# NEURAL CORRELATES OF ADOLESCENT BEHAVIOR

by

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Bachelor of Arts, Vassar College, 2002

Submitted to the Graduate Faculty of

Arts and Sciences in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

University of Pittsburgh

2011

UNIVERSITY OF PITTSBURGH

SCHOOL OF ARTS & SCIENCES

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Adolescence is a developmental stage between childhood and adulthood associated with numerous brain and behavioral changes. It is also a period of vulnerability, as adolescents tend to take more risks, and various psychiatric problems first typically manifest at this time. Yet little is known about the neuronal basis of these vulnerabilities. Although extracellular electrophysiological recording is a useful technique for measuring the neural activity of awake behaving animals, it had not yet been used to address the neural correlates of adolescent motivated behavior. This dissertation therefore had two primary objectives. The first was to characterize a novel behavioral task suitable for testing adolescent and adult rats. The second was to record the neural activity of brain regions involved in motivated behavior, as adolescents and adults performed it.

The behavioral task was a simple instrumental learning paradigm, in which rats associated poking into a hole with the delivery of a food pellet reward. While the learning and performance of this task was similar between the two groups, adolescents persisted in this activity more than adults when reward was withheld. It was determined that this was due to different age-related sensitivities to the presence of certain motivational factors.

After characterizing the task, it was performed by adolescent and adult rats that had electrode arrays implanted in their orbitofrontal cortex (OFC), nucleus accumbens (NAc), or dorsal striatum (DS). Neural activity was examined in the context of similar instrumental

behavior to determine whether adolescents processed salient events in a fundamentally different way from adults. Several interesting neural processing differences were observed, along with some notable similarities. The greatest phasic activity differences were found in the OFC and DS, particularly during the period immediately before reward. Local field potential oscillations also tended to differ, with particular disparities found in the DS. In contrast, NAc activity tended to look similar between adolescents and adults, with a few exceptions. In addition to demonstrating fundamental age-related neural processing differences during motivated behavior, these findings address existing hypotheses and raise new questions relevant to the neural basis of the increased vulnerabilities of adolescence.

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## PREFACE

The guidance and support I received during graduate school was immense. I consider myself incredibly fortunate to have worked in such a thriving scholarly environment and with so many caring and engaged faculty members and students. First and foremost I would like to thank my academic advisor and mentor Bita Moghaddam. Immediately after accepting me into her laboratory, she generously granted me the latitude to follow my own interests and try new things. Although not every idea I pursued was successful, I learned much from this process, and it has given me greater confidence to explore the unknown. All along the way Bita has offered sage advice and guidance. She has challenged me to think big, several steps ahead, and to not be too attached to conventional wisdom or popular hypotheses. “Do not be a mouthpiece for orthodoxy,” she has said. I have grown so much personally and intellectually from working in the Moghaddam lab, and will forever be grateful for this experience.

I would also like to thank my dissertation committee—Julie Fiez, Charlie Bradberry, Carol Colby, and Bea Luna—for their time, interest, and insightful feedback. I have come away from each meeting with new ideas and greater enthusiasm than before because of their attention to detail and excellent collective chemistry. They have also been incredibly supportive and positive, which has been a lift through this whole process.

Members of the Moghaddam lab—past and present—have taught me a great deal and also made me feel at home. Houman Homayoun, Yunbok Kim, and Alan Pehrson were more

than generous with their time and expertise. They taught me much of what I know about behavioral and electrophysiological methods. Discussions and debates with them and with Nelson Totah, Marguerite Matthews, Corina Bondi, Nick Simon, and especially Jesse Wood have helped me to flesh out, refine, and correct my own ideas and interpretations. I have also come to learn that the smooth operation of a laboratory, a department, and a program depend on the efforts of talented and dedicated staff. I am grateful to Talia Lisowitz, Jody Tock, and Karen Smithmyer within the Moghaddam lab; to the CNUP administrative staff, especially Patti Argenzio and Joan Blaney; and to the Pitt MSTP, especially Clayton Wiley, Manjit Singh, and Justin Markuss.

I have also benefited personally and intellectually from my relationships with John Saunders, Meghan Wilson, Andreea Bostan, Tristan Nixon, David Seidenfeld, Adam Greenberg, Christian Bikle, Heather Benno, and many others. Their friendships have made me a better and more well-rounded person, and they have made my successes more likely.

I would like to thank my sisters Rachel, Serena, and Nicole, and my parents Audrie and Larry for their life-long unconditional love and encouragement. Finally, my wife Emily has supported me in every way a true partner and a close friend can. She has listened to me practice virtually every talk and edited almost every manuscript, including this dissertation. She has believed in me more than anyone and has made my life much more interesting and fun.

*"I would there were no age between ten and three-and-twenty, or that youth would sleep out the rest; for there is nothing in the between but getting wenches with child, wronging the ancientry, stealing, fighting."* – William Shakespeare[1]

*"The imagination of a boy is healthy, and the mature imagination of a man is healthy; but there is a space of life between, in which the soul is in a ferment, the character undecided, the way of life uncertain, the ambition thick-sighted: thence proceeds mawkishness."* – John Keats[2]

[1] The Winter's Tale, III. iii.

[2] *The complete poetical works and letters of John Keats*. Cambridge Edition. New York: Houghton Mifflin Company. 1899. p.48

# 1.0 INTRODUCTION

Adolescence is a period in which individuals observe physical changes to their bodies, experience new interests and desires, and find themselves with greater freedom, independence, and responsibility. Although variably defined, adolescence is generally considered to begin with the onset of puberty and ends as one takes on adult social roles (Spear, 2000; Dahl, 2004). The span of puberty typically occurs from age 10 to 17 in girls and 12 to 18 in boys (Falkner and Tanner, 1986). From the mid-19$^{th}$ through the 20$^{th}$ century, an earlier average age of menarche has been observed in the western world (Falkner and Tanner, 1986; Tanner, 1990). The educational process is more prolonged and individuals are tending to wait longer before starting their careers, getting married, and having children (Dahl, 2004). Thus the length of adolescence is not fixed (and has been lengthening) and while the period correlates with many biological developmental processes, it is partially defined according to psychosocial and behavioral criteria.

Despite the definitional ambiguities, it is well recognized that during this period major transitions do occur, including a variety of characteristic behavioral changes seen across species. There is increased social behavior (Csikszentmihalyi et al., 1977), novelty and sensation seeking (Adriani et al., 1998; Stansfield et al., 2004; Stansfield and Kirstein, 2006), tendencies toward risk taking (Spear, 2000; Steinberg, 2008), emotional instability (Steinberg, 2005), and impulsivity (Fairbanks et al., 2001; Adriani and Laviola, 2003; Chambers et al., 2003; Vaidya et al., 2004). Peer relationships become dominant, and there are greater inclinations to seek out fun

and exciting experiences (Nelson et al., 2005). Increased novelty and sensation seeking may be evolutionarily adaptive, as these behaviors could improve the increasingly independent adolescent's chances of finding food and a mate (Spear, 2010). In modern society, however, these features can be associated with taking unnecessary risks. Therefore, adolescence is considered a period of behavioral vulnerability: teens are more likely to experiment with tobacco and illicit drugs and alcohol; drive recklessly; engage in unprotected sex; and have interpersonal conflicts (Arnett, 1992; Arnett, 1999; Spear, 2000; Chambers et al., 2003). Adolescent risk taking is more likely to occur in groups (e.g. vehicular accidents), when certain behaviors are perceived to be acceptable by one's peers (e.g. unprotected sex, drug use) (Steinberg, 2008), and in emotionally charged situations (Figner et al., 2009). Thus, while adolescents have survived the potential health problems of early childhood their morbidity and mortality rates are twice that of pre-pubescent children (Dahl, 2004).

In addition to the added risks of *normal* adolescent development, it is also the time when symptoms of a variety of mental illnesses often manifest, including mood disorders, eating disorders, and psychotic disorders such as schizophrenia (Volkmar, 1996; Pine, 2002; Sisk and Zehr, 2005; Paus et al., 2008). During this period there is a vast array of neurobiological changes that drive everything from a cascade of hormonal signals that initiate puberty (Sisk and Zehr, 2005), to increased cognitive ability and motivational changes (Luna et al., 2004; Doremus-Fitzwater et al., 2009b). Understanding precisely how the brain develops through adolescence, and relating such changes to both normal behavioral tendencies and pathological conditions, is critically important to public health.

## 1.1 MOTIVATED BEHAVIOR

Before discussing the specific brain and behavioral changes of adolescence in greater detail, it is worthwhile to briefly present a more general behavioral framework and mention some relevant neuroanatomy. This dissertation is concerned with *motivated* behavior (and in particular, instrumental behavior, described below), as opposed to reflexes and actions that are not directly related to stimuli. Motivation is presently defined narrowly as those brain states that cause an organism to "regulate the probability, proximity and availability of stimuli" (Salamone and Correa, 2002, p. 5). "Motivated behavior" is motor output derived from these brain states, and a "motivational factor" is simply some internal body state or external stimulus that facilitates such activity. It is obvious that this sort of behavior is critical to the survival of animals that must find food, reproduce, and avoid harm. The brain systems that allow organisms to flexibly interact with their environment in this way are fundamental to much more complex human actions and decisions, and are affected in many psychopathologies. This framework therefore depends upon several interacting systems, as organisms must be able to detect the presence and proximity of stimuli (sensory); imbue such stimuli with value (emotional and cognitive); learn and remember associations between stimuli or modify previously held associations (cognitive); and act to approach or avoid such stimuli (motor) (Figure 1-1). In recent years, neuroscientists have identified brain regions involved in each of these processes. This dissertation is primarily concerned with the cognitive and emotional systems, as age-related neural processing differences in these components are more likely to be responsible for the specific behavioral and psychiatric vulnerabilities of adolescence. In the past 120 years, psychologists have described various processes involved in association learning and the production of motivated behavior. More recently, neuroscientists have worked to identify the neural underpinnings of these phenomena.

The following is a brief description of one such learning phenomenon, followed by a simplified model of its neural substrates.

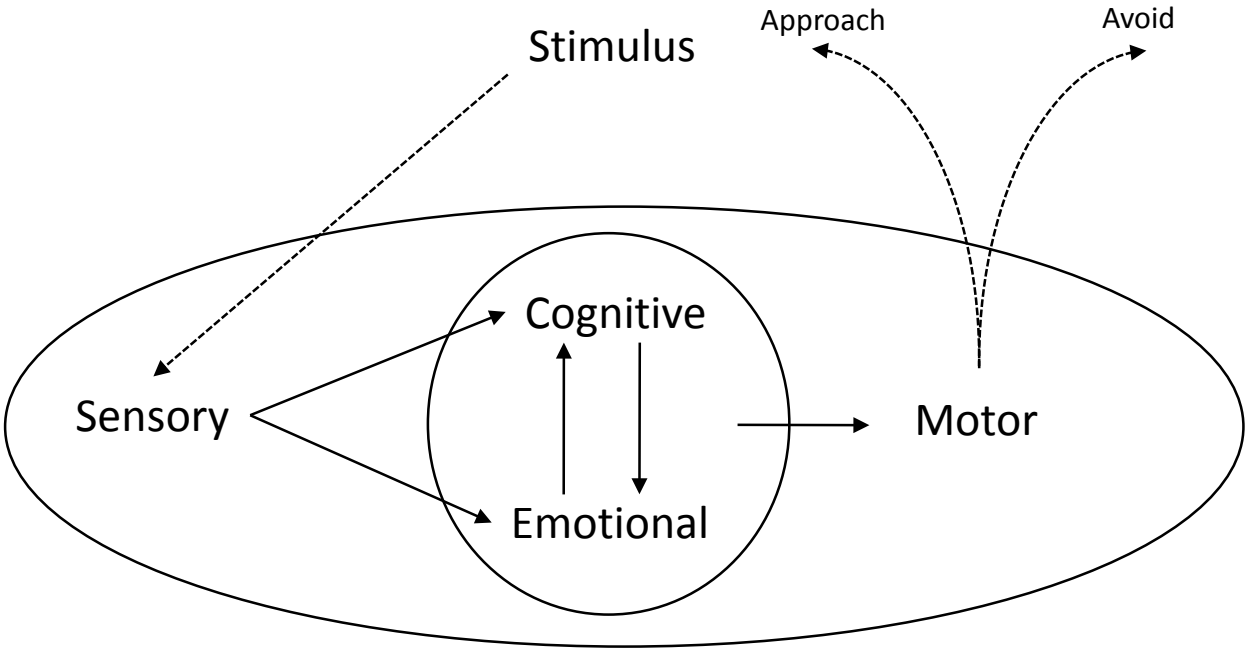


**Figure 1-1 Schematic framework for systems that underlie motivated behavior**
Each system—sensory, emotional, cognitive, and motor—work together to produce motivated behavior.

In one form of association learning an animal's behavior is necessary for the attainment of a particular outcome. For example, an animal can learn to press a lever to receive food. The lever press is therefore instrumental to the outcome, and this form of (action-outcome) learning is called instrumental or operant conditioning. While instrumental associations are initially learned based on particular goals (outcomes), performance can become more automatic to the point where behaviors occur even if the outcomes are devalued. These are called (stimulus-response) habits, and are maintained through separate neural systems from those of goal-directed instrumental behavior. Thus a shift occurs during the performance of instrumental behavior, from those structures that more flexibly guide actions depending upon their outcomes to those that do not. Figure 1-2 shows a schematic representation of a portion of the neural circuitry involved in

the cognitive and emotional systems that underlie the learning and performance of instrumental behavior.

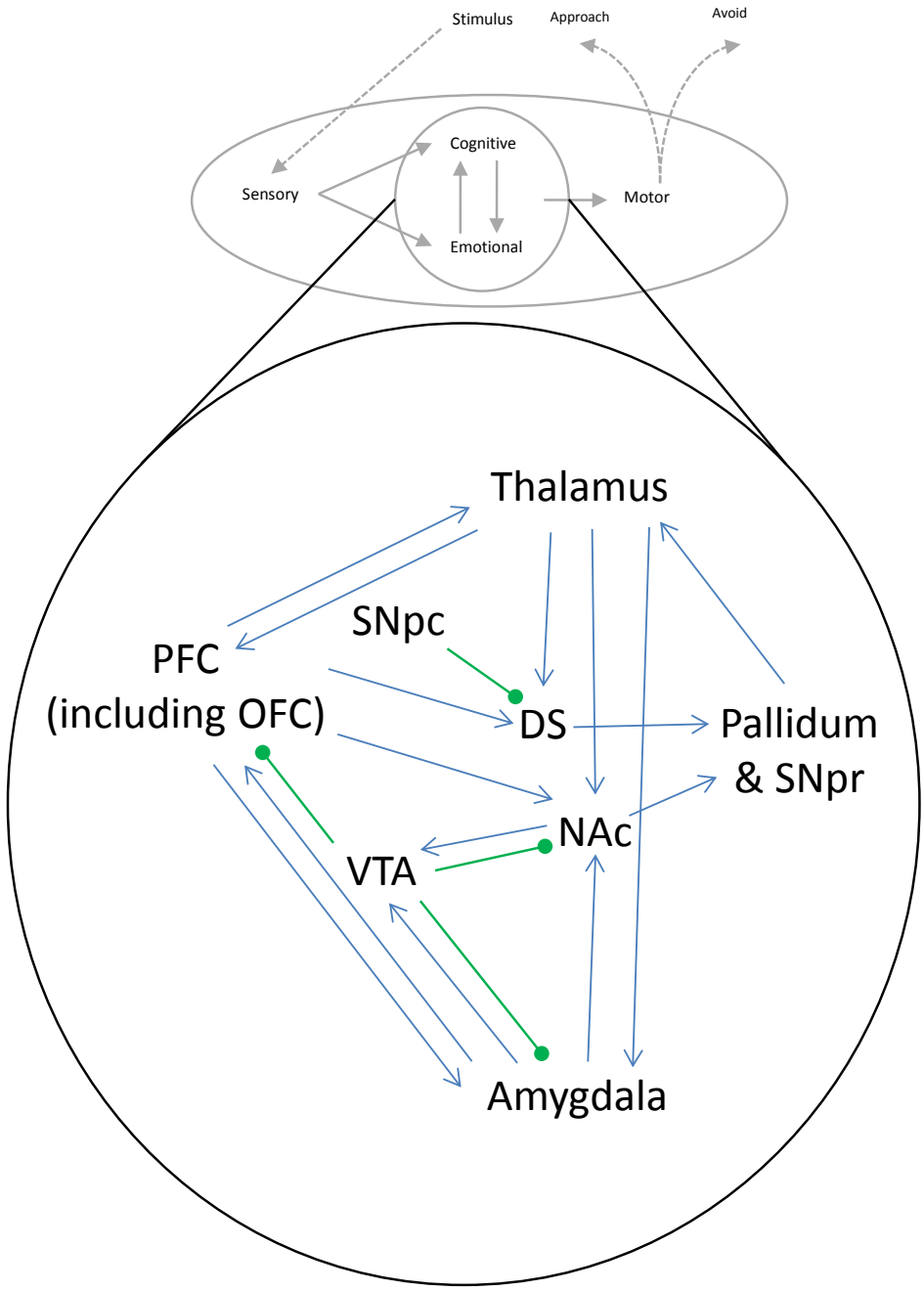


**Figure 1-2 Simplified neural circuitry for instrumental behavior**
The original schematic framework for motivated behavior is represented on top with a sampling of several critical brain structures that participate in cognitive and emotional processing. These regions receive sensory signals, process them, and ultimately send output to motor systems. Instrumental behavior depends upon the extensive integration of signals among these and other structures. Excitatory or inhibitory connections are represented with blue arrows and dopaminergic modulation is indicated with green dotted connections. DS = dorsal striatum; NAc = nucleus accumbens; SNpc = substantia nigra pars compacta; SNpr = substantia nigra pars reticulata; VTA = ventral tegmental area.

While it is highly simplistic [others have produced more detailed representations (Ikemoto and Panksepp, 1999; Kelley, 2004; Balleine et al., 2009; Kringelbach and Berridge, 2009; Koob and Volkow, 2010)] it illustrates the convergence of sensory, emotional, and cognitive systems at the dorsal striatum (DS) and nucleus accumbens (NAc), components of the main input to the basal ganglia. Several of the represented structures are necessary for aspects of goal-directed action (i.e., lesions disrupt learning, performance, and/or behavioral flexibility in certain contexts). Thalamo-cortico-basal ganglia loops, modulated by dopamine afferents from the ventral tegmental area and substantia nigra pars compacta, are thought to separately promote the learning of action sequences, the selection of relevant behavioral patterns (with the inhibition of irrelevant ones), and the execution and continued performance of motivated behavior (Graybiel et al., 1994; Packard and Knowlton, 2002; Graybiel, 2005; Costa, 2007). The amygdala provides emotion and attention signals critical to aspects of associative learning (Gallagher and Holland, 1994). For example, dopamine D1 signaling in the amygdala is necessary for the acquisition of an instrumental response (Andrzejewski et al., 2005). The prefrontal cortex (PFC), which includes the orbitofrontal cortex (OFC), is also important for these behaviors insofar as it facilitates attention, promotes learning, and permits behavioral flexibility (Corbit and Balleine, 2003; Ostlund and Balleine, 2005; Rolls and Grabenhorst, 2008; Takahashi et al., 2009). The OFC is thought to integrate emotion signals from the amygdala with sensory information as a basis of forming value expectations (Schoenbaum et al., 2009), although it is not necessary for the initial acquisition or performance of a simple instrumental response (McKee et al., 2010). The NAc is involved in the initial learning and level of performance of instrumental behavior (Sutherland and Rodriguez, 1989; Ploeger et al., 1994; Setlow, 1997; Ikemoto and Panksepp,

1999; Cardinal et al., 2001; Corbit et al., 2001; Day et al., 2011), and encodes cues related to both appetitive and aversive outcomes (Setlow et al., 2003). Recent work has shown that within the DS a medial portion is necessary for instrumental learning, while outcome-insensitive habitual behavior depends upon more lateral portions (Yin et al., 2004, 2005a, 2006). Different structures within the PFC also mediate the shift from goal-directed to habitual behavior (Coutureau and Killcross, 2003). Together, these regions provide an initial (albeit incomplete) picture of some of the brain networks involved instrumental behavior. They are presented for context as we examine adolescent behavioral vulnerabilities and neurodevelopment, and ultimately use an instrumental task to further investigate the neural correlates of adolescent motivated behavior.

## 1.2 ADOLESCENT BEHAVIOR

Adolescence begins with the onset of puberty, which is induced by neuroendocrine processes and involves a complex set of biological transitions including increased growth, changes in body composition, the development of gonads and secondary sexual organs and characteristics, and cardiovascular and respiratory changes (Falkner and Tanner, 1986). As this occurs the adolescent undergoes a variety of cognitive, behavioral, and psychosocial transitions. The various changes of adolescence do not all start and end together, and thus the puzzle of relating adolescent brain changes with behavior is challenging. Studying adolescence is like shooting at a moving target, with researchers designating “adolescent” groups of different ages and levels of development. With this caveat in mind, the literature reviewed here has primarily defined adolescent periods in

humans as the teenage years, in rhesus monkeys as age two to four years, and in rodents as week four to week six or seven.

Studies in rodents and humans have shown that adolescents exhibit greater "impulsive choice," defined as the preference for smaller rewards that occur sooner over larger delayed rewards, as measured with delay-discounting tasks (Adriani and Laviola, 2003; Steinberg et al., 2009). It is notable that in human studies only younger adolescents exhibit this difference; with delay discounting reaching adult levels by age 16-17 (Steinberg et al., 2009). Adolescent humans also score higher on the Sensation-Seeking Scale than adults, with males exhibiting higher levels than females (Zuckerman et al., 1978). Sensation seeking is "the need for varied, novel, and complex sensations and experiences…" (Zuckerman, 1979, p. 10), which may occur independently, or together with impulsivity. Sensation seeking is greatest during early- to mid-adolescence and lower thereafter, while impulse control appears to steadily improve through the teenage years, suggesting that they are subserved by different biological processes (Steinberg et al., 2008). Consistent with human evidence of heightened adolescent sensation seeking, adolescent rodents prefer novelty (Adriani et al., 1998; Douglas et al., 2003; Stansfield et al., 2004), exhibit greater novelty-induced locomotion (Stansfield and Kirstein, 2006; Sturman et al., 2010), and spend more time exploring open arms in an elevated plus maze than adults (Macrì et al., 2002; Adriani et al., 2004).

Adolescents' tendencies to seek novel experiences, even at the risk of physical or social harm, might be expected if their capacity to assess risk or compute outcome probability is underdeveloped. Cognitive abilities do continue to develop at this time (Spear, 2000). According to Piaget, the formal operation period, which is associated with more abstract reasoning, reaches full maturity during adolescence (Schuster and Ashburn, 1992), and may be less well developed

in some individuals. Also, the persistence of egocentrism, in which teenagers experience an 'imaginary audience' along with the 'personal fable' of unique feelings, may cause them to believe they are exceptional and give them a sense of invulnerability (Elkind, 1967; Arnett, 1992). However, only modest cognitive improvements appear from mid-adolescence onward (Spear, 2000; Luna et al., 2004), and even young children exhibit an accurate implicit understanding of probability (Acredolo et al., 1989). Furthermore, there is little evidence that adolescents actually perceive themselves as invulnerable or underestimate risk; in fact, they often overestimate risk, such as the chance they will become pregnant within a year, go to jail, or die young (de Bruin et al., 2007). Finally, any cognitive explanation for adolescent risk taking must account for the fact that children take fewer risks and yet are less cognitively developed than adolescents.

Alternatively, adolescent behavioral disparities could relate to differences in cognitive strategies. One hypothesis, called "fuzzy trace theory," states that far from lacking in cognitive ability, adolescents process the risk/benefit details of choices more explicitly than adults. Paradoxically, adolescents may behave more rationally than adults by more explicitly computing the expected values of different options, but this could lead to greater risk taking (Rivers et al., 2008). According to Rivers and colleagues (2008), through development we progress from doing more literal "verbatim" to a "fuzzy" gist-level heuristic that captures the essence or bottom line without details. This presumably improves the efficiency of decision making and tends to bias us away from risky choices as we tend to avoid potential adverse outcomes without assessing the actual probabilities involved. For example, unlike adolescents, adults favor choices that attach certainty to increased gains or reduced losses over probabilistic alternatives with identical expected values (Rivers et al., 2008). Overall, the idea that adolescent choices could reflect

differences in cognitive strategy—but not deficiencies in outcome prediction—is intriguing. Future neuroimaging and physiology studies of adolescent decision making might benefit from considering the possibility that differences in the precise pattern of neural activity, even within the same brain regions, along with the level of integration between different regions, could facilitate alternative styles of cognitive deliberation.

Adolescents' greater recklessness could be due to differences in how they experience risk and reward. One explanation is that human adolescents experience more negative affect and depressed mood, and may feel less pleasure from stimuli of low or moderate incentive value. Adolescents would therefore seek stimuli of greater hedonic intensity to satisfy a deficiency in their experience of reward (see Spear, 2000). This is supported by studies showing differences in the hedonic value of sucrose solutions to adults versus adolescents. Once sucrose concentrations exceed a critical point, the hedonic value sharply decreases; however such decreases are less pronounced or non-existent in children and adolescents (De Graaf and Zandstra, 1999; Vaidya et al., 2004). An alternative explanation is that adolescents have greater sensitivity to the reinforcing properties of pleasurable stimuli. Either possibility is consistent with animal models in which adolescents consume more sucrose solution (Vaidya et al., 2004), prefer chambers previously associated with social interaction (Douglas et al., 2004), and exhibit evidence of higher incentive value for drugs such as nicotine, alcohol, amphetamine, and cocaine than adults (Vastola et al., 2002; Badanich et al., 2006; Shram et al., 2006; Brenhouse and Andersen, 2008; Spear and Varlinskaya, 2010). This is not always seen, however, (Frantz et al., 2007; Mathews and McCormick, 2007; Shram et al., 2008), and increased adolescent drug preference could also be related to reduced sensitivity to aversive side-effects and withdrawal (Little et al., 1996; Moy et al., 1998; Schramm-Sapyta et al., 2007; Schramm-Sapyta et al., 2009). Similarly, adolescents

might perform more risky behaviors if their assessment of possible aversive consequences is less motivating or salient (or if the excitement of risk-taking itself makes such behavior more likely).

Another factor that could account for some adolescent behavioral differences is the impact of emotions (valence, feelings, arousal, and specific emotional states) on behavior. Behavioral disparities may arise if adolescents experience emotions differently, or if emotions differently influence decision making during this period of heightened emotional intensity and volatility (Buchanan et al., 1992; Arnett, 1999). Emotion is often thought to cloud rational decision making. While this may be true in some cases (especially when emotional content is unrelated or irrelevant to a decision context), recent work has examined how emotions may improve certain decisions. For example, the somatic marker hypothesis states that in ambiguous situations, emotional processes can advantageously guide behavior (Damasio, 1994). The Iowa Gambling Task was designed to test decision making under conditions of uncertainty (Bechara et al., 1994). Individuals with lesions of the ventromedial PFC or amygdala have difficulty favoring the advantageous risk-avoiding strategy, suggesting that deficiencies in integrating emotional information can lead to poor decisions (Bechara et al., 1996; Bechara et al., 1999). Adolescents and adults may differ in the way they integrate emotional information in decisions: adolescents may be less adept at interpreting or integrating relevant emotional content, or less effective at forming such associations. Cauffman et al. (2010) recently tested children, adolescents, and adults on a modified version of the Iowa Gambling Task; they observed that while both adolescents and adults improved their decision-making over time, adults did this more rapidly. Another study demonstrated that only by mid- to late- adolescence did subjects improve their gambling task performance, and that this improvement coincided with the appearance of physiological correlates of arousal (Crone and van der Molen, 2007). These results suggest that

adolescents may be less effective at forming or interpreting the sort of relevant affective information necessary to avoid risky decisions.

According to Rivers and colleagues (2008) differences in effective gist processing make adolescents more susceptible to potentially deleterious effects of arousal on decision making. In conditions of heightened arousal, a reduction in behavioral inhibition may cause one to switch from a "reasoned" to a "reactive" or impulsive mode. They further argue that the adolescent tendency to perform more verbatim-analytical processing makes this more likely, while the values and biases of the simpler adult "gist" processing is more impervious to arousal state (Rivers et al., 2008). Others have also argued that adolescent behavior may be particularly sensitive to conditions of high emotional arousal (see Dahl, 2001; Spear, 2010). A recent study by Figner and colleagues (2009) directly tested this hypothesis using a task that measured risk taking under different affective conditions. Adolescents and adults performed the Columbia Card Task, in which the level of tolerated risk was examined under conditions of greater/lesser arousal and while varying factors that could be used to make more informed decisions (such as the magnitude of gains/losses and their probability). Adolescents took more risks than adults only in the high-arousal condition, and in this context, adolescents were less affected by gain/loss magnitude and probability, suggesting simplified information usage by adolescents under conditions of heightened arousal (Figner et al., 2009).

Collectively these studies indicate that although adolescents often reason and behave like adults, in certain contexts there are differences in their cognitive strategy and/or in their response to risk and reward, especially under conditions of heightened emotional arousal. These behavioral changes likely reflect the substantial development of brain networks—including

structures in the PFC, basal ganglia, and neuromodulatory systems (e.g. dopamine) — that are critical to motivated behavior.

## 1.3 ADOLESCENT STRUCTURAL NEURODEVELOPMENT

The adolescent brain undergoes dramatic changes in gross morphology. Human structural imaging studies have demonstrated that throughout the cerebral cortex there is a loss of gray matter during adolescence, with gray-matter reductions in portions of the temporal lobe and dorsolateral PFC occurring in late adolescence (Sowell et al., 2001; Sowell et al., 2002; Sowell et al., 2003; Gogtay et al., 2004). Gray matter reductions are also apparent in the striatum and other subcortical structures (Sowell et al., 1999; Sowell et al., 2002). These changes may be related to a massive pruning of synapses observed during this period from animal studies (Rakic et al., 1986; Rakic et al., 1994), although some question this connection as synaptic boutons make up only a small proportion of cortical volume (Paus et al., 2008). Human imaging has also revealed that white matter increases through adolescence in cortical and subcortical fiber tracts (Benes et al., 1994; Paus et al., 1999; Paus et al., 2001; Asato et al., 2010), resulting from increased myelination, axon caliber, or both (Paus, 2010). Changes in the patterns of connectivity also occur during adolescence. For example, axonal sprouting and growth have been observed in circuits connecting the amygdala to cortical targets (Cunningham et al., 2002), and increasing measures of white matter are observed between the PFC and striatum and other areas (Sowell et al., 1999; Paus et al., 2001; Giedd, 2004; Gogtay et al., 2004; Liston et al., 2006; Asato et al., 2010).

At a finer scale, rat and primate studies have demonstrated numerous differences in adolescent neurotransmitter systems. Adolescents tend to over-express dopaminergic, adrenergic, serotonergic and endocannabinoid receptors across many regions followed by pruning to adult levels (Lidow and Rakic, 1992; Rodriguez de Fonseca et al., 1993). They express D1 and D2 dopamine receptors at higher levels in subcortical targets such as the dorsal striatum and nucleus accumbens, although some have not found reduced adult expression in this latter region (Gelbard et al., 1989; Teicher et al., 1995; Tarazi et al., 1999; Tarazi and Baldessarini, 2000). During adolescence, there are also changes in dopamine production and turnover, as well as evidence for changes in downstream effects of receptor-ligand binding (Coulter et al., 1996; Tarazi et al., 1998; Laviola et al., 2001; Badanich et al., 2006; Cao et al., 2007). Functionally, there is evidence from anesthetized rats that the spontaneous activity of midbrain dopamine neurons peaks during adolescence and then decreases (McCutcheon and Marinelli, 2009). Developmental changes in mesocorticolimbic dopamine circuitry and activity may underlie some differences in motivated behavior generally, as well as risk taking and addiction vulnerability in particular. Several studies have observed reduced psychomotor effects of stimulant drugs in adolescent animals but enhanced or similar reinforcing effects (Spear and Brake, 1983; Adriani et al., 1998; Bolanos et al., 1998; Laviola et al., 1999; Adriani and Laviola, 2000; Badanich et al., 2006; Frantz et al., 2007; Mathews and McCormick, 2007). In contrast, adolescents are more sensitive to the cataleptic effects of neuroleptics (e.g., haloperidol), which are antagonists for dopamine receptors (Spear et al., 1980; Spear and Brake, 1983; Teicher et al., 1993). Some have proposed that this pattern, along with the increased exploration and novelty-seeking, indicates that the adolescent dopamine system is near a “functional ceiling” at baseline (Chambers et al., 2003).

