus accumbens (Acb), medial prefrontal cortex (mPFC), and ventral tegmental area (VTA) because they are primary components of the mesocorticolimbic dopamine circuitry (Fibiger and Phillips, 1988); the lateral hypothalamus (LH) because of its orexinergic cell population (Aston-Jones *et al.*, 2009); the ventromedial hypothalamus (VMH) because of its role in gating reproductive and defensive behaviors (Choi *et al.*, 2005); and the posterior medial amygdala (MeP) as a positive control region known to express Fos in response to VS in both juvenile and adult male hamsters (Romeo *et al.*, 1998).

Regions were subdivided according to the hamster brain atlas (Morin and Wood, 2001), as indicated by previous research demonstrating distinct functional and anatomical characteristics of the subregions (Groenewegen *et al.*, 1999, Bradley and Meisel, 2001, Heidbreder and Groenewegen, 2003, Balfour *et al.*, 2006, Ikemoto, 2007). The mPFC included the anterior cingulate (Cg1), prelimbic (PrL), and infralimbic (IL) subregions; the Acb included the core (AcbC) and medial portion of the shell (AcbSh); the MeP included the dorsal (MePD) and ventral (MePV) subregions; the VMH included medial (VMHM) and lateral (VMHL) portions; and the VTA included interfasicular (IF), paranigral (PN), parabrachial pigmented (PBP), and Tail nuclei (Figure 3.1). The hypothalamic area containing orexinergic cells was subdivided into the dorsomedial hypothalamus and perifornical area (DM/PeF) and lateral hypothalamus (LH), relative to the lateral edge of the fornix because of functional specificity of these two populations of orexinergic neurons (Harris *et al.*, 2005), Figure 3.1).

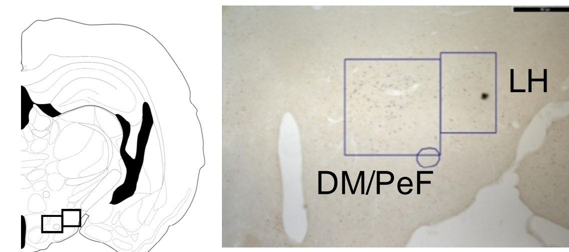
The VTA was further subdivided along its rostrocaudal extent because of previous reports of functional specificity in rats and mice (Olson *et al.*, 2005, Ikemoto, 2007) and a relative lack of region-specific analysis in the hamster. Rostral sections were defined as having TH cells adjacent to the fasciculus retroflexus prior to the onset of the interpeduncular nucleus; caudal sections were defined as having interpeduncular nucleus present prior to the medial lemniscus merging with the cerebral peduncle; tail sections were defined as having a rounded interpeduncular nucleus prior to the oral part of the pontine nuclei (Figure 3.1). Upon completion of microscopic inspection and analysis, similar effects of age and swab exposure were found in the rostral and caudal portions of each subregion; therefore, data from rostral and caudal IF, PN, and PBP sections were combined within subregion for statistical analysis and presentation here.

Anatomically matched tissue sections throughout the extent of each ROI (2-5 sections per subregion, depending on size) were selected at 4x objective. In the Acb, Me, and VMH, subregion contours were manually traced bilaterally according to the atlas and cytoarchitecture in Nissl-stained sections and then overlaid onto corresponding immunohistochemistry-treated tissue sections for cell counting. In the mPFC, 600 x 600 μm boxes were placed in the mPFC relative to brain contour and corpus callosum landmarks. In the hypothalamus, boxes were drawn to surround all Orx-ir cells medial or lateral to the lateral edge of the fornix in immunohistochemistry tissue sections. In the VTA, contours were drawn unilaterally in immunohistochemistry tissue sections.

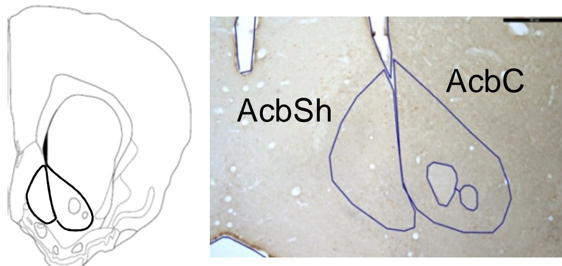
A. Prefrontal Cortex (PFC)
3.2 – 2.4 mm from bregma



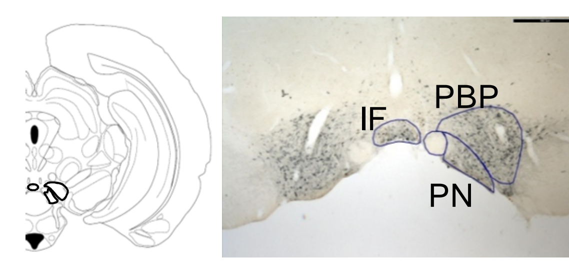
E. Hypothalamus
-1.8 – -2.3 mm from bregma



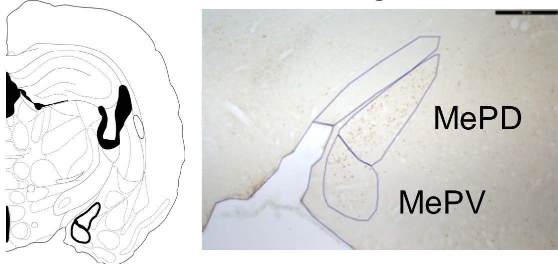
B. Nucleus Accumbens (Acb)
2.4 – 1.8 mm from bregma



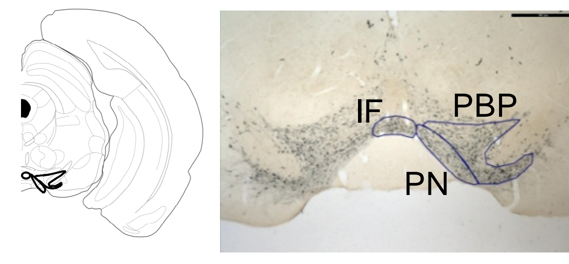
F. Rostral Ventral Tegmental Area (VTA)
-3.7 mm from bregma



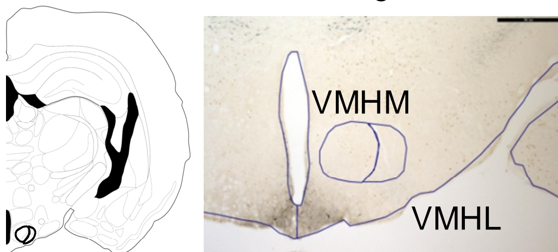
C. Medial Amygdala (Me)
-1.5 – -2.3 mm from bregma



G. Caudal Ventral Tegmental Area (VTA)
-4.0 – -4.3mm from bregma



D. Ventromedial hypothalamus (VMH)
-1.8 – -2.3 mm from bregma



H. Tail Ventral Tegmental Area (VTA)
-4.6 – -4.9 mm from bregma

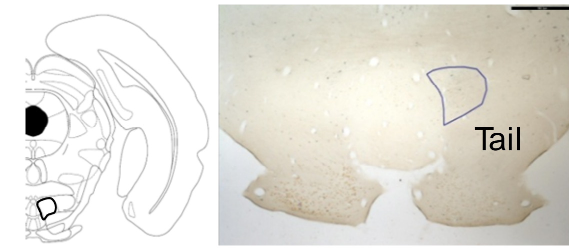


Figure 3.1. Figures and images of brain regions of interest. Representative subregion contours drawn over atlas diagrams (Morin and Wood, 2001) and low-magnification photomicrographs of immunohistochemically-treated tissue sections from VS-exposed

adult animals; scale bar is 500μm. For color images, the reader is referred to the electronic version of this dissertation.

Cells counts were made within a contour by a single experimenter blind to hamster treatment with an UPlanSApo 40x (0.9NA) objective on double-labeled immunohistochemistry tissue. Cells were considered Fos-ir if they had a distinct nucleus with visible puncta stained dark red-brown and TH- or Orx-ir if the cytoplasm was stained gray-blue. In all regions, single-labeled Fos-ir cells were counted, and measures describing Fos-ir cells do not include double-labeled cells. The number of Fos-ir cells within each subregion contour was divided by the area of that contour to create a measure of cell density within a section. These density data control for any change in subregion area with age, and generally detect similar effects of treatment as do cell count data. In the VTA, single-labeled TH-ir cells and cells double-labeled for both TH and Fos, here called TH/Fos-ir, were counted and used in the same density calculations as the Fos-ir cells. In the LH, single-labeled Orx-ir cells and cells double-labeled for both Orx and Fos, here called Orx/Fos-ir, were counted within an area defined by the presence of the Orx-ir cells. Therefore number, not density, of Orx-ir and Orx/Fos-ir cells per section are reported here. Measurements from each tissue section were averaged across sections to create one measurement per subregion per hamster. All analyses were performed on an Olympus BX51 microscope under brightfield illumination using Neurolucida (version 7; Microbrightfield, Williston, VT).

Statistical Analysis. With data from so many subregions within each hamster, one goal of our statistical approach was to simplify the data and present it at a circuit level by identifying clusters of regions that showed similar patterns of Fos expression

across animals. To do so, we used a combination of factor analysis and descriptive correlational analyses to inform our expectations given previous functional and anatomical findings. Factor analysis, with principle axis factoring and a promax rotation, identified two clusters of subregions. Cluster 1 included Cg1, PrL, IL, AcbC, AcbSh, MePD, MePV, IF, PN, PBP, and Tail, and we refer to regions in this cluster as mesocorticolimbic. Cluster 2 included DM/PeF, LH, VMHM, and VMHL, and we refer to regions in this cluster as hypothalamic.

Given the potential problems of using factor analysis with a small sample, we computed the correlations among the regions within each cluster as well as between the two clusters. The average within-cluster correlation was 0.34 in the MCL cluster and 0.42 in the hypothalamic cluster, based on 55 and 6 correlations, respectively. These indicate that Fos expression in subregions within the same cluster were consistently correlated with one another. We also examined correlations between regions falling into the two different clusters, and here the average of the 44 between-cluster correlations was 0.05 supporting the idea that Fos responses in these two clusters are relatively independent.

Fos-ir cell density was next analyzed with multilevel modeling treating animal as the upper-level sampling unit and brain region as the lower-level sampling unit. In this analysis, the cluster the region belonged to (mesocorticolimbic vs. hypothalamic) was treated as a within-subject variable, and age (juvenile vs. adult) and swab (blank vs. VS) were treated as between-subject independent variables. Multilevel modeling provides a more powerful analysis than a traditional repeated measures analysis of variance (ANOVA) because it allows for analysis even if data from all subregions were

unavailable for each hamster (as was the case in two juvenile and one adult hamsters due to poor quality tissue sections). The error structure was modeled to impose the traditional homoscedasticity assumption used in ANOVA. Our hypotheses predicted that swab (blank vs. VS) will differentially affect Fos expression in adults and juvenile animals in some subregions. Therefore we also tested a set of planned comparisons within each subregion, comparing swab vs. blank groups within juvenile and adult animals separately.

A different statistical approach was required when analyzing densities of TH-ir and TH/Fos-ir cells and numbers of Orx-ir and Orx/Fos-ir cells.because 1) they were only quantified in one region per animal and 2) we were interested in both age and swab effects. Therefore, two way ANOVAs were used to analyze the effects of age (juvenile vs. adult) and swab (blank vs. VS) on these variables.

Plasma testosterone measures. Duplicate 50 μl samples of plasma testosterone were analyzed within a single assay using the Coat-A-Count Total T Kit (Diagnostic Products, Los Angeles, CA). The minimum detectable concentration was 0.1ng/ml. The intra-assay coefficient of variation was 6.4% and 6.7% for Experiments 1 and 2, respectively. Two way ANOVA (age x swab) was used to analyze plasma testosterone concentrations between groups.

Results

Experiment 1: CPP for potential rewards

Expt 1a. CPP for VS. Adult hamsters showed a CPP for VS (Figure 3.2a). Paired *t*-tests showed that the preference score increased significantly for the adult VS group ($t_{(10)} = 2.74$, $p < 0.05$) but not the control group, which did not receive pairings of VS during conditioning trials ($t_{(9)} = .78$, *NS*). Likewise, the difference score decreased significantly for the adult VS group ($t_{(10)} = 3.74$, $p < 0.01$), but not controls ($t_{(9)} = .46$, *NS*). On the other hand, juvenile hamsters did not show a CPP for VS (Figure 3.2b). In juveniles, paired *t*-tests showed that neither the preference scores for the VS group ($t_{(8)} = -1.08$, *NS*) and control group ($t_{(8)} = 0.19$, *NS*), nor the differences score for the VS group ($t_{(8)} = 1.82$, *NS*) and control group ($t_{(8)} = -0.53$, *NS*) changed between pretest and test.

Expt 1b. CPP for palatable food. Juvenile hamsters showed a CPP for palatable food (Figure 3.2c). Paired *t*-tests showed that the preference score increased significantly for the palatable food group ($t_{(10)} = -2.275$, $p < 0.05$) but not the controls ($t_{(9)} = -1.39$, *NS*), and the difference score decreased significantly for the palatable food group ($t_{(10)} = 3.145$, $p < 0.01$) but not the controls ($t_{(9)} = 1.99$, *NS*). Exposure to palatable food did not affect chow intake or body weight (data not shown).

Expt. 1c. CPP for cocaine. Juvenile hamsters showed a CPP for cocaine (Figure 3.2d). Paired *t*-tests showed that the preference score increased significantly for the cocaine group ($t_{(7)} = -3.38$, $p < 0.01$) but not the control group ($t_{(7)} = -1.67$, *NS*), just as the difference score decreased significantly for the cocaine group ($t_{(7)} = 3.48$, $p < 0.01$) but not the control group ($t_{(7)} = 1.60$, *NS*).

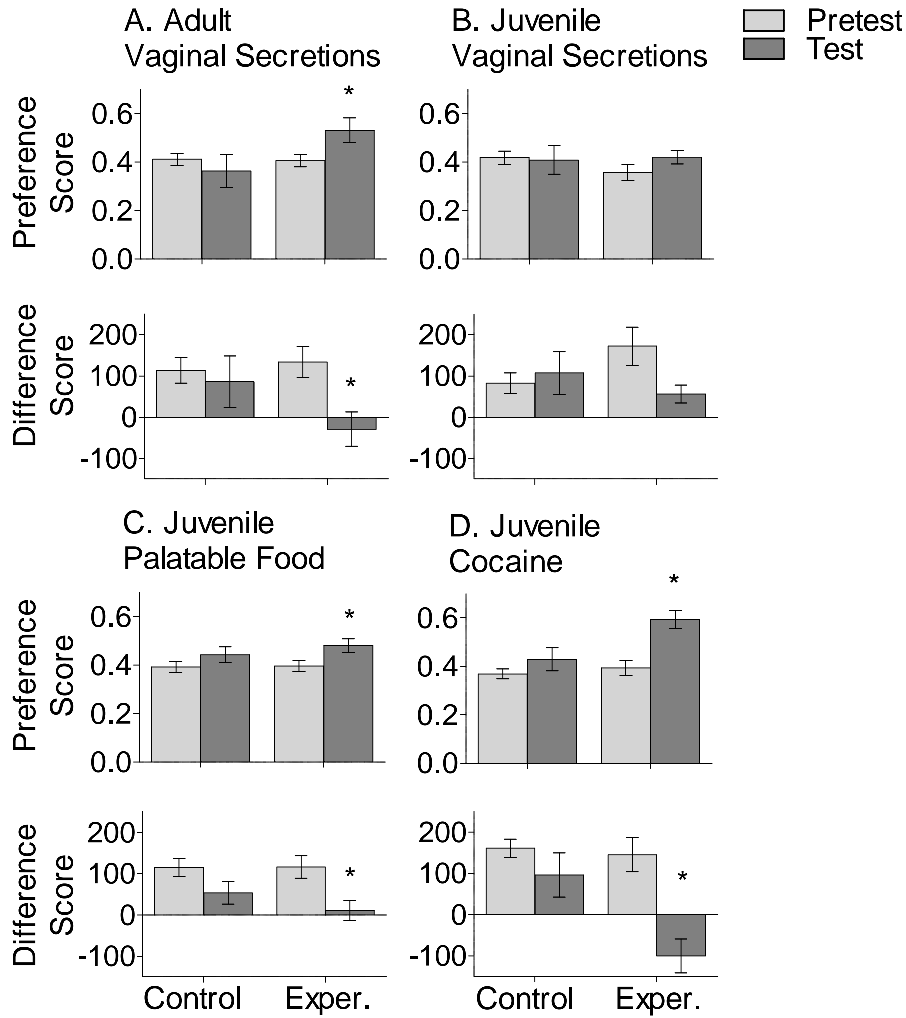


Figure 3.2. Preference and difference scores for control and experimental groups when tested for a CPP for VS (A and B), palatable food (C), and cocaine (D). Adult males showed a CPP for VS, whereas juvenile males did not; however, juvenile males did show a CPP for palatable food and cocaine. * indicates a significant difference between pretest and test within a group, $p < 0.05$ with paired t-test.

Experiment 2: Neural Responses to VS

Fos-ir. Multilevel modeling revealed a main effect of cluster ($F_{(1,429)} = 13.86$, $p < 0.01$), but no main effect of age or swab on Fos-ir cell density (Figure 3.3). This main effect of cluster was qualified by an interaction between cluster and swab ($F_{(1,429)} = 10.53$, $p < 0.01$), such that the effect of swab varied depending on the cluster (Figure 3.3). Follow-up multilevel modeling, analyzing Cluster 1 and 2 separately, indicated an increase in Fos-ir cell density in response to VS in the mesocorticolimbic cluster ($F_{(1,30)} = 20.366$, $p < 0.01$), but no effect of swab in the hypothalamic cluster ($F_{(1,28)} = 2.41$, *NS*).

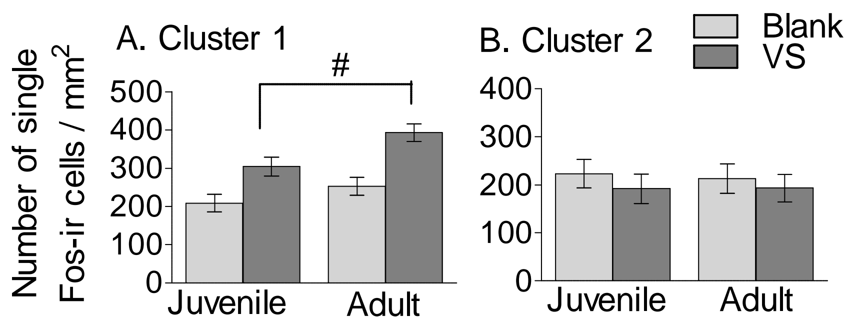


Figure 3.3. Fos-ir cell density in mesocorticolimbic (A) and hypothalamic (B) clusters. There was a greater density of Fos-ir cell in VS exposed hamsters compared to blank hamsters in the mesocorticolimbic but not hypothalamic clusters. # indicates a main effect of swab, $p < 0.01$ with multilevel modeling analysis.

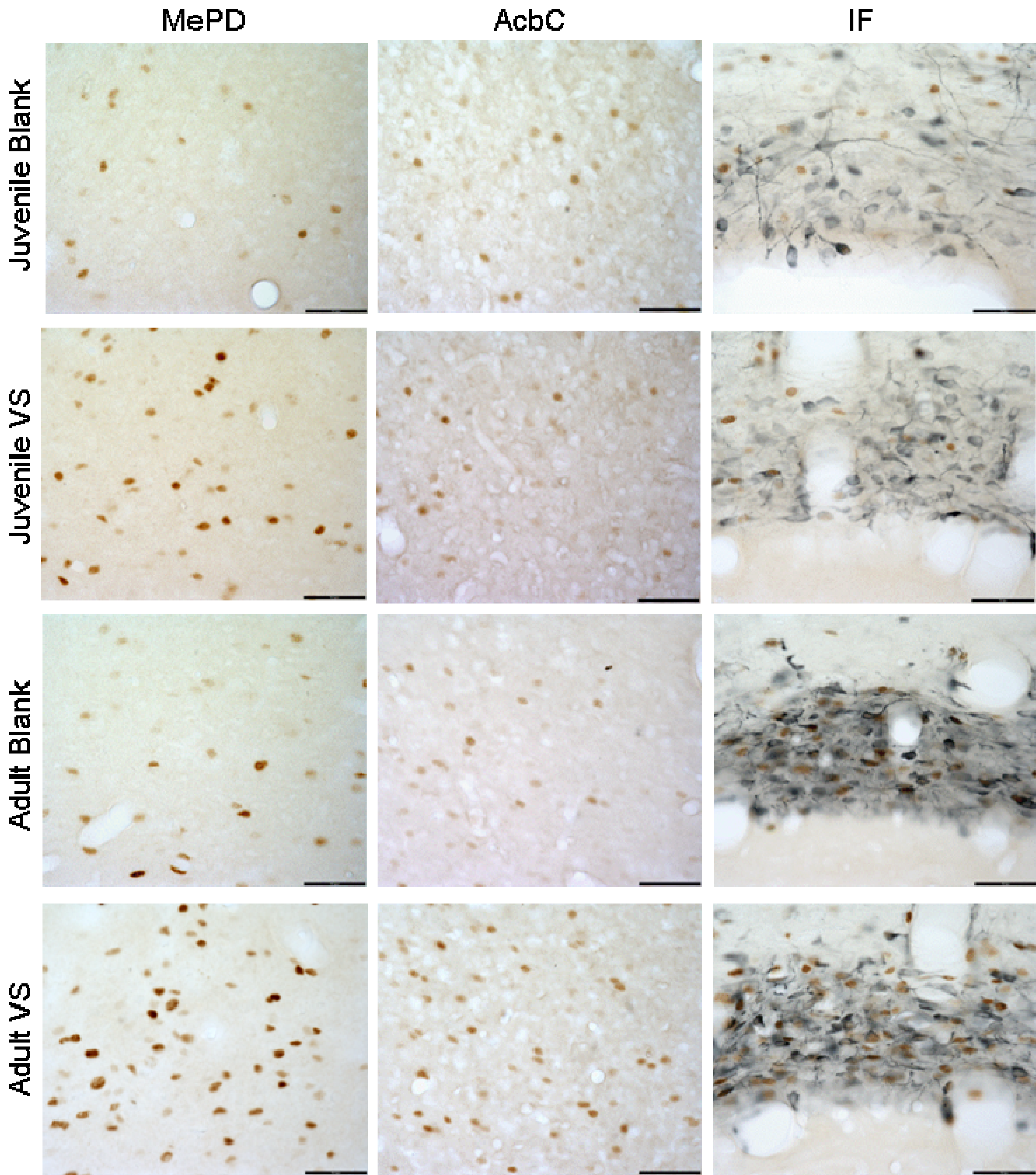


Figure 3.4. Images of Fos/TH immunohistochemistry. High magnification photomicrographs of representative Fos- (brown nuclei) and TH- (blue cytoplasm) immunoreactive tissue sections from juvenile and adult animals exposed either blank or VS swabs in the AcbC, MePD, and IF. Scale bar is 50µm.

Planned contrasts were performed to analyze differences in Fos-ir cell density between blank and VS-exposed animals within an age for each ROI, because the *a priori* hypotheses predicted that adult and juvenile hamsters would show different responses to VS. Within the mesocorticolimbic cluster, in both juvenile and adult hamsters, VS elicited an increase in Fos-ir cell density in the MePD ($t_{(26)}$ =5.33, $p < 0.01$ and $t_{(26)} = 6.61$, $p < 0.01$, respectively; Figure 3.4 and 3.5C), MePV ($t_{(26)}$ =5.49, $p < 0.01$ and $t_{(26)} = 5.06$, $p < 0.01$, respectively; Figure 3.5C), and PN ($t_{(28)}$ =2.16, $p < 0.05$ and $t_{(28)} = 2.490$, $p < 0.05$, respectively; Figure 3.5D). In contrast, the Fos response to VS in other mesocorticolimbic cluster subregions between adult and juvenile responses diverged. Adult, but not juvenile, hamsters showed greater Fos-ir cell density when exposed to VS compared to blank swabs in the IL ($t_{(26)} = 2.26$, $p = 0.03$ and $t_{(26)} = 1.35$, *NS*, respectively, Figure 3.5A), AcbC ($t_{(26)} = 2.33$, $p = 0.03$ and $t_{(26)} = 0.78$, *NS*, respectively, Figure 3.4 and 3.5B), IF ($t_{(28)} = 4.61$, $p < 0.01$ and $t_{(28)} = 1.746$, *NS*, respectively, Figure 3.4 and 3.5D), and PBP ($t_{(28)} = 3.56$, $p < 0.01$ and $t_{(28)} = 1.53$, *NS*, respectively, Figure 3.5D). VS did not elicit a Fos response in either juvenile or adult hamsters in the remaining mesocorticolimbic cluster subregions, which included Cg1, PrL, AcbSh, and VTA Tail (Figure 3.5). VS did not evoke a Fos response in any hypothalamic cluster subregions in either age group, as indicated by similar Fos-ir cell densities in the blank- and VS-exposed groups in both ages (Figure 3.6).

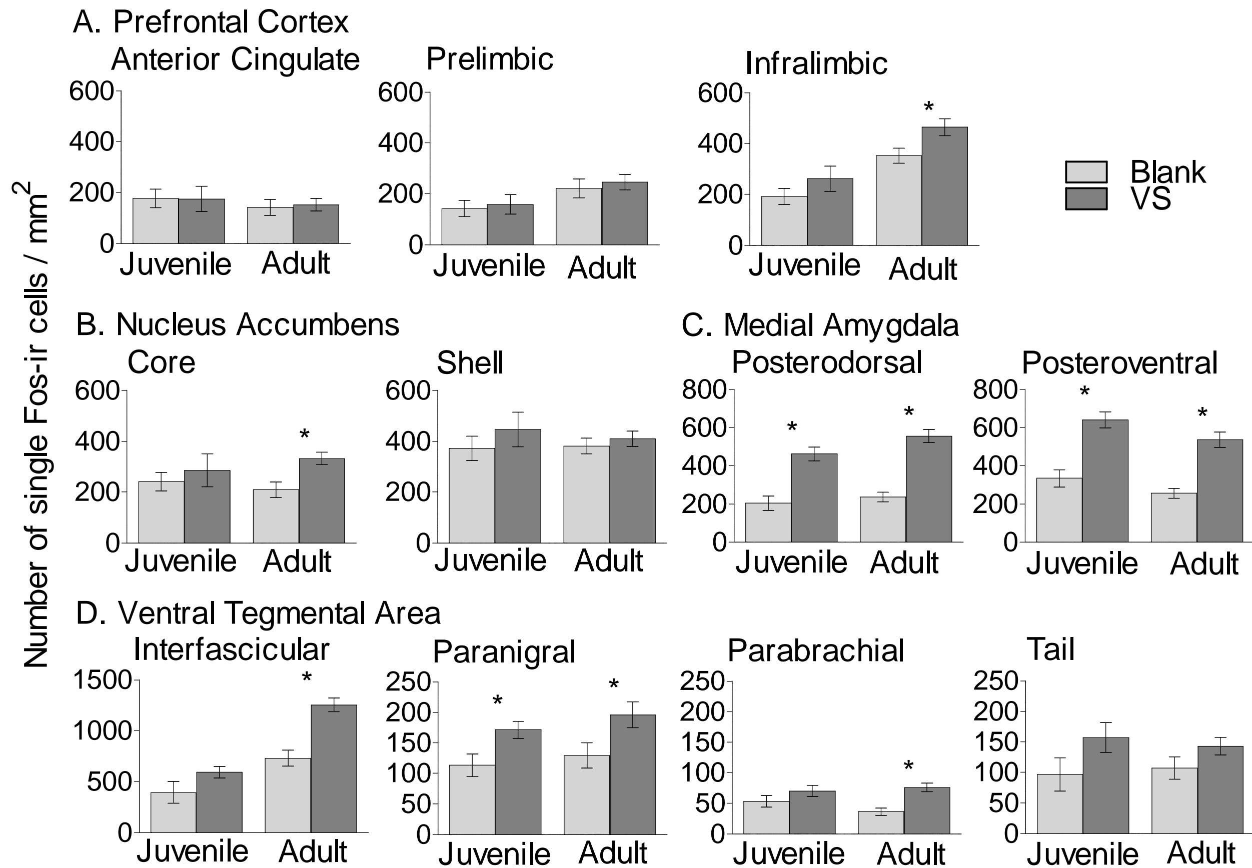


Figure 3.5. Single-labeled Fos-ir cell density in mesocorticolimbic cluster regions, PFC (A), Acb (B), MeP(C), and VTA (D). There was a greater density of Fos-ir cells in animals exposed to VS swabs compared to those exposed to blank swabs in the MeP and PN in both juveniles and adults, and in the AcbC, IL, and IF in only adults.
* indicates difference between Blank and VS swab with planned contrasts within an age group, $p < 0.05$. Note differences in y-axis in D.

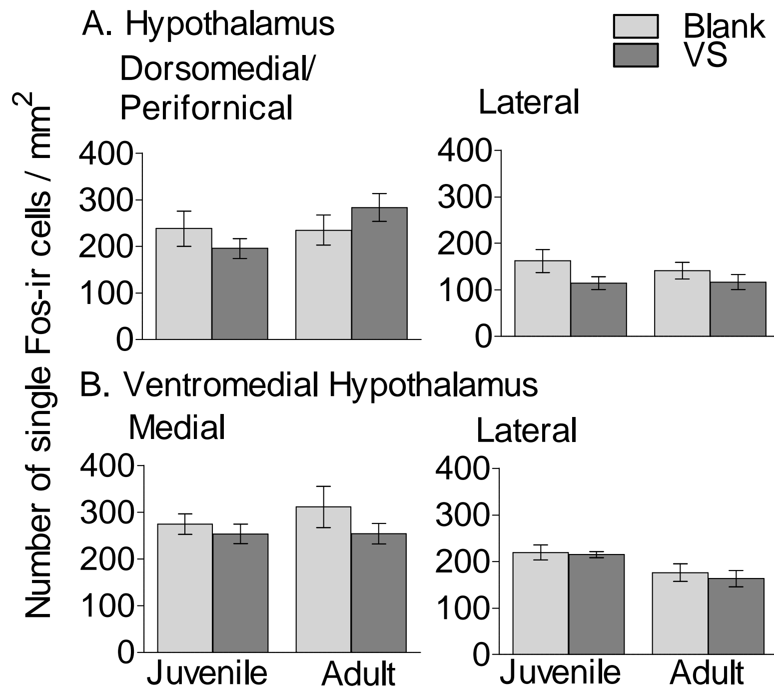


Figure 3.6. Fos-ir cell density in hypothalamic cluster regions, DM/PeF and LH (A) and VMH (B). No significant differences between blank- and VS-exposed groups were observed in either age with planned contrasts within an age.

TH-ir and TH/Fos-ir cells. The densities of TH-ir and TH/Fos-ir cells were calculated for VTA and analyzed by two-way ANOVA. In IF, a main effect of age was observed on the density of TH/Fos-ir cells ($F_{(1,28)}$ =88.246, $p < 0.01$, Figure 3.7a). Specifically, adults showed greater density of double-labeled cells independent of swab exposure. No effect of age was observed on density of TH-ir cells, and no significant effects of swab or age x swab interaction was observed on TH-related measures in IF.

In PN and PBP, a main effect of swab was observed on the density of TH/Fos-ir cells ($F_{(1,28)}$ = 12.51, $p < 0.01$, Figure 3.7b and $F_{(1,28)}$ = 23.63, $p < 0.01$, Figure 3.7c, respectively), such that hamsters exposed to VS expressed a greater density of double-

labeled cells compared to those exposed to a blank swab. No effect of swab was observed on density of TH-ir cells, and no effect of age or age x swab interaction was observed on any TH-related measure in PN or PBP.

In Tail, a main effect of age was observed on TH-ir cell density ($F_{(1,28)} = 4.524$, $p < 0.05$), such that juvenile hamsters expressed a greater TH-ir cell density than adults, regardless of swab condition (Figure 3.7d). No effect of age was observed on density of TH/Fos-ir cells, and no effect of swab or age x swab interaction was observed on any TH-related measure in Tail.

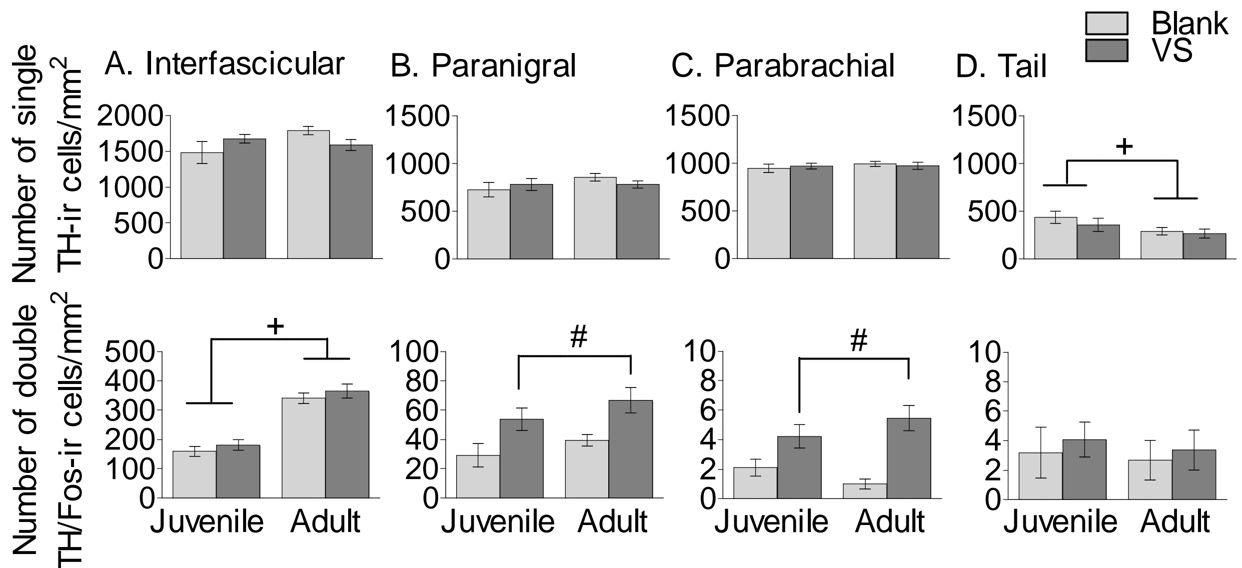


Figure 3.7. TH-ir measures in IF (A), PN (B), PBP (C) and Tail (D) VTA. First row shows number of single-labeled TH-ir cells and second row shows number of double-labeled TH/Fos-ir cells. In IF and Tail, adults and juveniles differ in immunoreactivity, independent of swab exposure; in PN and PBP, both adults and juveniles show greater immunoreactivity when exposed to a VS swab compared to a blank swab. + indicates main effect of age, # indicates main effect of swab, $p < 0.05$ with a two-way ANOVA. Note different y axis scales in throughout.

Orx-ir and Orx/Fos-ir cells. The number of Orx-ir cells and Orx/Fos-ir cells were determined in the LH and analyzed by two-way ANOVA (Figure 3.8). A main effect of age was observed on number of Orx-ir cells in both LHM ($F_{(1,25)} = 35.80$, $p < 0.01$) and LHL ($F_{(1,25)}=17.79$, $p < 0.01$), such that juvenile hamsters expressed a greater number of Orx-ir cells than adults, independent of swab condition. There was also a main effect of age on the number ($F_{(1,25)} = 7.12$, $p = 0.01$) of Orx/Fos-ir cells in LHM, such that adults expressed greater levels than juvenile hamsters on both measures. There was no effect of age on number of Orx/Fos-ir cells in the LHL, nor was there an effect of swab or age x swab interaction on any measure in LHM and LHL.

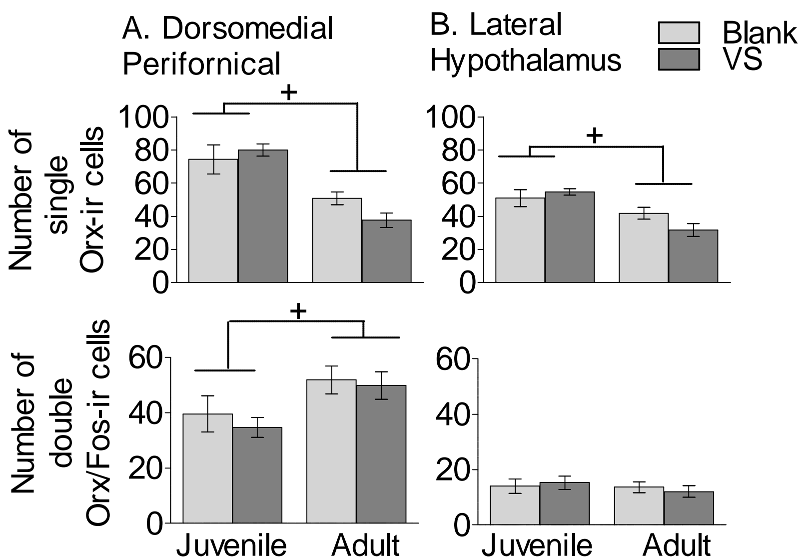


Figure 3.8. Orexin measures in the DM/PeF (A) and LH (B). First row shows number of single-labeled orexin cells and second row shows number of double-labeled Orx/Fos-ir cells. Adults and juveniles show differences in immunoreactivity, independent of swab exposure. + indicates an effect of age, $p < 0.01$ with a two-way ANOVA.

