

**EFFECTS OF THE NOVEL ATYPICAL ANTIPSYCHOTIC, ARIPIPRAZOLE, ON
RATS PERFORMING SIGNALLED AND UNSIGNALLED TEMPORAL
DISCRIMINATION TASKS.**

By
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ABSTRACT

In contrast to other atypical antipsychotics, aripiprazole (ARZ) acts as a partial agonist, rather than an antagonist, at dopamine D2 receptors (Burriss et al, 2002; Jordan et al, 2002). This unique pharmacology is thought to be essential to its multi-faceted use in the treatment of other disorders, particularly in its use as an adjunctive treatment for Major Depressive Disorder (Biler & Blondeau, 2011).

Currently, little treatment is available to attenuate cognitive dysfunction in schizophrenic individuals (Schatzberg et al, 2010). However, clinical and neurocognitive studies have reported ARZ to improve cognition in schizophrenic subjects (Leuchet et al, 2003; Schatzberg et al, 2010), as well as attenuate working memory impairments and attentional deficits in animal models of psychosis (Carli et al, 2010; Nordquest et al, 2008; Nagi et a, 2008). Further evaluation of ARZ is needed to assess its ability to ameliorate higher-order cognitive processes in a laboratory behavioral pharmacological context.

Differential-reinforcement-of-low-rates (DRL) is an operant timing schedule that requires animals to produce inter-response-times (IRTs) greater than a criterion temporal interval to obtain a reward. Because animals must temporally regulate their responses and internally estimate the criterion duration, successful performance on the DRL task is dependent on both temporal processing and controlled timing behaviors. Since individuals with schizophrenia exhibit temporal processing deficits (Carroll et al, 2008; Elveag et al, 2008; Tysk et al, 1990), the DRL schedule can be utilized to evaluate the effects of ARZ to attenuate timing behaviors in a laboratory animal model of psychosis.

In the studies presented here, water-deprived male Sprague-Dawley rats were trained to perform a DRL-72 second schedule that was either signaled (DRLS-72; $n = 8$) or unsignaled (DRLU-72; $n = 8$). Upon making a correct response ($IRT \geq 72s$), rats were given a small amount (0.06mL) of distilled water as reinforcement. Subjects were trained to perform the task in operant chambers with force-plate technology (Fowler et al, 2001), which allowed for high-precision measurements of locomotor activity (e.g., spatial patterning, distance traveled) and response characteristics (e.g., response durations, peak force of responses). Before drug treatment, DRLS-72 rats were significantly more successful at operant performance (e.g., higher reinforcement rates, longer IRTs) than DRLU-72 rats. In addition, DRLS-72 rats were less restricted in their spatial patterning and engaged in more locomotion, suggesting rats under the signaled contingency were less likely to engage in timing behaviors than DRLU-72 rats. These results reflect a difference of task difficulty between the two DRL schedules: The ability to perceive and respond to an external stimulus (DRLS-72 condition) was less demanding and difficult than internally estimating the temporal criterion (DRLU-72 condition).

The acute administration of ARZ (1.0, 3.0, and 6.0mg/kg) at the beginning of the session induced right-ward shifts in IRT frequency distributions and lengthened median IRT values, though this effect was greater in the DRLU-72 group. This latter finding is likely due to the increased reliance of DRLU-72 rats on timing behaviors and temporal processing—behaviors and processes that are sensitive to the pharmacological modulation of brain dopamine systems (Hinton & Meck, 1997; Meck, 1983). Similar to other antipsychotics on DRL-mediated behavior (Wiley et al, 2000; O'Donnell & Seiden, 1983), ARZ generally altered operant behavior by dose-dependently reducing non-burst and burst response rates and reinforcement rates in both DRL

groups. However, the 1mg/kg dose improved DRLU-72 operant performance by significantly increasing reinforcement rates and decreasing response rates—an effect that is similar to antidepressants (O’Donnell & Seiden, 1983).

In a separate experiment, 16 naive rats were again trained to perform the DRLS-72 (n = 8) and DRLU-72 (n = 7) task. After completing seven 4-hr sessions of DRLS-72 or DRLU-72 (28 hours of training), rats were administered 5.0mg/kg amphetamine (AMPH) over several sessions to model a state of psychosis (Young et al, 2010). To evaluate the effects of ARZ to attenuate schizophrenic-like cognitive and behavioral deficits, rats were first administered AMPH immediately preceding the operant session, followed 30 minutes later by acute injections of 1.0, 3.0, or 6.0mg/kg ARZ. AMPH treatment produced a consistent pattern of behavior in rats: Subjects engaged in focused stereotypy during the first portion (approximately 1.5-hr) of the session followed by hyperlocomotion and operant-directed behavior in the latter portion (approximately 2.5-hr). AMPH induced timing deficits in DRLU-72 rats by shifting IRT distributions to the left and reducing median IRT values. In addition, AMPH treatment significantly impaired DRLU-72 performance by increasing non-burst responses rates, reducing reinforcement rates, and disrupting space usage relative to non-AMPH behavioral sessions. However, AMPH treatment did not induce timing deficits in DRLS-72 rats. AMPH did not significantly alter DRLS-72 IRT distributions, median IRTs, space usage, and reinforcement rates when compared to non-AMPH treatment sessions. This result implies that AMPH treatment induced schizophrenic-like timing deficits in DRLU-72 rats, but not DRLS-72 rats. It is likely that AMPH induced greater impairments in the DRLU-72 condition because the

unsigned DRL contingency was substantially more cognitively demanding than the DRLS-72 condition.

In AMPH-treated rats, ARZ administration dose-dependently reduced hyperlocomotion and hastened recovery time from focused stereotypy. ARZ partially reversed the effects of AMPH in both DRL groups by significantly reducing non-burst responses, increasing median IRT values, and shifting IRT distributions to the right. However, ARZ could not completely attenuate AMPH-induced impairments in operant performance and collateral activity for DRLU-72 rats, suggesting that ARZ was limited in its capacity to attenuate schizophrenic-like cognitive and behavioral deficits that were induced by AMPH.

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TABLE OF CONTENTS

| | |
|---|-----------|
| CHAPTER 1: THE INVESTIGATION OF THERAPEUTIC COMPOUNDS IN PHARMACOLOGICAL ANIMAL MODELS OF PSYCHOSIS..... | 1 |
| 1.1 INTRODUCTION | 1 |
| 1.2 SCHIZOPHRENIA..... | 2 |
| 1.2a Prevalence, Incidence, and Estimated Costs..... | 2 |
| 1.2b Clinical Profile of Schizophrenia..... | 2 |
| 1.2c Positive Symptoms..... | 3 |
| 1.2d Negative Symptoms | 4 |
| 1.3 NEUROCHEMICAL-BASED HYPOTHESES OF SCHIZOPHRENIA..... | 4 |
| 1.3a The Dopamine Hypothesis | 5 |
| 1.3b The NMDA Receptor Hypofunction Hypothesis | 7 |
| 1.3c Serotonin-based Hypothesis | 8 |
| 1.4 ANTISYPCHOTIC DRUGS | 9 |
| 1.5 TRADITIONAL ANTIPSYCHOTICS | 10 |
| 1.5a History..... | 10 |
| 1.5b Classification of Traditional Antipsychotics | 12 |
| 1.5c Mechanism of Action | 12 |
| 1.5d Side-effects | 13 |
| 1.6 ATYPICAL ANTIPSYCHOTICS..... | 15 |
| 1.6a History..... | 15 |
| 1.6b Classification of Atypical Antipsychotics..... | 16 |
| 1.6c Clinical Efficacy..... | 16 |
| 1.6d Mechanism of action: Dopamine D2 Receptor Blockade or Partial Agonism..... | 17 |
| 1.6e Mechanism of action: Dual 5-HT2a Receptor Antagonism and D2 Receptor Blockade | 18 |
| 1.6f Adjunctive use of antipsychotics in Depression..... | 19 |
| 1.6g Side-effects | 20 |
| 1.7 ARIPIPRAZOLE | 21 |
| 1.7a Pharmacological Profile | 21 |
| 1.7b Clinical Profile..... | 22 |

| | | |
|---|---|-----------|
| 1.8 | INTERM SUMMARY | 23 |
| 1.9 | PHARMACOLOGICAL ANIMAL MODELS OF SCHIZOPHRENIA | 24 |
| 1.9a | Psychostimulants | 25 |
| 1.9b | Phencyclidine and other NMDA Receptor Antagonists | 28 |
| 1.9c | LSD and other Serotonergic Hallucinogens | 30 |
| 1.10 | SUMMARY | 31 |
| CHAPTER 2: TIMING BEHAVIOR IN ANIMALS: THE ROLE OF INTERVAL TIMING | | 34 |
| 2.1 | INTRODUCTION | 34 |
| 2.2 | INTERVAL TIMING | 34 |
| 2.2a | Impairments of Interval Timing in Schizophrenic Subjects | 35 |
| 2.2b | The Role of Dopaminergic Neurotransmission in Temporal Processing..... | 37 |
| 2.3 | EXPERIMENTAL ASSESSMENT OF TIMING BEHAVIOR IN ANIMALS | 38 |
| 2.3a | Operant Conditioning Schedules | 38 |
| 2.3b | Fixed Interval Procedure | 39 |
| 2.3c | Peak Interval Procedure..... | 40 |
| 2.3d | Differential-Reinforcement-of-Low-Rates Procedure..... | 41 |
| 2.3e | Bi-section Procedure | 42 |
| 2.4 | DIFFERENTIAL-REINFORCEMENT-OF-LOW-RATES | 43 |
| 2.4a | <i>Inner-Response Time</i> (IRT) Frequency Distributions | 43 |
| 2.4b | Collateral Activity | 45 |
| 2.4c | Signaled DRL Schedules..... | 46 |
| 2.5 | SUMMARY AND RATIONALE OF EXPERIMENTAL STUDIES | 47 |
| 2.6 | DIFFERENCES IN BEHAVIOR MAINTAINED BY SIGNALLED VERSUS UNSIGNALLED DRL SCHEDULES | 48 |
| 2.7 | METHODS | 49 |
| 2.7a | Subjects | 49 |
| 2.7b | Apparatus..... | 50 |
| 2.7c | Behavioral Procedure..... | 52 |
| 2.7d | Drugs | 53 |
| 2.7e | Statistical Analysis..... | 53 |
| 2.8 | RESULTS | 54 |
| 2.9 | DISCUSSION | 57 |

| | | |
|-------------------|--|-----------|
| 2.10 | FIGURES AND TABLES..... | 63 |
| CHAPTER 3: | EFFECTS OF ARIPIPRAZOLE IN RATS PERFORMING SIGNALLED OR UNSIGNALLED DRL TIMING SCHEDULES | 74 |
| 3.1 | INTRODUCTION | 74 |
| 3.1a | Interval Timing | 76 |
| 3.1b | Task Difficulty..... | 76 |
| 3.1c | Uncertainty of Reward..... | 77 |
| 3.2 | METHODS | 77 |
| 3.2a | Subjects | 77 |
| 3.2b | Apparatus..... | 78 |
| 3.2c | Behavioral Procedure..... | 78 |
| 3.2d | Drugs | 78 |
| 3.2e | Data Analysis | 78 |
| 3.3 | RESULTS..... | 79 |
| 3.3a | Average Response and Reinforcement Rates | 79 |
| 3.3b | IRT Frequency Distributions and Med IRTs..... | 81 |
| 3.3c | Distance Traveled during the pre-72 s Reinforcement Interval..... | 82 |
| 3.3d | Quadrant Usage | 82 |
| 3.4 | DISCUSSION | 83 |
| 3.5 | FIGURES AND TABLES..... | 87 |
| CHAPTER 4: | EFFECTS OF ARIPIPRAZOLE IN D-AMPHETAMINE-TREATED RATS PERFORMING SIGNALLED OR UNSIGNALLED DRL TIMING SCHEDULES..... | 93 |
| 4.1 | INTRODUCTION | 93 |
| 4.1a | The Influence of AMPH on interval timing..... | 94 |
| 4.6b | Task difficulty: Differential effects of AMPH..... | 95 |
| 4.2 | METHODS | 95 |
| 4.2a | Subjects | 95 |
| 4.2b | Apparatus..... | 95 |
| 4.2c | Behavioral Procedure..... | 96 |
| 4.2d | Drugs | 96 |
| 4.2e | Data Analysis | 97 |
| 4.3 | RESULTS..... | 98 |

| | | |
|------------|--|------------|
| 4.3a | Duration of Amphetamine-induced FS | 99 |
| 4.3b | Comparison of Operant Response Measures between AMPH and non-AMPH Treatment Sessions..... | 100 |
| 4.3c | IRT Frequency Distributions and Median IRTs | 101 |
| 4.3d | Non-burst response rates | 103 |
| 4.3e | <i>Burst</i> response rates | 105 |
| 4.3f | Reinforcement Rates..... | 106 |
| 4.3g | Unreinforced Response Rates..... | 108 |
| 4.3h | Distance traveled during the Pre-72 s reinforcement interval | 109 |
| 4.3i | AMPH-induced disruption of quadrant usage | 111 |
| 4.3j | Effects of ARZ + AMPH on quadrant usage..... | 112 |
| 4.4 | DISCUSSION | 114 |
| 4.4a | Effects of AMPH Treatment on DRL-Mediated Behavior..... | 114 |
| 4.4b | Effects of AMPH + ARZ on DRL-Mediated Behavior..... | 117 |
| 4.4c | The Ability of ARZ to Reverse Deficits in an AMPH Model of Psychosis..... | 119 |
| 4.5 | FUTURE DIRECTIONS | 124 |
| 4.5a | Analysis of DRLU-72 and DRLS-72 behavior during extinction | 124 |
| 4.5b | Analysis of DRLU-72 and DRLS-72 rats during training | 127 |
| 4.5c | Further examination of ARZ's Ability to Attenuate Behavioral and Cognitive Deficits in the DRL procedure and other Operant Schedules | 128 |
| 5.6 | FIGURES AND TABLES..... | 132 |
| | REFERENCES..... | 147 |

TABLE OF FIGURES

| | |
|---|-------------------------------------|
| Figure 2-1. Contingencies of the DRL operant procedure. | 63 |
| Figure 2-2. Movement trajectories and operant responses of a single rat for a 4-hr session. | 64 |
| Figure 2-3. Group mean IRT frequency distributions following saline administration. | 65 |
| Figure 2-4. Individual rat mean forelimb response force waveforms and tongue force waveforms for reinforced responding. | 67 |
| Figure 2-5. Locomotor activity (distance traveled) in saline-treated DRLU-72 and DRLS-72 rats during the pre-72 second reinforcement interval. | 72 |
| Figure 2-6. Group mean time spent in each quadrant during pre-72 s reinforcement interval. | 73 |
| Figure 3-1. Operant response measures under ARZ administration. | 87 |
| Figure 3-2. IRT frequency distributions under ARZ treatment. | 89 |
| Figure 3-3. Median IRT values for VECH and ARZ treatment sessions. | 90 |
| Figure 3-4. Distance traveled during pre-reinforcement interval under ARZ treatment. | 91 |
| Figure 3-5. Group mean (+/- SEM) time spent in each quadrant for ARZ treatment sessions. | 92 |
| Figure 4-1. Movement trajectories and operant responses of a single (A) AMPH -treated DRLU-72 and (B) AMPH-treated DRLS-72 rats in the operant chamber. | 132 |
| Figure 4-2. Group mean (+/- SEM) duration of FS expression within the 4-hr session. | 134 |
| Figure 4-3. Group mean floor sector usage in d-amphetamine-treated rats. | 135 |
| Figure 4-4. Comparison of operant response measures between AMPH and non-AMPH treatment sessions. | Error! Bookmark not defined. |
| Figure 4-5. IRT Frequency distributions for 5.0mg/kg d-amphetamine sessions. | 138 |
| Figure 4-6. Median IRT values for AMPH and ARZ treatment sessions. | 139 |
| Figure 4-7. Response and reinforcement rates for AMPH treatment sessions. | 141 |
| Figure 4-8. Group mean (+/- SEM) unreinforced response rates for AMPH and non-AMPH sessions. ... | 143 |
| Figure 4-9. Distance traveled during pre-reinforcement interval for AMPH treatment sessions. | 144 |
| Figure 4-10. Quadrant usage during the pre-reinforcement interval for AMPH and ARZ treatment sessions. | 146 |
| Table 2-1. Group mean values for operant response measures under saline conditions. | 66 |

CHAPTER 1: THE INVESTIGATION OF THERAPEUTIC COMPOUNDS IN PHARMACOLOGICAL ANIMAL MODELS OF PSYCHOSIS

1.1 INTRODUCTION

Schizophrenia (SZ) is a complex mental disorder that induces impairments in judgment and distorted perceptions of reality. Numerous structural, functional, and neurochemical brain abnormalities have been reported in the literature, as well as environmental and genetic factors that influence its occurrence (Feldman et al, 2007). Despite these findings, the etiology of SZ is still unknown, and no treatment is available to cure the disease.

Although the introduction of antipsychotic drugs in the 1950's revolutionized treatment available to schizophrenic individuals, these drugs remain limited in their capacity to ameliorate cognitive impairments associated with the disease (Davis et al, 1991; Andreasen et al, 1995). The effects of currently available antipsychotic drugs and experimental therapeutics on cognitive-mediated behaviors have been routinely evaluated in preclinical laboratory studies, which assess the ability of these compounds to produce therapeutic-like changes and attenuate schizophrenic-like cognitive deficits in animal models of psychosis (Young et al, 2010; Nordquest et al, 2008; Marcotte et al, 2001). In the series of experiments presented here (Chapters 2-4), we investigated the ability of the novel partial dopamine (DA) receptor agonist, aripiprazole, to attenuate schizophrenic-like timing deficits in rats.

In the present chapter, the clinical profile of SZ is discussed, followed by a review of the neurochemical hypotheses and abnormalities reflected in the disorder. Next, the development of antipsychotic drugs and their therapeutic mechanisms of action are described, along with the rationale for investigating the effects of aripiprazole. Lastly, a brief review of popular pharmacological animal models of psychosis is presented, followed by a delineation of the reasoning for selecting the psychostimulant, d-amphetamine, to model a state of psychosis in the rat.

1.2 SCHIZOPHRENIA

1.2a Prevalence, Incidence, and Estimated Costs

SZ is estimated to occur in 1% of the world population (Regier et al, 1993). Despite this relatively small rate of prevalence, the World Health Organization has listed SZ as one of the top 10 causes of disability in developed countries. Approximately 30,000 new cases are diagnosed annually in the United States (McEvoy, 2007), where the estimated cost for treatment was estimated over \$60 billion in 2002 (McEvoy, 2007; Wu et al, 2005).

1.2b Clinical Profile of Schizophrenia

Schizophrenic individuals do not usually express clinical symptoms until early adulthood (late teens to early twenties). Some schizophrenic patients will show continuous deterioration, but most often, schizophrenic individuals experience periodic relapses and remissions.

Schizophrenic patients suffer from profound disturbances within a wide range of perceptual, emotional and cognitive functions that ultimately disrupt normal thought processes and behavior. These symptoms are traditionally categorized into *positive* or *negative*

symptoms (Andreasen, 1990). Positive symptoms include behaviors that are in “addition” to normal functioning, such as hallucinations, delusions, and bizarre behaviors. Negative symptoms, on the other hand, describe behaviors that are impaired or absent from normal functioning, such as lack of emotional expression, social withdrawal, and cognitive impairments. It should be noted, however, that while these symptoms are common place in SZ, the manifestation and intensity of symptoms can vary greatly across patients. This is true for not only the type of symptoms experienced, but the actual content and nature of each symptom. This latter point is particularly true for positive symptoms, where the subject matter of hallucinations and delusions are often tailored to the unique experiences or cultural environment of the individual. For example, schizophrenic individuals in industrialized countries are more prone to delusions regarding technology (i.e., electronic tracking devices secretly implanted in citizens), whereas patients in development countries are more likely to experience positive symptoms concerning spiritual beings or demons. Thus, while schizophrenic individuals universally experience hallucinations and delusions, the nature of those symptoms are typically associated with the patient’s intrinsic experiences.

1.2c Positive Symptoms

Hallucinations are perhaps the most well-known symptom of SZ. They are a common occurrence in the disease, and can occur in several sensory modalities, such as visual, auditory, tactical, or olfactory. However, auditory hallucinations are the most dominant form, and patients will often hear voices that threaten, insult, or make demands of them. Delusions are also present, and range from feasible, irrational beliefs (*nonbizarre* delusions) to completely implausible, unfounded beliefs (*bizarre* delusions). Bizarre delusions are more common, and

are predominately persecutory in nature. Disturbances in thought and speech process can also occur, and are characterized by fragmented sentences, loosening of ideological associations, and a lack of organization in speech. Emotionally, schizophrenic individuals may react inappropriately to social cues and engage in bizarre behavior or postures.

1.2d Negative Symptoms

Negative symptoms include social and emotional deficits, such as diminished interest in social bonding (*Austism*), lack of motivation (*Avolition*), and diminished emotional responsiveness (*Anhedonia*). Cognitive impairments are also considered to be a subtype of negative symptoms, though recent literature has emphasized the substantial influence of these disturbances in terms of overall life quality and functioning (Leucht et al, 2003). These impairments are generally related to executive functioning, and involve difficulties in abstraction, strategic problem solving, goal-orientated behavior, complex and sustained attention, spatial and verbal learning, and performing sequential tasks. There is also evidence that schizophrenic individuals have deficits in temporal processing, and are less accurate at estimating and perceiving temporal intervals relative to healthy individuals (Rammsayer, 1990; Elvevag et al, 2003; Carroll et al, 2008)

1.3 NEUROCHEMICAL-BASED HYPOTHESES OF SCHIZOPHRENIA

Several neurotransmitters have been linked to abnormal neurotransmission in SZ, including dopamine, serotonin, glutamate, norepinephrine, gamma-aminobutyric acid (GABA), and acetylcholine (Young et al, 2010; Marcotte et al, 2001). Currently, dopamine and glutamate have garnered the most attention and support from the SZ literature (Young et al,

2010; Marcotte et al, 2001). This review will briefly summarize findings for dopamine, glutamate, and serotonin-related neurochemical hypothesis for SZ, as these neurotransmitters are associated with popular pharmacological animal models of psychosis (Young et al, 2010) and pharmacological treatments for SZ (Biler & Blondeau, 2011).

1.3a The Dopamine Hypothesis

Since its initial development in the late 1960's, the dopamine (DA) hypothesis remains the most prominent and enduring theory of schizophrenia. In the 20 years preceding its arrival, a series of historical discoveries converged to provide indirect evidence in support of the DA theory (Baumeister & Francis, 2002). Among these landmark findings was the discovery of the mechanism of action of psychomotor stimulants (amphetamine, cocaine, methamphetamine). During this period, researchers determined that the stimulant effect of amphetamine (AMPH) was mediated by DA (Rossum & Hurkmans, 1964). Additionally, it was known that the administration of psychostimulants could induce schizophrenic-like symptoms in nonpsychotic individuals, and exacerbate symptoms in schizophrenic individuals (Baumeister & Francis, 2002). Another finding was the discovery that Parkinson's disease involves a deficiency of DA, and that L-dopa, a DA precursor, could reverse Parkinsonian symptoms (Birkmayer & Hornykiewicz, 1961). This led to an increased understanding of DA and its relation to the extrapyramidal motor system (Baumeister & Francis, 2002). Support for the DA hypothesis also came from the discovery that antipsychotics were primarily DA D2 receptor antagonists, and that D2 receptor occupancy correlated with clinical efficacy (Davis et al, 1991).

When the DA hypothesis was initially proposed, it was thought that SZ was predominately caused by hyperactivity of dopaminergic neurotransmission (Davis et al, 1991). However, it was concluded that enhanced levels of DA could not account for all SZ symptoms, as antipsychotics have a low propensity to ameliorate negative symptoms and cognitive deficits (Leucht et al, 2003; Feldman et al, 2007). Criticism also came from clinical findings that showed antipsychotics could not alleviate symptoms in all SZ patients (Davis et al, 1991). The DA theory was later revised, claiming that SZ symptoms were primarily caused by hyperdopaminergia within subcortical (mesolimbic) structures and hypodopaminergia within cortical (mesocortical) structures (Stone et al, 2007; Davis et al, 1991). This theory suggests that reduced DA transmission within the mesocortical pathway contributes to the diminished functioning of the prefrontal cortex, leading to cognitive impairments and negative symptoms (hypofrontality).

Currently, the strongest support for the DA hypothesis is still derived from the mechanism of action of antipsychotics, and that all clinically effective antipsychotics modulate DA transmission in some capacity. Antipsychotics also exert effects on other neurotransmitter systems, but substantial variation exists among them. Furthermore, additional support for abnormal DA transmission has emerged from neuroimaging studies. These experiments have found a significant (but modest) increase in DA receptor density in schizophrenic individuals compared to healthy controls (Laruelle et al, 1996). Single photon emission computerized tomography (SPECT) studies have found significantly higher levels of AMPH-induced dopamine release in the striatum of schizophrenic individuals relative to controls (Laruelle et al, 1996). Abi-Dargham et al (2000) demonstrated that schizophrenic individuals had increased dopamine receptor occupancy at baseline and acute dopamine depletion states when compared to

nonpsychotic controls. These findings suggest a heightened sensitivity of dopamine receptors in SZ. (Abi-Dargham et al, 2000; Laruelle et al, 1996; Stone et al, 2007).

1.3b The NMDA Receptor Hypofunction Hypothesis

An alternative hypothesis based on impaired functioning of *N*-methyl-D-aspartate (NMDA) receptors (a subtype of glutamate receptors) has gained substantial attention over the last decade. Originally proposed by Olney and Farber (1995), the NMDA receptor hypofunction hypothesis states that a reduction of NMDA receptor function contributes to the pathology of SZ. They argued that abnormal glutamate dysfunction, via NMDA receptor antagonism, induces changes in dopaminergic transmission. Thus, abnormal glutamate transmission is seen as the primary cause of SZ, while dopaminergic changes are a secondary effect.

The NMDA receptor hypofunction theory was initially formulated after the clinical observation that phencyclidine (PCP), a non-competitive NMDA receptor antagonist, can induce psychotic symptoms in humans that closely resembles the disease (Luby et al, 1959). Currently, the majority of support for the NMDA dysfunction in SZ is primarily derived from behavioral pharmacological studies in both humans and animals (Allen & Young, 1978; Jentsch & Roth, 1999; Javitt, 2007; Amitai et al, 2007; Olney & Farber, 1995; Stone et al, 2007). An oft-cited finding from these reports is that, unlike psychostimulants, PCP and its related compounds (ketamine, MK-801) can induce the full spectrum of the disorder, including negative and cognitive symptoms (Sams-Dodd, 1995; Lee et al 2005). A more detailed discussion of NMDA receptor antagonists as an animal model of psychosis is presented later in this chapter (Section 1.9b).