Several lines of evidence suggest that the balance of large-scale excitatory and inhibitory neurotransmission is vastly different in adolescents compared to adults. Levels of GABA, the main inhibitory neurotransmitter in the brain, increases linearly through adolescence in rat forebrain (Hedner et al., 1984). The expression of the activating glutamate NMDA receptors on fast-spiking neurons (thought to be inhibitory interneurons) changes dramatically in the PFC of adolescents. At this time the vast majority of fast-spiking interneurons exhibit no synaptic NMDA receptor-mediated currents (Wang and Gao, 2009). Additionally the modulatory impact of dopamine-receptor binding shifts during adolescence (O'Donnell and Tseng, 2010). It is only by this time that the activation of dopamine D2 receptors increases interneuron activity (Tseng and O'Donnell, 2007). Furthermore, the synergistic interaction between dopamine D1 receptor activation and the NMDA receptor changes during adolescence, allowing for plateau depolarizations which may facilitate context-dependent synaptic plasticity (Wang and O'Donnell, 2001; O'Donnell and Tseng, 2010). These adolescent dopamine, glutamate, and GABA signaling changes suggest fundamental neural activity differences in the adolescent brain. All of these systems are essential to cognitive and emotional processes. Their dysfunction is implicated in numerous psychiatric illnesses ranging from mood disorders and addiction to schizophrenia.

## 1.4 ADOLESCENT FUNCTIONAL NEURODEVELOPMENT

Neuroimaging studies have shown differences in human adolescent functional activity in several forebrain regions. Compared to adults, adolescents have a reduced hemodynamic response in lateral orbitofrontal cortex and increased activity in ventral striatum to rewards (Ernst et al., 2005; Galvan et al., 2006). Others have found reduced activity in right ventral striatum and right

extended amygdala during reward anticipation, with no observed age-related activity differences after gain outcome (Bjork et al., 2004). In a decision-making task, adolescents had reduced right anterior cingulate and left orbitofrontal/ventrolateral PFC activation compared to adults during risky choices (Eshel et al., 2007).

Several studies have observed immaturity of adolescent cognitive control systems, along with poorer behavioral performance (Luna et al., 2010). For example, during tasks that require the inhibition of a prepotent response (the performance of which improves with age), adolescents have increased PFC activity in some subregions and decreased activity in others (Rubia et al., 2000; Bunge et al., 2002; Tamm et al., 2002). During an antisaccade cognitive control task, adolescent (but not adult) ventral striatum activity was reduced while viewing a cue that indicated if reward was available during a given trial, but it was more activated than its adult counterpart during reward anticipation (Geier et al., 2009). Thus adolescents generally activate similar cognitive and affective structures as adults, although often with different magnitudes or spatial and temporal patterns, or levels of functional interconnectivity (Hwang et al., 2010).

Maturation of intra- and inter-regional connectivity and neuronal coordination may play a central role in adolescent behavioral development. There is a direct relationship between measures of frontostriatal white matter, which increases through adolescence, and inhibitory control performance (Liston et al., 2006). White-matter development is also directly related to improved functional integration of gray matter regions, suggesting more-distributed network activity through development (Stevens et al., 2009). This is corroborated by a study that, using resting state functional connectivity MRI along with graph analyses, observed a shift from greater connectivity with anatomically proximal nodes to networks that were more extensively integrated across all nodes in adulthood regardless of distance (Fair et al., 2009). Similarly, age-

related increases in the functional integration of frontal and parietal regions support improved top-down inhibitory control performance in an antisaccade task (Hwang et al., 2010). White matter development, the rapid pruning of synapses (which are largely local excitatory connections), and developmental shifts in local interneuron activity may together facilitate more extensive functional coordination between brain regions through development. Less widely distributed activity in adolescents has also been demonstrated in another cognitive control task (Velanova et al., 2008). At the same time, diffuse functional signal uncorrelated with task-performance decreases through development (Durston et al., 2006). Thus, the adult pattern of utilizing more-distributed networks is coincident with reduced task-irrelevant activity, indicating greater efficiency in the pattern and extent of cortical processing.

Electrophysiological studies have also found evidence of further development of neuronal responses and greater local and long-range coordinated activity through adolescence. For example, the Contingent Negative Variation, which is a negative voltage event-related potential during response preparation, only develops in late childhood and continues to become larger through adolescence (Segalowitz and Davies, 2004; Bender et al., 2005). This is thought to reflect age-related differences in the distribution of PFC processing of attention and executive motor control (Segalowitz et al., 2010). Another age-related electrophysiological change is the development of strong positive peak (P300) approximately 300 ms after attending to a stimulus. A mature P300 pattern does not appear until approximately age 13 (Segalowitz and Davies, 2004). Finally, the Error-Related Negativity is a negative voltage centered over the anterior cingulate cortex during error trials of different tasks. Although there is some variability in the age of its appearance, it seems to arrive around mid-adolescence (Segalowitz and Davies, 2004). These findings provide additional evidence for the continued maturation of prefrontal cortical

processing during adolescence. Segalowitz and colleagues also found that the signal-to-noise ratio of the electrical signals of children and adolescents were often lower than that of adults. This could be due to functional immaturity or intra-individual instability of brain regions producing these signals (Segalowitz et al., 2010). It might also reflect reduced adolescent neural coordination within and between brain regions. This interpretation is consistent with work performed by Uhlhaas and colleagues (2009b), in which electroencephalograms (EEGs) were recorded in children, adolescents, and adults during a facial recognition task. They observed reduced theta (4-7 Hz) and gamma band (30-50 Hz) oscillatory power in adolescents compared to adults. Additionally there was greater long-range phase-synchrony in theta, beta (13-30 Hz), and gamma bands, along with improved task performance in adults. EEG oscillations are due to fluctuations in neuronal excitability and are thought to fine-tune the timing of spike output (Fries, 2005). Measures of synchrony in specific frequency bands facilitate communication between neuronal groups, and may be critical to numerous perceptual and cognitive processes (Uhlhaas et al., 2009c). Thus, these findings are evidence of enhanced coordinated local processing and improved inter-regional communication from adolescence to adulthood (Uhlhaas et al., 2009a).

## 1.5 CURRENT NEUROBEHAVIORAL MODELS

With all of the neurodevelopmental changes of adolescence, what accounts for the particular behavioral differences and vulnerabilities of this period? There are several hypotheses that seek to connect adolescent differences in motivated goal-directed behavior, social development, and behavioral inhibition with the maturity of specific neural circuits.

Adolescent refinement of a social information processing network is one model connecting adolescent social development with brain changes (Nelson et al., 2005). This framework describes three interconnected functional nodes with distinct neural structural underpinnings: the detection node (inferior occipital cortex, inferior and anterior temporal cortex, intraparietal sulcus, fusiform gyrus, and superior temporal sulcus), the affective node (amygdala, ventral striatum, septum, bed nucleus of the stria terminalis, hypothalamus, and orbitofrontal cortex in some conditions), and the cognitive-regulatory node (portions of the PFC). The detection node determines whether stimuli contain social information, which is further processed by the affective node which imbues such stimuli with emotional significance. The cognitive-regulatory node further processes this information, performing more complex operations related to perceiving the mental states of others, inhibiting prepotent responses, and generating goal-directed behavior (Nelson et al., 2005). Adolescent changes in the sensitivity and interaction of these nodes are hypothesized to intensify social and emotional experiences, strongly influence adolescent decision making, and contribute to the emergence of psychopathologies during this period (Nelson et al., 2005).

The triadic node model (Ernst et al., 2006) posits that the specific developmental trajectory of brain regions subserving affective processing and cognitive control, and the balance between them, may underlie the risk-taking propensity of adolescents. This model is also based on the activity of three nodes corresponding to specific brain regions. In this case a node responsible for reward approach (ventral striatum) is in balance with a punishment-avoidance node (amygdala). A modulation node (PFC) affects the relative influence of these countervailing forces, and risky behavior will result from a final calculus favoring approach. According to this model, in situations involving some probabilistic trade-off between appetitive and aversive

stimuli, the approach node is more dominant in adolescents. Hyperactivity or hypersensitivity of a reward-approach system might otherwise be adjusted by activity in portions of the PFC, however its underdevelopment in adolescents does not permit adequate self monitoring and inhibitory control (Ernst and Fudge, 2009).

Casey and colleagues hypothesize that differences in the developmental trajectory of adolescent PFC versus subcortical structures (e.g. ventral striatum and amygdala), along with the connections between them, might account for adolescent behavioral propensities (Casey et al., 2008; Somerville and Casey, 2010; Somerville et al., 2010). During a task involving the receipt of different reward values, the extent of adolescent activity in the nucleus accumbens was similar to that of adults (although with greater magnitudes) whereas the pattern of orbitofrontal cortical activity looked more like that of children than adults (Galvan et al., 2006). The relative maturity of subcortical systems and the immaturity of the PFC, which is critical to cognitive control, may lead to a greater adolescent propensity toward sensation seeking and risk taking. The key here, as in the triadic node model, is the concept of a relative inter-regional imbalance during adolescence, in contrast to childhood when these regions are all relatively immature and adulthood when they are all mature (Somerville et al., 2010). This model is also similar to Steinberg's framework, in which the relative decrease in risk taking from adolescence to adulthood is due to the development of cognitive control systems, connections facilitating the integration of cognition and affect among cortical and subcortical regions, and differences in reward salience or sensitivity (Steinberg, 2008).

## 1.6 PURPOSE OF DISSERTATION

The models described above explain adolescent behavioral changes and vulnerabilities in terms of specific underdeveloped neural systems. At this point, however, little is known of the physiology of these putative underdeveloped systems, especially at the level of neurons (rather than correlates of much larger regional activity). In order to develop more specific and mechanistic hypotheses of adolescent behavioral and psychiatric vulnerabilities, we must learn more about how critical regions process salient and motivational stimuli differently in the developing brain.

One useful approach for examining neural activity is with *in vivo* electrophysiological recordings from implanted electrode arrays in behaving animals. This technique enables one to record the activity of individual neurons as well as larger-scale field potentials with high temporal resolution. These measures allow the researcher to determine the profile of neuronal activation and inhibition, along with oscillations that coordinate the activity of different neural groups, time-locked to salient events during a task. Prior to the work presented here, there had been no published accounts of electrophysiological recording in awake, behaving adolescent animals. This could be due to various technical concerns. For example, as mentioned earlier, the period of adolescence is only around 2-3 weeks in rodents. Therefore, any electrophysiology study in adolescent rats requires a period of surgical recovery prior to testing and a behavioral paradigm that is both simple enough to be learned and performed in the remaining time, and that can yield insights into the potentially unique neuronal processing of this period.

This project therefore contained two main components. The first was to design a behavioral task and the second was to record the neural activity of adolescent and adult rats as they performed it. The task would have to be suitable for the brief period of rat adolescence and

contain specific time-lockable windows during which the neural processing of salient events could be examined. This task is introduced and characterized in Chapter 2, and the behavioral performance of adolescents is compared with that of adults. Chapters 3 and 4 examine the neural activity of adolescent and adult orbitofrontal cortex, dorsal striatum, and nucleus accumbens as rats performed this task. These regions were selected because of their involvement in aspects of motivated behavior, and in some cases, previous work suggesting their underdevelopment during adolescence (Ernst et al., 2006; Galvan et al., 2007). Thus, while the models described in the previous section hypothesize "immature" adolescent brain regions and networks, this dissertation represents a step toward more precisely identifying what such immaturity means in terms of neuronal processing differences during motivated behavior. In Chapter 5 these hypotheses are revisited in the context of the findings from this dissertation, and a hypothesis of reduced adolescent neural processing efficiency is introduced, along with suggestions for future work that might further delineate the mechanisms of adolescent behavioral and psychiatric risks.

# 2.0 ADOLESCENT VERSUS ADULT MOTIVATED BEHAVIOR*

## 2.1 ABSTRACT

Adolescence is associated with the development of brain regions linked to cognition and emotion. Such changes are thought to contribute to the behavioral and neuropsychiatric vulnerabilities of this period. We compared adolescent (Postnatal Days 28-42) and adult (older than Postnatal Day 70) rats as they performed a simple instrumental task and extinction. Animals were trained to poke into a hole for a food-pellet reinforcer. After six days of training, animals underwent extinction sessions in which the previously rewarded behavior was no longer reinforced. During extinction we examined the effects of continued presentation of a cue light and food restriction. Adults and adolescents exhibited similar performance during training, although adolescents made more task-irrelevant pokes, consistent with increased exploration. Adults made more premature pokes, which could indicate a more exclusive focus on the task. During extinction, adolescents made more perseverative (previously reinforced) pokes than adults. This behavior was strongly modulated by the combination of motivational factors present (food restriction and cue light), indicating that adolescents were differentially sensitive to them. Furthermore, food restriction induced greater open-field activity in adolescents but not adults.

* Adapted from Sturman DA, Mandell DR, Moghaddam B (2010) Adolescents exhibit behavioral differences from adults during instrumental learning and extinction. Behav Neurosci 124:16-25.

Thus, as the neural circuitry of motivated behavior develops substantially during adolescence, so too does the behavioral sensitivity to motivational factors. Understanding how such factors differently affect adolescents may shed light on mechanisms that lead to the development of disorders that first manifest during this period.

## 2.2 INTRODUCTION

Adolescence is a major transitional period between childhood and adulthood. It encompasses puberty, a time of reproductive development, and is characterized in humans and rodents by numerous non-reproductive socio-behavioral changes (Spear, 2000). It is during adolescence that the symptoms of several psychiatric disorders typically arise, including depression, eating disorders, and schizophrenia (Volkmar, 1996; Pine, 2002; Sisk and Zehr, 2005). Characteristic adolescent behavioral changes include elevated social interaction (Csikszentmihalyi et al., 1977) and increased novelty-seeking and risk-taking behavior (Adriani et al., 1998; Spear, 2000; Macrì et al., 2002; Stansfield and Kirstein, 2006; Steinberg, 2008). These latter behaviors correlate with drug and alcohol use (Andrucci et al., 1989), and adolescence is often considered a period of increased addiction vulnerability (Chambers et al., 2003; Adriani and Laviola, 2004). Furthermore, as in human adolescents, adolescent mice exhibit greater impulsivity than adults, as measured by reduced preference for larger delayed food reinforcers over more immediate smaller ones (Adriani and Laviola, 2003).

Along with these behavioral changes, the adolescent brain undergoes extensive remodeling (McCutcheon and Marinelli, 2009), with neurogenesis (Pinos et al., 2001), axonal growth (Benes et al., 2000; Cunningham et al., 2002), myelination (Benes et al., 1994), apoptosis

(Nunez et al., 2002), and synaptic and receptor pruning (Meyer et al., 1978; Teicher et al., 1995; Andersen et al., 2000) accompanying shifts in white-matter density and cortical grey-matter volume (Benes et al., 1994; Giedd et al., 1999; Paus et al., 2001; Juraska and Markham, 2004; Paus, 2005). The mesocortical dopaminergic circuitry, considered broadly relevant to motivated behavior [for reviews see Cools (2008), Salamone & Correa (2002), Berridge (2007), and Floresco & Magyar (2006)] also undergoes considerable development during adolescence (Lewis, 1997; Spear, 2000; Chambers et al., 2003; Ernst and Fudge, 2009).

Much of the literature on adolescence has focused on drug-related behavioral differences such as ethanol or psychostimulant sensitivity differences (Spear and Brake, 1983; Little et al., 1996; Bolanos et al., 1998; Moy et al., 1998; Varlinskaya and Spear, 2006; Badanich et al., 2008; Pautassi et al., 2008). For example, adolescents tend to exhibit less amphetamine- and cocaine-induced locomotor stimulation and stereotypy (Spear and Brake, 1983; Bolanos et al., 1998; Laviola et al., 1999; Mathews and McCormick, 2007). Conversely, adolescent rats show greater sensitivity to the cataleptic effects of neuroleptics (Shalaby and Spear, 1980; Spear and Brake, 1983). Less is known about developmental differences in motivated behavior more generally, such as how various non-pharmacological factors might affect adolescents differently than adults. The aim of this study was to characterize several similarities and differences in adolescent motivated behavior during an instrumental learning task and extinction. While motivation can be a somewhat elusive concept, others have defined it as “the set of processes through which organisms regulate the probability, proximity and availability of stimuli” (Salamone and Correa, 2002). Here we use the term “motivational factor” to identify elements that increase behavioral manifestations of such processes in an organism. We tested adolescent and adult rats in a simple paradigm where they learned to pair a particular action (nose poke) with a desired outcome (food

pellet), and followed the training period with extinction, at which point the action-outcome association was no longer reinforced. During extinction we examined how continued food restriction and presentation of a task cue may differently affect adolescents. We further compared the impact of one of these motivational factors, food restriction, on adolescent and adult open-field activity. Studying such differences may inform our understanding of how adolescent neurodevelopment leads to typical age-specific behavioral propensities and disease processes

## 2.3 METHODS

### 2.3.1 Subjects

Adolescent (Postnatal Days 28-42; n = 42) and adult (older than Postnatal Day 70; n = 42) male Sprague-Dawley rats (Harlan, Frederick, MD) were used. Pre-adolescent juvenile rats (Postnatal Day 21) and adults were received four days before beginning handling and operant box habituation. Training on the instrumental task began immediately after habituation, corresponding to one week after arrival (Postnatal Day 28 for adolescents). All subjects were housed in pairs under 12 hour light/dark cycle conditions (lights on at 7 pm), and testing was performed during animals' active phase. Food restriction was imposed during the habituation period, at which time pre-adolescents received 5 g and 8 g chow on consecutive days and then were maintained at 10 g chow/day on the final day of habituation and throughout training. This level of food restriction was chosen after observing that not all food was consumed from the previous day in some cages prior to testing when early adolescents were fed 5 g, 8 g, and then

sustained at 12 g chow/day. Adults were given 15g chow/day during habituation and training. All rats had *ad-lib* access to water except during testing. Experimental protocols were approved by the University of Pittsburgh Animal Care and Use Committee.

### 2.3.2 Instrumental Task

Operant chambers (Coulbourn Instruments) were equipped with a house light that illuminated the chamber during the task, three nose-poke holes, a food trough, and a food pellet delivery system. Nose-poke holes were arrayed horizontally on the wall opposite the food trough. Entries into the nose-poke holes or the food trough were detected by infrared photosensors. A PC-based controller and *Graphic State* software (Coulbourn Instruments) were used to run the task and record the rats' behavior.

During the first day of habituation, rats were placed in the operant chamber for 20 min with the house light on. During the second and third days of habituation, rats were placed in the operant box for 20 min with the house light on and food pellets (fortified dextrose pellets, 45 mg, Bio-Serv) were delivered into the food trough every 30 sec.

Rats were then trained on a reinforcement schedule in which a single instrumental nose poke was reinforced with the delivery of a single food pellet. The house light was continuously illuminated for the duration of each session. Trials began with the illumination of a light cue in the center (and only the center) nose-poke hole. After an animal poked into that hole, the light turned off and one pellet was delivered to the food trough, along with the illumination of a food trough light. The trial-onset cue would remain illuminated until the rat performed the nose poke (instrumental response). In order for the next trial to begin, the rat was required to poke into the food trough to retrieve the pellet, which turned off the food-trough light, and then wait for a

fixed 5 sec inter-trial interval (ITI). Animals received daily training sessions over six consecutive days. Each session was terminated upon the delivery of 99 pellets or the passage of 30 min. Nose pokes into either of the non-illuminated (left and right) modules were not reinforced, although this behavior was recorded and categorized as "task-irrelevant pokes." Video cameras allowed behavior to be monitored by the experimenter during testing. Rats that did not learn the instrumental task after three days were hand-shaped to the center hole and performed the task for three full sessions after this. These animals ($n$ = 5) were excluded from all instrumental training analyses. However, as their extinction and open-field behavior was not statistically different from that of their peers, these data were combined with their corresponding groups.

### 2.3.3 Extinction

The day after the completion of the last training session, rats began one of four extinction paradigms, during which the instrumental behavior was no longer reinforced. In the first group (adult $n$ = 18; adolescent $n$ = 18) rats remained food-restricted. Additionally the trial-onset cue that was previously associated with the beginning of each trial during training continued to be presented during extinction. A poke to the illuminated hole would turn off the cue light as before, but no food pellet was delivered. If the animal then poked in the food trough, after a 5 sec ITI the cue light was presented again. If it did not poke into the food trough, the cue light would reappear after a 15 sec delay. In a second group (adult $n$ = 6, adolescent $n$ = 6) animals underwent extinction exactly as in the first group, except that these animals were given *ad lib* access to food in their home cages each day (beginning immediately after the last training session). In a third extinction group (adult $n$ = 12; adolescent $n$ = 12) food restriction was maintained; however, no cue light was presented to the animals during extinction. In a final

group (adult $n$ = 6; adolescent $n$ = 6) animals were given *ad lib* access to food in their home cages and no cue was presented during extinction sessions.

### 2.3.4 Open Field

After operant-box testing during the second and third days of extinction, a subset of food-restricted and *ad lib* adult ($n$ = 24) and adolescent ($n$ = 24) rats were placed in the center of an open field arena (1 m × 1 m, divided into 25 squares) under normal white lighting and given 5 min to explore while being videotaped. An experimenter, unaware of the rats' food-restriction status, rated the number of total square entries and entries into the central grids. A square entry was counted when a rat's hind legs passed from one square into the next.

### 2.3.5 Statistical Analysis

To delineate age-related differences in task performance during training, Age (between) × Session (within) repeated-measures analyses of variance (ANOVAs) were performed on total trials per session, task-irrelevant pokes (left- and right-hole pokes), latency from cue onset to instrumental poke, and latency from instrumental poke to food-trough poke. During extinction, repeated-measures ANOVAs were used to examine the effects of age, food-restriction status, cue-light presence, and their interactions on perseverative and task-irrelevant pokes. These models were broken up to more easily interpret potential 3-way between-factor interactions. The following analyses were performed separately on animals for which a cue was or was not presented during extinction: Age × Food-Restriction Status (between) × Session (within); on food-restricted and *ad lib*-fed rats, Age × Cue-Light Presence (between) × Session (within); and

for adults and adolescents, Food-Restriction Status × Cue-Light Presence (between) × Session (within). To assess open-field behavior, Age × Food-Restriction Status ANOVAs were performed on total grid entries and central grid entries. When necessary, significant ANOVA results were supplemented with Fisher's least significant difference post hoc tests. In all repeated-measures ANOVAs for which the assumption of sphericity was violated, the lower-bound correction was used for a maximally conservative degrees of freedom adjustment. Pearson's correlation coefficient and analysis of covariance (used in models with fixed factors such as age or food-restriction status) were used to determine the relationship between continuous variables (e.g., total trials performed during training or total perseverative pokes during extinction) and to test for potential relationships within each age group between body weight and training trials, task-irrelevant pokes, and perseveration during extinction. We also used Pearson's correlation coefficient to examine the relationship between task-irrelevant pokes (during training and during extinction) and open-field behaviors, training trials, and perseveration during extinction. To avoid the potential problem of singular poking events registering as multiple pokes (e.g., several pokes within 1 s as a rat retrieves a food pellet), a distinct poke was defined as one that was not immediately preceded by a previous poke within 1 sec. The exception to this was counting an instrumental response as such even immediately after a premature poke.

## 2.4 RESULTS

### 2.4.1 Instrumental Performance

A significant interaction effect was observed for the total number of trials performed by adults versus adolescents across training sessions $F(1,1) = 7.05$, $p = 0.01$. This was due to adults performing a significantly greater number of trials in Sessions 3-6, but not Sessions 1-2, when most of the initial learning took place. Total trial performance was generally stable for adolescents and adults beginning in sessions 2-3 (Figure 2-1A). Examination of the average cumulative trials performed over time within sessions demonstrated that the difference between adolescents and adults in total trials in sessions 3-6 was associated with an early drop in the rate of adolescent trial performance after initially being similar to that of adults (Figure 1B). The rate of adolescent trial performance in the first session was very slightly faster (a steeper line) than that of adults. This suggests that on average, adolescents either learned the task slightly earlier or were simply slightly more active during this period (Figure 2-1B).

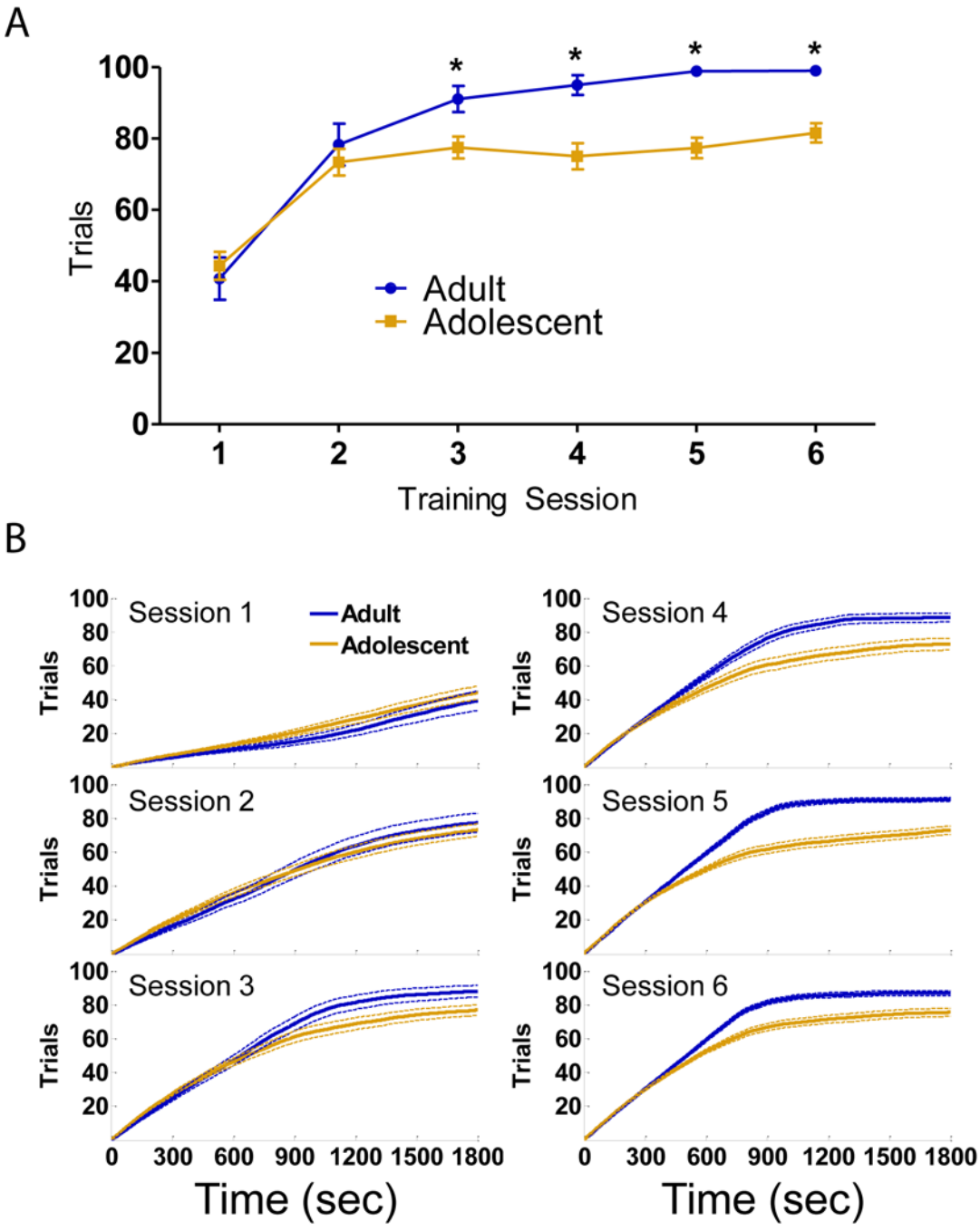


**Figure 2-1 Adolescent and adult trial performance across training sessions**
A) Adolescents perform similar total trials during the first two training sessions. From Session 3 onward adults perform more total trials than adolescents. B) Within-session average cumulative trial performance over time plus and minus standard error (dashed lines). Sessions 1 and 2 indicate similar or slightly faster performance (steeper slope) by adolescents. The rate of performance is nearly equal during the early portion of Sessions 3-6. The drop in trial performance rate after about 5-10 min into those sessions by adolescents contributes to their lower total trials, and may reflect earlier satiety. * = significant difference between adolescents and adults.

A significant Age × Session interaction was found for the mean latency from cue onset to instrumental poke $F(1,1) = 5.64$, $p < 0.05$. Although the latency from trial-onset cue to center-hole poke was initially lower for adolescents than adults, in sessions 4-6 adults had a shorter average latency (Figure 2-2A). Because adolescents more readily reduced their response rate in the latter portion of most training sessions (Figure 2-1B), the age-related latency differences in sessions 4-6 could be due to this effect. We therefore examined this latency during the first 5 min of each session, when adolescents and adults performed trials at the highest rate. A significant age difference was still present $F(1,77) = 9.03$, $p < 0.01$; however, there was no significant difference in latency for sessions 3-6 (Figure 2-2B). There was also no significant main effect or interaction for the average latency from instrumental poke to food-trough entry $F(1,77) = 3.37$, $p > 0.05$ and $F(1,1) = 2.49$, $p > 0.05$, although adolescents appeared to exhibit a shorter latency during the first training session (Figure 2-2C). Despite these latency similarities (and even slightly shorter cue-to-poke latencies for adolescents in early sessions), adults consistently performed more premature pokes across sessions, defined as pokes prior to the trial-onset cue during the ITI $F(1,77) = 21.72$, $p < 0.001$ (Figure 2-3A). Conversely, adolescents consistently performed more task-irrelevant (left and right) hole pokes than adults during training $F(1,77) = 191.31$, $p < 0.001$ (Figure 2-3B).

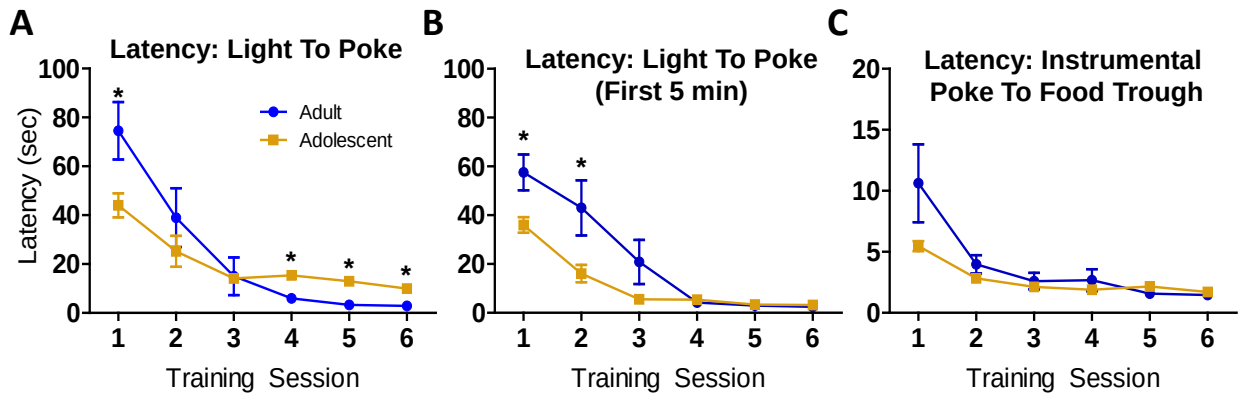


**Figure 2-2 Similar or slightly faster adolescent task acquisition followed by parity of performance**
A) The mean latency from trial onset cue to central poke (instrumental response) was shorter in the first training session. During sessions 4-6 this latency became shorter in adults than adolescents. B) Taking only the first 5 minutes of each session, prior to the drop off in within-session cumulative trial performance seen in Figure 1B, we see that adolescent latencies are still shorter in early sessions, but there were no significant latency differences in later sessions. C) There was also no significant age difference in the mean latency from the central poke to the retrieval of the food pellet. * = significant difference between age groups.