Plasma testosterone concentration. Plasma testosterone measures revealed a main effect of age in both Expt 1a ($F_{(1,35)} = 30.164$, $p < 0.01$) and Expt 2 ($F_{(1,26)} = 40.52$, $p < 0.01$), such that adult hamsters had greater testosterone concentrations than juvenile hamsters (Figure 3.9). In addition in Exp 2, a main effect of swab was observed ($F_{(1,26)} = 5.16$, $p = 0.03$), in which hamsters exposed to VS had greater testosterone concentrations than those exposed to blank swabs. This main effect appears to be driven solely by an increase in testosterone in VS-exposed adults, although no statistically significant age x swab interaction was detected.

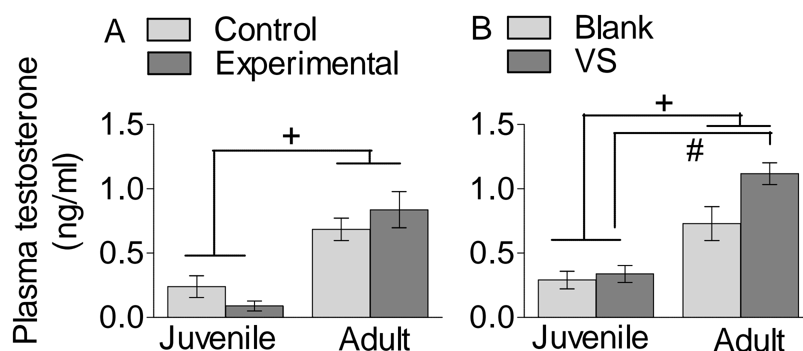


Figure 3.9. Plasma testosterone concentrations from animals in Exp 1a (CPP for VS, A) and Exp 2 (neural responses to VS, B). Adults have higher concentrations of testosterone than do juveniles in both experiments, and particularly when exposed to VS in Exp 2. + indicates a main effect of age, $p < 0.01$. # indicates a main effect of swab, $p < 0.05$; with a two-way ANOVA.

Discussion

This report is the first demonstration that adolescent maturation of social information processing includes a transformation of a species-specific, socially-relevant sensory signal from a neutral stimulus to an unconditioned reward in the absence of social experience. This perceptual shift is accompanied by a gain in the ability of the social stimulus to activate midbrain, accumbens, and prefrontal components of the mesocorticolimbic reward pathway, indicating that these particular regions are recruited to mediate the adolescent gain in the perception of VS as rewarding (Figure 3.10).

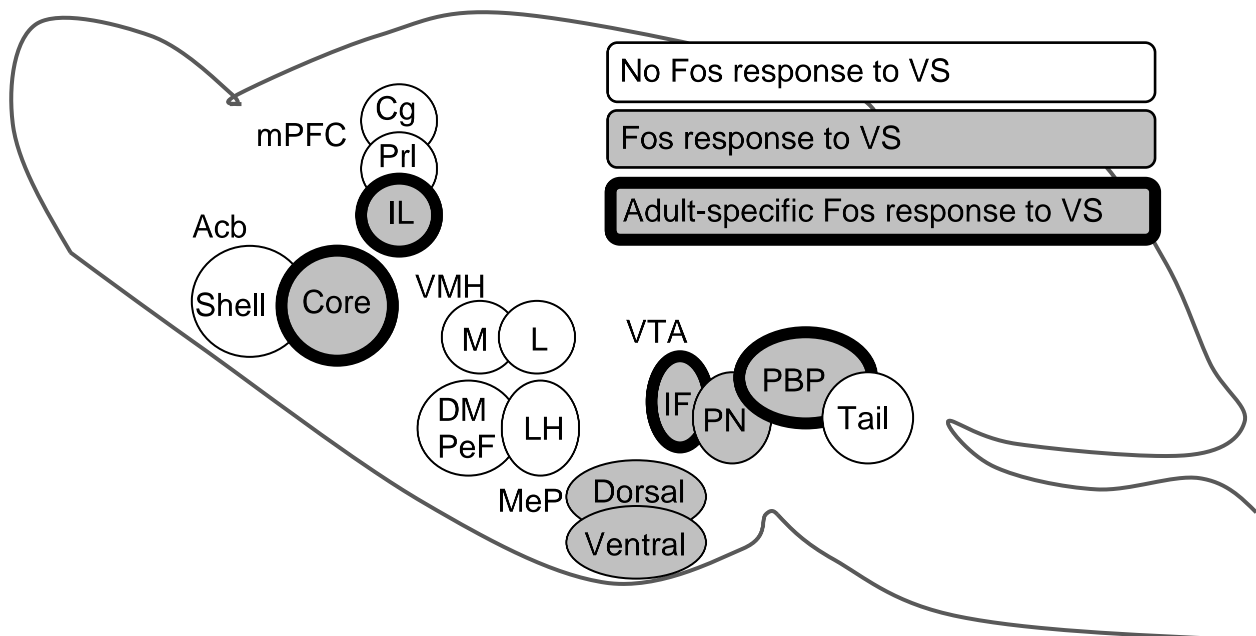


Figure 3.10. Schematic diagram of adolescent changes in VS-induced Fos responses (indicated by shading). VS activated the posterior medial amygdala and paranigral VTA neurons similarly in juvenile and adult male hamsters, but activated non-dopaminergic interfascicular and parabrachial VTA neurons, nucleus accumbens core, and infralimbic medial prefrontal cortex only in adults (bold outline). This pattern of selective neural activation in adulthood correlates with the ability of VS to serve as an unconditioned

stimulus for reward in adult, but not juvenile males, indicating recruitment of these specific cell groups in the adolescent development of social reward.

Adolescent gain in the positive valence of VS

Juvenile male hamsters failed to show a CPP for VS. However, they did show a CPP to both palatable food and cocaine, demonstrating a pre-adolescent ability to show place preferences for both natural and pharmacological rewards, and consistent with previous reports that peri-adolescent rodents form a CPP to these types of stimuli (Tzschentke, 2007). Adult males did form a CPP for VS, leading to the conclusion that adult, but not prepubertal, male hamsters perceive VS as rewarding. These results provide strong evidence that in the absence of sexual experience, a species-specific social stimulus that is a relatively weak reward or neutral in valence to juveniles becomes a potent unconditioned reward as a consequence of adolescent maturation. This report extends earlier studies on the development of hamsters' attraction to VS, where adults, but not juveniles, spend significantly more time investigating VS than control stimuli. Preferences for VS are present only after males reach 40 days of age, by which time circulating levels of testosterone are elevated as a result of puberty onset (Johnston and Coplin, 1979). Whether or not elevated testosterone levels influence the perception of VS as rewarding is an open and testable question. In either case, the transformation of VS as a rewarding social stimulus during adolescence is likely critical for successful social interactions in adulthood.

Adolescent maturation of VS-induced neural activation patterns

The adolescent gain of rewarding properties of VS was correlated with different patterns of VS-induced neural activation within the mesocorticolimbic reward circuitry in adults and juveniles. Factor analysis of Fos expression in the fifteen brain areas analyzed in this study identified two functionally related clusters of cell groups. One cluster included the MeP and members of a complex network of limbic, tegmental, and cortical projections that coordinate reward, incentive motivation and adaptive behavior (reviewed in (Berridge and Robinson, 1998, Ikemoto and Panksepp, 1999, Wise, 2004). This cluster was characterized by neural responsiveness to VS. The second cluster included the hypothalamic subregions, and was characterized by an absence of responsiveness to VS. Thus, developmental dynamics within the mesocorticolimbic cluster appear to underlie the developmental gain in positive valence of VS.

The mesocorticolimbic reward system includes extensive dopaminergic and non-dopaminergic projections from the VTA to the Acb, mPFC, and MeP, all of which are complexly and reciprocally connected via recurrent circuits (Swanson, 1982a, Oades and Halliday, 1987, Thompson and Swanson, 2010). In rodents, the flow of social chemosensory information to this circuit begins with direct projections from the main and accessory olfactory bulbs to the MeP, which integrates sensory information with the internal hormonal milieu for initial evaluation of the social stimulus (Wood and Newman, 1995a). This first pass evaluation can then be relayed either directly or via preoptic and hypothalamic cell groups to the VTA (Phillipson, 1979, Kevetter and Winans, 1981a, Coolen and Wood, 1998, Geisler and Zahm, 2005). Placing our data within the framework of this circuitry, we propose that VS acquires positive valence through

experience-independent alterations in reward circuit responses to initial evaluation of a social stimulus by the amygdala. We base this hypothesis first on the observation that early stage evaluation of VS by the MeP appears to be in place in juveniles and similar to that of adults, because VS elicited similar Fos responses in the amygdala and one of the downstream areas, the VTA PN. Subsequently, over the course of adolescence and in the absence of social experience, VS stimulation comes to engage the IF and PBP nuclei in the VTA, IL of the mPFC, and core of the Acb. This observation suggests that the responses of IF, PBB, IL, and AcbC to evaluative transmissions from the amygdala are altered by developmentally programmed maturational changes, thus associating these cell groups with a positive valence of VS in adulthood.

The neurobiological mechanisms that produce the adult-typical behavioral and neural responses to VS are unknown, but our results offer some testable hypotheses. First, it is important to note that within VTA, the populations of cells that were activated by VS only in adulthood were not dopaminergic, and therefore are likely GABAergic projection- or inter-neurons (Van Bockstaele and Pickel, 1995, Carr and Sesack, 2000a, Olson and Nestler, 2007, Ferreira *et al.*, 2008). In fact, dopaminergic (TH-ir) cells in PN and PBP were activated by VS in both juveniles and adults, indicating that these cell groups are involved in social information processing of VS, but in and of themselves do not encode whether VS is rewarding or not. However, dopamine release in Acb is associated with sexual behavior and reward (Wenkstern *et al.*, 1993, Meisel *et al.*, 1996, Becker *et al.*, 2001b, Robinson *et al.*, 2001). Thus, if VS-induced Fos expression in TH-ir VTA neurons is a proxy for dopamine release in corticolimbic regions, then our results suggest that non-dopaminergic VTA projection neurons, activated by VS only in

adulthood, may modulate Acb and/or mPFC responses to dopamine release differently in juveniles and adults On the other hand, Fos expression in TH-ir neurons may not reflect a change in dopamine synthesis or release, in which case differential modulation of VTA dopamine neurons by VTA interneurons may underlie the ability of adults, but not juveniles, to form a CPP to VS.

The above hypotheses do not preclude the possibility that age-related changes at the accumbens and mPFC projection sites of these dopaminergic neurons may also contribute to the observed pattern of Fos expression. Indeed, a number of adolescent changes in dopaminergic circuitry have been documented, including increased dopamine innervation of the PFC, dopamine synaptic dynamics in the Acb, dopamine receptor expression in the Acb and PFC, and dopamine modulation of PFC GABAergic interneurons (reviewed in (Doremus-Fitzwater *et al.*, 2010, Wahlstrom *et al.*, 2010b).   It is also important to note that this corticolimbic restructuring occurs concomitantly with hormonal changes during puberty, and further study is needed to determine the role of gonadal hormones in adolescent maturation of social information processing (Kuhn *et al.*, 2010).

The apparent lack of involvement of the hypothalamic cluster cell groups in mediating adolescent change in social information processing is surprising, given their roles in expression of social behaviors and reward. The VMH is involved in sexual behavior (Harding and McGinnis, 2005) and shows increased Fos expression in response to estrous odors in adult male rats (Kippin *et al.*, 2003). However, the rats in this study were sexually experienced, whereas hamster in the current study were sexually naïve, suggesting that a VMH response to estrous odors may be conditioned

as a result of previous experience. Hypothalamic orexin is involved in expression of sexual behavior and reward (Muschamp *et al.*, 2007, Di Sebastiano *et al.*, 2011) but the finding that orexinergic neurons were not responsive to VS suggests that the rewarding value of VS is somehow distinct from general sexual reward.

Adolescent maturation of mesolimbic dopamine and orexin

The number of single-labeled Tail VTA TH-ir and Orx-ir neurons was greater in juveniles than in adults. These results are somewhat difficult to interpret because a reduction in cytoplasmic immunoreactivity could be indicative of either reduced neuropeptide expression or reduced cytoplasmic levels of neuropeptide secondary to enhanced neuropeptide release. The current study also found that, compared with juveniles and independently of VS exposure, adults had greater numbers of Fos-expressing TH-ir and Orx-ir cells in Tail VTA and DM/PeF, respectively. These results may be indicative of heightened vigilance or sensitivity to non-specific stimuli in adulthood (e.g., a clean cotton swab in this study), as both dopamine and DM/PeF orexin have been implicated in general arousal as reviewed in (Harris and Aston-Jones, 2006, Ikemoto, 2007, Boutrel *et al.*, 2010).

Conclusion

Previous studies have documented adolescent changes in the rewarding properties of drugs of abuse in animals, but less attention has been paid to natural or social rewards (Doremus-Fitzwater *et al.*, 2010). The present study demonstrates an experience-independent gain in the unconditioned rewarding value of a social stimulus

over the course of adolescent development, and provides a neuroanatomical basis for the hypothesis that maturational changes within the mesocorticolimbic system mediate this shift in behavioral responses to VS. Because maturation of social information processing is a decisive component of mammalian adolescence, and perturbations in this aspect of development are associated with maladaptive behaviors and certain mental illnesses associated with adolescence, further research into the mechanisms by which social stimuli gain rewarding properties during this critical period of development is warranted.

Chapter 4: Adolescent maturation of mesocorticolimbic responses to a rewarding social cue is gonadal hormone independent

Introduction

The perception of and responses to social cues mature during puberty and adolescence. In particular, interactions with peers become more salient and gain sexual connotations (Spear, 2000, Forbes and Dahl, 2010). Thus, the sources of social reward evolve during the juvenile-to-adult transition, but little is known about the underlying neurobiological mechanisms of this vital aspect of adolescence. To address this question, we have studied developmental changes in endocrine, neural, and behavioral responses of sexually naïve male Syrian hamsters to female hamster vaginal secretions (VS) (Chapter 3 (Romeo *et al.*, 1998). VS signal female receptivity, and must be detected and neurally integrated with internal hormonal information by the male for a successful sexual interaction to occur (Wood and Newman, 1995b, Wood and Coolen, 1997). Importantly, adult, but not juvenile, hamsters are attracted to, and form a conditioned place preference (CPP) for, VS (Johnston and Coplin, 1979) and Chapter 3), demonstrating a shift toward rewarding evaluation of female pheromones contained in VS during reproductive maturation. Sexual reward is often associated with dopaminergic action within the mesocorticolimbic reward circuit (Meisel *et al.*, 1996, Becker *et al.*, 2001b), and this circuit is remodeled during puberty and adolescence (Doremus-Fitzwater *et al.*, 2010, Wahlstrom *et al.*, 2010a). Therefore, the present study investigated the effects of adolescent development and gonadal hormone manipulation on neural responses to VS within the reward circuit.

Immediate early gene expression, specifically Fos immunoreactivity, has been used as a proxy for neural activity to reveal neural correlates of male hamster behavioral responses to VS. Initial studies focused on hormone-sensitive brain regions previously implicated in chemosensory responses to social cues, including the bed nucleus of the stria terminalis (BNST), magnocellular region of the medial preoptic nucleus (MPNmag), and the posterior medial amygdala (MeP) (Fiber *et al.*, 1993, Kollack-Walker and Newman, 1997, Wood, 1997). Both gonad-intact adult and juvenile hamsters express more Fos in all of these regions after exposure to VS compared with unexposed age-matched controls (Romeo *et al.*, 1998). However, more recently, we have found that juvenile and adult responses to VS diverge in the mesocorticolimbic circuit (Chapter 3). In Chapter 3, VS again elicited a Fos response in the MeP of both juvenile and adult hamsters, and also in ventral tegmental area (VTA) dopamine-producing neurons. However, in non-dopamine VTA cells, nucleus accumbens (Acb) core, and the infralimbic region of the medial prefrontal cortex (mPFC), VS elicited a Fos response only in adults (Chapter 3). These data suggest that during adolescent development, VS acquires positive valence through alterations in reward circuit processing of sensory information relayed from the medial amygdala.

The increase in gonadal hormone secretion that occurs during puberty modulates behavioral responses to VS. While testosterone treatment is sufficient for juveniles to become attracted to VS, it is not sufficient for juveniles to perform adult-like levels of sexual behaviors with a receptive female (Johnston and Coplin, 1979, Meek *et al.*, 1997). Testosterone also affects a range of dopamine-modulated behaviors (Hull *et al.*, 1995, Putnam *et al.*, 2001, Kritzer *et al.*, 2007). These behavioral changes may be

mediated by gonadal hormone-induced alterations in the mesocorticolimbic system. For example, in adult rats, gonadectomy results in both short- and long-term effects on basal extracellular dopamine and glutamate-induced dopamine in the mPFC (Aubele and Kritzer, 2011b, a). On the other hand, gonadal hormone-*independent* changes in dopamine receptor expression in the rat accumbens and mPFC have been observed across adolescence (Andersen *et al.*, 2002). Therefore, adult-like Fos responses to VS may develop as the result of pubertal testicular hormonal influences or developmentally programmed hormone-independent changes within the mesocorticolimbic circuit. The goal of the present study is to determine if treating juvenile hamsters with testosterone results in an adult-like pattern of neural activation within the reward circuit in response to VS.

Methods

Animals

Sexually naïve male Syrian hamsters (*Mesocricetus auratus*) were obtained from Harlan Laboratories (Madison, WI) and singly housed in clear polycarbonate cages (30.5 x 10.2 x 20.3 cm) in temperature- and humidity-controlled vivaria with a shifted light:dark cycle (14:10, dark phase began at 2:00pm). They were provided with cotton nestlet enrichment and *ad libitum* access to food (Teklad Rodent diet 8640, Harlan, Madison, WI) and water. Juveniles arrived on postnatal day 18 (P18) or P19 and adults on P49-56. Ten-month old adult female hamsters were housed under similar conditions in separate vivaria and used as the source of VS. All experiments were conducted under <4 lux red light 1-5 hours into the dark phase. Hamsters were treated in accordance

with the National Institute of Health Guide for Care and Use of Laboratory Animals, and protocols were approved by the Michigan State University Institutional Animal Care and Use Committee.

Experimental design

The experiment used a 2 x 2 x 2 x 2 factorial design, with age (juvenile or adult), hormone status (testosterone or blank capsule treated), stimulus exposure (VS or clean oil), and brain region as independent variables. Because of the large number of animals, stimulus exposure and tissue collection were performed in cohorts on two consecutive days, with all groups represented evenly each day. Two or three days after arrival, on P21 (juveniles) and P51-58 (adults), hamsters were gonadectomized and received subcutaneous implants of two blank or testosterone-containing silastic capsules (one 7 mm and one 15 mm of testosterone, sealed on each end with 4mm of silastic adhesive; inner diameter 1.98 mm; outer diameter 3.18 mm). One week after capsule implants, on P28 (juveniles) and P58-66 (adults), half of each age/hormone group was randomly assigned to be exposed to either clean or VS-containing mineral oil. Hamsters were weighed and moved into a behavior testing room 3 hours prior to exposure.

Stimulus exposure

Thirty female hamsters were ovariectomized on P70 and used as stimulus animals in other experiments for seven months before hormone administration and collection of VS for use in the two exposure days in this study. They were injected subcutaneously with

10 µg estradiol benzoate and 500 µg progesterone in sesame oil 52 and 4 hours, respectively, prior to collection of VS. VS were collected by gentle vaginal palpation and mixed together (total of 0.5ml VS) and stored at -20℃ for two weeks prior to thawing and mixing with 1.5 ml of mineral oil one hour prior to exposure. Clean mineral oil was used as the control for VS exposure. To ensure equivalent exposure to nonvolatile components of VS across groups, approximately 20 µl of this mixture were applied with a metal spatula directly onto the nose of hamsters. To prevent control hamsters from smelling volatile components of VS, they were exposed to the clean oil and then removed from the room for tissue collection prior to exposing the VS group to the VS-oil. Clean- and VS-oil were delivered 1-2 and 3-4 hours after lights off, respectively.

Tissue collection and analysis

Hamsters were euthanized with an overdose of sodium pentobarbital (150 mg/kg, intraperitoneal) sixty minutes after stimulus exposure to allow for peak Fos expression (Sheng and Greenberg, 1990, Hughes and Dragunow, 1995). Flank gland lengths and seminal vesicle weights were recorded as indicators of peripheral testosterone effects (Vandenbergh, 1973). A terminal blood sample was collected via cardiac puncture, held on ice in a heparinized tube for less than four hours, and centrifuged to isolate plasma, which was later used for radioimmunoassay of circulating plasma testosterone. Hamsters were perfused transcardially with heparinized and buffered saline rinse followed by 4% paraformaldehyde. Brains were post-fixed in 4% paraformaldehyde for 12 hours and stored in 20% sucrose/phosphate buffered saline solution until sectioning.

Histological procedures. Brains were sectioned with a cryostat into 4 series of 40 µm thick sections and stored in cryoprotectant at -20℃ until histological processing. One series of sections was used to double-label Fos and tyrosine hydroxylase (TH) immunoreactivity by free-floating immunohistochemistry. TH is the rate-limiting enzyme for catecholamine production; dopamine-β-hydroxylase, the enzyme that converts dopamine to norepinephrine, is absent in the VTA in hamsters (Vincent, 1988), thus TH immunoreactivity in the VTA was used here to identify dopaminergic cells.

Immunohistochemistry was performed as in Chapter 3. Briefly, all work occurred at room temperature unless otherwise noted, rinses in Trizma buffered saline (TBS, 0.05M, pH = 7.6) occurred initially and between steps, and all antibodies were diluted in 2% normal goat serum (Pel-Freez Biologicals, Rogers, AR) and 0.3% Triton-X TBS. To visualize Fos and TH, residual aldehydes were removed and endogenous peroxidase activity was quenched before tissue was blocked and made permeable with 20% goat serum and 0.3% Triton-X TBS. Tissue was then incubated in the cFos primary antibody (c-Fos (4): rabbit, sc-52. 1:10,000, 0.02µg IgG/ml solution, Santa Cruz Biotech, Santa Cruz, CA) for 48 hours at 4℃, cFos secondary antibody (Biotinylated goat anti-rabbit IgG (H+L), 1:500, 3µg IgG/ml solution, Vector Laboratories, Burlingame, CA) for one hour, and avidin-biotin complex (Peroxidase- Vectastain ABC Kit PK-6100, Vector) for one hour, consecutively. Then, tissue was reacted with 3,3'-Diaminobenzidine tetrahydrochloride (Sigma-Aldrich, St. Louis, MO) to produce a dark brown reaction product in the nucleus of Fos-immunoreactive (ir) cells. After rinsing, tissue was again blocked and made permeable and then incubated overnight in TH primary antibody (Mouse anti-TH monoclonal antibody, 1:2,000, Millipore-Chemicon, Billerica, MA). TH

secondary antibody (Biotinylated goat anti-mouse IgG (H+L), 1:500, 3µg IgG/ml solution, Vector) and avidin-biotin complex (same as above) were then each applied consecutively for 1 hour, and sections were reacted with SG enzyme substrate (Kit SK-4700, Vector) to produce a cytoplasmic blue reaction product in TH-ir cells. Primary and secondary antibody deletion control studies were run on separate sections; non-specific background staining was low or absent in these sections. Tissue sections were mounted onto glass slides and dehydrated with a series of ethanols before coverslipping.

Microscopic analysis. Acb, mPFC and VTA were subdivided according to the hamster brain atlas (Morin and Wood, 2001), as indicated by previous research demonstrating distinct functional and anatomical characteristics of the subregions (Groenewegen *et al.*, 1999, Bradley and Meisel, 2001, Heidbreder and Groenewegen, 2003, Balfour *et al.*, 2006, Ikemoto, 2007). The mPFC included the anterior cingulate (Cg1), prelimbic (PrL), and infralimbic (IL) subregions; the Acb included the core (AcbC) and medial portion of the shell (AcbSh); and the VTA included interfasicular (IF), paranigral (PN), and parabrachial pigmented (PBP) nuclei (Figure 4.1). The VTA was further subdivided along its rostro-caudal extent by the presence of the interpeduncular nucleus in caudal sections because of previous reports of rostral/caudal functional differences in rats, mice, and hamsters (Olson *et al.*, 2005, Ikemoto, 2007). Upon completion of microscopic inspection and analysis, virtually all main effects and interactions were observed in the caudal portion of each VTA subregion, and therefore only data from caudal sections are presented here.

Anatomically matched tissue sections throughout the extent of the Acb (3 sections), mPFC (3 sections), caudal VTA (cVTA; 2 sections), and posterodorsal medial amygdala (MePd, 1 section, as a control region) were selected at 4x objective and indicated in Figure 4.1. In all regions, $0.064mm^2$ rectangular contours were placed bilaterally and consistently according to neuroanatomical landmarks visible in immunohistochemistry treated tissue. Because of the large size of Acb and MePd regions, two boxes were placed in each of those subregions and counts from them were added together within a hemisection.

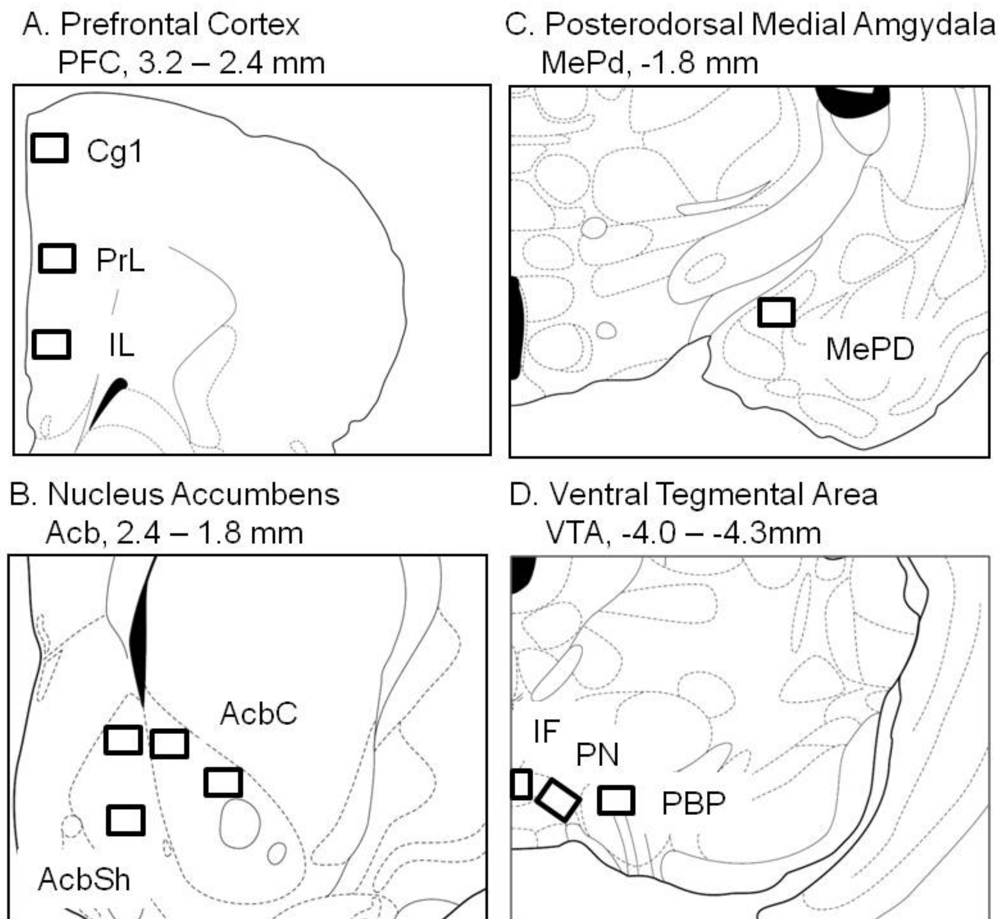


Figure 4.1. Representative subregion contours placed over atlas diagrams (Morin and Wood, 2001).

Images were taken within the contour with an UPlanSApo 40x (0.9NA) objective. In the MePd, Acb, and mPFC, only single-labeled Fos-ir cells were observed, and ImageJ (NIH Bethesda, MD) was used to quantify Fos-ir cells. Color images were converted to grayscale, and the image threshold was adjusted to be three standard deviations above and below the minimum and maximum gray values for each image, which consistently detected Fos-ir cells. Particle size was defined as above 200 and below 2000 pixels^2, excluding ones on the edge of the image. All particles above threshold were visually confirmed by an experimenter familiar with Fos-ir staining but blind to group assignment. In the VTA, both Fos- and TH-ir cells were present, which precluded ImageJ analysis. Therefore, cells were counted manually using Neurolucida (version 9; Microbrightfield, Williston, VT). Cells were considered Fos-ir if they had a distinct nucleus with visible puncta stained dark red-brown and TH-ir if the cytoplasm was stained gray-blue. All analyses were performed on an Olympus BX51 microscope under brightfield illumination, and images captured with an Optronics MicroFire camera. The number of single-labeled Fos-ir cells (excluding double-labeled cells in the VTA) within each subregion contour was divided by the area of that contour to create a measure of cell density within each hemisection. In the VTA, single-labeled TH-ir cells and cells double-labeled for both TH and Fos, here called TH/Fos-ir, were also counted and converted to density measures as with the Fos-ir cells. In all subregions, density measures were averaged across hemispheres and sections to create one measurement per subregion per hamster.

Plasma testosterone measures

Plasma testosterone was measured in duplicate 50 μl plasma samples in a single radioimmunoassay using the Coat-A-Count Total Testosterone Kit (Diagnostic Products, Los Angeles, CA). The minimum detectable concentration was 0.1ng/ml and the intra-assay coefficient of variation was 4.7%.

Statistical analysis

To provide an integrated assessment of independent variable effects on Fos expression across all brain regions studied, multilevel modeling (MLM) was used as in Chapter 3. The model treated hamster as the upper-level sampling unit and brain region as the lower-level sampling unit, with age, hormone status, and stimulus exposure, and brain region as independent variables and Fos-ir cell density as the dependent variable. The error structure was modeled to impose the traditional homoscedasticity assumption used in ANOVA. MLM provides a more powerful analysis than a traditional repeated measures analysis of variance (ANOVA) that uses a within-subject factor, because it integrates non-independence between samples from the same subject in the model. Interactions were followed up by separate MLMs; interactions that involved age were followed up by determining effects within juveniles and adults separately. Region interactions were followed-up by separate analyses for effects of age, hormonal status, or stimulus within a region. However in these analyses, there is just one sample per subject, in which case ANOVA provides the exact same modeling and statistical outcome as MLM. ANOVA was therefore used for analyses within region. Three way ANOVAs were also used to examine effects of age, hormone, and stimulus on TH and

Fos/TH-ir in the VTA. P < 0.05 was considered significant, and all statistical analyses were done with SPSS software (PASW Statistics 18; SPSS: An IBM Company, Chicago, IL).

Results

Fos-ir analysis. Multilevel modeling revealed multiple main effects and two-way interactions that are shown in Table 4.1. Notably, neither main effects of hormone status nor any interactions with hormone status were observed. Of the three two-way interactions observed, only the age x stimulus interaction was independent of brain region and could be followed-up without analyzing brain regions separately. Therefore, the age x stimulus interaction was probed to assess how the Fos response to VS was affected by age, taking all brain regions of interest into account. This analysis revealed an effect of stimulus in adults ($F_{(1,30)} = 18.61$, $p < 0.01$) but not juveniles ($F_{(1,28)} = 1.55$, NS), such that adults, but not juveniles, expressed more Fos-ir cells in response to VS than to clean oil (Figure 4.2). A three-way interaction of age x stimulus x region was also observed and followed-up with separate two-way ANOVAs (age x stimulus) for each region. For every region other than MePD, all main effects were qualified by an age x stimulus interaction (shown in Table 4.1); the appropriate interaction follow-up is described for each region below.

Region	Effect	F value	df	p value
all 9	age	63.66	1, 54	< 0.01
	hormone	0.55	1, 54	ns
	stimulus	4.32	1, 54	ns
	region	360.89	7, 378	< 0.01
	age x hormone	0.30	1, 54	ns
	age x stimulus	14.829	1, 54	< 0.01
	age x region	49.66	7, 378	< 0.01
	hormone x stimulus	1.87	1, 54	ns
	hormone x region	0.48	7, 378	ns
	stimulus x region	6.14	7, 378	< 0.01
	age x hormone x stimulus	0.03	1, 54	ns
	age x hormone x region	0.34	7, 378	ns
	age x stimulus x region	6.41	7, 378	< 0.01
	hormone x stimulus x region	1.74	7, 378	ns
	age x hormone x stimulus x region	0.11	7, 378	ns
MePD	age	5.80	1,49	0.02
	stimulus	166.19	1,49	<0.01
	age x stimulus	0.40	1,49	ns
AcbC	age	1.24	1, 54	ns
	stimulus	0.01	1, 54	ns
	age x stimulus	11.56	1, 54	<0.01
AcbSh	age	0.37	1, 54	ns
	stimulus	0.15	1, 54	ns
	age x stimulus	4.54	1, 54	0.04
Cg1	age	1.81	1, 54	ns
	stimulus	4.28	1, 54	0.04
	age x stimulus	6.78	1, 54	0.01
PrL	age	0.17	1, 54	ns
	stimulus	0.76	1, 54	ns
	age x stimulus	16.62	1, 54	<0.01
IL	age	0.82	1, 54	ns
	stimulus	0.85	1, 54	ns
	age x stimulus	9.13	1, 54	<0.01

Table 4.1. Complete list of age, hormone, stimulus and region effects with interactions. Salient interactions are followed up and described in text.

Table 4.1 (con't)

IF	age	56.70	1, 54	<0.01
	stimulus	6.62	1, 54	0.01
	age x stimulus	7.99	1, 54	<0.01
PN	age	26.60	1, 54	<0.01
	stimulus	0.04	1, 54	ns
	age x stimulus	4.62	1, 54	0.04
PBP	age	1.62	1, 54	ns
	stimulus	0.01	1, 54	ns
	age x stimulus	1.11	1, 54	ns

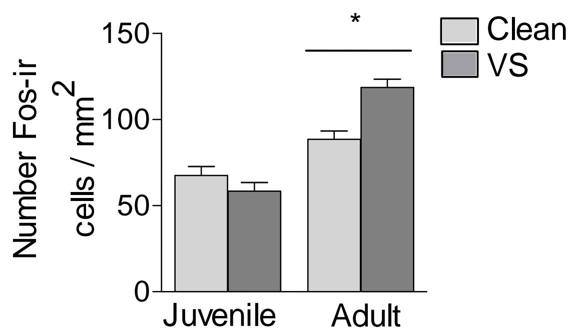


Figure 4.2. Cluster analysis of Fos responses to VS. Fos-ir cell density using data from all brain regions analyzed as sampling units as in MLM, mean +/- SE, * indicates p < 0.01. An age x stimulus interaction was found, such that only in adults was there a significant increase in the number of Fos-ir cells in response to VS.

In the IL and IF (Figure 4.3, left column), the age x stimulus follow-ups revealed an effect of stimulus in adults ($F_{(1,28)} = 5.88$, $p = 0.02$ and $F_{(1,30)} = 15.51$, $p < 0.01$, respectively), such that adults expressed more Fos-ir cells when in response to VS compared to clean oil. The effect of stimulus in juveniles was not significant in IF, and

fell just short of statistical significance in IL ($F_{(1,28)} = 0.03$, NS and $F_{(1,28)} = 3.963$, $p = 0.056$, respectively).

In the AcbC and PrL (Figure 4.3, middle column), the age by stimulus follow-ups revealed an effect of stimulus in both adults ($F_{(1,28)} = 5.57$, $p = 0.03$ and $F_{(1,28)} = 11.62$, $p = 0.02$, respectively), and juveniles ($F_{(1,30)} = 6.75$, $p = 0.01$ and $F_{(1,30)} = 5.01$, $p = 0.03$, respectively). However, the effects of VS were opposite at the two ages: juveniles expressed fewer Fos-ir cells in response to VS compared to clean oil, but adults expressed more Fos-ir cells in response to VS compared to clean oil. In the PN (Figure 4.3, middle column), no significant effects of stimulus were detected in either age group, however the results follow the same general trend as in AcbC and PrL, with juveniles expressing fewer, and adults more, Fos-ir cells in response to VS.

In the AcbSh (Figure 4.3, right column), the age x stimulus follow-up revealed no effect of stimulus in either age group. Therefore, the effect of age (juvenile or adult) was determined in clean oil- and VS- exposed hamsters separately. In clean oil-exposed hamsters, an effect of age was observed, $F_{(1,28)} = 4.55$, $p = 0.04$, such that juveniles expressed more Fos-ir cells than did adults. In VS-exposed hamsters, no effect of age was detected.