Additional evidence for the NMDA theory has come from clinical findings demonstrating the therapeutic effects of NMDA modulators, such as glycine, d-serine, and d-cycloserine (Singh & Singh, 2011; Javitt, 2007). These compounds bind to allosteric binding sites on NMDA receptors and modulate ion channel activity. A meta-analysis of 29 clinical trials found NMDA modulators significantly attenuate negative symptoms when used as adjunctive treatment with antipsychotics (Singh & Singh, 2011). However, none of these drugs have been found to be clinically effective when used as monotherapy (Javitt, 2007).

Recently, the NMDA receptor antagonism model has also gained support from neuroimaging studies. A SPECT study found that acute administration of ketamine reduced NMDA receptor binding, and that NMDA receptor antagonism within the middle and inferior frontal lobes was positively correlated with the severity of negative symptoms (Stone et al, 2007). However, ketamine-induced changes in NMDA receptors did not correlate with positive symptoms. Another imaging study reported reduced NMDA receptor binding in the left hippocampus of drug-free SZ patients compared to healthy controls, although it should be noted researchers used a relatively small sample size (Pilowsky et al, 2006).

1.3c Serotonin-based Hypothesis

Substantial evidence exists for serotonergic irregularities in schizophrenic individuals (Hashimoto et al, 1993; Gurevich & Joyce, 1997; Roth & Meltzer, 1995). Postmortem studies have found increased densities of 5-HT_{1a} receptors (Hashimoto et al, 1993), as well as decreased densities of 5-HT_{2a} receptors (Gurevich & Joyce, 1997; Arora & Meltzer, 1991) within the frontal lobe of schizophrenic individuals when compared to healthy controls.

Recently, reduced 5-HT_{2a} densities in drug-naïve SZ subjects have been confirmed *in vivo* using neuroimaging techniques (Rasmussen et al, 2010).

Further support for serotonin dysregulation comes from the pharmacological profile of atypical antipsychotics, which act as dual dopamine D₂ and serotonin 5-HT_{2a} receptor antagonists (Newman-Tancredi & Kleven, 2011). While 5-HT_{2a} antagonism is thought to contribute to the therapeutic effect of atypicals (Biler & Blondeau, 2011; Leuchet et al, 2003; Newman-Tancredi & Kleven, 2011; Meltzer et al, 2003), 5-HT_{2a} agonism has been proposed to elicit schizophrenic-like symptoms in nonpsychotic individuals (Vollenweider et al, 2007; Hoffman et al, 1968; Hollister et al, 1968). Indeed, the development of the serotonergic hypothesis of SZ was based on the finding that LSD could induce psychotic symptoms in healthy individuals (Young et al, 2010; Marcotte et al, 2001).

Although the role of serotonin dysfunction in psychosis is unclear, it appears that modulation of serotonergic transmission can produce therapeutic effects in SZ (Ichikawa et al, 2001; Akhondzadeh et al, 2008). It has been suggested that serotonin abnormalities can exert effects on other neurotransmitter systems, including dopamine and glutamate (Roth & Meltzer, 1995). Thus, while abnormal serotonergic transmission may not be a primary cause of SZ symptoms, it remains a key component in the overall disruption of neurotransmission in the disease.

1.4 ANTISYPCHOTIC DRUGS

Currently, the most clinically effective treatment for schizophrenia is antipsychotic drugs. Prior to their development in the 1950's, the most popular forms of treatment for

psychosis were ineffective or crude, ranging from psychotherapy, electroconvulsive shock therapy (ECT), prefrontal lobotomies, and the administration of barbiturates or other powerfully sedating drugs (Shorter & Healy, 2007; Kucharski, 1984; Crilly, 2007). The introduction of antipsychotics produced dramatic changes in health care by allowing schizophrenic patients to leave hospital wards and function within society (Synder & Banerjee, 1974). In tandem with their rapid, wide-spread use, extensive research was conducted to determine their mechanism of action, and it was eventually concluded that neuroleptics are predominantly dopamine receptor antagonists (Carlsson & Linqvist, 1963; van Rossum, 1966; Baumeister and Francis, 2002).

1.5 TRADITIONAL ANTIPSYCHOTICS

1.5a History

One of the earliest drugs to demonstrate antipsychotic potential was reserpine, an alkaloid derived from the roots of the Indian tropical plant *Rauwolfia Serpentina* (Snake root). Before its discovery by North America and Europe, *Rauwolfia* had been utilized in Asia for centuries to treat various ailments, and its sedating effect was used to calm mental patients and put children to sleep (Baumeister & Francis, 2002). Interest from Western medicine emerged in the late 1940's, when a clinical study demonstrated its usefulness as an antihypertensive drug (Vakil, 1949; Baumeister & Francis, 2002). Several investigators made note of its sedative properties, and reports of Indian physicians using reserpine to treat psychotic patients lead American psychiatrist Nathan Kline to conduct a clinical trial examining its antipsychotic potential (Feldman et al, 1997; Kline et al, 1954). Kline's study (1954) found reserpine attenuated psychiatric symptoms, and the drug was introduced in North America in

1953 (Feldman et al, 1997; Baumeister & Francis, 2002). Following its initial reception into the United States, extensive work in pharmacological research attributed reserpine's antipsychotic ability to its inhibition of vesicular storage for several monoamines, including norepinephrine, serotonin, and dopamine (Feldman et al, 1997). The prevention of vesicular storage allowed monoamine oxidase to breakdown neurotransmitters, thus causing a reduction in serotonin and catecholamines.

Coinciding with the rise of reserpine in Western psychiatry, chlorpromazine was discovered to exhibit a similar therapeutic effect in schizophrenic patients (Curzon, 1990; Kapur & Mamo, 2003). Interest in the use of antihistamines to lower presurgical stress prompted French surgeon Henri Laborit to examine the effects of chlorpromazine in his patients (Curzon, 1990; Baumeister & Francis, 2002). Laborit noticed chlorpromazine's ability to produce a tranquil and calm state in patients that was distinct from its sedative effects (Laborit & Huguenard, 1951). Shortly after Laborit's findings, psychiatrists John Delay and Pierre Deniker administered chlorpromazine in their own psychiatric patients (Baumeister & Francis, 2002; Kapur & Mamo, 2003). They found the drug antagonized their patients' psychotic symptoms, and subsequently reported chlorpromazine's antipsychotic potential (Delay, 1952). In 1953, the U.S. Food & Drug administration approved the use of chlorpromazine as an antipsychotic after its efficacy was demonstrated in state hospitals (Kane et al, 2010). Shortly after the introduction of chlorpromazine to clinical practice, several other neuroleptics quickly followed, such as flupenthixol (Depixol), thioridazine (Mellaril), trifluoperazine (Stelazine), thiothixene (Navane), trifluopromazine (Vesprin), Haloperidol (Haldol), zuclopenthixol (Clopixol), fluphenazine (Prolixin), chlorprothixene (Taractan) and mesoridazine (Serentil).

1.5b Classification of Traditional Antipsychotics

Traditional antipsychotics are categorized by their chemical structure, which also provides general information on their receptor selectivity and potency. Phenothiazines make up the largest and most commonly utilized class of traditional antipsychotics (Feldman et al, 1997). Compared to other neuroleptics, they are relatively non-selective in their receptor targets, demonstrating antagonistic activity at histamine (H1), peripheral and central muscarinic acetylcholine, serotonin, and noradrenaline receptors (Feldman et al, 1997). Thioxanthenes exhibit a strong affinity for central muscarinic receptors and are less likely than other traditional antipsychotics to induce extrapyramidal side-effects (EPS). The third major chemical class, the butyrophenones, has a high potency and selectivity for D2 receptors (Feldman et al, 1997).

1.5c Mechanism of Action

The therapeutic effect of antipsychotics is largely attributed to the blockade of dopamine D2 receptors within the mesolimbic pathway (Carlsson and Lindqvist, 1963; van Rossum, 1967; Seeman et al, 1975). Receptor binding studies have shown traditional antipsychotics bind to DA postsynaptic receptors and DA presynaptic autoreceptors with high affinity (Creese et al, 1976; Kapur & Seeman, 2000). The affinities for different DA receptor subtypes vary amongst traditional antipsychotics; however, D2 receptor occupancy appears to be directly related with their clinical potency (Seeman & Lee, 1975). The therapeutic effects of antipsychotics are reported to occur at 65-80% D2 receptor occupancy (Schatzberg et al, 2010; Miyamoto et al, 2005).

Though neuroleptics readily bind to D2 receptors within a matter of hours, their antipsychotic effect does not occur for 2-3 weeks (Johnstone et al, 1978). While this latency is

poorly understood, it is thought that chronic administration of antipsychotics—and therefore, sustained blockade of DA D2 receptors—gradually induce clinically relevant changes in DA transmission (Grace et al, 1992; Grace et al, 1997). Electrophysiological studies have shown that acute administration of antipsychotics leads to increases in DA cell firing, and changes the DA cell firing pattern from a single spike to multiple spikes (Feldman et al, 1997). However, chronic administration leads to reduced firing of DA cells and a decrease in the number of spontaneously active DA cells. Based on these findings, researchers have suggested that chronic administration of antipsychotics overexcite DA cells (depolarization block), which leads to a reduced firing of DA cells (Grace et al, 1997; Grace & Bunney, 1995).

1.5d Side-effects

Traditional antipsychotics can produce a number of different adverse-side effects in patients. The most common side-effect of neuroleptics seen in schizophrenic individuals are extrapyramidal symptoms (EPS), which has been estimated to occur in 70% of patients receiving therapeutic doses of traditional antipsychotics (Davis et al, 2003). These symptoms include akinesia, akathisia, rigidity, and tremors. It is generally believed that EPS symptoms are caused by D2 receptor blockade within the nigrostriatal pathway, specifically in the basal ganglia (Feldman et al, 1997). Receptor binding and position emission tomography (PET) studies have shown neuroleptic-treated patients with acute EPS have higher percentages of D2 receptor occupancy in the basal ganglia than patients without EPS (Farde et al, 1992).

The severity of EPS symptoms appears to be inversely related to antipsychotic-induced antagonism of muscarinic acetylcholine receptors; neuroleptics that bind to muscarinic

cholinergic receptors with relatively high affinity are less likely to induce EPS than antipsychotics with weaker affinity for these receptor sites (Synder et al, 1974). Therefore, common treatment for antipsychotic-induced EPS is the administration of anticholinergic drugs, such as trihexyphenidyl and benztropine mesylate (Cogentin). Treatment with these drugs, however, can produce anticholinergic side-effects, such as constipation, impaired vision, dry mouth, and memory problems.

Prolonged administration of traditional antipsychotics can also lead to tardive dyskinesia. These symptoms usually involve quick movements (tics) of the face and mouth, involuntary protrusions of the tongue, and can eventually lead to spastic movement of limbs and distorted posturing of the body. Unlike EPS, discontinued use of antipsychotics does not diminish symptoms, and no treatment is currently available (Feldman et al, 1997). It has been proposed that chronic usage of neuroleptics—and therefore, chronic blockade of D2 receptors—may induce dopamine receptor supersensitivity (Snyder et al, 1997). Indeed, in rats chronically administered fluphenazine, reserpine, or haloperidol for 3 weeks produced significant increases in DA receptor binding a week after receiving their last antipsychotic treatment (Snyder et al, 1977). Clinical findings have also shown that reinstating antipsychotic drug treatment or increasing doses of antipsychotics can provide transient relief from symptoms. However, the pathology of tardive dyskinesia is still poorly understood.

In addition to EPS and tardive dyskinesia, traditional antipsychotics can induce a vast array of side-effects related to their non-therapeutic actions on other receptor sites. Blockade of D2 receptors within the tuberoinfundibular pathway prevents dopamine-induced blockade

of the pituitary gland. Without the inhibitory actions of dopamine, the pituitary gland can produce excessive amounts of prolactin (hyperprolactinemia), which in turn can result in several other endocrinologic side-effects, such as galactorrhoea, amenorrhoea, breast enlargement, weight gain and reduced sex drive. Cardiovascular complications, such as tachycardia and arrhythmias, can be induced by anticholinergic and antiadrenergic actions of traditional antipsychotics. Finally, sedation caused by neuroleptic's antihistaminic abilities also interferes with patients' quality of life.

1.6 ATYPICAL ANTIPSYCHOTICS

1.6a History

The first atypical antipsychotic, clozapine, was synthesized by a Swedish pharmaceutical company in 1958 (Crilly, 2007). Clozapine differed radically from other first generation antipsychotics during its time; its low propensity for EPS was initially met with high criticism from the medical community, since it was believed that EPS was a compulsory side-effect of drugs with antipsychotic potential (Crilly, 2007). However, reports of clozapine-treated patients developing agranulocytosis, a deficiency of white blood cells, negatively impacted its use as a therapeutic agent (Idanpaan-Heikkila et al, 1975). Interest in clozapine reemerged a decade later, when a clinical study demonstrated clozapine to have a higher efficacy than traditional antipsychotics in attenuating symptoms in treatment-resistant patients (Kane et al, 1992; Breier et al, 1994). Clozapine was also the first antipsychotic drug to demonstrate significant attenuation of negative symptoms and schizophrenic-related cognitive deficits (Meltzer, 1994). Though still used today, its use is limited to severe and treatment-resistant cases. Since clozapine, several other atypical antipsychotics have been developed within the last two

decades: risperidone (Risperidal), olanzapine (Zyprexa), ziprasidone (Geodon), sertindole (Flexyx), aripiprazole (Abilify), quetiapine (Seroquel), and asenapine (Saphris).

1.6b Classification of Atypical Antipsychotics

Unlike first generation antipsychotics, atypicals are classified by their pharmacodynamic properties; chiefly their shared mechanism of serotonin 5-HT_{2a} and dopamine D₂ receptor antagonism. Serotonergic modulation is not unique to atypicals; several traditional antipsychotics, such as chlorpromazine, also antagonize 5-HT_{2a} receptors. The distinguishing feature of atypicals from original neuroleptics is their higher affinity for 5-HT_{2a} receptors over D₂ receptors.

1.6c Clinical Efficacy

Clinically, atypicals have two important properties that differ from traditional antipsychotics: First, they are generally perceived to be more efficacious in attenuating schizophrenic-like symptoms, particularly negative and cognitive impairments (Leucht et al, 2003). This superior clinical profile of atypicals has been attributed to their capacity to increase DA release within the cortex and hippocampus via modulation of serotonergic neurotransmission (Meltzer et al, 2003). Second, they are associated with a lower incidence of EPS and other adverse side-effects than traditional antipsychotics. This effect has been linked with their actions on serotonin receptor sites and their receptor heterogeneity—features that are thought to enable atypicals to produce a therapeutic response with lower D₂ receptor occupancy than traditional antipsychotics (Newman-Tancredi & Kleven, 2011).

1.6d Mechanism of action: Dopamine D2 Receptor Blockade or Partial Agonism

Similar to first generation antipsychotics, most atypicals are DA D2 receptor antagonists, with the notable exception of aripiprazole, which is a DA D2 partial agonist (See section 1.4). Although the therapeutic window of optimal D2 receptor occupancy is not as clear for atypicals, they can produce an antipsychotic effect with less D2 receptor occupancy than conventional neuroleptics (Seeman et al, 1997). In addition, atypicals exhibit faster dissociation rates from the DA D2 receptor (Seeman et al, 1997; Pani et al, 2007). This feature has been the focus of much attention within the literature, and it has been argued to be a fundamental property in producing an “atypical” therapeutic effect: Atypicals can bind to DA D2 receptors long enough to produce an antipsychotic effect, but not long enough to induce EPS (Feldman et al, 2007). It has also been speculated that drugs with faster dissociation rates may be more effective in attenuating DA transmission because they are more sensitive to transient dopaminergic activity (Pani et al, 2007). In fact, the mechanism of action of the DA D2 partial agonist, aripiprazole, is dependent on the endogenous level of dopamine near the synapse: Aripiprazole acts as an agonist when DA concentrations are low, and an antagonist when DA concentrations are high (Sharpiro et al, 2003).

The degree to which atypical-induced changes in DA activity alone contribute to their superior clinical profile is still under debate (Feldman et al, 2007). Nonetheless, all antipsychotic drugs possess some ability to modulate DA neurotransmission, and this appears to be essential in producing an effective therapeutic response.

1.6e Mechanism of action: Dual 5-HT2a Receptor Antagonism and D2 Receptor Blockade

Most published literature has attributed the low EPS liability of atypicals to the combined effects of potent 5-HT2a antagonism and weak D2 antagonism (for review, Meltzer et al, 2003). A 5-HT2a antagonist, M100907, was able to prevent haloperidol-induced catalepsy, but only when haloperidol was administered at low doses (Ishikane et al; 1997). It is theorized that 5-HT2a antagonism potentiates DA D2 receptor blockade within the mesolimbic pathway but does not affect the nigrostriatal pathway (Newman-Tancredi & Kleven, 2011). Furthermore, 5-HT2a antagonism of DA transmission may explain why atypical antipsychotics can produce therapeutic responses at lower D2 receptor occupancy than traditional neuroleptics (Newman-Tancredi & Kleven, 2011).

Dual 5-HT2a and D2 receptor antagonism is also thought to be responsible for enhanced DA transmission in the frontal cortex. Administration of the 5-HT2a and 5-HT2c antagonist, ritanserin, did not affect dopaminergic activity when administered alone, but increased DA activity in the rat medial prefrontal cortex (mPFC) when combined with the D2 antagonist raclopride (Anderson et al, 1995). Westerink et al (2001) found similar results with the selective 5-HT2a antagonist, M100907, which had no significant effects alone but potentiated the effects of raclopride to increase DA release in the mPFC.

Recently, research has increasingly focused on the importance of 5-HT1a receptor stimulation by antipsychotics. Several studies have shown that agonism of 5-HT1a receptors can increase DA release in cortical areas (Bardwin et al, 2007; Newman-Tancredi & Kleven, 2010; Bortolozzi et al, 2007; Bortolozzi et al, 2010). An elegant study by Bortolozzi et al (2010) showed antipsychotic-induced

increases of cortical DA is abolished in 5-HT1a KO mice, but not 5-HT2a KO mice. Likewise, the 5-HT1a antagonist, Way 100835, blocked atypical-induced DA release in the medial frontal cortex of rats (Ichikawa, 2001). Some studies have also suggested that 5-HT2a antagonism stimulates 5-HT1a receptors (Newman-Tancredi & Kleven, 2011). For example, olanzapine and risperidone are 5-HT2a antagonists, but have been shown to indirectly activate 5-HT1a receptors to increase DA release in the frontal cortex (Ichikawa et al, 2001).

Although the function and relationship between 5-HT1a and 5-HT2a receptors is not fully understood, both receptors have been implicated in regulating dopaminergic activity by enhancing DA transmission in the prefrontal cortex—an effect that may alleviate schizophrenic-induced hypofrontality (Bortolozzi et al, 2010; Newman-Tancredi & Kleven, 2011). In a double-blind clinical trial, ritanserin was shown to enhance the effect of risperidone in treating negative symptoms (Akhondzadeh et al, 2008). Primavanserin, an inverse 5-HT2a antagonist, has also demonstrated potential in increasing the clinical efficacy of risperidone (Abbas & Roth, 2008). Anxiolytics busprione and tandospirone, selective partial 5-HT1a agonists, have been reported to improve cognitive and negative symptoms in clinical trials when administered alone (Akimova et al, 2009).

1.6f Adjunctive use of antipsychotics in Depression

It should be noted that it has become increasingly common to prescribe low doses of atypicals as an adjunctive treatment in major depressive disorder (Biler & Blondeau, 2011; Khan, 2011). Clinical trials have shown atypicals to augment the clinical efficacy of serotonin reuptake inhibitors (SSRIs; Ostroff & Nelson 1999; McIntyre et al, 2007; Mahmoud et al, 2007). In 2007, Aripiprazole was the first antipsychotic drug approved by the FDA for adjunctive treatment in depression, followed two years later by quetiapine (Sequel). Symbyax—a

combination of the atypical antipsychotic Olanzapine (Zyprexa) and SSRI fluoxetine (Prozac)—has earned FDA approval for use as monotherapy in unipolar depression.

Because the doses of atypicals used in adjunctive treatment do not induce marked antagonism of D2 receptors, it is assumed that their effects on other receptors, particularly serotonergic receptor targets, are responsible for enhancing SSRI-induced therapeutic effects (Biler & Blondeau, 2011; Biler & Szabo, 2003). The beneficial effect of atypical and SSRIs may be related to their effects on norepinephrine (NE) neurotransmission (Biler & Blondeau, 2011; Khan, 2008). Electrophysiological studies have shown that atypicals reverse SSRI-induced reductions in NE neuronal firing activity (Chernoloz et al, 2009; Dremencov et al, 2007), and it is hypothesized that atypicals may increase NE in the prefrontal cortex via 5-HT_{2c} antagonism (Millan et al, 2003).

1.6g Side-effects

Clinical trials have repeatedly reported fewer incidences of EPS, tardive dyskinesia and hyperprolactinaemia induced by atypicals than conventional antipsychotics (Leuchet et al, 2003; Davis et al, 2003). Although atypical antipsychotics are linked to fewer neurological side-effects, they are associated with adverse metabolic changes that are somewhat unique to their drug class (Melkersson et al, 2004). Weight gain is a side-effect of both first and second generation antipsychotics, but occurs more frequently with atypicals, particularly olanzapine and clozapine (Lieberman et al, 2005; Baptista et al, 2008). However, clinical studies have found aripiprazole and ziprasidone to have a lower propensity to induce weight gain than other atypical antipsychotics (Zimbroff et al, 2007; Lieberman et al, 2004; Baptista et al, 2008). In

addition, lipid abnormalities and elevated blood glucose levels have also been reported, and are related to an increased risk of developing Diabetes mellitus (Baptista et al, 2008).

1.7 ARIPIPRAZOLE

Aripiprazole (ARZ) is classified as an atypical antipsychotic, though its mechanism of action is uniquely different from other neuroleptics. It is the first antipsychotic approved for clinical use that acts as a partial agonist at DA D2 receptors (Burriss et al, 2002). This attribute allows ARZ to normalize DA transmission according to the endogenous levels of DA; exerting agonist activity when surrounding extracellular concentrations of DA are low, and antagonist activity when DA levels are high. In addition, ARZ has demonstrated partial agonistic activity at 5-HT1a receptors and antagonism at 5-HT2a receptors (Jordon et al, 2002). Due to these properties, ARZ has often been referred to as a *dopamine-serotonin stabilizer* (Schatzberg et al, 2010; Burriss et al, 2002). In theory, ARZ's pharmacological characteristics support its use beyond treating psychosis. As previously mentioned, ARZ was approved as an adjunctive treatment for unipolar depression in 2007, and ongoing research is currently evaluating its usefulness in substance and alcohol abuse (Backstrom et al, 2010; Ingman et al, 2006).

1.7a Pharmacological Profile

ARZ is a potent partial agonist at dopamine D2 and D3 receptors, with moderate affinity for D4 receptors (Burriss et al, 2002; Jordon et al, 2002; Sharpiro et al, 2003). Receptor binding studies indicated ARZ is capable of both pre- and post- synaptic stimulation of D2 receptors (Kikuchi et al, 1995; Sharpiro et al, 2003). ARZ has affinity for several serotonin receptors; it is a potent 5-HT1a partial agonist, as well as a potent 5-HT2a and 5-HT2b antagonist. ARZ also shows moderate affinity for 5-HT2c and 5-HT7 receptor sites (Jordon et al, 2002; DeLeon et al,

2004). However, unlike other atypical drugs, ARZ has a low 5-HT₂:D₂ affinity ratio (Sharpiro et al, 2003; Kessler, 2007). Additionally, ARZ binds with moderate affinity to alpha-adrenergic and histamine H₁ receptors (Sharpiro et al, 2003; Kessler, 2007; DeLeon et al, 2004).

Animal studies have demonstrated ARZ's ability to mediate D₂-neurotransmission. Under hyperdopaminergic conditions, ARZ acts as a dopamine antagonist, blocking apomorphine and AMPH-induced stereotypies and locomotor activity (Backstrom et al, 2010; Nordquist et al, 2008; Fowler et al, 2008; Kikuchi et al, 1995). When administered alone, ARZ did not induce stereotypy or increase locomotion (Nordquist et al, 2008, Backstrom et al, 2010).

1.7b Clinical Profile

Several randomized, double-blind clinical trials found ARZ to be significantly more effective than placebo in attenuating schizophrenia-related symptoms (DeLeon et al, 2004; Kane et al, 2002; Potkin et al, 2003). ARZ also exhibited similar effectiveness to risperidone and haloperidol on positive and negative symptom rating scales (PANSS; Kane et al, 2002; Potkin et al, 2003; Schatzberg et al, 2010). Other clinical studies have demonstrated its usefulness in treating acute mania in bipolar disorder (Young et al, 2009; Lyseng-Williamson & Perry, 2004). ARZ was found to be significantly more effective than placebo or haloperidol in treating acute mania, and was confirmed to significantly delay the onset of relapse compared to haloperidol (Young et al, 2009; Lyseng-Williamson & Perry, 2004).

Currently, ARZ—along with quetiapine—are the only atypical drugs approved by the FDA for adjunctive treatment in unipolar depression. Clinical studies have reported that adjunctive treatment of ARZ significantly reduced depressive symptoms and increased remission relative

to placebo (Marcus et al, 2008; Berman et al, 2007). It is postulated that ARZ's effects on serotonergic receptors are key to enhancing the therapeutic effect of SSRIs (Pae et al, 2008).

Compared to other atypicals, ARZ may possess a superior side-effect profile. ARZ has a low propensity to induce EPS, weight gain, and cardiovascular abnormalities (DeLeon et al, 2004). In contrast to other neuroleptics, ARZ does not induce hyperprolactinemia (DeLeon et al, 2004; Schatzberg et al, 2010). Commonly reported side effects during clinical trials included headache, akinesia and agitation (Schatzberg et al, 2010, Blier & Blondeau, 2011).

1.8 INTERM SUMMARY

While traditional antipsychotics are therapeutically effective at reducing positive symptoms (e.g., hallucinations, delusions), they lack efficacy in attenuating negative symptoms—in particular, cognitive impairments related to executive functioning (Andreasen et al, 1995). The unique pharmacological profile of ARZ may prove useful in treating SZ-related cognitive disorders; as a dopamine-serotonin stabilizer, ARZ may attenuate hypofrontality by increasing and decreasing dopaminergic transmission in prefrontal and subcortical areas, respectively (Burriss et al, 2002; Jordan et al, 2002). Neurocognitive studies have reported ARZ to ameliorate working memory deficits in schizophrenic or schizo-affective individuals (Levoyer et al, 2007), as well as to increase in verbal learning (Kern et al 2006). While few in number, behavioral pharmacological studies have found that ARZ administration in rats improved cognitive function in areas such as attention, recognition memory, and spatial learning (Carli et al, 2010; Nagai et al, 2009; Nordquest et al, 2008; Burda et al, 2011).