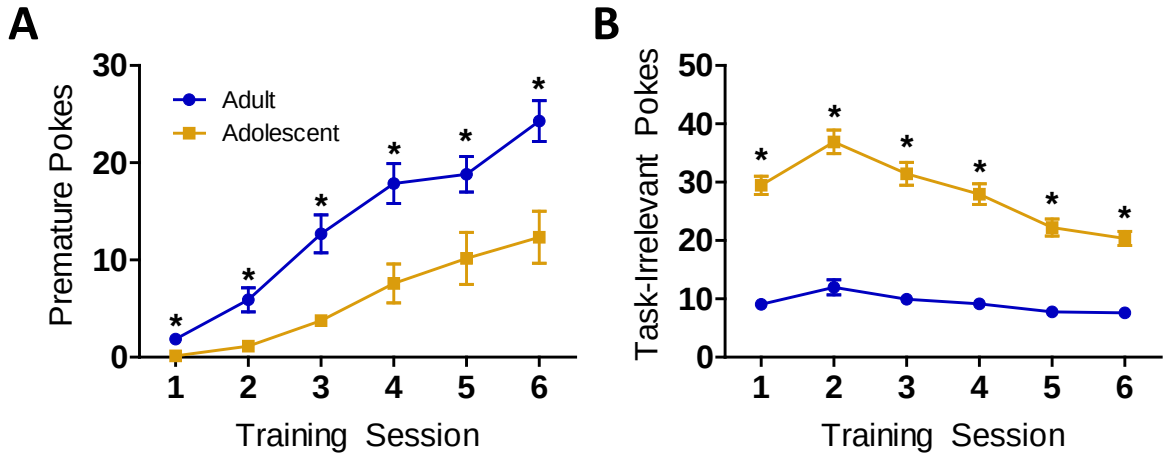


**Figure 2-3 Adolescents and adults perform different behaviors between trials**
A) Adults consistently performed more premature (precue) pokes during the intertrial interval than adolescents. In both age groups the number of premature pokes increased, but this increase was more pronounced in adults than adolescents. B) Adolescents consistently performed more task-irrelevant (left- and right-hole) pokes than adults. * = significant age-related differences.

### 2.4.2 Extinction

During extinction sessions when the trial-onset cue was present, food-restricted adolescents performed significantly more perseverative (previously reinforced center hole) pokes than adults $F(1,33) = 33.16$, $p < 0.001$. No Age × Session interaction was present $F(1,1) = 1.28$, $p > 0.05$ (Figure 2-4A). In food-restricted rats for which no cue light was presented during extinction, a main effect of age was still observed $F(1,22) = 32.14$, $p < 0.001$ and again no interaction was present $F(1,1) = 0.535$, $p > 0.05$ (Figure 2-4B). To test the hypothesis that the difference in perseverative pokes between food-restricted adolescents and adults was larger when the cue was present than when it was absent, we ran an Age × Cue-Light Presence (between) × Session (within) repeated-measures ANOVA. This model indicated via a significant Age × Cue-Light presence interaction that food-restricted adolescents performed disproportionately more perseverative pokes than food-restricted adults when the cue light was present than when it was absent $F(1,55) = 4.41$, $p < 0.05$ (Figure 2-5A). Rats not food-restricted during extinction performed significantly fewer perseverative pokes than food-restricted rats when the cue was present $F(1,43) = 35.07$, $p < 0.01$. No differences were observed between *ad-lib*-fed adolescents and adults when the cue light was present $F(1,10) = 1.73$, $p > 0.05$ (Figure 2-4C) and no significant Age × Cue Light Presence interaction was present in *ad-lib*-fed rats $F(1,20) = 1.95$, $p > 0.05$ (Figure 2-5B). An Age × Food-Restriction Status interaction was observed, $F(1,43) = 6.15$, $p < 0.05$, indicating that among rats to which the cue was presented (Figures 4A and 4C), food restriction more strongly increased perseverative pokes in adolescents than in adults (Figure 2-5C). When the cue light was absent (Figures 4B and 4D), food-restricted rats still performed more perseverative pokes than *ad-lib*-fed rats, $F(1,32) = 11.57$, $p < 0.01$. However, no Age × Food-Restriction Status interaction was observed when the cue light was absent $F(1,32) = 0.148$,

$p > 0.05$, indicating that food restriction did not have a stronger effect on adolescents than adults when the cue was absent (Figure 2-5D). When rats were no longer food-restricted and the cue was omitted, adults performed fewer perseverative pokes than adolescents $F(1,10) = 39.79$, $p < 0.001$ (Figure 2-4D). We performed a Cue Presence × Food-Restriction Status (between) × Session (within) ANOVA separately on adolescents and adults. Adolescents exhibited a significant Food-Restriction Status × Cue Presence interaction, $F(1,38) = 11.96$, $p = 0.001$ (Figure 2-5E), indicating that cue presence interacted with food restriction to further increase perseveration in these younger rats. In adults, however, no such interaction was observed $F(1,37) = 2.43$, $p > 0.05$ (Figure 2-5F).

Total trials performed during training was a significant covariate for adults and adolescents predictive of total perseverative pokes during extinction $F(1,75) = 11.49$, $p = 0.001$. This indicates a positive linear relationship between training trials and perseveration within age groups, although adolescents tended to perform fewer total trials during training but more perseverative pokes during extinction than did adults. No statistically significant relationship was observed for trial performance, task-irrelevant poking, or perseverative pokes during extinction as a function of the covariate body weight when food-restriction status was included as a fixed factor ($p > 0.05$; data not shown).

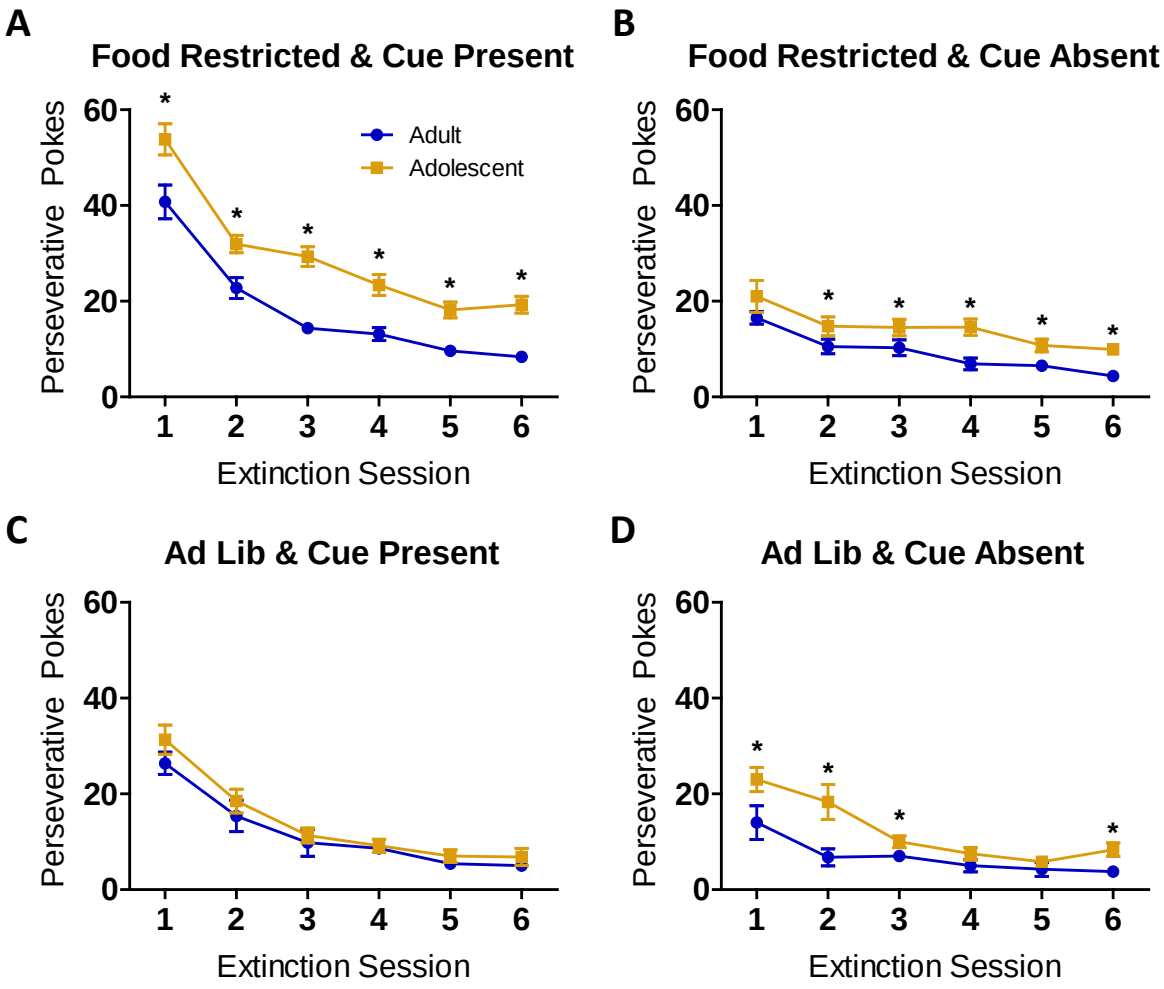


**Figure 2-4 Age-related differences in perseverative poking were modulated by the presence of motivational factors**

A) When animals remained food restricted and the cue light continued to be presented during extinction, adolescents consistently performed more perseverative pokes than adults. B) When the cue light was omitted but animals were still food restricted, adolescents still perseverated more but this difference was smaller than in A. C) When the rats had *ad lib* access to food outside of the task and the cue was still presented there were no perseveration differences between adolescents and adults. D) When animals were neither food restricted nor presented the cue, adults performed fewer perseverative pokes. Adolescents in this condition were more similar in their perseveration to their counterparts exposed to a single motivational factor as in B and C. * = significant age-related differences.

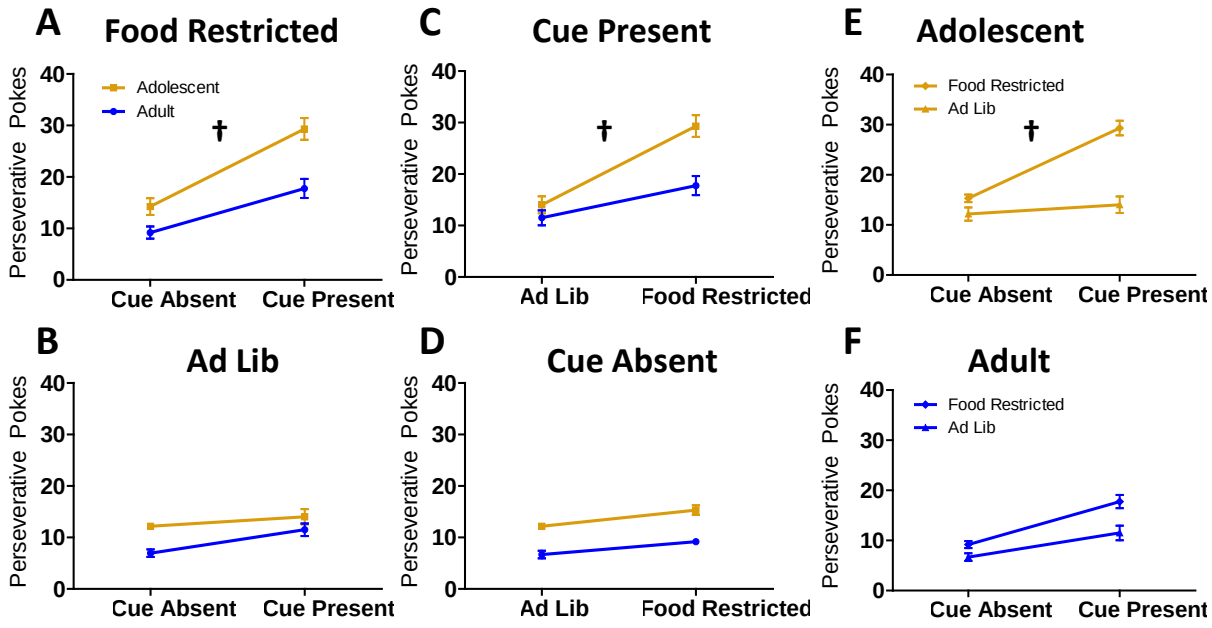


**Figure 2-5 Interaction plots showing differences in adolescent and adult sensitivities to motivational factors across extinction sessions**

A) Among food-restricted animals a significant Age × Cue Presence interaction was observed. Adolescents perseverated more than food-restricted adults generally, but importantly, the presence of the cue had a stronger activating effect on adolescents than adults. B) There was no Age × Cue Presence interaction in *ad lib* rats. C) A significant Age × Food-Restriction Status interaction was observed for rats presented the cue. Among these rats, only the food-restricted adolescents perseverated more than adults. D) No Age × Food-Restriction Status interaction was observed in animals for which the cue was omitted. E) In adolescents, the combination of both motivational factors interacted to further increase perseveration. A single or no motivational factor led to similar lower levels. F) Unlike adolescents, in adults the combination of motivational factors did not interact synergistically to further increase perseverative pokes. † = significant interactions.

Adolescents continued to perform significantly more task-irrelevant pokes than adults during extinction, $F(1,76) = 124.31$, $p < 0.001$ (Figure 2-6A). A main effect of food restriction on total task-irrelevant pokes across extinction sessions was observed, $F(1,80) = 7.25$, $p < 0.01$. However, post-hoc comparisons indicated that while *ad-lib*-fed adults performed significantly fewer total task-irrelevant pokes than food-restricted adults, this effect was not significant for adolescents (Figure 2-6B). There was no statistically significant correlation between task-irrelevant pokes and open-field grid entries, total training trials, or perseveration during extinction for adolescents or adults ($p > 0.05$; data not shown).

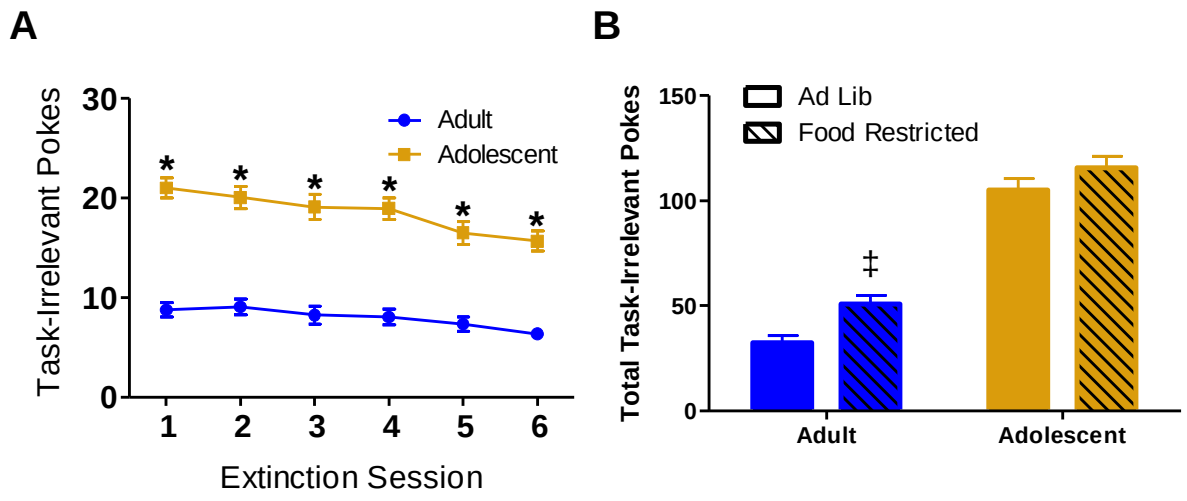


**Figure 2-6 Task-irrelevant poking behavior during extinction**
A) Adolescents consistently performed more task-irrelevant pokes during extinction. B) A main effect of food-restriction status was observed, although only *ad-lib*-fed adults performed significantly fewer task-irrelevant pokes. * = Significant age-related difference; ‡ = significant difference in task-irrelevant pokes as a function of food-restriction status within an age group.

Food-restricted adolescents gained weight throughout the experiment. On the 1st day of testing, food-restricted adolescents (Postnatal Day 28) weighed (*M* ± *SD*) 74.9 ± 6.1 g. By the 1st day of extinction, food-restricted adolescents (Postnatal Day 34) weighed 110.4 ± 7.2 g. On the 5th day of extinction food-restricted adolescents (Postnatal Day 38) weighed 116.9 ± 8.2 g.

### 2.4.3 Open field

In the open field, adolescents performed more total grid entries than adults, $F(1,43) = 90.48$, $p < 0.001$, and an Age × Food-Restriction Status interaction was significant $F(1,43) = 5.55$, $p < 0.05$. Food restriction increased adolescents' total grid entries, but adults were unaffected (Figure 2-7A). The effect was similar for central grid entries, with adolescents entering the central grids more than adults $F(1,43) = 25.38$, $p < 0.001$, and an Age × Food-Restriction Status interaction

was observed $F(1,43) = 5.03$, $p < 0.05$. As with total grid entries, food restriction increased central grid entries in adolescents but not adults (Figure 2-7B).

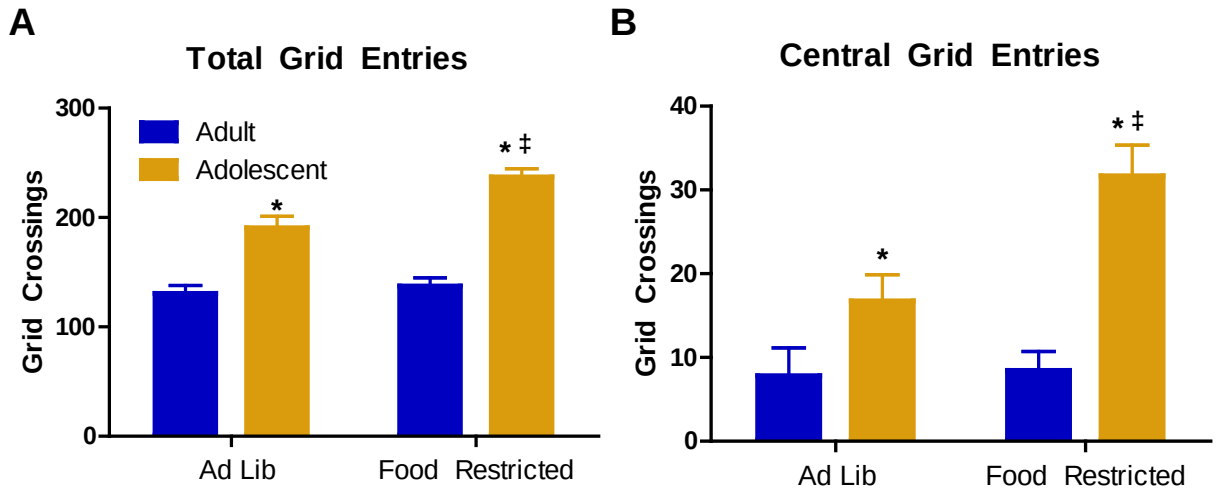


**Figure 2-7 Adolescent open field activity was modulated by food restriction**
A) Adolescents performed more total grid entries than adults. This behavior was increased in food-restricted adolescents. There was no significant difference in total grid entries as a function of food-restriction in adult rats. B) Adolescents entered the central grid area more than adults and again food restriction increased this behavior in the younger rats. Adult central-grid entries were unaffected by food-restriction status. * = Significant age-related difference; ‡ = significant difference as a function of food-restriction status within an age group.

## 2.5 DISCUSSION

In this study, we compared the behavior of adolescent and adult rats during a simple instrumental task and extinction. There was no age-related difference in the total number of trials performed during the first two sessions, although the within-session rate of trial performance and the latencies from the trial-onset cue to the instrumental response may have been slightly faster in adolescents during those sessions. By sessions 3-4 adults and adolescents reached a stable maximum in the total number of trials performed, with adults performing more total trials from session 3 onward. We also found that the rate of trial performance was similar in the early portions of these sessions and an absence of latency differences indicate similar performance

once the task was well learned. We did observe persistent differences in task-irrelevant pokes (performed more by adolescents) and premature pokes (performed more by adults). During extinction we found that adolescents tended to perform more perseverative pokes than adults, although the presence and extent of this difference was modulated by the combination of food-restriction and continued cue presentation. Finally, we observed that adolescents were more active in an open field than adults generally, and that this activity was increased by food restriction in the younger rats only.

Adolescents and adults perform different behaviors during the ITI. The increased task-irrelevant pokes by adolescents may be consistent with greater exploration and general activity in adolescents, described by others (Shalaby and Spear, 1980; Spear, 2000) and observed in our open-field experiment. It is notable, however, that we found no significant predictive relationship among individual animals between open-field activity and task-irrelevant poking. As others have interpreted premature pokes in a different task as a measure of impulsivity (Carli et al., 1983; Robbins, 2002), we were initially surprised to observe this behavior more in adults than adolescents. One important difference between those studies and this one is that in this study there was no penalty for premature poking, and so rats had no reason to resist a desire to poke early. Thus, the behavior probably does not reflect poor impulse control in the present task. Both adolescents and adults increased premature poking across training sessions; this suggests that premature pokes do not indicate a weaker cue-action association on the part of adults, since we would expect such an association to improve over time. The observed age-related differences in these behaviors could represent a greater exclusive focus on the task at hand by adults whereas adolescents are more inclined to shift their attention to the task-irrelevant holes during that period. It is noteworthy that once the trial-onset cue was presented, adolescents were at least as

quick to respond as adults. Thus, premature poking may indeed reflect the single-mindedness of task-performing rats, with adolescents tending to divert themselves more often (although to a lesser extent over time) to explore task-irrelevant holes, although this propensity does not impair task performance.

During extinction adolescents tended to perform more perseverative (previously reinforced) pokes. Others have demonstrated that adolescents exhibit resistance to extinction of cocaine-seeking behavior in a conditioned place preference paradigm (Brenhouse and Andersen, 2008); we observe this pattern in the context of a natural (food) reinforcer. Although this may suggest behavioral inflexibility or cognitive impairment on the part of adolescents, it appears unlikely, as the extent of this age-related difference was modulated by motivational factors (food-restriction status and cue presence). When rats remained food restricted and the cue light continued to be presented (i.e., the same circumstances as training), the difference between adolescents and adults in perseverative pokes was the greatest. If only one of these factors was present the difference was either small (e.g. when animals remained food-restricted but no cue was presented) or absent altogether (when animals had *ad lib* home cage food access but the cue was presented during extinction). When neither factor was present adolescents performed more perseverative pokes than adults, but this was due to a reduction in adult perseveration; adolescents with one motivational factor perseverated to a similar degree as those with neither motivational factor. This pattern of results suggests that adolescents have a higher ceiling for behavioral activity when these motivational factors are present and a higher floor when they are absent. Conversely, adults have a lower ceiling when these factors are present and a lower behavioral floor when they are absent. Similarly, *ad-lib*-fed adults performed even fewer task-irrelevant pokes during extinction than their food-restricted counterparts; *ad-lib*-fed adolescents

exhibited no significant reduction in this behavior. Thus, motivational factors, separately and in combination, differently affect the extinction behavior of adolescents and adults.

We observed greater total open-field grid entries among adolescents, which is consistent with the majority of studies that compare adolescent and adult locomotion and novelty-induced activity (Spear and Brake, 1983; Darmani et al., 1996; Stansfield and Kirstein, 2006); however see Philpot, (2008). We also observed greater central grid entries by adolescents regardless of food-restriction status. As central grid entries are thought to be a function of an animal's anxiety state, these results are consistent with adolescents spending more time exploring the open arms of an elevated plus maze (Macrì et al., 2002) [however see Doremus-Fitzwater et al. (2009a)], and displaying a greater propensity for either risk-taking behavior (Ernst et al., 2006; Steinberg, 2008) or exploration and novelty-seeking (Spear and Brake, 1983; Darmani et al., 1996; Stansfield and Kirstein, 2006; Philpot and Wecker, 2008). It is interesting that food restriction increased total grid entries and central grid entries in adolescents while it had no effect on adult open field activity. Even in this novel environment not previously associated with food reinforcement, food restriction has an activating effect on adolescents. Food restriction is known to increase dopamine receptor signaling and the rewarding and motor activating effects of drugs (Carr et al., 2003); it also increases motivation to work for food, which is why food-reinforced instrumental tasks such as ours use it. It is possible that the novelty of the open field environment maximally activated adults, such that food restriction did not further increase activity. In addition to exhibiting a higher baseline of open-field activity, the increased food-restriction-induced activity of adolescents may be due to a higher potential behavioral ceiling in these younger rats.

We cannot be certain that the level of food restriction and its motivational effects were equal between age groups. This is particularly difficult because adolescents gain a great deal of

weight (and must do so to be healthy, even under food restriction) while adults lose weight under this condition. Although we cannot equate adolescent and adult food-restriction, our lack of observed latency differences and the similar early trial-performance rate in sessions 3-6 indicate that whatever baseline motivational differences might exist, they were not large enough to cause differences in these behavioral measures once the task was well learned. The exception to this is the within-session drop in trial-performance rate by adolescents, which could reflect earlier satiety by these animals. Such differences, however, do not adequately explain the sustained disparities in task-irrelevant poking and perseveration during extinction. Similarly, behavioral differences in the open field could partially reflect unequal food restriction. If this were to entirely account for the age-related differences we might still expect to observe adult open field activity to be affected to a lesser or greater extent. The lack of any change in adult open field behavior suggests that the age-related behavioral differences are not solely due to differences in the degree of food-restriction severity. Finally, the pattern of perseveration during extinction depended strongly on the combination of both food restriction and cue presence. In fact, those adolescents that were *ad lib* while the cue was presented, were food-restricted with the cue absent, or lacked both the cue and food restriction all exhibited similar levels of perseveration. It was only the combination of food restriction and cue presence that substantially increased adolescent perseveration. Thus, age-related differences in the motivational consequences of food restriction appear insufficient to account for all of these results, although we acknowledge that this is a difficulty in using any task that food restricts adolescents and adults.

Although care was taken to expose adolescents and adults to similar conditions prior to and during behavioral testing, there is always the possibility that adolescents could respond differently to housing, shipping, or other conditions differently and in such a way that might

affect measures of behavioral performance. For example, adolescents and adults were shipped four days before handling. If their transport was stressful, our findings could reflect age-related differential effects of shipping stress on performance. Similarly, food restriction reduces the rate of normal adolescent weight-gain. The weights of our adolescent rats were within the normal to low-normal free-feeding range of adolescent Sprague-Dawley rats at corresponding ages described by McCutheon and Marinelli (2009). Nevertheless, it is possible that reduced growth rate or various external factors could differently affect adolescent physiology and alter behavior.

The connection between adolescent neurdevelopmental and behavioral changes are of great clinical relevance, especially in light of the associated increased risk taking (Spear, 2000; Steinberg, 2008) and addiction vulnerability of this period (Khuder et al., 1999; Chambers et al., 2003; Compton et al., 2005). Significant components of the circuitry that underlies motivated, goal-directed behavior undergo substantial changes during adolescence (Spear, 2000; Doremus-Fitzwater et al., 2009b). These regions subserve aspects of instrumental learning and extinction. The dorsal striatum is critical to the expression of action-outcome associations of the sort formed during instrumental learning (Balleine et al., 2009). The prefrontal cortex (PFC) mediates the initial encoding of action-outcome learning, and plays a central role in cognitive flexibility, such as during extinction (Corbit and Balleine, 2003; Jung et al., 2008). The amygdala and extended amygdala are thought to provide valence information necessary for learning the initial rewarding or anti-rewarding contingencies of an operant behavior, and for allowing flexibility when contingencies change (Koob, 2009). Finally, the nucleus accumbens (NAc), which receives a convergence of information from the PFC, amygdala, thalamus, and other regions, is critical to incentive-motivated behavior, instrumental learning, and food intake (Mogenson et al., 1980; Kelley, 2004). These crucial brain regions undergo numerous changes during adolescence. There

is increasing dopaminergic innervation of the PFC during adolescence (Rosenberg and Lewis, 1994; Benes et al., 2000). Dopamine D1, D2, and D4 receptor expression peaks during adolescence in the dorsal striatum (Seeman et al., 1987; Teicher et al., 1995; Tarazi and Baldessarini, 2000; Teicher et al., 2003) and PFC (Andersen et al., 2000) before being pruned to lower adult levels. Others have also found a similar pattern for the NAc (Tarazi and Baldessarini, 2000) although this has not always been observed (Teicher et al., 1995). During adolescence there is also increasing glutamatergic connectivity from the basolateral amygdala to the PFC (Cunningham et al., 2002, 2008) and from the PFC to the NAc among dopamine D1-expressing neurons, before this is reduced in adulthood (Brenhouse et al., 2008).

Recently, the triadic node hypothesis has been proposed to explain the elevated risk-taking behavior of adolescents in terms of underlying neurodevelopment (Ernst et al., 2006; Ernst and Fudge, 2009). This hypothesis posits that in adolescents, NAc-mediated approach is out of balance with amygdala-mediated avoidance. The third "node" of this model is the PFC, which normally maintains equilibrium between these countervailing forces. This intriguing framework may account for adolescent proclivities to both take risks and behave impulsively. The idea that these circuits associated with reward and motivation are shifted in their sensitivities during adolescence is consistent with age-related regional neural activation differences in anticipation, receipt, and omission of reward observed in fMRI studies (Galvan et al., 2006; Van Leijenhorst et al., 2009) and greater adolescent preferences for natural (Douglas et al., 2004; Wilmouth and Spear, 2009) and drug reinforcers (Vastola et al., 2002; Philpot et al., 2003; Badanich et al., 2006; Shram et al., 2006; Brenhouse and Andersen, 2008; Brenhouse et al., 2008). Thus both the neural circuitry of motivated behavior and the sensitivity and preference for reinforcing stimuli is altered during adolescence. This framework is consistent with our finding

that adolescent perseveration during extinction is differentially affected by the presence of certain motivational factors.

Human adolescence is often considered a period of storm and stress because of tendencies toward heightened interpersonal conflict, emotional reactivity, and risk behavior (Arnett, 1999). Although most adolescents do not experience psychiatric problems, it is at this time that problems often arise (Volkmar, 1996; Spear, 2000; Pine, 2002; Sisk and Zehr, 2005). The changing cognitive and affective milieu of the developing brain may cause adolescents to process and react to internal and external stimuli differently. This in turn could be relevant to their increased neuropsychiatric vulnerabilities and tendencies toward risk behavior. We observed that while certain measures of cognitive performance were similar among adolescent rats performing an instrumental learning task, persistent differences were observed during training (e.g. task-irrelevant and premature pokes) and extinction, with adolescents exhibiting more perseverative behavior and more sensitivity to the activating effects of internal and external motivational factors. By studying how adolescents respond to such stimuli differently we may learn more about the unique propensities and neuropsychiatric vulnerabilities of the period.

Acknowledgements—This work was supported by National Institute of Mental Health grants MH48404 and MH065468 and the Andrew Mellon Foundation for a predoctoral fellowship.

# 3.0 ADOLESCENT VERSUS ADULT NEURAL ACTIVITY IN ORBITOFRONTAL CORTEX DURING MOTIVATED BEHAVIOR*

## 3.1 ABSTRACT

Adolescence is a time of both cognitive maturation and vulnerability to several major psychiatric illnesses and drug dependence. There is increasing awareness that behavioral or pharmacological intervention during this period may be critical for disease prevention in susceptible individuals. Therefore, we must attain a deeper understanding of how the adolescent brain processes salient events relevant to motivated behavior. To do this, we recorded single unit and local field potential activity in the orbitofrontal cortex of rats as they performed a simple reward-driven operant task. Adolescents encoded basic elements of the task differently than adults indicating that neuronal processing of salient events differs in the two age groups. Entrainment of local field potential oscillations, variance in spike timing and relative proportions of inhibitory and excitatory responses differed in an event-specific manner. Overall adolescent phasic neural activity was less inhibited and more variable through much of the task. Cortical inhibition is essential for efficient communication between neuronal groups, and reduced inhibitory control of cortical activity has been implicated in schizophrenia and other disorders.