In the Cg1 (Figure 4.3, right column), the age x stimulus follow-up revealed an effect of stimulus in juveniles, $F_{(1,28)} = 9.90$, $p < 0.01$, such that they expressed fewer Fos-ir cells in response to VS compared to clean oil. No effect of stimulus was detected in adult hamsters.

In the MePD (Figure 4.3 left column) and PBP (Figure 4.3, right column), no age x stimulus interactions were observed. A main effect of stimulus was observed in the MePD, such that hamsters expressed more Fos-ir cells in response to VS than to clean oil. A main effect of age was also observed in the MePD, such that juveniles expressed more Fos-ir cells than adult hamsters.

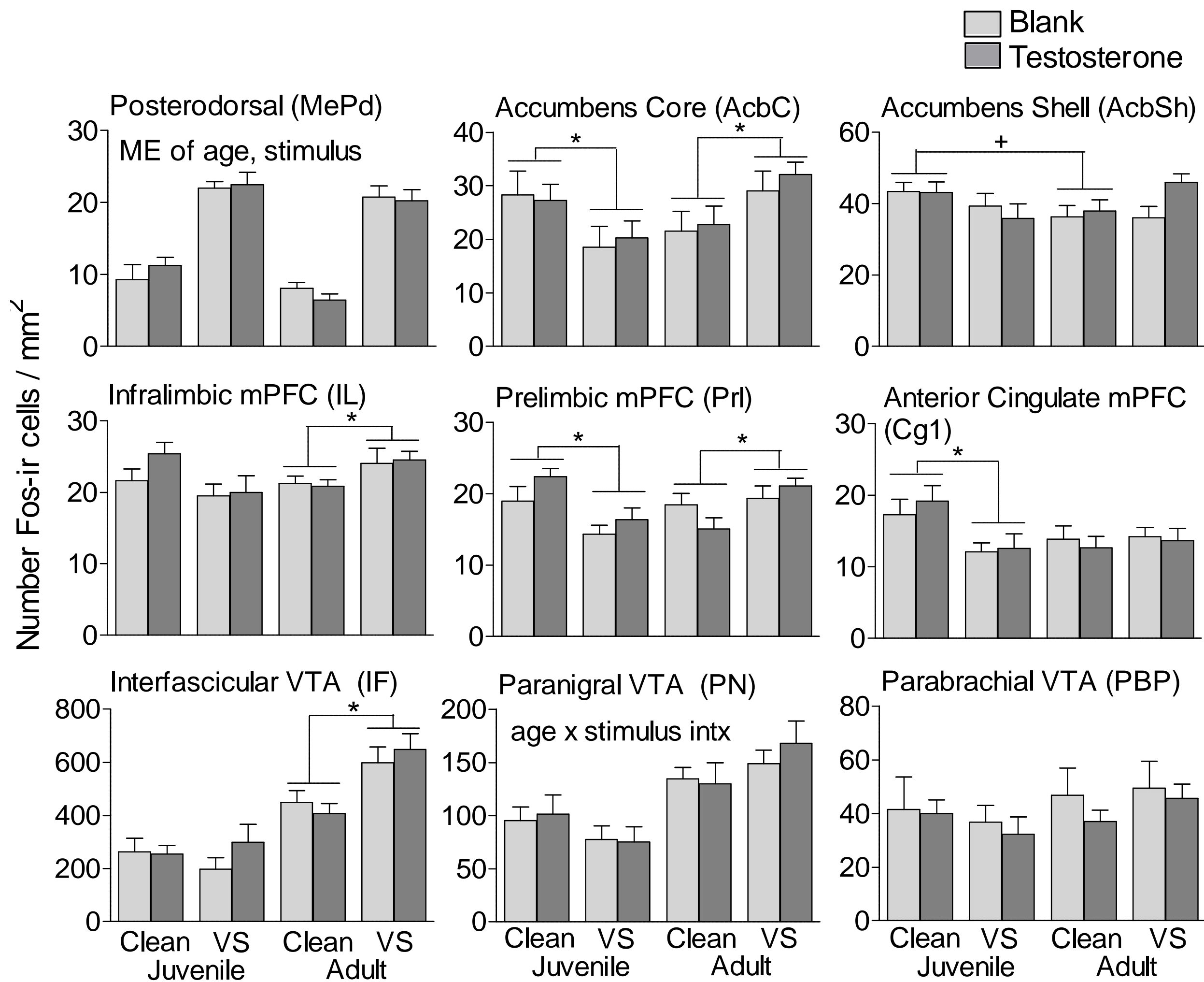


Figure 4.3. Fos-ir cell density in all brain regions analyzed, in blank- or testosterone (T)-treated juveniles and adults exposed to clean or VS-oil. Mean +/- SE, $p < 0.05$. In the MePD, main effects of age and stimulus were detected. In all other regions but PB, interactions between age and stimulus were detected. To probe these interactions,

effects of stimulus on hamsters within age (*), or effects of age within stimulus (+) were assessed. Interactions occurred either when the effect of stimulus was opposite for the two age groups (AcbC, PrL), when an effect of stimulus was present only in one age (IL, IF, Cg), or when an effect of age was present in only one stimulus group (AcbSh). In the PN, an age x stimulus interaction was detected, but no significant effects of stimulus were found upon follow-up within each age. An interaction was likely detected because the effects of VS were in opposite directions in juveniles and adults.

TH-ir and Fos/TH-ir analysis

TH-ir. Main effects of age were observed on TH-ir cells in the IF and PN, such that juveniles expressed more TH-ir cells than did adults, $F_{(1,54)} = 28.08$, $p < 0.01$ and $F_{(1,54)} = 8.23$, $p < 0.01$ respectively (Fig 4.4, top row). A main effect of hormone was also observed on TH-ir cells in the IF, such that blank capsule treated hamsters expressed more TH-ir cells than did T-treated hamsters, $F_{(1,54)} = 4.27$, $p = 0.04$. No effects of stimulus or other interactions were observed in any of the three VTA subregions.

Fos/TH-ir. Main effects of age were observed on Fos/TH-ir cells in the IF, PN, and PBP such that adults expressed more Fos/TH-ir cells than did juveniles, $F_{(1,54)} = 45.96$, $p < 0.01$; $F_{(1,54)} = 6.31$, $p = 0.02$; and $F_{(1,54)} = 10.68$, $p < 0.01$ respectively (Fig 4.4, bottom row). An age x stimulus interaction was also observed on Fos/TH-ir cells in the PN, $F_{(1,54)} = 7.72$, $p < 0.01$. Follow-up revealed an effect of age in VS-exposed

hamsters, $F_{(1,30)} = 16.28$, $p < 0.01$, but not in clean oil exposed hamsters; when exposed to VS, adults expressed more Fos/TH-ir cells than did juveniles.

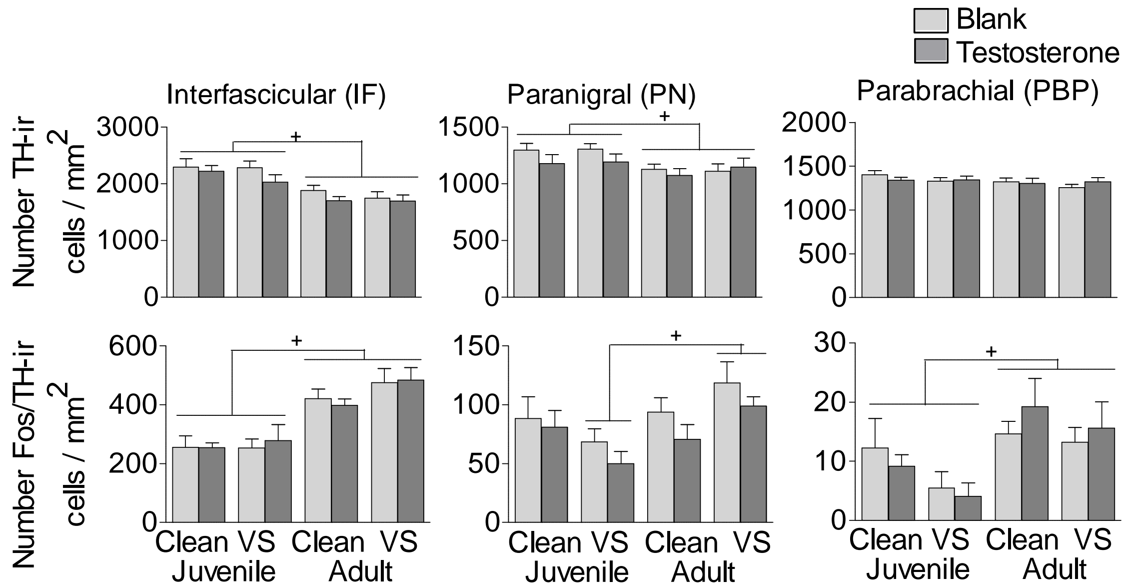


Figure 4.4. TH- and Fos/TH-ir cell density in the VTA, in blank- or testosterone- treated juveniles and adults exposed to clean or VS-oil. Mean +/- SE, p < 0.05. In the IF and PN, main effects of age were detected (+) on TH-ir cells such that juveniles expressed more than did adults. In the IF, a main effect of hormone was detected on TH-ir cells, such that blank-treated hamsters expressed more than did testosterone-treated. In all three regions, main effects of age were detected (+) on Fos/TH-ir cells, such that adults expressed more than did juveniles. Finally, in the PN, an age x stimulus interaction was detected on Fos/TH-ir cells, such that an effect of age was observed in VS exposed hamsters but not clean oil exposed hamsters.

Peripheral and testosterone measures

Physiological measures are shown in Table 4.2, and confirm efficacy of testosterone capsules in raising circulating testosterone and increasing flank glands in both ages. Gonadectomy resulted in a decrease in seminal vesicle weight only in adults. Groups of the same age did not differ in body weight.

				Body Weight (g)		Flank gland length (mm)		Seminal Vesicle Weight (g)		Plasma T (ng/ml)	
Age	Capsule	Stimulus	N	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Juv.	Empty	Blank	7	51.03	4.04	3.39	0.82	< 0.01	-	<0.10	-
Juv.	Empty	VS	8	50.60	4.63	3.30	2.03	< 0.01	-	<0.10	-
Juv.	T	Blank	7	50.27	3.03	4.99	1.27	0.03	0.05	7.76	0.87
Juv.	T	VS	8	50.56	5.41	4.64	0.71	0.01	0.04	7.99	1.19
Adult	Empty	Blank	8	99.28	13.87	6.44	2.21	0.03	0.05	<0.10	-
Adult	Empty	VS	8	96.95	10.57	7.04	1.20	0.08	0.09	<0.10	-
Adult	T	Blank	8	93.05	8.63	8.41	1.85	0.25	0.27	6.19	1.34
Adult	T	VS	8	89.34	18.41	7.28	0.86	0.21	0.20	6.19	1.19

Table 4.2. Peripheral measures of hamsters at time of sacrifice. Mean and standard deviations (SD) of body weight, flank gland length, seminal vesicle weight, and plasma testosterone (T). Adults are heavier, have longer flank glands, heavier seminal vesicles, and less circulating testosterone. Testosterone treated hamsters have longer flank glands, heaver seminal vesicles, and more circulating testosterone.

Discussion

This study demonstrates that elevating circulating testosterone to adult-like levels in juvenile hamsters is not sufficient to induce adult-like mesocorticolimbic responses to VS. That is, VS elicited a Fos response only in adults, and not in juveniles, whether they were treated with testosterone or not (Figure 4.2). In fact, testosterone treatment

did not affect the VS-induced Fos response in adults either. These findings indicate that some developmental process other than the pubertal rise in testosterone accounts for the observed age x stimulus interactions, in which either a VS-induced Fos response occurred in only one age group (as in the infralimbic mPFC, interfascicular VTA, and anterior cingulate mPFC), or the direction of the VS-induced Fos response was in opposite directions in juveniles and adults (as in the accumbens core, prelimbic mPFC, and paranigral VTA). In addition, differences between adults and juveniles in the density of Fos/TH-ir cells only emerged when hamsters were exposed to VS, independent of testosterone treatment, demonstrating that dopaminergic cell responses to VS also mature with age. A one-week period of testosterone treatment, similar to that used here, is sufficient to cause juvenile males to be attracted to VS (Johnston and Coplin, 1979), but not to induce adult-typical sexual behavior (Meek *et al.*, 1997, Schulz and Sisk, 2006). Therefore, the adolescent, but hormone-independent changes in VS-induced Fos expression appear to reflect those observed for sexual behavior, instead of reflecting rewarding effects of VS exposure. Given the involvement of this neural circuitry in linking motivation with motor output (Sesack and Grace, 2009), we propose that the differential Fos responses to VS in adult and juvenile males reflect differences in their drive to perform motor components of sexual behavior.

The mesocorticolimbic reward system includes extensive dopaminergic and non-dopaminergic projections from the VTA to the Acb, mPFC, and MeP, all of which are complexly and reciprocally connected via recurrent circuits (Swanson, 1982a, Oades and Halliday, 1987, Thompson and Swanson, 2010). In rodents, the MeP is responsible for initial evaluation of a chemosensory social stimulus and integration with hormonal

information (Wood and Newman, 1995a), and then relays this information to the VTA, mPFC, and Acb (Phillipson, 1979, Kevetter and Winans, 1981a, Coolen and Wood, 1998, Geisler and Zahm, 2005). Results from this experiment again demonstrate that MeP evaluation of VS in juveniles is similar to that of adults. However, over the course of adolescence and independent of social experience, VS exposure comes to engage non-dopamingeric cells in the IF and PN nuclei in the VTA, and downstream targets in the IL and PrL of the mPFC, and core of the Acb. Therefore, we propose that neural responses to VS mature across adolescence in the mesocorticolimbic system, independently of hormone exposure and sexual experience.

Neural mechanisms of adolescent shift in VS response

Adolescent development of mesocorticolimbic neural circuitry could explain changes in the pattern of Fos responses to VS across adolescence. Although VS exposure was in general associated with an increase in Fos-ir in adult hamsters, it was unexpectedly associated with reduced constitutive Fos expression in juvenile hamsters throughout the corticolimbic regions. A reduction in Fos in response to a sensory stimulus is uncommon (or at least not often reported), but could be explained mechanistically via specific dopamine receptor activation. D1 receptors are associated with excitatory G proteins and their activation generally causes an increase in Fos expression via activation of the AC/PKA/CREB pathway (Missale *et al.*, 1998). In contrast, D2 receptors are coupled with inhibitory G proteins that have the opposite effect on the intracellular cascade, including intracellular calcium, an important regulator of Fos (Missale *et al.*, 1998, Luo *et al.*, 2011). Synergistic activation of D1 and D2 receptors

typically increase Fos-ir within a given brain region, but D2 specific agonists have been observed to reduce Fos expression (Wirtshafter and Asin, 1994, Keefe and Gerfen, 1995)). Likewise, D2 antagonists often induce an increase in Fos expression, which suggests tonic inhibitory effects of DA on D2 receptor activation and Fos expression (Bertran-Gonzalez *et al.*, 2008). Therefore, the reduced constitutive Fos expression in response to VS may result from D2 receptor activation in juveniles and D1/D2 receptor activation in adult animals.

There are several possible explanations for D2-shifted activation in juveniles. The lack of increased Fos/TH-ir cells in response to VS in the current study may suggest a lack of burst dopamine cell firing in response to VS. However, this observation does not preclude VS-induced dopamine release in corticolimbic sites, as glutamateric release on to dopaminergic axon terminals has been shown to induce phasic dopamine release (Grace, 1991, David *et al.*, 2005). Release of glutamate in response to VS, either from the amygdala or prefrontal cortex, could therefore induce dopamine release in response to VS. Glutamatergic terminal innervation of the prefrontal cortex and accumbens increases dramatically during adolescence (Cunningham *et al.*, 2002, Brenhouse *et al.*, 2008). Therefore, perhaps glutamate in adult, but not juvenile, hamsters causes a local phasic dopamine release. Previous studies have shown that tonic release of DA at basal activity states can activate high-affinity D2 receptors, while phasic release in response to a stimulus activates both D1 and D2 (Creese *et al.*, 1983, Richfield *et al.*, 1989, Grace, 1991). Thus, perhaps a phasic dopamine release in response to VS in the adult hamsters allows for activation of

D1 receptors to increase Fos, while a less robust DA release preferentially activates D2 receptors and causes reduced Fos in juveniles.

Additionally, developmental profiles of dopamine receptor expression could explain D2-biased activation in juveniles. Although receptor binding studies have yielded somewhat inconsistent results on developmental profiles of D1 and D2 receptor expression in the striatum and cortex, most find an increase in expression that either peaks at mid-adolescence or continues into adulthood (Andersen *et al.*, 1997b, Andersen *et al.*, 2000, Tarazi and Baldessarini, 2000). Importantly, studies that directly compared D1 and D2 developmental profiles show that the D2:D1 binding ratios are much higher in juveniles than adults in the accumbens (Tarazi and Baldessarini, 2000). This heightened expression of D2 receptors in juvenile animals, paired with sub-adult levels of dopamine and glutamate innervation of the accumbens and cortex (Giorgi *et al.*, 1987, Kalsbeek *et al.*, 1988, Cunningham *et al.*, 2002, Brenhouse *et al.*, 2008, Mathews *et al.*, 2009), would favor expression of D2 receptor activation compared to adults.

Voluntary exposure affects neural responses to VS

In the previous study, increased Fos expression in response to VS was observed in AcbC, IL mPFC, non-dopamingeric cells in the IF VTA, PN VTA and PBP VTA in adult hamsters (Chapter 3). A similar pattern was observed in the current study, with a few exceptions: an adult response in the PrL was a new observation, and adult Fos responses in the PN and PBP were not present. In addition, both juvenile and adult hamsters also showed Fos/TH-ir responses to VS in the VTA in Chapter 3, and these

increases in Fos/TH were not observed in the current study. These inter-study differences may result from methodological differences between the studies. In Chapter 3, immunoreactivity was quantified throughout the regions of interest manually, while in Chapter 4, analyzed smaller rectangular portions of the brain regions. However, these smaller contours either detected similar responses to VS in adult animals (as in the Acb and mPFC), or covered almost the entire extent of the subregion as defined in Chapter 3 (as in the IF and PN). Instead, a more important difference between the studies in Chapter 3 and 4 may be the way in which hamsters were exposed to VS.

In the current study, in order to more completely control for equivalent exposure of volatile and non-volatile components of VS across the groups, VS or oil was applied directly to hamsters' nares, instead of presented on a cotton swab dropped into their home cage as in the previous study. It is possible that more robust VTA responses may be observed when VS exposure is gained voluntarily, as in investigating swabs in Chapter 3, as opposed to involuntarily, as in oil application in the current study. In fact, a similar phenomenon is seen when in response to electrical stimulation of the medial forebrain bundle in male rats. Animals voluntarily seeking self-stimulation showed greater Fos-ir in the VTA than yoked animals; no differences were seen between active and yoked rats in the mPFC and Acb (Hunt and McGregor, 1997). Voluntary exposure may also promote burst firing of dopaminergic VTA cells. Thus, voluntary approach to VS swabs may activate psychological or motor responses not seen in the current study. Therefore, these differential results present an interesting line of questions for future work.

Hormone dependent and independent effects of adolescent maturation

In general, one week of gonadectomy did not inhibit VS-induced Fos-ir in MePd, NA, mPFC, or VTA in adult hamsters. The lack of an effect in the MePd is not surprising in light of a previous study in which 12 weeks of gonadectomy also failed to block VS-induced Fos-ir in MeP (Fiber and Swann, 1996). However, an effect was seen in a medial preoptic nucleus in that study, demonstrating that responses to VS are hormone sensitive in at least some brain regions. Testosterone has also been previously shown to modulate dopamine-dependent PFC function in male rats, but in a temporally specific pattern: GDX causes a decrease in TH innervation and extracellular dopamine when measured after 4 days of hormone absence, but causes an increase in the same measures after 28 days of hormone absence (Aubele and Kritzer, 2011b). Short-term (10-14 days) castration in the ventral striatum does not affect tissue concentrations of dopamine and DOPAC (Engel *et al.*, 1979, Mitchell and Stewart, 1989), however long-term (28-56 days) gonadectomy generally reduces dopamine and DOPAC concentrations in NA tissue (Alderson and Baum, 1981, Mitchell and Stewart, 1989) but see (Baum *et al.*, 1986). Taken together, it is possible that 1) mesocorticolimbic responses to VS are not modulated by circulating testosterone, as is the case in the MePd, or 2) a longer hormonal absence in adult hamsters could have prevented VS responses in the mPFC and NA. One week of testosterone treatment was selected here to parallel the treatment sufficient to induce CPP responses to VS in juvenile hamsters (Chapter 5), however extending the length of testosterone treatment is an interesting question for future work.

Several age-related, VS-independent, changes in Fos expression in VTA dopaminergic cells were observed. The number of Fos/TH-ir cells was greater in adults than juveniles, which may be indicative of heightened vigilance or sensitivity to a range of stimuli in adulthood (mineral oil, in this study). The same effect of age on constitutive Fos expression of dopaminergic cells was observed in gonad intact hamsters (Chapter 3); therefore, this study demonstrates that this change is dependent on adolescent maturation and not circulating hormones. Age also affected the number of single-labeled VTA TH-ir cells, such that juvenile hamsters expressed more than did adults. This differential labeling could indicate either reduced TH expression within existing cells or reduced TH-expressing cell number in adult hamsters. One mechanism of differential TH expression could be increased dopamine release in adulthood, which then feeds back to reduce TH-expression (as observed in (Stork *et al.*, 1994), or adolescent changes in ion channel activity known to regulate TH-expression (Aumann *et al.*, 2011).

An effect of hormone was also observed on TH-ir cells in IF, in that testosterone-exposed hamsters expressed fewer TH-ir cells than did blank-treated hamsters, independent of age. There is some evidence for testosterone suppressing TH expression, as 30 days of gonadectomy causes an increase in the number of TH-ir cells in adult male rats (Johnson *et al.*, 2010). This effect was not seen after 14 days of gonadectomy (McArthur *et al.*, 2007), a time-span that more closely matches 7 days of treatment in this study. However, the temporal pace of responses to hormone removal is often slower than that of responses to hormone replacement (Putnam *et al.*, 2003). Greater numbers of TH-ir cells were previously observed in gonad-intact juveniles

compared to adults (Chapter 3), and could be explained by either the developmental or hormonal effects seen here.

Conclusion

The present study demonstrates that testosterone does not activate adult-typical Fos responses to a rewarding social cue in juvenile male hamsters, indicating that adolescent developmental programming underlies activation of mesocorticolimbic brain regions by female chemosensory cues in adults. These Fos responses may be a neural correlate for motivation or enactment of sexual behavior, which also is not activated by testosterone prior to adolescent development. Normal expression of sexual behaviors is dependent on exposure to testosterone during puberty, as it organizes the brain for adult-typical expression of behavior. Therefore, mesocorticolimbic structures may be a target for these organizational effects of testosterone, and ongoing studies will investigate responses of the Acb to sexual experience in hamsters deprived of pubertal testosterone. As perturbations in adolescent maturation are associated with maladaptive behaviors and certain mental illnesses, these studies are important in expanding our knowledge of this sensitive period of development.

Chapter 5: Dopamine mediates testosterone-induced social reward in juvenile male hamsters.

Introduction

Given the importance of appropriately interpreting social stimuli in successful adult social interactions, determining the neural underpinnings of responses to social cues is essential to understanding social behavior. While social stimuli serve to communicate a range of social information, many salient cues relate to sexual behavior (O'Connell and Hofmann, 2011). Male Syrian hamsters provide a useful model with which to study responses to these cues, as their sexual behavior is dependent on both neural processing of female hamster vaginal secretions (VS) and recent exposure to testosterone (Murphy and Schneider, 1970, Whalen and Debold, 1974, Petrulis, 2009). VS are an unconditioned reward, as sexually-naïve adult male hamsters will form a conditioned place preference (CPP) for them (Bell *et al.*, 2010). Attraction to VS, like the performance of sexual behavior, is dependent on activational effects of testosterone (Gregory and Bishop, 1975). However, it is currently unknown whether the reinforcing value of VS is similarly testosterone-dependent. Therefore, this study seeks to determine the necessity of testicular hormones for VS CPP.

An important neural response to chemosensory stimuli and copulation in rodents is the release of dopamine in the medial preoptic area (MPOA) and nucleus accumbens (Acb) (Malmnas, 1976, Mas *et al.*, 1990, Pfaus *et al.*, 1990, Louilot *et al.*, 1991, Damsma *et al.*, 1992, Mitchell and Gratton, 1992, Meisel *et al.*, 1993, Wenkstern *et al.*, 1993, Hull *et al.*, 1995, Schulz *et al.*, 2003). In fact, the MPOA dopamine release in response to sexual interactions requires intact olfactory bulbs in hamsters (Triemstra *et*

al., 2005), further emphasizing the importance of this chemosensory stimuli for sexual responses in this species. Dopamine has been implicated in multiple aspects of sexual reward. For example, systemic administration of haloperidol, a D2 dopamine receptor antagonist, decreases unconditioned motivation for primary female visual, auditory, and chemosensory cues in sexually-naïve male rats, as well as conditioned motivation for olfactory cues previously associated with sexual behavior (Lopez and Ettenberg, 2000, 2002). In addition, formation of conditioned place preference for sexual behavior in female hamsters is blocked by administration of a D2 receptor antagonist (Meisel *et al.*, 1996). However, other studies have found that dopamine receptor activation is not required for CPP for sexual rewards in male rats and mice (Agmo and Berenfeld, 1990, Agustin-Pavon *et al.*, 2007, Ismail *et al.*, 2009). Therefore, this study seeks to determine if dopamine is necessary for CPP to VS in male hamsters.

Testosterone regulates dopaminergic circuitry and tone, but with spatial and temporal specificity. Castration causes a decrease in sexual behavior after 2-8 weeks, which coincides with decreases in basal dopamine levels and turnover in the Acb and MPOA (Mitchell and Stewart, 1989). These effects of testosterone on dopaminergic circuitry for the performance on sexual behavior have not been well studied in the Acb; however, testosterone has repeatedly been shown to regulate dopamine in the MPOA. The absence of a precopulatory MPOA dopaminergic response to a stimulus female is predictive of the extinction of copulatory behavior after gonadectomy (Hull *et al.*, 1995). Likewise, the presence of a preoptic dopaminergic response to a stimulus female prior to copulation is predictive of the reinstatement of copulatory behavior upon testosterone replacement in long-term castrated male rats (Putnam *et al.*, 2001). Moreover, sexual

behavior can be partially restored in male rats castrated for 3-8 months by systemic and intra-MPOA injections of apomorphine, a dopamine agonist (Scaletta and Hull, 1990). Thus, testosterone may allow for dopaminergic responses to sexual stimuli that are essential for the expression of sexual behavior.

Puberty is a time of dramatic change in circulating testicular hormones and mesocorticolimbic maturation (Spear, 2000). Not surprisingly, then, puberty in male Syrian hamsters is coincides with changes in endocrine, neural, and behavioral responses to VS (Meek *et al.*, 1997, Romeo *et al.*, 1998). Juvenile male hamsters do not show adult-typical attraction or CPP to VS (Johnston and Coplin, 1979) and Chapter 3). This behavioral differences between gonad-intact juvenile and adult hamsters are mirrored by their dopaminergic responses to VS. Adult, but not juvenile, hamsters show an increase in dopamine release and metabolism in response to VS in the MPOA (Schulz *et al.*, 2003). Similarly, there are adolescent changes in expression of immediate early gene in response to VS in the dopaminergic mesocorticolimbic system. Adult, but not juvenile hamsters express Fos in response to VS in the ventral tegmental area (VTA), medial prefrontal cortex (mPFC) and Acb in adult but not juvenile hamsters (Chapter 3). However, if juvenile male hamsters are treated with testosterone, they become attracted to VS (Johnston and Coplin, 1979). Given the involvement of testosterone and dopamine in rewarding aspects of sexual behavior, and the changes observed in these endogenous signalers during puberty, I propose that responses to VS will be affected by interactions of the two. This next series of studies tested the effects of testosterone and dopamine manipulations in CPP for VS in adult and juvenile male hamsters. Specifically, the experiments first asked whether testosterone treatment of

juvenile males enables formation of a CPP to VS. Finding that this was the case, I then tested the hypothesis that dopamine receptor activation mediates the ability of testosterone to induce rewarding properties of VS in juvenile hamsters.

Methods

Animals

Syrian hamsters (*Mesocricetus auratus*) were obtained from Harlan Laboratories (Madison, WI) and were housed in temperature- and humidity-controlled vivaria with a light:dark cycle of 14 hr light:10 hr dark, and *ad libitum* access to food (Teklad Rodent diet 8640, Harlan, Madison, WI) and water. Upon arrival, juvenile males were housed with their male littermates and biological mothers until weaning at P18. Weanling and adult males were singly housed in clear polycarbonate cages (30.5 x 10.2 x 20.3 cm) and were all sexually naïve at the time of study. Sixty adult female hamsters, approximately 12 months old, were housed under similar conditions in separate vivaria and used as the source of VS. Female hamsters were ovariectomized several weeks before hormone administration and collection of VS. They were injected subcutaneously with 10 µg estradiol benzoate and 500 µg progesterone in sesame oil, 52 and 4 hours respectively, prior to collection of VS by gentle vaginal palpation. All experiments were conducted under <4 lux red light 1-5 hours into the dark phase. Hamsters were treated in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animals, and protocols were approved by the Michigan State University Institutional Animal Care and Use Committee.

Surgery and hormone implantation

In some experimental groups, hamsters were gonadectomized under isoflurane anesthesia. Bilateral longitudinal scrotal incisions were made, and the testes were removed with a cut distal to ligature or cauterization, in adults and juveniles, respectively. Some groups were also subcutaneously implanted with two blank or testosterone-containing silastic capsules (one 5 mm and one 13 mm of testosterone, Sigma-Aldrich, St. Louis, MO, sealed on each end with 4mm of silastic adhesive; inner diameter 1.98 mm; outer diameter 3.18 mm). These capsules have been used previously in both juveniles and adults to produce adult physiological levels of circulating testosterone (~2-7 ng/ml). Subjects received a subcutaneous injection of ketoprofen analgesic at time of surgery and again 24 hours after.

Conditioned place preference (CPP) experimental design

Place preference conditioning occurred as described previously (Bell *et al.*, 2010) in an apparatus with one middle compartment and two outer compartments (Med Associates, St. Albans, VT). These outer compartments were designed to allow for compartment-specific associations: one outer compartment had black walls, scalloped Plexiglas floor, and was scented with 4% acetic acid, while the other had white walls, mesh grid floor, and was scented with pine pellets underneath the floor. To acclimate subjects to handling and novel chambers, male hamsters were placed in glass aquaria in the behavioral testing room for 10 minutes each day, starting two days prior to beginning the CPP regimen. The CPP regimen included a pretest, 10 conditioning sessions, and

test, which all occurred at the same time of day (+/- an hour) for each hamster. In order to reduce the number of cohorts required and prevent exposing control animals to the smell of the stimuli, control animals were housed in a separate room in which the dark phase began at 8:00am, and testing at 9:00am. Experimental animals were housed in rooms in which the dark phase began at 2:00pm, and testing at 3:00.

A pretest (2 minutes in the middle compartment followed by 15 minutes access to all compartments) was used to determine each hamster's initial compartment preference. The outer compartment in which the hamster spent more time was defined as the initially preferred compartment. A preference score, defined as [time in the initially non-preferred compartment/(time in the initially preferred compartment + time in the initially non-preferred compartment)], and a difference score, defined as [time in the initially-preferred compartment – time in the initially non-preferred compartment] were calculated for each animal (Bell *et al.*, 2010). Hamsters that did not enter each compartment at least 5 times were excluded from further training. To the extent possible, animals were assigned to experimental and control groups so as to equate groups for initial chamber preferences and preference scores, and also to have equal representation of litters in the different groups.

Following the pretest, the hamsters received a total of ten 30-minute conditioning sessions in the side compartments, one session per day on consecutive days, alternating five no-stimulus and five stimulus-paired sessions. During the no-stimulus conditioning sessions, hamsters in both the experimental and the control groups were placed in their initially preferred compartments, where they remained alone. During stimulus-paired conditioning sessions, hamsters in the experimental group were placed

in the initially nonpreferred compartments with the stimulus. The hamsters in the control groups were also placed in their initially nonpreferred compartments but were not given the stimulus. This group served to demonstrate that any change in preference or difference score across tests seen in the experimental group was not due to chance or habituation during conditioning. The CPP apparatus was cleaned thoroughly with 25% ethanol between each animal, and with 75% ethanol at the end of each conditioning day.

In Experiments 1-3, VS were used as the conditioning stimulus. An hour before use, VS were collected from 30 females and mixed together to total approximately 500µl. Approximately 15 µl of VS were applied to water-moistened cotton gauze packed into a 2-ml Eppendorf tube, one tube for each male. Immediately before testing, the tube was placed out of reach from the male at the top of the back wall in the initially nonpreferred compartment in VS-paired conditioning sessions for the VS group. Empty Eppendorf tubes were used for the control group in all conditioning sessions and for the VS group in the no-stimulus conditioning sessions. To ensure exposure to nonvolatile components of VS, the remaining ~200 µl of VS were mixed with 1.5 ml of mineral oil, and approximately 20 µl of this mixture was applied with a metal spatula directly onto the nose of hamsters in the VS group immediately before being placed in the VS-paired compartment. Clean oil was applied to the nose of hamsters in the control group for all conditioning sessions and in the VS group for no-stimulus conditioning sessions.

Twenty-four hours after the last conditioning session, hamsters were tested for their place preference following the same procedure used for the pretest. As in the pretest, preference and difference scores were calculated for each animal. One hour

after completion of the CPP test, hamsters were euthanized with an overdose of sodium pentobarbital (150 mg/kg, ip) and a terminal blood sample was collected via cardiac puncture for radioimmunoassay of circulating plasma testosterone.

Experiment 1: Are testicular hormones necessary for formation of a CPP to VS in adult hamsters? This first experiment tested whether circulating testicular hormones are required for the display of a CPP to VS in adult hamsters. Pilot studies in this laboratory indicated that male hamsters formed a CPP to VS when conditioning began one week after gonadectomy (Bell *et al.*, 2011), suggesting that putative activational effects of testicular hormones do not wash out acutely, as indicated by the gradual decline in sexual behavior over many weeks following gonadectomy in male rodents (Hull and Dominguez, 2007). Therefore, in this experiment, we studied hamsters that had been gonadectomized (GDX) 10 weeks prior to the start of conditioning. All adults arrived at postnatal day P56-63, but staggered so that they could be tested within the same CPP cohort. No-stimulus control animals were left gonad-intact and were pretested at P64-71, while the two experimental groups received conditioning with VS. The GDX+0 group was GDX at P57-64, remained unmanipulated for 10 weeks, and were then implanted with blank capsules at P127-134, one week prior to pretest at P134-141. The GDX+T group was GDX and given testosterone capsules at P57-64, one week prior to pretest at P64-71 to serve as positive controls to demonstrate a significant CPP. This arrangement required different ages of animals. However, we have never observed age-related differences in behavioral or neural responses to testosterone in prior experiments that controlled for this variable in young adults (Schulz *et al.*, 2009).

Experiment 2: Are adult levels of testosterone sufficient to activate a CPP to VS in juvenile hamsters? This experiment determined whether testosterone treatment of juvenile hamsters is sufficient for formation of a CPP to VS. One week of testosterone is sufficient to induce attractive responses to VS, and this duration of testosterone treatment was therefore used here (Johnston and Coplin, 1979). Juveniles arrived at P6 with their mothers, and were either left gonad-intact as no-stimulus controls or were GDX and given a testosterone capsule at P14. All juveniles were pretested at P21. In addition, age-matched gonad-intact adults were included as positive control and no-stimulus control groups; they arrived at P56-63 and were pretested at P70-77. Here the chamber characteristics were modified from that described above in an effort to bring the initial preference scores closer to 0.50. Instead of the mesh floor, a solid Plexiglas floor was used in the white side, without pine pellets as a scent.

Experiment 3: Does dopamine receptor activation mediate testosterone-dependent CPP to VS in juvenile hamsters? This experiment tested the involvement of dopamine in testosterone-facilitated CPP to VS in juvenile male hamsters. The same chamber stimuli were used as in Experiment 1 and previously published studies, as the changes used in Experiment 2 failed to improve the preference scores. All animals arrived at P12, were pretested at P20, and were run in 3 cohorts. Gonad-intact hamsters were used as no-stimulus controls, while other groups were GDX and given blank or testosterone capsules at P13, one week before testing. The GDX+0 group was included to confirm that juveniles with low levels of testosterone (as in gonad-intact animals) do not show a CPP to VS. A GDX+T was included as a positive control to

show that testosterone treatment can induce a CPP to VS. The remaining groups were all GDX+T, and were given injections of haloperidol (a D2 antagonist, 0.05, 0.15, 0.45mg/kg, ip,) or propylene glycol vehicle 30 minutes prior to VS and no-stimulus conditioning sessions, respectively. No-stimulus, GDX+0 and GDX+T control groups received propylene glycol vehicle injections 30 minutes prior to both conditioning sessions. A final group was included to test for any aversive properties of haloperidol. These animals were gonad-intact, and received 0.45mg/kg haloperidol or propylene glycol ip 30 minutes prior to conditioning sessions in the initially preferred and non-preferred chamber, respectively. Thus, any aversive properties of haloperidol would reduce their initial preference. No VS pairings were made in this group.