The experiments presented in later chapters (Chapters 2-4) were intended to evaluate the ability of ARZ to attenuate schizophrenic-like cognitive impairments in an animal model of psychosis. In the next section, different pharmacological models of schizophrenia are presented, as well as the rationale for choosing the psychostimulant, d-amphetamine, to model a putative schizophrenic-like state in rats for our current study. In Chapter 2, the selection of interval timing as a cognitive measure in our studies is discussed, including its relation to schizophrenia, dopaminergic transmission, and usefulness in measuring overall cognitive function in test subjects. In the remaining chapters, the therapeutic-like efficacy of ARZ is evaluated in drug-free and AMPH-treated rats trained to perform an operant timing schedule.

1.9 PHARMACOLOGICAL ANIMAL MODELS OF SCHIZOPHRENIA

In hopes of delineating the pathophysiology and etiology of schizophrenia, several animal models have been developed using surgical, pharmaceutical and genetic approaches to mirror the behavioral phenotype and neurological changes that occur in the human form of the disease. These models have been evaluated in terms of their ability to produce schizophrenic-like symptoms (face validity), identify effective therapeutic agents (predictive validity), and abide by a specific etiological hypothesis (etiological validity; Young et al, 2010; Marcotte et al, 2001).

Due to the complex heterogeneity of schizophrenia, it is difficult, if not impossible, to produce the full spectrum of the disorder within animals. Even amongst schizophrenic individuals there is substantial variation in the severity and behavioral expression of schizophrenic symptoms. However, the face validity of these models has been evaluated in

animal behavioral studies designed to evaluate deficits in specific cognitive or sensory paradigms where schizophrenic individuals are known to be impaired.

A notable animal model of schizophrenia is the neonatal ventral hippocampus (VH) lesion model (Lipska & Weinberger, 2000), where lesions interfere with the development of the hippocampus and its projections to the prefrontal cortex. These excitotoxic lesions in the rat or monkey hippocampus have been shown to induce abnormalities in DA-related behaviors (e.g., increased responsiveness to psychostimulants, impaired social functioning) during early adulthood (Sams-Dodd et al, 1997; Lipska & Weinberger, 2000; Lipska et al, 1993). However, the most frequently employed models of schizophrenia utilize psychotomimetic pharmacological compounds to induce a presumed psychotic-like state in animals (Young et al, 2010; Marcotte et al, 2001; Bubeníková-Valesová et al, 2008). These drug models were inspired, in part, by the schizophrenic behavioral phenotype they induce in nonpsychotic human individuals (face validity). Furthermore, these animal models have demonstrated, to an extent, predicative validity in evaluating the therapeutic value of antipsychotics as well as experimental compounds (Young et al, 2010; Marcotte et al, 2001). The most commonly utilized drugs to model schizophrenia are psychostimulants, non-competitive NMDA receptor antagonists, and serotonin 5-HT_{2a} agonists (Young et al, 2010; Bubeníková-Valesová et al, 2008).

1.9a Psychostimulants

Psychostimulants (cocaine, AMPH, apomorphine) are the most commonly used drug class to produce psychotic-like symptoms in animals. As direct (apomorphine) and indirect

(cocaine, AMPH) dopamine agonists, they are useful for examining the therapeutic effects of drugs with D2 receptor antagonistic properties (Young et al, 2010). Their etiological validity is derived from the dopamine hypothesis (Section 1.3).

Acute administration of psychostimulant drugs in rodents can produce hyperlocomotion at moderate doses and stereotypies at high doses (Young et al, 2010; Fowler et al, 2007; Marcotte et al, 2001). Because these effects are induced by enhanced dopaminergic transmission within mesolimbic and nigrostriatal structures, they are thought to model positive-like symptoms (Mavrikaki et al, 2010; Backstrom et al, 2010). In accordance with the dopamine hypothesis, antipsychotics have been shown to dose-dependently antagonize psychostimulant-induced hyperlocomotion and stereotypies in rats (Backstrom et al, 2010; Nordquist et al, 2008; Fowler et al, 2007; Fowler et al, 2011; Pijnenburg et al, 1975).

Similar to schizophrenic subjects, psychostimulant-treated animals exhibit prepulse inhibition (PPI) deficits in sensory motor paradigms (Swerdlow et al, 1994; Nordquist et al, 2008; Wan & Swerdlow, 1993). It is hypothesized that psychostimulant-induced PPI impairments are primarily caused by D2 receptor activation (Wan & Swerdlow, 1993; Wan et al, 1996). For example, treatment with the D2 receptor agonist, quinpirole, but not the D1 agonist, SKF 38393, induced PPI deficits in rats (Swerdlow et al, 1993). Furthermore, pretreatment with antipsychotic drugs ameliorated apomorphine-induced PPI deficits, and this effect was positively correlated with D2 receptor affinity (Young et al, 2010; Wan & Swerdlow, 1993; Swerdlow et al, 1994).

In addition, psychostimulant treatment has been shown to interfere with temporal processing and impair performance on operant timing schedules (Fowler et al, 2009, Balcells-Olivero et al, 1997; Cheung et al, 2007; Meck, 1983). Specifically, psychostimulant administration distorts temporal processing by causing animals and humans to perceive temporal intervals as longer than baseline temporal perceptions (Fowler et al, 2009, Balcells-Olivero et al, 1997; Cheung et al, 2007; Meck, 1983). Clinical studies have found schizophrenic individuals to exhibit similar distortions in temporal processing (Rammsayer, 1990; Tysk, 1990). In the next chapter (Chapter 2), the role of dopamine in temporal processing is discussed in detail, as well as the operant timing schedules that can be used to assess timing behavior.

Cognitive impairments related to attention have been reported following repeated AMPH treatment. Martinez & Sarter (2008) found rats pretreated with low doses of AMPH exhibited significant impairments in an operant-sustained attention task relative to controls, and that haloperidol and clozapine significantly attenuated AMPH-induced disruption in comparison to saline. Similarly, Brockel and Fowler (1995) found the chronic administration of haloperidol (0.02-0.12mg/kg) over 23 days in rats prevented AMPH-induced (2.0mg/kg) increases in errors of omission in a type of sustained attention task. Another study found repeated administration (3 times a week for 5 weeks) with AMPH, but not PCP, produced deficits in an attentional set-shifting task in rats (Fletcher, 2005). Fletcher and colleges (2005) also found AMPH-induced impairments were reversed by intracranial infusion of the D1 agonist, SKF38393, into the medial prefrontal cortex. Similarly, SKF38393 attenuated deficits induced by AMPH sensitization in rats performing a 5-choice serial time reaction task (Fletcher, 2007).

1.9b Phencyclidine and other NMDA Receptor Antagonists

Non-competitive NMDA receptor antagonists (ketamine, PCP, MK-801) have emerged as a popular model of psychosis in contemporary behavioral pharmacological research (Amitai et al, 2007; Olney & Farber, 1995; Stone et al, 2007). From this drug class, phencyclidine (PCP) is the most frequently employed compound used to model psychosis in animal studies. Similar to AMPH and other dopamine agonists, acute administration of PCP induces hyperlocomotion and stereotypies when administered at high doses (Fowler et al, 2003; Lehman-Mastan & Geyer, 1991; Benneyworth et al, 2011), which can be attenuated by the administration of antipsychotics (Murray, 1973; Jackson et al, 1994). These findings imply that PCP, similar to AMPH, also exerts some of its effects on dopaminergic transmission (Murray, 1973). It should be noted, however, that the expression of hyperlocomotion and stereotyped behaviors are distinctly different for each drug. At high doses, PCP can induce specific locomotor stereotypies in animals not seen following AMPH treatment, such as backpeddling, repetitive circling/rotational behavior and ataxia (Murray, 1973; Lehman-Masten & Geyer, 1991; Castellani & Adams, 1981).

While both psychostimulants and NMDA receptor antagonists produce positive-like schizophrenic symptoms in animals (e.g., hyperlocomotion, stereotypies), PCP and its related compounds are claimed to induce the “full spectrum” of the disorder. Ketamine and PCP have been shown to induce social withdrawal in animals; a now increasingly popular model of negative symptoms (Sams-Dodd, 1995; Lee et al, 2005). In addition, NMDA receptor antagonists can induce schizophrenic-like cognitive impairments, including reduced attentional

performance (Amitai et al, 2007), abnormal sensorimotor gating (Mansbach, 1991), disruption of reversal learning (Abdul-Monim et al, 2003; Abdul-Monim et al, 2006; Jentsch & Taylor, 2001), decreased recognition memory (Nagi et al, 2009), and altered timing behaviors (Sanger, 1992; Wiley & Willmore, 2000; McClure & McMillan, 1997; Sanger & Jackson, 1988).

Furthermore, it has been proposed that chronic—rather than acute—exposure of PCP produces an animal model of psychosis that better reflects the neuropathological and clinical abnormalities seen in the disease (Jentsch & Roth, 1999; Morris et al, 2005), and that these drug-induced changes persist during and after withdrawal (Jentsch et al, 1997, Mouri et al, 2007). Long-term exposure to NMDA antagonists have been reported to decrease regional cerebral blood flow and reduce dopamine utilization in the cortex (Jentsch et al, 1997; Jentsch & Roth, 1999); a state that may be useful for modeling hypofrontality in animals (Jentsch & Roth, 1999). Repeated exposure to PCP has also been shown to sensitize responses to AMPH, indicating sensitization of dopamine systems within the mesolimbic pathway (Jentsch et al, 1997).

Jentsch et al (1997) were one of the first teams to utilize a “subchronic” dosing model using PCP. In the following experiment, investigators trained vervet monkeys to perform a variation of the object retrieval task—a behavioral paradigm dependent on prefrontal lobe functioning. Monkeys were then administered 0.3mg/kg PCP bi-daily for 14 days, and given a drug-free week before experimental testing resumed. Jentsch and colleagues (1997) found sub-chronic PCP treatment significantly impaired dopaminergic function within the prefrontal cortex and impaired task performance in the object retrieval task. Since Jentsch’s finding,

variations of the subchronic dosing model are becoming increasingly utilized to produce models of schizophrenic-like cognitive deficits in rats and monkeys (Abdul-Monim et al, 2003; Abdul-Monim et al, 2006; Jentsch & Taylor, 2001; Rodefer et al, 2005; Enomoto et al, 2005; Mandillo et al, 2003; Elsworth et al, 2011). Several of these studies have also found atypical antipsychotics, but not traditional antipsychotics, to attenuate cognitive impairments (Abdul-Monim et al, 2003; Abdul-Monim et al, 2006; Rodefer et al, 2008; Nagai et al, 2009).

Despite the usefulness of NMDA receptor antagonists—particularly, PCP—to induce a wide range of putative psychotic-like symptoms in animals, a few studies have been published criticizing their etiological validity (Seillier & Giuffrida, 2009; Gilmour et al, 2009). Seillier & Giuffrida (2009) demonstrated withdrawal effects from sub-chronic treatment of PCP, but not MK-801, manifested social withdrawal and working memory deficits in variable-delayed alternation task. These studies suggest that PCP may induce cognitive deficits and schizophrenic-like symptoms through mechanisms other than, or in combination with, NMDA receptor antagonism.

1.9c LSD and other Serotonergic Hallucinogens

Support for serotonin-based theories of SZ was initially prompted by the similarities of schizophrenic symptoms to LSD-induced behavioral effects (Hoffmann, 1968). However, it is now thought that LSD induces symptoms that mimic the early stages of SZ symptoms, such as euphoria, exhilaration, and a lost sense of self (Keeler et al, 1965; Gouzoulis-Mayfrank et al, 1998a). Other 5-HT_{2a} agonists, such as psilocybin, mescaline, and *N,N*-dimethyltryptamine (DMT), have also been reported to induce psychotic symptoms similar to SZ (Gouzoulis-

Mayfrank et al, 1998b). These drugs have been shown to disrupt sensory gating measures in animals and humans, inducing deficits on PPI and habituation tasks (Krebs-Thomson et al, 2006; Johansson et al, 1995; Vollenweider et al, 2007; Geyer & Vollenweider, 2008). These effects are hypothesized to occur through stimulation of 5-HT_{2a} receptors (Geyer et al, 1998; Marcotte et al, 2001). Interestingly, it is thought that NMDA receptor antagonists induce PPI deficits by indirect stimulation of 5-HT_{2a} receptors (Geyer et al, 1984; Marcotte et al, 2001).

However, the usefulness of serotonin animal drug models is weakened by findings demonstrating rapid tolerance to the psychotomimetic properties of LSD and related serotonergic hallucinogens (Hollister, 1968; Marcotte et al, 2001). These effects conflict with clinical observations of SZ symptoms, which usually occur throughout the afflicted individual's lifetime. In addition, LSD predominately induces visual hallucinations, whereas schizophrenic individuals tend to experience auditory hallucinations (Gouzoulis-Mayfrank et al, 1998a).

1.10 SUMMARY

Out of the 3 most popular pharmacological models, we chose to utilize the psychostimulant, AMPH, to induce a psychotic-like state in animals. The use of LSD and other serotonergic drugs were not an ideal model, as little evidence supports serotonin as the primary disrupted neurotransmitter in SZ. In addition, tolerance rapidly develops with chronic LSD treatment (Hollister, 1968; Marcotte et al, 2001) whereas repeated administration of NMDA receptor antagonists and psychostimulants induce sensitization in animals (Jentsch et al, 1997; Fletcher, 2005; Fowler et al, 2009).

Previous findings within our lab have found acute doses of PCP to induce variable behavioral effects across subjects (Latif et al, 2010). When administered at moderate to high doses, PCP –treated rats vary in the expression of stereotypy behavior (Latif et al,2010). Within our lab, we evaluated the effects of 2.0, 4.0, and 8.0mg/kg PCP in rats trained to perform the DRL-72 operant schedule. At the 4mg/kg and 8mg/kg doses, individual differences were observed in the onset and duration of operant responding. Following the 4mg/kg dose, responding in some rats was suppressed during the first half of the session (n=7), suppressed during the latter half (n=2), completely suppressed (n=1), or not at all (n = 13). Indeed, similar findings of response inconsistency have been reported in DRL behavior following acute PCP treatment (Sanger, 1992; Sanger, 1988; Freeman et al, 1984). In Wistar rats treated with 4mg/kg PCP, Sanger reported that PCP-induced effects on response rates were inconsistent across rats, with some demonstrating increases and others decreases (Sanger, 1988). In a chronic PCP dosing experiment, Freeman et al, reported that response rates varied in ICR mice when administered their first dose of 10mg/kg PCP, with 7 mice demonstrating mild to substantial increases in responses, and 2 mice showing a strong suppression of responding. While this variability in both response rate and the occurrence of operant responding in DRL schedules is an interesting phenomenon, it suggests PCP has low reliability in terms of inducing similar behaviors across subjects.

We have previously shown AMPH to induce timing deficits similar to schizophrenic individuals on the DRL-72 task (Fowler et al, 2008; Fowler et al, 2009). Following sensitization, AMPH produces reliable, consistent effects on behavior across subjects (Fowler et al, 2008; Fowler et al, 2009). Additionally, AMPH has strong predictive validity in evaluating the

therapeutic efficacy of DA-related drugs (Young et al, 2010). Thus, the AMPH model of schizophrenia was chosen to evaluate the effects of the partial D2 agonist, aripiprazole, on the timing behavior in rats.

CHAPTER 2: TIMING BEHAVIOR IN ANIMALS: THE ROLE OF INTERVAL TIMING

2.1 INTRODUCTION

Our subjective experience of time influences cognitive, emotional, and motor-related components of behavior. It is evident that the capacity to process time and temporal durations is essential to engaging in biologically relevant behavior (Hinton & Meck, 1997). Most organisms possess the cognitive ability to translate or represent the temporal characteristics of their environment (Fetterman & Dreyfus, 1987; Taylor, 2004). Long-term durations, such as seasonal cycles and circadian rhythms, inform animals when to mate, hibernate, or forage for food (Taylor et al, 2004; Hinton & Meck, 1997). Shorter durations, in the second to milliseconds range, influence speech and motor-related activity (Hinton & Meck, 1997).

Activities that are guided within the seconds to minutes range (interval timing) allow animals to utilize environmental contingences and stimuli to predict the outcomes of future events (Nevin & Reynolds, 1973; Staddon & Cerutti, 2003; Hinton & Meck, 1997). The regulation of behavior involving short temporal durations (second to minutes) has been extensively investigated in operant conditioning studies that have trained animals to perform time-based schedules of reinforcement (Nevin & Reynolds, 1973; Gallistel and Gibbon, 2002).

2.2 INTERVAL TIMING

Interval timing is the cognitive ability to estimate short temporal durations (in the seconds to minute range) with a precision well above random performance. The capacity to estimate brief intervals governs key components of behavior and decision-making, allowing

organisms to process relevant stimuli and anticipate the outcome of future events (Hinton & Meck, 1997; Balci et al, 2009). Research on optimal foraging behavior has strongly supported the notion that animals utilize interval timing when judging the costs and benefits of acquiring and consuming food (Bateson, 2003; Hills & Adler, 2002; Matell & Meck, 2000). In addition, interval timing influences the subjective value of delayed rewards and affects the occurrence and rate of operant responding (Gallistel & Gibbon, 2000; Gallistel et al, 2004). Indeed, interval timing exerts subtle but pervasive influences on daily functioning, regulating behavior in routine tasks such as estimating when a television program will return from commercial, or choosing which grocery checkout lane will lead to the quickest end to a shopping episode. Thus, interval timing allows organisms to process environmental contingencies to predict relationships between significant events and responses (Balci et al, 2009).

2.2a Impairments of Interval Timing in Schizophrenic Subjects

It has been postulated that its functional dependence on other cognitive processes, such as attention and memory, may allow the experimental examination of interval timing to serve as a window into overall cognitive functioning (Meck, 1996, Balci et al, 2009). In recent decades, interval timing has been useful in characterizing cognitive deficits in neurological diseases, aging, and drug-intoxicated states (Balci et al, 2009). Clinical studies have reported temporal processing deficits in several dopamine-related neurological disorders, such as Parkinson's disease (Lange et al, 1995; Malapani et al, 1997), schizophrenia (Carroll et al, 2008, Elveag et al, 2003; Rammsayer, 1990) and Huntington's disease (Paulsen, 2004).

When administered time production and estimation tasks, schizophrenic subjects have been shown to “overestimate” the passage of time, and perceive temporal durations as longer than objective time (Rammsayer, 1990; Tysk, 1983). Other studies have found schizophrenic subjects to have lower accuracy and increased variability when producing and estimating temporal intervals relative to healthy controls (Carroll et al, 2008; Elveag et al, 2003). Additionally, schizophrenic individuals have exhibited deficits in both auditory and visual timing tasks (Tysk, 1990; Tysk, 1983; Schwatz et al, 1988; Carroll et al, 2008).

However, the methodologies and experimental procedures employed in these studies have been criticized in the literature (Davalos et al, 2005). Some studies required subjects to attend to both temporal and non-temporal information; a condition that may have influenced performance on timing estimation tasks (Davalos et al, 2005; Poynter & Homa, 1983). For example, in subjects estimating the duration of visual stimuli presented on a light board, Poynter & Homa (1983) suggested the complexity of visual stimuli in the experimental procedure affected the accuracy of judgment durations. Thus, temporal processing deficits found in schizophrenic subjects in visual timing tasks may be related to other cognitive impairments (e.g., reduced attentional performance) present in the disease. Another limitation is that the medication regime of schizophrenic subjects was either not addressed or described in detail by the experimenter (Tysk, 1983; Tysk, 1990; Rammsayer, 1990; Schwatz et al, 1988). It is possible that antipsychotic administration may have influenced temporal processing in schizophrenic individuals, because antipsychotic treatment in preclinical studies has been shown to alter timing performance in animals performing timed schedules (Meck, 1996; Meck 2006). Overall, little work has been done to assess timing deficits in unmedicated schizophrenic

individuals, or if significant differences in temporal processing exist between medicated and unmedicated patients.

2.2b The Role of Dopaminergic Neurotransmission in Temporal Processing

Extensive work in preclinical studies has demonstrated the sensitivity of interval timing to pharmacological modulation of dopaminergic neurotransmission. The administration of psychostimulants and dopamine agonists, such as cocaine (Chang et al, 2006), d-amphetamine (Fowler et al, 2009; Balcells-Olivero et al, 1997), metamphetamine (Meck, 1983; Marcicq and Church, 1983) and quinpirole (Cheng et al, 2007) produce premature responding in animals performing timed operant schedules, and induce a leftward shift of timing response functions. These findings have led investigators to conclude that pharmacological enhancement of dopaminergic transmission cause subjects to overestimate the passage time, and perceive durations as longer than baseline temporal perceptions. Conversely, dopamine antagonists, such as haloperidol (Marcq & Church, 1983; Buhusi & Meck, 2001) are thought to have the reverse effect, producing a rightward shift of timing functions and causing subjects to perceive temporal intervals as shorter than normal temporal perceptions.

Additional support for dopamine's involvement in interval timing has been described in lesion studies. Meck et al (2006) found lesions within subcortical dopaminergic cell bodies (e.g., Substantia nigra, SN) or their projections to the striatum (e.g., Caudate-Putamen, CPu; nucleus accumbens, NAS) can disrupt temporal processing. Meck (2006) investigated the performance of rats with lesions in the SN or CPu in a bi-section procedure, which requires subjects to define durations as either "short" (e.g., right lever press) or "long" (e.g., left lever press). While both

SN and CPu lesions abolished temporal regulation in rats, the administration of L-DOPA restored timing behavior in SN lesioned rats but not rats with CPu lesions (Meck et al, 2006), suggesting intact dopaminergic functioning in the striatum is critical for temporal processing. Similarly, imaging studies have highlighted the importance of the basal ganglia in interval timing (Jahanshai et al, 2006; Coull & Nobre, 2008). A PET study reported increased activity in the left caudate nucleus and right putamen during time reproduction tasks of 500 milliseconds and 2 seconds, respectively (Coull & Nobre, 2008). The implicated role of dopamine and striatal functioning in interval timing is consistent with the idea that dopamine-dysregulation induces temporal processing deficits in schizophrenia, as well as Parkinson's disease and Huntington's disease.

2.3 EXPERIMENTAL ASSESSMENT OF TIMING BEHAVIOR IN ANIMALS

2.3a Operant Conditioning Schedules

Operant conditioning is a type of instrumental learning where an animal learns about environmental contingencies to receive a reward (Richelle and Lejeune, 1980; Staddon & Cerutti, 2003; Nevin & Reynolds, 1973). However, operant conditioning is distinct from other forms of instrumental learning in that it requires a subject to emit a specific response (e.g., lever press, nose poke, key peck) to obtain a reward (Davey, 1981). Several different *schedules of reinforcement* are available to examine operant behavior. In these types of schedules, a reward is delivered only if a response conforms to specific contingencies determined by the schedule selected by the experimenter (Staddon & Cerutti, 2003; Richelle and Lejeune, 1980). For example, *fixed ratio* schedules require a set number of responses in order to obtain a

reward. Thus, a schedule of reinforcement dictates specific response requirements for receiving a reward. (Davey, 1981).

Several operant schedules are useful for examining timing behavior in animals, and have provided key insights into the variables that govern interval timing mechanisms. The schedules discussed below require animals to regulate the timing of their responses (*temporal regulation*), estimate a temporal duration of a presented stimulus (*temporal discrimination*), or both (Davey, 1981; Gallistel and Gibbon, 2004; Gallistel and Gibbon, 2000; Richelle & Lejeune, 1980).

2.3b Fixed Interval Procedure

In this operant schedule, reinforcement is delivered on the first response made after a specified duration has passed (e.g., 1 min). Responses that occur before the end of the required duration do not produce a reinforcer and have no effect on the schedule's contingences. Fixed Interval (FI) schedules generate low response rates near the beginning of the specified interval, and rates gradually increase towards the end of the set duration; a pattern that is referred to as *scalloping* (Skinner, 1938). However, some subjects cease responding at the beginning of the interval, and resume responding at a steady rate for the remainder of the duration (Schneider, 1969). Schneider (1969) coined this response pattern as *break-and-run*. It is unknown what factors induce either scalloping or break-and-run response patterns, but the capacity to sense the passage of time is presumed to be one factor influencing FI behavior.

A focal point of analysis in FI operant performance is the time a subject waits to resume responding during the specified duration (Hinton & Meck, 1997; Richelle & Lejeune, 1980). This behavior is thought to reflect a temporal regulation of responses, where longer pauses in responding after the onset of the required interval are associated with better timing behavior (Richelle & Lejeune, 1980). Furthermore, the resumption of responding after reinforcer delivery is influenced by the length of the required interval: as the length of the duration increases, the initial pause at the beginning of the interval also increases (Schneider et al, 1969).

2.3c Peak Interval Procedure

Under FI schedules, operant responding usually occurs before the required duration has passed and terminates once the reinforced response is made. Thus, important information on the subject's ability to estimate temporal durations is obscured (Hinton & Meck, 1997). The peak interval (PI) procedure evolved from this limitation in an effort specifically aimed at studying timing behavior (Staddon & Cerutti, 2003).

PI schedules combine FI trials with unreinforced "probe" trials. During probe trials, subjects receive a prolonged duration, and responses are never reinforced. Thus, these probe trials provide information on when the subject anticipated reinforcement delivery and the end of the delay (Gallistel & Gibbon, 2002). The distributions of responses produced by probe trials mirrors a normal distribution that is slightly skewed to the right. The three variables that are derived from this distribution are *peak rate*, *peak time*, and *spread*. Peak rate refers to the maximum rate of responding, which usually occurs near the learned FI trial duration. The peak

time is the mode of the distribution, and is interpreted as the time within trials when the subject had the highest expectation of reward. The spread is a measurement of temporal precision, and is calculated from the width of the distribution at 50% of its maximum response rate.

Dopaminergic drugs have been shown to distort temporal regulation and the expectation of reward delivery in subjects performing PI procedures (Drew et al, 2003; Meck et al, 1986; Meck et al, 1996; Matell et al, 2004). Studies have reported dopamine agonists (methamphetamine, cocaine) increase responding and shift peak times to the left in comparison to drug-free subjects (Matell et al, 2004; Maricq et al, 1981). In a two-interval (12s, 36s) PI task, the D2 antagonist haloperidol significantly increased rats' response start and stop times during probe trials relative to their drug-free performance. Interestingly, this same study found the D1 antagonist SCH-23390 did not distort temporal regulation, although it significantly decreased response rates (Drew et al, 2003). This result is in agreement with an earlier publication by Meck et al (1986), that reported drugs with high affinity for dopamine D2 receptors are positively correlated with distortions in temporal perception (Drew et al, 2003).