* Adapted from Sturman DA, Moghaddam B (2011) Reduced neuronal inhibition and coordination of adolescent prefrontal cortex during motivated behavior. J Neurosci 31:1471-1478.

Thus, these results suggest that reduced inhibitory responses of adolescent cortical neurons to salient events could be a critical mechanism for some of the increased vulnerabilities of this period.

## 3.2 INTRODUCTION

Adolescence is a time of adjustment as one completes the physical and psychosocial transitions to adulthood (Arnett, 1999). It is also considered a period of vulnerability as it coincides with the onset of symptoms for several major psychiatric problems, including mood disorders, schizophrenia, and drug abuse (Volkmar, 1996; Pine, 2002; Johnston et al., 2008). In recent years, studies in adolescent humans and animal models have described age-related shifts in cellular and molecular brain architecture and disparities in the pharmacological effects of various drugs (Spear and Brake, 1983; Spear, 2000; Adriani et al., 2004; Brenhouse et al., 2008; Paus, 2010). Age-related behavioral differences have also been examined and are often focused upon, although adolescent behavior tends to be quite similar to that of adults in most contexts with only modest changes in decision-making capacity from mid-adolescence onward (Spear, 2000; Luna et al., 2004; Doremus-Fitzwater et al., 2009a; Figner et al., 2009; Cauffman et al., 2010). Nevertheless, adolescents may process salient events differently from adults. For example, a recent study observed greater adolescent than adult c-fos protein expression in dorsal striatum and nucleus accumbens after exposure to a reward-associated odor cue (Friemel et al., 2010). Differences in measures of adolescent prefrontal cortex (PFC) neural activity and connectivity have also been described (Ernst et al., 2006; Galvan et al., 2006; Liston et al., 2006;

Geier et al., 2009; Uhlhaas et al., 2009b; Hwang et al., 2010). However, little is known of the precise nature of these age-related disparities at the neuronal level.

To directly compare the dynamic processing of cortical neurons in adolescents with that of adults, we recorded single-unit and local field potential (LFP) activity from the orbitofrontal cortex (OFC) of rats as they performed a reward-motivated behavior. The OFC was targeted because of its central role in processing value expectation and previous evidence of its under-development in adolescents (Schultz et al., 2000; Galvan et al., 2006; Schoenbaum et al., 2009). The behavioral task involved acting upon a learned action-outcome association (Chapter 2; Sturman et al., 2010), which is a fundamental building-block of complex motivated behavior. The simplicity of this task allowed for behavioral measures to be very similar between groups. We could therefore test the hypothesis that even with similar task performance, the adolescent OFC encodes salient task-related information differently than adults. Characterizing such fundamental neural activity differences—and doing so at the neuronal level—is critical for identifying developmental processes that may be associated with the increasing neuropsychiatric risks of adolescence, and for the future design of intervention strategies to prevent and treat such problems.

## 3.3 METHODS

### 3.3.1 Subjects

Adolescent (Postnatal Days 28-42; $n$ = 8) and adult (older than Postnatal Day 70; $n$ = 4) male Sprague-Dawley rats (Harlan, Frederick MD) were used. Juvenile (Postnatal Day 21) and

adult rats were received one week before surgery. Subjects were housed in a climate-controlled vivarium under 12 h light-dark conditions (lights on at 7 pm), with *ad lib* access to chow and water before training. All animal use procedures were approved by the University of Pittsburgh Animal Care and Use Committee.

### 3.3.2 Surgery and Electrophysiology

Rats underwent electrode array implantation surgeries as described previously (Totah et al., 2009). Briefly, microelectrode arrays (NB Labs), consisting of eight Teflon-insulated stainless-steel wires arranged in a 2 × 4 pattern, were implanted in the OFC. Adults were implanted bilaterally 2.8-3.8 mm anterior to bregma, 3.1-3.5 mm lateral to bregma, and 4.5 mm ventral to the dura surface. Adolescents (Postnatal Day 28-29) were implanted unilaterally (because of size limitations) 2.8-3.2 mm anterior to bregma, 2.8-3.2 mm lateral to bregma, and 4.0 mm ventral to the dura surface. During recordings, a unity-gain junction field-effect transistor headstage attached to a light-weight cable (NB Labs) was connected to a commutator (NB Labs) that allowed rats to move freely within the testing box. Recorded single-unit activity was amplified at 1000× gain and analog band-pass filtered at 300 – 8000 Hz; LPFs were band-pass filtered at 0.7 – 170 Hz. Single-unit activity was digitized at 40 kHz and LFPs were digitized at 40 kHz and downsampled to 1 kHz by Recorder software (Plexon). Single-unit activity was digitally high-pass filtered at 300 Hz, and LFPs were low-pass filtered at 125 Hz. Behavioral event markers from the operant box were sent to Recorder to mark events of interest. Single units were isolated in Offline Sorter (Plexon) using a combination of manual and semi-automatic sorting techniques as described previously (Homayoun and Moghaddam, 2008; Totah et al., 2009).

### 3.3.3 Behavior

Adult and adolescent rats were tested in an operant box apparatus (Coulbourn Instruments) that contained a house light, a pellet magazine that could deliver food pellets (fortified dextrose, 45 mg; Bio-serv) into a food trough, and three nose-poke holes arrayed horizontally on the wall opposite the food trough. After 5-6 d of surgical recovery, animals were mildly food restricted, underwent habituation to the behavioral testing apparatus, and began training on the behavioral task, which has been characterized previously (Sturman et al., 2010). Briefly, rats learned to poke into an illuminated center nose-poke hole for food-pellet reinforcement. Trials began with the onset of a cue light inside the center nose-poke hole. When the rat poked into that hole the light immediately turned off and a single pellet was delivered to the food trough, which was then illuminated. Poking into the food trough to receive the pellet turned off the food trough light and triggered a 5 s inter-trial interval (Figure 1A). Each session was terminated after 100 trials or the passage of 30 min. Main task-performance measures included the number of total trials completed during each session, the latency from cue to instrumental poke, and the latency from instrumental poke to food trough entry (pellet retrieval). Age × Session repeated-measures ANOVAs were performed on all outcome measures in SPSS ($\alpha = 0.05$). In all cases where the assumption of sphericity was violated, the lower-bound corrections were used for a maximally conservative degrees-of-freedom adjustment.

### 3.3.4 Histology

Upon completion of the experiment, rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and a 200 μA current was passed through recording electrodes for 5 s to mark

electrode tip placements. Animals were perfused with saline and 10% buffered formalin. Brains were then removed and placed in 10% formalin. Brains were sectioned in coronal slices, stained with cresyl violet, and mounted to microscope slides. Electrode-tip placements were examined under a light microscope. Only rats with correct placements within the OFC (Figure 3-1B) were included in electrophysiological analyses.

### 3.3.5 Electrophysiology Analysis

Electrophysiological data were analyzed with custom-written scripts, executed in Matlab (MathWorks), along with the Chronux toolbox (http://chronux.org/) for LFP analyses and firing-rate variability functions graciously made available by Churchland et al. (2010) (http://www.stanford.edu/~shenoy/GroupCodePacks.htm). In general, neural activity was time-locked to specific task events: trial-onset cue, instrumental nose-poke response, and food-trough entry. Raw LFP traces were time-locked to these task events, and trials with clipping artifacts were excluded prior to averaging. Each subject's trial-averaged power spectrum in the several seconds around each task event was calculated by fast Fourier transform. This was done using 13 leading tapers, a time-bandwidth product of 7, and a 1 s spanning moving-window (in 250 ms steps). These parameters, compared to others that we had examined allowed for a frequency resolution of ~2 Hz, which generally allowed for multiple frequency bins in each band of interest. A multitaper approach was used because it improves spectrogram estimates when dealing with non-infinite time series data (Mitra and Pesaran, 1999), although using one, three, and nine tapers led to very similar spectrograms. Each frequency bin (row) in the power spectrum was Z-score normalized to the average spectral power during the baseline period (a 2 s

window beginning 3 s before the cue). Normalized power spectra were averaged for adolescents and adults.

Peri-event time firing-rate histograms were produced for each unit in windows around task events. The cross-trial average firing rate of each unit was *Z*-score normalized to that of its baseline period. Units were categorized as "activated" or "inhibited" within windows of interest based on whether their average normalized activity contained three consecutive 50 ms bins with $Z \geq 2$ or $Z \leq -2$, respectively. These criteria were validated using a nonparametric bootstrap analysis on the baseline period of each unit. For each unit, the baseline window was randomly sampled with replacement 10,000 times. The proportion of 2 s windows whose resampled activity reached significance criteria is a measure of the expected false-positive rate for that unit during any 2 s window. This led to an overall expected false-positive rate of $\alpha = 0.0034$ for all adolescent units and $\alpha = 0.0038$ for all adult units. These low $\alpha$ values indicate that unit false-categorization would be rare enough as to not unduly impact statistical comparisons of category proportions between adolescents and adults. To compare the time course of unit responses, the categorization analysis was performed with a moving window around task events (moving-window size 500 ms in 250 ms steps). For time windows of particular interest for age-related statistical comparisons (e.g., in the 1 s after the cue), $X^2$ analyses were performed which included the number of adult and adolescent activated, inhibited, and non-significant units. Significant $X^2$ tests were followed by *post hoc* comparisons of proportions for each category (e.g. inhibited units between adolescents and adults) using a *Z*-test for two proportions (Table 1). Except where otherwise noted, electrophysiological analyses are presented for sessions 3-6, at which point the action-outcome association is well-learned by both groups. Here and elsewhere, the null hypothesis was rejected when $p < 0.05$.

Analyses of firing-rate variability were calculated as Fano factors (spike count variance/mean) using an 80 ms moving window in 50 ms steps. For each unit, spike count variance and mean spike count were computed at each time point. The slope of the regression relating variance and mean for all units was determined at each window step, providing a Fano factor time course around task events. To examine whether observed changes in Fano factor over time (and age-related Fano factor differences) were due to changes in mean firing rate rather than variance, we performed a mean-matching technique devised by Churchland et al. (2010). In the first analysis we performed mean-matching separately for adolescent and adult units. This technique held the mean firing-rate distribution constant at each time-point, by randomly and repeatedly discarding units. Fano factor estimates for each time point were based on the average of 10 iterations of this process. This procedure has been validated as an effective approach to avoiding artifacts due to firing-rate changes (Churchland et al., 2010). In addition to this, a separate mean-matching analysis was performed, in which the greatest common mean firing-rate histogram was used both across time within an age group (as above) and also between age groups. The observation of similar raw and mean-matched Fano factors would confirm that the time courses and age-related differences in Fano factor reflected spike-timing variability and were not merely artifacts of differences in mean firing-rate. Adolescent and adult Fano factors were statistically compared using rank-sum tests in Matlab.

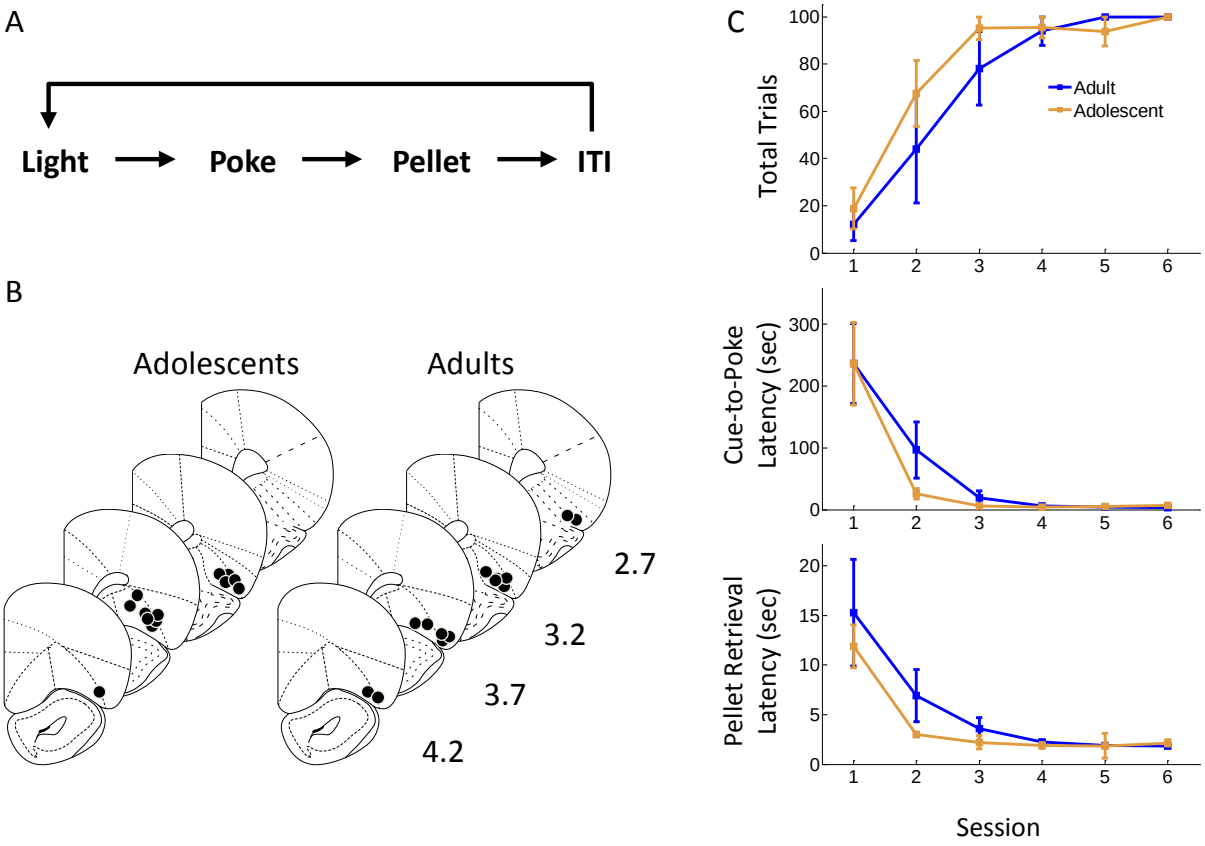


**Figure 3-1 Task paradigm, electrode placements, and behavioral performance**
A) Adolescent and adult rats were trained on a simple instrumental learning paradigm in which they associated a nose poke (instrumental response) into a light-cued hole with the subsequent delivery of a food-pellet reinforcer (Sturman et al., 2010). Trials began with the onset of the light cue. Once cued, animals could poke into the lit hole, which turned off that light and led to the immediate delivery of a pellet into a food trough on the opposite side of the box. As soon as they poked into the food trough to retrieve the pellet a 5 s inter-trial interval was triggered, followed by the next trial. Sessions were terminated after rats performed 100 trials or 30 minutes elapsed. Rats performed this task in six sessions on consecutive days. B) Electrode placement for rats included in the study. Electrodes were placed in the left or right OFC, corresponding to the lateral orbital and agranular insular cortices. Black dots represent the approximate location of lesions associated with electrode placements, overlaid on standard rat atlas images (Paxinos and Watson, 1998). Numbers represent distance (in millimeters) anterior to bregma for adult animals. C) No significant behavioral differences were observed between adolescents and adults in the initial learning or performance of this task, with comparable between-age-group total trials (top), latencies from trial-onset cue to the instrumental response (middle), and latencies from instrumental response to food pellet retrieval (bottom).

## 3.4 RESULTS

### 3.4.1 Behavior

During the behavioral task, adolescents poked into a light-cued hole to receive a food-pellet reinforcer (Figure 3-1A). No significant differences were observed between adolescents and adults in the total number of trials $F(1,1) = 1.3$, $p = 0.28$; latency from trial onset cue to the instrumental response $F(1,1) = 0.34$, $p = 0.57$; or the latency from the instrumental response to food pellet retrieval $F(1,1) = 1.2$, $p = 0.31$. The task was consistently and maximally performed by adult and adolescent animals by the third training session (Figure 3-1C).

### 3.4.2 Local Field Potentials

Electrophysiological recording of LFPs, a measure thought to reflect the activity of regional afferents, revealed somewhat similar patterns for adolescents and adults through much of the task, with notable differences in spectral power immediately after food trough entry to receive reinforcement (Figure 3-2A). At that time, adults exhibited greater alpha (8-12 Hz) and beta (13-30 Hz) power. Theta (4-7 Hz) and low gamma (31-75 Hz) power were similar between groups, whereas adolescents had greater high gamma (76-100 Hz) power than adults (Figure 3-2B).

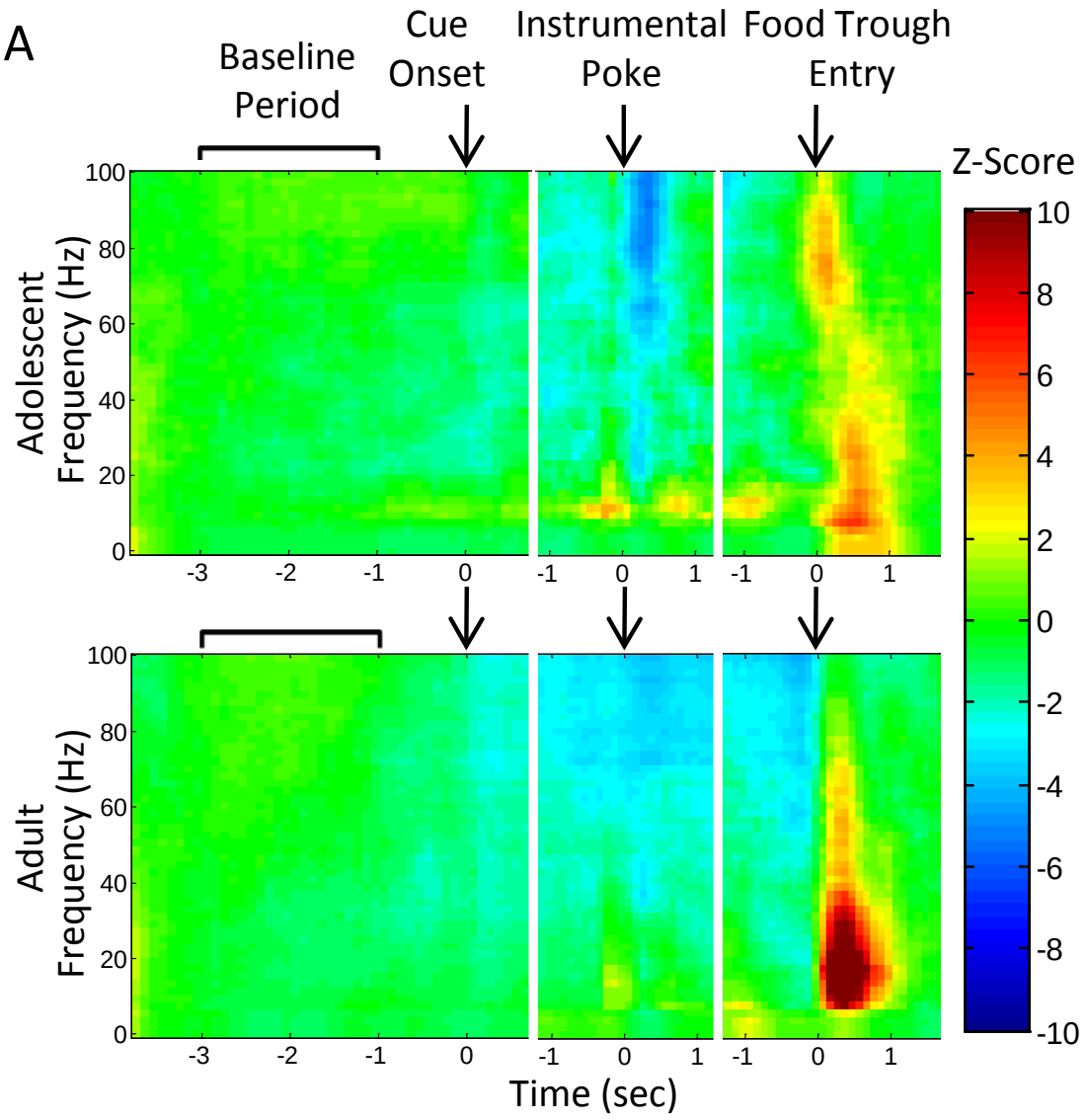
A
Baseline Period
Cue Onset
Instrumental Poke
Food Trough Entry
Z-Score
Adolescent Frequency (Hz)
Adult Frequency (Hz)
Time (sec)
B
Theta (4-7 Hz)
Alpha (8-12 Hz)
Beta (13-30 Hz)
Low Gamma (31-75 Hz)
High Gamma (76-100 Hz)
Adult
Adolescent
Z-Score
Time (sec)

**Figure 3-2 Adolescent and adult local field potentials in the orbitofrontal cortex**
A) LFP power spectra for adolescents and adults in windows around key task events were normalized to the baseline period (3 to 1 second prior to cue onset) for each frequency. The time course of normalized LFP power was primarily similar between adolescents and adults. At cue onset both groups exhibited slight reductions in gamma (>30 Hz) power. This was also observed around the instrumental response. Adults and adolescents both exhibited slightly increased beta (13-30 Hz) power at this time. Immediately following reinforcement adolescents and adults had increases in theta (4-7 Hz), alpha (8-12 Hz), beta, and gamma power, with adults showing greater increases in the alpha and beta bands. Adolescents had greater increases in high gamma power (above ~75 Hz). B) Time course of adolescent and adult normalized LFP power around reinforcement. Line graphs correspond to baseline-normalized adolescent and adult LFP power averaged across discrete frequency bands as labeled.

### 3.4.3 Fano Factor

Age-related differences were observed in firing-rate variability associated with specific task events. The Fano factor, which is the slope of the relationship between spike-count variance and spike-count mean (Churchland et al., 2010), was computed to examine the variability of spike timing across trials (Figure 3-3). Adolescents (8 rats, 265 units) had significantly larger Fano factors than adults (4 rats, 184 units) during sessions 3-6 (comparisons performed with rank-sum tests) during the 2 s baseline period $Z = 6.90$, $p < 0.01$, in a 1 sec window immediately after the trial-onset cue $Z = 5.48$, $p < 0.01$, in a 1 s window centered around the instrumental response $Z = 3.12$, $p < 0.01$, and in the one second leading up to reinforcement retrieval $Z = 3.77$, $p < 0.01$ (Figure 3-3). Because Fano factor calculations depend upon window size and step we varied these parameters in the period around the instrumental poke to demonstrate that, although the magnitude and smoothness of the calculations are affected, the general time course and age-related differences remain (Figure 3-4).We performed a mean-matching technique (Churchland et al., 2010) to hold the mean firing rate approximately constant over time so that temporal firing-rate changes would not obscure our interpretation of the Fano factor as a measure of variability (Figure 3-5A). We similarly equalized firing-rate distributions between age groups (Figure 3-5B). Raw Fano factors were very similar to those computed with either mean-matching method, confirming that the observed Fano factor time course reflects the variability in spike

timing regardless of mean firing-rate dynamics. One exception to this was after reinforcement retrieval, at which time adults exhibited greater raw Fano factors (Figure 3-3). This difference was due at least in part to changes in mean firing rate, as there was no statistically significant difference in the mean-matched Fano factors during that period (Figure 3-5). These findings indicate that salient events lead to a reduction in the variability of spike timing for both adolescents and adults, and that interestingly, adolescent OFC neural spike timing is generally more variable than that of adults throughout much of the task. Stimulus-driven Fano factor reductions are thought to be a general property of cortical architecture (Churchland et al., 2010). Thus, higher Fano factors may suggest an intrinsic tendency for spike timing to be less tightly-controlled in the OFC of adolescents compared with adults.

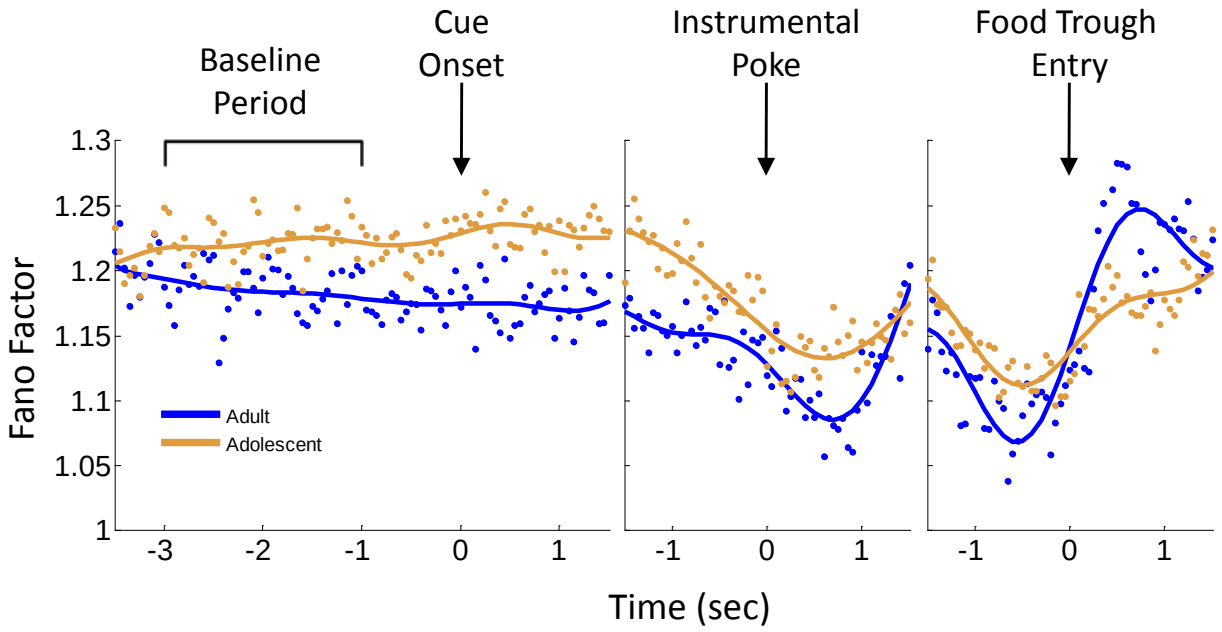


**Figure 3-3 Fano factor analysis of adolescent and adult firing-rate variability**
The Fano factor is the slope of the trial-by-trial spike-count variance and spike-count mean for all units. Using a sliding window, this variability estimate was computed at time points around task events of interest. Fitted polynomial lines are plotted over raw Fano factor values. For both groups, the instrumental response and the period preceding entry into the food trough were accompanied by reductions in the Fano factor. Adolescents tended to have higher Fano factors than adults. Specifically, adolescents had greater Fano factors during the baseline period, in the 1 s after the trial onset cue, in a 1 s window around the instrumental poke, and in the 1 s leading up to reinforcement retrieval. These results were not due to time- or age-dependent firing-rate differences, because this pattern survived a mean-matching procedure that controls for changes in firing-rate (See Figure 3-6).

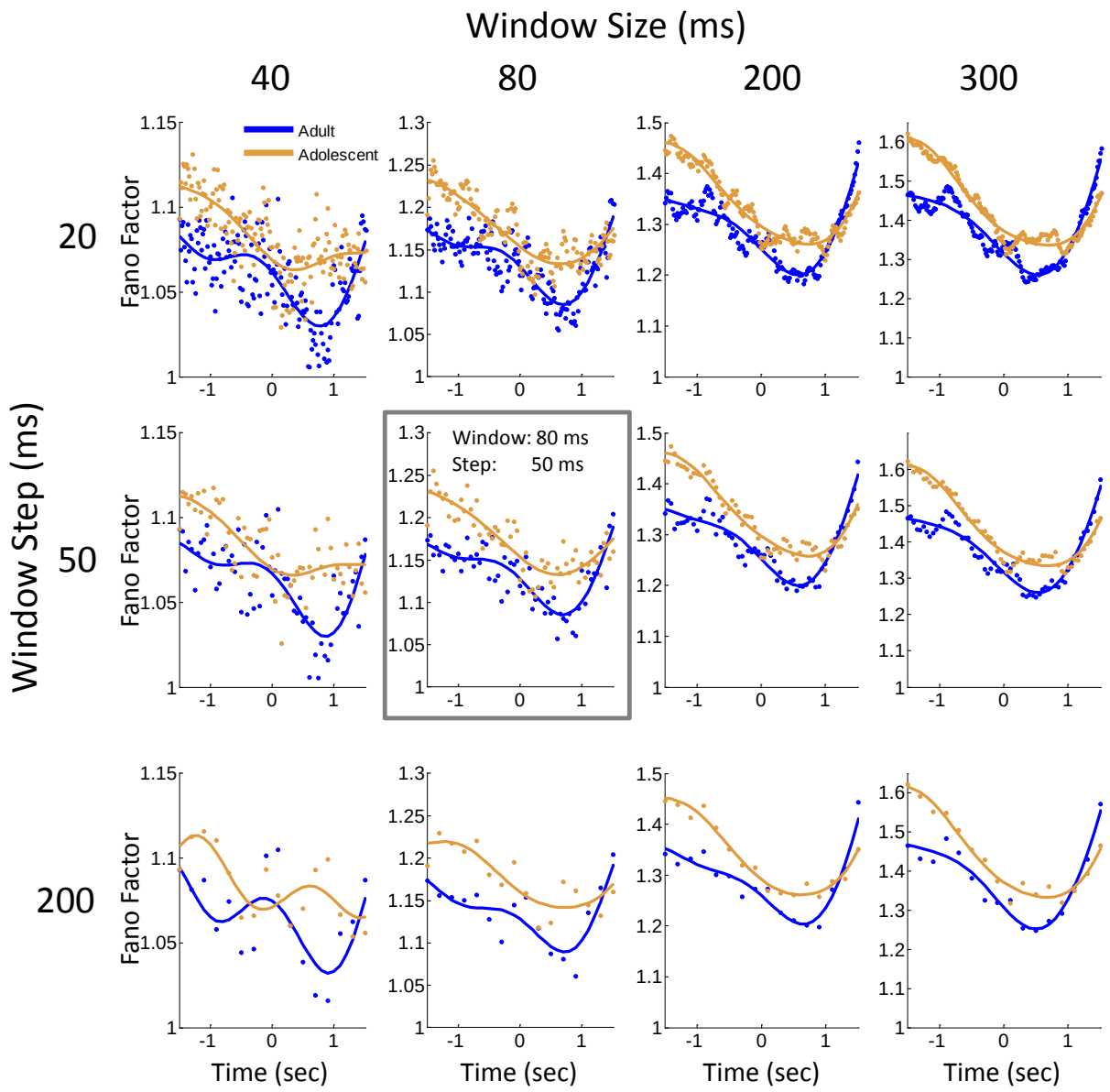


**Figure 3-4 Effects of window size and window step on Fano factor calculations**
Each plot represents the period around the instrumental poke. Although changing parameters did affect the magnitude and smoothness of the series of Fano factor values, the general time courses and age-related differences remained. The parameters used throughout the paper (window size 80 ms, window step 50 ms) are indicated with the grey box.