Experiment 4: Does dopamine receptor antagonism alone alter place preference in juvenile hamsters? This experiment was designed to more completely determine if the doses of haloperidol used in Experiment 3 had any intrinsic aversive qualities in testosterone-treated hamsters. If this were shown to be the case, then the results of Experiment 3 would be difficult to interpret, as an observed prevention of CPP for VS might instead just be an overriding avoidance of the haloperidol-conditioned environment. All animals arrived at P12, were GDX+T at P13, pretested at P20, and run in one cohort. A similar conditioning paradigm was used as above, but haloperidol was given in the initially preferred chamber in an attempt to reduce initial preferences, and no VS were used. Preference scores were converted to aversion scores, and indicated time in initially preferred compartment over time in initially preferred and non-preferred. Locomotor activity (number of infra-red beam breaks) and fecal boli output

during conditioning sessions were also quantified as indicators of physiological effects of haloperidol.

Experiment 5: Does dopamine receptor antagonism affect food detection in juvenile hamsters? This experiment determined the potential of haloperidol to block olfactory abilities in testosterone-treated juveniles. Animals from Experiment 4 were used, and tested on P32. Two plastic dishes were placed at opposite ends of glass aquaria (51 x 26 x 31.5 cm) that either contained moistened food pellets or were left empty. Both dishes were covered with opaque foil with small holes punched in the top, so that hamsters could smell but not see the food. Thirty minutes before the test, hamsters were injected with their assigned dose of haloperidol or propylene glycol vehicle. Hamsters were placed in the middle of the aquaria and the time spent in nose-contact with the two dishes was recorded in a five minute test. Aquaria were cleaned with 25% ethanol between tests, and the side of the food was alternated between tests so that half the animals in each group were presented food on the right side of the aquaria.

Plasma testosterone measures

Duplicate 50 µl samples of plasma testosterone were analyzed within a single assay using the Coat-A-Count Total Testosterone Kit (Diagnostic Products, Los Angeles, CA). The minimum detectable concentration was 0.08ng/ml and the intra-assay coefficient of variation was 7.9%. Five hamsters removed their testosterone capsules mid-experiment and were excluded from behavioral or testosterone analysis. Final group

sizes are expressed in Table 5.1. Testosterone concentrations from animals in Experiment 4 are not available at this time.

Statistical analysis

To assess whether VS (or haloperidol) induced a change in place preference, paired t-tests within each group were used to evaluate the change in preference (or aversion) score and difference score from pretest to test (Bell *et al.*, 2010). A significant change in both preference score and difference score within a group was required to indicate a CPP.

To assess effects of haloperidol on locomotor activity in Experiment 4, a one-way ANOVA and Dunnett post-hoc test were used to compare number of infrared beam breaks in the initially preferred compartment between groups. A separate ANOVA was performed with data from the initially non-preferred compartment (propylene glycol-paired) as an un-drugged control. Because locomotor activity is generally greater in the black compartment than that in the white, only animals that received haloperidol in the white compartment were included in the locomotor analysis, n = 5-6/group. To assess haloperidol effects on fecal boli output, paired t-tests were used to compare number of fecal boli while in haloperidol or vehicle conditioning sessions, within each group. To assess whether haloperidol affected preferences for food smells, paired t-tests were used to compare time spent investigating the empty or food containing dish within each group. $P < 0.05$ was considered significant, and all statistical analyses were done with SPSS software (PASW Statistics 18; SPSS: An IBM Company, Chicago, IL).

Results

Experiment 1 – Are testicular hormones necessary for formation of a CPP to VS in adult hamsters?

Long-term gonadectomy prevented a CPP for VS in adult hamsters (Figure 5.1). No changes in preference or difference score of the GDX+0 group were seen as a result of conditioning ($t_{(9)}$ = 1.14, NS and $t_{(9)}$ = 0.06, NS, respectively). However, paired t-tests did indicate a significant increase in the preference score and decrease in the difference score for the GDX+T group as a result of conditioning ($t_{(9)}$ = -4.88, $p < 0.05$ and $t_{(9)}$ = 5.64, $p < 0.05$, respectively). No changes in preference or difference score between pretest and test were seen in age-matched no-stimulus control group ($t_{(11)}$ = -0.76, NS and $t_{(11)}$ = 1.09, NS, respectively). Therefore, exposure to testicular hormones is necessary for VS-induced CPP.

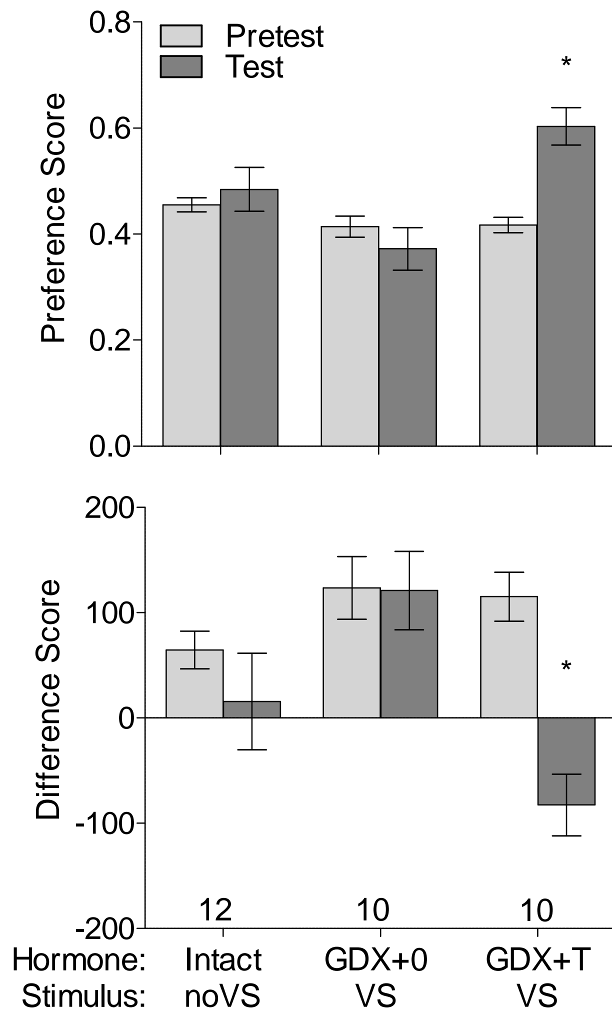


Figure 5.1. CPP to VS in hormone manipulated adult hamsters. Preference and difference scores at pretest and test, mean +/- SE, $p < 0.05$, with group sample sizes above the x axis. Long-term GDX with blank hormone capsules prevented the CPP for VS observed in the GDX + testosterone group.

Experiment 2: Are adult levels of testosterone sufficient to activate a CPP to VS in juvenile hamsters?

Testosterone treatment was sufficient to permit a CPP to VS in juvenile animals (Figure 5.2). Paired *t*-tests showed a significant increase in the preference score and decrease in the difference score for the juvenile GDX+T group as a result of conditioning ($t_{(6)} = -2.90$, $p < 0.05$ and $t_{(6)} = 2.66$, $p < 0.05$, respectively). No changes in preference or difference score were seen in age-matched no-stimulus control group from pretest to test ($t_{(6)} = -1.38$, NS and $t_{(6)} = 2.20$, NS, respectively). Gonad-intact adults also showed a CPP to VS, replicating previous results (Figure 5.2). Paired t-tests showed an increase in the preference score and a decrease in the difference score for the adult gonad-intact group as a result of conditioning ($t_{(7)} = -3.88$, $p < 0.05$ and $t_{(7)} = 3.95$, $p < 0.05$, respectively). No changes in preference or difference score were seen in age-matched no-stimulus control group from pretest to test ($t_{(6)} = -1.76$, NS and $t_{(6)} = 1.98$, NS, respectively).

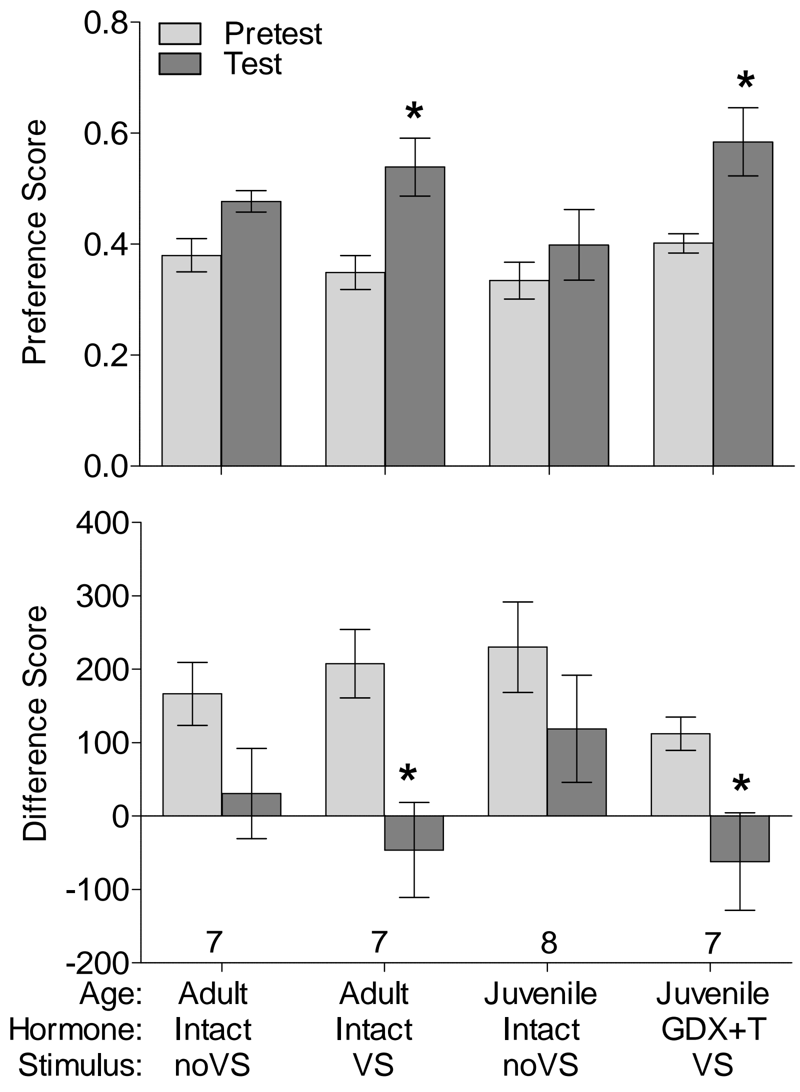


Figure 5.2. CPP to VS in hormone manipulated juvenile and adult hamsters. Preference and difference scores at pretest and test; mean +/- SE, $p < 0.05$, with group sample sizes above the x axis. Testosterone treatment in juvenile hamsters facilitated an adult-like CPP for VS.

Experiment 3: Does dopamine receptor activation mediate testosterone-dependent CPP to VS in juvenile hamsters?

Dopamine receptor antagonism blocked the CPP for VS in T-treated juvenile hamsters (Figure 5.3). The GDX+T VS group that received vehicle injection showed a CPP to VS, as both preference and difference scores showed a significant change as a result of conditioning ($t_{(5)} = -4.043$, $p < 0.05$ and $t_{(5)} = 4.18$, $p < 0.05$, respectively). This effect was blocked by haloperidol at all three doses: neither the 0.05mg/kg, 0.15mg/kg, nor 0.45mg/kg GDX+T VS groups showed a significant change in preference score ($t_{(7)} = -1.11$, NS; $t_{(6)} = -1.52$, NS; and $t_{(7)} = -0.68$, NS, respectively) or difference score ($t_{(7)} = 1.64$, NS; $t_{(6)} = 1.79$, NS; and $t_{(7)} = 0.92$, NS, respectively) as a result of conditioning. The GDX+0 VS group did not show a significant change in either preference or difference score as a result of conditioning ($t_{(6)} = -0.82$, NS and $t_{(6)} = 1.32$, NS, respectively), replicating effects seen in gonad-intact juveniles with similar concentrations of circulating hormone (Chapter 3). The no-stimulus control group did not show a significant change in either preference or difference score from pretest to test ($t_{(10)} = -1.33$, NS and $t_{(10)} = 1.97$, NS, respectively). However, the 0.45mg/kg noVS group showed a CPA to haloperidol, as both preference and difference scores showed a significant change across conditioning ($t_{(7)} = -2.39$, $p < 0.05$ and $t_{(7)} = 2.74$, $p < 0.05$, respectively). This suggests that this high dose is aversive, and precludes interpretation of the results from the GDX+T VS group at that dose.

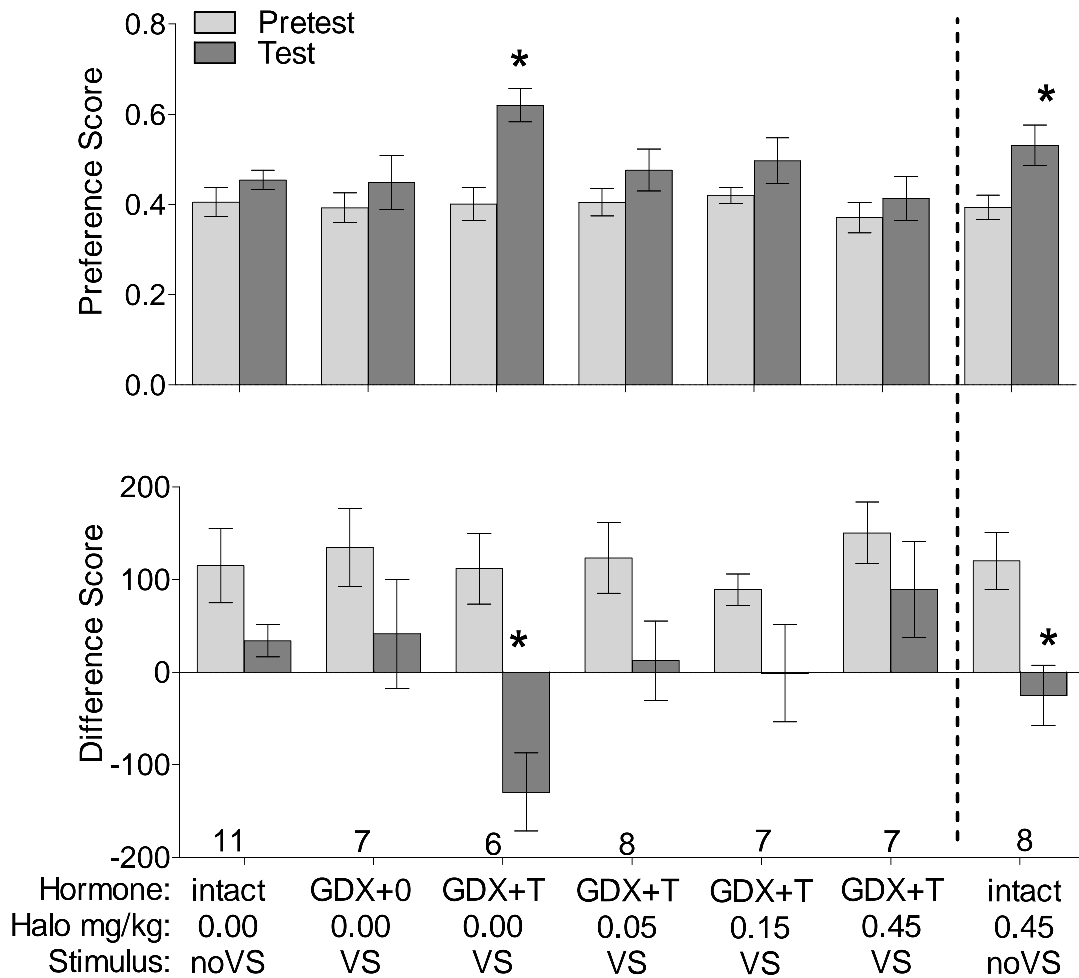


Figure 5.3. CPP to VS in hormone and dopamine manipulated juvenile hamsters. Preference and difference scores at pretest and test; mean +/- SE, $p < 0.05$, with group sample sizes above the x axis. Testosterone treatment in juvenile hamsters facilitated an adult-like CPP for VS, as in Experiment 2. Dopamine antagonism prevented the CPP for VS at multiple doses. However, the highest haloperidol dose conditioned a place aversion.

Experiment 4: Does dopamine receptor antagonism alone alter place preference in juvenile hamsters?

Potential aversive affects of haloperidol at different doses were unable to be determined (Figure 5.4). Most groups, including the no-stimulus control group, 0.15mg/kg group, and 0.45mg/kg group, showed significant changes in preference score ($t_{(6)} = -2.78$, $p < 0.05$; $t_{(6)} = -5.77$, $p < 0.01$; and $t_{(6)} = -3.63$, $p < 0.01$, respectively) and difference score ($t_{(6)} = 2.96$, $p < 0.05$; $t_{(6)} = 5.57$, $p < 0.01$; and $t_{(6)} = 3.29$, $p < 0.05$, respectively). The 0.05mg/kg group showed a significant change in difference score ($t_{(6)} = 2.74$, $p < 0.05$) but not preference score ($t_{(6)} = -2.16$, NS). The observed change in no-stimulus control group indicates that changes in the haloperidol groups are likely not due to the haloperidol, and thus this experiment does not provide evidence one way or the other about the potential aversiveness of haloperidol.

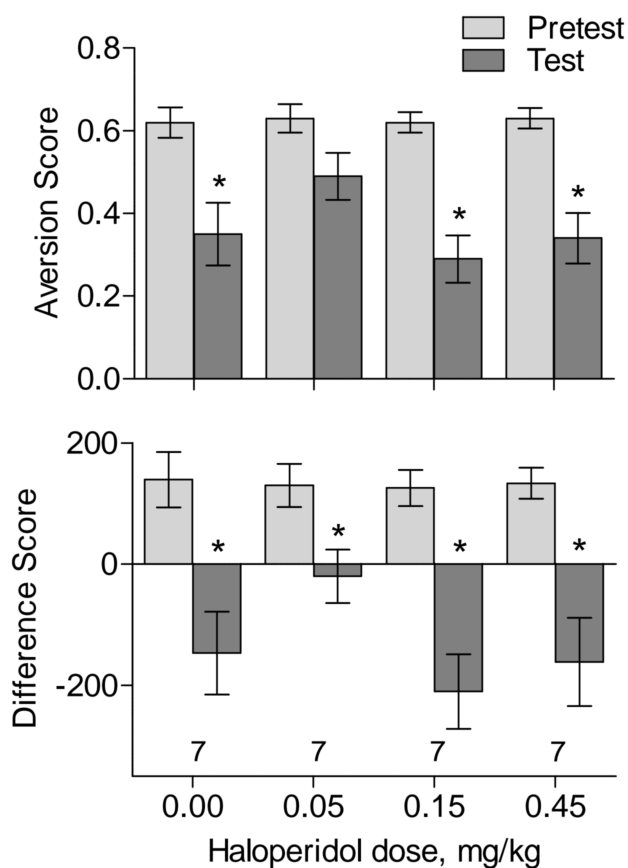


Figure 5.4. CPA to dopamine antagonist in juvenile hamsters. Aversion and difference scores at pretest and test; mean +/- SE, $p < 0.05$, with group sample sizes above the x axis. Groups showed a significant change in preference and/or difference scores, independent of haloperidol exposure, precluding interpretation.

Dopamine receptor antagonism affected activity and fecal boli output, but only at the 0.45mg/kg dose. An effect of dose was found on locomotor activity in the initially-preferred compartment that was paired with haloperidol, $F_{(19)} = 6.44$, $p < 0.01$. The post-hoc test revealed that haloperidol significantly reduced locomotor activity compared to controls, but only at the highest dose, $p < 0.01$ (Figure 5.5). No differences were found between groups in locomotor activity in the vehicle-paired compartment, $F_{(19)} = 2.16$, NS.

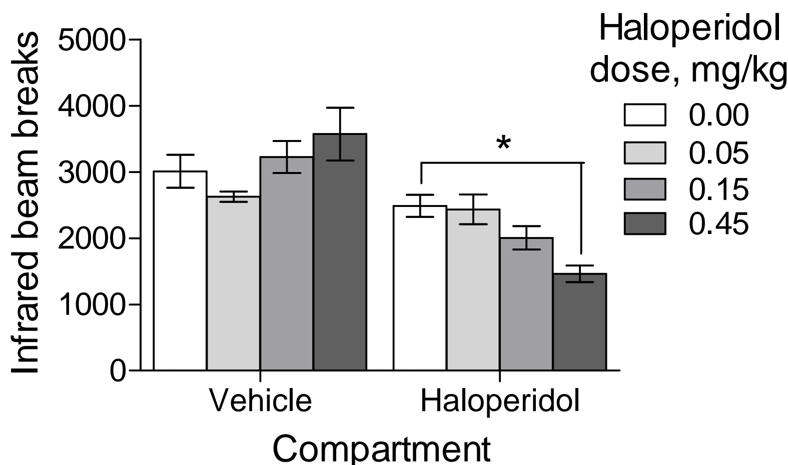


Figure 5.5. Locomotor activity in dopamine manipulated juvenile hamsters. Infrared beam breaks, indicating locomotor activity, mean +/- SE, $p < 0.01$. Animals assigned to 0.00 – 0.45mg/kg haloperidol doses did not differ in locomotor activity when assessed in the vehicle-paired compartment. However, locomotor activity was reduced in the highest haloperidol dose group while under the effects of the drug in the haloperidol-paired compartment.

No differences were found in within-animal fecal boli output in initially preferred or non-preferred compartments in the 0.00, 0.05, or 0.15mg/kg haloperidol groups ($t_{(6)}$ = 0.53, NS; $t_{(6)}$ = -0.14, NS; and $t_{(6)}$ = -1.74, NS, respectively. However, the 0.45mg/kg haloperidol group did produce more fecal boli in the initially preferred compartment during haloperidol-paired conditioning sessions, as compared to vehicle-paired conditioning sessions, $t_{(6)}$ = -5.10, $p < 0.01$ (Figure 5.6).

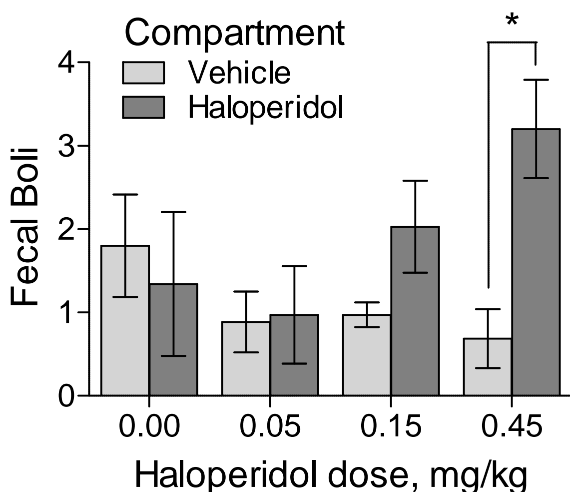


Figure 5.6. Fecal boli in dopamine manipulated juvenile hamsters. Number of fecal boli produced while in vehicle or haloperidol paired compartments, mean +/- SE, $p < 0.01$. Animals assigned to 0.00 – 0.15mg/kg haloperidol doses did not differ in fecal boli output when in the vehicle- or haloperidol-paired compartment. However, fecal boli output was increased in the highest haloperidol dose group while under the effects of the drug in the haloperidol-paired compartment.

***Experiment 5: Does dopamine receptor antagonism affect food detection in juvenile hamsters*?**

Dopamine receptor antagonism did not affect preferences for the food-containing dish over the control dish, indicating olfactory abilities are intact in haloperidol treated animals (Figure 5.7). The 0.00mg/kg, 0.05mg/kg, 0.15mg/kg and 0.45mg/kg all spent more time investigating the food dish over the control ($t_{(6)} = 5.50$, $p < 0.01$; $t_{(6)} = 5.80$, $p < 0.01$; $t_{(6)} = 4.06$, $p < 0.01$; and $t_{(6)} = 6.30$, $p < 0.01$, respectively).

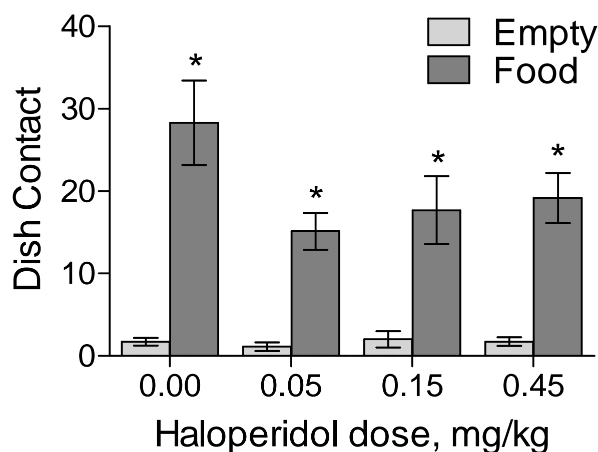


Figure 5.7. Food detection in dopamine manipulated juvenile hamsters. Time spent in nose-contact with the empty or food-containing dish, mean +/- SE, $p < 0.01$. All doses spent more time in contact with the food dish, indicating intact olfactory abilities even with haloperidol treatment.

Physiological measures

Physiological measures are shown in Table 5.1, and confirm efficacy of testosterone capsules in raising circulating testosterone in both ages. Groups of the same age did not differ in body weight.

				Body Weight (g)		Plasma T (ng/ml)	
Age	Hormone	Stimulus	N	Mean	SD	n	SD
Experiment 1							
Adult	Intact	noVS	12	132.50	13.67	1.41	0.53
Adult	GDX+0	VS	10	150.72	11.95	< 0.08	-
Adult	GDX+T	VS	10	116.77	12.09	2.60	0.87
Experiment 2							
Adult	Intact	noVS	7	126.51	11.00	2.76	1.21
Adult	Intact	VS	8	124.81	9.06	2.24	0.80
Juvenile	Intact	noVS	7	65.70	5.99	0.94	0.66
Juvenile	GDX+T	VS	7	63.39	3.88	4.06	0.68
Experiment 3							
Juvenile	Intact	noVS + 0.00	11	58.76	3.90	0.89	0.50
Juvenile	GDX+0	VS + 0.00	7	54.19	4.48	< 0.08	-
Juvenile	GDX+T	VS + 0.00	6	56.05	5.69	3.87	1.21
Juvenile	GDX+T	VS + 0.05	8	56.01	6.77	2.70	1.47
Juvenile	GDX+T	VS + 0.15	7	56.51	6.91	4.09	0.65
Juvenile	GDX+T	VS + 0.45	8	52.34	4.59	4.09	1.15
Juvenile	Intact	noVS + 0.45	8	59.15	5.70	0.89	0.62
Experiment 4 & 5							
Juvenile	GDX+T	VS + 0.00	7	54.39	7.04	n/a	
Juvenile	GDX+T	VS + 0.05	7	53.06	7.05		
Juvenile	GDX+T	VS + 0.15	7	51.24	5.61		
Juvenile	GDX+T	VS + 0.45	7	52.33	5.06		

Table 5.1. Group size, body weight, and plasma testosterone concentration. Adults were consistently larger than juveniles, but groups within an age generally did not differ in body weight. Testosterone pellets produced circulating testosterone concentrations within adult-typical physiological range.

Discussion

These studies demonstrate that testosterone is necessary for male hamsters to evaluate VS as rewarding. Specifically, long-term gonadal-hormone absence does not permit a CPP to VS in adults, while testosterone treatment of juveniles is sufficient to enable them to form a CPP to VS. These effects of testosterone are similar to those seen for activation of sexual behavior in adulthood and general attraction to VS in juveniles, behaviors that normally increase during adolescence (Whalen and Debold, 1974, Morin and Zucker, 1978, Johnston and Coplin, 1979). The testosterone-mediated gain in VS reward may be dependent on dopaminergic action, as a D2 receptor antagonist prevented expression of a CPP to VS. However, conclusions about the role of dopamine receptor activation in CPP formation must be postponed until completion of important experimental control experiments, which are discussed below.

Experimental Controls

A dopamine receptor antagonist was used to block dopaminergic responses to VS that may facilitate reward or reinforcement to allow for a CPP, and to keep dopaminergic receptor activity at the usual basal state. However, high doses of dopamine antagonists have the potential to cause aversive or unpleasant psychological or physiological effects. Such an aversive response to haloperidol could conceal an existing CPP by inducing the animal to avoid the haloperidol-associated CPP compartment. Experiment 3 gave some indication that the high dose of haloperidol may indeed be aversive in gonad-intact juveniles, and Experiment 4 was designed to more completely test aversive properties of VS in testosterone-treated juveniles. Unfortunately, the control

group that never received haloperidol (and instead received vehicle injections in both initially preferred and non-preferred chambers) showed a significant change in aversion and difference scores from pretest to test. It is unclear what caused this shift in preference in the control group, but regardless, it prevents interpretation of similar changes in haloperidol exposed animals, and the experiment will be repeated.

Some useful indicators of physiological and perhaps psychological effects of haloperidol were gleaned from the experiment. Haloperidol reduced locomotor behavior and increased fecal boli output, but only at the highest dose. Fecal boli output has classically been used as an indicator of anxiety and aversion (Sanberg, 1989), however D2 receptors also serve to inhibit gut motility in the enteric nervous system (Li *et al.*), thus reducing the utility of this measure in this particular case. While doses of 0.2mg/kg have been previously shown to cause aversive side effects (Risinger *et al.*, 1992, Manzanedo *et al.*, 2001), the doses between 0.05mg/kg and 1mg/kg have not cause a conditioned place aversion in other studies that specifically checked for them in mice and rats (Martin-Iverson *et al.*, 1985, Spyraki and Fibiger, 1988, Di Scala and Sandner, 1989, Suzuki and Misawa, 1995, Wu and Zhu, 1999, Imaizumi *et al.*, 2000, Adams *et al.*, 2001, Manzanedo *et al.*, 2001, Tzschentke, 2004, Manzanedo *et al.*, 2005, Miyata *et al.*, 2011). While I acknowledge that effects of drugs can differ among rodent species, I am cautiously hopeful that the next experiment will demonstrate no aversive qualities of 0.05mg/kg haloperidol. Therefore, to allow for a more robust integration of this study with the other data contained in my dissertation, I will discuss the results of this study under the assumption that the lowest dose of haloperidol is not aversive.

One other possible mechanism by which haloperidol might block a CPP to VS is by simply blocking the ability of the animals to smell VS. This possibility was a realistic one, as dopamine is a prominent neurotransmitter in the olfactory bulb and modulates the transmission of odorant sensory information from the receptor cells to the mitral cell at the level of the glomerulus (Cave and Baker, 2009). However, Experiment 5 demonstrated that hamsters exposed to even the highest dose of haloperidol can still detect food olfactory cues, as they showed a preference for the food-containing dish over the empty dish. Therefore, we can conclude that haloperidol did not prevent a CPP to VS simply by blocking olfactory detection of the cue. An interesting secondary observation from this study was that haloperidol treated hamsters tended to show less a less dramatic preference for the food. As these effects were present at lower doses of haloperidol that do not otherwise reduce locomotor activity, they may support dopaminergic involvement in motivation for food (Richard and Berridge, 2011).

Mechanism of haloperidol action

Because we have found that multiple dopamine-sensitive brain regions, including the AMY, Acb, PFC, and VTA, are involved in behavioral responses to VS, systemic intervention was used to antagonize dopamine receptors at multiple putative sites of action. Haloperidol is a potent D2 antagonist, but can also bind the adrenergic and sigma receptor less effectively (NIMH Psychoactive Drug Screening Program). D2 binding attenuates cellular excitability in both pyramidal neurons in the mPFC and medium spiny neurons in the Acb (Cepeda *et al.*, 1999, Tseng and O'Donnell, 2004, Benoit-Marand and O'Donnell, 2008). At these sites, D2 receptor activation serves to

reduce the impact of irrelevant information in the integration of corticolimbic inputs, thus allowing more specific learned associations (O'Donnell, 2003). It is likely this aspect of D2 receptor function that is disrupted by haloperidol to block the CPP for VS. Additionally, haloperidol can increase dopamine release from terminals in the accumbens by acting 1) post-synaptically in the “indirect” ventral pallidum pathway that provides feedforward input to the VTA (Valenti and Grace, 2010) and 2) pre-synpatically as an autoreceptor to regulate release and reuptake at the site of dopamine release (Wu *et al.*, 2002). These aspects of D2 receptor activation emphasize the complexity of dopaminergic action, and that it is overly simplistic to think that more dopamine equals more reward. Dopamine generally modulates responses to other neurotransmitters, and may serve to facilitate the formation of specific associations.

Dopamine and sexual reward

As mentioned previously, dopamine has been implicated in multiple aspects of sexual behavior, including anticipatory or appetitive behaviors (Pfaus and Phillips, 1989), copulatory or consummatory behaviors (Arteaga *et al.*, 2002), and the reinforcing responses to sexual interaction (Meisel *et al.*, 1996). In addition, dopamine is likely important for associating sociosexual stimuli with the environment or other cues. Systemic low doses of a non-specific dopamine antagonist block conditioned mate preference in female rats (Coria-Avila *et al.*, 2008), and a D2 agonist during co-habitation with a scented same-sex partner induces a same-sex partner preference for similarly scented males in male rats (Triana-Del Rio *et al.*, 2011). Work in monogamous prairie voles further supports the importance of D2 receptor in associating sexual

reward with stimuli or individuals, as systemic injections of D2, but not D1, receptor agonist and antagonist facilitate and disrupt partner preference in male voles, respectively (Wang *et al.*, 1999). The current study supports the role for dopamine in reinforcing responses to unconditioned social cues in sexually-naïve animals, and parallels the effects of haloperidol in reducing motivation for primary female visual, auditory, and chemosensory cues in sexually-naïve male rats (Lopez and Ettenberg, 2001).

The possible site(s) of action of dopamine cannot be determined from this study, however there are several likely candidates. Dopamine agonists and antagonists into MPOA facilitate and reduce the performance of sexual behavior, respectively, in male and female rats (Hull *et al.*, 1986, Bitran *et al.*, 1988, Pehek *et al.*, 1988, Graham and Pfaus, 2010). In addition, the MPOA is also implicated in anticipatory sexual behaviors and female preferences (Pfaus and Phillips, 1991, Moses *et al.*, 1995). The mesolimbic system does not seem to be involved in the performance of copulatory behaviors, except for general motor abilities (Hull *et al.*, 1991, Moses *et al.*, 1995). However, dopaminergic action in the Acb may be involved in anticipatory sexual behavior, such as increased locomotor activity and erections in response to female cues, independent of motor effects (Pfaus and Phillips, 1991, Liu *et al.*, 1998). In addition, the Abc is important in pair bonding and mate-cue association, as evidenced by work in the voles (Gingrich *et al.*, 2000, Aragona *et al.*, 2006). Thus, dopamine in the MPOA, Acb, or both regions may be important for conditioned place preferences to VS.

Testosterone and sexual reward

Like dopamine, testosterone probably has multiple sites and mechanisms of action in contributing to social reward. Several studies have demonstrated that long-term (2-8 weeks) gonadectomy results in an increase in several measures of dopaminergic tone in the MPOA, including tissue content and amphetamine-induced dopamine release, but a decrease in extracellular dopamine (Engel *et al.*, 1979, Simpkins *et al.*, 1983, Gunnet *et al.*, 1986, Mitchell and Stewart, 1989, Du *et al.*, 1998). These changes occur without a corresponding change in dopaminergic cell number in the MPOA or the A14 periventricular cells (Du and Hull, 1999). Importantly, MPOA dopaminergic responses to female stimuli in male rats are also modulated by testosterone (Hull *et al.*, 1995, Putnam *et al.*, 2001).

While effects of castration in the ventral striatum are less consistent than those in the MPOA, 28 day gonadectomy generally reduces dopamine and DOPAC concentrations in Acb tissue (Alderson and Baum, 1981, Baum *et al.*, 1986, Mitchell and Stewart, 1989). In the mPFC, GDX causes a decrease in TH innervation and extracellular dopamine when measured 4 days after hormone absence, but causes an increase in the same measures 28 days after hormone absence (Aubele and Kritzer, 2011b). Interestingly, the mPFC is also the most androgen receptor-enriched source of input to the VTA and, as the VTA itself has few androgen receptors, may indirectly impart hormone sensitivity to the VTA dopaminergic cell population (Aubele and Kritzer, 2011a). Finally, 30, but not 14 days of gonadectomy causes an increase in the number of TH-ir cells in adult male rats (McArthur *et al.*, 2007, Johnson *et al.*, 2010). Taken

together, the withdrawal of gonadal hormones affects dopamine systems in site specific ways.