2.3d Differential-Reinforcement-of-Low-Rates Procedure

Differential-reinforcement-of-low-rates (DRL) is an operant timing schedule where the delivery of a reward occurs after the absence of a specific operant response for a specified interval of time (Mallot & Cumming, 1964). Under these circumstances, subjects learn, to some extent, to withhold responses until the predetermined interval has elapsed. Premature responses reset the required temporal duration, and only response times that meet or exceed

the specified interval are reinforced (Figure 2-1). Since no external cues are provided to signal the end of the required interval, animals must internally regulate the timing of their responses. As a result of these contingencies, subjects tend to produce low response rates in DRL schedules (Richelle & Lejeune, 1980), and well-trained animals produce frequency distributions of interresponse times (IRTs) that peak near the specified temporal criterion. A more detailed discussion of DRL schedules, which was the operant conditioning schedule selected for the timing research reported here, is provided in the next section of this chapter.

2.3e Bi-section Procedure

In the bi-section procedure, subjects are trained to discriminate two signal durations by emitting responses on one of 2 levers (Balci et al, 2009; Church & Deluty, 1977). These durations comprise one short interval (e.g., 2 s) and one long interval (e.g. 8 s). After presentation of either signal, reinforcement is contingent upon the subject's making a correct response on the lever assigned to the duration. In testing, subjects are again presented with short and long durations, but receive unreinforced trials (probe trials) where a new, intermediate duration is presented (Balci et al, 2009). These signal durations range between the two reference durations (e.g., 2.5, 3.5, 4.0, 6.5 s), and animals are forced to interpret the new signal as either "short" or "long". Responses can be plotted as a psychometric function that depicts the probability of a long response choice by the probe signal durations (Balci et al, 2009). The signal duration that elicits a "long" response 50% of the time is referred to as the point of subjective equality (PSE; Balci et al, 2009; Church & Deluty, 1977).

Under drug-free or saline conditions, the PSE value is usually close to the geometric mean of the original short and long intervals (Church & Deluty, 1977; Maricq et al, 1981; Balci et al, 2009). For example, if the short reinforced interval was 2 s, and the long reinforced interval was 8 s, then the PSE value will be close to 4 s. Consistent with the role of dopaminergic neurotransmission in temporal processing, methamphetamine (Maricq et al, 1981; Maricq & Church, 1983; Bizot, 1997) has been shown to reduce PSE values in rats, suggesting subjects “overestimated” the duration of temporal intervals. Likewise, the administration of dopamine antagonists, such as the D2 receptor blocker haloperidol, tended to increase PSE values in rats performing the bi-section procedure (Maricq & Church, 1983).

2.4 DIFFERENTIAL-REINFORCEMENT-OF-LOW-RATES

Out of all the operant timing schedules, the DRL schedule is one of the most thoroughly studied in regards to timing behaviors (Richelle & Lejeune, 1980). Under this timing schedule, the temporal regulation of a subject’s behavior can be assessed by both operant (e.g, IRT frequency distributions, response rates) and non-operant (e.g., collateral activity, distance traveled) performance. In the following section, DRL-mediated timing behaviors are discussed in further detail. Lastly, differences in timing behaviors reported between “unsigned” (no external cues) and “signed” (external cues) DRL schedules are presented.

2.4a *Inter-Response Time (IRT) Frequency Distributions*

The temporal pattern of responses is often described by a frequency distribution of response times, or *inter-response times* (IRTs), produced by the subject within the experimental session. These distributions have been described as bi-modal, with one peak occurring at very short IRTs and another near the temporal criterion (Richelle & Lejeune, 1980). Often, a mean

or median IRT value is calculated from the IRT distribution to describe overall timing behavior; these statistical measures usually exclude very short IRT values.

The occurrence of very short IRTs, or *burst* responses, is not fully understood. It is thought that they do not reflect temporal regulation of responses, but are governed by other behavioral or physiological processes (Richards et al, 1993). Some have argued bursting behavior is indicative of impulsivity (an inability to inhibit responses) or emotional frustration (Cheng et al, 2007; Ardayfio et al, 2008; Pattij et al, 2004; Sanger, 1992). Kramer and Rilling (1970) observed burst responses infrequently occur after a reinforced response, but are more likely to occur after unreinforced responses. More specifically, these types of unreinforced responses contain IRTs that were very close to the required temporal criterion (“near-miss” responses). However, Ferraro et al (1965) reported bouts of bursting activity to occur before reinforced activity; implying burst responses may have a useful purpose in timing behavior.

Investigators have also described patterns in the temporal adjustment of non-burst responses to the criterion interval (Richelle & Lejeune, 1980). One such finding is that reinforced responses are more likely to occur in succession or long sequences instead of being intermixed with unreinforced responses (Richelle & Lejeune, 1980; Farmer & Schoenfeld, 1964). It is thought that that recently produced IRTs guide the subsequent IRT; thus, timing behavior is temporarily improved after emitting a reinforced response (Richelle & Lejeune, 1980; Farmer & Schoenfeld, 1964; Ferraro et al, 1965). Figure 2-2 depicts this phenomenon, and shows the operant responses and movement trajectories of a single rat engaged in a DRL-72 task. Spatial location and movement during the operant task was measured by force plate

actometers (Fowler et al, 2001) that can track the position of a single rat. Each “box” in the figure represents a 3-min time frame within the operant chamber. Throughout the session, the majority of reinforced responses (blue triangles) appear clustered together in sequences of 2 to 8 in a row (Figure 2-2a).

Similarly, others have noticed temporal regulation gradually improves from the beginning of the session (Richelle & Lejeune, 1980). In rats performing 4 hr sessions of DRL-72, Fowler et al (2009) noted substantial improvement in reinforcement rates following the first hour. This behavioral pattern is also shown in figure 2-2a; for each hour, the rat earned 17, 22, 27, and 25 reinforcers.

2.4b Collateral Activity

While operant responses measures (e.g., response rates, reinforcement rates, median IRT values) are useful for assessment of timing behavior, investigators have found similar value in studying behavior when animals are not actively engaged in operant responding (Richelle & Lejeune, 1980). During these periods, animals have been reported to engage in “timing mechanisms” (Richelle & Lejeune, 1980) to guide temporal responding. These specialized behaviors, or *collateral activities*, are thought to regulate operant behavior and promote successful DRL performance (McIntire et al, 1987; Laties et al, 1965; van Hest et al, 1986).

A range of controlled or facilitative behaviors have been associated with collateral activity. Laties and colleagues (1965) observed a tendency for some rats to nibble their tails between emitting responses on a DRL schedule. Other researchers have found DRL performance to significantly improve in animals when objects (e.g., wood blocks, running wheel) were provided

to facilitate collateral activities (McClure et al, 1983; van Hest et al; 1987). In rats trained to a DRL 72 second (DRL-72) schedule, Fowler et al (2009) found rats engaged in minimal locomotion and spatially confined themselves away from the operandum between operant responding (Fowler et al, 2009). Fowler and colleagues (2009) argued that minimal movement was required to successfully estimate the required temporal duration, and the tendency of rats to wait outside the space near the operandum deterred premature responding.

Conversely, impairment of collateral activity has been shown to adversely affect temporal regulation of operant responding. When rats were forced to remain in front of the operandum, response rates increased and DRL performance worsened (McIntire, et al, 1983). In the previously discussed study by Fowler et al (2009), the administration of 5.0mg/kg d-amphetamine impaired DRL performance and disrupted spatial patterning by increasing locomotion and space occupied within the chamber during the pre-reinforcement interval. In an unpublished study, our lab has also found phencyclidine (2mg/kg-8mg/kg) to disrupt collateral activity (Latif et al, in preparation). Taken together, these findings suggest that collateral behaviors (or lack thereof) may systematically vary during the IRTs of lever pressing behavior maintained by DRL schedules of reinforcement.

2.4c Signaled DRL Schedules

Few studies have examined DRL-mediated timing behavior under signaled schedules (Wiley et al, 2000; Carery & Kritkauskys, 1972; Wiley & Wilmore, 2000). In the signaled DRL contingency, an external stimulus (e.g., tone, light) was presented to signal the end of the temporal criterion. These studies have found that rats trained on signaled DRL schedules

produce higher reinforcement rates and longer median IRTs than rats engaged in unsignaled schedules (Wiley et al, 2000; Carery & Kritkauskys, 1972; Wiley & Wilmore, 2000). In addition, rats performing signaled DRL schedules are less prone to drug-induced disruption of DRL performance. Wiley et al (2000) examined the effects of amphetamine, diazepam, pimozide, desipramine, and tetrahydrocannabinol in rats trained in a multiple signaled-unsignaled DRL-15 schedule. They reported that for each drug, rats earned significantly more reinforcers under signaled components than unsignaled components. Similarly, Carery & Kritkauskys (1972) found amphetamine treatment (1.0mg/kg) did not significantly alter response rates relative to saline treatment in rats trained to a signaled DRL-22 schedule.

It is thought that signaled schedules promote optimal DRL performance by diminishing dependency on timing mechanisms (Wiley et al, 2000). Thus, rather than requiring the animal to temporally regulate its responses, DRL performance is dependent on the animal's ability to attend to the external stimulus (Wiley et al, 2000). However, the role of collateral behavior under signaled schedules has not yet been investigated. In addition, the previously mentioned studies used relatively low temporal requirements (15-22 s). Studies with higher temporal requirements are likely to be increasingly dependent on collateral activity due to increased task difficulty.

2.5 SUMMARY AND RATIONALE OF EXPERIMENTAL STUDIES

Because temporal processing deficits have been reported in schizophrenic individuals, interval timing can be used as a cognitive measure in preclinical behavioral studies to (1) examine the validity of different animal models of psychosis and (2) investigate the effect of

therapeutic drugs within these models. The DRL operant procedure is useful in this pursuit: it requires subjects to utilize interval timing and allows the expression of collateral activities to successfully estimate temporal durations. Thus, timing proficiency is not only assessed by operant performance, but also by non-operant response measures, such as the spatial patterning and locomotor-related behavior of subjects, when the floor of the operant chamber serves as a sensor (i.e., force plate actometer).

In the following set of experiments, we sought to evaluate the effects of an atypical antipsychotic, aripiprazole (ARZ), in amphetamine treated-rats trained to either signaled (DRLS) or unsignaled (DRLU) DRL-72 schedules. These experiments have been divided into 3 sections. In the current chapter, we investigated fundamental differences in operant performance and timing-related behaviors between DRLS-72 and DRLU-72 operant schedules. Next, we examined the effects of ARZ on rats trained to DRLU-72 and DRLS-72 schedules (Chapter 4). In the final experiment, rats were administered the psychostimulant, d-amphetamine, to create a schizophrenic-like state in the animal. The resulting timing deficits from amphetamine treatment, as well as the ability of ARZ to attenuate the impairments, were assessed (Chapter 5).

2.6 DIFFERENCES IN BEHAVIOR MAINTAINED BY SIGNALLED VERSUS UNSIGNALLED DRL SCHEDULES

Before investigating the effect of amphetamine and aripiprazole, we evaluated basic behavioral differences in DRL operant performance and collateral activity in rats between signaled (DRLS-72) and unsignaled (DRLU-72) schedules. Two hypotheses were of specific interest. These were derived from the facts that 1) the relatively difficult 72-s schedule had not

heretofore been studied in the context of *signaled* DRL nor 2) had collateral behaviors been previously studied in this context. Therefore, **our first hypothesis** was that with a 72-s parameter the difference between the unsignaled and signaled conditions would be quite large with the signaled rats earning significantly more reinforcers and displaying a more precise and more peaked IRT distribution than the unsignaled rats. **Our second hypothesis** was that the evidence for well-defined collateral behaviors during pre-reinforcement intervals would be greater for the unsignaled than for the signaled conditions. We expect signaled rats to be more efficient (i.e., earn more reinforcers) on the DRL-72 task than unsignaled rats because the signaled DRL contingency diminishes the need for rats to internally time and regulate behavior. Thus, signaled rats will be less dependent on interval timing and exhibit less stringent timing-related behaviors (e.g., collateral activity, reduced locomotor activity) than their unsignaled counterparts. In addition, the difficulty of the DRL-72 task is reduced in signaled rats compared to unsignaled rats. Signaled rats will need to perceive an external stimulus—a task that is less demanding on cognitive-related behaviors (attentional demands) than internally estimating a temporal duration (temporal discrimination). Because DRLS-72 rats are given “increased stimulus control” (Wiley et al, 2000), we expected DRLS-72 rats to exhibit superior operant performance; earning more reinforcers, emitting less burst responses, and producing longer IRTs than DRLU-72 rats.

2.7 METHODS

2.7a Subjects

Sixteen male, Sprague-Dawley rats (Harlan, Indianapolis) were used in this study. Animals were housed individually in plastic cages that contained Aspen wood shavings for

bedding. The housing room was maintained on a 12:12-h light/dark cycle. The lights remained on in the housing room from 6:00AM to 6:00PM. Food was freely available within their home cages. Prior to operant training procedures, animals were gradually adjusted to a water restriction schedule of 15mL daily. If a rat obtained more than 15mL within a training or experimental session, additional water was not given. Rats began operant training at approximately 2.5 months of age, and experimental testing at approximately 4 months of age. When experimental testing began, rats had a mean body weight of 356.25g (SEM +/- 4.70). Behavioral measurements were performed from 8:30am to 6:00pm, during the light portion of the light/dark cycle. Animals were used in accordance to the University of Kansas Institutional Animal Care and Use Committee. Experimental procedures followed guidelines and regulations established by the National Institutes of Health 1996 edition of the *Guide for the Care and Use of Laboratory Animals*.

2.7b Apparatus

All training and testing procedures were conducted in four specialized “hybrid” chambers, designed with force-plate actometers in the flooring and operant fixtures (Fowler et al, 2001). The force-sensing capabilities of the actometers provided detailed measurements of operant response measures (response force, response duration) and motor behavior (location within the chamber, movement trajectory). Each chamber was housed in a sound-attenuating box (Med Associates, ENV-018MD) and was governed by its own computer. Computers were integrated with LabMaster interface (Scientific Solutions, Mentor, OH, USA) and contained customized pascal programs that controlled the operant procedure and record collateral behavior. For the present study, data were collected with a temporal resolution of 0.01 s.

The floor of the chamber (28 x 28 cm) acts as a sensory load plate that rests on four transducers, with one transducer per corner. A 23 cm high Plexiglas cage covering (the “chamber”) was elevated 2 mm above the floor and was supported by four vertical pillars. A house light was located centrally above the chamber on top of the Plexiglas cage.

One side of the Plexiglas case has two openings separated by 18 cm. The first opening (2.5cm X 3.0cm) was located near the back of the chamber, with its left ledge 3.0cm away from the adjacent wall and its lower edge 5.0cm above the floor. Two green LED lights were positioned in a single row along the right side of the opening, and one green LED light was positioned on its left. This opening provided access the 1.8-cm diameter disk (operandum), which was mounted on an isometric force transducer and was capable of detecting changes in forelimb force. To emit a response, the rat had to extend its forepaw through the opening and depress the operandum with a criterion force of 12g. If a rat pressed the operandum with a force less than 12g, it was not registered as a response. The operandum was located 1.6cm outside the chamber. The second opening (7cm X 7cm) was raised 1.3cm above the chamber floor, and was located 2.5cm from the adjacent wall. This opening provided access to a 1.8-cm diameter lick disk that dispensed 0.06 mL distilled water when a reinforced response was made. A Manostat peristaltic pump (model 72-410-014) situated outside the sound-attenuating cabinet transferred water to the lick disk. Delivery of a reward was simultaneously presented with an audible click from a relay located 5cm outside of the chamber, as well as a small indicator light that illuminated the lick disk during water delivery. The lick port was positioned 4 cm outside the chamber within a polycarbonate covering.

2.7c Behavioral Procedure

When rats were first introduced to hybrid chambers, they were given a 30-minute session of a variable timing schedule of 60-s (VT-60). Access to the operandum was blocked by a Plexiglas-covering (5.0cm X 6.5cm) fastened over the original Plexiglas opening for the operandum. The pseudo-random timing of reinforcement delivery was calculated using the Fleshler-Hoffman procedure (Fleshler & Hoffman, 1962). The purpose of the VT-60 session was to allow rats to habituate to the chambers and become familiar with the site of reinforcement delivery (i.e., the lick disk).

After completing the VT-60 session, rats were divided into signaled (DRLS; n=8) or unsignaled (DRLU; n=8) groups. In the following sessions, rats were switched to either DRLS-1 s or DRLU-1s schedules of reinforcement and were gradually trained (see below) to emit a response on the operandum. For rats in the DRLS condition, a 1-s tone sounded and the 3 green LED lights illuminated when the temporal criterion was met. Sessions were conducted once daily for each rat in 30-minute sessions given 5 times a week. For operant training, the Plexiglas covering blocking access to the operandum was removed, and an aluminum metal beam (1.8cm x 6.0cm x 0.2cm) was bolted onto the operandum. The metal beam protruded approximately 2.5cm into the chamber. If a rat earned a minimum of 50 reinforcers in a single session, the metal extension piece was retracted by approximately 0.5 cm for the next session. Once a rat met the 50 reinforcer criterion for a single session, the extension piece was systematically retracted for the next session until the rat learned to extend its paw through the opening to emit a response on the operandum. In the final step, the metal extension piece was

removed from the force transducer and replaced with the original 1.8cm diameter operandum disc covering.

During the next 30 days, rats were progressively trained to perform the DRL-72 task. DRL training began with 30 minutes sessions of DRL-1, DRL-2, DRL-4, DRL-8, DRL-16, followed by 2-hr sessions of DRL-36 and DRL-72. At the final stage, rats were given six 4-hr sessions of DRL-72. Once 4-hr sessions began, rats were run three times a week. This experimental procedure was implemented because only 8 rats could be run a day during the light portion of the light/dark cycle. Therefore, one group of rats (n=8) were run on Mondays, Wednesdays, and Fridays, while the second group (n = 8) was run on Tuesdays, Thursdays, and Saturdays.

2.7d Drugs

Although no drugs (i.e., d-amphetamine, aripiprazole) were given in the pre-drug experiment comparing DRLU-72 and DRLS-72, drug-naïve rats received a single injection of vehicle (VECH) immediately before entering the operant chambers. VECH was a solution of physiological saline containing 2.5% Tween-80 solution. All injections were administered intraperitoneally (i.p.) and in a volume of 1mg/kg.

2.7e Statistical Analysis

Data were collected from hybrid chambers (Fowler et al, 2009) and processed through customized PASCAL programs and SYSTAT (Systat software, San Jose, CA). For analysis, behavioral measurements from VECH sessions were used, and differences between DRLU-72 and DRLS-72 groups were assessed (between-groups differences). Statistical significance ($\alpha = 0.05$) was generally determined using repeated measures (RM) ANOVAs and post hoc ANOVAs.

Operant response variables included the number of responses, number of reinforcements, median interresponse times (IRTs), response duration, and peak force. Responses were divided into *burst* responses (IRT < 3 s) or *non-burst* responses (IRT > 3 s). Behavioral measurements derived from the force plate actometer include locomotor activity and spatial location within the chamber (e.g., quadrant usage). Rather than assessing these measurements for the entire session, analysis concentrated on the 72-s time period before rats produced a reinforced response. Locomotor and movement trajectory data were averaged for each individual rat's reinforced responses before calculating DRLS-72 and DRLU-72 group means. Locomotor activity was assessed by recording the distance traveled between coordinates every 0.50 s throughout the 4-hr session.

2.8 RESULTS

DRLU-72 and DRLS-72 rats exhibited significant differences in operant performance and locomotor activity. Figure 2-3 depicts the IRT frequency distributions for VECH-treated DRLU-72 and DRLS-72 rats. Percentage—rather than the number—of total responses were sorted into 2-s bins. For the DRLU-72 IRT frequency distribution, the majority of non-burst responses (IRT > 3 s) occurred near the 72-s temporal requirement, peaking several seconds below it. The DRLU-72 group had a median IRT value of 66.06 s (SEM +/- 2.19); similar findings have been reported by other studies (Fowler et al, 2009; Balcells-oliveo et al, 2009). The DRLS-72 group IRT frequency distribution is largely defined by a sharp peak in the 73-74-s bin, where rats made 38.43% of their total responses. This value is in striking contrast to the DRLU-72 group, which made only 1.61% of their total responses in the same IRT bin. Over 50% of the DRLS-72 group IRTs occurred between 71-78-s, whereas DRLU-72 rats made only 7.57% of their total responses

within the same IRT range. A one-way ANOVA for median IRTs confirmed that DRLS-72 rats produced significantly higher IRTs than DRLU-72 rats [F-ratio (1,14) = 11.31, $p < 0.01$]. These findings suggest that IRT frequency distributions were substantially influenced by the presence or absence of an external signal, with DRLS-72 IRTs being heavily dependent on the occurrence of a cue.

Table 2-1 summarizes DRLU-72 and DRLS-72 group mean values for operant response measures. A one-way ANOVA found VECH-treated DRLS-72 rats obtained significantly more reinforcers than the VECH-treated DRLU-72 group [F-ratio (1,14) = 60.45, $p < 0.0001$]. Thus, DRLS-72 rats produced more reinforced responses than the DRLU-72 group. However, neither non-burst [F-ratio (1,14) = 2.44, $p < ns$] nor burst response rates [F-ratio (1,14) = 0.15, $p < ns$] significantly differed between signaled and unsignaled groups. The lack of difference in response rates—particularly burst response rates—between the two DRL groups is interesting, as it implies that rats given external cues to help guide responding (DRLS-72) still produced the same amount of burst responses as rats without exteroceptive signals.

Further analysis of operant responding was conducted by examining the force waveforms for reinforced responses. Figure 2-4 depicts the mean reinforced response forelimb and tongue waveforms for each DRLS-72 (Figure 2-4A) and DRLU-72 (Figure 2-4B) rat. The X axis spans a 16-s window that shows the 8 s preceding and following reinforcement delivery. The 1-s time frame where reinforcer was delivered is represented by the vertical grey box. For several DRLU-72 rats, the response waveforms appear comparatively prolonged, starting 1-3 s earlier than the DRLS-72 group (Figure 2-4). It is likely that the contingencies of the DRL schedule

influenced DRLU-72 waveforms, as the IRT of a response was not registered until forelimb force both exceeded *and then* dropped below the required force requirement. Thus, prolonged response duration increased the likelihood of a reward by lengthening the duration of the IRT. This effect has not been previously reported.

Additionally, DRLU-72 schedules differentially influenced burst response characteristics following a reinforced response, although statistical analysis of burst median IRTs and response durations indicated these effects were insignificant (data not shown). Figure 2-4 shows the majority of DRLS-72 rats produced response waveforms with sharp, bimodal peaks, while DRLU-72 response waveforms had broad, single peaks.

Because DRLS-72 rats earned significantly more reinforcements than DRLU-72 rats, differences in locomotor activity could be attributed to DRLS-72 rats making more trips to and from the operandum and lick disk. Therefore, task-related locomotor activity (collateral behavior) was assessed during the 72-s preceding a reinforced response, where group means contained each individual subject's mean distance regardless of the number of reinforced responses made by each subject. As previously demonstrated in our lab, DRLU-72 rats engage in minimal locomotor activity and remain spatially confined near the lick disk until the temporal criterion had nearly elapsed (Fowler et al, 2009). Figure 2-5 depicts the temporal gradient of DRLU-72 and DRLS-72 groups' locomotor activity (distance traveled) during the pre-reinforcement interval. Relative to the DRLU-72 group, DRLS-72 mean locomotor activity was consistently higher throughout the 72-s interval, with a shallow peak near the 10-s mark (Figure 2-5). At approximately 69 s, locomotor activity rapidly increased for both groups as they

moved closer to the operandum to initiate a response. A one-way ANOVA confirmed that DRLS-72 rats engaged in substantially more locomotion than DRLU-72 rats during the pre-reinforcement 72-s interval under VECH conditions [$F\text{-ratio}(1,14)=51.60, p < 0.0001$].

To determine group differences in space usage (location), the chamber floor was divided into 4 quadrants, and the percentage of time spent in each quadrant during the 72 s pre-reinforcement interval was calculated for DRLU-72 and DRLS-72 groups. Figure 2-6A shows the mean percent time each quadrant was occupied for both DRL groups. A two-way RM ANOVA indicated a significant effect of quadrant [$F(3,42) = 40.89, p < 0.001$], confirming that rats—regardless of DRL schedule—preferred to spatially confine themselves to QI and QIV, where they could readily engage the operandum and lick disk, respectively. Though DRLU-72 and DRLS-72 groups did not significantly differ in quadrant usage [$F(1,14) = 1.97, p = ns$], a significant interaction quadrant X DRL schedule was found [$F(3,42) = 4.82, p < 0.01$], indicating that DRL schedule influenced space usage during the pre-72 s reinforcement interval. Post hoc analysis using one-way ANOVAs found DRLU-72 rats spent significantly more time in QIV [$F(1,14) = 5.14, p < 0.05$] and less time in QIII [$F(1,14) = 17.47, p < 0.001$] relative to DRLS-72 rats. These results suggest that signaled rats were less likely to spatially restrict themselves within the chamber before making a reinforced response, as the DRLS-72 group spent less time waiting in QIV and more time positioned in the remaining quadrants.

2.9 DISCUSSION

In the present analysis, we sought to compare basic behavioral differences in DRL-mediated behavior between signaled and unsignaled schedules. Not unexpectedly, DRLS-72

rats produced higher IRTs and earned more reinforcers than the DRLU-72 group. Similar findings were reported by Wiley et al (2000) for rats engaged in a multiple signaled-unsigned DRL-15 schedule. The superior operant performance of DRLS-72 rats can largely be attributed to the presence of an external signal, which served as a stronger predictor of reward than the IRT of a response. The analysis of operant response measures, locomotor activity, and spatial trajectories suggest that the signaled condition promoted optimal DRL performance while diminishing dependency on timing behaviors observed in unsigned schedules.

The IRT frequency distributions (Figure 2-3) demonstrated that DRLU-72 rats were more reliant on interval timing and internally estimating the criterion interval, as the percentage of non-burst IRTs gradually peaked towards the 72-s mark. In contrast, DRLS-72 rats were heavily dependent on signal presentation, with the majority of non-burst IRTs occurring 1-6 s after the external cues were presented. For the DRLS-72 IRT distribution, the occurrence of IRTs outside of this range remained relatively constant, suggesting the external cue reduced the rats' need to time responses.