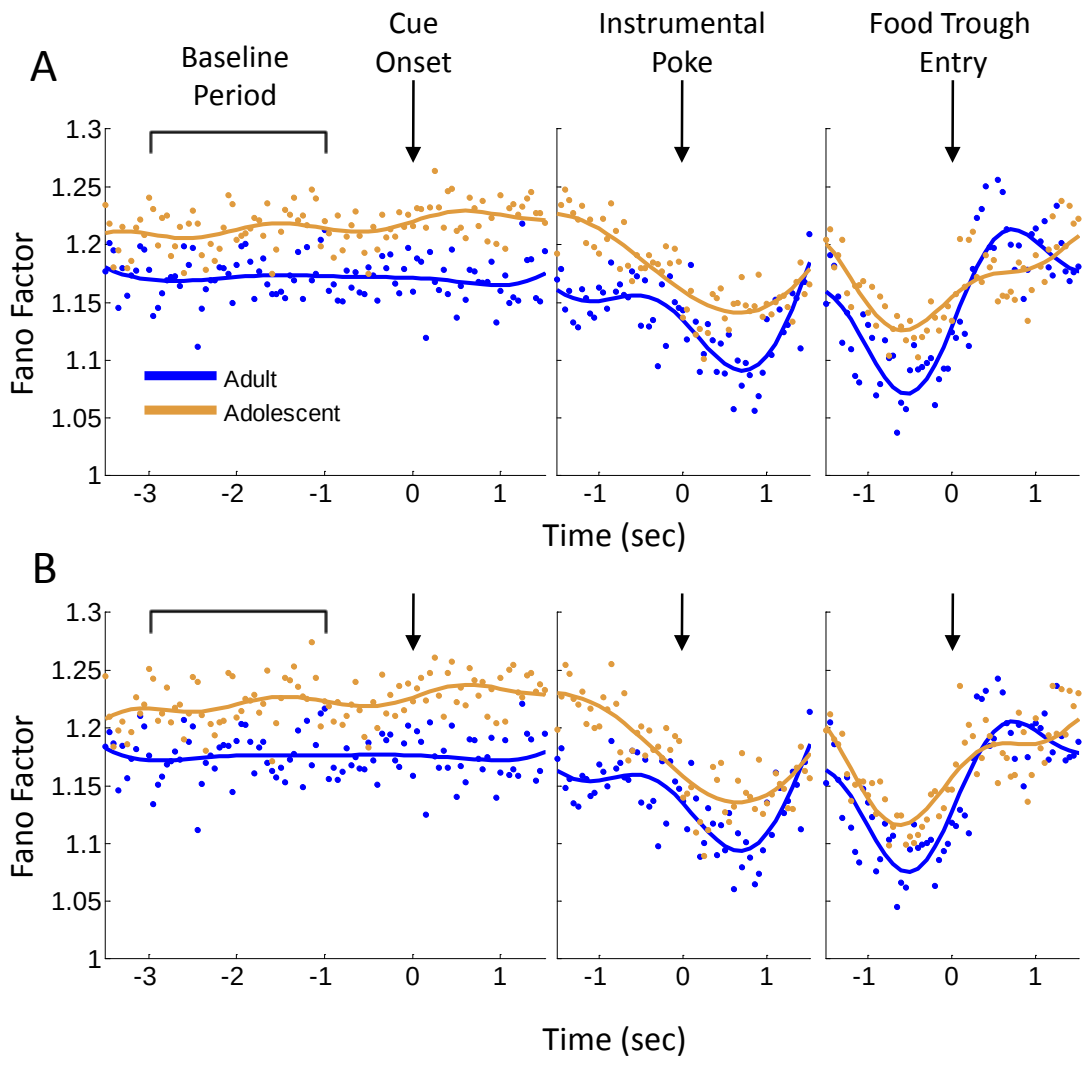


**Figure 3-5 Mean-matched Fano factor analyses**

This figure uses the same conventions as in Figure 3-3. A) Fano factors were computed as before except that a mean-matching technique was used to hold the firing-rate distributions constant over time [see Methods and Churchland et al. (2010)]. During the baseline and cue period (-3.5 to +1.5 s relative to cue-onset; left) 71% of adult units and 77% of adolescent units survived the mean-matching procedure. During the 3-s period around the instrumental poke (center) 63% of adult units and 74% of adolescent units survived. In the 3-s period around entry into the food trough (right) 63% of adult units and 72% of adolescent units survived. The mean-matching procedure did little to change the general time course of adolescent and adult Fano factors. Adolescents still exhibited significantly greater Fano factors during the baseline period ($Z = 7.09$, $p < 0.001$), in the 1 s after the cue ($Z = 5.53$, $p < 0.001$), in a 1 s window around the instrumental poke ($Z = 3.25$, $p = 0.001$), and in the 1 s before entry into the food trough ($Z = 4.60$, $p < 0.001$). However, whereas adults had greater raw Fano factors in the 1 s after reinforcement retrieval (Figure 3-3), after mean-matching this difference was no longer significant ($Z = -1.46$, $p = 0.14$). These findings indicate that the time course of raw Fano factors primarily reflects changes in fire-rate variability rather than changes in mean firing-rate, except after food-trough entry. B) Between-age group mean-matching. In this analysis the same mean-matching procedure was used as before, except that in addition to mean-matching firing-rate histograms for each time point within age groups, common firing-rate histograms were also shared across time for each event window between age groups. As there were more adolescent units ($n = 265$) than adult units ($n = 184$), smaller percentages of adolescent units survived mean-matching. During the baseline-cue window (left) 65% of adult and 45% of adolescent units survived mean matching. In the window around the

instrumental poke (center) 61% of adult and 42% of adolescent units survived. In the window around entry into the food trough 59% of adult and 41% of adolescent units survived mean-matching. Age-mean-matched Fano factors were remarkably similar to both raw and within-group mean-matched Fano factors. Again age-related significant differences were observed in the same windows computed earlier corresponding to the baseline period ($Z = 7.33$, $p < 0.001$), post-cue ($Z = 5.53$, $p < 0.001$), around the instrumental poke ($Z = 3.12$, $p = 0.002$) and before entry into the food trough ($Z = 4.15$, $p < 0.001$). As seen in A, there was no significant age difference in Fano factor after entry into the food trough ($Z = -0.73$, $p = 0.47$). These findings demonstrate that the tendency for adolescents to have higher raw Fano factors (Figure 3-3) truly reflect greater firing-rate variability across trials in these younger rats.

### 3.4.4 Unit Activity

Analysis of single-unit neural activity during the task revealed substantial event-specific differences between adolescents and adults. During session 1, before learning the action-outcome associations, unit activity changed little to task events in either group. Once the task was well-learned (training sessions 3-6), however, task events elicited consistent patterns of neural activity (Figure 3-6). The baseline-normalized firing rates of each unit time-locked to task events are shown in Figure 3-7A, illustrating the range and extent of phasic neural activity. In adults (4 rats, 184 units), but not adolescents (8 rats, 265 units), average activity was reduced at the cue and preceding the instrumental response (Figure 3-7B). After the response, the normalized population activity of both groups similarly dropped, with adolescents rebounding more than adults. Around the time of reinforcement population activity increased, with adults peaking earlier and at a lower level than adolescents. Maximal adolescent activity was reached at the time of food trough entry; at which point average adult activity was far lower. Comparisons of the proportion of excitatory and inhibitory phasic activity to task events (Figure 3-7C) generally revealed reduced inhibitory responses and similar or enhanced excitatory responses in adolescents. In the 1 s following the cue, adults had a significantly larger proportion of inhibited units than adolescents with comparable proportions of activated units (Table 3-1). After the

instrumental response, when adolescents and adults had similar reductions in population activity, similar proportions of activated and inhibited units were observed. A moving-window categorization analysis, used to visualize the time course of neural recruitment, demonstrated that around the instrumental response, adult inhibited units became inhibited earlier and were sustained longer than in adolescents (Figure 3-7C). This is confirmed by examining proportions of inhibited units in time windows 0.5 s before and 1-1.5 s after the instrumental response (Table 3-1). Although adult activated units also appear to be recruited before those of adolescents, these differences were not statistically significant. The proportions of units categorized as activated and inhibited differed substantially around reinforcement, with adults having greater proportions of inhibited units and adolescents having greater proportions of activated units. By 0.5-1 s after reinforcement, there were no age-related differences in unit categorization. These findings demonstrate that although similar proportions of adolescent and adult units can become activated or inhibited at different times (e.g., instrumental poke), through much of the task adolescents had smaller proportions of inhibited units.

Although too few in number to draw a strong conclusion, adolescent ($n$ = 8 units) and adult ($n$ = 5 units) putative fast spiking (FS) interneurons exhibited a similar general pattern of activity around events of interest as the general population of units during sessions 3 – 6 (Figure 3-8).

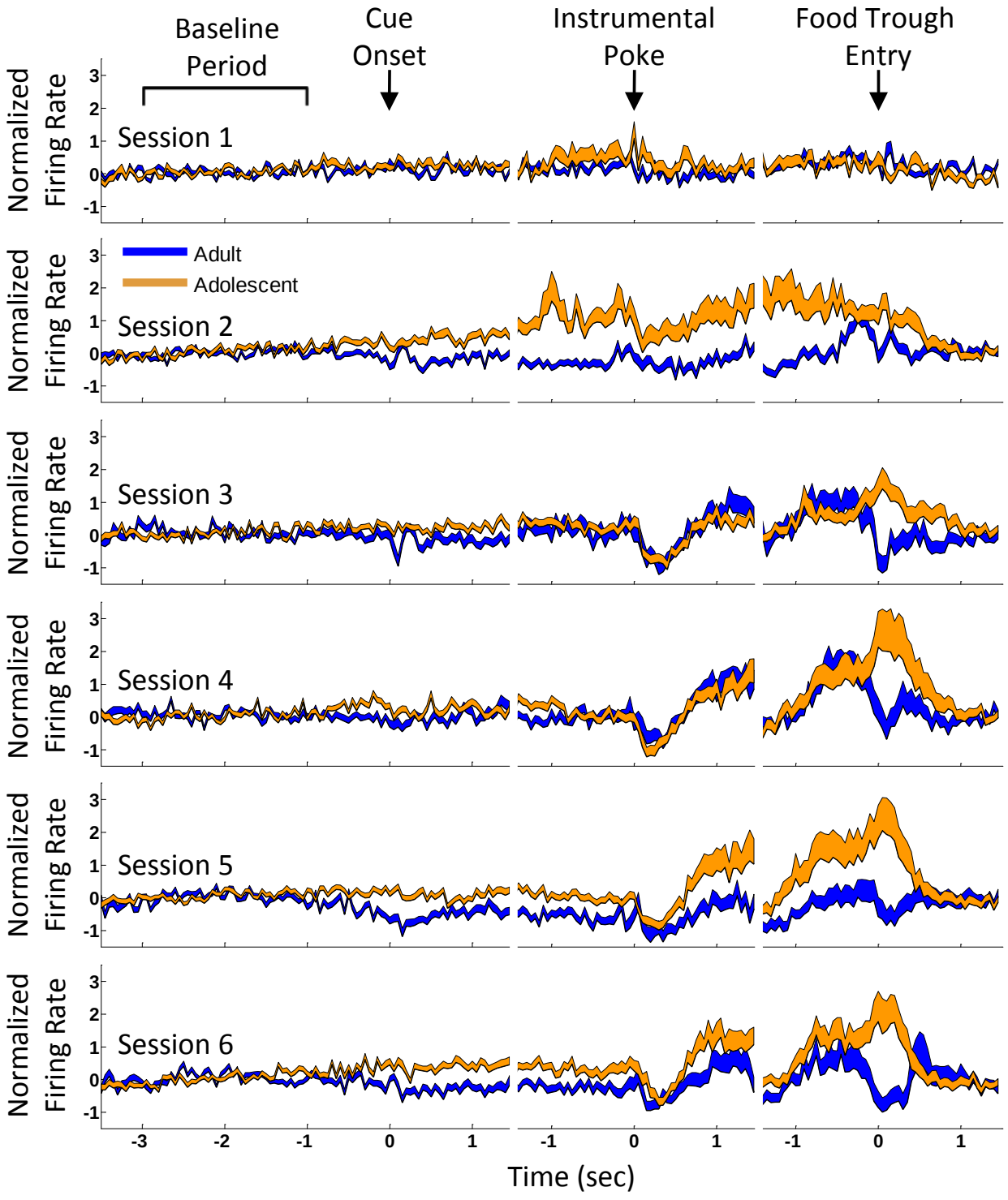


**Figure 3-6 Session-by-session firing-rate activity in the orbitofrontal cortex**
Average baseline-normalized firing-rate + 1 *SEM* (shading) for all adult and adolescent units, time-locked to task events during each of six sessions. The median task-wide firing rate for all adolescent units was 4.66 Hz and all adult units was 5.18 Hz. Although slight, their corresponding firing-rate distributions were significantly different (rank-sum test, $Z = 2.18$, $p = 0.03$). This figure demonstrates that in session 1 (adult $n = 47$, adolescent $n = 60$; top

row), when the action-outcome association was not yet learned (Figure 3-1C) there was little task-related activity to the cue (left) instrumental pokes (center) or rewarded food-trough entries (right) in either group. By session 2 (adult $n$ = 59, adolescent $n$ = 60; second row), as rats learned at different rates, and performed the task to varying extents, average OFC neural activity began to change around task events in both groups. From session 3 onward (session 3: adult $n$ = 49, adolescent $n$ = 64; session 4: adult $n$ = 46, adolescent $n$ = 67; session 5: adult $n$ = 41, adolescent $n$ = 72; session 6: adult $n$ = 48, adolescent $n$ = 62; third to sixth rows), average normalized neural activity settled into somewhat stable patterns in both age groups.

**Table 3-1 Comparison of adolescent and adult orbitofrontal cortex unit activity in selected time windows**
Windows of interest are time-locked to the cue, instrumental poke (Poke) or entry into the food trough (FT). The proportion of adolescent (Adol) and adult units that met criteria for significant activation or inhibition (see Methods) are indicated out of the total number of units along with the categorized percentages in parentheses. In each case, significant ($p < .05$) $X^2$ tests (that include number of activated, inhibited and non-significant) units were followed up by direct age-related comparisons with $Z$ tests of two proportions. Significant age-related proportional difference are indicated with bold type.

| Task Event (Window) | Activated Units | Inhibited Units |
|---|---|---|
| Cue (0 to 1 s) | Adult: 14/184 (7.6%)<br>Adol: 11/265 (6.4%) | **Adult: 23/184 (12.5%)**<br>**Adol: 7/265 (2.6%)** |
| Poke (-0.5 to 0 s) | Adult: 21/184 (11.4%)<br>Adol: 19/265 (7.2%) | **Adult: 34/184 (18.5%)**<br>**Adol: 21/265 (7.9%)** |
| Poke (0 to 0.5 s) | Adult: 25/184 (13.6%)<br>Adol: 32/265 (12.1%) | Adult: 46/184 (25.0%)<br>Adol: 56/265 (21.1%) |
| Poke (1 to 1.5 s) | Adult: 28/184 (15.2%)<br>Adol: 51/265 (19.3%) | **Adult: 46/184 (25.0%)**<br>**Adol: 28/265 (10.6%)** |
| FT Entry (-0.5 to 0 s) | **Adult: 35/184 (19.0%)**<br>**Adol: 80/265 (30.2%)** | **Adult: 53/184 (28.8%)**<br>**Adol: 42/265 (15.9%)** |
| FT Entry (0 to 0.5 s) | **Adult: 34/184 (18.5%)**<br>**Adol: 79/265 (29.8%)** | **Adult: 59/184 (32.1%)**<br>**Adol: 51/265 (19.3%)** |
| FT Entry (0.5 to 1 s) | Adult: 31/184 (16.9%)<br>Adol: 38/265 (14.3%) | Adult: 43/184 (23.4%)<br>Adol: 46/265 (17.4%) |

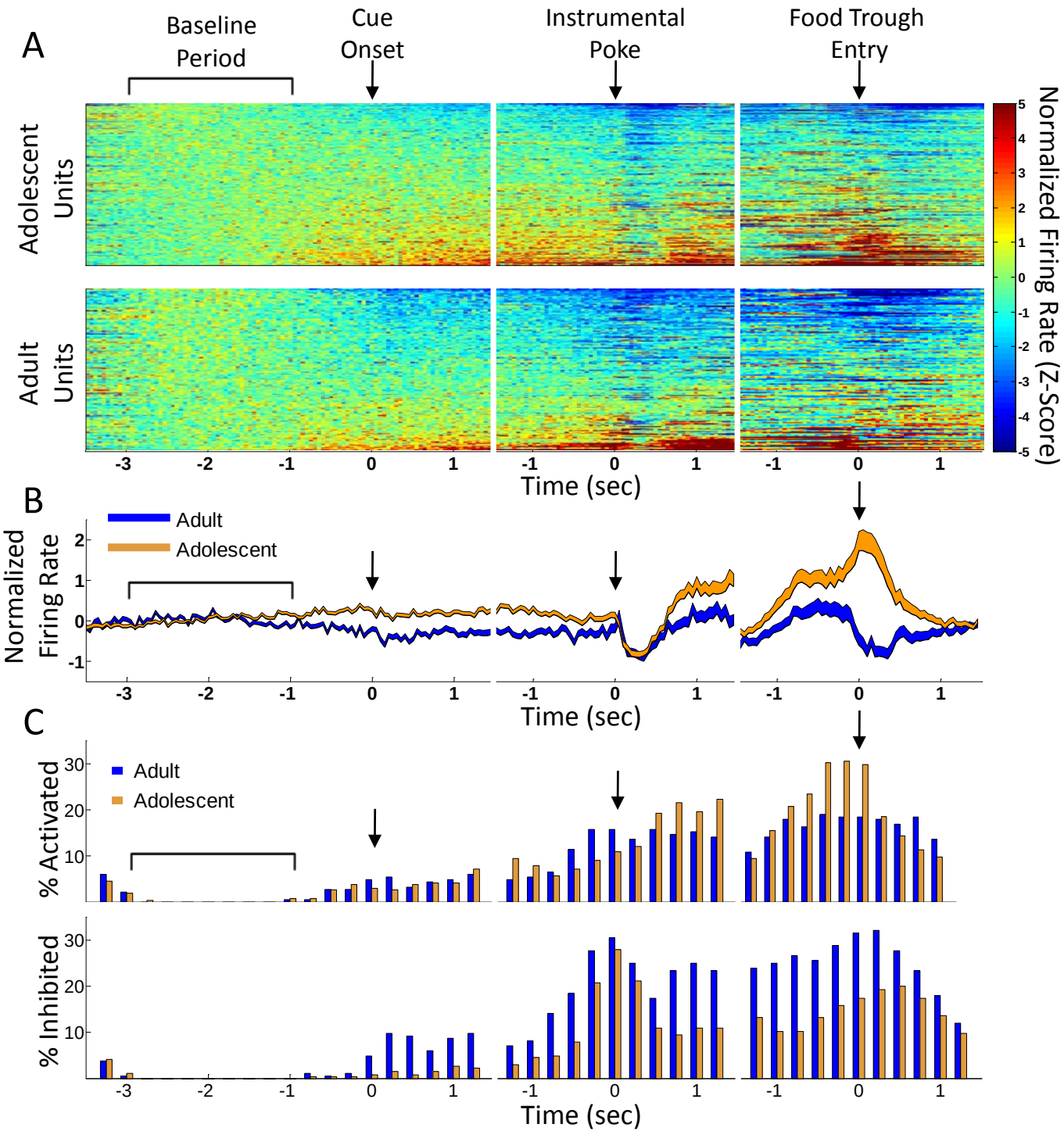


**Figure 3-7 Phasic orbitofrontal cortex population and single-unit activity**
A) Heat plots represent the baseline-normalized firing rate for each adolescent (*n* = 265; top plots) and adult (*n* = 184; bottom plots) unit during Sessions 3-6. Each row is the activity of an individual unit in 50 ms time bins aligned to corresponding events of interest, and sorted from lowest to highest average normalized firing-rate. Arrows indicate the timing of task events. B) Average normalized firing rate (across all units) + 1 *SEM* (shading) for adults (blue) and adolescents (orange) during task events. The general population activity is lower for adults than adolescents leading up to, during, and after cue onset. This continues until animals perform the instrumental

response (middle). At that time average unit activity is strikingly similar for adolescents and adults. After the instrumental response, as rats approach the food trough adolescent average unit activity rises to a greater extent than adults, and peaks at the time of food trough entry (right). In contrast, average adult unit activity peaks prior to entry into the food trough. C) Time course of unit activation and inhibition. The percentage of units that are categorized as activated (top) or inhibited (bottom) are identified over time using a moving-window analysis (window-size, 500 ms in 250 ms steps), and locked to task events. Adolescents had a similar percent of activated units to the cue, but significantly fewer inhibited units than adults (left) (see Table 3-1). During the instrumental poke (middle), adolescents and adults had similar profiles of activated and inhibited units, although adult units responded with inhibition earlier and in a more sustained manner. Around the time of reinforcement (right), adolescents ultimately had higher percentages of activated units whereas adults consistently had higher percentages of inhibited units.

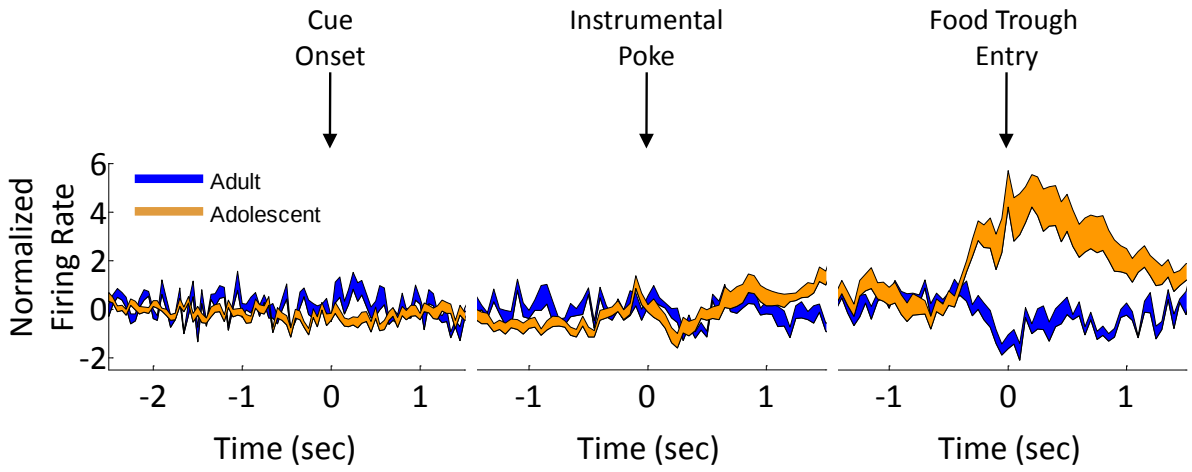


**Figure 3-8 Average population activity of putative fast-spiking interneurons**
These units were categorized as putative fast-spiking interneurons based on narrow peak-to-trough time (< 0.5 ms) and a session-wide average firing-rate > 10 Hz. A strong interpretation of their activity should be avoided because of the very small number of units. However, it appears as though their general pattern of activity during salient events was somewhat similar to that of the overall population of units recorded during this task.

## 3.5 DISCUSSION

At both population and single-unit levels, the adolescent OFC processed reward-motivated behavior differently than that of adults, with the most prominent distinction being less pronounced adolescent reductions in neural activity during reward and other salient events. Adolescents also exhibited greater cross-trial spike-timing variability throughout much of the

task. During reinforcement, in addition to less-reduced activity, there was a larger proportion of adolescent units that increased their activity, as well as differences in alpha, beta and gamma LFP power compared to adults. It is important that these age-related neural processing differences were observed although task performance was similar, which indicates that such differences do not simply reflect a behavioral confound (Schlaggar et al., 2002; Yurgelun-Todd, 2007). Even if adding additional subjects were to reveal behavioral differences during early training, both adolescents and adults performed the task at a maximal level from the third session onward. Our electrophysiology analyses focused on these later sessions, when the action-outcome association was well learned by both groups. We chose a behavioral task that, although simple enough to be learned in the short timeframe of rat adolescence, could be considered a basic building-block of more complex motivated behavior. Thus, these findings indicate that even as adolescents perform the same motivated behavior as adults, their neural encoding of salient events and apparent processing efficiency (as it relates to spike-timing variability) fundamentally differ.

Adolescent neurons tended to have less reduced activity than adults during important behavioral events such as the trial-onset cue, before the instrumental response, and before and during reward. Such age-related differences could be due to less OFC neuronal inhibition at these times. Neuronal inhibition plays a critical role in synchronizing oscillatory activity (Fries et al., 2007; Cardin et al., 2009; Sohal et al., 2009), controlling precise spike-timing, and improving the efficiency of neuronal communication (Buzsaki and Chrobak, 1995). Such oscillations, as measured with EEG and LFP, are rhythmic fluctuations in neuronal excitability, thought to reflect the interactions of intrinsic cellular and circuit properties (Buzsaki and Draguhn, 2004), which fine-tune the timing of spike output (Fries, 2005). Synchrony of oscillations may provide a

conduit for the communication of neuronal groups (Fries, 2005), and may be central to perceptual binding and other processes (Uhlhaas et al., 2009c). Measures of neuronal synchrony in specific frequency bands correlate with cognitive performance in numerous contexts (Basar et al., 2000; Hutcheon and Yarom, 2000) and are reduced in several pathological states, such as schizophrenia (Uhlhaas and Singer, 2010). Uhlhaas and colleagues found differences in task-related EEG oscillations between human adolescents and adults (Uhlhaas et al., 2009b). Consistent with these findings, we found smaller increases in alpha and beta power in the OFC of adolescents during reinforcement. These frequency bands are thought to be important for neural communication over longer distances (Pfurtscheller et al., 2000; Brovelli et al., 2004; Klimesch et al., 2007), which could be less efficient in adolescents. This interpretation is consistent with studies showing that functional connectivity changes from being more local to more distributed through development (Fair et al., 2009; Hwang et al., 2010; Somerville and Casey, 2010).

We also observed age-related differences in firing-rate variability across trials, assessed using a Fano factor analysis. Recent work has demonstrated that, in many cortical regions, neuron spiking activity is stabilized by stimuli or instrumental behavior, as reflected in reduced Fano factors (Churchland et al., 2010). Indeed we observed that in the OFC, instrumental behavior, reward approach/anticipation, and reinforcement (in adults) led to reductions in our measure of firing-rate variability. The largest reductions in variability occurred as rats performed the instrumental response and in the period before reinforcement. Greater firing-rate variability would be expected if the timing of phasic neural activity was less tightly controlled, as may be the case in the OFC of adolescents. Adolescents had greater Fano factors than adults through much of the task, with the exception of the 1 s period immediately following food-trough entry.

These results indicate that adolescents tend to have greater firing-rate variability, which may suggest reduced efficiency in neural coding. That is, greater Fano factors indicate that adolescent OFC neurons encode the same salient events with more variability, from trial to trial, which could in turn mean lower signal-to-noise ratios in the corresponding rate code compared with that of adults. This is consistent with the finding that the event-related potentials of children and adolescents have lower signal-to-noise ratios than adults, which could be attributable to "intra-individual instability" of brain regions producing these signals (Segalowitz et al., 2010). Just as neural inhibition is critical for entraining oscillations, inhibitory networks provide precision timing for the spiking of principal cells (Buzsaki and Chrobak, 1995). Thus, a connection may exist between the tendency for adolescent units to exhibit less phasic inhibition to salient events, and the greater firing-rate variability of adolescent units. We must express caution, however, that such a connection is not likely direct, as the timing of the greatest Fano factor disparities was not also the timing of largest differences in phasic inhibition.

Vast neurodevelopmental changes occur during adolescence. There is a reduction in gray matter and augmentation of white matter during this period (Benes et al., 1994; Paus et al., 1999; Paus et al., 2001; Sowell et al., 2001; Sowell et al., 2002; Sowell et al., 2003; Gogtay et al., 2004). Receptors for several neuromodulators such as dopamine are expressed at higher levels in adolescents than in adults in PFC and basal ganglia (Gelbard et al., 1989; Lidow and Rakic, 1992; Teicher et al., 1995; Tarazi et al., 1999; Tarazi and Baldessarini, 2000). In anesthetized rats, the spontaneous neural activity of dopamine neurons is greater in adolescents than juveniles or adults (McCutcheon and Marinelli, 2009). In cortical slices activating effects of a dopamine D2 receptor agonist are only present by late-adolescence or early adulthood, at which time a sudden shift is observed (Tseng and O'Donnell, 2007). The expression of NMDA receptors on

fast-spiking (FS) neurons also changes dramatically in the PFC of adolescents. The majority of adolescent FS interneurons exhibit no synaptic NMDA receptor-mediated currents. Those cells that do have them exhibit a far-reduced NMDA/AMPA ratio (Wang and Gao, 2009). These studies demonstrate fundamental differences in the architecture and physiology of adolescent brain regions and transmitters associated with both normal motivated behavior and mental illnesses. The present study, which to our knowledge is the first to use extracellular electrophysiological recording in awake, behaving adolescent animals, advances the functional relevance of these cellular and molecular findings by demonstrating that task-related neural activity is fundamentally different in adolescents during the processing of salient events.

Human fMRI studies have found that adolescents process reward and reward-anticipation differently than adults at a larger-scale regional level (Ernst et al., 2005; Galvan et al., 2006; Geier et al., 2009; Van Leijenhorst et al., 2009). Current explanations for some adolescent behavioral vulnerabilities include the notion that the PFC is "underdeveloped" in terms of its activity and/or its functional connectivity with and modulation of subcortical structures (Ernst et al., 2006; Casey et al., 2008; Steinberg, 2008). The present study finds that developmental differences are observable even during very basic reward-motivated behavior, and are fundamentally manifested at the single-unit level by a reduced propensity for reduced neural activity in adolescent OFC to most, but not all, salient events. Although future work is needed to establish such a connection, differences at the single-unit level in the proportions of inhibitory responses may be the source of some of the adolescent differences observed in oscillatory power and spike-timing variability. Because of the importance of inhibition in controlling the precise timing of spikes, entraining oscillations, and thus facilitating efficient communication of neuronal groups (Buzsaki and Chrobak, 1995; Fries et al., 2007), reduced adolescent PFC

inhibition is consistent with the observation of large-scale differences in cortical processing seen in this study and others. However, the tendency for adolescents to have less-reduced unit activity around salient events may result from lower reductions in the activity of excitatory afferents as well as reduced inhibition.

Altered cortical inhibitory activity may influence behavioral inhibition (Chudasama et al., 2003; Narayanan and Laubach, 2006) and has been associated with some pathological states (Chamberlain et al., 2005; Lewis et al., 2005; Behrens and Sejnowski, 2009; Lewis, 2009). For example, individuals with schizophrenia have reduced GAD67 mRNA expression, an enzyme involved in the synthesis of the inhibitory neurotransmitter GABA (Akbarian et al., 1995). Schizophrenia patients also have reduced GABA membrane transporter (GAT-1)-immunoreactive axon cartridges in the PFC (Woo et al., 1998). This is of particular relevance to research in adolescents, as GAT-1 immunoreactive cartridges (which are also immunoreactive to parvalbumin) peak just before adolescence and then undergo a dramatic reduction through late adolescence (Cruz et al., 2003), the typical onset time for schizophrenia. Future work delineating the precise source of age-related phasic activity differences during normal development may be directly relevant to the pathophysiology and symptomatic time course of psychiatric illnesses that arise during adolescence.

Acknowledgements—This work was supported by National Institute of Mental Health, the Pittsburgh Life Sciences Greenhouse, and the Andrew Mellon Foundation for a predoctoral fellowship. Thanks to Jesse Wood and Yunbok Kim for insightful discussions, and Churchland et al. (2010) for making Matlab variability functions available.

# 4.0 ADOLESCENT VERSUS ADULT NEURAL ACTIVITY IN DORSAL STRIATUM AND NUCLEUS ACCUMBENS DURING MOTIVATED BEHAVIOR

## 4.1 ABSTRACT

Adolescence is a time of adjustment as one completes the physical and psychosocial transitions to adulthood. It is also considered a period of vulnerability as it coincides with the onset of symptoms for several major psychiatric problems. While studies in adolescent humans and animal models have described age-related shifts in neural architecture, little is known of the functional consequences of these changes at the neuronal level in the context of motivated behavior. In this study, single-unit activity and local field potentials were recorded from the nucleus accumbens (NAc) and dorsal striatum (DS) of adolescent and adult rats as they performed an instrumental behavior. While several measures of neural activity were similar in the NAc, activity was generally quite different between the two groups in the DS. In particular, during the period between the instrumental response and reward retrieval, adolescent neurons became more activated, peaking well after those of adults and providing a neural correlate of reward anticipation/approach only in the DS of these younger rats. These findings indicate fundamental adolescent processing differences in these basal ganglia nuclei, particularly in the DS, which may contribute to several behavioral differences and vulnerabilities.