The above studies are informative as to the hormonal sensitivity of a brain region, and suggest possible brain regions in which long-term testosterone absence could induce functional deficits that prevent adult hamsters from showing a CPP to VS. However, the application of these studies in understanding mechanisms by with testosterone treatment permits the display of a CPP to VS in juvenile hamsters is limited by several factors. Most of the above studies assessed the effects of gonadal-hormone removal, which can occur in different temporal patterns that gonadal-hormone exposure or replacement. In one study, sexual behavior was recovered after 10 days of testosterone treatment in males castrated for 21 days (Putnam *et al.*, 2001). Moreover, the above studies were performed in adult animals; as neural structures are generally more plastic and responsive to the effects of gonadal-steroids earlier in development, the effects of testosterone exposure in juvenile animals may be different from those in adults (Schulz *et al.*, 2009).

Some insights into testosterone and dopaminergic action in VS reward can also be gained from comparisons between juvenile and adult hamsters. Upon VS application to the nose in mineral oil, juvenile hamsters showed Fos immunoreactivity indicative of D2 receptor activation in corticolimbic regions (Chapter 4). This Fos response to VS was observed in both blank and testosterone treated gonadectomized juveniles, suggesting that testosterone was not modulating dopaminergic activity there. In a separate study, gonad-intact juveniles failed to show adult-typical dopamine responses to VS in the MPOA (Schulz *et al.*, 2003). While it is not clear whether or not

this preoptic area response to VS is dependent on testosterone, the medial preoptic area is known to be hormone responsive, and thus is a good candidate for testosterone-facilitated, dopamine-dependent, reward.

Conclusion

Taken together, these studies demonstrate the importance of testosterone and dopamine in rewarding responses to an unconditioned social stimulus. Both testosterone and dopamine systems mature during adolescence, when VS reward is typically acquired. It should be noted that the dopaminergic circuit could be functional in juvenile animals to mediate CPP to VS, but that testosterone-dependent maturation of some other neural circuitry is also necessary for VS reward. Opioids have been implicated in sexual reward, but the literature on hormone-sensitively is less clear. However, the most parsimonious explanation, given the supporting evidence, is that testosterone treatment in juvenile animals mimics the normative elevation in pubertal testosterone, which in turn affects the dopaminergic system to permit VS reward.

Chapter 6: Discussion

Experiments contained in this dissertation identify an adolescent shift in behavioral and neural responses to an unconditioned chemosensory social reward, female hamster vaginal secretions. The present set of experiments demonstrates both hormone dependent and independent aspects of adolescent and pubertal development: we have observed 1) age, but not testosterone, dependent changes in mesocorticolimbic Fos responses to VS; and 2) testosterone, but not age, dependent CPP to VS. The testosterone-dependent CPP for VS is also dependent on dopamine receptor activation. The normal rise in testosterone during adolescence likely permits the rewarding response to VS in adulthood, as recent exposure to testosterone is necessary for VS CPP then, as well. The various responses to VS in testosterone-treated juveniles in these studies, i.e., the absence of a Fos response to VS in VTA TH neurons, and the efficacy of the dopamine receptor antagonist to block a CPP for VS, may seem contradictory at first. However, upon further reflection on the nuances of Fos expression and dopamine receptor activation, the studies cohesively imply that the balance of dopamine receptor activation in response to VS shifts from being D2-biased in juveniles, to D1-biased in adults.

The logic underlying the proposition that D2 receptor activation is the predominant response to VS in juvenile hamsters is as follows. Dopamine must be released from terminals in some motivationally-relevant brain region in testosterone-treated juveniles in response to VS in mineral oil, or else haloperidol would not have blocked the CPP to VS. However, there is no obvious evidence of activation of TH

neurons by VS, either in the VTA of testosterone-treated juveniles that received VS on their noses (Chapter 4), or in the PVN/PeVN, SuM, or MeP of adult or juvenile hamsters (Chapter 3, appendix). As the regions (to our knowledge) include the populations of dopaminergic cells that project to the mPFC, Acb, and/or MPOA and are relevant to behavioral responses to VS, either it appears that dopaminergic responses to VS in juveniles are not detected as increases in Fos/TH-immunoreactivity. Failure to detect juvenile dopamine responses to VS with Fos/TH double label could occur in (at least) two ways. The first possibility is that the juvenile response to VS occurs in a sub-population of TH cells that projects to a specific downstream target. In the VTA, such sub-populations tend to be spatially diffuse throughout the region, and in this case a Fos response within this specific sub-population could be lost in a low signal-to-noise ratio within the whole-VTA analysis. The second possibility is simply that Fos expression is not an index of TH cell activation and/or is not a correlate of dopamine release. This second option is logically appealing for several reasons that are detailed below.

Fos expression in TH-ir cells may not always correlate with dopamine release, for several reasons. Dopaminergic release in response to VS in juveniles is suggested by the observed reductions in Fos in PFC and Acb in response to VS that may be the result of D2 receptor activation via their association with inhibitory G-proteins, as discussed in Chapter 4. D2 receptor activation in response to VS is also suggested by the effectiveness of haloperidol to block a CPP to VS in Chapter 5. As mentioned previously, D2 receptor activation can occur in response to tonic levels of dopamine release, when DA cells are firing at relatively low (not coordinated, phasic) rates (Richfield *et al.*, 1989, Goto *et al.*, 2007). These tonic dopamine levels may result from

graded increase in dopaminergic cell activity, above baseline but not reaching phasic rates, or glutamate evoked release of dopamine from local axon terminals (David *et al.*, 2005), in response to VS in juveniles. These mechanisms of dopamine release may be correlated with an increase in expression of some other immediate early gene, or is insufficient to evoke Fos expression in TH neurons. Our theoretical understanding of Fos expression emphasizes this latter point. Fos expression is the result of cellular metabolic processes and changes in gene expression in response to some input, and does not reflect changes in membrane potential (Herdegen and Leah, 1998). In other words, Fos cannot indicate neural activity, moment to moment. Instead, it reveals the residual effects of whatever the cell experienced 60 minutes ago. Thus, the absence of Fos does not indicate a lack of cellular activity, or in our case, dopaminergic responses to VS in juvenile hamsters.

This nuanced appreciation of Fos expression does not limit our ability to interpret all Fos/TH measures. While the absence of a response does not indicate the lack of cellular activity, the presence of Fos/TH response to VS probably does indicate that a cell released dopamine. Many studies have demonstrated an increase in Fos/TH-ir that occurs in contexts that also cause an increase in dopamine release, including sexual behavior, electrical intracranial self-stimulation, and drug exposure (Asmus, 1994, Hunt and McGregor, 1997, Shim *et al.*, 2000, Thiele *et al.*, 2000, Balfour *et al.*, 2004). Phasic dopamine release might require increased TH (or some other gene product) synthesis to keep up with all the energy needed for generation of action potentials, fill depleted stores of dopamine, etc. This more robust increase in action potential firing and dopamine release would also activate D1 receptors downstream, and cause an

increase in Fos in the Acb and mPFC (Herdegen and Leah, 1998, Goto *et al.*, 2007). Importantly, we observe these both an increase in Fos/TH in VTA cells, and an increase in Fos in corticolimbic areas in adult animals in response to VS when allowed voluntary approach and investigation of VS (Chapter 3). Therefore, while further study is needed to confirm these findings, the current studies support the activation of VTA cells release of corticolimbic dopamine in response to VS

Even though we cannot determine exactly how much dopamine is being released in response to VS in juvenile animals, we can still compare Fos responses across ages to determine that juveniles are not showing adult-like responses to VS. Taken together, these studies suggest an adolescent maturation in mesocorticolimbic circuitry to allow for a shift in dopaminergic responses to VS towards D1 receptor activation in adulthood. It is worth noting that a relatively small proportion of TH-IR cells are activated by VS in adulthood. While somewhat unsatisfying, this seems to be a common finding in many species and dopaminergic cell populations in copulatory behavior (Balfour *et al.*, 2004, Bharati and Goodson, 2006, Vittoz *et al.*, 2008, Northcutt and Lonstein, 2009). Nonetheless, activity in even a small population of cells can be behaviorally-relevant, as these cells are known to have extensive dendritic branching that can amplify effects of cellular activity (Lindvall and Bjorklund, 1978, Arbuthnott and Wickens, 2007).

Age-dependent maturation of D1:D2 responses to VS.

Given the number of known adolescent changes in mesocorticolimbic circuitry, there are many possible mechanisms by which responses to VS could shift to favor corticolimbic D1 activation across adolescence. We can rule out some possibilities by interpreting

reward responses that are present in juvenile animals. For example, CPP to cocaine is intact in juvenile male hamsters (Chapter 2), and may actually be enhanced in juvenile rats compared to adults (Brenhouse *et al.*, 2008). In both juvenile and adult rats, CPP to cocaine seems to be more dependent on D1 than D2 receptor activation, as indicated by selective D1 receptor optogenetic activation promoting the CPP (Lobo *et al.*, 2010). Thus, D1-dependent neural circuitry is sufficiently mature and functional in juvenile hamsters, and itself not the reason for differential Fos responses to VS across adolescence. However, continued development of the mesocorticolimbic system might still shift the balance of receptor activation, and dopaminergic responses specific to VS may still only be sufficient to activate D1 responses in adult hamsters.

We begin to explore these mechanisms at the source of dopamine in the VTA. Here, greater activation of dopaminergic cells would favor D1 receptor activation downstream because of the more robust release in dopamine. There is little evidence in the literature for hypo-responsive dopaminergic cells during adolescence: most studies suggest that basal firing rates are achieved by 20 days of age, while some purport a peak in firing rates mid adolescence but with similar rates early adolescence and in adulthood (Pitts *et al.*, 1990, Tepper *et al.*, 1998, Marinelli *et al.*, 2006). No studies, to my knowledge, have investigated the possibility of increased MPOA or amygdalar projections to the VTA during adolescence that could relate to increased activation of transmission of VS-relevant input. However, if we can apply our Fos expression data to compare juveniles and adults, we see VTA cells in juveniles and adults actually respond to VS in similar ways. Voluntary investigation of VS induces Fos expression in non-dopaminergic cells in the PN, and dopaminergic cells in the PN

and PBN in both ages, while nose application of VS does not. The one cell group that differs between juveniles and adults in its response to VS in the VTA is GABAergic cells in the IF, that responds to VS only in adults regardless of exposure method. However, there are relatively few GABAergic projections to corticolimbic sites, and GABA typically serves to reduce dopaminergic cell activity. Therefore, neither dopaminergic nor non-dopaminergic cell populations in the VTA likely serve as the neural correlate for changes in circuitry responses to VS during adolescence.

Two primary targets of dopaminergic innervation, the accumbens and prefrontal cortex, also show dramatic changes during adolescence that could account for increased D1 receptor activation. In the accumbens, while both basal and electrically stimulated levels extracellular dopamine peak mid adolescence, adults show greater levels of these measures and TH expression than do P30 juveniles, a comparison similar to ages used in this dissertation (Philpot and Kirstein, 2004, Marinelli *et al.*, 2006, Walker and Kuhn, 2008, Mathews *et al.*, 2009, Philpot *et al.*, 2009). In the mPFC, dopaminergic innervation increases progressively until P50 – 60, as evidenced by TH fiber density (Kalsbeek *et al.*, 1988, Benes *et al.*, 2000). Recently, fast-scan cyclic voltametry has been used to study dopamine transients that provide high temporal resolution as to burst firing of dopaminergic cells (Robinson *et al.*, 2011). In the Acb core, P28 juvenile and P70 adult animals show similar incidents of spontaneous and same-sex social-contact induced bursting. However, adults show greater burst firing in response to unconditioned odor, visual, and auditory cues. Thus, in both the Acb and PFC, more robust dopamine innervation and burst firing could preferentially activate D1 receptors in adulthood.

In addition to an increase in dopaminergic innervations, there are adolescent changes in glutmatatergic projections that could affect neural responses to VS. Basolateral amygdalar input to the mPFC increases during adolescence (Cunningham *et al.*, 2002), which could indirectly relay chemosensory information from the MeP to induce glutamate release in response to VS. Also, there is a dramatic increase in glutamatergic projection from the mPFC to the Acb during adolescence (Brenhouse *et al.*, 2008). This increase in glutamate is if interest because of its ability to induce local release of dopamine from synaptic terminals in the Acb and mPFC (David *et al.*, 2005). Thus, greater glutamate release in response to VS in corticolimbic sites could evoke phasic dopamine release, which could activate D1 receptors only in adult hamsters. Lower levels of both glutamate and dopamine innervations in corticolimbic sites could be associated with tonic dopamine that preferentially activates D2 receptors in juvenile hamsters.

Coincident with these increases in dopaminergic tone, there is also a shift in dopamine receptor expression. In the Acb, prior to P28 in male rats, D2 receptors are almost twice as prominent as D1 receptors; by P60, D1 receptors are expressed slightly more than D2 receptors (Tarazi and Baldessarini, 2000). In the mPFC, D2-like autoreceptors are present in juvenile but not adult male rats (Andersen *et al.*, 1997b); as autoreceptors typically serve to modulate DA update and release, the lack of this feedback in adult animals could allow for a more prolonged or robust dopamine release, thereby activating D1 receptors in adulthood. In summary, there are multiple mesocorticolimbic changes during adolescence, including an increase in amygdalar

input, an increase in dopaminergic innervation, and a shift in dopamine receptor expression, that could serve as mechanisms for adult-specific Fos responses to VS.

Testosterone-dependent CPP to VS

We have observed that one week of circulating testosterone is sufficient to allow for formation of a CPP to VS in juvenile hamsters. At the same time, pilot studies suggested that gonadectomized adult hamsters can form a CPP to VS up to at least nineteen days following removal of testosterone, and it is only long term gonadectomized hamsters that fail to form a CPP to VS. Testosterone and gonadectomy could be affecting the gain and loss of CPP to VS via the same mechanism at a different rate, or via different mechanisms in juveniles and adult animals. Perhaps the long-term (28-56 day) effects of castration to reduce Acb tissue content (Alderson and Baum, 1981, Mitchell and Stewart, 1989) correlate with the loss of CPP to VS in 10 week gonadectomized adult males in Chapter 5. Similarly, long-term castration-induced increases TH innervation of the mPFC in male rats (Aubele and Kritzer, 2011b) may relate to the loss of CPP to VS in gonadectomized adults. However, the relationship of an increase in mPFC TH and a decline in performance on mPFC and dopamine-dependent cognitive tasks is still unclear (Kritzer *et al.*, 2007).

There are many examples of gonadal hormones regulating dopaminergic circuitry so as to allow for rewarding responses to drugs; however many of them concern estradiol in female rats (Festa and Quinones-Jenab, 2004, Becker and Hu, 2008). For example, cocaine sensitivity and amphetamine sensitization are regulated by estradiol in ovariectomized female rats (Forgie and Stewart, 1994). While T could theoretically be

aromatized to E in males, E does not sensitize males' responses to cocaine in the same manner (Jackson *et al.*, 2005). This result, coupled with the findings that gonadectomy does not affect cocaine or amphetamine sensitivity in adult males (Robinson *et al.*, 1982, Becker, 1999, Becker *et al.*, 2001a, Hu and Becker, 2003, Russo *et al.*, 2003), reduces the likelihood of that T is aromatized to E to affect mesolimbic responses to dopaminergic drugs. However, testosterone withdrawal could affect responses to VS differently than it affects responses to dopaminergic drugs.

The studies on the effects of gonadectomy on dopaminergic tissue described throughout this dissertation may not be informative as to the mechanism behind the gain in CPP to VS with testosterone treatment in juveniles. As stated previously, the temporal pattern in functional gains after testosterone replacement can be quite different from the loss of function after testosterone removal. However, our results in Chapter 4 can indicate some sites of testosterone action in responses to VS. Specifically, both blank and testosterone treated juveniles showed a reduction in Fos in response to VS, suggesting that testosterone is likely not affecting the overall balance of D1/D2 receptor activation in the mesocorticolimbic system. While our Fos analysis may not be sensitive enough to detect hormonal effects in specific cell types or populations, these current results and a survey of the literature suggest that testosterone is modulating neural function outside the mesocorticolimbic system. Specifically, I propose that testosterone is modulating dopaminergic function in the MPOA for several reasons. The MPOA Fos response to VS is testosterone dependent (Fiber and Swann, 1996), as is dopaminergic activity within the area (Hull *et al.*, 1995). Gonadectomy tends to reduce MPOA extracellular DA while increasing intracellular DA in the

terminals, but only after 20-30 days of hormone absence (Engel *et al.*, 1979, Simpkins *et al.*, 1983, Du *et al.*, 1998, Du and Hull, 1999). Importantly, one study that showed gradual and synchronous decline in both precopulatory dopamine responses to female cues and sexual behavior over 21 days, and then partial and full recovery of both dopamine responses and sexual behavior after 5 and 10 days of testosterone treatment (Putnam *et al.*, 2001). The gradual decline and rapid recovery parallel observations in this dissertation of CPP to VS. Therefore, while MPOA has not been previously implicated in CPP for reward, its hormone sensitivity, dopaminergic function, and responsiveness to VS makes the MPOA an excellent candidate for the effects of T in facilitating a CPP to VS.

It should be noted that conclusions about the sources of this MPOA dopamine are incomplete. The TH-ir cells in the MeP, PVN, and SuM all fail to show Fos responses to VS in either juveniles or adults. However, as we know from earlier discussions about Fos/TH-ir cells in the VTA, the absence of Fos/TH expression does not preclude the release of dopamine from terminals. Indeed, like the corticolimbic sites, dopamine release can be regulated at the terminals by glutamate in the MPOA. This MPOA effect occurs via a glutamate-induced increase in local nitric oxide which then causes the release of dopamine from terminals (Dominguez and Hull, 2001, Dominguez *et al.*, 2004). Importantly, testosterone regulates nitric oxide synthase, the enzyme used to produce nitric oxide (Du and Hull, 1999). However, the effect of testosterone has only been shown by gonadectomizing animals and observing a decline in the enzyme (Putnam *et al.*, 2005). Therefore, further study is needed to demonstrate the ability of testosterone to upregulate nitric oxide in juvenile animals.

Disconnect between VS reward and sexual behavior

When we began this series of studies, we had initially hypothesized that as VS became rewarding to the hamster, it would promote sexual behavior, thus explaining the gain in sexual behavior across adolescence. However, these studies demonstrate that VS can be rewarding, without the associated increase in sexual behavior, in juvenile males treated with testosterone. As such, the question remains: why do juveniles hamsters fail to show adult-like sexual behavior when treated with testosterone and with functional gonadal hormone receptors and enzymes (Meek *et al.*, 1997, Romeo *et al.*, 1998)? Below, I describe several possible explanations for minimal sexual behavior in juvenile animals. While no single factor can explain the behavioral transition, they may all contribute in some degree.

As juvenile hamsters tend to show more submissive and fear-related behaviors than adults, it is possible that females (even though they maintain a strict lordosis posture for most of the interaction) are perceived as an aversive or anxiogenic stimulus. To test this in a small pilot study, we determined if sexual behaviors in testosterone treated juvenile hamsters could be increased by treatment with diazepam, an anxiolytic previously shown to reduce anxiety like behavior in an elevated plus maze and to reduce submissive behaviors in a conditioned defeat paradigm in male hamsters (Yannielli *et al.*, 1996, Moise *et al.*, 2008, Gannon *et al.*, 2011). Four groups of animals were used: three groups were gonadectomized and given testosterone pellets one week prior to behavior tests on P31, while a fourth group remained gonad intact. Two of the GDX+T groups received injections of diazepam (2mg/kg and 5mg/kg, ip, doses shown to be affective in above studies), while the other two groups received vehicle injections

30 minutes prior to test. Sexually experienced and hormonally-primed females were used as stimuli. Unfortunately, due to video recording malfunction, only data from three different live-scorers are available; therefore, only qualitative descriptions of the results are presented here. In general, both testosterone and diazepam treatment reduced defensive-like behaviors, including flank-marks and escape-dashes. However, neither dose of diazepam paired with testosterone increased sexual behaviors like mounts and intromissive-like behaviors. Therefore, while testosterone may serve to reduce aversive responses to females and dis-inhibit the display of sexual behavior, some other factors are necessary to promote the behaviors.

These behavior tests did reveal another possible, though less exciting, explanation for minimal sexual behavior in juvenile hamsters. As the male:female body weight ratio increased, number of mounts also increased. Thus, it is possible that juvenile males, who weighed 50-70g in this study, simply have trouble positioning themselves appropriately at the females' hindquarters (who weighed 130-160g in this study), to allow for an adult-like mount. However, more exhaustive tests are needed to confirm this possibility. The larger males could also be further along in their pubertal development, and would have shown more sexual behavior regardless the size of the female. To best assess this possibility, juvenile males of similar body should be tested with relatively smaller females. Again, body size ratio may be one of several factors allowing the promotion of sexual behavior. However, it certainly cannot explain immature Fos responses to VS in testosterone-treated juveniles.

One other interesting possibility for adult-specific sexual behavior is suggested by increases in luteinizing hormone in response to female urine in adult but not juvenile

male mice (Maruniak *et al.*, 1978). Luteinizing hormone release is promoted by gonadotrophin releasing hormone (GnRH) cells scattered throughout the hypothalamus that project to the median eminence (Sisk and Foster, 2004). GnRH cell size (but not number) increases during adolescence (Urbanski *et al.*, 1992), perhaps explaining differential LH responses to female cues during adolescence. In addition, prepubertal hamsters are more sensitive to negative feedback actions of testosterone to reduce GnRH release (Sisk and Turek, 1983). Therefore, testosterone treatment reduces GnRH release in juvenile hamsters, instead of increasing GnRH release in adult animals. This may affect sexual behavior in a meaningful way, as GnRH cells also project through the BNST, Me, and MPOA to facilitate sexual behavior in adult male hamsters whose VNO had been removed (Lehman *et al.*, 1987, Meredith and Howard, 1992). Therefore, the lack of GnRH activation in response to testosterone may explain low levels of sexual behavior prior to adolescence and the disinhibition of the GnRH cells. GnRH is known to amplify Fos responses to VS in the MPOA and MeP (Westberry and Meredith, 2003), regions previously observed to show adult-like Fos expression in response to VS in juvenile animals. It is possible that quantification of Fos expression cannot detect differences between juveniles and adults in VS-induced neural activity, or GnRH may have actions outside these brain regions that have not yet been studied, including mesocorticolimbic regions implicated in this dissertation.

An additional insight into the disconnect between VS reward and sexual behavior comes from the Fos expression patterns in response to VS that are age, but not testosterone, dependent. This pattern does not perfectly align with CPP responses to VS, which can be induced by testosterone prepubertally. Instead, the pattern matures

across adolescent development, independent of circulating testosterone, as does the expression of sexual behavior. Distinct aspects of sexual behavior have also been observed to parallel Fos expression in female hamsters when challenged by food deprivation (Klingerman *et al.*, 2011). In that study, food deprivation reduced appetitive behaviors like vaginal marking and male preferences, while copulatory behavior, mating-induced CPP, and Acb Fos responses to mating were unaffected. Rewarding qualities of VS may in fact, represent an appetitive sexual behavior, as does anogenital investigation. Thus, Fos responses to sexual stimuli may reflect the motivational drive to perform sexual behaviors.

The interpretation of Fos as an indicator for sexual drive is in agreement with our understanding of Fos as an overall readout of mental state. VS reward may be necessary, but not sufficient, for sexual behaviors; the adolescent shift in dopamine receptor activation towards D1, discussed above, might also be necessary for motivated behavior (Seamans and Yang, 2004). Indeed, the mesolimbic system parallels and is interconnected with the nigrostriatal circuit, known to be so important for motor output (Sesack and Grace, 2009). Moreover, the core of the accumbens, where we have observed Fos responses to VS, is more connected with the substantia nigra and ventral pallidum than is the shell. Therefore, Fos expression in the mesocorticolimbic system may best represent the mental state of an animal prepared to execute a behavior in response to a cue. Accordingly, only adult animals show mesocorticolimbic Fos in response to VS, and only adult animals show sexual behavior. In contrast, amygdalar Fos responses to VS may represent chemosensory detection and perception, and are similar between juvenile and adult animals.

Adolescence and reward

Effects of adolescent development on reward-seeking have been observed in practically every form of drug and natural reward, but not always along the same temporal pattern (Doremus-Fitzwater *et al.*, 2010). Sensitivity to cocaine is generally greater in adolescents compared to adults, and the age of peak sensitivity somewhere between P21-P40 (Laviola *et al.*, 1992, Badanich *et al.*, 2006, Brenhouse *et al.*, 2008). On the other hand, sensitivity to amphetamine, also a dopamine-dependent stimulant, is greater in adulthood than in adolescence at low doses, and vice versa at high doses (Tirelli *et al.*, 2003, Mathews *et al.*, 2009). Reward sensitivity to nicotine is consistently greater in adolescence than in adulthood (Vastola *et al.*, 2002, Belluzzi *et al.*, 2004, Torrella *et al.*, 2004), while sensitivity to ethanol has been more difficult to determine because of age-specific responses to aversive components of the drug and dose-dependent effects (Philpot *et al.*, 2003, Dickinson *et al.*, 2009). Characterization of adolescent changes in drug reward is complicated by doses, testing paradigms, and aversive components of the stimuli.

In contrast to drug reward, sensitivity to natural rewards, sucrose and social interactions with a same-sex conspecific, is consistently heightened during adolescence compared to adulthood (Douglas *et al.*, 2004, Wilmouth and Spear, 2004, Varlinskaya and Spear, 2008, Friemel *et al.*, 2010). However, the sensory modality used to detect the reward (e.g. olfactory vs visual input), may produce reward-specific temporal patterns due to maturation of neural connectivity between the particular sensory systems with brain regions important in motivation. Indeed, one possible neural

mechanism of a gain in VS reward during adolescence is MeP input to the mPFC and Acb.

Together, this literature demonstrates the importance in studying a range of motivated stimuli. Male hamster response to VS is an excellent model with which to study changes in adolescent reward for several reasons: 1) they are unconditioned, and does not require learning or sensitization; 2) they are hormone sensitive, a feature useful to tap into behavioral changes that occur during a period of hormonal change; and 3) they are socially relevant, without the confound of a stimulus animal whose behavior could depend on that of the experimental subject. As social behaviors show the most dramatic changes during adolescence, and many other risk-taking behaviors are ultra-sensitive to social input during adolescence, this third feature is perhaps the most important.

APPENDIX

Appendix: Select populations of dopaminergic cells do not show an immediate early gene response to VS

Introduction

In addition to the ventral tegemental area (VTA) dopaminergic cell population discussed in the preceding pages, tyrosine hydroxylase (TH)-ir cells also reside in the hamster posterior medial amygdala (MeP), bed nucleus of the stria terminalis (BNST) and medial preoptic area (MPOA). Dopamine beta-hydroxylase and phenylethanolamine N-methyltransferase are not expressed in these regions, indicating that norepinephrine and epinephrine are not the final end-products of TH activity (Vincent, 1988, Asmus *et al.*, 1992, Asmus, 1993). The MeP and BNST are also immunoreactive for dopamine, confirming their identity as dopaminergic cells (Asmus *et al.*, 1992, Asmus, 1993). However, the MPOA TH-ir cell population does not contain L-amino acid decarboxylase and is not dopamine-ir, suggesting that L-3,4-dihydroxyphenylalanine, and not dopamine, is the end product of TH activity (Vincent and Hope, 1990, Asmus, 1993). Like rats, hamsters also express TH (and not dopamine beta-hydroxylase and phenylethanolamine N-methyltransferase) in the periventricular nucleus (PeVN), paraventricular nucleus (PVN), posterior hypothalamus (PH), and supramammilary nucleus (SuM) (Vincent, 1988).

Chapter 1 previously emphasized the importance of these regions in sociosexual behavior: the MeP is an important site for chemosensory cue and gonadal hormone integration (Wood and Coolen, 1997); the PVN is important in sexual arousal in response to female cues, and interconnected with the mesolimbic dopamine circuit

(Succu et al 2007); and the PH/SuM are perhaps extensions of the VTA (Ikemoto 2010). In addition, these regions may be sources of dopaminergic input to the medial preoptic area (Miller and Lonstein, 2009 and Lookingland and Moore 1984). Some work has characterized responses of the MeP and PVN TH-ir cells to hamster sexual behavior (Asmus et al 1994), however, little is known about responses specific to VS exposure. Therefore, this appendix contains data from the MeP, PVN, PH, SuM from animals used in Chapter 3, but were not submitted for publication with Chapter 3 data.

Methods

The methodology is exactly the same as that in Chapter 3, except for details specific to the brain regions below.

In the MePD and MePV, contours were drawn as in Chapter 3. Fos-ir cells were presented in the preceding chapter, so only single-labeled TH and double-labeled Fos/TH data are given here. Because so few cells were observed (0-3 per animal), the number of single-labeled TH or double-labeled Fos/TH-ir cells in all Pd and PV sections were summed within an animal.

The PVN was partitioned in two different ways. In the first, all tissue sections containing the PVN (Bregma -0.3 to -1.5mm, 6-8 sections per animal) were traced in Nissl-stained sections to also include some adjacent PeVN, and were overlaid onto immunohistochemistry sections. Additionally, a smaller subsection of the PVN was quantified to parallel analyses of PVN Fos responses to sexual behavior in a previous study (Kollack-Walker and Newman, 1997). Therefore, a smaller section of the PVN including the magnocellular cell group along the ventrolateral edge of the previously

drawn traces, was re-quantified in two sections per animal and 4 animals per group; these data are also shown below. In both portions of the PVN, the number of single-labeled Fos-, single-labeled TH-, and double-labeled Fos/TH-ir cells was divided by the area (mm^2) of the contour in each section to create a density measurement; density was used for analysis and presentation here.

For both the PH and SuM, a $0.064mm^2$ rectangular contour was placed in the middle of the section just above and below the supramammillary decussation, respectively, in two anatomically matched immunohistochemistry tissue sections (Bregma -3.2 and -3.5mm). As in the PVN, density of single-labeled Fos-, single-labeled TH-, and double-labeled Fos/TH-ir cells were analyzed and presented.

Results

In the MeP, a main effect of age was observed on the number of TH-ir cells ($F_{(1,26)} = 9.30$, $p < 0.01$), such that adults had a larger number of cell than did juveniles (Figure A.1). Likewise, a main effect of age was observed on the number of Fos/TH-ir cells ($F_{(1,26)} = 24.67$, $p < 0.01$), such that adults expressed more than juveniles. No effects of swab or age x swab interactions were observed on TH-ir or Fos/TH-ir cell number. In adults, approximately 50% of the TH-ir cells were double-labeled.

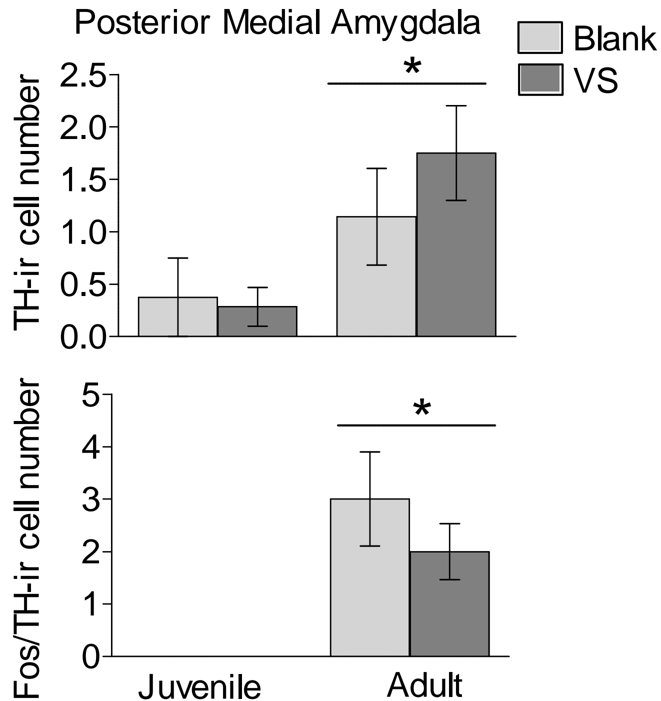


Figure A.1. Total number of TH-ir and Fos/TH-ir cells in the posterior medial amygdala, Mean +/- SEM, * $p < 0.01$. Adults expressed more TH-ir and Fos/TH-ir cells than did juveniles.

In the full quantification of the PVN, main effects of age were observed on Fos-ir, TH-ir, and Fos/TH-ir cell density ($F_{(1,27)} = 52.07$, $p < 0.01$; $F_{(1,27)} = 33.60$, $p < 0.01$; $F_{(1,27)} = 17.48$, $p < 0.01$, respectively), such that adults expressed more of each cell type than did juveniles (Figure A.2). In the more limited quantification of the magnocellular PVN cell group, similar main effects of age were observed on Fos-ir, TH-ir, and Fos/TH-ir cell density ($F_{(1,12)} = 5.93$, $p < 0.05$; $F_{(1,12)} = 15.29$, $p < 0.01$; $F_{(1,12)} =$

5.58, $p < 0.05$, respectively), such that adults expressed more of each cell type than did juveniles (Figure A.2). In addition, a main effect of swab was observed on Fos-ir cell density ($F_{(1,12)} = 10.19$, $p < 0.01$), such that VS-exposed hamsters expressed more Fos than blank-swab exposed hamsters. This finding replicated the Fos response to VS in adult males observed in Kollack-Walker and Newman, 1997, which was the attempt of this secondary partitioning of the PVN. No other effects of swab or age x swab interactions were found.

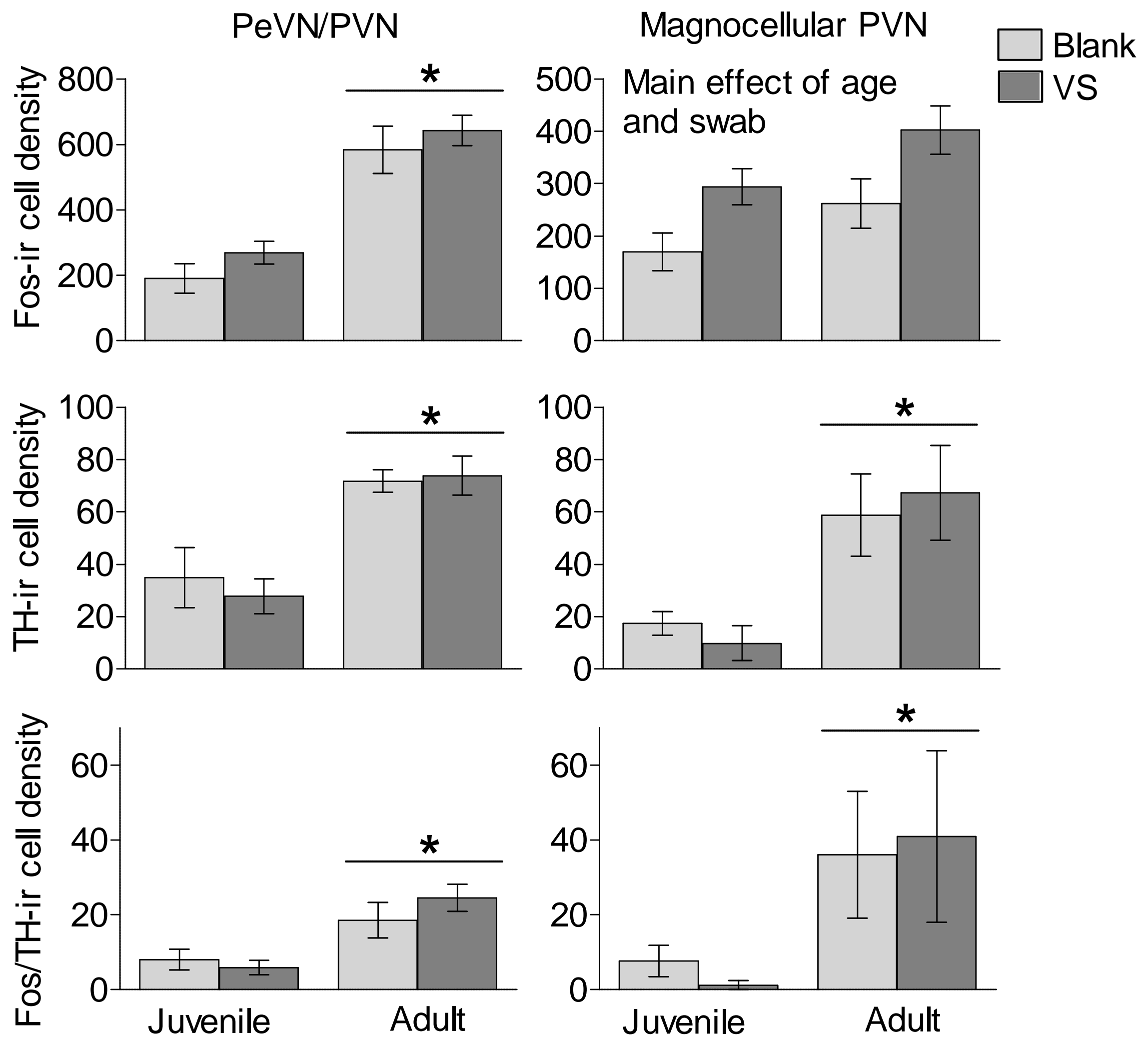


Figure A.2. Fos-ir, TH-ir, and Fos/TH-ir cell density in the full extent (left) or magnocellular region (right) of the paraventricular nucleus, Mean +/- SEM, * $p < 0.01$. Adults expressed greater Fos-ir, TH-ir, and Fos/TH-ir cell density than did juveniles in both portions of the region. In addition, VS-exposed hamsters expressed more Fos than did blank swab-exposed hamsters in the magnocellular region.