Though non-burst response rates did not significantly differ between DRL groups, intrinsic differences in response characteristics were found. Firstly, DRLS-72 rats produced more *reinforced* responses than DRLU-72 rats; thereby demonstrating an increased accuracy of responding. This result is not surprising, as it is complemented by other measurements of DRLS-72 operant performance, i.e., higher reinforcement rates and longer median IRTs than DRLU-72 rats. This finding is also in agreement with Wiley et al (2000), who found rats produced similar response rates under signaled and unsigned contingencies, but had higher

response efficiencies under signaled DRL components. Secondly, DRLU-72 rats had significantly longer non-burst response durations than DRLS-72 rats (Table 2-1). The plots of each subject's mean response waveforms before and after reinforcement delivery (Figure 2-4) revealed that several DRLU-72 rats were inclined to initiate responses significantly earlier than DRLS-72 rats. The tendency for DRLU-72 rats to produce longer response durations is most likely influenced by the DRL schedule response parameters: the IRT of a response was not registered when forelimb force initially exceeded the criterion force requirement, but occurred when force returned below it. Therefore, prolonged response durations increased the likelihood of a reward because they increased the IRT of a response. We hypothesize that DRLU-72 rats were more likely to utilize this response contingency because they were more dependent on interval timing than DRLS-72 rats. For DRLS-72 rats, the signal presentation was sufficient for accurate responding and reduced the need to produce long response durations. Thus, the association of the external cue to reward supplanted (rather than strengthened) the association between the temporal properties of a response to a reward.

Interestingly, both DRL groups produced similar burst response rates. The occurrence of burst responses have been implicated in other behavioral processes; most notably "impulsive" behavior (Ardayfio et al, 2008; Sanger et al, 1992; Pattij et al, 2004). Specifically, burst responses are thought to be associated with impairments in response inhibition—an inability to withhold premature responses that further delays the onset of a reinforcement opportunity (Richards, 1991). Thus, high burst response rates on DRL schedules are thought to reflect impulsive behaviors such as impaired inhibitory control or intolerance to the delay of a reward (Winstanley, 2003; Ardayfio et al, 2008). If burst responses were indicative of

impulsivity, DRLS-72 rats would be expected to produce lower rates of burst responding than DRLU-72 rats, as the signaled contingency provides rats with additional stimulus control. Our findings, however, indicate that DRL groups did not significantly differ in burst response rates (Table 2-1), suggesting that bursting behavior cannot solely be interpreted as an impairment in inhibitory control. Furthermore, the response waveforms in Figure 2-4 demonstrated that several rats routinely made burst responses after making a reinforced response. This behavior appears more abundant in DRLS-72 rats: after making a reinforced response, several burst responses with subcriterion force values occurred during the 1-s reinforcement delivery period (grey bar, Figure 2-4). The finding that burst responding frequently occurred after reinforced responding suggests that that burst responses may not be indicative of impulsive behavior but rather, reflect a probing of the schedule's contingencies and the effect (or lack of effect) of their response.

Analysis of locomotor activity data (distance traveled, quadrant usage) was restricted to the pre-72 s interval of a reinforced response, when rat locomotor behavior was most likely to be associated with collateral activity. Temporal gradients of DRLU-72 and DRLS-72 rats' locomotor movement indicated relatively consistent levels of locomotion that quickly escalated 3-4 s before the temporal criterion elapsed, as rats began to approach the operandum to initiate a response (Figure 2-5). However, the DRLS-72 condition significantly increased baseline locomotor activity relative to the DRLU-72 condition. Furthermore, measurements of quadrant usage indicated that while rats under either DRL schedule spent more time near operant fixtures, DRLU-72 rats were more likely to spatially distance themselves away from the operandum and position themselves near the lick disk than DRLS-72 rats (Figure 2-6A). These

results are in agreement with previously published findings showing that collateral behavior improved operant performance by relocating the animal away from response-related stimuli (Fowler et al, 2009; McIntire et al, 1983), and that timing requires the animal to wait and abstain from excessive locomotion (Richelle & Lejone, 1980; Fowler et al, 2009). Under the DRLS-72 schedule, however, rats displayed more movement in the chamber than DRLU rats, possibly because the signal reduced the need for quiet attentiveness and sustained attention, which are required for optimal timing performance in the absence of exteroceptive cueing.

In the current study, rats trained on signaled DRL schedules demonstrated greatly improved operant performance relative to rats on unsignaled schedules. Consistent with Wiley et al's (2000) conclusions, our results suggest that the signaled DRL schedule diminished the utilization of timing behaviors. Additionally, the signaled DRL condition appeared to decrease the salience of temporal response-reward associations. Similar hypothesis have been established for variable interval (VI) operant schedules by Pearce & Hall (1978) and St.Claire-Smith (1979), who found that rats trained to cued-reinforcement VI schedules responded at lower rates than rats given random cues independent of reinforcement delivery. Coined "Overshadowing theory", researchers proposed that stimulus-reward associations overshadowed response-reward associations. This theory has been disputed, however, by findings that response rates under signaled VI schedules were resistant to extinction and satiation compared to rats in the random cued condition (Roberts et al, 1984; Tarpy et al, 1985; Hall, 1982). Roberts et al (1984) argued that these results imply the signal-reward pairing actually *strengthened* the response-reward pairing rather than superseding it. Similar findings were later published by Tarpy et al (1985), who reported that rats trained to signaled DRL-20 schedules were more resistant to satiation

than their unsignaled counterparts. However, the present study showed that DRLS-72 rats did not engage in the usual timing behavior that normally accompanies or promotes strong temporal response-reward associations under unsignaled DRL schedules, such as restricted collateral activity and prolonged response durations.

2.10 FIGURES AND TABLES

Figure 2-1. Contingencies of the DRL operant procedure. In order to successfully obtain a reward, an animal must withhold a response for a set period of time (e.g., 72 s) from its previous response. Premature responses reset the temporal criterion, and only responses that meet or exceed the temporal criterion are reinforced.

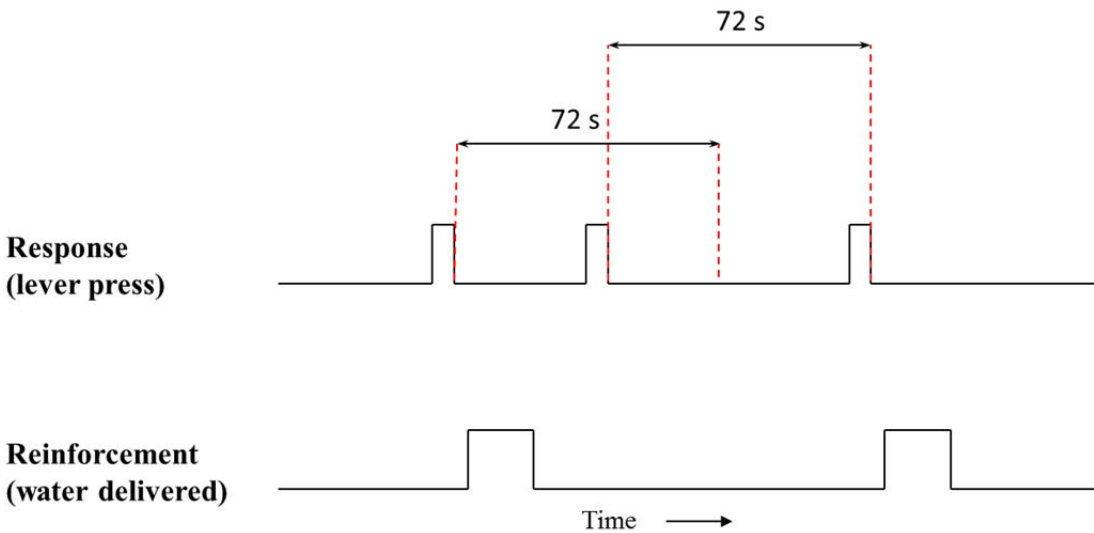
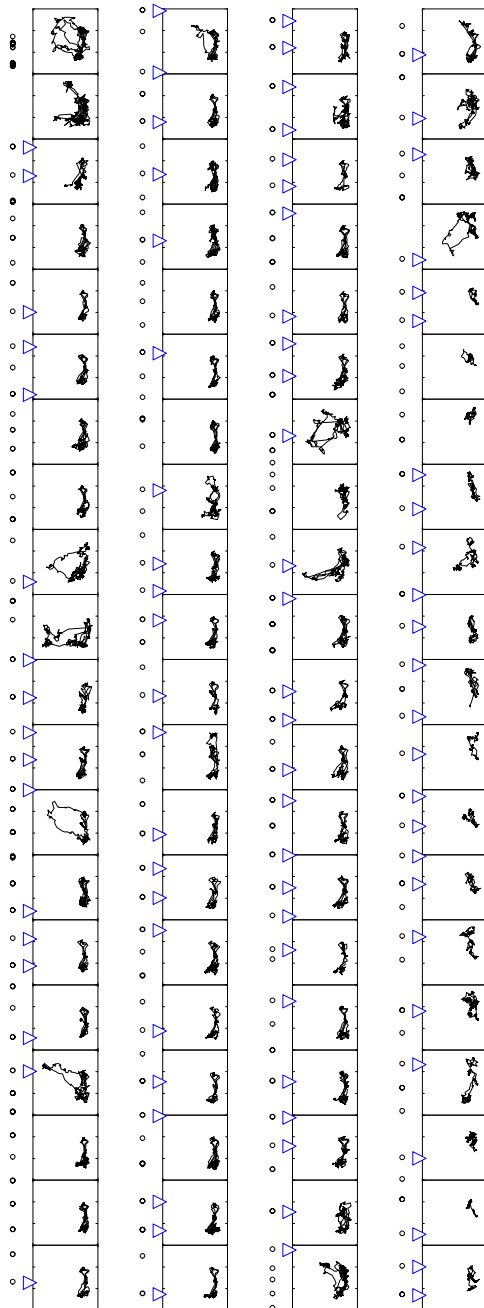


Figure 2-1 Movement trajectories and operant responses of a single rat for a 4-hr session. (A) The plot to the left depicts the movement trajectories and operant responses for a single rat performing a DRL-72 task during a 4-hr session. Each box represents a 3-min frame of the session. (B) The location of the operandum and lick disk are depicted in the diagram on the right. Open circles represent an emitted response, and reinforced responses are indicated by the blue triangles.

A.



B.

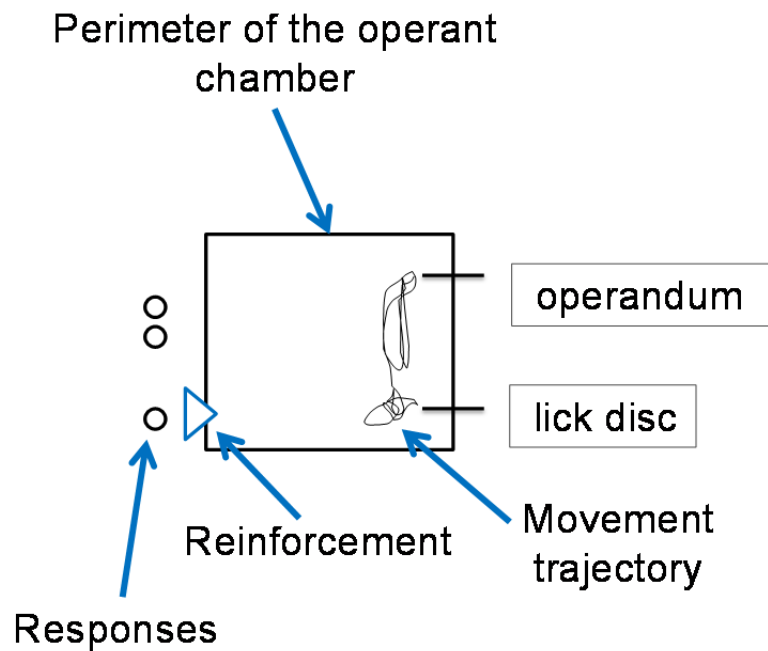


Figure 2-3. Group mean IRT frequency distributions following saline administration.

Percentage of responding was used to normalize data across subjects. Each bin size is 2 s, and the right-most data point represents IRTs ≥ 121 s. The vertical dashed line signifies the 72-s temporal requirement. The y-axis was log₁₀ transformed in order to emphasize differences in IRT response frequency at each bin. Except for the smallest and largest values, the IRT frequency distribution for DRLU-72 follows a curve that peaks approximately six seconds below the 72-s criterion. The DRLS-72 IRT frequency plot contains a large, sharp peak in the 73-74 s bin.

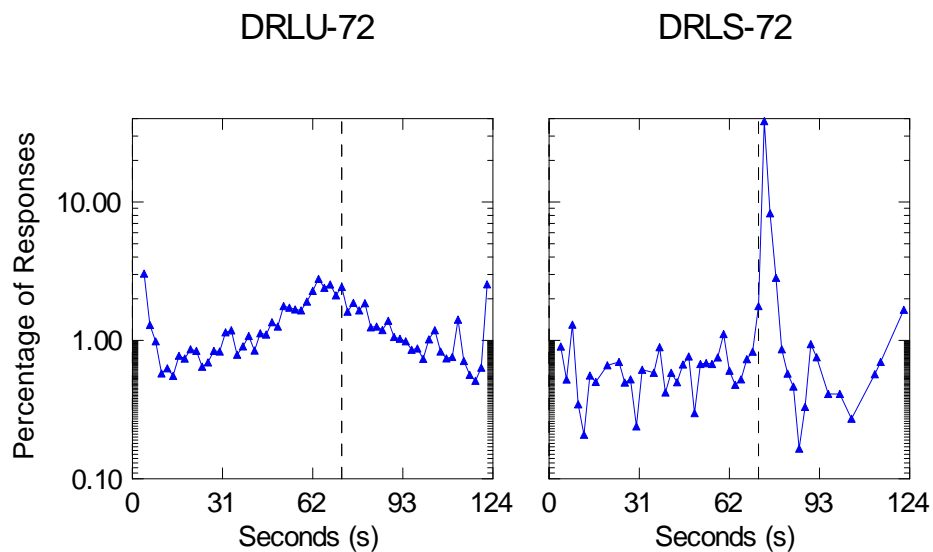


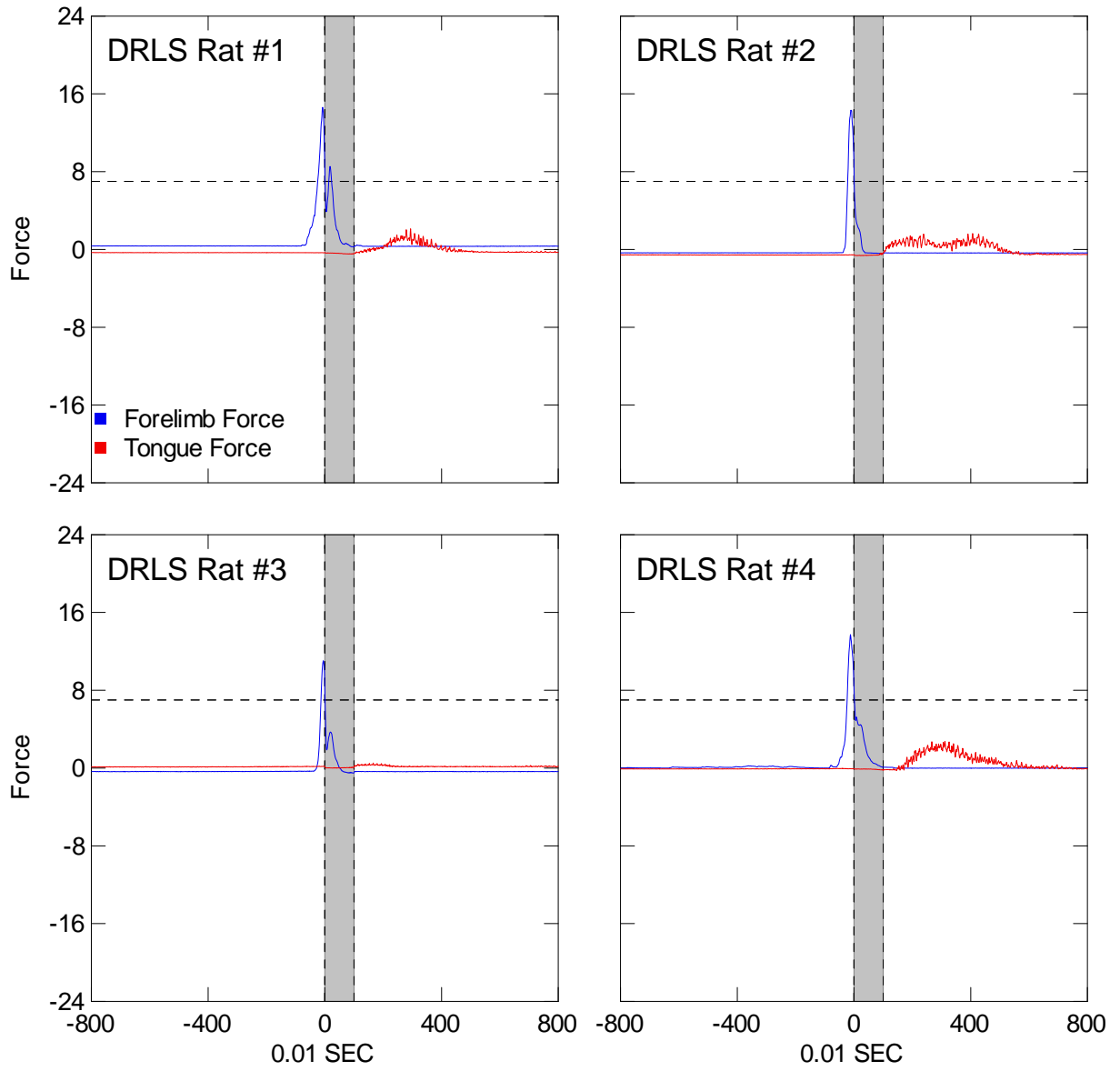
Table 2-1. Group mean values for operant response measures under saline conditions.

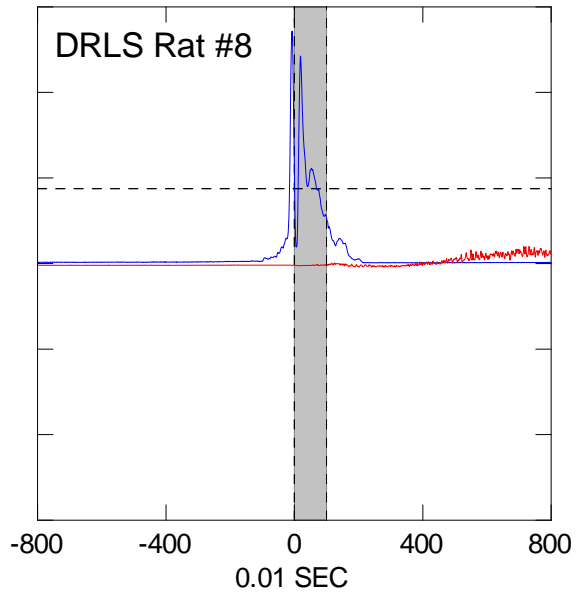
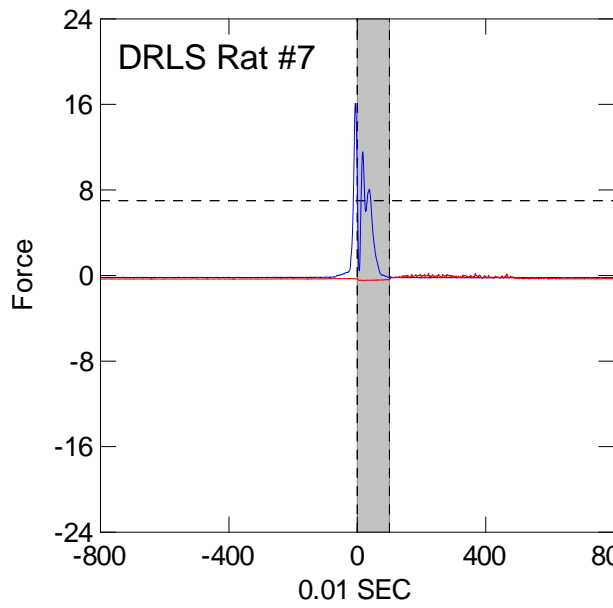
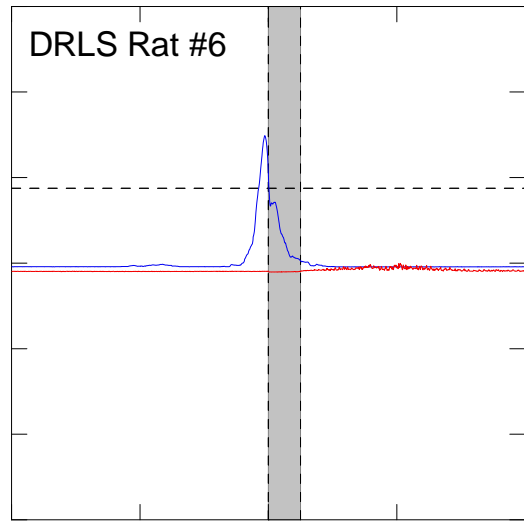
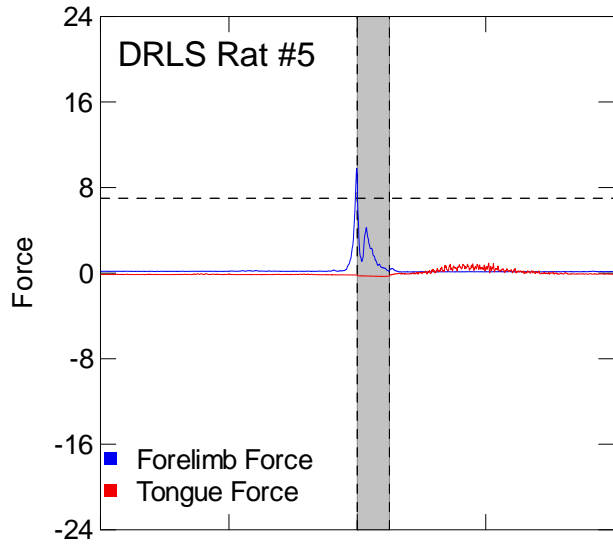
Standard errors of the mean are listed in parenthesis.

| Response Measures | DRLU | DRLS | Significance |
|--|--------------|---------------|---------------------|
| Median IRT (sec) | 66.06 (2.19) | 73.46 (0.24) | $p < 0.01$ |
| Reinforcements Rates (Rein/hr) | 17.41 (1.47) | 39.84 (2.48) | $p < 0.001$ |
| Response Rate (Resp/hr) | 51.16 (4.34) | 43.22 (2.64) | $p = ns$ |
| Burst Response Rate (BR/hr) | 46.16 (8.81) | 40.81 (10.85) | $p = ns$ |
| Response Duration of non-burst responses (0.01 s) | 45.8 (9.66) | 18.58 (2.65) | $p < 0.05$ |

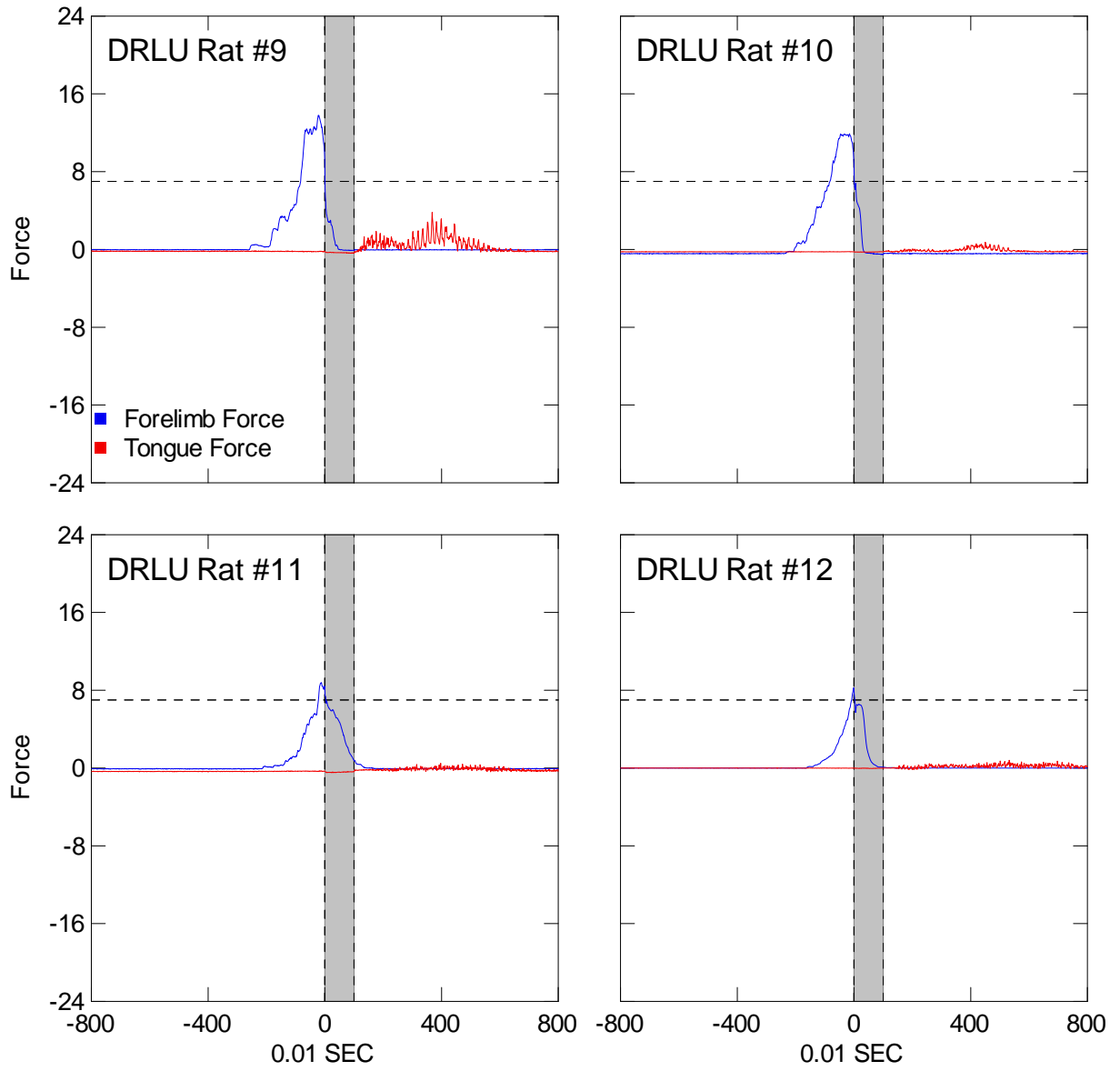
Figure 2-4 Individual rat mean forelimb response force waveforms and tongue force waveforms for reinforced responding. For each rat, the mean response force wave form (blue), and lick rhythms (red) were plotted across a 16-s window. The x-axis shows the 8 s preceding and following reinforcement delivery. The vertical grey bar indicates the 1-s frame when the reinforcer was delivered. (A) The first 8 plots depict DRLS-72 rats' force and lick waveforms, followed by (B) 8 plots for DRLU-72 rats.