## 4.2 INTRODUCTION

Adolescence is the developmental stage bridging childhood with adulthood. It is a time of numerous brain and behavioral transitions. It can also be a period of increased risk-taking, and is coincident with the symptomatic onset of mental illnesses such as mood disorders and schizophrenia (Volkmar, 1996; Pine, 2002). Human imaging studies along with rodent and primate work have helped to identify various neurodevelopmental changes. For example, during adolescence there is a reduction in gray matter and increased white matter, presumably representing pruning and myelination, respectively, especially in the frontal and temporal lobes (Sowell et al., 2003; Gogtay et al., 2004). The expression and distribution of receptors for various neurotransmitter systems changes at this time, often with an inverted-U pattern in which peak expression is seen during adolescence (Lidow and Rakic, 1992; Rodriguez de Fonseca et al., 1993; Teicher et al., 1995; Tarazi et al., 1999; Andersen et al., 2000; Tarazi and Baldessarini, 2000). Perhaps related to these changes, several studies have shown differential sensitivities to the behaviorally activating or inhibiting effects of a variety of drugs (Spear and Brake, 1983; Spear, 2000). Human neuroimaging studies have also been useful in identifying functional activity differences in adolescents, especially in brain regions involved in the production of motivated behavior and “executive” functions (Luna et al., 2010). While an emphasis is usually placed on differences, adolescents often behave remarkably like adults. Their risk tolerance does appear to be greater than that of adults, although this is generally observed under conditions of heightened emotional arousal (Figner et al., 2009). Both adolescents and adults will work for rewards, and such behavior may appear similar at times (e.g. smoking a cigarette). Nevertheless, the vast neurodevelopmental changes which occur during this period clearly seem to cause the adolescent brain to process behaviorally salient and motivational information differently from

adults, and to lead to age-specific vulnerabilities like addiction. Thus, for example, the transition from nicotine use to dependence appears more likely during adolescence than during adulthood (Khuder et al., 1999; Chambers et al., 2003).

The basal ganglia are large subcortical nuclei associated with the control of volitional action. They play a central role in association learning, habit formation, and adaptive control of behavioral patterns (Packard and Knowlton, 2002; Graybiel, 2005; Yin et al., 2008). The striatum is the main input structure of the basal ganglia, and receives vast projections from cortical regions involved in sensory, motor, and cognitive processes (Voorn et al., 2004), as well as dopaminergic input (Costa, 2007). During adolescence receptors for dopamine and endocannabinoids peak in the dorsal striatum (DS) (Gelbard et al., 1989; Teicher et al., 1995; Tarazi et al., 1999; Tarazi and Baldessarini, 2000). DS dopamine signaling is critical to association learning and behavioral flexibility, and both dopamine and endocannabinoid signaling in the DS are essential for the transition from goal-directed to automatic habitual behavior (Hilario et al., 2007; Ashby et al., 2010). Thus, these age-related transitions could suggest physiological differences in adolescent processing of salient stimuli in the production of goal-directed and habitual behavior. The nucleus accumbens (NAc), part of the ventral striatum, receives afferents from the amygdala (Kelley et al., 1982) and prefrontal cortex (PFC) (Powell and Leman, 1976), and is a major target of dopaminergic afferents from the ventral tegmental area (Moore et al., 1976). This pattern of anatomical inputs, along with its efferents to motor systems, has led some to hypothesize that the NAc is key to the translation of “motivation” to “action” (Mogenson et al., 1980). Thus, this structure is central to some current neurobehavioral hypotheses regarding adolescent risk taking and sensation seeking (Ernst et al., 2006; Casey et al., 2008; Steinberg, 2008).

We have recently demonstrated the feasibility of recording the single-unit and local field potential activity of adolescent rats as they performed an instrumental task (Sturman and Moghaddam, 2011). We observed that even during similar behavior, adolescents encode salient events—especially reward—differently from adults in the orbitofrontal cortex. In this study we record the neural activity in DS and NAc in the same behavioral context. We find that while there are modest differences in the pattern of adolescent neural activity in the NAc, more dramatic differences are observed in the DS, especially in the period leading up to reward.

## 4.3 METHODS

### 4.3.1 Subjects

Adult male (older than Postnatal Day 70, $n = 12$) and pregnant dam (Embryonic Day 16; $n = 4$) Sprague-Dawley rats (Harlan, Frederick MD) were received and housed in separate climate-controlled vivaria with 12 h light/dark cycle (lights on at 7 pm), and with *ad lib* access to chow and water. Pregnant dams had litters approximately one week after arrival. Litters were culled to no more than six male pups, which were then weaned on Postnatal Day 21 ($n = 16$). Animal procedures were approved by the University of Pittsburgh Animal Care and Use Committee.

### 4.3.2 Surgery and Electrophysiology

Adult surgeries were performed after a minimum of one week of habituation to housing. Adolescent surgeries were performed at Postnatal Day 28-30. Both purchased (stainless steel; NB Labs) and home-built (tungsten) 50 μm Teflon-coated eight-wire microelectrode arrays were implanted. Both array types had measured impedances ranging from 200-1000 kΩ. Microelectrode arrays were implanted in NAc or DS, with some animals having bilateral implants in the same region and others receiving unilateral implants in each target. Adult DS arrays were implanted 1.0 mm anterior to bregma, 2.2 mm lateral to bregma, and 4.3 mm ventral to the dura; adolescent DS arrays were implanted 0.7 mm anterior to bregma, 2.2 mm lateral to bregma, and 4.0 mm ventral to the dura. Adult NAc arrays were implanted 1.6 mm anterior to bregma, 1.4 mm lateral to bregma, and 7.0 mm ventral to the dura; adolescent NAc arrays were implanted 1.0 mm anterior to bregma, 1.2 mm lateral to bregma, and 6.5 mm ventral to the dura. Recordings were performed as previously described (Sturman and Moghaddam, 2011). Briefly, 10-pin connectors for each array were attached to a light-weight headstage cable (NB Labs) with a unity-gain junction field-effect transistor. The cable was then connected to a commutator (NB Labs) allowing rats to move freely within testing boxes without tangling. Single-unit activity was amplified at 1000× gain and analog band-pass filtered at 300 – 8000 Hz; LFPs were band-pass filtered at 0.7 – 170 Hz. Using Recorder software (Plexon), all signals were sampled at 40 kHz, although LFP data were then downsampled to 1 kHz prior to storage. Single-unit activity was digitally high-pass filtered at 300 Hz, and LFPs were low-pass filtered at 125 Hz. Behavioral testing boxes sent event markers to the same computer recording neural activity to permit the time-locking of events for our analyses. Single units were isolated using Offline Sorter (Plexon)

through a combination of manual and semi-automatic sorting techniques, as described previously (Homayoun and Moghaddam, 2008).

### 4.3.3 Behavior

Behavioral testing procedures were conducted as described previously (Sturman et al., 2010; Sturman and Moghaddam, 2011). Briefly, testing was done using an operant box apparatus (Coulbourn Instruments) that contained three nose-poke holes on one wall and a food trough connected to a pellet magazine, along with a house light, on the opposite wall. After 5 days of surgical recovery, rats were mildly food restricted and began habituation to the testing apparatus before the first of six training sessions. During each session rats learned to poke for individual food pellets (fortified dextrose, 45 mg; Bio-serv). Each trial started with the onset of a cue light in the center nose-poke manipulandum. If the rat poked into that hole, immediately that light would turn off and a pellet would be delivered to the now-illuminated food trough. Retrieval of the pellet triggered a 5 s inter-trial interval (Figure 4-1). The timing of behavior was recorded based on infrared beam breaks in the nose-poke hole or food trough. Rats could perform a maximum of 100 trials per session, which would otherwise terminate after 30 min. A rat's behavioral performance was assessed by examining for each session the total number of trials, the average latency from trial-onset cue to the instrumental response, and the latency from the instrumental response to pellet retrieval. Age × Session repeated-measures analyses of variance (ANOVAs) were performed using SPSS software on all of these measures ($\alpha = 0.05$), with lower-bound degrees-of-freedom corrections where the assumption of sphericity was violated.

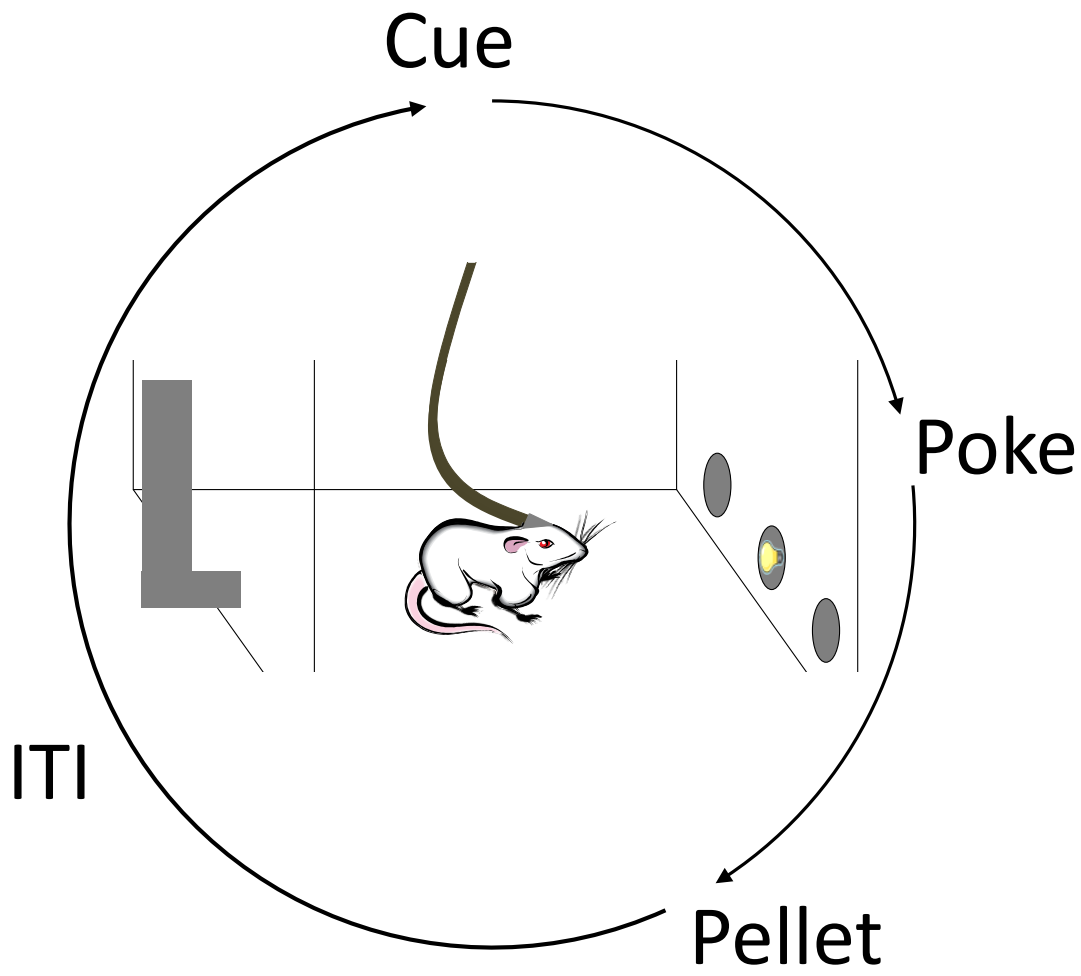


**Figure 4-1 Behavioral task**
Rats performed an instrumental behavior inside a standard operant chamber. Each trial began with the onset of a light cue within the center nose-poke hole (Cue). If the rat poked into that hole while the light was on (Poke) the light turned off and a food pellet was delivered to a food trough on the opposite wall. Once the rat poked into the food trough to retrieve the pellet (Pellet) a 5 sec inter-trial interval (ITI) was triggered, followed by the next trial. Rats could perform a maximum of 100 trials within each 30 min session.

### 4.3.4 Histology

Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and a 200 μA current was passed through recording electrode wires for 5 s to mark tip placements. Perfusions were performed with 0.9% saline and then 10% buffered formalin, in which brains were also stored

after the perfusions. Coronal sections were made for each brain around the region of interest, stained with cresyl violet, and mounted on microscope slides. Analyses were only performed on data from rats with confirmed electrode placements in DS and/or NAc (Figure 4-2).

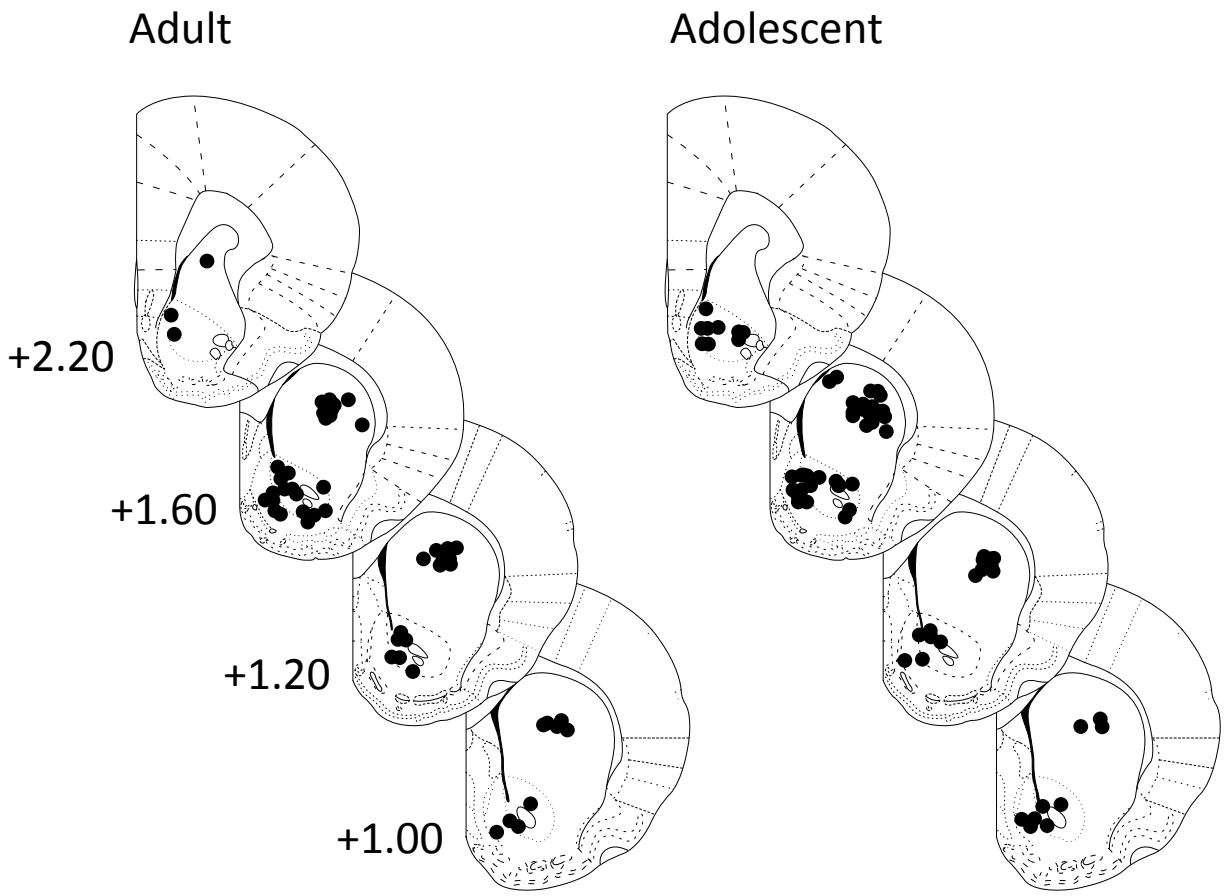


**Figure 4-2 Electrode placements**
The locations of electrode tips within dorsal striatum and nucleus accumbens are indicated for arrays included in the electrophysiological analyses overlaid standard atlas images (Paxinos and Watson, 1998). Numbers represent distance (in millimeters) from bregma in adult rats.

### 4.3.5 Electrophysiology Analysis

Electrophysiological data were analyzed using custom-written Matlab (MathWorks) scripts along with functions from the Chronux toolbox (http://chronux.org/). Except where stated otherwise, all electrophysiological analyses were performed on those sessions in which behavior

was maximal for both adolescents and adults (sessions 4-6; Figure 4-3). Neural activity was time-locked to signals sent from operant boxes with the start of three task events: cue onset, instrumental poke, and food trough entry. After removing trials in which the raw LFP voltage trace contained either clipping artifacts or outliers (± 3 SD from the mean voltage), trial-averaged power spectra were computed for each subject using fast Fourier transform. This was done using 5 leading tapers, a time-bandwidth product of 3, and a 500 ms moving window (in 100 ms steps). Power values at each frequency were *Z*-score normalized to the baseline period (a 2 s window beginning 3 s prior to cue onset). Normalized power spectra were then averaged for each group.

Single-unit analyses were based on peri-event time firing-rate histograms in windows around task events. As in the LFP analysis, single-unit activity was *Z*-score normalized based on the mean and standard deviation firing rates of each unit during the baseline period. Units were categorized as "activated" or "inhibited" within a window of interest if they contained three consecutive 50 ms bins with $Z \geq 2$ or $Z \leq -2$, respectively. We validated that these criteria gave low false-categorization rates by performing a nonparametric bootstrap analysis on the baseline period of each unit as described previously (Sturman and Moghaddam, 2011). Briefly, each unit's baseline period was randomly sampled with replacement 10,000 times. The false positive rate ($\alpha$) for a 2 s window is the number of instances that category criteria were reached divided by 10,000 (In the NAc, $\alpha$ = 0.0036 for adolescents and $\alpha$ = 0.0051 for adults; in the DS, $\alpha$ = 0.0040 for adolescents and $\alpha$ = 0.0038 for adults). Once units were categorized, $X^2$ analyses were performed on *a priori* windows of interest for all activated, inhibited, and non-significant units. Only significant $X^2$ tests were followed by *post hoc Z*-tests for two proportions to determine the underlying significant category differences. To visualize the time course of unit recruitment (i.e.

as “activated” or “inhibited”), category analyses were performed in 500 ms moving windows (in 250 ms steps) in larger windows time-locked to task events. The null hypothesis was rejected when $p < 0.05$.

## 4.4 RESULTS

### 4.4.1 Behavior

Both adolescent ($n = 16$) and adult ($n = 12$) rats learned the action-outcome association. As previously reported (Sturman and Moghaddam, 2011), no statistically significant age-related differences were observed across training in the number of trials per session $F(1,1) = 1.74$, $p = 0.20$; the latency from the cue to the instrumental poke $F(1,1) = 0.875$, $p = 0.36$; or the latency from the instrumental poke to the entry into the food trough $F(1,1) = 0.82$, $p = 0.36$ (Figure 4-3). The latency from cue onset to center poke did appear somewhat different in the early sessions, although this was not statistically significant and was clearly driven by three outlier animals that had not learned the association (Figure 4-3B inset). Unless otherwise stated, all electrophysiological analyses were performed on sessions 4-6, as by this point all animals clearly demonstrated a high level of performance, indicating well-learned action-outcome associations. During these sessions the average adult and adolescent latency from the instrumental response to entry into the food trough were ($M \pm SEM$) 2.47 ± 0.12 sec and 2.54 ± 0.17 sec, respectively.

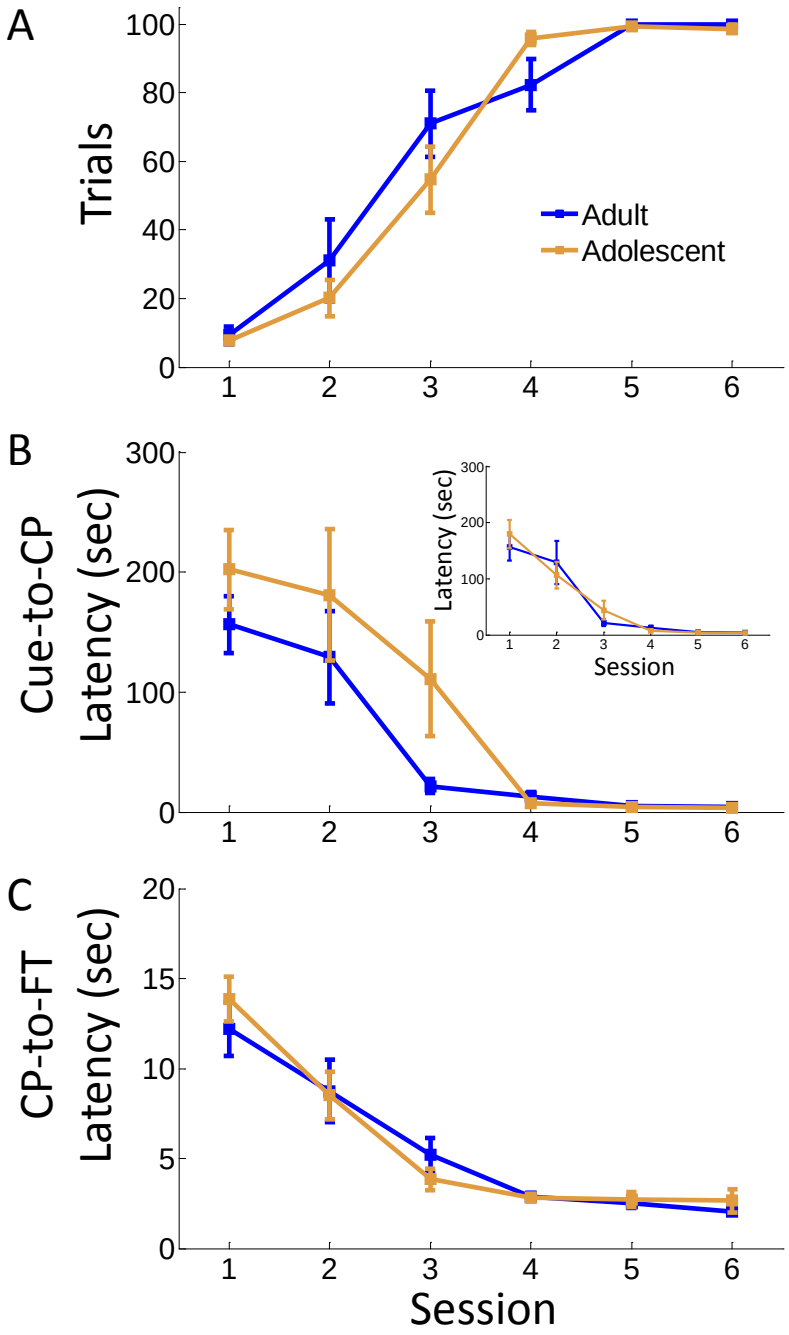


**Figure 4-3 Behavioral performance**
Similar performance between adolescents ($n$ = 16) and adults ($n$ = 12) in the total trials performed (A), the latency from cue-onset to center poke (CP; i.e., the instrumental response) (B), or the center poke to entry into the food trough (FT) (C). No Age × Session differences were detected, and the seemingly higher average cue-to-center poke latency during early sessions for adolescents was driven by a few adolescent outliers. With those removed, latencies looked even more similar (B; inset). Because rats in both groups clearly learned the association and performed the task at very high levels by session 4, electrophysiological analyses incorporate data from sessions 4-6.

### 4.4.2 Nucleus Accumbens

#### 4.4.2.1 Local Field Potentials

Average normalized LFP spectrograms were remarkably similar for adolescents and adult (Figure 4-4). Oscillatory power transiently decreased in both groups at the instrumental poke in beta (13-30 Hz) and gamma (>31 Hz) bands. Adolescents had increased oscillatory power before and after the instrumental poke in the alpha band (8-12 Hz), with adults exhibiting a hint of this as well, but to a lesser degree (Figure 4-4A). The most striking age-related differences in LFP were around reward (Figure 4-4B), with adolescents having more alpha power 1 s prior to food trough entry and also greater theta (4-7 Hz), alpha, and beta power, especially ~500 ms after reaching the food trough—coincident with reward consumption. Both groups had increased gamma oscillations (in the 50-60 Hz range) at this time.

#### 4.4.2.2 Unit Activity

During the course of training the single-unit activity of both adolescents and adults went from little or variable task-related activity to consistent patterns that were quite similar between the two age groups. At the population level, by session 4 (when all rats were performing the task at a high level) both adolescents and adults exhibited some increase and then decrease in phasic activity at the instrumental poke. This pattern was even more pronounced leading up to and following reward (food trough entry) (Figure 4-5).

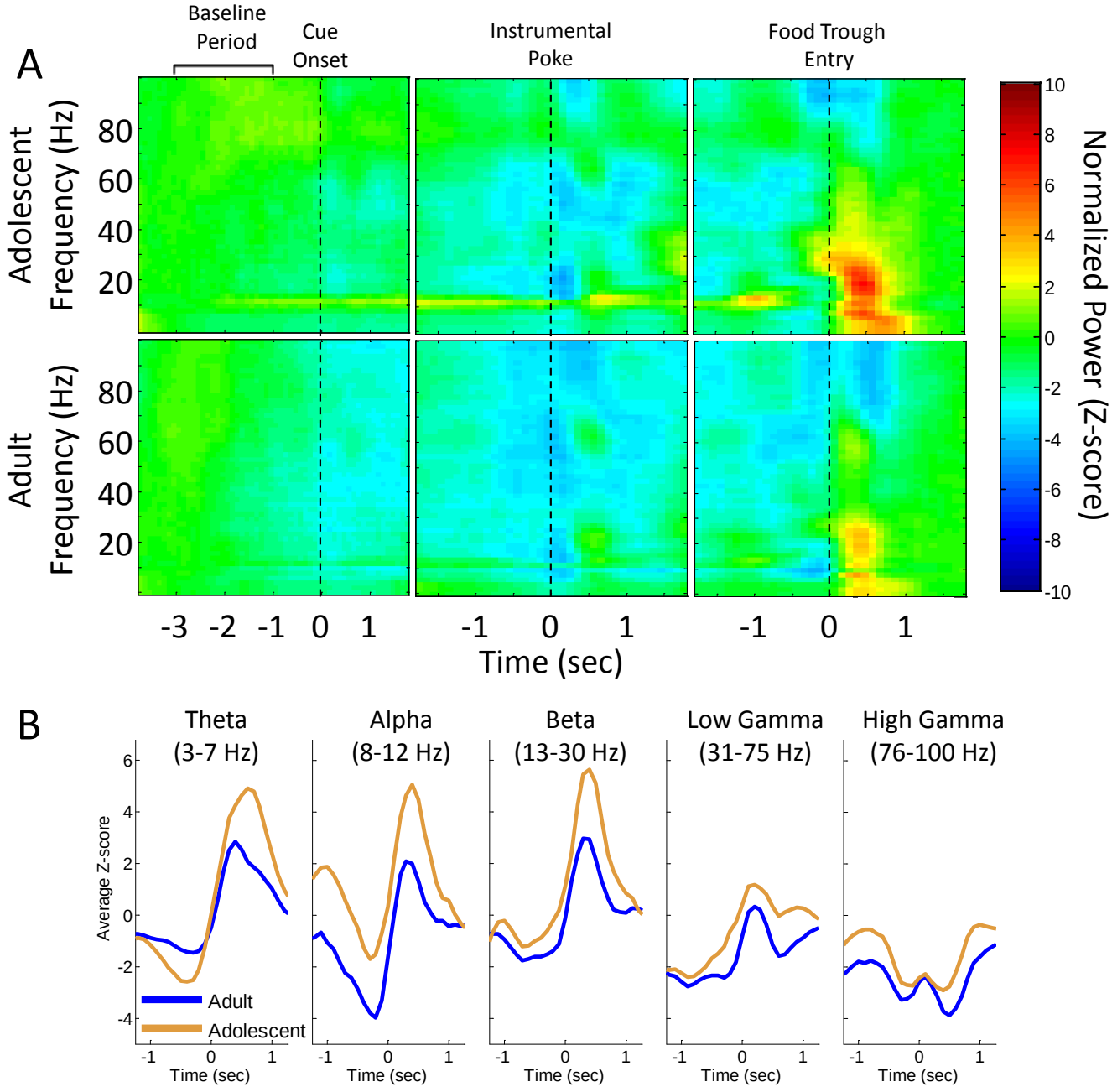


**Figure 4-4 Local field potentials in the nucleus accumbens**

The patterns of LFP oscillations were similar between adolescents and adults. A) In both groups trial-onset was associated with reductions in broad-range oscillatory power, with particularly low power in beta and gamma oscillations at the instrumental poke. The largest differences were seen around food trough entry, with larger increases in adolescent alpha 1 s prior to food trough entry. During reward retrieval (in the 1 s after entry into the food trough), adolescents had larger increases in theta, alpha, and beta power, although with similar time courses as adults (B).

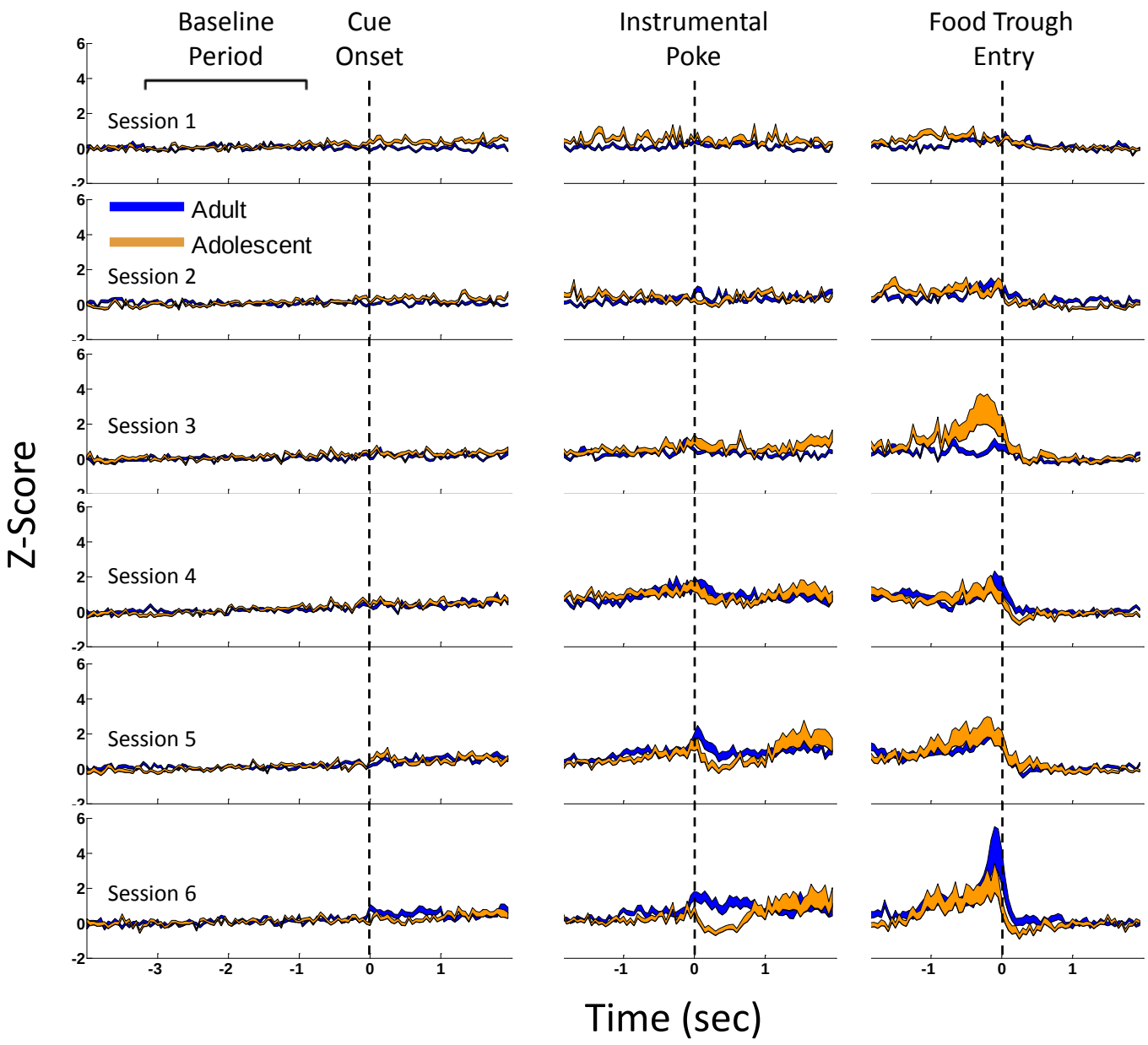


**Figure 4-5 Session-by-session firing-rate activity in the nucleus accumbens**
The average baseline-normalized adolescent and adult neural activity during each session +1 *SEM* (shading) are represented. In the early sessions, prior to learning the action-outcome association, task-related changes in neural activity were not apparent. By the time all subjects performed the task at high levels (session 4), consistent, although subtle, task-related changes were observed, which appeared similar for adolescents and adults. Session 1: Adolescent $n$ = 73, Adult $n$ = 79; Session 2: Adolescent $n$ = 66, Adult $n$ = 79; Session 3: Adolescent $n$ = 56, Adult $n$ = 77; Session 4: Adolescent $n$ = 62, Adult $n$ = 65; Session 5: Adolescent $n$ = 56, Adult $n$ = 70; Session 6: Adolescent $n$ = 47, Adult $n$ = 49.