In the PH, a main effect of swab was observed on Fos-ir cell density ($F_{(1,26)}$ = 4.88, $p < 0.05$, such that VS-exposed hamsters expressed more Fos-ir cells than blank-swab exposed hamsters (Figure A.3). No other effect of swab, no effect of age and no age x swab interaction was found on Fos, TH, or Fos/TH-ir cell density in PH. In the SuM, a main effect of age was observed on Fos/TH-ir cell density ($F_{(1,26)}$ = 4.576, $p < 0.05$, such that adult hamsters expressed more Fos/TH-ir cells than juveniles hamsters (Figure A.3). No other effect of age, no effect of swab, and no age x swab interactions was found on Fos, TH, or Fos/TH-ir cell density in SuM.

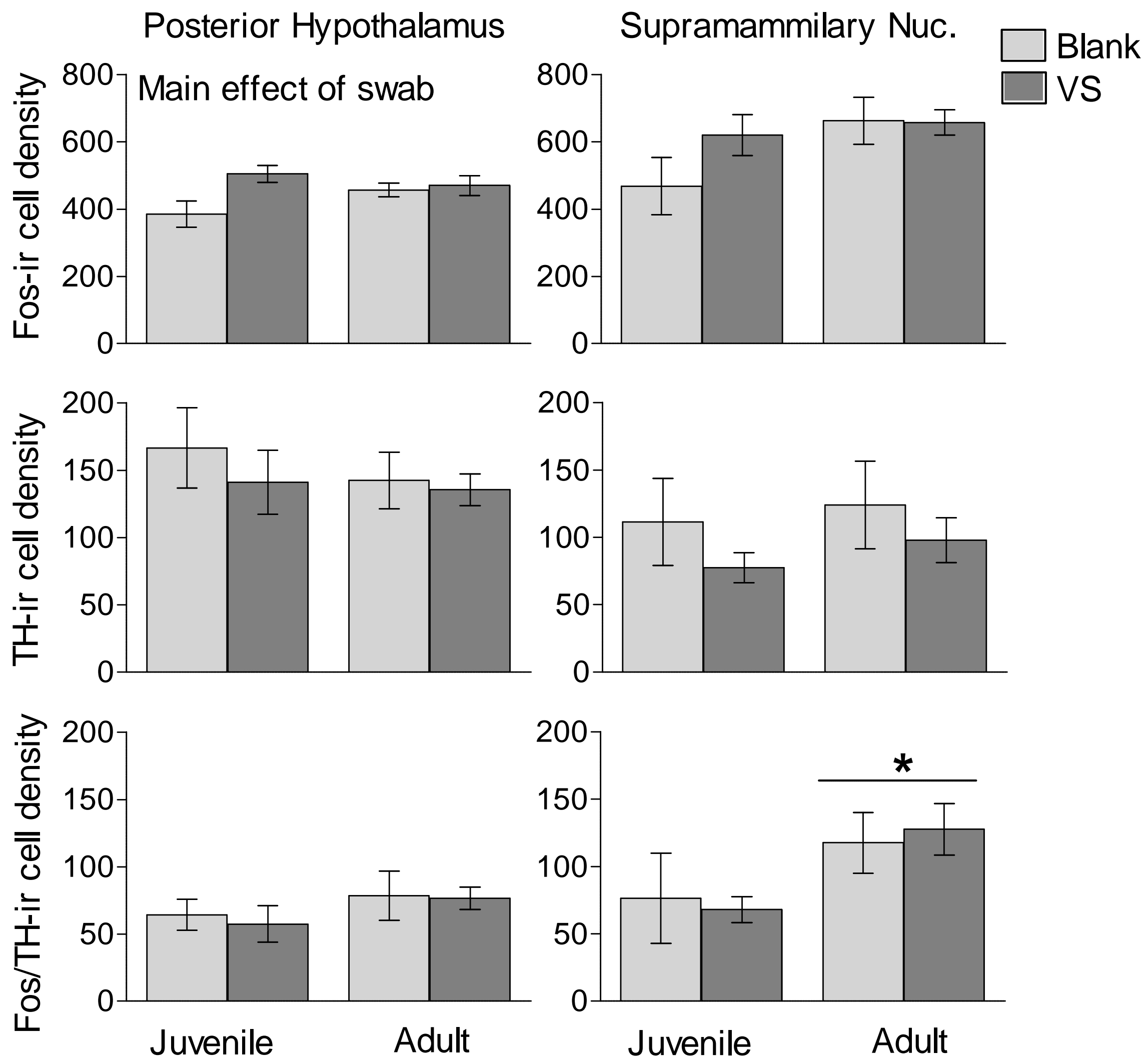


Figure A.3. Fos-ir, TH-ir, and Fos/TH-ir cell density in the posterior hypothalamus and supramammilary nucleus, Mean +/- SEM, * $p < 0.01$. Adults expressed greater Fos/TH-ir cell density than did juveniles in the supramammilary nucleus. In addition, VS-exposed hamsters expressed more Fos than did blank swab-exposed hamsters in the posterior hypothalamus.

Discussion

An increase in Fos/TH-ir cell expression with age was found in all but one of the brain regions analyzed in this study. A similar effect was observed previously in the MeP, such that approximately 50% of TH-ir cells were active in unhandled control male hamsters, leading the authors to suggest that Fos is involved in constitutive expression of TH (Asmus et al 1993; Hughes et al 1992; Herdegen et al 1993). Therefore, our data may indicate an increase in constitutively active dopaminergic cell population with adolescent development in the MeP, PVN, and SuM. In fact, the same effect was observed in the intrafascicular VTA, as demonstrated in Chapter 3.

The functions of these dopaminergic cell populations are not completely understood. The present study suggests that they are not involved in responses to VS, and are likely not the source of enhanced dopamine release in the MPOA upon VS exposure (Schulz et al 2003). This finding is consistent with those of previous studies that failed to functionally link MeP and PVN populations of TH cells to gonadal hormone manipulations (Asmus et al 1993) or sexual behavior (Asmus et al 1994). Instead, handling alone causes an increase of Fos/TH-ir in the PVN, which the authors attribute to arousal or stress functions of the region (Asmus et al 1994). Chronic stress from P28-35 does cause an increase in TH expression in MePD and BNST, but not in the PeVN (the PVN was not studied (Wommack *et al.*, 2004)). Dopamine in the PVN has been clearly implicated in erection (Melis and Argiolis, 2003), however, the TH cells analyzed in the present and Asmus et al 1994 study failed to co-express Fos in response to sexual behavior. It is possible that these analyses did not include the relevant A14 dopamine cells in the PeVN, which extends along the rostral and caudal

extent of the third ventricle. While the functions of these MeP, PVN, PH, and SuM dopaminergic cell populations are likely to be region-specific, they may play common roles in arousal states that increase during adolescence.

Fos expression, as a proxy for neural activation, was increased in response to VS only in the magnocellular portion of the PVN. This site-specific response to VS replicates previous observations (Kollack-Walker and Newman 1997), and this sub-region of PVN overlaps partially with sub-regions that contain oxytocin- and vasopressin-expressing neurons known to be important in female hamster sexual behavior (Morin and Blanchard, 1993, Albers and Bamshad, 1998) and penile erections (Argiolis and Melis, 2003). Thus, perhaps oxytocin responses to VS are of interest for future study, as oxytocin expression also increases during puberty in male rats (Chibbar *et al.*, 1990).

The present study demonstrated an increase in number of TH-ir cells between P28 to P56 in the MeP. However, this finding was opposite to that in Wommack et al 2004, that observed a decrease in TH expression in the BNST and MePd in unmanipulated control animals from P28 to P70. Perhaps one reason for this discrepancy is simply the small number of TH-ir cells in this region. Animals were not treated with colchicine in the present study, as that has been observed to affect Fos expression (Ceccatelli *et al.*, 1989); thus, the number TH-ir cells in the MeP are dramatically lower than that in other studies (Asmus et al 1992). The Wommack et al 2004 study observed more TH-ir cells than the present (3-7 per section, as opposed to ~1), and thus may be better able to detect changes across age.

Literature Cited

Literature Cited

Acquas E, Carboni E, Leone P, Di Chiara G (1989) SCH 23390 blocks drug-conditioned place-preference and place-aversion: anhedonia (lack of reward) or apathy (lack of motivation) after dopamine-receptor blockade? Psychopharmacology 99:151-155.

Acquas E, Di Chiara G (1994) D1 receptor blockade stero-specifically impairs the acquisition of drug-conditioned place preference and place aversion. Behavioral Pharmacology 5:555-569.

Adams JU, Careri JM, Efferen TR, Rotrosen J (2001) Differential effects of dopamine antagonists on locomotor activity, conditioned activity and conditioned place preference induced by cocaine in rats. Behavioural Pharmacology 12:603-611.

Adolphs R (2001) The neurobiology of social cognition. Curr Opin Neurobiol 11:231-239.

Adolphs R (2009) The Social Brain: Neural Basis of Social Knowledge. Annual Review of Psychology 60:693-716.

Afonso VM, Sison M, Lovic V, Fleming AS (2007) Medial prefrontal cortex lesions in the female rat affect sexual and maternal behavior and their sequential organization. Behav Neurosci 121:515-526.

Agmo A (2003) Unconditioned sexual incentive motivation in the male Norway Rat (Rattus norvegicus). Journal of Comparative Psychology 117:3-14.

Agmo A, Berenfeld R (1990) Reinforcing properties of ejaculation in the male rat: role of opioids and dopamine. Behav Neurosci 104:177-182.

Agmo A, Gomez M (1993) Sexual reinforcement is blocked by infusion of naloxone in to the medial preoptic area. Behav Neurosci 107:812-818.

Agmo A, Picker Z (1990) Catecholamines and the initiation of sexual behavior in male rats without sexual experience. Pharmacology, biochemistry, and behavior 35:327-334.

Agmo A, Villalpando A (1995) Central nervous stimulants facilitate sexual behavior in male rats with medial prefrontal cortex lesions. Brain Res 696:187-193.

Agustin-Pavon C, Martinez-Ricos J, Martinez-Garcia F, Lanuza E (2007) Effects of dopaminergic drugs on innate pheromone-mediated reward in female mice: A new case of dopamine-independent 'liking'. Behav Neurosci 121:920-932.

Aharon I, Etcoff N, Ariely D, Chabris CF, O'Connor E, Breiter HC (2001) Beautiful Faces Have Variable Reward Value: fMRI and Behavioral Evidence. Neuron 32:537-551.

Albers HE, Bamshad M (1998) Role of vasopressin and oxytocin in the control of social behavior in Syrian hamsters (Mesocricetus auratus). Prog Brain Res 119:395-408.

Albin RL, Young AB, Penney JB (1989) The functional anatomy of basal ganglia disorders. TRENDS in Neuroscience 12:366-375.

Alderson LM, Baum MJ (1981) Differential effects of gonadal steroids on dopamine metabolism in mesolimbic and nigro-striatal pathways of male rat brain. Brain Res 218:189-206.

Alexander GM, Packard MG, Hines M (1994) Testosterone has rewarding affective properties in male rats: implications for the biological basis of sexual motivation. Behav Neurosci 108:424-428.

Andersen SL, Dumont NL, Teicher MH (1997a) Developmental differences in dopamine synthesis inhibition by (±)-7-OH-DPAT. Naunyn-Schmiedeberg's Archives of Pharmacology 356:173-181.

Andersen SL, LeBlanc CJ, Lyss PJ (2001) Maturational increases in c-fos expression in the ascending dopamine systems. Synapse 41:345-350.

Andersen SL, Rutstein M, Benzo JM, Hostetter JC, Teicher MH (1997b) Sex differences in dopamine receptor overproduction and elimination. Neuroreport 8:1495-1498.

Andersen SL, Thompson AP, Krenzel E, Teicher MH (2002) Pubertal changes in gonadal hormones do not underlie adolescent dopamine receptor overproduction. Psychoneuroendocrinology 27:683-691.

Andersen SL, Thompson AT, Rutstein M, Hostetter JC, Teicher MH (2000) Dopamine receptor pruning in prefrontal cortex during the periadolescent period in rats. Synapse 37:167-169.

Aragona BJ, Liu Y, Yu YJ, Curtis JT, Detwiler JM, Insel TR, Wang Z (2006) Nucleus accumbens dopamine differentially mediates the formation and maintenance of monogamous pair bonds. Nat Neurosci 9:133-139.

Arbuthnott GW, Wickens J (2007) Space, time and dopamine. Trends Neurosci 30:62-69.

Arteaga M, Motte-Lara J, Velázquez-Moctezuma J (2002) Effects of yohimbine and apomorphine on the male sexual behaviour pattern of the golden hamster (Mesocricetus auratus). European Neuropsychopharmacology 12:39-45.

Asmus SE, Kincaid AE, Newman SW (1992) A species-specific population of tyrosine hydroxylase-immunoreactive neurons in the medial amygdaloid nucleus of the Syrian hamster. Brain Res 575:199-207.

Asmus SE, Sarah Winans Newman (1994) Colocalization of tyrosine hydroxylase and Fos in the male Syrian hamster brain following different states of arousal. J Neurobiol 25:156-168.

Asmus SESWN (1993) Tyrosine hydroxylase neurons in the male hamster chemosensory pathway contain androgen receptors and are influenced by gonadal hormones. The Journal of Comparative Neurology 331:445-457.

Aston-Jones G, Smith RJ, Moorman DE, Richardson KA (2009) Role of lateral hypothalamic orexin neurons in reward processing and addiction. Neuropharmacology 56, Supplement 1:112-121.

Aubele T, Kritzer MF (2011a) Androgen Influence on Prefrontal Dopamine Systems in Adult Male Rats: Localization of Cognate Intracellular Receptors in Medial Prefrontal Projections to the Ventral Tegmental Area and Effects of Gonadectomy and Hormone Replacement on Glutamate-Stimulated Extracellular Dopamine Level. Cereb Cortex.

Aubele T, Kritzer MF (2011b) Gonadectomy and Hormone Replacement Affects In Vivo Basal Extracellular Dopamine Levels in the Prefrontal Cortex but Not Motor Cortex of Adult Male Rats. Cereb Cortex 21:222-232.

Aumann TD, Egan K, Lim J, Boon WC, Bye CR, Chua HK, Baban N, Parish CL, Bobrovskaya L, Dickson P, Horne MK (2011) Neuronal activity regulates expression of tyrosine hydroxylase in adult mouse substantia nigra pars compacta neurons. J Neurochem 116:646-658.

Avanitogiannis A, Tzschentke TM, Riscaldino L, Wise RA, Shizgal P (2000) Fos expression following self-stimulation of the medial prefrontal cortex. Behav Brain Res 107:123-132.

Badanich KA, Adler KJ, Kirstein CL (2006) Adolescents differ from adults in cocaine conditioned place preference and cocaine-induced dopamine in the nucleus accumbens septi. Eur J Pharmacol 550:95-106.

Baird AA, Gruber SA, Fein DA, Maas LC, Steingard RJ, Renshaw PF, Cohen BM, Yurgelun-Todd D (1999) Functional magnetic resonance imaging of facial affect recognition in children and adolescents. Journal of the American Academy of Child and Adolescence Psychiatry 38:195-199.

Balfour ME, Brown JL, Yu L, Coolen LM (2006) Potential contributions of efferents from medial prefrontal cortex to neural activation following sexual behavior in the male rat. Neuroscience 137:1259-1276.

Balfour ME, Yu L, Coolen LM (2004) Sexual Behavior and Sex-Associated Environmental Cues Activate the Mesolimbic System in Male Rats. Neuropsychopharmacology 29:718-730.

Baum MJ, Melamed E, Globus M (1986) Dissociation of the effects of castration and testosterone replacement on sexual behavior and neural metabolism of dopamine in the male rat. Brain Res Bull 16:145-148.

Becker JB (1999) Gender Differences in Dopaminergic Function in Striatum and Nucleus Accumbens. Pharmacol Biochem Behav 64:803-812.

Becker JB, Hu M (2008) Sex differences in drug abuse. Frontiers in Neuroendocrinology 29:36-47.

Becker JB, Molenda H, Hummer DL (2001a) Gender Differences in the Behavioral Responses to Cocaine and Amphetamine. Ann N Y Acad Sci 937:172-187.

Becker JB, Rudick CN, Jenkins WJ (2001b) The Role of Dopamine in the Nucleus Accumbens and Striatum during Sexual Behavior in the Female Rat. J Neurosci 21:3236-3241.

Been LE, Petrulis A (2011) Chemosensory and hormone information are relayed directly between the medial amygdala, posterior bed nucleus of the stria terminalis, and medial preoptic area in male Syrian hamsters. Horm Behav 59:536-548.

Been LE, Petrulis A (2012) Dissociated functional pathways for appetitive and consummatory reproductive behaviors in male Syrian hamsters. Horm Behav 61:204-211.

Bell MR, De Lorme KC, Meerts SH, Sisk CL (2011) Testosterone-treated juvenile male Syrian hamsters show a conditioned place preference for vaginal secretions. Society for Neuroscience abstract.

Bell MR, Meerts SH, Sisk CL (2010) Male Syrian hamsters demonstrate a conditioned place preference for sexual behavior and female chemosensory stimuli. Horm Behav 58:410-414.

Belluzzi JD, Lee AG, Oliff HS, Leslie FM (2004) Age-dependent effects of nicotine on locomotor activity and conditioned place preference in rats. Psychopharmacology 174:389-395.

Benes FM, Taylor JB, Cunningham MC (2000) Convergence and Plasticity of Monoaminergic Systems in the Medial Prefrontal Cortex during the Postnatal Period: Implications for the Development of Psychopathology. Cereb Cortex 10:1014-1027.

Benoit-Marand M, O'Donnell P (2008) D2 dopamine modulation of corticoaccumbens synaptic responses changes during adolescence. Eur J Neurosci 27:1364-1372.

Berridge K (2007) The debate over dopamine’s role in reward: the case for incentive salience. Psychopharmacology 191:391-431.

Berridge KC, Robinson TE (1998) What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Res Rev 28:309-369.

Berridge KC, Robinson TE (2003) Parsing reward. Trends Neurosci 26:507-513.

Bertran-Gonzalez J, Bosch C, Maroteaux M, Matamales M, Hervé D, Valjent E, Girault J-A (2008) Opposing Patterns of Signaling Activation in Dopamine D1 and D2 Receptor-Expressing Striatal Neurons in Response to Cocaine and Haloperidol. The Journal of Neuroscience 28:5671-5685.

Bharati IS, Goodson JL (2006) Fos responses of dopamine neurons to sociosexual stimuli in male zebra finches. Neuroscience 143:661-670.

Bitran D, Hull EM, Holmes GM, Lookingland KJ (1988) Regulation of male rat copulatory behavior by preoptic incertohypothalamic dopamine neurons. Brain Res Bull 20:323-331.

Bjork JM, Knutson B, Fong GW, Caggiano DM, Bennett SM, Hommer DW (2004) Incentive-Elicited Brain Activation in Adolescents: Similarities and Differences from Young Adults. J Neurosci 24:1793-1802.

Bjorklund A, Lindvall O, Norbin A (1975) Evidence of an incerto-hypothalamic dopamine neurone system in the rat. Brain Res 89.

Blakemore SJ (2008) The social brain in adolescence. Nat Rev Neurosci 9:267-277.

Boonyaratanakornkit V, Edwards DP (2007) Receptor Mechanisms Mediating Non-Genomic Actions of Sex Steroids. Semin Reprod Med 25:139,153.

Boutrel B, Cannella N, de Lecea L (2010) The role of hypocretin in driving arousal and goal-oriented behaviors. Brain Res 1314:103-111.

Bradley KC, Meisel RL (2001) Sexual Behavior Induction of c-Fos in the Nucleus Accumbens and Amphetamine-Stimulated Locomotor Activity Are Sensitized by Previous Sexual Experience in Female Syrian Hamsters. The Journal of Neuroscience 21:2123-2130.

Brenhouse HC, Sonntag KC, Andersen SL (2008) Transient D1 Dopamine Receptor Expression on Prefrontal Cortex Projection Neurons: Relationship to Enhanced Motivational Salience of Drug Cues in Adolescence. J Neurosci 28:2375-2382.

Briand L, Trotier D, Pernollet J-C (2004) Aphrodisin, an aphrodisiac lipocalin secreted in hamster vaginal secretions. Peptides 25:1545-1552.

Broaddus WC, Bennett JP (1990) Postnatal development of striatal dopamine function. I. An examination of D1 and D2 receptors, adenylate cyclase regulation and presynaptic dopamine markers. Dev Brain Res 52:265-271.

Bruce HM (1959) An exteroceptive block to pregnancy in the mouse. Nature 184:105.

Brudzynski SM, Pniak A (2002) Social contacts and production of 50-kHz short ultrasonic calls in adult rats. Journal of Comparative Psychology 116:73-82.

Buijs RM, Geffard M, Pool CW, Hoorneman EMD (1984) The dopaminergic innervation of the supraoptic and paraventricular nucleus. A light and electron microscopy study. Brain Res 323:67-72.

Canteras NS, Simerly RB, Swanson LW (1994) Organization of projections from the ventromedial nucleus of the hypothalamus: a Phaseolus vulgaris-leucoagglutinin study in the rat. J Comp Neurol 348:41-79.

Cao J, Lotfipour S, Loughlin SE, Leslie FM (2007) Adolescent Maturation of Cocaine-Sensitive Neural Mechanisms. Neuropsychopharmacology 32:2279-2289.

Carlezon WA, Haile CN, Coppersmith R, Hayashi Y, Malinow R, Neve RL, Nestler EJ (2000) Distinct Sites of Opiate Reward and Aversion within the Midbrain Identified Using a Herpes Simplex Virus Vector Expressing GluR1. The Journal of Neuroscience 20:RC62.

Carlezon WA, Thomas MJ (2009) Biological substrates of reward and aversion: A nucleus accumbens activity hypothesis. Neuropharmacology 56:122-132.

Carr DB, Sesack SR (2000a) GABA-containing neurons in the rat ventral tegmental area project to the prefrontal cortex. Synapse 38:114-123.

Carr DB, Sesack SR (2000b) Projections from the Rat Prefrontal Cortex to the Ventral Tegmental Area: Target Specificity in the Synaptic Associations with Mesoaccumbens and Mesocortical Neurons. J Neurosci 20:3864-3873.

Casey BJ, Galvan A, Hare TA (2005) Changes in cerebral functional organization during cognitive development. Curr Opin Neurobiol 15:239-244.

Cave JW, Baker H (2009) Dopamine systems in the forebrain. Adv Exp Med Biol 651:15-31.

Ceccatelli S, Villar MJ, Goldstein M, Hokfelt T (1989) Expression of c-Fos immunoreactivity in transmitter-characterized neurons after stress. Proc Natl Acad Sci U S A 86:9569-9573.

Cepeda C, Li Z, Cromwell HC, Altemus KL, Crawford CA, Nansen EA, Ariano MA, Sibley DR, Peacock WJ, Mathern GW, Levine MS (1999) Electrophysiological and Morphological Analyses of Cortical Neurons Obtained from Children with Catastrophic Epilepsy: Dopamine Receptor Modulation of Glutamatergic Responses. Dev Neurosci 21:223-235.

Chambers RA, Taylor JR, Potenza MN (2003) Developmental Neurocircuitry of Motivation in Adolescence: A Critical Period of Addiction Vulnerability. Am J Psychiatry 160:1041-1052.

Charara A, Grace AA (2003) Dopamine Receptor Subtypes Selectively Modulate Excitatory Afferents from the Hippocampus and Amygdala to Rat Nucleus Accumbens Neurons. Neuropsychopharmacology 28:1412-1421.

Chen J, Randeva HS (2010) Genomic organization and regulation of the human orexin (hypocretin) receptor 2 gene: identification of alternative promoters. Biochem J 427:377-390.

Cheung S, Ballew JR, Moore KE, Lookingland KJ (1998) Contribution of dopamine neurons in the medial zona incerta to the innervation of the central nucleus of the amygdala, horizontal diagonal band of Broca, and hypothalamic paraventriculuar nucleus. Brain Res 808:174-181.

Chibbar R, Toma JG, Mitchell BF, Miller FD (1990) Regulation of Neural Oxytocin Gene Expression by Gonadal Steroids in Pubertal Rats. Mol Endocrinol 4:2030-2038.

Choi GB, Dong HW, Murphy AJ, Valenzuela DM, Yancopoulos GD, Swanson LW, Anderson DJ (2005) Lhx6 Delineates a Pathway Mediating Innate Reproductive Behaviors from the Amygdala to the Hypothalamus. Neuron 46:647-660.

Clancy AN, Macrides F, Singer AG, Agosta WC (1984) Male hamster copulatory responses to a high molecular weight fraction of vaginal discharge: Effects of vomeronasal organ removal. Physiol Behav 33:653-660.

Coolen LM, Wood RI (1998) Bidirectional connections of the medial amygdaloid nucleus in the Syrian hamster brain: Simultaneous anterograde and retrograde tract tracing. The Journal of Comparative Neurology 399:189-209.

Coppola DM, O'Connell RJ (1988) Are pheromones their own reward? Physiol Behav 44:811-816.

Coria-Avila GA, Gavrilla AM, Boulard B, Charron N, Stanley G, Pfaus JG (2008) Neurochemical basis of conditioned partner preference in the female rat: II. Disruption by flupenthixol. Behav Neurosci 122:396-406.

Coulter CL, Happe HK, Murrin LC (1996) Postnatal development of the dopamine transporter: a quantitative autoradiographic study. Dev Brain Res 92:172-181.

Crawford LL, Holloway KS, Domjan M (1993) The nature of sexual reinforcement. J Exp Anal Behav 60:55-66.

Creese I, Sibley DR, Hamblin MW, Leff SE (1983) The Classification of Dopamine Receptors: Relationship to Radioligand Binding. Annu Rev Neurosci 6:43-71.

Cunningham MG, Bhattacharyya S, Benes FM (2002) Amygdalo-cortical sprouting continues into early adulthood: Implications for the development of normal and abnormal function during adolescence. The Journal of Comparative Neurology 453:116-130.

Damasio A, Damasio H, Tranel D (2012) Persistence of Feelings and Sentience after Bilateral Damage of the Insula. Cereb Cortex.

Damsma G, Pfaus JG, Wenkstern D, Phillips AG, Fibiger HC (1992) Sexual Behavior Increases Dopamine Transmission in the Nucleus Accumbens and Striatum of Male Rats: Comparison with Novelty and Locomotion. Behav Neurosci 106:181-191.

David HN, Ansseau M, Abraini JH (2005) Dopamine–glutamate reciprocal modulation of release and motor responses in the rat caudate–putamen and nucleus accumbens of “intact” animals. Brain Res Rev 50:336-360.

Davis BJ, Macrides F, Youngs WM, Schneider SP, Rosene DL (1978) Efferents and centrifugal afferents of the main and accessory olfactory bulbs in the hamster. Brain Res Bull 3:59-72.

Day TA, Blessing W, Willoughby JO (1980) Noradrenergic and dopamine projections to the medial preoptic area of the rat. A combined horseradish peroxidase/catecholamine fluorescence study. Brain Res 193:543-548.

De Beun R, Jansen E, Slangen JL, van de Poll Ne (1992) Testosterone as appetitive and discriminative stimulus in rats: sex- and dose-dependent effects. Physiol Behav 52:629-634.

de Lecea L, Kilduff TS, Peyron C, Gao X-B, Foye PE, Danielson PE, Fukuhara C, Battenberg ELF, Gautvik VT, Bartlett FS, Frankel WN, van den Pol AN, Bloom FE, Gautvik KM, Sutcliffe JG (1998) The hypocretins: Hypothalamus-specific peptides with neuroexcitatory□activity. Proceedings of the National Academy of Sciences 95:322-327.

DeBold JF, Clemens LG (1978) Aromatization and the induction of male sexual behavior in male, female, and androgenized female hamsters. Horm Behav 11:401-413.

delBarco-Trillo J, LaVenture AB, Johnston RE (2009) Male hamsters discriminate estrous state from vaginal secretions and individuals from flank marks. Behav Processes 82:18-24.

delBarco-Trillo J, McPhee ME, Johnston RE (2011) Syrian hamster males below an age threshold do not elicit aggression from unfamiliar adult males. Aggressive Behav 37:91-97.

Di Scala G, Sandner G (1989) Conditioned place aversion produced by FG 7142 is attenuated by haloperidol. Psychopharmacology 99:176-180.

Di Sebastiano AR, Wilson-Pérez HE, Lehman MN, Coolen LM (2011) Lesions of orexin neurons block conditioned place preference for sexual behavior in male rats. Horm Behav 59:1-8.

Di Sebastiano AR, Yong-Yow S, Wagner L, Lehman MN, Coolen LM (2010) Orexin mediates initiation of sexual behavior in sexually naive male rats, but is not critical for sexual performance. Horm Behav 58:397-404.

Dickinson SD, Kashawny SK, Thiebes KP, Charles DY (2009) Decreased Sensitivity to Ethanol Reward in Adolescent Mice as Measured by Conditioned Place Preference. Alcoholism: Clinical and Experimental Research 33:1246-1251.

Dobi A, Margolis EB, Wang H-L, Harvey BK, Morales M (2010) Glutamatergic and Nonglutamatergic Neurons of the Ventral Tegmental Area Establish Local Synaptic Contacts with Dopaminergic and Nondopaminergic Neurons. The Journal of Neuroscience 30:218-229.

Dominguez J, Riolo JV, Xu Z, Hull EM (2001) Regulation by the Medial Amygdala of Copulation and Medial Preoptic Dopamine Release. The Journal of Neuroscience 21:349-355.

Dominguez JM, Gil M, Hull EM (2006) Preoptic Glutamate Facilitates Male Sexual Behavior. The Journal of Neuroscience 26:1699-1703.

Dominguez JM, Hull EM (2001) Stimulation of the medial amygdala enhances medial preoptic dopamine release: implications for male rat sexual behavior. Brain Res 917:225-229.

Dominguez JM, Muschamp JW, Schmich JM, Hull EM (2004) Nitric oxide mediates glutamate-evoked dopamine release in the medial preoptic area. Neuroscience 125:203-210.

Doremus-Fitzwater TL, Varlinskaya EI, Spear LP (2010) Motivational systems in adolescence: Possible implications for age differences in substance abuse and other risk-taking behaviors. Brain and Cognition 72:114-123.

Douglas LA, Varlinskaya EI, Spear LP (2004) Rewarding properties of social interactions in adolescent and adult male and female rats: Impact of social versus isolate housing of subjects and partners. Dev Psychobiol 45:153-162.

Du J, Hull EM (1999) Effects of testosterone on neuronal nitric oxide synthase and tyrosine hydroxylase. Brain Res 836:90-98.

Du J, Lorrain DS, Hull EM (1998) Castration decreases extracellular, but increases intracellular, dopamine in medial preoptic area of male rats. Brain Res 782:11-17.

Eaton RC, Markowski VP, Lumley LA, Thompson JT, Moses J, Hull EM (1991) D2 receptors in the paraventricular nucleus regulate genital responses and copulation in male rats. Pharmacol Biochem Behav 39:177-181.

Eluvathingal TJ, Hasan KM, Kramer L, Fletcher JM, Ewing-Cobbs L (2007) Quantitative Diffusion Tensor Tractography of Association and Projection Fibers in Normally Developing Children and Adolescents. Cereb Cortex 17:2760-2768.

Engel J, Ahlenius S, Almgren O, Carlsson A, Larsson K, Sodersten P (1979) Effects of gonadectomy and hormone replacement on brain monoamine synthesis in male rats. Pharmacology, Biochemistry, and Behavior 10:149-154.

Ernst M, Fudge JL (2009) A developmental neurobiological model of motivated behavior: Anatomy, connectivity and ontogeny of the triadic nodes. Neuroscience and Biobehavioral Reviews 33:367-382.

Ernst M, Nelson EE, Jazbec S, McClure EB, Monk CS, Leibenluft E, Blair J, Pine DS (2005) Amygdala and nucleus accumbens in responses to receipt and omission of gains in adults and adolescents. NeuroImage 25:1279-1291.

Ernst M, Pine DS, Hardin M (2006) Triadic model of the neurobiology of motivated behavior in adolescence. Psychol Med 36:299-312.

Ernst M, Romeo RD, Andersen SL (2009) Neurobiology of the development of motivated behaviors in adolescence: A window into a neural systems model. Pharmacol Biochem Behav 93:199-211.

Everitt BJ (1990) Sexual motivation: A neural and behavioural analysis of the mechanisms underlying appetitive and copulatory responses of male rats. Neurosci Biobehav Rev 14:217-232.

Fadel J, Deutch AY (2002) Anatomical substrates of orexin-dopamine interactions: lateral hypothalamic projections to the ventral tegmental area. Neuroscience 111:379-387.

Fahrbach SE, Morrell JI, Pfaff DW (1989) Studies of ventromedial hypothalamic afferents in the rat using three methods of HRP application. Exp Brain Res 77:221-233.

Fallon JH, Moore RY (1978) Catecholamine innervation of the basal forebrain. IV. Topography of the dopamine projection to the basal forebrain and neostriatum. J Comp Neurol 180:545-580.

Fernandez-Fewell GD, Meredith M (1994) c-fos expression in vomeronasal pathways of mated or pheromone- stimulated male golden hamsters: contributions from vomeronasal sensory input and expression related to mating performance. J Neurosci 14:3643-3654.

Fernandez-Gausti A, Omana-Zapata I, Lujan M, Condres-Lara M (1994) Actions of sciatic nerve ligature on sexual behavior of sexually experience and inexperience male rats: effects of frontal pole decortication. Physiol Behav 55:577-581.

Ferreira JGP, Del-Fava F, Hasue RH, Shammah-Lagnado SJ (2008) Organization of ventral tegmental area projections to the ventral tegmental area–nigral complex in the rat. Neuroscience 153:196-213.

Festa ED, Quinones-Jenab V (2004) Gonadal hormones provide the biological basis for sex differences in behavioral responses to cocaine. Horm Behav 46:509-519.

Fiber JM, Adames P, Swann JM (1993) Pheromones induce c-fos in limbic areas regulating male hamster mating behavior. Neuroreport 4:871-874.

Fiber JM, Swann JM (1996) Testosterone Differentially Influences Sex-Specific Pheromone-Stimulated Fos Expression in Limbic Regions of Syrian Hamsters. Horm Behav 30:455-473.

Fibiger HC, Phillips AG (1988) Mesocorticolimbic Dopamine Systems and Reward. Ann N Y Acad Sci 537:206-215.

Forbes EE, Dahl RE (2010) Pubertal development and behavior: Hormonal activation of social and motivational tendencies. Brain and Cognition 72:66-72.

Forbes EE, Ryan ND, Phillips ML, Manuck SB, Worthman CM, Moyles DL, Tarr JA, Sciarrillo SR, Dahl RE (2010) Healthy Adolescents' Neural Response to Reward: Associations with Puberty, Positive Affect, and Depressive Symptoms. J Am Acad Child Adolesc Psychiatry 49:162-172e161-165.

Forgie ML, Stewart J (1994) Sex differences in the locomotor-activating effects of amphetamine: role of circulating testosterone in adulthood. Physiol Behav 55:639-644.

Friemel CM, Spanagel R, Schneider M (2010) Reward sensitivity for a palatable food reward peaks during pubertal developmental in rats. Frontiers in Behavioral Neuroscience 4:39.

Frye CA, Rhodes ME, Rosellini R, Svare B (2002) The nucleus accumbens as a site of action for rewarding properties of testosterone and its 5alpha-reduced metabolites. Pharmacol Biochem Behav 74:119-127.

Galvan A (2010) Adolescent Development of the Reward System. Frontiers in Human Neuroscience 4:6.

Galvan A, Hare T, Voss H, Glover G, Casey BJ (2007) Risk-taking and the adolescent brain: who is at risk? Developmental Science 10:F8-F14.

Gannon RL, Lungwitz E, Batista N, Hester I, Huntley C, Peacock A, Delagrange P, Millan MJ (2011) The benzodiazepine diazepam demonstrates the usefulness of Syrian

hamsters as a model for anxiety testing: Evaluation of other classes of anxiolytics in comparison to diazepam. Behav Brain Res 218:8-14.

Gardner M, Steinberg L (2005) Peer Influence on Risk Taking, Risk Preference, and Risky Decision Making in Adolescence and Adulthood: An Experimental Study. Developmental Psychology 41:625-635.

Geier C, Luna B (2009) The maturation of incentive processing and cognitive control. Pharmacology, Biochemistry, and Behavior 93:212-221.