A.





B.



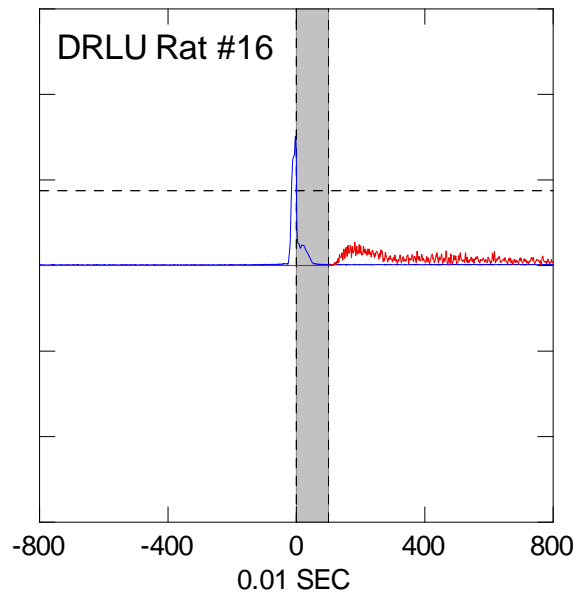
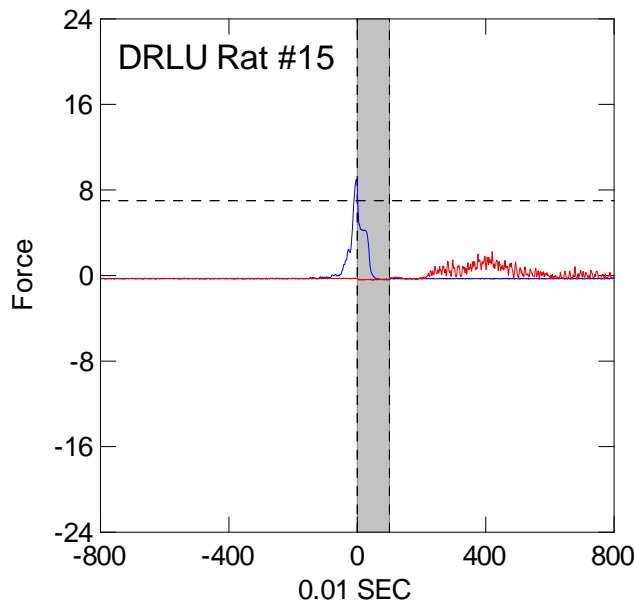
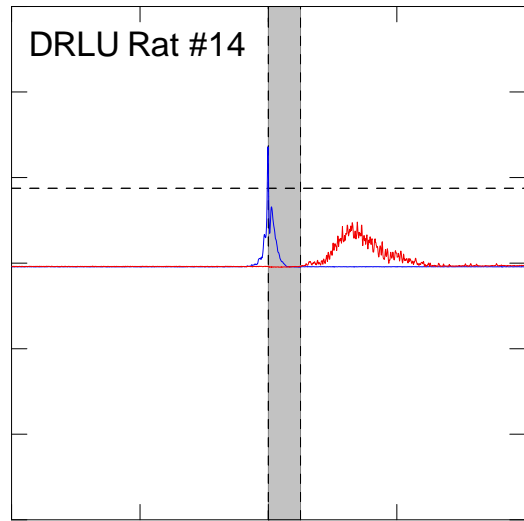
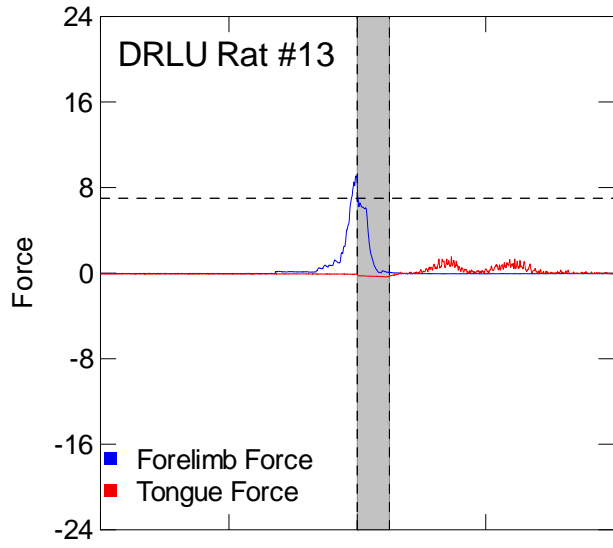


Figure 2-5. Locomotor activity (distance traveled) in saline-treated DRLU-72 and DRLS-72 rats during the pre-72 s reinforcement interval. The plot below displays locomotor activity for DRLS-72 (*red*) and DRLU-72 (*blue*) across time. *Thick* lines represent locomotor activity, and *thin* lines represent standard error. The vertical dashed line represents the 72-s temporal criterion. For each DRL group, the mean distance traveled (+/-SEM) is indicated within the figure.

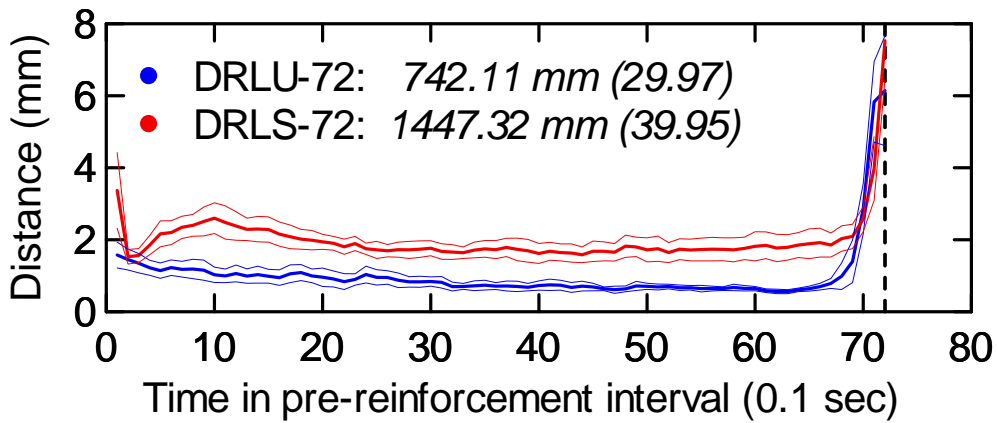
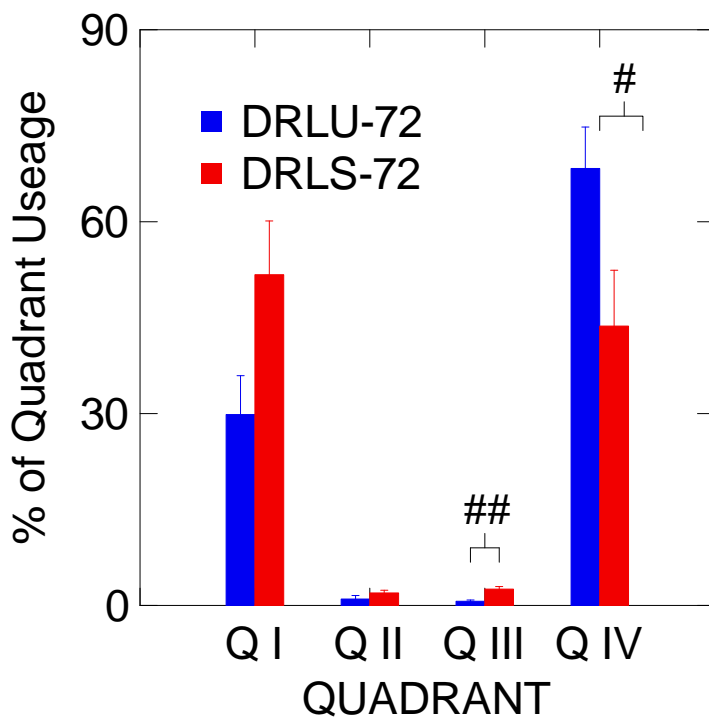


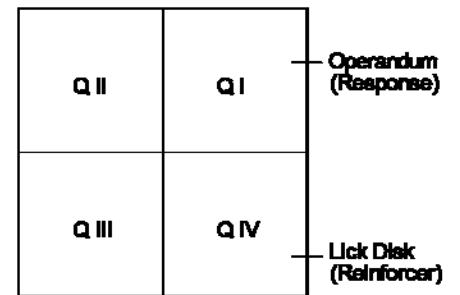
Figure 2-6. Group mean time spent in each quadrant during pre-72 s reinforcement interval.

(A) The plot below depicts the group mean (\pm SEM) percentage of time DRLU-72 (*blue*) and DRLS-72 (*red*) rats spent in each quadrant. Quadrant location was measured in hundredths of a second. (B) The schematic diagram on the right describes the location of each quadrant within the operant chamber. Note that Q I contains the operandum window, and Q IV contains access to the lick disk. A two-way RM ANOVA found a significant interaction of DRL schedule (signaled or unsignaled) and quadrant ($p < 0.01$), suggesting that DRL schedule influenced temporal-spatial collateral activity. The # symbol indicates a significant effect of schedule. Post-hoc significant levels are as follows: # = $p < 0.05$, ## = $p < 0.01$.

A.



B.



CHAPTER 3: EFFECTS OF ARIPIPRAZOLE IN RATS PERFORMING SIGNALLED OR UNSIGNALLED DRL TIMING SCHEDULES

3.1 INTRODUCTION

Cognitive related-behaviors that can be assessed by the DRL schedule, such as interval timing, sustained attention, and response inhibition (Meck, 2006; Richelle & Lejenué; 1980; Ardayfio et al, 2008) have been reported to be disrupted in schizophrenic individuals (Rammsayer, 1990; Balci et al, 2009; Carroll et al, 2008). In the previous chapter, DRLS-72 rats demonstrated less dependency on timing-related behaviors than DRLU-72 rats. Analysis of collateral activity indicated DRLS-72 rats were less spatially restricted and engaged in significantly more locomotion during the pre-72 s reinforcement interval than DRLU-72 rats. This suggests that signaled rats were less likely to maintain sustained attention and inhibit motor-related behavior while waiting for the 72-s interval to elapse, as collateral activity was not as crucial for temporally regulating operant responses as it was for unsignaled rats. In addition, IRT frequency distributions indicated DRLS-72 rats were heavily reliant on the presence of an external cue to time responses, whereas DRLU-72 rats were solely dependent on interval timing. Taken together, these findings suggest that DRLS-72 performance was less dependent on cognitive functions and behaviors that are known to be impaired in schizophrenia. Moreover, these cognitive functions—specifically, interval timing—are dopamine-related processes (Meck, 2006; Balci et al, 2009). Thus, we assume that DRLU-mediated behavior would be more susceptible to disruption by dopaminergic pharmacological

agents because interval timing is more essential to successful DRL performance in unsignaled rats than in their signaled counterparts.

In the research work presented in this chapter, we examined the effects of the atypical antipsychotic, aripiprazole (ARZ), on DRL-mediated behavior in rats performing signaled (DRLS-72) and unsignaled (DRLU-72) schedules. To our knowledge, the effects of ARZ have not been evaluated in the DRL operant procedure. Other antipsychotic drugs have been shown to dose-dependently reduce operant response rates and reinforcement rates in unsignaled DRL schedules, as well as prolong IRTs and induce a rightward shift of IRT response frequency distributions (McGuire & Seiden, 1980; Cheng & Liao, 2007; Liao, 2005). This is in contrast to antidepressants, which have been consistently shown to improve response efficiency in DRL schedules by reducing response rates while increasing reinforcement rates of DRL-72 schedules (O'Donnell & Seiden, 1983).

As a partial dopamine agonist, ARZ is thought to normalize brain dopaminergic transmission depending on the endogenous levels of dopamine: ARZ acts as a dopamine agonist when extracellular dopamine concentrations are low, and as a dopamine antagonist when extracellular dopamine concentrations are high (Burriss et al, 2002; Schatzberg et al, 2010). Theoretically, ARZ's pharmacological profile could attenuate schizophrenic-like cognitive deficits (hypofrontality) while reducing positive symptoms (Bolonna & Kervin, 2005). ARZ has also proven useful as an adjunctive treatment in depression (Marcus et al, 2008; Berman et al, 2007). Thus, evaluating its effect on the DRL-72 schedule may provide insights regarding its therapeutic potential. The purpose of the current experiment was to investigate the effects of

ARZ on cognitive-related behavior in rats performing DRL schedules. Expected outcomes (hypothesis) are couched in terms of three behavioral processes: interval timing, task difficulty, and uncertainty of reward.

3.1a Interval Timing

As previously stated in Chapter 2, rats in the unsignaled condition are expected to be more reliant on interval timing than DRLS-72 rats. However, interval timing and temporal processing is sensitive to the pharmacological modulation of dopamine (Balci et al, 2009; Matell & Meck, 2000; Hinton & Meck, 1997), particularly at the dopamine D2 receptor (Meck, 1984). Since DRLU-72 performance is increasingly dependent on dopamine-related cognitive processes, we hypothesize that DRLU-72 rats will be more sensitive to ARZ-induced behavioral effects on DRL-mediated behavior than DRLS-72 rats.

3.1b Task Difficulty

Again, we expect DRLS-72 rats to exhibit superior operant performance (i.e., increased response efficiency) than DRLU-72 rats, since the ability to perceive an external stimulus is less demanding than internally timing a temporal interval. Thus, we expect DRLS-72 rats to exhibit less stringent collateral behaviors than DRLU-72 trained rats. If the effects of ARZ are similar to those of other antipsychotics (e.g., reduce operant responses and reinforcement rates), we believe it is more likely to impair DRLU-72 operant performance, as these rats are more dependent on dopamine-related cognitive processes. Thus, DRLS-72 rats are expected to be more resistant to the effects of ARZ than DRLU-72 rats.

3.1c Uncertainty of Reward

In the signaled condition, rats are given an external cue to guide responding; thus, there is less uncertainty in the delivery of a reinforcer. Extensive research has investigated the neuronal firing rate of dopamine in response to a reward (Schultz, 2010). These experiments suggest that the size of a dopamine neuronal discharge (phasic increase in firing rate) is inversely related to the expectation of a reward, such that unpredicted rewards will elicit a dopamine neuronal response that is larger in magnitude than a predicted reward (Schultz, 2010; Fiorillo et al, 2003). Currently, the dopamine transient signals during DRLU- and DRLS-mediated behavior are being investigated in our lab in collaboration with Dr. Michael A. Johnson's lab at the University of Kansas. Because there is a higher uncertainty of reward in the DRLU-72 contingency, we expect these rats to exhibit larger dopamine neuronal responses (accompanying reward delivery) that are larger in magnitude than in the DRLS-72 contingency. If this hypothesis is supported by data, ARZ may be more likely to exert disruptive and dopamine antagonistic effects on DRLU-72 rats than DRLS-72 rats, because DRLU-72 rats.

3.2 METHODS

3.2a Subjects

The original sixteen rats from the first experiment (Chapter 2) were used in this study. Additional information on how rats were housed and cared for is described in the previous chapter.

3.2b Apparatus

Testing procedures were conducted with the same apparatus (i.e., hybrid chambers) as the previous study discussed in Chapter 2.

3.2c Behavioral Procedure

For detailed explanation on training procedures for DRLU-72 and DRLS-72 rats, see Chapter 2. Rats were given six 4-hr sessions of DRL-72 before experimental testing began. Sessions were run three times a week; the first group (DRLS-72; n = 8) were run on Mondays, Wednesdays, and Fridays, while the second group (DRLU-72; n=8) were run on Tuesdays, Thursdays, and Saturdays.

3.2d Drugs

ARZ was purchased from Toronto Research Chemicals, Inc (Toronto, Canada). All injections were administered by i.p. and in a volume of 1.0 ml/kg. ARZ was suspended in physiological saline containing 2.5% Tween-80 solution (VECH). Similar to drug-free sessions, each drug session was separated by 1-2 days. VECH was administered immediately before rats began their 7th 4-hr session, followed by 3.0mg/kg ARZ, 1.0mg/kg ARZ, and then 6.0mg/kg ARZ.

3.2e Data Analysis

As in the previous experiment (Chapter 2), data were collected from hybrid chambers (Fowler et al, 2001) and processed through customized PASCAL programs and SYSTAT (Systat software, San Jose, CA). For analysis, behavioral measurements from VECH sessions were compared with ARZ treatment sessions (within-subjects differences), and differences between DRLU and DRLS groups were assessed (between-group differences). Statistical significance ($\alpha =$

0.05) was generally determined using multivariate repeated measures (RM) ANOVAs and post hoc ANOVAs. Operant response variables included the number of responses, number of reinforcements, and median interresponse times (IRTs). Responses were divided into *burst* responses (IRT < 3 s), *non-burst* responses (IRT > 3 s), and *unreinforced non-burst* (IRT > 3 s and IRT < 72 s) responses. For each rat, the operant response measures (e.g., median IRTs, number of responses, number of reinforcements) were assessed for the entire 4-hour session. For rats emitting less than 5 responses in a session, scores were replaced with the group mean for that treatment day in order to perform RM ANOVA without omitting data for several rats. Similarly, for statistical analysis of timing behavior, the median IRT scores were calculated for each rat emitting at least 50 responses within a session. Behavioral measures derived from the force plate actometer were locomotor activity and spatial location within the chamber (e.g., quadrant usage). As in Chapter 2, locomotor analysis was performed during the 72-s time period before rats produced a reinforced response. Locomotor and movement trajectory data were averaged for each individual rat's reinforced responses before calculating DRLS-72 or DRLU-72 group means.

3.3 RESULTS

3.3a Average Response and Reinforcement Rates

Similar to other antipsychotics examined on DRL schedules, the predominant effect of ARZ was to decrease the number of responses and reinforcements earned in a dose-dependent manner (Wiley et al, 2000; O'Donnell & Seiden, 1983). Response rates for vehicle and ARZ sessions are shown in Figure 3-1A. For non-burst responses, a two-way RM ANOVA confirmed a significant effect of DRL schedule [$F(1,14)=6.15, p < 0.05$], where DRLU-72 rats consistently

produced more responses than DRLS-72 rats at every ARZ dose. However, post hoc tests conducted with one-way ANOVAs found this effect significant only for the 3.0mg/kg ARZ dose ($F(1,14) = 10.02, p < 0.01$). Additionally, a two-way ANOVA of non-burst responses yielded a significant effect of ARZ dose, where ARZ administration decreased non-burst response rates for both DRL groups [$F(3,42) = 28.56, p < 0.0001$; Figure 3-1A]. An interaction of DRL schedule X ARZ dose was insignificant.

A two-way RM ANOVA of burst response rates indicated an insignificant effect of DRL schedule [$F(1,14) = 0.47, p = ns$], a significant effect of ARZ dose [$F(3,42) = 24.88, p < 0.0001$], and no interaction. Thus, despite having “increased stimulus control” (Wiley et al, 2000), DRLS-72 rats did not substantially differ from DRLU-72 rats in burst response rates, but ARZ dose-dependently reduced burst responding in both DRL groups (Figure 3-1A).

Statistical analysis of reinforcement rates using a two-way RM ANOVA yielded an insignificant effect of DRL schedule [$F(1,14) = 1.00, p = ns$], significant effect of ARZ dose [$F(3,42) = 8.79, p < 0.001$], and significant interaction of DRL schedule x dose [$F(3,42) = 8.35, p < 0.001$]. Post-hoc testing using one-way RM ANOVAs indicated a significant decrease of DRLS-72 reinforcement rates from the VECH session for all doses [for 1.0mg/kg ARZ, $F(1,7) = 6.29, p < 0.05$; for 3.0mg/kg ARZ, $F(1,7) = 23.04, p < 0.01$, for 6.0mg/kg ARZ, $F(1,7) = 29.09, p < 0.01$]; However, DRLU-72 reinforcement rates significantly *improved* following 1.0mg/kg ARZ administration [$F(1,7) = 9.96, p < 0.05$], and did not significantly differ from VECH values for the 3.0mg/kg [$F(1,7) = 4.51, p = ns$] and 6.0mg/kg dose [$F(1,7) = 0.22, p = ns$]. While ARZ treatment

tended to decrease reinforcement rates, post hoc comparisons suggest this effect was stronger for the DRLS-72 group (Figure 3-1B).

To better understand the differential effects of ARZ on DRLU-72 and DRLS-72 reinforcement rates, unreinforced non-burst responses (IRTs > 3 s and < 72 s) were calculated for each treatment session. Figure 3-1D shows ARZ dose dependently reduced unreinforced responding, and that DRLU-72 rats produced substantially more unreinforced responses than DRLS-72 rats at every dose. A two-way RM ANOVA indicated all effects were significant: DRL Schedule [$F(1,14) = 34.96, p < 0.0001$], ARZ dose [$F(3,42) = 17.69, p < 0.0001$], and interaction [$F(3,42) = 13.72, p < 0.0001$]. Post hoc testing using one-way ANOVAS confirmed significant differences in unreinforced response rates between DRL schedules for every dose: [for VECH, $F(1,14) = 43.27, p < 0.001$; for 1.0mg/kg ARZ, $F(1,14) = 17.71, p < 0.001$; for 3.0mg/kg ARZ, $F(1,14) = 19.00, p < 0.001$; for 6.0mg/kg ARZ, $F(1,14) = 13.66, p < 0.01$]. Additionally, post-hoc testing for either DRL group confirmed a significant reduction of unreinforced response rates at each ARZ dose (post-hoc significance levels are reported in Figure 3-1D). However, it should be noted that DRLU-72 unreinforced responses accounted for a larger percentage of overall responding relative to DRLS-72 unreinforced responses. Thus, ARZ-induced reductions in unreinforced responses may have had more beneficial effects (i.e., increased rates of reinforcement) on DRLU-72 operant performance than on DRLS-72 maintained behavior.

3.3b IRT Frequency Distributions and Med IRTs

Aripiprazole produced a right-ward shift of IRT distributions in a dose-dependent fashion (Figure 3-2). The magnitude of the ARZ-induced right-ward shift, however, was different for

both DRL groups. With increasing dose, the DRLU-72 group median IRT value increased by 16.67%, 17.15%, and 35.41% from VECH values, whereas DRLS-72 rats had a 1.74%, 1.42%, and 3.40% increase. A two-way RM ANOVA of median IRTs indicated an insignificant effect of DRL schedule [$F(1,14) = 1.40, p = ns$], a significant effect of ARZ dose [$F(3,42) = 8.74, p < 0.001$], and significant interaction [$F(3,42) = 5.69, p < 0.01$]. Post-hoc analysis using one-way ANOVAs, however, indicated a significant effect of DRL schedule for the 6.0mg/kg dose [$F(1,14) = 11.67, p < 0.01$]. Figure 3-3 shows the group mean value of median IRTs for all treatment conditions, as well as significant post-hoc results.

3.3c Distance Traveled during the pre-72 s Reinforcement Interval

After ARZ treatment, DRLS-72 rats engaged in substantially more pre-reinforcement activity than DRLU-72 rats (Figure 3-4). A two-way ANOVA for distance traveled during pre-reinforcement intervals yielded a significant main effect of DRL schedule [$F(1,14)=59.82, p < 0.0001$], an insignificant effect of dose [$F(3,42)=2.75, p = ns$], and no interaction. Relative to vehicle, ARZ administration produced modest declines in locomotion at the 1.0mg/kg and 3.0mg/kg dose, followed by a larger but non-significant increase at the 6.0mg/kg dose (Figure 3-4).

3.3d Quadrant Usage

Figure 3-5 shows the mean percent time each quadrant was occupied for both DRL groups. Statistical analysis of quadrant usage during the pre-72 s reinforcement interval was conducted using a three-way RM ANOVA of DRL Schedule X Quadrant X ARZ dose. An insignificant effect of DRL schedule [$F(1,14) = 0.54, p = ns$], insignificant effect of dose [$F(3,42) = 1.28, p = ns$], and significant effect of quadrant [$F(3,42) = 55.82, p < 0.0001$] were obtained,

with no 3-way interaction. All two-way interactions were insignificant except Quadrant x DRL Schedule [$F(3,42) = 7.64, p < 0.001$], which was a replication of data reported in the previous chapter. Regardless of DRL schedule and dose, rats spent the majority of the pre-reinforcement interval positioned in QI (operandum) and QIV (Lick disk). However, the distribution of time spent in QI and QIV differed between DRL groups. Post hoc testing using one-way ANOVAs confirmed that DRLU-72 rats spent significantly more time in QIV than the DRLS-72 group [for 1.0mg/kg ARZ, $F(1,14) = 8.77, p < 0.05$; for 3.0mg/kg ARZ, $F(1,14) = 8.26, p < 0.05$; for 6.0mg/kg ARZ, $F(1,14) = 5.51, p < 0.05$] and significantly less time in QI for the 1.0mg/kg and 3.0mg/kg dose [for 1.0mg/kg ARZ, $F(1,14) = 7.18, p < 0.05$; for 3.0mg/kg ARZ, $F(1,14) = 6.89, p < 0.05$; for 6.0mg/kg ARZ, $F(1,14) = 4.26, p = ns$]. These group differences in quadrant usage suggest that DRLS-72 rats are less inclined to spatially distance themselves away from the operandum regardless of ARZ's behavioral effects.

3.4 DISCUSSION

To our knowledge, this is the first study to evaluate the effects of ARZ on DRL-mediated behavior. Other typical and atypical antipsychotics, however, have been investigated under the DRL schedule and other timing operant schedules, such as the bi-section task and peak procedure (Wiley et al, 2000; Frederick & Allen, 1996). Reviews on antipsychotics within the timing literature have consistently shown these drugs to increase the temporal latency of responses and shift IRT distributions to the right; an effect popularly attributed to depletion of available dopamine or blockade of dopamine D2 receptors in the brain (Backstrom et al, 2011, Nordquist et al, 2008, Wiley et al, 2000). Most studies have reported that antipsychotics drugs dose-dependently decrease response and reinforcement rates on DRL schedules (Wiley et al,

2000; O'Donnell & Seiden, 1983; Jackson et al, 1995), though a few conflicting reports exist (Pollard & Howard, 1986; Britton & Koob, 1989).

ARZ acted similarly to other antipsychotic drugs on DRL schedules by dose-dependently shifting median IRT values to the right and reducing non-burst and burst-response rates relative to VECH (Wiley et al, 2000; O'Donnell & Seiden, 1983). However, DRLS-72 IRT distributions were less affected by ARZ administration than those of the DRLU-72 group, suggesting that the presence of an external signal largely maintained the temporal regulation of responses in the DRLS-72 group.