A closer examination of the phasic neural activity reveals several close similarities in the pattern and extent of neuronal activation and inhibition, along with some notable differences (Figure 4-6). Specifically, the onset of the cue light led to the activation of about 10% of NAc neurons in both adolescents and adults, with few neurons becoming inhibited, and no statistically significant age-related difference in the proportion of activated or inhibited neurons at this time. Once neurons activated for a trial they tended to remain activated until the animal's entry into the food trough. The temporal dynamics were such that some proportion of neurons became more strongly activated around both the instrumental poke and food trough entry. Additionally, adults activated more neurons briefly after the instrumental poke and leading up to entry into the food trough. These differences were modest but statistically significant (Table 4-1). The time course of neurons becoming inhibited was virtually identical between adolescents and adults around the instrumental poke, with an increase in inhibitory responses immediately after it. There were no age-related differences in the proportions of inhibited neurons (Table 4-1), although the magnitude of neural inhibition was greater in some adolescent units. In contrast, after entry to the food trough, while both adolescent and adult neural inhibition peaked, there was more adolescent inhibition, both in proportion of units and in the extent of inhibition (Figure 4-6; Table 4-1). After entry into the food trough, both adolescent and adult neuronal activity rapidly returned to baseline. Thus, while there were some modest differences between the groups, the general pattern of neural responses (and average normalized firing-rate activity across units) was remarkably similar in the NAc.

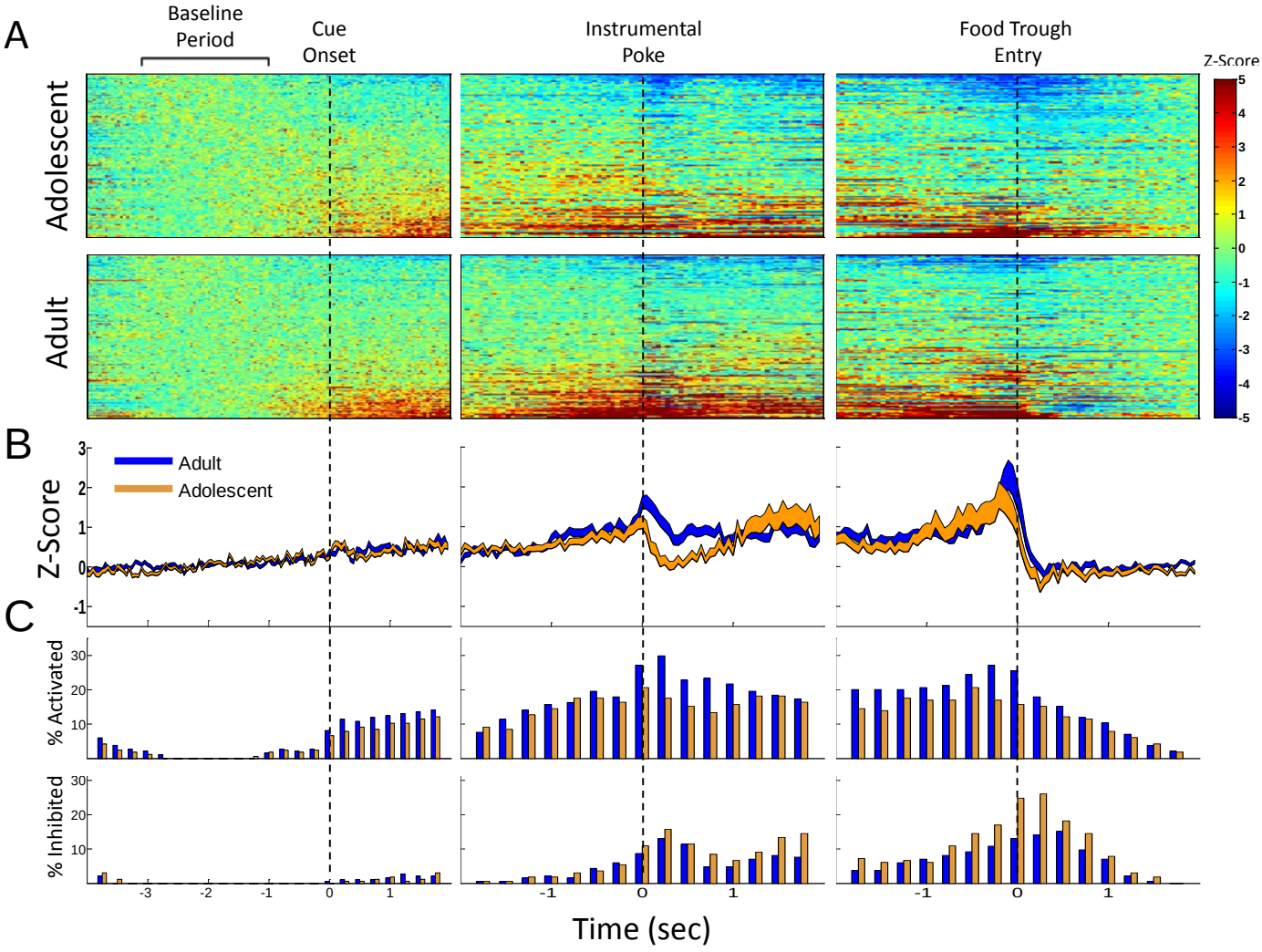


**Figure 4-6 Population and single unit activity in the nucleus accumbens**
A) Heatplots represent the adolescent (*n* = 165; top) and adult (*n* = 184; bottom) normalized firing-rate activity of each neuron (row) of Sessions 4-6, time-locked to task events, and arranged from lowest to highest magnitude. B) Average normalized firing-rate activity across all adolescent and adult neurons during these sessions. C) The percentage of activated (top) and inhibited (bottom) neurons based on category criteria (see Methods) within 500 ms moving windows of 250 ms steps. Neurons in both groups became activated to the cue and activation continued through the instrumental response until food trough entry. Adults exhibited a ramping up in the proportion of activated units at the instrumental poke and just prior to food trough entry, and these increases were not as strong in adolescents. The time course of neuronal inhibition was similar between age groups, although adolescents had larger proportions of inhibited neurons after entry into the food trough. In spite of these modest magnitude differences, the general pattern and time course of activation and inhibition was strikingly similar between adolescents and adults.

**Table 4-1 Comparisons of adolescent and adult nucleus accumbens and dorsal striatum unit activity in selected time windows**

The number and percentage of adult and adolescent (Adol) activated and inhibited units are given for specific windows of interest. Significant age-related proportional differences are indicated with bold type.

| Task Event (Window) | Nucleus Accumbens Activated Units | Nucleus Accumbens Inhibited Units | Dorsal Striatum Activated Units | Dorsal Striatum Inhibited Units |
|---|---|---|---|---|
| Cue (0 to 0.5 s) | Adult: 21 (11.4%)<br>Adol: 13 (7.9%) | Adult: 2 (1.1%)<br>Adol: 1 (0.6%) | Adult: 22 (8.9%)<br>Adol: 30 (9.3%) | Adult: 11 (4.4%)<br>Adol: 7 (2.2%) |
| Poke (-0.5 to 0 s) | Adult: 33 (17.9%)<br>Adol: 27 (16.4%) | Adult: 11 (6.0%)<br>Adol: 9 (5.5%) | Adult: 60 (24.1%)<br>Adol: 80 (24.8%) | **Adult: 38 (15.3%)**<br>**Adol: 23 (7.1%)** |
| Poke (0 to 0.5 s) | **Adult: 55 (28.8%)**<br>**Adol: 29 (17.5%)** | Adult: 24 (12.6%)<br>Adol: 26 (15.7%) | **Adult: 63 (24.8%)**<br>**Adol: 49 (15.1%)** | Adult: 61 (24.0%)<br>Adol: 76 (23.5%) |
| FT Entry (-0.5 to 0 s) | **Adult: 50 (27.2%)**<br>**Adol: 28 (17.0%)** | Adult: 20 (10.9%)<br>Adol: 28 (17.0%) | **Adult: 50 (19.9%)**<br>**Adol: 141 (43.1%)** | **Adult: 83 (33.1%)**<br>**Adol: 54 (16.5%)** |
| FT Entry (0 to 0.5 s) | Adult: 33 (17.8%)<br>Adol: 25 (15.2%) | **Adult: 26 (14.1%)**<br>**Adol: 43 (26.1%)** | **Adult: 28 (11.2%)**<br>**Adol: 87 (26.7%)** | **Adult: 89 (35.6%)**<br>**Adol: 63 (19.3%)** |

### 4.4.3 Dorsal Striatum

#### 4.4.3.1 Local Field Potentials

In contrast to the NAc, LFP activity in the DS was considerably different between adolescents and adults. While adolescents exhibited increased oscillatory power across a wide range of frequencies, especially prior to the instrumental poke, adults primarily decreased oscillatory power during this period (Figure 4-7). Immediately after the instrumental response adolescents showed strong increases in theta and alpha power, while much smaller increases were observed in adults. After entry into the food trough, adolescents had higher theta power, while adults had greater beta power. Alpha activity was virtually identical between the two groups during this period (Figure 4-7B).

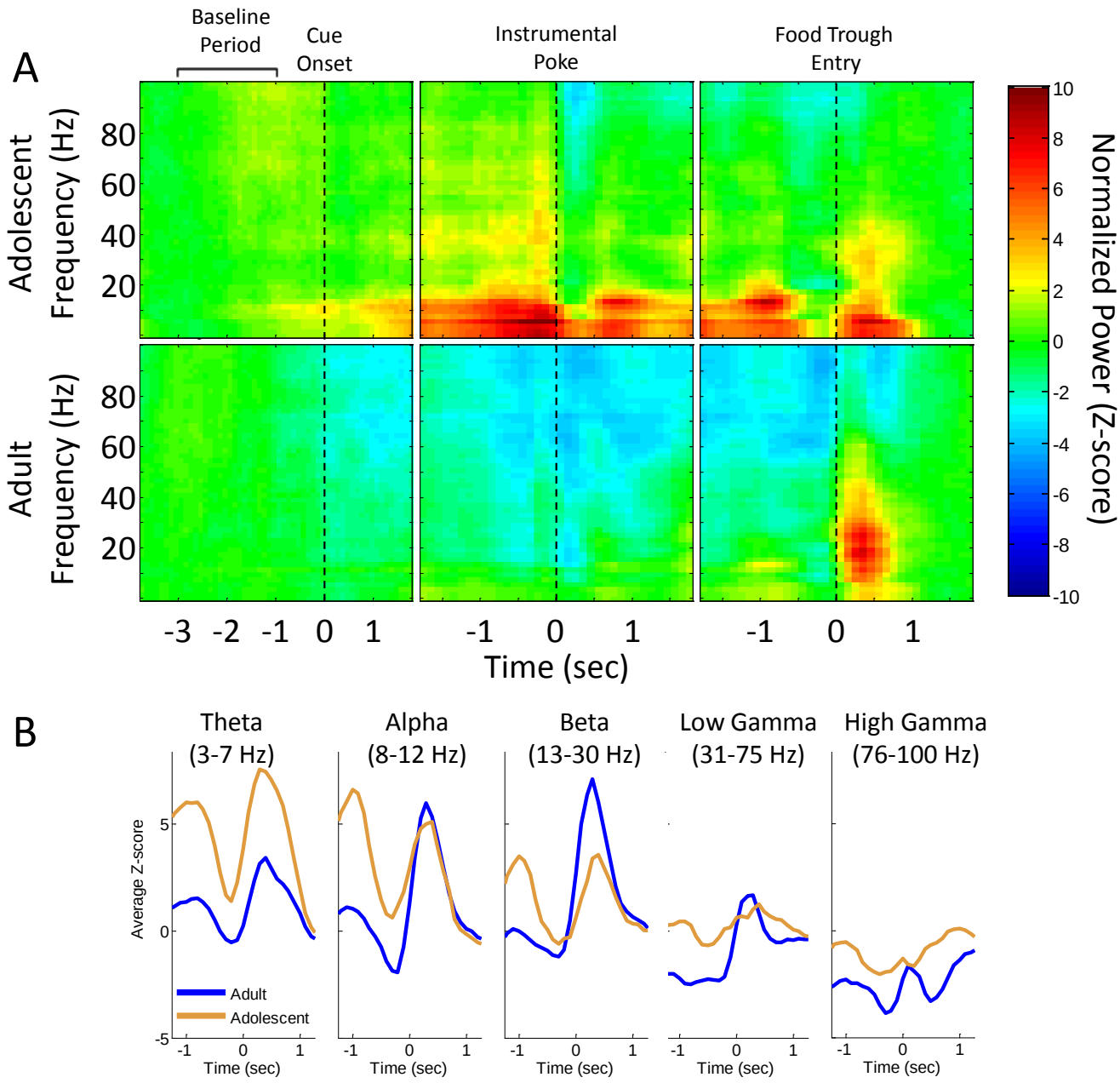


**Figure 4-7 Local field potentials in the dorsal striatum**

A) In DS, adolescents and adults exhibited different patterns of baseline-normalized LFP oscillatory power time-locked to task events. Adolescents generally had increased theta, alpha, and beta power from the beginning of each trial, with gamma increases prior to the instrumental poke and after food trough entry. In contrast, adults showed little change or modest decreases in oscillatory power through a wide range of frequencies until food trough approach. 1 s prior to food trough entry they exhibited modest increases in theta and alpha power, although less dramatically than adolescents. B) In the 1 s after the instrumental response, adolescents exhibited larger increases in theta power, with similar increases in alpha. Adults had stronger increases in beta power at this time.

#### 4.4.3.2 Unit Activity

As with single-unit activity in the NAc, consistent population responses to task events were only seen once rats had learned the action-outcome association and performed numerous trials in each session. Unlike activity in the NAc, dramatic differences in the time course and extent of population activity were observed between adolescents and adults (Figure 4-8). A closer examination of this activity during sessions 4-6 reveals similarities in the activity of some neuronal groups, but considerable differences in others (Figure 4-9). As in the NAc, about 10% of recorded neurons became activated at the trial-onset cue, with few cells becoming inhibited. No age-related statistically significant differences in the proportions of activated, inhibited, and non-significant neurons were observed at this time (Table 4-1). The proportion of activated cells and their magnitude of activity increased in both groups prior to the instrumental response, although activation increases were more substantial in adolescents. Immediately after the instrumental response, cells that were previously activated became inhibited, as did many units that were not previously engaged. The time course of neuronal inhibition around the instrumental poke peaked at the same time in both groups, although a portion of adult units became inhibited slightly earlier than those of adolescents prior to the instrumental response. The post-instrumental response neuronal inhibition in neurons that were previously activated lasted longer in adolescents than adults. Those same neurons then increased their activity again, peaking earlier (and to a lower extent) in adults than adolescents. A sizeable proportion of adolescent neurons continued to activate until immediately prior to receiving the reward. Such reward-anticipation/approach neurons were sparse in adults. Similarly, after reaching the food trough neuronal activation was followed by inhibition in many of those same neurons, and simultaneously a large additional contingent of neurons became inhibited. The time course of

neuronal inhibition was similar between the two age groups, although adults had larger proportions of inhibited neurons at reward. In contrast, adolescents had larger proportions of activated neurons immediately prior to the reward, and this difference persisted for 500 ms after reaching the food trough (Figure 4-9; Table 4-1).

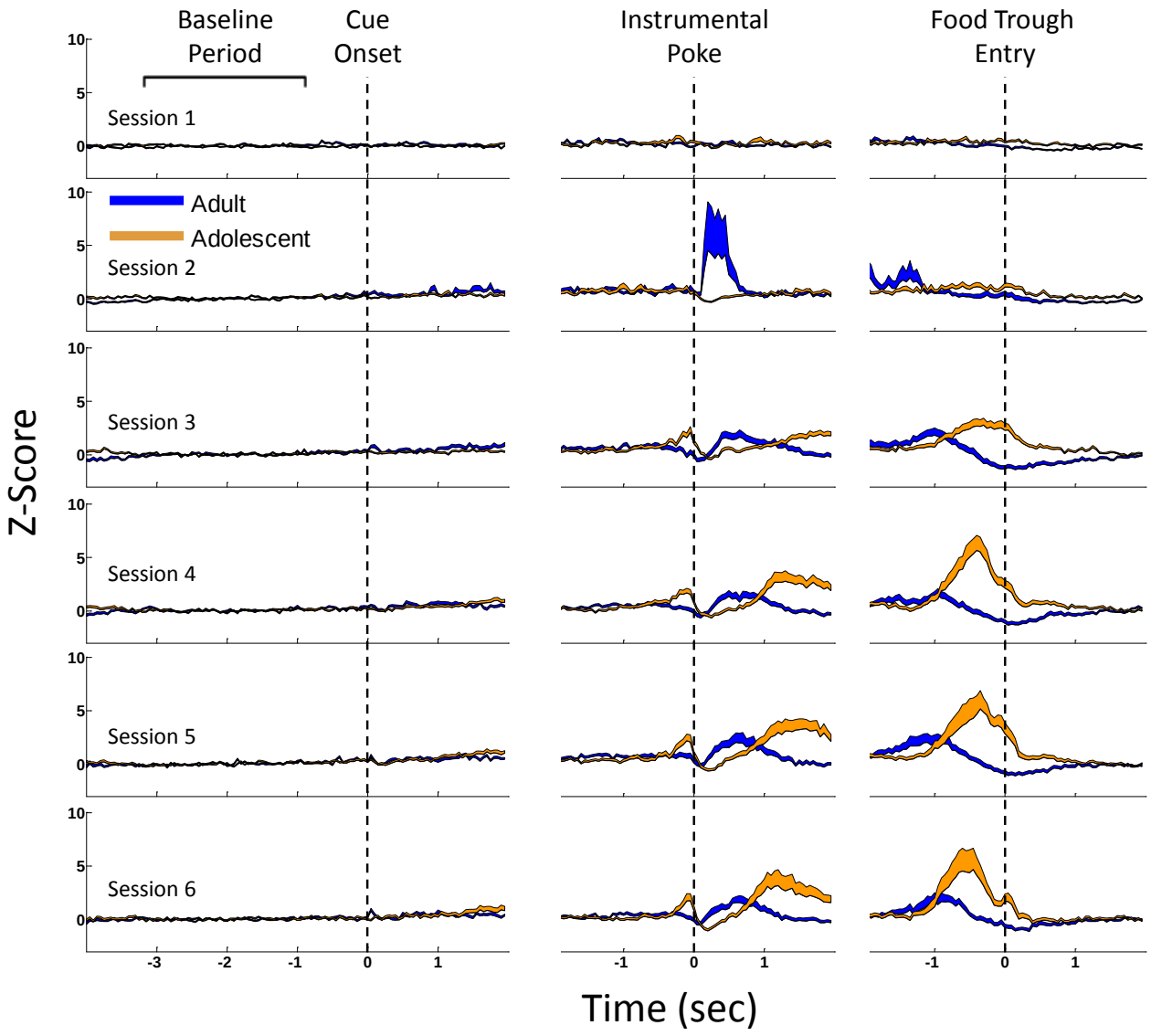


**Figure 4-8 Session-by-session firing-rate activity in the dorsal striatum**
The average baseline-normalized adolescent and adult neural activity during each session + 1 *SEM* (shading) is shown. As in the NAc, consistent neural responses were apparent in adolescents and adults by the time action-outcome associations were learned and the task was performed at a high level. Unlike the NAc, however, age-related differences in magnitude and time course of activity are readily apparent, and consistently seen in later sessions. Session 1: Adolescent $n$ = 172, Adult $n$ = 126; Session 2: Adolescent $n$ = 148, Adult $n$ = 112; Session 3: Adolescent $n$ = 158, Adult $n$ = 78; Session 4: Adolescent $n$ = 117, Adult $n$ = 80; Session 5: Adolescent $n$ = 110, Adult $n$ = 76; Session 6: Adolescent $n$ = 95, Adult $n$ = 92.

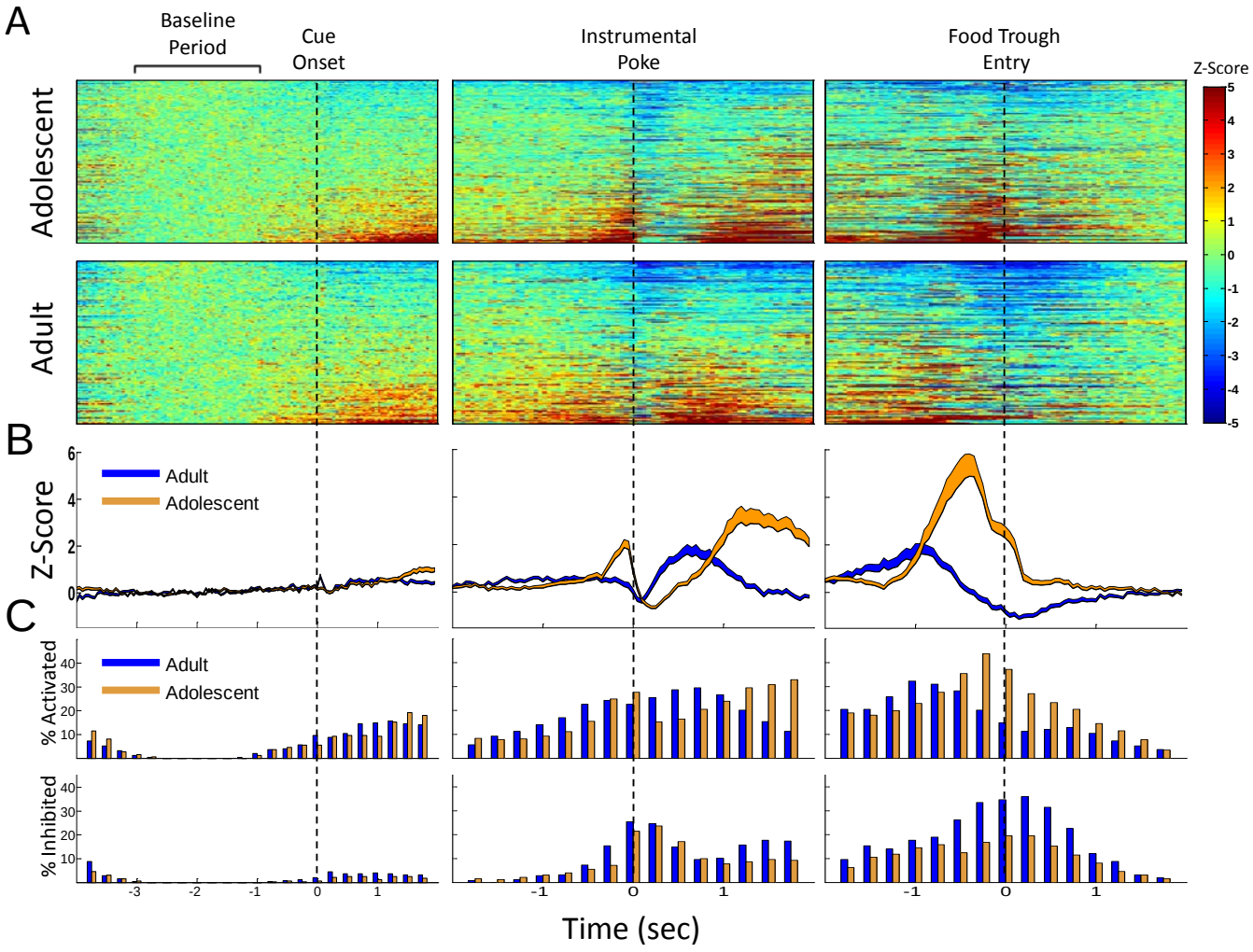


**Figure 4-9 Population and single unit activity in the dorsal striatum**
The same conventions are followed as in Figure 4-6. A) Heatplots represent the phasic single-unit activity of each adolescent ($n$ = 322) and adult ($n$ = 248) neuron of Sessions 4-6, time-locked to task events. A portion of adolescent and adult neurons became activated with the beginning of each trial. These neurons continued their activity until the instrumental poke. Some portion of (especially adolescent) neurons ramped up their activity immediately prior to this poke and a large proportion of neurons (including those previously activated) became inhibited. This inhibition, which in adults was shorter in duration, gave way to activation again as rats approached the food trough. While many adolescent neurons continued to become activated all the way up to food trough entry, adult activated units peaked much earlier and very few remained activated to this point. These neurons (along with others) again became inhibited. While both age groups had this pattern of activation-to-inhibition, the time course was such that only adolescents had what might be considered reward-anticipation/approach neurons. In contrast, the time course of neuronal inhibition was far more similar between the groups, with adult inhibition beginning slightly earlier leading up to the instrumental poke, but peaking at the same time (500 ms afterwards). B) Average population activity + 1 *SEM* (shading) demonstrate the age-related differences in phasic neural activity. C) While the time course of adolescent and adult inhibition was similar at reward (peaking around the food trough entry), adults had significantly greater proportions of inhibited neurons while adolescents had significantly more activated neurons at this time (Table 4-1).

## 4.5 DISCUSSION

In the present study we found that while the general patterns of NAc phasic and field potential activity were quite similar in adolescents and adults, substantial differences were observed in the DS during the performance of well-learned instrumental behavior. Adolescent DS phasic activation occurred later and peaked higher after the instrumental response, providing a neural correlate for reward anticipation/approach only in these younger animals. The activation of similar-appearing neuronal groups in adults peaked far earlier and did not carry their activity to reinforcement. Dramatic age-related differences were also observed in DS LFP oscillations, especially around the instrumental response. These findings indicate adolescent functional immaturities in both structures, but particularly in the DS. Thus, even as adolescent rats performed the same behavior as adults, critical basal ganglia nuclei process salient events differently.

In the NAc, the trial-onset cue was associated with the activation of about 10% of neurons in both age groups. Activated neurons continued their increased activity through the trial until rats poked into the food trough. Adults tended to recruit more activated units at the instrumental poke and immediately before food trough entry, while adolescents had more inhibited units after entry into the food trough. Aside from these modest differences, the proportions of recruited activated and inhibited units, and the time course of their responses, were generally quite similar, as reflected in the average normalized population activity. The patterns of NAc LFP power time-locked to task events were also similar, although immediately after entry into the food trough adolescents had larger increases in theta, alpha, and beta power than adults. As LFP oscillations reflect the weighted average dendrosomatic components of synaptic signals (Logothetis, 2002), these findings indicate more similar regional afferent

activity in NAc than in DS between adolescents and adults. The greater adolescent NAc alpha and beta power after entry into the food trough could indicate somewhat stronger inter-regional coordination, as these bands are associated with communication over longer distances (Pfurtscheller et al., 2000; Brovelli et al., 2004; Klimesch et al., 2007). Stronger adolescent LFP responses to reward are consistent with human fMRI evidence of increased adolescent hemodynamic responses in NAc (Ernst et al., 2005; Galvan et al., 2006) [although see (Bjork et al., 2004)]—a fair comparison as the fMRI signal is also thought to reflect regional afferents and local processing, and correlates far better with LFPs than single or multi-unit activity (Logothetis et al., 2001; Logothetis, 2002).

In contrast to the NAc, phasic neural activity in the DS was remarkably different between adolescents and adults. As in the NAc, cue onset was associated with the activation of approximately 10% of DS neurons in both age groups. However, the pattern of activity was quite different for a sizeable contingent of neurons that became activated, then briefly inhibited at the time of the instrumental response, and then activated again. Adult units were re-activated far earlier (with a shorter period of inhibition) than their adolescent counterparts, whose activation persisted all the way to the time of food trough entry. The degree (proportion and average magnitude) of activation was also greater in adolescents than in adults leading up to reinforcement, while the inhibition of activity seen at the end of each trial was far greater in adults than adolescents. This pattern of adolescent and adult phasic activity differences is reminiscent of those recently observed in the orbitofrontal cortex (Chapter 3; Sturman and Moghaddam, 2011), which directly projects to this region of DS in the rat (Schilman et al., 2008). It is notable, however, that peak activation occurs earlier in the DS than we previously found in the orbitofrontal cortex, hinting that this pattern may not be due simply to excitatory

cortico-striatal interactions. Others have also observed that the activation of neurons in striatum tend to precede those of PFC (Pasupathy and Miller, 2005). In both DS and orbitofrontal cortex, peak activation occurred approximately 500 ms earlier in adults than adolescents (Sturman and Moghaddam, 2011). It remains unclear, however, whether the activation seen in the orbitofrontal cortex study is related to that found slightly earlier in the DS. Future work with simultaneous recording in DS, orbitofrontal, and other PFC regions could address age-specific functional relationships between them.

Age-related differences in LFPs were also more impressive in the DS. Adolescents had broad-spectrum power increases around the instrumental response, while adults had decreases in the same frequency bands. The pattern of oscillatory activity was more similar in the DS during the period around reward, although adolescents had greater increases in theta, alpha, and beta prior to food trough entry and greater theta afterwards. In contrast adults had greater beta power after food trough entry. Such striking age-related LFP differences in DS suggest that during the instrumental response afferent activity may be quite different, especially prior to reinforcement. Striatum LFP power is modulated by dopamine D2 receptor activity (Burkhardt et al., 2009): D2 receptor blockade decreases gamma and beta power, and increases delta and theta power. The dramatic differences observed in DS (but not NAc) LFP power might be related to age-specific patterns in D2 receptor expression. During adolescence the expression of D2 (and D1) receptors peaks in DS and a pronounced lateral-to-medial expression gradient appears, both of which are reduced in adulthood (Teicher et al., 1995). Microdialysis studies have found similar or reduced dopamine release in adolescent DS, although in very different behavioral contexts from the present study (Laviola et al., 2001; Cao et al., 2007). Thus, with age-specific differences in the pattern of both dopamine receptor expression and dopamine release, it is difficult to interpret

how such factors might precisely affect LFP oscillations during this period. Overexpression of D1 and D2 receptors has also been reported in NAc, but the effect is smaller, and some researchers have not found such reductions in NAc dopamine receptor expression after adolescence (Teicher et al., 1995; Tarazi and Baldessarini, 2000). Furthermore, D2 receptor blockade does not appear to have the same impact on LFP power in NAc (Matulewicz et al., 2010). Finally, our measure of LFP power is normalized to each rat's baseline period in each frequency bin, so if these age-related differences do reflect immaturities in adolescent dopamine signaling, they would likely be due to fast changes in dopamine release as these are within-trial oscillation power changes. It is not clear that such putative dopamine signaling differences would affect LFP power at this time scale.

The DS, in coordination with the PFC via cortico-basal ganglia loops, is greatly involved in both the learning and performance of goal-directed actions. Lesions or NMDA receptor blockade of the medial (associative) DS disrupt instrumental learning (Yin et al., 2005a; Yin et al., 2005b). Lesions, or GABA-mediated inactivation of the lateral (sensorimotor) DS, on the other hand, disrupt stimulus-response (habit) learning, and cause animals to become more sensitive to action-outcome associations (Yin et al., 2004, 2006). Together, these sub-regions operate in parallel, allowing behavior to become more automatic and less cognitively expensive over time, with the ability to snap back into a more goal-directed mode when outcome contingencies or motivational contexts change (Balleine et al., 2009). The DS electrode placements in the present study were central-to-lateral in both adolescents and adults. Recorded activity could therefore reflect more automatic stimulus-response associations or an ongoing transition from goal-directed to more habitual behavior. Future work may detect differences in the formation of habits during adolescence. If these DS processing differences do indeed reflect

unique adolescent habit formation timing or efficacy/strength, they might be directly relevant to the increased addiction vulnerability—particularly in the transition from more casual to habitual drug use—of this period (Khuder et al., 1999; Chambers et al., 2003).