Geisler S, Zahm DS (2005) Afferents of the ventral tegmental area in the rat-anatomical substratum for integrative functions. The Journal of Comparative Neurology 490:270-294.

Giedd JN, Blumenthal J, Jeffries NO, Castellanos FX, Liu H, Zijdenbos A, Paus T, Evans AC, Rapoport JL (1999) Brain development during childhood and adolescence: a longitudinal MRI study. Nat Neurosci 2:861-863.

Giedd JN, Clasen LS, Lenroot R, Greenstein D, Wallace GL, Ordaz S, Molloy EA, Blumenthal JD, Tossell JW, Stayer C, Samango-Sprouse CA, Shen D, Davatzikos C, Merke D, Chrousos GP (2006) Puberty-related influences on brain development. Mol Cell Endocrinol 254-255:154-162.

Gingrich B, Liu Y, Cascio C, Wang Z, Insel TR (2000) Dopamine D2 receptors in the nucleus accumbens are important for social attachment in female prairie voles (Microtus ochrogaster). Behav Neurosci 114:173-183.

Giorgi O, De Montis G, Porceddu ML, Mele S, Calderini G, Toffano G, Biggio G (1987) Developmental and age-related changes in D1-dopamine receptors and dopamine content in the rat striatum. Brain Res 432:283-290.

Giuliano F, Bernabé J, Brown K, Droupy S, Benoit G, Rampin O (1997) Erectile response to hypothalamic stimulation in rats: role of peripheral nerves. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology 273:R1990-R1997.

Gogtay N, Giedd JN, Lusk L, Hayashi KM, Greenstein D, Vaituzis AC, Nugent TF, III, Herman DH, Clasen LS, Toga AW, Rapoport JL, Thompson PM (2004) Dynamic mapping of human cortical development during childhood through early adulthood. Proceedings of the National Academy of Sciences 101:8174-8179.

Gomez DM, Newman SW (1992) Differential projections of the anterior and posterior regions of the medial amygdaloid nucleus in the Syrian hamster. J Comp Neurol 317:195-218.

Gotlib IH, Krasnoperova E, Yue DN, Joormann J (2004) Attentional Biases for Negative Interpersonal Stimuli in Clinical Depression. Journal of Abnormal Psychology 113:127-135.

Goto Y, Grace AA (2005) Dopaminergic modulation of limbic and cortical drive of nucleus accumbens in goal-directed behavior. Nat Neurosci 8:805-812.

Goto Y, Otani S, Grace AA (2007) The Yin and Yang of dopamine release: a new perspective. Neuropharmacology 53:583-587.

Grace A, Bunney B (1984a) The control of firing pattern in nigral dopamine neurons: burst firing. The Journal of Neuroscience 4:2877-2890.

Grace A, Bunney B (1984b) The control of firing pattern in nigral dopamine neurons: single spike firing. The Journal of Neuroscience 4:2866-2876.

Grace AA (1991) Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: A hypothesis for the etiology of schizophrenia. Neuroscience 41:1-24.

Graham MD, Pfaus JG (2010) Differential regulation of female sexual behaviour by dopamine agonists in the medial preoptic area. Pharmacol Biochem Behav 97:284-292.

Greco B, Edwards DA, Michael RP, Clancy AN (1998) Androgen receptors and estrogen receptors are colocalized in male rat hypothalamic and limbic neurons that express Fos immunoreactivity induced by mating. Neuroendocrinology 67:18-28.

Gréco B, Edwards DA, Michael RP, Clancy AN (1996) Androgen receptor immunoreactivity and mating-induced Fos expression in forebrain and midbrain structures in the male rat. Neuroscience 75:161-171.

Gregory EH, Bishop A (1975) Development of olfactory-guided behavior in the golden hamster. Physiol Behav 15:373-376.

Groenewegen HJ, Wright CI, Beijer AVJ, Voorn P (1999) Convergence and Segregation of Ventral Striatal Inputs and Outputs. Ann N Y Acad Sci 877:49-63.

Gulia KK, Mallick HN, Kumar VM (2003) Orexin A (hypocretin-1) application at the medial preoptic area potentiates male sexual behavior in rats. Neuroscience 116:921-923.

Gunnet JW, Lookingland KJ, Moore KE (1986) Comparison of the effects of castration and steroid replacement on incertohypothalamic dopaminergic neurons in male and female rats. Neuroendocrinology 44:269-275.

Guyer AE, Lau JYF, McClure-Tone EB, Parrish J, Shiffrin ND, Reynolds RC, Chen G, Blair RJR, Leibenluft E, Fox NA, Ernst M, Pine DS, Nelson EE (2008) Amygdala and Ventrolateral Prefrontal Cortex Function During Anticipated Peer Evaluation in Pediatric Social Anxiety. Arch Gen Psychiatry 65:1303-1312.

Guyer AE, McClure EB, Shiffrin ND, Pine DS, Nelson EE (2009) Probing the Neural Correlates of Anticipated Peer Evaluation in Adolescence. Child Dev 80:1000-1015.

Hadeishi Y, Wood RI (1996) Nitric oxide synthase in mating behavior circuitry of male Syrian hamster brain. J Neurobiol 30:480-492.

Harding SM, McGinnis MY (2004) Androgen receptor blockage in the MPOA or VMN: effects on male sociosexual behaviors. Physiol Behav 81:671-680.

Harding SM, McGinnis MY (2005) Microlesions of the ventromedial nucleus of the hypothalamus: effects on sociosexual behaviors in male rats. Behav Neurosci 119:1227-1234.

Harris GC, Aston-Jones G (2006) Arousal and reward: a dichotomy in orexin function. Trends Neurosci 29:571-577.

Harris GC, Wimmer M, Aston-Jones G (2005) A role for lateral hypothalamic orexin neurons in reward seeking. Nature 437:556-559.

Harris GC, Wimmer M, Randall-Thompson JF, Aston-Jones G (2007) Lateral hypothalamic orexin neurons are critically involved in learning to associate an environment with morphine reward. Behav Brain Res 183:43-51.

Haycock JW, Becker L, Ang L, Furukawa Y, Hornykiewicz O, Kish SJ (2003) Marked disparity between age-related changes in dopamine and other presynaptic dopaminergic markers in human striatum. J Neurochem 87:574-585.

Hayward C, Sanborn K (2002) Puberty and the emergence of gender differences in psychopathology. J Adolesc Health 30:49-58.

Heidbreder C, Groenewegen H (2003) The medial prefrontal cortex in the rat: evidence for a doros-ventral distinction based upon functional and anatomical characteristics. . Neurosci Biobehav Rev 27:555-579.

Heimer L (1972) The olfactory connections of the diencephalon in the rat. An experimental light- and electron-microscopic study with special emphasis on the problem of terminal degeneration. Brain Behavior and Evolution 6:484-523.

Heimer L, Zahm DS, Churchill L, Kalivas PW, Wohltmann C (1991) Specificity in the projection patterns of accumbal core and shell in the rat. Neuroscience 41:89-125.

Hendricks SE, Scheetz HA (1973) Interaction of hypothalamic structures in the mediation of male sexual behavior. Physiol Behav 10:711-716.

Henzel WJ, Rodriguez H, Singer AG, Stults JT, Macrides F, Agosta WC, Niall H (1988) The primary structure of aphrodisin. J Biol Chem 263:16682-16687.

Herba C, Phillips M (2004) Annotation: Development of facial expression recognition from childhood to adolescence: behavioural and neurological perspectives. Journal of Child Psychology and Psychiatry 45:1185-1198.

Herdegen T, Leah JD (1998) Inducible and constitutive transcription factors in the mammalian nervous system: control of gene expression by Jun, Fos and Krox, and CREB/ATF proteins. Brain Res Rev 28:370-490.

Hoffman DC, Beninger RJ (1988) Selective D1 and D2 dopamine agonists produce opposing effects in place conditioning but not in conditioned taste aversion learning. Pharmacol Biochem Behav 31:1-8.

Hoffman DC, Beninger RJ (1989) The effects of selective dopamine D1 or D2 receptor antagonists on the establishment of agonist-induced place conditioning in rats. Pharmacol Biochem Behav 33:273-279.

Hoffman EA, Haxby JV (2000) Distinct representations of eye gaze and identity in the distributed human neural system for face perception. Nat Neurosci 3:80-84.

Hoffman GE, Lyo D (2002) Anatomical Markers of Activity in Neuroendocrine Systems: Are we all 'Fos-ed out'? J Neuroendocrinol 14:259-268.

Hokfelt T, Everitt BJ, Theodorsson-Norheim E, Goldstein M (1984) Occurence of neurotensinlike immunoreactivity in subpopulations of hypothalamic, mesencephalic, and medullary catecholamine neurons. J Comp Neurol 222:543-559.

Hoover W, Vertes R (2007) Anatomical analysis of afferent projections to the medial prefrontal cortex in the rat. Brain Structure and Function 212:149-179.

Horvath TL, Naftolin F, Leranth C (1993) Leuteinizing hormone-releasing hormone and gamma-aminobutyric acid neurons in the medial preoptic area are synaptic targets of dopamin axons originating in anterior periventricular areas. J Neuroendocrinol 5:71-79.

Hu M, Becker JB (2003) Effects of Sex and Estrogen on Behavioral Sensitization to Cocaine in Rats. The Journal of Neuroscience 23:693-699.

Huck U, Lisk R, Kim S, Evans A (1989) Olfactory discrimination of estrous condition by the male golden hamster (Mesocricetus auratus). Behavioral Neural Biology 51:1-10.

Hughes AM, Everitt BJ, Herbert J (1990) Comparative effects of preoptic area infusions of opioid peptides, lesions and castration on sexual behaviour in male rats: studies of instrumental behavior, conditioned place preference and partner preference. Psychopharmacology 102:243-256.

Hughes P, Dragunow M (1995) Induction of immediate-early genes and the control of neurotransmitter-regulated gene expression within the nervous system. Pharmacol Rev 47:133-178.

Hull EM, Bitran D, Pehek EA, Warner RK, Band LC, Holmes GM (1986) Dopaminergic control of male sex behavior in rats: Effects of an intracerebrally-infused agonist. Brain Res 370:73-81.

Hull EM, Dominguez JM (2007) Sexual behavior in male rodents. Horm Behav 52:45-55.

Hull EM, Du J, Lorrain DS, Matuszewich L (1995) Extracellular dopamine in the medial preoptic area: implications for sexual motivation and hormonal control of copulation. J Neurosci 15:7465-7471.

Hull EM, Eaton RC, Markowski VP, Moses J, Lumley LA, Loucks JA (1992) Opposite influence of medial preoptic D1 and D2 receptors on genital reflexes: implications for copulation. Life Science 51:1705-1713

Hull EM, Weber MS, Eaton RC, Dua R, Markowski VP, Lumley L, Moses J (1991) Dopamine receptors in the ventral tegmental area affect motor, but not motivational or reflexive, components of copulation in male rats. Brain Res 554:72-76.

Hunt GE, McGregor IS (1997) Rewarding brain stimulation induces only sparse Fos-like immunoreactivity in dopaminergic neurons. Neuroscience 83:501-515.

Huttenlocher PR (1984) Synapse elimination and plasticity in developing human cerebral cortex. American Journal of Mental Deficiencies 88:488-496.

Ikemoto S (2007) Dopamine reward circuitry: Two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex. Brain Res Rev 56:27-78.

Ikemoto S (2010) Brain reward circuitry beyond the mesolimbic dopamine system: A neurobiological theory. Neurosci Biobehav Rev 35:129-150.

Ikemoto S, Kohl RR (1997) GABAa Receptor Blockade in the Anterior Ventral Tegmental Area Increases Extracellular Levels of Dopamine in the Nucleus Accumbens of Rats. J Neurochem 69:137-143.

Ikemoto S, Panksepp J (1999) The role of nucleus accumbens dopamine in motivated behaviors: a unifying interpretation with special reference to reward-seeking. Brain Res Rev 31:6-41.

Imaizumi M, Takeda M, Fushiki T (2000) Effects of oil intake in the conditioned place preference test in mice. Brain Res 870:150-156.

Inglis FM, Moghaddam B (1999) Dopaminergic Innervation of the Amygdala Is Highly Responsive to Stress. J Neurochem 72:1088-1094.

Ismail N, Girard-Beriault F, Nakanishi S, Pfaus JG (2009) Naloxone, but not flupenthixol, disrupts the development of conditioned ejaculatory preference in the male rat. Behav Neurosci 123:992-999.

Jackson LR, Robinson TE, Becker JB (2005) Sex Differences and Hormonal Influences on Acquisition of Cocaine Self-Administration in Rats. Neuropsychopharmacology 31:129-138.

Johnson ML, Day AE, Ho CC, Walker QD, Francis R, Kuhn CM (2010) Androgen Decreases Dopamine Neurone Survival in Rat Midbrain. J Neuroendocrinol 22:238-247.

Johnston RE (1974) Sexual Attraction Function of Golden Hamster Vaginal Secretion. Behavioral Biology 12:111-117.

Johnston RE (1986) Effects of female odors on the sexual behavior of male hamsters. Behavioral and Neural Biology 46:168-188.

Johnston RE, Coplin B (1979) Development of Responses to Vaginal Secretion and Other Substances in Golden Hamsters. Behavioral and Neural Biology 25:473-489.

Kalsbeek A, Voorn P, Buijs RM, Pool CW, Uylings HB (1988) Development of the dopaminergic innervation in the prefrontal cortex of the rat. J Comp Neurol 269:58-72.

Kebabian JW, Calne DB (1979) Multiple receptors for dopamine. Nature 277:93-96.

Keefe KA, Gerfen CR (1995) D1–D2 dopamine receptor synergy in striatum: effects of intrastriatal infusions of dopamine agonists and antagonists on immediate early gene expression. Neuroscience 66:903-913.

Keller M, Baum MJ, Brock O, Brennan PA, Bakker J (2009) The main and the accessory olfactory systems interact in the control of mate recognition and sexual behavior. Behavioral Brain Research 200:268-276.

Kelley AE, Berridge KC (2002) The Neuroscience of Natural Rewards: Relevance to Addictive Drugs. The Journal of Neuroscience 22:3306-3311.

Keverne EB (2004) Importance of olfactory and vomeronasal systems for male sexual function. Physiol Behav 83:177-187.

Kevetter GA, Winans SS (1981a) Connections of the corticomedial amygdala in the golden hamster. I. Efferents of the “vomeronasal amygdala”. The Journal of Comparative Neurology 197:81-98.

Kevetter GA, Winans SS (1981b) Connections of the corticomedial amygdala in the golden hamster. II. Efferents of the "olfactory amygdala". J Comp Neurol 197:99-111.

Kevetter GA, Winans SS (1981c) Connections of the cotricomedial amygdala in the golden hamster. I. Efferents of the "vomeronasal amygdala". J Comp Neurol 197:81-98.

King BE, Packard MG, Alexander GM (1999) Affective properties of intra-medial preoptic area injections of testosterone in male rats. Neurosci Lett 269:149-152.

Kipke MD, Editor (1999) Adolescent Development and the Biology of Puberty: Summary of a workshop on New Research. Washington, D.C.: National Academy Press.

Kippin TE, Cain SW, Pfaus JG (2003) Estrous odors and sexually conditioned neutral odors activate separate neural pathways in the male rat. Neuroscience 117:971-979.

Kippin TE, Sotiropoulos V, Badih J, Pfaus JG (2004) Opposing roles of the nucleus accumbens and anterior lateral hypothalamic area in the control of sexual behaviour in the male rat. Eur J Neurosci 19:698-704.

Kippin TE, van der Kooy D (2003) Excitotoxic lesions of the tegmental pedunculopontine nucleus impair copulation in naive male rats and block the rewarding effects of copulation in experienced male rats. Eur J Neurosci 18:2581-2591.

Klingerman CM, Patel A, Hedges VL, Meisel RL, Schneider JE (2011) Food restriction dissociates sexual motivation, sexual performance, and the rewarding consequences of copulation in female Syrian hamsters. Behav Brain Res 223:356-370.

Kollack-Walker S, Newman SW (1995) Mating and agonistic behavior produce different patterns of Fos immunolabeling in the male Syrian hamster brain. Neuroscience 66:721-736.

Kollack-Walker S, Newman SW (1997) Mating-induced expression of c-fos in the male Syrian hamster brain: Role of experience, pheromones, and ejaculations. J Neurobiol 32:481-501.

Kollack S, Newman SW (1992) Mating behavior induces selective expression of Fos protein within the chemosensory pathways of the male Syrian hamster brain. Neurosci Lett 143:223-228.

Kondo Y (1992) Lesions of the medial amygdala produce severe impairment of copulatory behavior in sexually inexperience male rats. Physiol Behav 51:939-943.

Kondo Y, Arai Y (1995) Functional association between medial amygdala and the medial preoptic area in regulation of mating behavior in the male rat. Physiol Behav 57:69-73.

Koob GF, Nestler EJ (1997) The neurobiology of drug addiction. Journal of Neuropsychiatry and Clinical Neurosciences 9:482-497.

Korotkova TM, Sergeeva OA, Eriksson KS, Haas HL, Brown RE (2003) Excitation of Ventral Tegmental Area Dopaminergic and Nondopaminergic Neurons by Orexins/Hypocretins. J Neurosci 23:7-11.

Kritzer MF (1997) Selective colocalization of immunoreactivity for intracellular gonadal hormone receptors and tyrosine hydroxylase in the ventral tegmental area, substantia nigra, and retrorubral fields in the rat. The Journal of Comparative Neurology 379:247-260.

Kritzer MF, Brewer A, Montalmant F, Davenport M, Robinson JK (2007) Effects of gonadectomy on performance in operant tasks measuring prefrontal cortical function in adult male rats. Horm Behav 51:183-194.

Kritzer MF, Creutz LM (2008) Region and Sex Differences in Constituent Dopamine Neurons and Immunoreactivity for Intracellular Estrogen and Androgen Receptors in Mesocortical Projections in Rats. J Neurosci 28:9525-9535.

Kudwa AE, Dominguez-Salazar E, Cabrera DM, Sibley DR, Rissman EF (2005) Dopamine D5 receptor modulates male and female sexual behavior in mice. Psychopharmacology 180:206-214.

Kuhn C, Johnson M, Thomae A, Luo B, Simon SA, Zhou G, Walker QD (2010) The emergence of gonadal hormone influences on dopaminergic function during puberty. Horm Behav 58:122-137.

Lagoda G, Muschamp JW, Vigdorchik A, Hull EM (2004) A nitric oxide synthesis inhibitor in the medial preoptic area inhibits copulation and stimulus sensitization in male rats. Behav Neurosci 118:1317-1323.

Lajtha A, Sershen H (2010) Heterogeneity of Reward Mechanisms. Neurochem Res 35:851-867.

Lammel S, Hetzel A, Häckel O, Jones I, Liss B, Roeper J (2008) Unique Properties of Mesoprefrontal Neurons within a Dual Mesocorticolimbic Dopamine System. Neuron 57:760-773.

Landauer MR, Banks EM, Carter CS (1977) Sexual preferences of male hamsters (Mesocricetus auratus) for conspecifics in different endocrine conditions. Horm Behav 9:193-202.

Laviola G, Dell'Omo G, Alleva E, Bignami G (1992) Ontogeny of cocaine hyperactivity and conditioned place preference in mice. Psychopharmacology 107:221-228.

LeDoux J (2007) The amygdala. Curr Biol 17:R868-R874.

Lehman MN, Newman SW, Silverman A-J (1987) Luteinizing Hormone-Releasing Hormone in the Vomeronasal System and Terminal Nerve of the Hamstera. Ann N Y Acad Sci 519:229-240.

Lehman MN, Winans SS (1983) Evidence for a ventral non-striatal pathway from the amygdala to the bed nucleus of the stria terminalis in the male golden hamster. Brain Res 268:139-146.

Lehman MN, Winans SS, Powers JB (1980) Medial nucleus of the amygdala mediates chemosensory control of male hamster sexual behavior. Science 210:557-560.

Lenroot RK, Gogtay N, Greenstein DK, Wells EM, Wallace GL, Clasen LS, Blumenthal JD, Lerch J, Zijdenbos AP, Evans AC, Thompson PM, Giedd JN (2007) Sexual dimorphism of brain developmental trajectories during childhood and adolescence. NeuroImage 36:1065-1073.

Li ZS, Schmauss C, Cuenca A, Ratcliffe E, Gershon MD (2006) Physiological Modulation of Intestinal Motility by Enteric Dopaminergic Neurons and the D2 Receptor: Analysis of Dopamine Receptor Expression, Location, Development, and Function in Wild-Type and Knock-Out Mice. The Journal of Neuroscience 26:2798-2807.

Lindvall O, Bjorklund A (1978) Anatomy of the dopaminergic neuron syntheis in the rat brain. Advances in Biochemical psychopharmacology 19:1-23.

Lindvall O, Bjorklund A, Skagerberg G (1984) Selective istochemical deomonstration of dopaine terminal systems in rat di- and telencephalon: new evidence for dopaminergic innervation of hypothalamic neurosecretory nuclei. Brain Res 306:19-30.

Liu YC, Sachs BD, Salamone JD (1998) Sexual behavior in male rats after radiofrequency or dopamine-depleting lesions in nucleus accumbens. Pharmacol Biochem Behav 60:585-592.

Liu YC, Salamone JD, Sachs BD (1997) Impaired sexual response after lesions of the paraventricular nucleus of the hypothalamus in male rats. Behav Neurosci 111:1361-1367.

Lobo MK, Covington HE, Chaudhury D, Friedman AK, Sun H, Damez-Werno D, Dietz DM, Zaman S, Koo JW, Kennedy PJ, Mouzon E, Mogri M, Neve RL, Deisseroth K, Han M-H, Nestler EJ (2010) Cell Type–Specific Loss of BDNF Signaling Mimics Optogenetic Control of Cocaine Reward. Science 330:385-390.

Lopez HH, Ettenberg A (2000) Haloperidol challenge during copulation prevents subsequent increase in male sexual motivation. Pharmacology, biochemistry, and behavior 67:387-393.

Lopez HH, Ettenberg A (2001) Dopamine antagonism attenuates the unconditioned incentive value of estrous female cues. Pharmacology, biochemistry, and behavior 68:411-416.

Lopez HH, Ettenberg A (2002) Sexually conditioned incentives:attenuation of movitational impact during dopamine receptor antagonism. Pharmacology, biochemistry, and behavior 72:65-72.

Lorrain DS, Hull EM (1993) Nitric oxide increases dopamine and serotonin release in the medial preoptic area. Neuroreport 5:87-89.

Lorrain DS, Matuszewich L, Howard RV, Du J, Hull EM (1996) Nitric oxide promotes medial preoptic dopamine release during male rat copulation. Neuroreport 8:31-34.

Louilot A, Gonzalez-Mora JL, Guadalupe T, Mas M (1991) Sex-related olfactory stimuli induce a selective increase in dopamine release in the nucleus accumbens of male rats. A voltammetric study. Brain Res 553:313-317.

Luo Z, Volkow ND, Heintz N, Pan Y, Du C (2011) Acute Cocaine Induces Fast Activation of D1 Receptor and Progressive Deactivation of D2 Receptor Striatal Neurons: In Vivo Optical Microprobe [Ca2+]i Imaging. The Journal of Neuroscience 31:13180-13190.

Macrides F, Clancy AN, Singer AG, Agosta WC (1984a) Male hamster investigatory and copulatory responses to vaginal discharge: An attempt to impart sexual significance to an arbitrary chemosensory stimulus. Physiol Behav 33:627-632.

Macrides F, Johnson PA, Schneider SP (1977) Responses of the male golden hamster to vaginal secretion and dimethyl disulfide: attraction versus sexual behavior. Behavioral Biology 20:377-386.

Macrides F, Singer AG, Clancy AN, Goldman BD, Agosta WC (1984b) Male hamster investigatory and copulatory responses to vaginal discharge: relationship to the endocrine status of females. Physiol Behav 33:633-637.

Malmnas CO (1976) The significance of dopamine, versus other catecholamines, for L-dopa induced facilitation of sexual behavior in the castrated male rat. Pharmacology, biochemistry, and behavior 4:521-526.

Manzanedo C, Aguilar MA, Rodríguez-Arias M, Miñarro J (2005) Sensitization to the rewarding effects of morphine depends on dopamine. Neuroreport 16:201-205.

Manzanedo C, Aguilar MaA, Rodríguez-Arias M, Miñarro J (2001) Effects of dopamine antagonists with different receptor blockade profiles on morphine-induced place preference in male mice. Behav Brain Res 121:189-197.

Maras PM, Petrulis A (2006) Chemosensory and steroid-responsive regions of the medial amygdala regulate distinct aspects of opposite-sex odor preference in male Syrian hamsters. Eur J Neurosci 24:3541-3552.

Marcus JN, Aschkenasi CJ, Lee CE, Chemelli RM, Saper CB, Yanagisawa M, Elmquist JK (2001) Differential expression of orexin receptors 1 and 2 in the rat brain. J Comp Neurol 435:6-25.

Margolis EB, Mitchell JM, Ishikawa J, Hjelmstad GO, Fields HL (2008) Midbrain Dopamine Neurons: Projection Target Determines Action Potential Duration and Dopamine D2 Receptor Inhibition. J Neurosci 28:8908-8913.

Marinelli M, Rudick CN, Hu XT, White FJ (2006) Excitability of dopamine neurons: modulation and physiological consequences. CNS and Neurological Disorders Drug Targets 5:49-97.

Markham JA, Morris JR, Juraska JM (2007) Neuron number decreases in the rat ventral, but not dorsal, medial prefrontal cortex between adolescence and adulthood. Neuroscience 144:961-968.

Marowsky A, Yanagawa Y, Obata K, Vogt KE (2005) A Specialized Subclass of Interneurons Mediates Dopaminergic Facilitation of Amygdala Function. Neuron 48:1025-1037.

Martin-Iverson MT, Ortman R, Fibiger HC (1985) Place Preference Conditioning with Methylphenidate and Nomifensin. Brain Res 332:59-67.

Martínez-Hernández J, Lanuza E, Martínez-García F (2006) Selective dopaminergic lesions of the ventral tegmental area impair preference for sucrose but not for male sexual pheromones in female mice. Eur J Neurosci 24:885-893.

Martínez-Hernández J, Lanuza E, Martínez-García F (2012) Lesions of the dopaminergic innervation of the nucleus accumbens medial shell delay the generation of preference for sucrose, but not of sexual pheromones. Behav Brain Res 226:538-547.

Martínez-Ricós J, Agustín-Pavón C, Lanuza E, Martínez-García F (2007) Intraspecific communication through chemical signals in female mice: reinforcing properties of involatile male sexual pheromones. Chem Senses 32:139-148.

Martínez I, Paredes RG (2001) Only Self-Paced Mating Is Rewarding in Rats of Both Sexes. Horm Behav 40:510-517.

Maruniak JA, Coquelin A, Bronson FH (1978) The Release of LH in Male Mice in Response to Female Urinary Odors: Characteristics of the Response in Young Males. Biol Reprod 18:251-255.

Mas M, Fumero B, Gonzalez-Mora JL (1995) Voltammetric and microdialysis monitoring of brain monoamine neurotransmitter release during sociosexual interactions. Behav Brain Res 71:69-79.

Mas M, Gonzalez-Mora JL, Louilot A, Sole C, Guadalupe T (1990) Increased dopamine release in the nucleus accumbens of copulating male rats as evidenced by in vivo voltammetry. Neurosci Lett 110:303-308.

Maskos U (2008) The cholinergic mesopontine tegmentum is a relatively neglected nicotinic master modulator of the dopaminergic system: relevance to drugs of abuse and pathology. Br J Pharmacol 153:S438-S445.

Mathews IZ, Waters P, McCormick CM (2009) Changes in hyporesponsiveness to acute amphetamine and age differences in tyrosine hydroxylase immunoreactivity in the brain over adolescence in male and female rats. Dev Psychobiol 51:417-428.

McArthur S, Murray HE, Dhankot A, Dexter DT, Gillies GE (2007) Striatal susceptibility to a dopaminergic neurotoxin is independent of sex hormone effects on cell survival and DAT expression but is exacerbated by central aromatase inhibition. J Neurochem 100:678-692.

McCutcheon JE, Marinelli M (2009) Age matters. Eur J Neurosci 29:997-1014.

McGinnis MY, Williams GW, Lumia AR (1996) Inhibition of male sex behavior by androgen receptor blockade in preoptic area or hypothalamus, but not amygdala or septum. Physiol Behav 60:783-789.

Meek LR, Romeo RD, Novak CM, Sisk CL (1997) Actions of Testosterone in Prepubertal and Postpubertal Male Hamsters: Dissociation of Effects on Reproductive Behavior and Brain Androgen Receptor Immunoreactivity. Horm Behav 31:75-88.

Meerts SH, Clark AS (2007) Female rats exhibit a conditioned place preference for nonpaced mating. Horm Behav 51:89-94.

Meerts SH, Clark AS (2009a) Artificial vaginocervical stimulation induces a conditioned place preference in female rats. Horm Behav 55:128-132.

Meerts SH, Clark AS (2009b) Conditioned place preference for mating is preserved in rats with pelvic nerve transection. Behav Neurosci 123:539-546.

Meerts SH, Clark AS (2009c) Lesions of the medial preoptic area interfere with the display of a conditioned place preference for vaginocervical stimulation in rats. Behav Neurosci 123:752-757.

Mehrara BJ, Baum MJ (1990) Naloxone disrupts the expression but not the acquisition by male rats of a conditioned place preference response for an oestrous female. Psychopharmacology 101:118-125.

Meisel RL, Camp DM, Robinson TE (1993) A microdialysis study of ventral striatal dopamine during sexual behavior in female Syrian hamsters. Behavioral Brain Research 55:151-157.

Meisel RL, Joppa MA (1994) Conditioned place preference in female hamsters following aggressive or sexual encounters. Physiol Behav 56:1115-1118.

Meisel RL, Joppa MA, Rowe RK (1996) Dopamine receptor antagonists attenuate conditioned place preference following sexual behavior in female Syrian hamsters. Eur J Pharmacol 309:21-24.

Melis MR, Argiolas A (2003) Central Oxytocinergic Neurotransmission: A drug target for the therapy of psychogenic erectile dysfunction. Curr Drug Targets 4:55-66.

Melis MR, Argiolas A, Gessa GL (1987) Apomorphine-induced penile erection and yawning: site of action in brain. Brain Res 415:98-104.

Melis MR, Melis T, Cocco C, Succu S, Sanna F, Pillolla G, Boi A, Ferri G-L, Argiolas A (2007) Oxytocin injected into the ventral tegmental area induces penile erection and increases extracellular dopamine in the nucleus accumbens and paraventricular nucleus of the hypothalamus of male rats. Eur J Neurosci 26:1026-1035.

Melis MR, Succu S, Iannucci U, Argiolas A (1997) Prevention by morphine of apomorphine- and oxytocin-induced penile erection and yawning: involvement of nitric oxide. Naunyn-Schmiedeberg's Archives of Pharmacology 355:595-600.

Melis MR, Succu S, Mascia MS, Cortis L, Argiolas A (2003) Extra-cellular dopamine increases in the paraventricular nucleus of male rats during sexual activity. Eur J Neurosci 17:1266-1272.

Melis MR, Succu S, Mauri A, Argiolas A (1998) Nitric oxide production is increased in the paraventricular nucleus of the hypothalamus of male rats during non-contact penile erections and copulation. Eur J Neurosci 10:1968-1974.

Meredith GE (1999) The Synaptic Framework for Chemical Signaling in Nucleus Accumbens. Ann N Y Acad Sci 877:140-156.

Meredith M (1986) Vomeronasal organ removal before sexual experience impairs male hamster mating behavior. Physiol Behav 36:737-743.

Meredith M (1991) Sensory processing in the main and accessory olfactory systems: comparisons and contrasts. The Journal of Steroid Biochemistry and Molecular Biology 39:601-614.

Meredith M, Howard G (1992) Intracerebroventricular LHRH relieves behavioral deficits due to vomeronasal organ removal. Brain Res Bull 29:75-79.

Miller LL, Whitsett JM, Vandenbergh JG, Colby DR (1977) Physical and Behavioral Aspects of Sexual Maturation in Male Golden Hamsters. Journal of Comparative and Physiological Psychology 91:245-259.

Miller RL, Baum MJ (1987) Naloxone inhibits mating and conditioned place preference for an estrous female in male rats soon after castration. Pharmacol Biochem Behav 26:781-789.

Miller SM, Lonstein JS (2009) Dopaminergic projections to the medial preoptic area of postpartum rats. Neuroscience 159:1384-1396.

Missale C, Nash SR, Robinson SW, Jaber M, Caron MG (1998) Dopamine Receptors: From Structure to Function. Physiol Rev 78:189-225.

Mitchell JB, Gratton A (1992) Mesolimbic dopamine release elicited by activation of the accessory olfactory system: A high speed chronoamperometric study. Neurosci Lett 140:81-84.

Mitchell JB, Stewart J (1989) Effects of castration, steroid replacement, and sexual experience on mesolimbic dopamine and sexual behaviors in the male rat. Brain Res 491:116-127.

Miyata H, Itasaka M, Kimura N, Nakayama K (2011) Decreases in Brain Reward Function Reflect Nicotine- and Methamphetaine- Withdrawal Aversion in Rats. Current Neuropharmacology 9:63-67.

Mogg K, Bradley BP (1998) A cognitive-motivational analysis of anxiety. Behav Res Ther 36:809-848.

Moise A, Eisenstein S, Astarita G, Piomelli D, Hohmann A (2008) An endocannabinoid signaling system modulates anxiety-like behavior in male Syrian hamsters. Psychopharmacology 200:333-346.

Monk CS, McClure EB, Nelson EE, Zarahn E, Bilder RM, Leibenluft E, Charney DS, Ernst M, Pine DS (2003) Adolescent immaturity in attention-related brain engagement to emotional facial expressions. NeuroImage 20:420-428.

Morin LP, Blanchard J (1993) Organization of the hamster paraventricular hypothalamic nucleus. J Comp Neurol 332:341-357.

Morin LP, Wood RI (2001) A stereotaxic atlas of the golden hamster brain. San Diego, CA: Academic Press.

Morin LP, Zucker I (1978) Photoperiodic regulation of copulatory behaviour in the male hamster. J Endocrinol 77:249-258.

Moses J, Hull EM (1999) A Nitric Oxide Synthesis Inhibitor Administered Into the Medial Preoptic Area Increases Seminal Emissions in an Ex Copula Reflex Test. Pharmacol Biochem Behav 63:345-348.

Moses J, Loucks JA, Watson HL, Matuszewich L, Hull EM (1995) Dopaminergic drugs in the medial preopic area and nucleus accumbens: effects on motor activity, sexual motivation, and sexual performance. Pharmacology, Biochemistry, and Behavior 51:681-686.

Mrzljak L, Uylings HB, Van Eden CG, Judas M (1990) Neuronal development in human prefrontal cortex in prenatal and postnatal stages. Prog Brain Res 85:185-222.

Murphy MR (1973) Effects of Female Hamster Vaginal Discharge on the Behavior of Male Hamsters. Behavioral Biology 9:367-375.

Murphy MR, Schneider GE (1970) Olfactory Bulb Removal Eliminates Mating Behavior in the Male Golden Hamster. Science 167:302-304.

Muschamp JW, Dominguez JM, Sato SM, Shen R-Y, Hull EM (2007) A Role for Hypocretin (Orexin) in Male Sexual Behavior. J Neurosci 27:2837-2845.

Nair-Roberts RG, Chatelain-Badie SD, Benson E, White-Cooper H, Bolam JP, Ungless MA (2008) Stereological estimates of dopaminergic, GABAergic and glutamatergic neurons in the ventral tegmental area, substantia nigra and retrorubral field in the rat. Neuroscience 152:1024-1031.

Narita M, Nagumo Y, Hashimoto S, Narita M, Khotib J, Miyatake M, Sakurai T, Yanagisawa M, Nakamachi T, Shioda S, Suzuki T (2006) Direct Involvement of Orexinergic Systems in the Activation of the Mesolimbic Dopamine Pathway and Related Behaviors Induced by Morphine. J Neurosci 26:398-405.

Nelson EE, Leibenluft E, McClure EB, Pine DS (2005) The social re-orientation of adolescence: a neuroscience perspective on the process and its relation to psychopathology. Psychol Med 35:163-174.

Niikura S, Yokoyama O, Komatsu K, Yotsuyanagi S, Mizuno T, Namiki M (2002) A causitive factor of copulatory disorder in rats following social stress. Journal of Urology 168:843-849.

Northcutt KV, Lonstein JS (2009) Social contact elicits immediate-early gene expression in dopaminergic cells of the male prairie vole extended olfactory amygdala. Neuroscience 163:9-22.

Nussey S, Whitehead S (2001) Endocrinology: An Integrated Approach. Oxford: BIOS Scientific Publishers.

O'Connell LA, Hofmann HA (2011) The Vertebrate mesolimbic reward system and social behavior network: A comparative synthesis. The Journal of Comparative Neurology 519:3599-3639.

O'Connell RJ, Meredith M (1984) Effects of volatile and nonvolatile chemical signals on male sex behaviors mediated by the main and accessory olfactory systems. Behav Neurosci 98:1083-1093.