Interestingly, ARZ differentially affected DRLU-72 and DRLS-72 reinforcement rates. While ARZ dose-dependently decreased reinforcers earned in the DRLS-72 condition, post-hoc testing confirmed a significant improvement in DRLU-72 reinforcement rates at the 1.0mg/kg ARZ dose and slight, non-significant decreases at the 3.0mg/kg and 6.0mg/kg dose (Figure 3-1B). Differences in reinforcement rates between DRL groups may be explained by ARZ-induced decreases in response rates, particularly unreinforced response rates. Prior to ARZ administration, DRLS-72 rats exhibited high response efficiency, producing low rates of unreinforced responses (13.50 +/- 1.71 SEM) compared to DRLU-72 rats (135.0 +/- 18.39 SEM). For both groups, ARZ dose-dependently increased response efficiency; however, this had a stronger effect on DRLU-72 performance, as DRLS-72 rats were already approaching optimal response efficiency under VECH (Figure 3-1D). Thus, the ARZ-induced reduction in response rates improved DRLU-72 performance by decreasing extraneous responding (i.e., unreinforced

responding), whereas it worsened DRLS-72 performance by decreasing the opportunity to produce responses that were likely to be reinforced.

The 1.0mg/kg ARZ dose improved DRLU-72 operant performance by significantly decreasing response rates and increasing reinforcement rates. This therapeutic effect is similar to the effects of antidepressant drugs, which have consistently been shown to improve operant responding (i.e., decrease response rates and increase reinforcement rates) and temporal discrimination in rats performing DRLU-72 schedules (McGuire & Seiden, 1980; Seiden et al, 1985; O'Donnell et al, 2005). The neurochemical mechanisms by which antidepressants induce these effects on DRLU-72 mediated behavior are not fully understood; however, several studies have indicated an involvement of serotonergic neurotransmission (O'Donnell et al, 2005). Specifically, several studies published by Marek and colleagues have shown that drugs that act as 5-HT_{1a} agonists or 5-HT_{2a} antagonists, or both, dose-dependently induce antidepressant-like effects on sprague dawley rats performing DRLU-72 schedules (Marek & Seiden, 1987; Marek et al, 1989a, Marek et al, 1989b). ARZ has similar pharmacodynamics properties to the serotonergic compounds mentioned above: it is a partial agonist of 5-HT_{1a} receptors and an antagonist of 5-HT_{2a} receptors (Jordan et al, 2002). However, antidepressant drugs and compounds selective for the 5HT_{2a} or 5HT_{1a} receptor site usually produce these therapeutic effects in a dose-dependent manner (O'Donnell et al, 2005), unlike ARZ, which continued to decrease DRLU-72 response rates with increasing dose but produced modest and non-significant declines in reinforcement rates at the 3.0mg/kg and 6.0mg/kg ARZ doses. Similarly, Pollard & Howard (1986) reported traditional antipsychotics chlorpromazine and haloperidol to induce antidepressant-like effects on DRLU-72 schedules when administered at moderate

doses. Overall, ARZ's general effects on operant performance share qualities associated with both antidepressant and antipsychotics on DRLU schedules.

For the present study, ARZ did not substantially alter DRL-mediated locomotor activity relative to the VECH session as assessed in the pre-72 s reinforcement interval. Rather, locomotion and spatial positioning was predominately influenced by DRL condition: For all treatment sessions, DRLS-72 rats continued to exhibit significantly higher levels of locomotor activity (Figure 3-4) and stayed in closer proximity to the operandum than DRLU-72 rats. Thus, ARZ did not disrupt locomotor activity, apparently leaving collateral behavior intact. ARZ had a tendency to produce moderate declines in locomotion at the 1.0mg/kg and 3.0mg/kg dose, and a non-significant increase at the highest dose. This is in contrast to other studies that have reported large, significant decrease in locomotion following ARZ treatment within the same dose range of the current experiment (Ingman et al, 2006; Fellensten et al, 2007; Nordquist et al, 2008). These conflicting results may partially be explained by differences in methodologies; particularly the timing of ARZ treatment and locomotor assessment (Nordquist et al, 2008; Ingman et al, 2006). Furthermore, our analysis focused on task-directed locomotor activity (movement during the pre-72 s reinforcement interval), when minimal levels of locomotion are associated with successful DRLU-72 operant performance (Fowler et al, 2009). Our findings are comparable to Backstrom et al (2010) who found ARZ administration (0.03-3.0mg/kg) induced non-significant decreases in spontaneous locomotor activity in Wistar rats. The propensity of ARZ to decrease locomotion is in line with its DA antagonistic properties, as DA antagonists have been consistently shown to suppress locomotor activity (O'Neill et al, 1999; Fowler et al, 2008).

3.5 FIGURES AND TABLES

Figure 3-1. Operant response measures under ARZ administration.

Plots depicted on the left and right describe operant response measures for DRLU-72 and DRLS-72 groups, respectively. The bar graphs for each row depicted the group mean (\pm SEM) values for (A) non-burst responses, (B) burst responses, (C) reinforcements, and (D) unreinforced non-burst response rates for all treatment conditions. Post hoc tests using one-way RM ANOVAS were performed to determine if the value for the drug dose was significantly different from vehicle dose. Post-hoc test significance levels are as follows: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

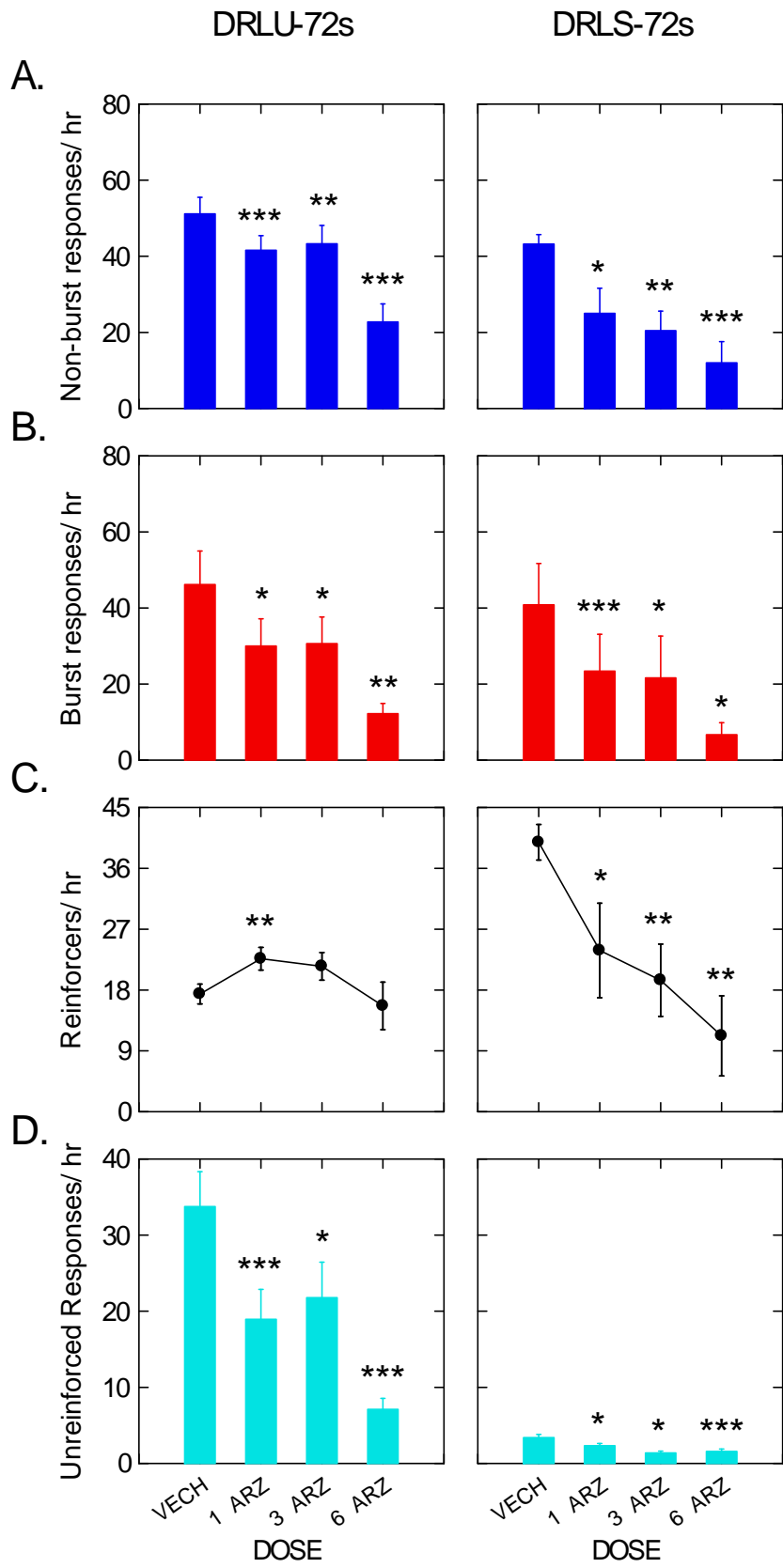


Figure 3-2. IRT frequency distributions under ARZ treatment.

The plots below depict the group mean percentage of responses made at each 2-sec bin for VECH (*blue*) and ARZ (*orange*) treatment sessions. The top and bottom rows reflect DRLU-72 and DRLS-72 frequency distributions, respectively. The vertical dotted line indicates the 72-sec temporal requirement. The y-axis was log₁₀ transformed in order to emphasize the differences in IRT response frequency at each bin. Note that the DRLS-72 IRT values for the 3mg/kg and 6mg/kg appear unstable because the low number of responses.

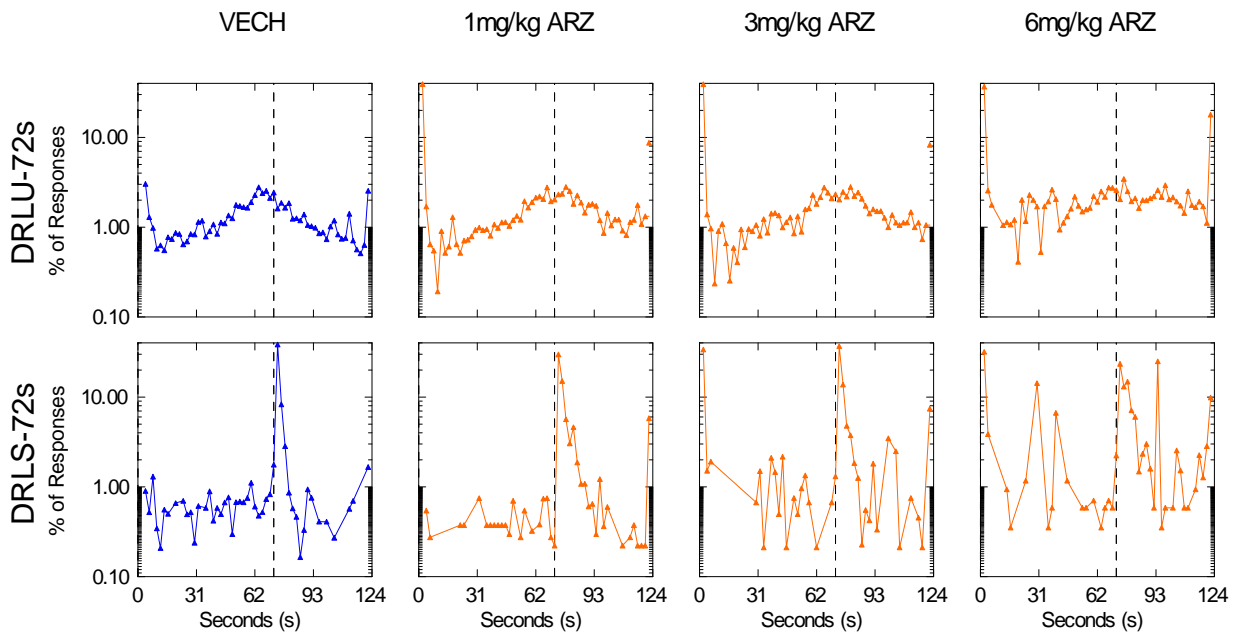


Figure 3-3. Median IRT values for VECH and ARZ treatment sessions.

The DRLU-72 (*blue*) and DRLS-72 (*red*) group means (+/- SEM) for median IRTs are plotted for each drug dose. The dashed line represents the 72-sec criterion interval. Post hoc analysis were conducted with one-way ANOVAs at each dose to determine a significant effect of schedule (DRLU-72 vs DRLS-72; #). One-way RM ANOVAs were performed to indicate a significant difference of the drug dose value from the vehicle dose (*). Post hoc test significance levels are as follows: # = $p < 0.01$, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

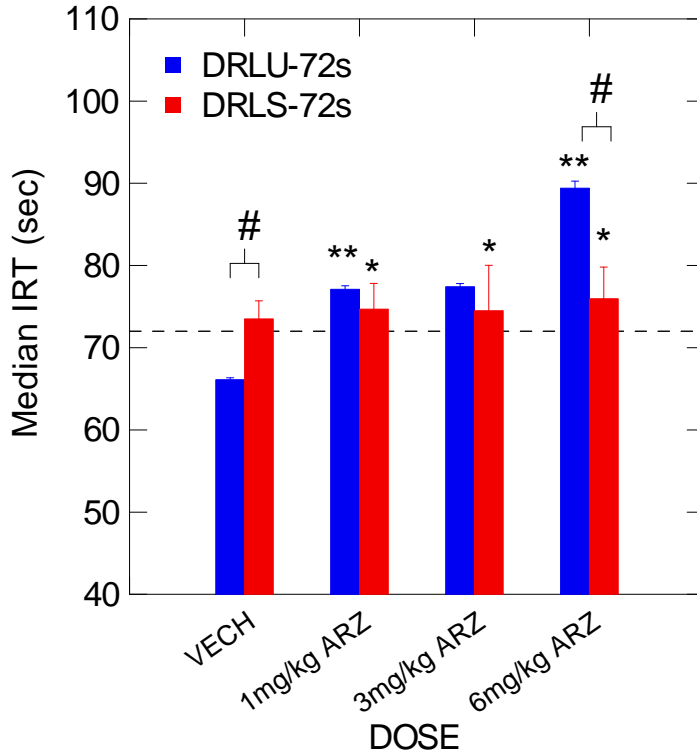


Figure 3-4. Distance traveled during pre-reinforcement interval under ARZ treatment conditions. The group mean (\pm SEM) for distance traveled during the pre-72 sec reinforcement interval are plotted for each drug dose. While DRL schedule significantly influenced locomotor activity, ARZ did not significantly alter locomotor movement.

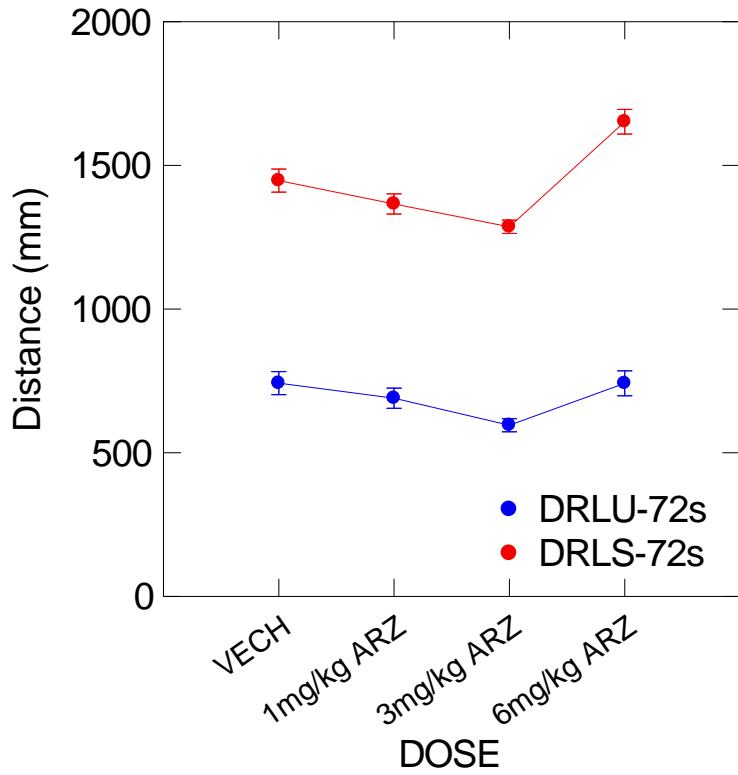
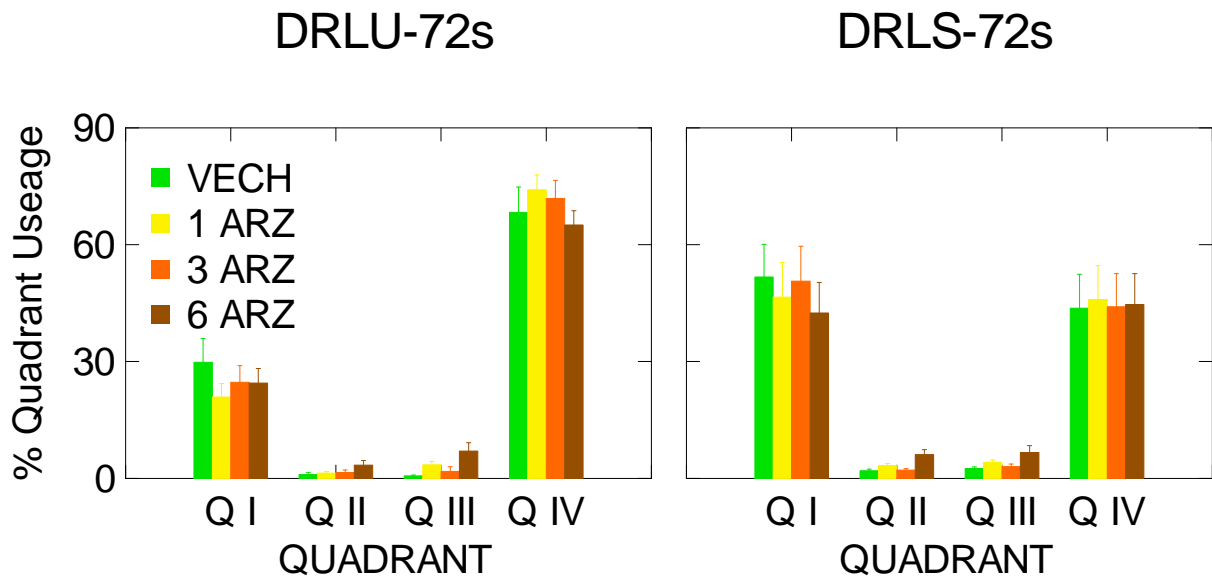


Figure 3-5. Group mean (+/- SEM) time spent in each quadrant for ARZ treatment sessions. Quadrant usage during the pre-72 sec reinforcement interval is shown for DRLU-72 and DRLS-72 groups. Quadrant location was measured in hundredths of a second. The distribution of time spent in Q1 and QIV differed between DRL groups.



CHAPTER 4: EFFECTS OF ARIPIPRAZOLE IN D-AMPHETAMINE-TREATED RATS PERFORMING SIGNALLED OR UNSIGNALLED DRL TIMING SCHEDULES

4.1 INTRODUCTION

In the current experiment, rats trained to either DRLU-72 or DRLS-72 schedules were administered 5.0mg/kg AMPH to induce a putative schizophrenic-like timing deficit. Previous studies have shown that AMPH treatment distorts temporal processing in DRLU rats by decreasing median IRT values and shifting IRT distributions to the left (Fowler et al, 2008; Fowler et al, 2009; Balcells-Olivero et al, 1997). AMPH treatment has also been shown to disrupt DRLU operant performance by increasing response rates while decreasing reinforcement rates (Fowler et al, 2008; Fowler et al, 2009; Balcells-Olivero et al, 1997). In studies that have compared DRLU and DRLS performance, signaled rats were resistant to AMPH-induced impairment, earning more reinforcers and producing longer median IRTs than unsignaled rats (Wiley et al, 2000; Carey & Kritkauskys et al, 1972). However, these studies examined low doses of AMPH treatment (1.0 -3.0mg/kg) in rats that were not previously sensitized to AMPH treatment. In addition, these studies investigated AMPH-induced impairments in DRLU and DRLS schedules with low temporal requirements (15-22 s); as opposed to the current study, where rats were trained to adhere to a higher temporal criterion (72 s).

The capacity of ARZ to attenuate timing deficits in an animal model of psychosis has not yet been examined. Previous laboratory animal behavioral experiments have demonstrated the ability of aripiprazole (ARZ) to attenuate cognitive impairments in animal models of

schizophrenia. Carli et al (2010) found ARZ to attenuate attentional impairments induced by the competitive NMDA receptor antagonist, carboxypiperazin-4-propyl-1-phosphonic acid (CPP) in rats performing the 5-choice serial reaction time task (5-CSRTT). ARZ attenuated PCP-induced cognitive impairments in recognition memory (Nagi et al, 2008) and PPI tasks in rats (Nordquest et al, 2008). In addition, ARZ has been shown to reduce AMPH-induced hyperlocomotion (Nordquest et al, 2008; Mavrikaki et al, 2010) in rats.

The goals of the present study were to (1) evaluate the severity of AMPH-induced impairment in signaled and unsignaled DRL schedules and (2) to assess the ability of ARZ, a partial dopamine D2 agonist, to reverse these deficits. Thus, this study is intended to assess the ability of the antipsychotic, ARZ, to attenuate schizophrenic-like cognitive impairments induced by AMPH. Listed below are our hypotheses about behavioral processes that are expected to be differentially affected by ARZ on DRL-trained rats pretreated with AMPH.

4.1a The Influence of AMPH on interval timing

Past experiments have shown AMPH treatment to distort temporal processing: AMPH causes both animals and humans to perceive temporal durations as passing more rapidly than objective time (Fowler et al, 2009; Balcells-Olivero et al, 1997). In accordance with the literature, we have previously shown amphetamine treatment to impair DRLU-72 performance by inducing left-ward shifts in IRT frequency distributions and significantly decreasing median IRTs (Fowler et al, 2009; Fowler et al, 2008; Balcells-Olivero et al, 1997). While temporal processing may be similarly disrupted in DRLS-72 rats, we hypothesize that the presence of an external signal for reward availability will lessen AMPH-induced impairments, because it

reduces dependency on interval timing and shifts behavioral control from internal to exteroceptive stimuli.

4.6b Task difficulty: Differential effects of AMPH

Because interval timing without external cueing is a more demanding and complex cognitive task than perceiving and responding to an external stimulus, we expect DRLU-72 rats to be significantly more impaired by AMPH than DRLS-72 rats. As previously stated above, we anticipate DRLS-72 rats to be resistant to AMPH-induced disruption of operant performance, although AMPH treatment is still expected to induce unconditioned behaviors such as hyperlocomotion and focused stereotypy, given the doses studied.

4.2 METHODS

4.2a Subjects

For the present study, sixteen new rats that had not previously received any drug treatment were used. Similar to the experiments discussed in Chapters 2 and 3, subjects were male, Sprague-Dawley rats (Harlan, Indianapolis). Rats began operant training at approximately 2.5 months of age, and experimental testing at approximately 4.5 months of age. When experimental testing began, rats had a mean body weight of 350.87g (SEM +/- 5.01). Additional information on how rats were housed and cared for is described in Chapter 2.

4.2b Apparatus

All training and testing procedures were conducted in four specialized “hybrid” chambers, designed with force-plate actometers in the flooring and operant fixtures (Fowler et al, 2001). Detailed descriptions of the hybrid chambers are provided elsewhere (Chapter 2).

4.2c Behavioral Procedure

Training procedures were carried on in a similar manner to previous experiments (Chapters 2 and 3). One rat trained to the DRLU-72 condition died of unknown causes before drug treatment began. Once the remaining rats (DRLS-72, n = 8; DRLU-72, n = 7) had demonstrated stable operant performance, drug testing began.

4.2d Drugs

Aripiprazole (ARZ) and d-amphetamine sulfate were purchased from Toronto Research Chemicals, Inc (Toronto, Canada) and Sigma-Aldrich (St.Louis, Mo, USA), respectively. All injections were administered i.p. and in a volume of 1.0 ml/kg. D-amphetamine sulfate was mixed in physiological saline and ARZ was suspended in a physiological saline containing 2.5% Tween-80 solution.

Similar to drug-free sessions, each drug session was separated by 1-2 days. Each rat received an injection for 5.0mg/kg d-amphetamine at the start of the next 10 sessions (Sessions #7-17). The rationale for using a dose of 5.0mg/kg d-amphetamine for several sessions has been previously described in an earlier paper (Fowler et al, 2009), where our lab demonstrated repeated treatments of AMPH in rats led to an increased behavior response (i.e., reduced latency to express focused stereotypy and more pronounced focused stereotypy) to the drug. Following sensitization to d-amphetamine, rats received VECH-ARZ (2.5 % Tween-80 solution), 0.1.0mg/kg ARZ, 0.3 mg/kg ARZ, or 1.0mg/kg ARZ 30 minutes into the session. However, because these doses of ARZ did not produce effects sufficient for a dose-effect analysis, it was decided to use higher doses of ARZ (1.0 mg/kg, 3.0 mg/kg, 6.0 mg/kg). Therefore, rats received 8 additional sessions of 5.0mg/kg d-amphetamine (Sessions 22-29) before receiving sessions

that examined the effects of VECH-ARZ, 3.0 mg/kg ARZ, 1.0 mg/kg ARZ, and 6.0 mg/kg ARZ (Sessions 30-33). ARZ was administered according to a within-subject dose effect design. Again, VECH-ARZ and ARZ were administered 30 minutes after receiving AMPH treatment.