Manipulations of the NAc affect motivation, baseline behavioral activity, and the learning and execution of instrumental behavior (Sutherland and Rodriguez, 1989; Ploeger et al., 1994; Setlow, 1997; Ikemoto and Panksepp, 1999; Cardinal et al., 2001; Corbit et al., 2001; Day et al., 2011). Lesions and dopamine depletion of this structure reduce instrumental responding, although they do not abolish action-outcome associations or the ability to devalue such associations (Balleine and Killcross, 1994; Sokolowski and Salamone, 1998; de Borchgrave et al., 2002). A lesion to the core (but not shell) region of the NAc does remove the specificity of devaluation in a paradigm with more than one operant (Corbit et al., 2001). This suggests that only the NAc core may play a direct role in connecting a specific instrumental action with the incentive value of a specific outcome. In the present study both adolescents and adults had arrays with electrodes spread across both the core and shell, making specific electrophysiological core/shell distinctions impossible. Because the NAc is so critically involved in baseline behavioral activity (which is greater in adolescents), motivation, and reward-guided action, it was somewhat surprising that adolescent neural activity differences in NAc were modest and transient compared with those found in DS. A greater proportion of adult NAc neurons were excited at key points in the task such as the instrumental response and at reward anticipation/approach while a greater proportion of adolescent units were inhibited at the food trough entry. The pattern of reward-related adolescent NAc activity differences is particularly complex: similar proportions of adolescent and adult neurons change their firing patterns around entry into the food trough, but the relative proportion of activation versus inhibition tilts

modestly toward inhibition in adolescents. On the other hand, theta, alpha, and beta LFP power were greater in adolescents at reward consumption, demonstrating some non-linearity of afferent and efferent activity that has been long observed between oscillations and spike output (Buchwald et al., 1965; Logothetis, 2002). Therefore, during most of the task, adolescent and adult NAc phasic and field potential activity were quite similar; however, evidence of immaturities in adolescents was present in the precise balance of activation/inhibition and LFP power during short intervals immediately adjacent to the instrumental poke and food trough entry. The nearly identical responses seen at other times (e.g., in the time course of inhibited neurons from cue-onset until about 1 s prior to food trough entry) suggests that other aspects of NAc activity may be more fully mature by adolescence.

The age-related neural activity differences in the present study cannot be adequately explained based on behavioral differences. The period of greatest neural differences was the time between the instrumental response and entry into the food trough. The average latency of this behavior was essentially identical for the two age groups. Furthermore, while the time course of neuronal activation often differed substantially, neuronal inhibition in both brain regions appeared to peak at the same times for adolescents and adults: immediately after the instrumental response and at the time of entry into the food trough.

In this study we demonstrate that adolescent NAc, and especially DS, process aspects of motivated behavior differently than adults. Both age groups contained neurons with similar patterns of activity (e.g. especially inhibited neurons around the instrumental response), but the magnitude (in DS and NAc) and time course (in DS) were often strikingly different, especially during reward anticipation/approach and retrieval. These basal ganglia structures play a central role in normal learning and memory, habit formation, and other aspects of motivated behavior.

Their dysfunction is associated with psychiatric problems including depression, obsessive-compulsive disorder, and addiction (Fineberg et al., 2010; Koob and Volkow, 2010; Krishnan and Nestler, 2010). Therefore, learning how the activity of these regions changes through development—and doing so at the neuronal level—is critical to our understanding of the mechanisms of adolescent vulnerabilities and the future design of clinical interventions.

Acknowledgements—This work was supported by National Institute of Mental Health, the Pittsburgh Life Sciences Greenhouse, and the Andrew Mellon Foundation for a predoctoral fellowship. Thanks to Jesse Wood, Yunbok Kim, and Nicholas Simon for insightful discussions.

# 5.0 GENERAL DISCUSSION

## 5.1 SUMMARY AND INTERPRETATION OF MAIN FINDINGS

This dissertation 1) characterizes behavioral similarities and differences during a novel task designed for testing adolescent and adult rats, and 2) compares the neural activity in adolescent versus adult OFC, NAc, and DS as rats performed this task. The following is a summary of the main findings.

### 5.1.1 Behavioral results

The behavioral task was designed to allow adolescents and adults to learn and act upon a simple action-outcome association. Several age-related behavioral similarities and differences were found. Just as human adolescents may perform a goal-directed action in a fashion indistinguishable from that of adults (e.g., the simple lighting of a cigarette), it was not expected that all adolescent behavioral outcome measures would be different. In fact, in the context of electrophysiological recording, similar instrumental trial performance allowed for a kind of "behavioral clamp," enabling us to determine whether more basic neural processing differences exist in adolescents even as they perform the same motivated behavior. Such findings could relate directly to the age-specific vulnerabilities that are the principal impetus for studying adolescence (e.g., why should the adolescent brain, during the lighting of that cigarette, be more

vulnerable to developing nicotine dependence). Examining electrophysiological activity in the context of behavioral *differences* is still of interest, although it can be difficult to determine whether neural processing differences are merely reflective of differences in behavior or whether there is something additional and intrinsic to the adolescent brain that causes it to operate in a different fashion regardless of performance. This difficulty has been discussed in the context of fMRI work on adolescents, with careful analyses determining that if the confound of behavioral performance is controlled, adolescent neural signaling differences sometimes disappear (Schlaggar et al., 2002; Yurgelun-Todd, 2007). Thus, while distinct behavioral and psychiatric vulnerabilities of adolescence motivate much of this research, we sought to characterize potential neural processing differences in adolescents even as their behavioral performance was similar to adults. Determining the ways in which specific brain regions involved in motivated behavior are in a different functional state during adolescence (i.e., process the same salient events differently), allows for the development of more specific hypotheses regarding the mechanisms that underlie those vulnerabilities.

#### 5.1.1.1 Similar adolescent and adult instrumental performance

In the behavioral study (Chapter 2) adolescent instrumental performance was similar to that of adults, with a few notable exceptions. While both groups learned the association at a comparable rate, adolescents tended to complete fewer total trials after the task was well learned. Cumulative recorder plots demonstrated that unlike adults, adolescents reduced their rate of responding through the course of a session, suggesting a possible satiety effect. Both groups had similar latencies during key windows in the task (e.g., from the cue light to instrumental poke or from the instrumental poke to entry into the food trough), indicating that as adolescent and adult rats progressed through the different events of each trial, their behavior was quite similar.

Task performance during simultaneous recording in OFC, DS, and NAc, was again similar between adolescents and adults (Chapters 3 and 4), although there were some differences in those studies from the previous behavioral findings. The first and most obvious difference was that adolescents no longer performed fewer trials than adults. This could be due to the fact that in the electrophysiology studies, testing began approximately 1 week later (i.e., mid-way through adolescence), to allow rats to recover from surgery. During this period, the caloric needs (Spear, 2000) and weight (McCutcheon and Marinelli, 2009) of rats steeply increase. Another departure from the pure behavior study was the number of sessions needed to reach maximal performance. Some electrophysiology rats (especially those implanted in DS and NAc) took 1 day longer than non-implanted rats to reach maximal performance. It is yet unclear what contributed to this. Importantly, these differences were seen equally in the two age groups, such that task performance was still very similar among OFC-, DS-, and NAc-implanted adolescents and adults.

#### 5.1.1.2 Adolescents respond differently to motivational factors during extinction

During extinction adolescents performed more perseverative (previously reinforced) pokes than adults. At first, this appeared to suggest a learning deficit in these younger animals: did they struggle to learn or integrate this new information (i.e., that the previous instrumental action was no longer reinforced)? Were they acting more out of habit than their adult counterparts? After further testing, it became clear that whatever cognitive flexibility and/or habit formation differences may exist between adolescents and adults, they would not adequately explain our observations. Instead, motivational context accounted for the perseveration differences. Specifically, the continued presentation of the trial-onset light cue, along with continued food restriction, led adolescents to perseverate much more than adults. Removal of

either one of these factors caused their perseveration to look much more like that of adults. Removal of both motivational factors caused adolescents to perseverate almost as they would with a single factor, although adults dropped their level of poking further. From this, it was concluded that adolescents appear to have a higher behavioral ceiling when motivational factors are present—and that motivational factors can interact synergistically to increase poking in these younger rats. Adults in contrast had a lower behavioral floor when factors were absent. These findings have interesting potential implications for the sorts of contexts in which adolescents may exhibit persistent behavior even in the absence of direct reinforcement: the adolescent brain may be in a state such that motivational factors can lead to greater behavioral output. This may indicate that discontinuing previously rewarded behaviors, such as drug use, may be even more difficult for adolescents than adults if cues, cravings, and/or other motivational factors are present.

#### 5.1.1.3 Miscellaneous adolescent behavioral differences

In the behavior study (Chapter 2), adolescents performed more task-irrelevant (left- and right-hole) pokes than adults. Adults, on the other hand, performed more premature (pre-cue) pokes. Premature pokes were not punished in any way, and increased in both groups across sessions—indicating that instead of measuring impulsive action they could reflect a kind of single-mindedness of task-performance. By performing more task-irrelevant pokes, adolescents showed themselves to be more behaviorally active in general, and perhaps less singularly focused than adults. The other main age-related behavioral difference was activity in an open field. We found that adolescents were generally more active, and interestingly, food restriction further increased this disparity; adults did not increase their activity while food restricted. This may further support the interpretation of adolescents responding differently to motivational

factors: in this case hunger caused adolescents, but not adults, to become even more active than normal in a novel environment.

### 5.1.2 Adolescent processing differences in orbitofrontal cortex during motivated behavior

After characterizing the behavioral task it was used along with simultaneous electrophysiological recording. To do this, rats began training a week later than in the previous study to allow for their surgical recovery. This brief time window still permitted rats to perform six training sessions. Because of time limitations, extensive extinction testing was not performed (although some extinction data were acquired for each rat, they are not presented in this dissertation). The goal of electrophysiological experiments was to determine whether (and how) adolescents processed salient events differently than adults, even when behavior was similar. Our first target region was the OFC (Chapter 3).

#### 5.1.2.1 Age-related differences in the balance of excitatory and inhibitory responses in the orbitofrontal cortex

Adolescent OFC neurons tended to show less inhibition through much of the task. This was reflected in average normalized population activity, and was due primarily to smaller proportions of units with inhibitory responses. This was apparent around the cue, before and after the instrumental response, and in the period around reinforcement. Interestingly, there were no age-related differences in excitatory or inhibitory responses at the time of the instrumental poke for about 500 ms. This exception should be emphasized, as it indicates that adolescent OFC neural activity *can* respond in a very similar way to that of adults in certain contexts, but often it does not. In addition to reduced inhibition, adolescents had stronger reward

anticipation/approach excitatory activity, which peaked later than that of adults. These age-related differences in the balance of excitation and inhibition have profound implications for immature cortical neural architecture. For example, reduced inhibition could be a functional consequence of immature interneurons that are critically involved in controlling the precise spike-timing of pyramidal cells and the entrainment of oscillations (Buzsaki and Chrobak, 1995; Fries et al., 2007). If this is the case, it might also suggest reduced efficiency of cortical processing and coordination (see below).

#### 5.1.2.2 Greater firing-rate variability in adolescent orbitofrontal cortex

While a characteristic neural response pattern can be classified as activated, inhibited, or non-changing, due to the stochasticity of neural activity, the timing and extent of these responses vary somewhat from trial to trial. The Fano factor is a way of quantifying this variability. Recent work has demonstrated that a general property of cortical activity is stimulus-induced reductions in the Fano factor. Such reductions during salient events were observed in the OFC of both age groups, but critically, the Fano factor was generally higher in adolescents than adults. This greater variability indicates that adolescent single-unit activity is less consistent, and suggests noisier representations of cortical information and reduced processing efficiency in these younger rats.

#### 5.1.2.3 Age-related differences in orbitofrontal cortex oscillations

Although LFP oscillations in OFC were largely similar between adolescents and adults, during reward, adolescents had smaller increases in alpha and beta power. While the precise meaning of this large-scale regional activity difference is unclear, it could reflect reduced coordination of afferents from other regions. This interpretation is consistent with previous

findings of reduced inter-regional coordination in adolescent humans, as measured with fMRI and EEG.

### 5.1.3 Adolescent and adult nucleus accumbens and dorsal striatum processing during motivated behavior

As in the OFC, several similarities and differences were found in both the NAc and DS activity of adolescents versus adults (Chapter 4). While this was true for both structures, it was remarkable how dramatic the age-related differences were in DS, and how relatively modest they appeared in the NAc.

#### 5.1.3.1 Similar adolescent neural activity in nucleus accumbens

In both adolescents and adults, the performance of instrumental behavior was associated with the initial activation of a similar proportion of neurons followed by a gradual increase in neuronal recruitment, which peaked at the instrumental response and then again just prior to retrieving the reward. Although adults tended to recruit a greater proportion of activated neurons at the peak times, the time course was quite similar between groups. A group of neurons began to be inhibited just prior to the instrumental response (peaking in the proportion of inhibited units just afterwards) with nearly identical proportions and time courses between age groups, followed by a second peak after entry into the food trough. At this later point, adolescents tended to inhibit a greater proportion of units than adults, although again, with a similar time course.

The pattern of NAc LFP oscillations was also remarkably similar between adolescents and adults. During reinforcement, however, adolescents had modestly larger increases in theta, alpha, and beta power. Together these data demonstrate a nearly mature pattern of neural activity

at the levels of both single-units and field potentials. Differences in the proportions of activated/inhibited neurons and the power of LFP oscillations at specific times do suggest the presence of some remaining functional immaturities.

#### 5.1.3.2 Different adolescent neural activity in dorsal striatum

As in the NAc the onset of a trial was associated with a similar proportion of activated DS neurons. From the time of the instrumental response, however, the time course of activated neural recruitment was quite different for adolescents and adults. While a similar pattern of activation followed by inhibition and re-activation is seen in both groups, adults inhibit more briefly, re-activate sooner, and peak at a far earlier and lower level than adolescents. Adolescent activation persists all the way to entry into the food trough, indicating a DS neural correlate of reward anticipation/approach in adolescents, but not adults. As seen in the NAc, the overall time course of neuronal inhibition was actually remarkably similar for adolescents and adults, with nearly identical proportions of inhibited units around a peak in the period after the instrumental response, and a second peak in inhibition at food trough entry. Contrary to the NAc, however, where adolescents had a greater proportion of inhibited neurons at the food trough entry, in the DS adults had more inhibited neurons while adolescents had a greater proportion of activated neurons.

As with the phasic activity, LFP oscillations were more different between adolescents and adults in the DS. This was particularly pronounced in the period around the instrumental response, where theta, alpha, and beta power increased in adolescents, but decreased or showed no consistent change in adults. During reward, adolescents continued to show a stronger increase in theta, while alpha power was more similar and beta increases were greater in adults. These

stark differences suggest that in several ways, the DS is functionally quite immature in adolescents as they perform a goal-directed behavior.

## 5.2 NEURAL MECHANISMS OF ADOLESCENT BEHAVIOR

### 5.2.1 Synthesis of neurobehavioral models of adolescence

As discussed in Chapter 1, a central theme of several neurobehavioral models is that during adolescence, there are differences in the sensitivity, level, or effect of activity in brain networks that subserve emotional processing and cognitive control (Table 5-1). Much of the evidence for these models is from human neuroimaging studies that demonstrate that adolescents activate similar brain regions as adults during the performance of behavioral tasks. Differences that are found are generally in the precise pattern, time course, and extent of the neural activity in these regions. Thus, the adolescent brain is remarkably mature in its organization and engagement of specific networks to execute motivated behavior. The subtle differences that do exist are thought to be relevant to the behavioral differences and vulnerabilities observed in certain contexts. The convergence of these models in terms of the specific brain regions and/or connections between them is remarkable. While not identical, all of them propose that certain adolescent behavioral tendencies stem from functional underdevelopment of the PFC and NAc (and/or the connection between them) within networks that integrate activity in the amygdala and sometimes other emotion-processing regions (Figure 5-1).

**Table 5-1 Neurobehavioral hypotheses integrating adolescent behavioral changes with brain development**

| Hypothesis | Description | References |
|---|---|---|
| Social information processing network | Changes in adolescent social behavior reflect the development of specific brain networks that integrate the detection of social information with cognitive and affective processing regions. | Nelson et al. (2005) |
| Triadic node | Adolescent risky behavior can be explained in terms of the relative strength of ventral striatum-mediate approach versus amygdala-mediated avoidance and an immature supervisory prefrontal cortex. | Ernst et al. (2006) |
| Differential development of limbic reward versus top-down control systems | The relatively earlier development of bottom-up limbic regions versus the prefrontal cortex biases behavior toward risk and reward. | Casey et al. (2008) |
| Arousal of socio-emotional systems at puberty | Increased sensation-seeking and risk-taking during adolescence are due to changes in reward salience and sensitivity as a result of brain remodeling (e.g. changes in dopamine and oxytocin systems) and immature cognitive control systems. | Steinberg (2008) |
| Inefficient neuronal processing | Adolescent neural processing is less well-distributed and coordinated due to immature myelination, pruning, interneuron development, and other factors. This leads to imbalances in local activation and inhibition in systems that underlie motivated behavior, and precipitate both altered sensitivities to salient stimuli and less-effective top-down control in certain contexts. | Sturman and Moghaddam (2011) |

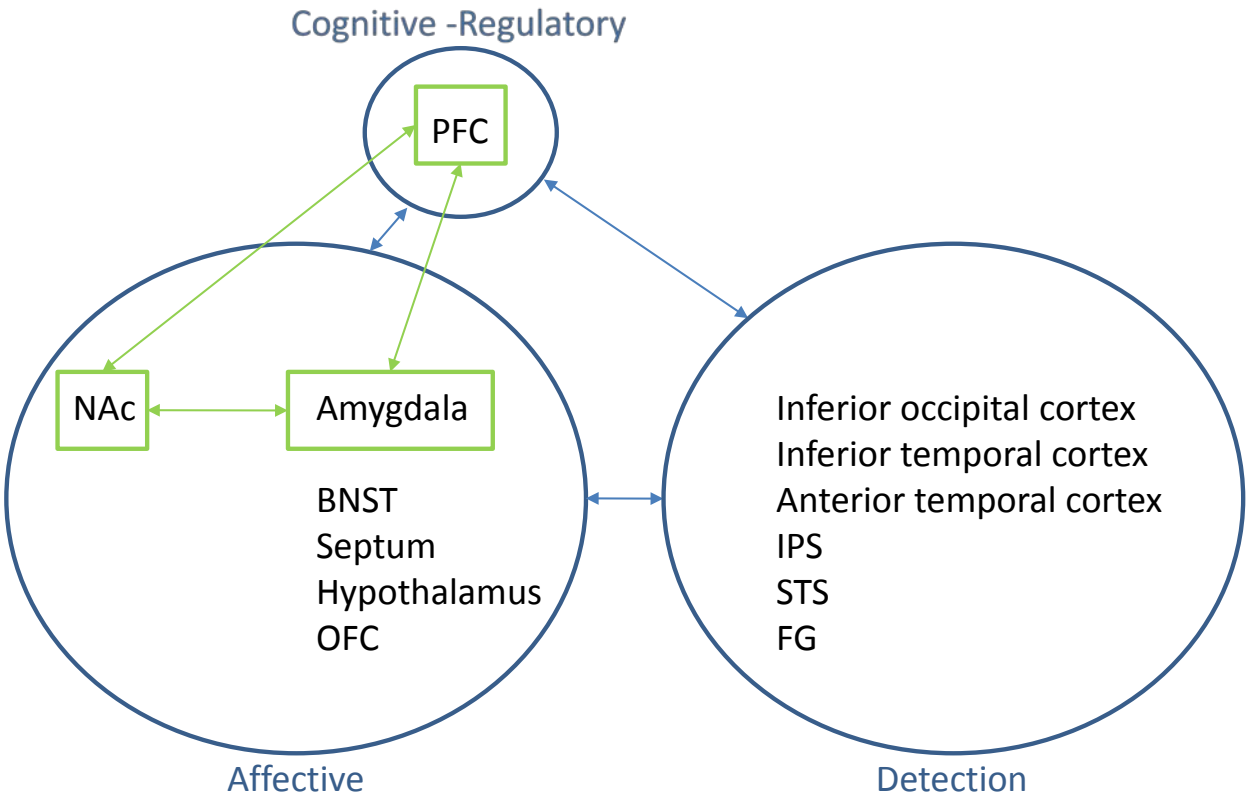


**Figure 5-1 Synthesis of neurobehavioral models**
Current models describing the neural underpinnings of adolescent behavioral tendencies reflect the underdevelopment of several specific brain regions and/or their connections. All brain regions presented are part of the social information processing network (blue). The triadic node model (green) encompasses the same brain regions and connections postulated by Casey (2008) and Steinberg (2008) in their hypotheses of adolescent sensation seeking and risk taking (Table 5-1). PFC = prefrontal cortex; NAc = nucleus accumbens; BNST = bed nucleus of the stria terminalis; IPS = intraparietal sulcus; STS = superior temporal sulcus; FG = fusiform gyrus.

The next step for neuroscientists is to address what is precisely meant by the "underdevelopment" or "immaturity" of these regions and networks. Human imaging studies have identified some of these neural activity differences at a large-scale regional level. With *in vivo* electrophysiological recording in behaving rats, we can further examine differences in regions of interest at both the levels of field potentials and individual neurons, and do so with high temporal resolution. In addition to determining *what* the neural activity differences are, we must develop more specific models that indicate the mechanisms that lead to them, and address

how these differences give rise to specific behavioral tendencies and psychiatric vulnerabilities. For example, Liston et al. (2006) demonstrated that increasing fronto-striatal white matter through development predicted inhibitory control performance in a go/no-go task. This could mean that through more effective inter-regional communication and coordination, the PFC can exert a stronger top-down influence on the striatum as the brain develops. Recent functional imaging evidence confirms such a relationship between inter-regional functional connectivity and age-related improvements in top-down inhibitory control (Hwang et al., 2010). Similarly, simultaneous recording in the PFC and striatum could reveal weaker coherence and/or phase-locking between these regions in adolescents. We might also expect reduced age-related differences in phasic striatum activity to correlate with measures of improved inter-regional coordination through development. This example demonstrates how large-scale observations can lead to specific hypotheses of mechanisms that relate the immaturity of brain networks to behavior. Refining these models will involve greater specificity in our identification of adolescent neural processing differences. This dissertation demonstrates several such differences in adolescents during motivated behavior, and future work using these techniques will lead to more detailed hypotheses regarding the neural mechanisms of adolescent vulnerabilities.

### 5.2.2 Reduced adolescent processing efficiency: a hypothesis

Based on our data and other evidence, we hypothesize that some neural processing differences (i.e., “immaturities”), mentioned in the neurobehavioral models, may be the result of reduced neuronal coordination and processing efficiency in adolescents. These differences manifest as a result of less-effective information transfer between regions and imbalances in neuronal excitation and inhibition within critical brain regions, such as the orbitofrontal cortex

and portions of the basal ganglia. As described earlier, others have found that during adolescence there are dramatic changes in both the expression patterns of various receptors and the effects of receptor activation, including in the response of inhibitory fast-spiking interneurons to dopamine and NMDA receptor stimulation in the PFC. Such changes would be expected to affect both the balance of excitation and inhibition and the coordination of neuronal groups. As fast-spiking interneuron activity is critical to controlling the precise timing of neural activity and the entrainment of oscillations, the developmental shifts in adolescent interneuron activity and their response to neuromodulators like dopamine may be central to some of these age-related processing differences. As a result of this, adolescent neural activity may be less well-coordinated, noisier, and more local, and also perhaps more sensitive to the behaviorally activating effects of rewards, novelty, or other salient stimuli. Our observations of increased adolescent Fano factor, reduced neuronal inhibition, and reduced reward-related alpha and beta oscillatory power in the OFC (Chapter 3) are consistent with this hypothesis. Reduced inter-regional oscillatory coordination, further hampered by incomplete myelination, could together account for the less-distributed functional activity observed in imaging studies. The previously mentioned tendency for adolescents to favor risky choices in emotionally charged contexts could also be related to a combination of reduced inter-regional communication (e.g., failure of the PFC to effectively dampen subcortical “go” signals in the basal ganglia), and exaggerated activation and/or reduced inhibition to salient cues in the context of motivated behavior, as we observed during reward anticipation in the OFC and DS.

### 5.2.3 Processing differences in dorsal striatum should be included in models of adolescent vulnerabilities

Adolescents exhibited clear differences from adults in how their DS encoded salient events during motivated behavior. As discussed earlier (Chapter 1), the DS, in conjunction with the PFC, facilitates the learning of action-outcome associations as well as the transitions to outcome-insensitive habits. The neural circuitry involved in reward-guided instrumental behavior and habit formation are essentially the same as those thought to be "hijacked" in addiction (Koob and Volkow, 2010). Age-related processing differences within this circuitry are therefore highly relevant to adolescent vulnerabilities.

Repeated performance of an action facilitates the development of habits, and is relevant to addiction and other "extreme habits" (Graybiel, 2008). While the task used in our study was not designed for habit formation, the fact that adolescents tended to perseverate more during extinction than adults could indicate a greater level of automaticity or tendency to express learned behavioral patterns in some situations. Future work may confirm that adolescents have a greater tendency to form habits, or more persistently act on them once they are in place. If this is true, it would point to a direct mechanism for increased adolescent addiction vulnerability—with processing differences in the DS (as well as subregions of the PFC) potentially playing a central role.

Casey et al. (2008) argued that adolescent risk-taking could be due to an imbalance between the level of development in the NAc and PFC (Table 5-1). In an fMRI study they observed that the pattern of neural activity in the NAc was far more similar between adolescents and adults than between children and adolescents. In contrast, activity in the OFC was more similar between children and adolescents than between adolescents and adults:

> "Our results suggest that there are protracted maturational changes in top–down control systems relative to subcortical regions implicated in appetitive behaviors… [D]isproportionate contributions of subcortical systems relative to prefrontal regulatory systems may underlie poor decision-making that predisposes adolescents to drug use and, ultimately, addiction." (Galvan et al., 2007)

The findings in this dissertation are somewhat consistent with this interpretation, as the pattern and time course of both large-scale (field potential) and single-unit neural activity was far more similar between adolescents and adults in the NAc than in the OFC. However, our data do not support the idea that this is a cortical vs. subcortical distinction, as adolescent neural activity in DS was so different from that of adults. Adolescent neurobehavioral models should therefore be modified to include the DS as a potential source of behavioral differences and vulnerabilities.

### 5.2.4 What do specific age-related neural activity differences mean?

This dissertation presents several age-related differences in unit activity and LFP oscillations, particularly in the OFC and DS. While it has been tempting to speculate about the precise meaning of these differences (e.g. reduced adolescent neuronal inhibition in the OFC), some caution should exercised. Here I would like to state why such caution is important and then briefly ignore this advice as a couple of possible interpretations are explored further.

Comparing the electrophysiological activity between adolescents and adults is a bit like comparing the sounds of two different fax machines as they send a fax. The static noises that are heard correlate with the transfer of specific information (i.e. the fax messages). So if the fax machines sound different it could mean that they are sending different faxes. Alternatively, they could be using different algorithms to encode and send the same information. Comparing the character of the sounds will not necessarily tell us how their messages and/or algorithms differ. This is true even though there is a direct relationship between the sounds and the messages they

carry. If we knew the algorithms we could theoretically compare all of the information, as well as the elegance of the algorithms themselves. Using various approaches and techniques, neuroscientists work to better understand what might be thought of as "neural algorithms" by examining various aspects of the brain's form and function. It is unclear whether the age-related neural differences described in this dissertation occur because they encode different information and/or the same information differently. The fact that adolescents and adults behaved similarly supports the notion that they encode similar information; however, at this point, finding differences in the relative balance of neural activation and inhibition or LFP oscillations does not address this.

What might the neural activity differences in our recordings indicate if both groups encoded the same information (i.e. their "messages" were the same but "algorithms" were different)? From this perspective, it appears that the adult OFC and DS were doing more with less: peak activations occurred earlier and were much lower than those of adolescents. This could further support the notion of reduced adolescent processing efficiency in these areas. Along these lines, others have shown that glucose utilization is greater in the forebrain of children and adolescents than that of adults (Chugani et al., 1987). The adolescent brain has more local excitatory connections that are not yet pruned, and thus it could be potentially more prone to hyperactivity. On a related note, one function of the basal ganglia is selection of cognitive and behavioral patterns, and thus the filtering out of others (Kropotov and Etlinger, 1999; Nicola, 2007). As described previously, both adolescent and adult DS exhibited neuronal activation leading up to the instrumental response, followed by inhibition, then again by activation leading up to reward retrieval. Adolescents tended to inhibit longer after the instrumental response before re-activating. It is intriguing to consider the possibility that the activity of these neurons reflect

switching representations of the various cognitive and motor steps involved in each trial. If this is the case, it could once again indicate a kind of reduced adolescent neural processing efficiency, as there is a greater lag from one representation to the next.

The similarities in NAc processing between adolescents and adults could very well indicate that this structure operates similarly in both groups: during the same behavior it encodes the same "message" and uses the same "algorithm." While lesions of the NAc reduce instrumental responding, they do not disrupt specific action-outcome associations (Balleine and Killcross, 1994). In this context, it may thus send very low-level incentive salience or other sorts of motivational signals related to the stimuli in this task (Berridge, 2007). These signals could be quite similar in the two groups. It should be noted, however, that different algorithms can lead to the same or similar solutions; similar neural activity does not necessarily indicate that the neural architecture in the NAc is fully mature in adolescence (indeed, there is some evidence to the contrary, as discussed previously).

It is still too early to state with much confidence the precise meaning of each adolescent neural activity difference. Collectively, there is some evidence for reduced adolescent neural processing efficiency of similar signals, particularly in the DS and OFC. Such differences may be of little consequence to the performance of a simple instrumental task. However, in complex decision-making contexts, particularly those with social and emotional factors (in which separating the relevant signal from noise could be more difficult), the consequences of such age-related processing differences may become more apparent.

## 5.3 CONCLUSION AND FUTURE DIRECTIONS

By utilizing electrophysiological recording from implanted arrays in behaving rats this dissertation represents a novel approach for comparing the neural processing of adolescents with that of adults during motivated behavior. Much remains to be done to more fully characterize the activity of regions relevant to the behavioral and psychiatric vulnerabilities of adolescence. For example, recording from the amygdala and medial PFC would provide insight into additional structures centrally involved in the learning and flexible performance of goal-directed behavior. Simultaneous recordings in different regions can lead to analyses that measure the efficient communication between regions (e.g. coherence and phase-locking), which could further test our hypothesis of reduced adolescent processing efficiency. Furthermore, it is possible that while the neural activity of adolescent NAc appears similar to that of adults, interactions between the NAc and the PFC or other structures could vastly differ.

While the first step has been to examine neural activity while behavior was similar, future electrophysiological studies could focus on behavioral differences. For example, differences in NAc activity could be expected in the context of extinction, a time as we have shown that adolescents often perseverate more when certain motivational factors are present (Chapter 2). Additional behavioral tasks that measure cognitive flexibility or which contrast habitual with goal-directed action could also yield interesting age-related neural processing differences. However, the development of such paradigms may be a challenge given the brief window for experimentation afforded by the short period of rat adolescence.

As we have learned more about the specific brain and behavioral changes of adolescence several neurobehavioral models have been proposed. Central to most of these is the notion that immature neuronal processing in the PFC and other cortical and subcortical regions, along with

their interaction, leads to behavior that is biased towards risk, reward, and emotional reactivity during adolescence. This dissertation reports that although adolescents performed an instrumental behavior very similarly to that of adults, they were more behaviorally sensitive to the presence of motivational factors. Furthermore, even during similar instrumental performance, the neural activity of critical brain regions that lie at the interface of sensory, emotional, and cognitive systems often process salient events quite differently in adolescents versus adults. By using techniques like electrophysiological recordings in laboratory animals, we can more precisely identify age-related processing differences, and develop and test hypotheses that relate them directly to both the increased risky behavior of normal adolescence and the onset of psychiatric problems that often arise at this time.

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