O'Donnell P (2003) Dopamine gating of forebrain neural ensembles. Eur J Neurosci 17:429-435.

O'Donnell P, Grace A (1995) Synaptic interactions among excitatory afferents to nucleus accumbens neurons: hippocampal gating of prefrontal cortical input. The Journal of Neuroscience 15:3622-3639.

O’Donnell P, Lewis BL, Weinberger DR, Lipska BK (2002) Neonatal Hippocampal Damage Alters Electrophysiological Properties of Prefrontal Cortical Neurons in Adult Rats. Cereb Cortex 12:975-982.

Oades RD, Halliday GM (1987) Ventral tegmental (A10) system: neurobiology. 1. Anatomy and connectivity. Brain Res 434:117-165.

Oldenburger WP, Everitt BJ, de Jonge FH (1992) Conditioned place preferenc einduced by sexual interaction in female rats. Horm Behav 26.

Olson VG, Nestler EJ (2007) Topographical organization of GABAergic neurons within the ventral tegmental area of the rat. Synapse 61:87-95.

Olson VG, Zabetian CP, Bolanos CA, Edwards S, Barrot M, Eisch AJ, Hughes T, Self DW, Neve RL, Nestler EJ (2005) Regulation of Drug Reward by cAMP Response Element-Binding Protein: Evidence for Two Functionally Distinct Subregions of the Ventral Tegmental Area. The Journal of Neuroscience 25:5553-5562.

Orsini JC (1982) Androgen influence on lateral hypothalamus in the male rat: possible behavioral significance. Physiol Behav 29:979-987.

Orsini JC, Jourdan F, Cooper HM, Monmaur P (1985) Influence of female odors on lateral hypothalamus in the male rat. Semiquantitative deoxyglucose analysis. Physiol Behav 35:509-516.

Packard MG, Schroeder JP, Alexander GM (1998) Expression of Testosterone Conditioned Place Preference Is Blocked by Peripheral or Intra-accumbens Injection of [alpha]-Flupenthixol. Horm Behav 34:39-47.

Palkovits M, Fekete M, Makara GB, Herman JP (1977) Total and partial hypothalamic deafferentations of topographical identification of catecholaminergic innervations of certain preoptic and hypothalamic nuclei. Brain Res 127:127-136.

Pankevich DE, Cherry JA, Baum MJ (2006) Accessory olfactory neural Fos responses to a conditioned environment area blocked in male mice by vomeronasal organ removal. Physiol Behav 87:781-788.

Papp M (1988) Different effects of short- and long-term treatment with imipramine on the apomorphine- and food-induced place preference conditioning in rats. Pharmacol Biochem Behav 30:889-893.

Papp M, Muscat R, Willner P (1993) Subsensitivity to rewarding and locomotor stimulant effects of a dopamine agonist following chronic mild stress. Psychopharmacology 110:152-158.

Parada M, Chamas L, Censi S, Coria-Avila G, Pfaus JG (2010) Clitoral stimulation induces conditioned place preference and Fos activation in the rat. Horm Behav 57:112-118.

Paredes RG (2009) Evaluating the Neurobiology of Sexual Reward. Institute for Laboratory Animals Research Journal 50:15-27.

Paredes RG, Alonso A (1997) Sexual behavior regulated (paced) by the female induces conditioned place preference. Behav Neurosci 111:123-128.

Paredes RG, Highland L, Karam P (1993) Socio-sexual behavior in male rats after lesions of the medial preoptic area: evidence for reduced sexual motivation. Brain Res 618:271-276.

Parfitt DB, Thompson RC, Richardson HN, Romeo RD, Sisk CL (1999) Differential neuroendocrine response to female pheromones in juvenile and adult male Syrian hamsters. Society for Neurosciety Abstracts 25: 1453.

Paus T, Nawaz-Khan I, Leonard G, Perron M, Pike GB, Pitiot A, Richer L, Susman E, Veillette S, Pausova Z (2010) Sexual dimorphism in the adolescent brain: Role of testosterone and androgen receptor in global and local volumes of grey and white matter. Horm Behav 57:63-75.

Pehek EA, Warner RK, Bazzett TJ, Bitran D, Band LC, Eaton RC, Hull EM (1988) Microinjection of cis-flupenthixol, a dopamine antagonist, into the medial preoptic area impairs sexual behavior of male rats. Brain Res 443:70-76.

Peper JS, Hulshoff Pol HE, Crone EA, van Honk J (2011a) Sex steroids and brain structure in pubertal boys and girls: a mini-review of neuroimaging studies. Neuroscience 191:28-37.

Peper JS, van den Heuvel MP, Mandl RCW, Pol HEH, van Honk J (2011b) Sex steroids and connectivity in the human brain: A review of neuroimaging studies. Psychoneuroendocrinology 36:1101-1113.

Perks SM, Clifton PG (1997) Reinforcer revaluation and conditioned place preference. Physiol Behav 61:1-5.

Perrin JS, Hervé P-Y, Leonard G, Perron M, Pike GB, Pitiot A, Richer L, Veillette S, Pausova Z, Paus T (2008) Growth of White Matter in the Adolescent Brain: Role of Testosterone and Androgen Receptor. The Journal of Neuroscience 28:9519-9524.

Peters KD, Hom SM, Wood RI (2004) Testosterone and chemosensory detection in male Syrian hamster. Horm Behav 46:341-348.

Petrulis A (2009) Neural mechanisms of individual and sexual recognition in Syrian hamsters (Mesocricetus auratus). Behav Brain Res 200:260-267.

Petrulis A, Johnston RE (1995) A reevaluation of dimethyl disulfide as a sex attractant in golden hamsters. Physiol Behav 57:779-784.

Peyron C, Tighe DK, van den Pol AN, de Lecea L, Heller HC, Sutcliffe JG, Kilduff TS (1998) Neurons Containing Hypocretin (Orexin) Project to Multiple Neuronal Systems. J Neurosci 18:9996-10015.

Pfaus JG, Damsma G, Nomikos GG, Wenkstern D, Blaha CD, Phillips AG, Fibiger HC (1990) Sexual behavior enhances central dopamine transmission in the male rat. Brain Res 530:345-348.

Pfaus JG, Phillips AG (1989) Differential effects of dopamine receptor antagonists on the sexual behavior of male rats. Psychophermacology (Berlin) 98:363-368.

Pfaus JG, Phillips AG (1991) Role of Dopamine in Anticipatory and Consummatory Aspects of Sexual Behavior in the Male Rat. Behav Neurosci 105:727-743.

Pfeiffer CA, Johnston RE (1992) Socially stimulated androgen surges in male hamsters: The roles of vaginal secretions, behavioral interactions, and housing conditions. Horm Behav 26:283-293.

Pfeiffer CA, Johnston RE (1994) Hormonal and behavioral responses of male hamsters to females and female odors: Roles of olfaction, the vomeronasal system, and sexual experience. Physiol Behav 55:129-138.

Phillips AG, Ahn S, Howland JG (2003) Amygdalar control of the mesocorticolimibc dopamine system: parallel pathways to motivated behavior. Neuroscience and biobehavioral reviews 27:543-554.

Phillipson OT (1979) Afferent projections to the ventral tegmental area of Tsai and interfascicular nucleus: A horseradish peroxidase study in the rat. The Journal of Comparative Neurology 187:117-143.

Philpot REX, Kirstein C (2004) Developmental Differences in the Accumbal Dopaminergic Response to Repeated Ethanol Exposure. Ann N Y Acad Sci 1021:422-426.

Philpot RM, Badanich KA, Kirstein CL (2003) Place Conditioning: Age-Related Changes in the Rewarding and Aversive Effects of Alcohol. Alcoholism: Clinical and Experimental Research 27:593-599.

Philpot RM, Wecker L, Kirstein CL (2009) Repeated ethanol exposure during adolescence alters the developmental trajectory of dopaminergic output from the nucleus accumbens septi. Int J Dev Neurosci 27:805-815.

Pierman S, Tirelli E, Douhard Q, Baum MJ, Bakker J (2006) Male aromatase knockout mice acquire a conditioned place preference for cocaine but not for contact with an estrous female. Behav Brain Res 174:64-69.

Pitts DK, Freeman AS, Chiodo LA (1990) Dopamine neuron ontogeny: electrophysiological studies. Synapse 6:309-320.

Popik P, Wrobel M, Rygula R, Bisaga A, Bespalov AY (2003) Effects of memantine, an NMDA receptor antagonist, on place preference conditioning with drug and nondrug reinforcers in mice. Behavioral Pharmacology 14:237-244.

Powers JB, Bergondy ML (1983) Androgenic regulation of chemoinvestigatory behaviors in male and female hamsters. Horm Behav 17:28-44.

Powers JB, Bergondy ML, Matochick JA (1985) Male hamster sociosexual behaviors: effects of testosterone and its metabolites. Physiol Behav 35:607-616.

Powers JB, Fields RB, Winans SS (1979) Olfactory and vomeronasal system participation in male hamsters' attraction to female vaginal secretions. Physiol Behav 22:77-84.

Powers JB, Winans SS (1975) Vomeronasal organ: critical role in mediating sexual behavior of the male hamster. Science 187:961-963.

Putnam SK, Du J, Sato S, Hull EM (2001) Testosterone Restoration of Copulatory Behavior Correlates with Medial Preoptic Dopamine Release in Castrated Male Rats. Horm Behav 39:216-224.

Putnam SK, Sato S, Hull EM (2003) Effects of testosterone metabolites on copulation and medial preoptic dopamine release in castrated male rats. Horm Behav 44:419-426.

Putnam SK, Sato S, Riolo JV, Hull EM (2005) Effects of testosterone metabolites on copulation, medial preoptic dopamine, and NOS-immunoreactivity in castrated male rats. Horm Behav 47:513-522.

Rayport S, Sulzer D, Shi WX, Sawasdikosol S, Monaco J, Batson D, Rajendran G (1992) Identified postnatal mesolimbic dopamine neurons in culture: morphology and electrophysiology. J Neurosci 12:4264-4280.

Richard JM, Berridge KC (2011) Nucleus Accumbens Dopamine/Glutamate Interaction Switches Modes to Generate Desire versus Dread: D1 Alone for Appetitive Eating But D1 and D2 Together for Fear. The Journal of Neuroscience 31:12866-12879.

Richfield EK, Penney JB, Young AB (1989) Anatomical and affinity state comparisons between dopamine D1 and D2 receptors in the rat central nervous system. Neuroscience 30:767-777.

Risinger FO, Dickinson SD, Cunningham CL (1992) Haloperidol reduces ethanol-induced motor activity stimulation but not conditioned place preference. Psychopharmacology (Berl) 107:453-456.

Robbins TW, Everitt BJ (1996) Neurobehavioural mechanisms of reward and motivation. Curr Opin Neurobiol 6:228-236.

Robinson DL, Phillips PEM, Budygin EA, Trafton BJ, Garris PA, Wightman RM (2001) Sub-second changes in accumbal dopamine during sexual behavior in male rats. Neuroreport 12:2549-2552.

Robinson DL, Zitzman DL, Smith KJ, Spear LP (2011) Fast dopamine release events in the nucleus accumbens of early adolescent rats. Neuroscience 176:296-307.

Robinson TE, Camp DM, Jacknow DS, Becker JB (1982) Sex differences and estrous cycle dependent variation in rotational behavior elicited by electrical stimulation of the mesostriatal dopamine system. Behavioral Brain Research 6:273-287.

Rodríguez-Manzo G (1999) Yohimbine interacts with the dopaminergic system to reverse sexual satiation: further evidence for a role of sexual motivation in sexual exhaustion. Eur J Pharmacol 372:1-8.

Romeo RD, Parfitt DB, Richardson HN, Sisk CL (1998) Pheromones Elicit Equivalent Levels of Fos-Immunoreactivity in Prepubertal and Adult Male Syrian Hamsters. Horm Behav 34:48-55.

Romeo RD, Richardson HN, Sisk CL (2002) Puberty and the maturation of the male brain and sexual behavior: recasting a behavioral potential. Neurosci Biobehav Rev 26:381-391.

Romeo RD, Wade J, Venier JE, Sisk CL (1999) Androgenic Regulation of Hypothalamic Aromatase Activity in Prepubertal and Postpubertal Male Golden Hamsters. Endocrinology 140:112-117.

Rosenberg DR, Lewis DA (1995) Postnatal maturation of dopaminergic innervation of monkey prefrontal and motor cortices: A tyrosine hydroxylase immunohistochemical analysis. J Comp Neurol 258:383-400.

Rowe FA, Edwards DA (1972) Olfactory bulb removal: influences on the mating behavior of male mice. Physiol Behav 8:37-41.

Russo SJ, Festa ED, Fabian SJ, Gazi FM, Kraish M, Jenab S, Quiñones-Jenab V (2003) Gonadal hormones differentially modulate cocaine-induced conditioned place preference in male and female rats. Neuroscience 120:523-533.

Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richardson JA, Kozlowski GP, Wilson S, Arch JRS, Buckingham RE, Haynes AC, Carr SA, Annan RS, McNulty DE, Liu W-S, Terrett JA, Elshourbagy NA, Bergsma DJ, Yanagisawa M (1998) Orexins and Orexin Receptors: A Family of Hypothalamic Neuropeptides and G Protein-Coupled Receptors that Regulate Feeding Behavior. Cell 92:573-585.

Salamone JD, Correa M, Mingote SM, Weber SM (2005) Beyond the reward hypothesis: alternative functions of nucleus accumbens dopamine. Curr Opin Pharm 5:34-41.

Sanberg PR (1989) Neuroleptic-induced emotional defecation: Effects of pimozide and apomorphine. Physiology & Behavior 46:199-202.

Santana N, Mengod G, Artigas F (2009) Quantitative Analysis of the Expression of Dopamine D1 and D2 Receptors in Pyramidal and GABAergic Neurons of the Rat Prefrontal Cortex. Cereb Cortex 19:849-860.

Saper CB, Swanson LW, Cowan WM (1976) The efferent connections of the ventromedial nucleus of the hypothalamus of the rat. J Comp Neurol 169:409-442.

Sato SM, Schulz KM, Sisk CL, Wood RI (2008) Adolescents and androgens, receptors and rewards. Horm Behav 53:647-658.

Sato Y, Christ GJ, Horita H, Adachi H, Suzuki N, Tsukamoto T (1999) The effects of alterations in nitric oxide levels in the paraventricular nucleus on copulatory behavior and reflexive erections in male rats. Journal of Urology 162:2182-2185.

Sato Y, Horita H, Kurohata T, Adachi H, Tsukamoto T (1998) Effect of the nitric oxide level in the medial preoptic area on male copulatory behavior in rats. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology 274:R243-R247.

Sato Y, Wada H, Horita H, Suzuki N, Shibuya A, Adachi H, Kato R, Tsukamoto T, Kumamoto Y (1995) Dopamine release in the medial preoptic area during male copulatory behavior in rats. Brain Res 692:66-70.

Sawai N, Ueta Y, Nakazato M, Ozawa H (2010) Developmental and aging change of orexin-A and -B immunoreactive neurons in the male rat hypothalamus. Neurosci Lett 468:51-55.

Scaletta LL, Hull EM (1990) Systemic or intracranial apomorphine increases copulation in long-term castrated male rats. Pharmacology, biochemistry, and behavior 37:471-475.

Schechter MD, Calcagnetti DJ (1993) Trends in place preference conditioning with a cross-indexed bibliography; 1957-1991. Neurosci Biobehav Rev 17:21-41.

Schmitt O, Usunoff K, Lazarov N, Itzev D, Eipert P, Rolfs A, Wree A (2012) Orexinergic innervation of the extended amygdala and basal ganglia in the rat. Brain Structure and Function 217:233-256.

Schoenfeld TA, Leonard CM (1985) Behavioral development in the Syrian golden hamster. In: The Hamster: Reproduction and Behavior (Siegel, H. I., ed), pp 289-321 New York: Plenum Press.

Schulz KM (2007) Sensitive Periods in the Development of Male Social Behavior: The Role of Gonadal Hormones During Adolescence. Michigan State University Dissertation.

Schulz KM, Richardson HN, Romeo RD, Morris JA, Lookingland KJ, Sisk CL (2003) Medial preoptic area dopaminergic responses to female pheromones develop during puberty in the male Syrian hamster. Brain Res 988:139-145.

Schulz KM, Sisk CL (2006) Pubertal hormones, the adolescent brain, and the maturation of social behaviors: Lessons from the Syrian hamster. Mol Cell Endocrinol 254-255:120-126.

Schulz KM, Zehr JL, Salas-Ramirez KY, Sisk CL (2009) Testosterone Programs Adult Social Behavior before and during, But Not after, Adolescence. Endocrinology 150:3690-3698.

Seamans JK, Yang CR (2004) The principal features and mechanisms of dopamine modulation in the prefrontal cortex. Prog Neurobiol 74:1-58.

Seeman P, Bzowej NH, Guan HC, Bergeron C, Becker LE, Reynolds GP, Bird ED, Riederer P, Jellinger K, Watanabe S (1987) Human brain dopamine receptors in children and aging adults. Synapse 1:399-404.

Seitz RJ, Nickel J, Azari NP (2006) Functional modularity of the medial prefrontal cortex: Involvement in human empathy. Neuropsychology 20:743-751.

Seroogy KB, Mehta A, Fallon JH (1987) Neurotensin and cholecystokinin coexistence within neurons of the ventral mesencephalon: projections to forebrain. Exp Brain Res 68:277-289.

Sesack SR, Grace AA (2009) Cortico-Basal Ganglia Reward Network: Microcircuitry. Neuropsychopharmacology 35:27-47.

Sheng M, Greenberg MF (1990) The Regulation and Function of c-fos and Other Immediate Early Genes in the Nervous System. Neuron 4:477-485.

Shim I-S, Won J-S, Lee J-K, Song D-K, Kim SE, Huh S-O, Kim Y-H, Suh H-W (2000) Modulatory effect of ginseng total saponin on dopamine release and tyrosine hydroxylase gene expression induced by nicotine in the rat. J Ethnopharmacol 70:161-169.

Shippenberg TS, Bals-Kubik R, Huber A, Herz A (1991) Neuroanatomical substrates mediating the aversive effects of D-1 dopamine receptor antagonists. Psychopharmacology 103:209-214.

Silveyra P, Cataldi NI, Lux-Lantos V, Libertun C (2009) Gonadal steroids modulated hypocretin/orexin type-1 receptor expression in a brain region, sex and daytime specific manner. Regulatory Peptides 158:121-126.

Simerly RB, Chang C, Muramatsu M, Swanson LW (1990) Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: an in situ hybridization study. J Comp Neurol 294:76-95.

Simerly RB, Swanson LW (1986) The organization of neural inputs to the medial preoptic nucleus of the rat. J Comp Neurol 246:312-342.

Simerly RB, Swanson LW (1988) Projections of the medial preoptic nucleus: a Phaseolus vulgaris leucoagglutinin anterograde tract-tracing study in the rat. J Comp Neurol 270:209-242.

Simmons DA, Yahr P (2002) Projections of the posterodorsal preoptic nucleus and the lateral part of the posterodorsal medial amygdala in male gerbils, with emphasis on cells activated with ejaculation. The Journal of Comparative Neurology 444:75-94.

Simpkins JW, Kalra SP, Kalra PS (1983) Variable Effects of Testosterone on Dopamine Activity in Several Microdissected Regions in the Preoptic Area and Medial Basal Hypothalamus. Endocrinology 112:665-669.

Singer AG, Agosta WC, Clancy AN, Macrides F (1987) The Chemistry of Vomeronasally Detected Pheromones: Characterization of an Aphrodisiac Protein. Ann N Y Acad Sci 519:287-298.

Singer AG, Agosta WC, O'Connell RJ, Pfaffmann C, Bowen DV, Field FH (1976) Dimethyl disulfide: an attractant pheromone in hamster vaginal secretion. Science 191:948-950.

Singer AG, Clancy AN, Macrides F, Agosta WC (1984a) Chemical studies of hamster vaginal discharge: effects of endocrine ablation and protein digestion on behaviorally active macromolecular fractions. Physiol Behav 33:639-643.

Singer AG, Clancy AN, Macrides F, Agosta WC (1984b) Chemical studies of hamster vaginal discharge: Male behavioral responses to a high molecular weight fraction require physical contact. Physiol Behav 33:645-651.

Singer AG, Macrides F, Clancy AN, Agosta WC (1986) Purification and analysis of a proteinaceous aphrodisiac pheromone from hamster vaginal discharge. J Biol Chem 261:13323-13326.

Sisk CL, Foster DL (2004) The neural basis of puberty and adolescence. Nat Neurosci 7:1040-1047.

Sisk CL, Turek FW (1983) Developmental Time Course of Pubertal and Photoperiodic Changes in Testosterone Negative Feedback on Gonadotropin Secretion in the Golden Hamster. Endocrinology 112:1208-1216.

Sowell ER, Trauner DA, Gamst A, Jernigan TL (2002) Development of cortical and subcortical brain structures in childhood and adolescence: a structural MRI study. Developmental Medicine & Child Neurology 44:4-16.

Spear LP (2000) The adolescent brain and age-related behavioral manifestations. Neurosci Biobehav Rev 24:417-463.

Spelsberg TC, Rories C, Rejman JJ, Goldberger A, Fink K, Lau CK, Colvard DS, Wiseman G (1989) Steroid action on gene expression: possible roles of regulatory genes and nuclear acceptor sites. Biol Reprod 40:54-69.

Spyraki C, Fibiger HC (1988) A role for the mesolimbic dopamine system in the reinforcing properties of diazepam. Psychopharmacology (Berl) 94:133-137.

Steel E (1982) Testosterone-dependent non-copulatory behaviour in male hamsters (Mesocricetus auratus). J Endocrinol 95:387-396.

Steel E, Hutchison JB (1986) Olfactory recognition in the male hamster: effect of non-aromatizable androgens, 17β-hydroxy-17α-methyl-estra-4,9,11-triene-3-one (R1881) and 5α-dihydrotestosterone, in combination with oestrogen. J Endocrinol 110:525-531.

Steel E, Hutchison JB (1988) Behavioral action of estrogen in male hamsters: Effect of the aromatase inhibitor, 1,4,6-androstatriene-3, 17-dione (ATD). Horm Behav 22:252-265.

Steel E, Keverne EB (1985) Effect of female odour on male hamsters mediated by the vomeronasal organ. Physiol Behav 35:195-200.

Steinberg L (2008) A social neuroscience perspective on adolescent risk-taking. Developmental Reviews 28:78-106.

Stork O, Hashimoto T, Obata K (1994) Haloperidol activates tyrosine hydroxylase gene-expression in the rat substantia nigra, pars reticulata. Brain Res 633:213-222.

Succu S, Sanna F, Cocco C, Melis T, Boi A, Ferri G-L, Argiolas A, Melis MR (2008) Oxytocin induces penile erection when injected into the ventral tegmental area of male rats: role of nitric oxide and cyclic GMP. Eur J Neurosci 28:813-821.

Succu S, Sanna F, Melis T, Boi A, Argiolas A, Melis MR (2007) Stimulation of dopamine receptors in the paraventricular nucleus of the hypothalamus of male rats induces penile erection and increases extra-cellular dopamine in the nucleus accumbens: Involvement of central oxytocin. Neuropharmacology 52:1034-1043.

Surmeier DJ, Ding J, Day M, Wang Z, Shen W (2007) D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons. Trends Neurosci 30:228-235.

Suzuki T, Misawa M (1995) Sertindole antagonizes morphine-, cocaine-, and methamphetamine-induced place preference in the rat. Life Sci 57:1277-1284.

Swanson LW (1982a) The projections of the ventral tegmental area and adjacent regions: a combined flourescent retrograde tracer and immunofluorescence study in the rat. Brain Res Bull 9:321-353.

Swanson LW (1982b) The projections of the ventral tegmental area and adjacent regions: A combined fluorescent retrograde tracer and immunofluorescence study in the rat. Brain Res Bull 9:321-353.

Swanson LW, Sawchenko PE (1980) Paraventricular nucleus: a site for the integration of neuroendocrine and autonomic mechanisms. Neuroendocrinology 31:410-417.

Tarazi FI, Baldessarini RJ (2000) Comparative postnatal development of dopamine D1, D2 and D4 receptors in rat forebrain. Int J Dev Neurosci 18:29-37.

Teicher MH, Andersen SL, Hostetter JC (1995) Evidence for dopamine receptor pruning between adolescence and adulthood in striatum but not nucleus accumbens. Dev Brain Res 89:167-172.

Teicher MH, Gallitano AL, Gelbard HA, Evans HK, Marsh ER, Booth RG, Baldessarini RJ (1991) Dopamine D1 autoreceptor function: possible expression in developing rat prefrontal cortex and striatum. Dev Brain Res 63:229-235.

Tenk CM, Wilson H, Zhang Q, Pitchers KK, Coolen LM (2009) Sexual reward in male rats: Effects of sexual experience on conditioned place preferences associated with ejaculation and intromissions. Horm Behav 55:93-97.

Tepper JM, Sharpe NA, Koos TZ, Trent F (1998) Postnatal development of the rat neostriatum: electrophysiological, light- and electron-microscopic studies. Dev Neurosci 20:125-145.

Thiele TE, Cubero I, Van Dijk G, Mediavilla C, Bernstein IL (2000) Ethanol-Induced c-Fos Expression in Catecholamine- and Neuropeptide Y-Producing Neurons in Rat Brainstem. Alcoholism: Clinical and Experimental Research 24:802-809.

Thompson R, Swanson L (2010) Hypothesis-driven structural connectivity analysis supports network over hierarchical model of brain architecture. Proc Natl Acad Sci U S A 107:15235-15239.

Tirelli E, Laviola G, Adriani W (2003) Ontogenesis of behavioral sensitization and conditioned place preference induced by psychostimulants in laboratory rodents. Neurosci Biobehav Rev 27:163-178.

Torrella TA, Badanich KA, Philpot RM, Kirstein CL, Wecker L (2004) Developmental Differences in Nicotine Place Conditioning. Ann N Y Acad Sci 1021:399-403.

Triana-Del Rio R, Montero-Domínguez F, Cibrian-Llanderal T, Tecamachaltzi-Silvaran MB, Garcia LI, Manzo J, Hernandez ME, Coria-Avila GA (2011) Same-sex cohabitation under the effects of quinpirole induces a conditioned socio-sexual partner preference in males, but not in female rats. Pharmacol Biochem Behav 99:604-613.

Triemstra JL, Nagatani S, Wood RI (2005) Chemosensory Cues are Essential for Mating-Induced Dopamine Release in MPOA of Male Syrian Hamsters. Neuropsychopharmacology 30:1436-1442.

Trivedi P, Yu H, MacNeil DJ, Van der Ploeg LHT, Guan X-M (1998) Distribution of orexin receptor mRNA in the rat brain. FEBS Lett 438:71-75.

Tsai H-C, Zhang F, Adamantidis A, Stuber GD, Bonci A, de Lecea L, Deisseroth K (2009) Phasic Firing in Dopaminergic Neurons Is Sufficient for Behavioral Conditioning. Science 324:1080-1084.

Tseng KY, O'Donnell P (2004) Dopamine–Glutamate Interactions Controlling Prefrontal Cortical Pyramidal Cell Excitability Involve Multiple Signaling Mechanisms. The Journal of Neuroscience 24:5131-5139.

Tseng KY, O'Donnell P (2005) Post-pubertal Emergence of Prefrontal Cortical Up States Induced by D1–NMDA Co-activation. Cereb Cortex 15:49-57.

Tseng KY, O'Donnell P (2007) D2 dopamine receptors recruit a GABA component for their attenuation of excitatory synaptic transmission in the adult rat prefrontal cortex. Synapse 61:843-850.

Tzschentke T (2004) Reassessment of buprenorphine in conditioned place preference: temporal and pharmacological considerations. Psychopharmacology 172:58-67.

Tzschentke TM (1998) Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. Prog Neurobiol 56:613-672.

Tzschentke TM (2000) The medial prefrontal cortex as part of the brain reward system. Amino Acids 19:211-219.

Tzschentke TM (2007) Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. Addict Biol 12:227-462.

Urbanski HF, Doan A, Pierce M, Fahrenbach WH, Collins PM (1992) Maturation of the hypothalamo-pituitary-gonadal axis of male Syrian hamsters. Biol Reprod 46:991-996.

Usunoff KG, Schmitt O, Itzev DE, Haas SJ, Lazarov NE, Rolfs A, Wree A (2009) Efferent Projections of the Anterior and Posterodorsal regions of the Medial Nucleus of the Amygdala in the Mouse. Cells, Tissues, Organs 190:256-285.

Uylings HBM, Groenewegen HJ, Kolb B (2003) Do rats have a prefrontal cortex? Behav Brain Res 146:3-17.

Valenti O, Grace AA (2010) Antipsychotic drug-induced increases in ventral tegmental area dopamine neuron population activity via activation of the nucleus accumbens–ventral pallidum pathway. The International Journal of Neuropsychopharmacology 13:845-860.

Van Bockstaele EJ, Pickel VM (1995) GABA-containing neurons in the ventral tegmental area project to the nucleus accumbens in rat brain. Brain Res 682:215-221.

van Furth WR, Wolterink G, van Ree JM (1995) Regulation of masculine sexual behavior: involvement of brain opioids and dopamine. Brain Res Rev 21:162-184.

Van Leijenhorst L, Moor BG, Op de Macks ZA, Rombouts SARB, Westenberg PM, Crone EA (2010a) Adolescent risky decision-making: Neurocognitive development of reward and control regions. NeuroImage 51:345-355.

Van Leijenhorst L, Zanolie K, Van Meel CS, Westenberg PM, Rombouts SARB, Crone EA (2010b) What Motivates the Adolescent? Brain Regions Mediating Reward Sensitivity across Adolescence. Cereb Cortex 20:61-69.

van Wingen G, Mattern C, Verkes RJ, Buitelaar J, Fernández G (2010) Testosterone reduces amygdala–orbitofrontal cortex coupling. Psychoneuroendocrinology 35:105-113.

Vandenbergh JG (1973) Acceleration and inhibition of puberty in female mice by pheromones. Journal of reproduction and fertility Supplement 19:411-419.

Varlinskaya EI, Spear LP (2008) Social interactions in adolescent and adult Sprague–Dawley rats: Impact of social deprivation and test context familiarity. Behav Brain Res 188:398-405.

Vastola BJ, Douglas LA, Varlinskaya EI, Spear LP (2002) Nicotine-induced conditioned place preference in adolescent and adult rats. Physiol Behav 77:107-114.

Vertes RP (2004) Differential projections of the infralimbic and prelimbic cortex in the rat. Synapse 51:32-58.

Vertes RP (2006) Interactions among the medial prefrontal cortex, hippocampus and midline thalamus in emotional and cognitive processing in the rat. Neuroscience 142:1-20.

Vincent SR (1988) Distributions of Tyrosine Hydroxylase-, Dopamine beta Hydroxylase-, and Phenylethanolamine-N-Methyltransferase- Immunoreactive Neurons in the Brain of the Hamster (Mesocricetus auratus). The Journal of Comparative Neurology 268:584-599.

Vincent SR, Hope BT (1990) Tyrosine hydroxylase containing neurons lacking aromatic amino acid decarboxylase in the hamster brain. J Comp Neurol 295:290-298.

Vittoz NM, Schmeichel B, Berridge CW (2008) Hypocretin /orexin preferentially activates caudomedial ventral tegmental area dopamine neurons. Eur J Neurosci 28:1629-1640.

Volman I, Toni I, Verhagen L, Roelofs K (2011) Endogenous Testosterone Modulates Prefrontal–Amygdala Connectivity during Social Emotional Behavior. Cereb Cortex 21:2282-2290.

Vomachka AJ, Greenwald GS (1979) The Development of Gonadotropin and Steroid Hormone Patterns in Male and Female Hamsters from Birth to Puberty. Endocrinology 105:960-966.

Wahlstrom D, White T, Luciana M (2010a) Neurobehavioral evidence for changes in dopamine system activity during adolescence. Neurosci Biobehav Rev 34:631-648.

Wahlstrom D, White T, Luciana M (2010b) Neurobehavioral evidence for changes in dopamine system activity during adolescence. Neuroscience and Biobehavioral Reviews 34:631-648.

Walker QD, Kuhn CM (2008) Cocaine increases stimulated dopamine release more in periadolescent than adult rats. Neurotoxicol Teratol 30:412-418.

Wang Z, Yu G, Cascio C, Liu Y, Gingrich B, Insel TR (1999) Dopamine D2 receptor-mediated regulation of partner preferences in female prairie voles (Microtus ochrogaster): a mechanism for pair bonding? Behav Neurosci 113:602-611.

Warner RK, Thompson JT, Markowski VP, Loucks JA, Bazzett TJ, Eaton RC, Hull EM (1991) Microinjection of the dopamine antagonist cis-flupenthixol into the MPOA impairs copulation, penile reflexes and sexual motivation in male rats. Brain Res 540:177-182.

Wenkstern D, Pfaus JG, Fibiger HC (1993) Dopamine transmission increases in the nucleus accumbens of male rats during their first exposure to sexually receptive female rats. Brain Res 618:41-46.

Westberry J, Meredith M (2003) The influence of chemosensory input and gonadotropin releasing hormone on mating behavior circuits in male hamsters. Brain Res 974:1-16.

Whalen RE, Debold JF (1974) Comparative Effectiveness of Testosterone, Androstenedione and Dihydrotestosterone in Maintaining Mating Behavior in the Castrated Male Hamster. Endocrinology 95:1674-1679.

White NM, Packard MG, Hiroi N (1991) Place conditioning with dopamine D1 and D2 agonists injected peripherally or into nucleus accumbens. Psychopharmacology 103:271-276.

Wilmouth CE, Spear LP (2004) Adolescent and Adult Rats' Aversion to Flavors Previously Paired with Nicotine. Ann N Y Acad Sci 1021:462-464.

Wirtshafter D, Asin KE (1994) Interactive effects of stimulation of D1 and D2 dopamine receptors on fos-like immunoreactivity in the normosensitive rat striatum. Brain Res Bull 35:85-91.

Wise RA (2004) Dopamine, learning and motivation. Nat Rev Neurosci 5:483-494.

Wommack JC, Salinas A, Melloni RH, Delville Y (2004) Behavioural and Neuroendocrine Adaptations to Repeated Stress during Puberty in Male Golden Hamsters. J Neuroendocrinol 16:767-775.

Wood RI (1997) Thinking about Networks in the Control of Male Hamster Sexual Behavior. Horm Behav 32:40-45.

Wood RI (2004) Reinforcing aspects of androgens. Physiol Behav 83:279-289.

Wood RI, Coolen LM (1997) Integration of chemosensory and hormonal cues is essential for mating in the male syrian hamster: role of the medial amygdaloid nucleus. Neuroscience 78:1027-1035.

Wood RI, Newman SW (1995a) Integration of chemosensory and hormonal cues is essential for mating in the male Syrian hamster. J Neurosci 15:7261-7269.

Wood RI, Newman SW (1995b) The Medial Amygdaloid Nucleus and Medial Preoptic Area Mediate Steroidal Control of Sexual Behavior in the Male Syrian Hamster. Horm Behav 29:338-353.

Wood RI, Newman SW (1999) Androgen receptor immunoreactivity in the male and female Syrian hamster brain. J Neurobiol 39:359-370.

Wood RI, Swann JM (2005) The bed nucleus of the stria terminalis in the Syrian hamster: Subnuclei and connections of the posterior division. Neuroscience 135:155-179.

Woodley SK, Baum MJ (2004) Differential activation of glomeruli in the ferret's main olfactory bulb by anal scent gland odours from males and females: an early step in mate identification. Eur J Neurosci 20:1025-1032.

Wu Q, Reith MEA, Walker QD, Kuhn CM, Carroll FI, Garris PA (2002) Concurrent Autoreceptor-Mediated Control of Dopamine Release and Uptake during Neurotransmission: An In Vivo Voltammetric Study. The Journal of Neuroscience 22:6272-6281.

Wu W-R, Zhu X-Z (1999) The amphetamine-like reinforcing effect and mechanism of l-deprenyl on conditioned place preference in mice. Eur J Pharmacol 364:1-6.

Xia Y, Driscoll JR, Wilbrecht L, Margolis EB, Fields HL, Hjelmstad GO (2011) Nucleus Accumbens Medium Spiny Neurons Target Non-Dopaminergic Neurons in the Ventral Tegmental Area. The Journal of Neuroscience 31:7811-7816.

Yamamoto Y, Ueta Y, Hara Y, Serino R, Nomura M, Shibuya I, Shirahata A, Yamashita H (2000) Postnatal development of orexin/hypocretin in rats. Mol Brain Res 78:108-119.

Yannielli PC, Kanterewicz BI, Cardinali DP (1996) Daily rhythms in spontaneous and diazepam-induced anxiolysis in Syrian hamsters. Pharmacol Biochem Behav 54:651-656.

Yoshida K, McCormack S, España RA, Crocker A, Scammell TE (2006) Afferents to the orexin neurons of the rat brain. The Journal of Comparative Neurology 494:845-861.