4.2e Data Analysis

Data were collected from hybrid chambers and processed through customized PASCAL programs and SYSTAT (Systat software, San Jose, CA). For analysis, behavioral measurements from ARZ-VECH sessions were compared with ARZ treatment sessions (within-subjects differences), and differences between DRLU-72 and DRLS-72 groups were assessed (between-groups differences). Statistical significance ($\alpha = 0.05$) was generally determined using multivariate repeated measures (RM) ANOVAs and post hoc ANOVAs. Operant response variables included the number of responses, number of reinforcements, and median interresponse times (IRTs). Responses were divided into *burst* responses (IRT < 3 s), *non-burst* responses (IRT > 3 s), and unreinforced non-burst responses (IRT > 3 s and IRT < 72 s). For AMPH sessions, operant response measures (e.g., median IRTs, number of responses, number of reinforcements) for each rat were assessed *after* focused stereotypy subsided. For comparison, operant responses from non-AMPH treatment sessions (Chapter 3) were calculated for similar time points where ARZ drug-induced behavioral changes would be similar (Figure 4-4). This analysis is discussed in more detail within the context of the results. Behavioral measures derived from the force plate actometer were focused stereotypy (FS) scores, locomotor activity and spatial location within the chamber (e.g., quadrant usage). Rather than assessing these measurements for the entire session, analysis concentrated during the 72- s time period before rats produced a reinforced response. Locomotor and movement

trajectory data were averaged for each individual rat's reinforced responses before calculating DRLS-72 or DRLU-72 group means. Locomotor activity was assessed by recording the distance traveled between two coordinates every 0.50 s throughout the 4-hr session. Focused stereotypy (FS) scores were obtained by measuring the number of sectors (1.75cm^2) used and power spectra of the rats' vertical force during movement on the chamber floor. The duration of the FS syndrome was calculated by finding the 3-min frame when FS scores dropped to 20% of their maximum value or the number of sectors used increased to 15.

4.3 RESULTS

For all AMPH treatment sessions, the 5.0mg/kg dose induced a pattern of behavior that was highly consistent among rats. Figure 4-1 shows the operant responses and movement trajectories of a single DRLU-72 rat (panel A) and DRLS-72 rat (panel B) for the AMPH + ARZ-VECH treatment session. AMPH-treated rats spent the first portion of the 4-hr session engaged in FS; they were unable to perform operant responses or interact with any operant fixtures. This can be seen in Figure 4-1, which shows both DRLU-72 and DRLS-72 rats spatially confined during the first half of the session, with exception of the 10th frame, when ARZ-VECH was administered. After FS subsided, AMPH-treated rats engaged in hyperlocomotion and operant-directed behavior. In Figure 4-1, the AMPH-treated DRLS-72 rat (Figure 4-1B) earned more reinforcers and made fewer unreinforced responses than the AMPH-treated DRLU-72 rat (Figure 4-1A), suggesting that AMPH administration did not severely disrupt DRLS-72 learned behaviors relative to the DRLU-72 group once operant responding resumed after FS.

When evaluating the effects of ARZ on AMPH-treated rats, we examined its ability to antagonize FS and hyperlocomotion (an unlearned behavior), as well as normalize operant performance and restore apparently normal cognitive function (a learned behavior). In other words, we assessed the ability of ARZ to attenuate schizophrenic-like impairments in both learned and unlearned behaviors induced by AMPH.

In the analysis presented below, we examined the effects of ARZ on unconditioned behaviors (FS, hyperlocomotion) and learned behaviors (operant performance and collateral activity) in AMPH-treated DRLU-72 and DRLS-72 rats. Then, we examined the effects of AMPH treatment on both DRL groups by comparing operant performance and locomotor activity from AMPH-treatment sessions with non-AMPH treatment sessions (Chapter 3).

4.3a Duration of Amphetamine-induced FS

For the AMPH + VECH-ARZ session, group mean FS durations were nearly identical: 105min (+/- 6.99) and 104.25min (+/-9.07) for DRLU-72 and DRLS-72, respectively. Administration of ARZ dose-dependently decreased the amount of time AMPH-treated rats engaged in FS (Figure 4-2) and restored locomotion earlier in the session relative to control (AMPH + ARZ-VECH session). Figure 4-3 shows the DRL group mean number of sectors used across 3-min time blocks for all AMPH treatment sessions. The red line represents locomotor activity for the 1.0 mg/kg (Figure 4-3A), 3.0 mg/kg (Figure 4-3B) and 6.0 mg/kg ARZ dose (Figure 4-3C), respectively. A two-way RM ANOVA of FS durations indicated an insignificant effect of DRL schedule [$F(1,13)=2.36$, $p = ns$], a significant effect of ARZ dose [$F(3,39) = 20.43$, $p <$

0.0001], and no interaction. Thus, ARZ dose-dependently reduced the duration of FS regardless of DRL contingency. Significant post hoc comparisons for group means are shown in Figure 4-1.

4.3b Comparison of Operant Response Measures between AMPH and non-AMPH Treatment Sessions

To examine the effects of AMPH treatment on DRLU-72 and DRLS-72 timing behaviors and cognitive-related locomotor activity (collateral activity), we compared AMPH-treatment sessions with non-AMPH treatment sessions. This analysis, however, was not straightforward: Because AMPH-treated rats did not initiate responses on the operandum until focused stereotypy subsided, averaging operant response measures across the entire 4-hr session would mask AMPH-induced changes in timing behavior. To assess ARZ's therapeutic-like effects in the current study, the drug was given 30 min into the session after the AMPH's effects were prominent. Therefore, operant response rates for AMPH sessions were calculated for each rat during the time period when operant responding resumed.

As described in the previous chapter, ARZ—when studied by itself—was administered at the beginning ($t = 0$ min) of the session. For these non-AMPH sessions, operant response measures were determined for the same *length* of time but not the same *time period* as AMPH sessions, because the pharmacokinetic effects of ARZ would differ. The schematic diagram in Figure 4-4 further explains how time periods within AMPH (*current study*) and non-AMPH sessions (*Chapter 3 study*) were selected for analysis of operant performance. For example, AMPH-treated DRLU rats resumed responding approximately 75 min (± 6.99 SEM) after receiving the ARZ-VECH dose (Figure 4-4A). Therefore, operant response measures for the

corresponding non-AMPH session were collected 75 min after receiving ARZ-VECH (Figure 4-4B). Similar calculations for operant response measures were performed for both DRL groups and ARZ-drug treatment sessions.

4.3c IRT Frequency Distributions and Median IRTs

The IRT frequency distributions and group mean IRT values for AMPH treatment sessions are depicted in figures 4-5 and 4-6A, respectively. While median IRT values for the DRLU-72 group varied from 30-35 s, the DRLS-72 group median IRTs were close to the 72-s temporal criterion, ranging from 71-75 s (Figure 4-6A). A two-way RM ANOVA of median IRTs confirmed a significant main effect of DRL schedule [$F(1,13) = 291.38, p < 0.0001$], a significant effect of ARZ dose [$F(3,39) = 3.22, p < 0.05$], and no interaction. Post hoc analysis using one-way ANOVAs indicated a significant effect of DRL schedule for all AMPH treatment sessions [for ARZ-VECH, $F(1,13) = 328.60, p < 0.0001$; for 1.0 mg/kg ARZ, $F(1,13) = 269.73, p < 0.0001$; for 3.0 mg/kg ARZ, $F(1,13) = 192.05, p < 0.0001$; for 6.0 mg/kg ARZ, $F(1,13) = 101.33, p < 0.0001$]. ARZ administration in AMPH sessions produced significant but modest right-ward shifts of group median IRTs (Figure 4-6A). A RM ANOVA for ARZ dose was performed on median IRTs for each DRL group separately; however, none of these post hoc tests were significant for any DRL group.

Relative to the non-AMPH sessions, AMPH treatment produced a left-ward shift of the DRLU-72 group mean IRT frequency distributions (Figure 4-5) and shortened median IRT values (Figure 4-6B). This finding is in agreement with other published reports on AMPH-induced disruption of DRLU timing behavior (Fowler et al, 2009; Carey & Kritkauskys, 1972; Wiley et al,

2000; Balcells-oliveo et al, 1997). In contrast to the DRLU-72 group, DRLS-72 median IRT values did not differ greatly between AMPH and non-AMPH sessions (Figure 4-6B).

Analysis of AMPH's effects on median IRT values were carried out using a 3-way RM ANOVA (DRL schedule X AMPH condition X ARZ dose). All main effects were significant [for DRL Schedule, $F(1,27) = 78.45, p < 0.0001$; for AMPH condition, $F(1,27) = 168.33, p < 0.0001$; for ARZ dose, $F(3,81) = 11.71, p < 0.0001$], including a 3-way interaction of ARZ dose X DRL schedule X AMPH condition [$F(3,81) = 3.37, p < 0.05$], suggesting that all 3 variables significantly influenced median IRTs and the temporal regulation of responding. In addition, DRL schedule significantly interacted with AMPH [$F(1,27) = 136.68$] and ARZ dose [$F(3,81) = 4.47, p < 0.01$]. The graphs in Figure 4-6B suggest AMPH and ARZ had little effect on DRLS-72 group median IRTs, while DRLU-72 median IRT values were greatly influenced by AMPH and ARZ treatment. To further investigate these interactions of AMPH and ARZ on median IRT values, a 2-way RM ANOVA for AMPH condition X ARZ dose was conducted separately for each DRL group. For DRLS-72, median IRTs did not significantly differ between AMPH and non-AMPH sessions [$F(1,14) = 2.23, p = ns$], but significantly increased by ARZ dose [$F(3,42) = 6.09, p < 0.01$]. No interaction was detected. For DRLU-72, all effects were significant [AMPH condition, $F(1,13) = 151.84, p < 0.0001$; ARZ dose, $F(3,39) = 7.88, p < 0.001$; ARZ dose X AMPH condition, $F(3,39) = 3.66, p < 0.05$]. This analysis suggests AMPH strongly influenced timing behavior in DRLU-72 rats, but had little effect on the DRLS-72 group. These findings are in agreement with Carey & Kritkauskys (1972), who showed rats trained to a DRLS-22s schedule are resistant to AMPH disruption in comparison to their unsignaled counterparts (DRLU-22s).

4.3d Non-burst response rates

As in the previously discussed experiments (Chapters 3 and 4), statistical analysis of non-burst and burst responses were segregated due to the different behavioral processes (i.e., interval timing vs “impulsive” behavior) that govern their occurrence (Richards, 1993). Non-burst responses are typically associated with temporal regulation, while burst responses have been associated with impulsivity and emotional responding (i.e., “frustration”) within the literature (Cheng et al, 2007; Ardayfio et al, 2008; Pattij et al, 2004; Sanger, 1992).

The operant response measures for all amphetamine treatment sessions are depicted in Figure 4-7. Past DRLU studies have shown antipsychotics and antidepressant drugs to dose-dependently decrease both non-burst and burst response rates (McGuire & Seiden, 1980; Seiden et al, 1985), while psychostimulants, such as AMPH, tend to increase both types of response rates (Fowler et al, 2009; Cheng & Liao, 2007). In the current study, ARZ administration dose-dependently decreased non-burst responding in both groups, though DRLU-72 rats produced higher rates of non-burst responding than the DRLS-72 group (Figure 4-7A). A two-way ANOVA for non-burst response rates indicated a significant main effect of DRL schedule [$F(1,13) = 48.35, p < 0.0001$] and ARZ dose [$F(3,39) = 5.31, p < 0.01$] with no interaction. Post hoc analysis confirmed DRLU-72 rats emitted significantly more non-burst responses than DRLS-72 rats, suggesting that the presence of an external signal was still effective for reducing responses under the AMPH condition [for VECH, $F(1,13) = 36.71, p < 0.0001$; for 1.0mg/kg ARZ, $F(1,13) = 47.36, p < 0.0001$; for 3.0mg/kg ARZ, $F(1,13) = 17.02, p < 0.01$; for 6.0mg/kg ARZ, $F(1,13) = 29.78, p < 0.001$]. ARZ administration dose-dependently decreased non-burst response rates, though post hoc testing suggests this effect was

predominately seen in the DRLS-72 group (Figure 4-6A). Polynomial contrasts confirmed a significant dose-related linear trend for non-burst response rates in DRLU-72 [$F(1,6) = 12.57, p < 0.05$] and DRLS-72 rats [$F(1,7) = 17.59, p < 0.01$].

As expected, AMPH treatment produced large increases in DRLU-72 non-burst response rates relative to non-AMPH treatment sessions. Similarly, AMPH increased DRLS-72 non-burst response rates in comparison to non-AMPH sessions, although this effect was not as pronounced. The effects of AMPH on non-burst response rates were evaluated in a 3-way ANOVA (DRL Schedule X AMPH condition X ARZ dose). A significant effect of DRL schedule [$F(1,27) = 45.64, p < 0.0001$], AMPH condition [$F(1,27) = 76.63, p < 0.0001$], and ARZ dose [$F(3,81) = 15.74, p < 0.0001$] were confirmed. An interaction of DRL schedule X AMPH condition was also detected [$F(1,27) = 24.75, p < 0.0001$], as AMPH-induced increases in non-burst response rates were less pronounced for signaled rats than for unsignaled rats (Figure 4-7A). No other interactions were significant. Additional analysis was carried out separately for each DRL group using a 2-way RM ANOVA for AMPH condition X ARZ dose. For the DRLU-72 group, a significant effect of AMPH condition [$F(1,13) = 57.21, p < 0.0001$] and ARZ dose [$F(3,39) = 6.36, p < 0.01$] was confirmed with no interaction. Similarly, all main effects were significant for DRLS-72 with no interaction [AMPH condition, $F(1,14) = 16.44, p < 0.01$; ARZ dose, $F(3,42) = 11.22, p < 0.001$]. Thus, ARZ partially reversed the AMPH-induced increases in non-burst response rates and acted similar to other antipsychotic and antidepressant drugs by dose-dependently decreasing their occurrence.

4.3e *Burst response rates*

In contrast to non-burst responses, burst response rates did not significantly differ between DRL groups, though ARZ dose-dependently reduced their occurrence (Figure 4-7B). A two-way ANOVA of burst responses resulted in an insignificant effect of DRL Schedule [$F(1,13) = 4.59, p = ns$], a significant effect of ARZ dose [$F(3,39) = 6.37, p < 0.01$], and no interaction. Post hoc tests performed separately for each DRL group indicated a significant effect of ARZ for the DRLS-72 group [$F(3,21) = 6.50, p < 0.01$], with a significant linear trend [$F(1,7) = 12.832, p < 0.01$]; however, this was not seen in the DRLU-72 group [$F(3,18) = 2.74, p = ns$]. Thus, similar to non-burst response rates, the dose-dependent effect of ARZ was more pronounced in DRLS-72 rats than the DRLU-72 group

In a 3-way ANOVA (DRL schedule X AMPH condition X ARZ dose) for burst responses, only ARZ dose was significant [$F(3,81) = 15.60, p < 0.0001$]. Thus, AMPH did not significantly alter the rates of burst responses across all ARZ treatment sessions. The effect of AMPH condition and DRL schedule was further examined using 2-way ANOVAs (AMPH X DRL Schedule) at each ARZ dose. These statistical tests were only significant for the 6.0mg/kg dose, which confirmed a significant effect of DRL Schedule [$F(1,27) = 12.82, p < 0.01$], AMPH condition [$F(1,27) = 6.95, p < 0.05$], and interaction [$F(1,27) = 16.00, p < 0.001$]. These results for AMPH and ARZ are in contrast to previous reports on DRL burst responding, which have repeatedly shown burst responding to increase when animals receive psychostimulants, such as AMPH and methamphetamine (McClure & McMillan, 1997; Wiley et al, 2000; Carey & Krakausky, 1972). Differences between published reports and the current study may be due to the time period where operant response measures were collected, which usually occurred after the first 30

minutes of the session. Past DRL studies have alluded to the presence of within session fluctuations of DRL performance (Richelle & Lejeune, 1980; Farmer and Schoenfeld, 1964; Ferraro et al, 1965). Within our lab, an oft-observed phenomenon during 4 –hr DRL sessions is that burst response rates are typically higher at the beginning of the session, when motivation and thirst is high (unpublished). Studies have suggested that operant performance tends to improve later in the session; when long sequences of reinforced IRTs are more likely to occur (Farmer and Schoenfeld, 1964).

4.3f Reinforcement Rates

Under AMPH conditions, ARZ administration resulted in a dose-dependent decline in of reinforcement rates for the DRLS-72 group, while DRLU-72 reinforcement rates appeared relatively unchanged (Figure 4-7C). At the 6.0mg/kg ARZ dose, the reinforcement rates for amphetamine-treated DRLS-72 rats dropped nearly 50% from the amphetamine ARZ-VECH session, whereas DRLU-72 reinforcement rates changed by less than 2% (Figure 4-6C).

A two-way ANOVA of reinforcement rates indicated a significant main effect of DRL schedule [$F(1,13) = 66.63, p < 0.001$] and a significant effect of ARZ dose [$F(3,39) = 3.09, p < 0.05$]. A significant interaction of DRL schedule and ARZ dose was also confirmed, [$F(3,39) = 3.83, p < 0.05$], suggesting ARZ's effects were more prominent in DRLS-72 rats than the DRLU-72 group. Post hoc tests using a RM ANOVA for ARZ dose indicated a significant, dose-dependent decrease for DRLS-72 rats [$F(3,21) = 4.01, p < 0.05$] but not DRLU-72 rats [$F(3,18) = 1.52, p = ns$]. In addition, a post hoc polynomial contrast for the DRLS-72 group confirmed a significant linear trend for DRLS-72 rats [$F(1,7) = 7.07, p < 0.05$].

As expected, DRLS-72 rats earned significantly more reinforcers than DRLU-72 rats for all amphetamine treatment sessions. Post hoc analysis indicated a significant difference between DRL groups for all doses but the 6.0mg/kg dose, when DRLS-72 reinforcement rates approached DRLU-72 values [for ARZ-VECH, $F(1,13) = 164.95, p < 0.001$; for 1.0mg/kg, $F(1,13) = 221.91, p < 0.001$; for 3.0mg/kg, $F(1,13) = 11.78, p < 0.01$; for 6.0mg/kg dose, $F = 1.32, p = ns$]. Thus, while DRLS-72 rats demonstrated better reward-procuring behavior than DRLU-72 rats (i.e., higher median IRTs; Figure 4-4), the number of reinforcements earned at the 6.0mg/kg ARZ dose did not statistically differ.

The effects of AMPH on reinforcement rates were assessed with a 3-way RM ANOVA (DRL schedule X AMPH condition X ARZ dose). Statistical analysis confirmed a significant effect of DRL schedule [$F(1,27) = 44.89, p < 0.0001$] and ARZ dose [$F(3,81) = 11.54, p < 0.001$] but not AMPH condition. A 3-way interaction was not significant, but DRL schedule significantly interacted with AMPH condition [$F(1,27) = 13.98, p < 0.001$] and ARZ dose [$F(3,81) = 7.04, p < 0.01$]. Further evaluation of these interactions was performed using a two-way RM ANOVA of AMPH condition X ARZ dose for each DRL group. For DRLS-72 rats, a significant effect of AMPH condition [$F(1,14) = 7.94, p < 0.05$] and ARZ dose [$F(3,32) = 8.22, p < 0.001$] were detected with no interaction. However, only AMPH condition was significant for DRLU-72 rats [$F(1,13) = 14.4, p < 0.01$], indicating that DRLU-72 reinforcement rates were predominately influenced by AMPH and not ARZ administration. Taken together, these statistical results imply that ARZ's effects on reinforcement rates were more prominent in rats trained to the signaled DRL contingency than to the unsignaled schedule.

4.3g Unreinforced Response Rates

Post hoc testing indicated that AMPH treatment significantly increased DRLS-72 non-burst response rates (Figure 4-7A, right panel), but not reinforcement rates (Figure 4-7C, right panel), in comparison to the non-AMPH session (ARZ-VECH session). This is an unusual finding for subjects performing a DRL schedule, because increases in non-burst response rates are usually accompanied by a decrease in reinforcement rates and a worsening of operant performance (Richelle & Lejeune, 1980). This relationship can be seen in the DRLU-72 group: post hoc testing confirmed that AMPH administration significantly increased non-burst response rates (Figure 4-7A, left panel) and decreased reinforcement rates (Figure 4-7C, left panel) relative to the non-AMPH session. These findings suggested that AMPH administration may have exerted differential effects on unreinforced responding in DRLU-72 and DRLS-72 groups.

To evaluate unreinforced responding, unreinforced response rates were calculated for the non-AMPH and AMPH ARZ-VECH sessions (Figure 4-8). A two-way ANOVA (AMPH condition X DRL schedule) confirmed a significant effect of AMPH [$F(1,27) = 64.96, p < 0.001$] and a significant effect of DRL schedule [$F(1,27) = 110.93, p < 0.001$]. A significant interaction was found [$F(1,27) = 34.23, p < 0.01$], confirming that AMPH treatment resulted in larger increases in unreinforced responding for the DRLU-72 group relative to the DRLS-72 group (Figure 4-8). Although AMPH treatment significantly increased DRLS-72 unreinforced response rates relative to the non-AMPH session, the magnitude of this increase was still relatively small [$F(1,14) = 4.91, p < 0.05$]. In contrast, AMPH administration produced a substantially large increase in DRLU-72 unreinforced response rates [$F(1,13) = 61.43, p < 0.0001$]. Similar to the non-AMPH

experiment (Chapter 3; Figure 3-1D), AMPH-treated DRLS-72 rats emitted significantly fewer unreinforced responses than the AMPH-treated DRLU-72 group [$F(1,13) = 80.79, p < 0.001$]. Altogether, these results indicate that while AMPH significantly elevated DRLS-72 and DRLU-72 non-burst response rates relative to the non-AMPH session (Figure 4-7A, right panel), AMPH-treated signaled rats still produced relatively low levels of unreinforced responding (Figure 4-8). Thus, cued-reinforcement enabled AMPH-treated DRLS-72 rats to maintain low rates of unreinforced responding; an effect that likely prevented an AMPH-induced decrease in DRLS-72 reinforcement rates.

4.3h Distance traveled during the Pre-72 s reinforcement interval

The first experiment (Chapter 2) showed that saline-treated DRLS-72 rats engaged in substantially more task-related locomotor activity than DRLU-72 rats (Figure 2-5). When ARZ was administered alone (Chapter 3), signaled rats continued to exhibit more locomotor activity than unsignaled rats, though ARZ had a non-significant tendency to decrease locomotion at low to moderate doses (Figure 3-4).

In the present study, AMPH abolished differences in locomotor activity between DRL groups. Figure 4-9 shows AMPH-treated DRLU-72 and DRLS-72 rats produced similar levels of locomotion, though ARZ administration dose-dependently decreased activity. A two-way ANOVA confirmed an insignificant effect of DRL schedule [$F(1,13) = 0.04, p = ns$], a significant effect of ARZ dose [$F(3,39) = 8.75, p < 0.001$], and no interaction. Post-hoc testing confirmed that AMPH-induced locomotion did not significantly differ between DRL schedules at any ARZ dose [for A + ARZ-VECH, $F(1,13) = 1.10, p = ns$; for A + 1.0mg/kg ARZ, $F(1,13) = 0.79, p = ns$; for A + 3.0mg/kg ARZ, $F(1,13) = 1.13, p = ns$; for A + 6.0mg/kg ARZ, $F(1,13) = 0.61, p = ns$].

Post-hoc comparisons also suggested ARZ had a more prominent effect in AMPH-treated DRLS-72 rats' locomotor activity than their unsignaled counterparts. Post hoc tests confirmed a significant decline in DRLS-72 locomotor activity at the 3.0mg/kg and 6.0mg/kg ARZ dose relative to the ARZ-VECH amphetamine treatment session [for 1.0mg/kg ARZ, $F(1,7) = 3.02$, $p = ns$; for 3.0mg/kg ARZ, $F(1,7) = 18.39$, $p < 0.01$; for 6.0mg/kg ARZ, $F(1,7) = 11.74$, $p < 0.05$]. However, none of the post hoc comparisons for the DRLU-72 group were significant [for 1.0mg/kg, ARZ $F(1,6) = 0.39$, $p = ns$; for 3.0mg/kg ARZ, $F(1,6) = 0.17$, $p = ns$; for 6.0mg/kg ARZ, $F(1,6) = 1.90$, $p = ns$]. Thus, while AMPH induced the same level of hyperlocomotion in DRLU-72 and DRLS-72 rats, the locomotor-suppressing effects of ARZ differed between DRL groups.

As expected, AMPH produced large increases in locomotor activity relative to non-AMPH sessions (Figure 4-9). A 3-way RM ANOVA (AMPH condition X DRL schedule X ARZ dose) yielded significant main effects for AMPH condition [$F(1,27) = 330.08$, $p < 0.0001$], DRL Schedule [$F(1,27) = 4.39$, $p < 0.05$], and ARZ dose [$F(3,81) = 6.20$, $p < 0.001$], with no three-way interaction. A significant interaction of DRL schedule X AMPH condition [$F(1,27) = 6.16$, $p < 0.05$] confirmed AMPH treatment eliminated differences in DRLU-72 and DRLS-72 locomotor activity previously seen in non-AMPH sessions (Figure 4-9b). Thus, similar to FS durations (Figure 4-2), AMPH induced similar levels of hyperlocomotion in both DRL groups. While ARZ dose-dependently reduced both unconditioned behavioral responses produced by AMPH, the results for the current study suggest that ARZ is much more effective at reducing FS durations than suppressing locomotion induced by AMPH.

4.3i AMPH-induced disruption of quadrant usage

The group mean percentage of time DRLU-72 and DRLS-72 rats spent in each quadrant while waiting for the criterion interval to elapse is depicted in Figure 4-10. Statistical significance levels for post-hoc comparisons between AMPH and non-AMPH sessions by quadrant are reported in Figure 4-10A. When ARZ-VECH was administered alone (*blue*), both DRL groups spent the majority of the pre-72 s interval positioned within quadrants close to operant fixtures, waiting near the operandum (QI) and lick disk (QIV). However, DRLU-72 rats spent the majority of time in QIV (68.34%), away from the operandum, while DRLS-72 rats preferred to wait in QI (51.72%), in close proximity to it (Figure 4-10A). Past studies have shown rats trained to unsignaled DRL schedules prefer to stay away from the operandum during the criterion interval (McIntire et al, 1983); a preference that may help inhibit premature responses and be essential to successful DRL performance (Richelle & Lejeune, 1980; Fowler et al, 2009). Because external cues provide rats with additional “stimulus control” (Wiley et al, 2000), DRLS-72 rats may be less dependent on collateral activity (i.e., spatially distancing themselves away from the operandum) than DRLU-72 rats.

In the current study, AMPH administration disrupted DRLU-72 quadrant usage by increasing the time spent near the operandum (QI) and significantly decreasing time spent waiting near the lick disk (QIV). In addition, AMPH significantly increased the time DRLU-72 rats spent in QII and QIII, away from operant fixtures (Figure 4-10A). Despite AMPH-induced changes in quadrant usage, AMPH-treated DRLU-72 rats still spent the majority of the pre-reinforcement interval waiting within QI and QIV combined, similar to non-AMPH quadrant usage. This suggests that AMPH did not completely abolish collateral activity in DRLU-72 rats.

Overall, these results are similar to previously reported findings from within our lab (Fowler et al, 2009).

AMPH treatment also altered DRLS-72 quadrant usage, although these effects appear more subtle in comparison to the DRLU-72 group (Figure 4-10A; *right-side*). AMPH significantly increased the time signaled rats spent in QII and QIII, and produced a small decrease in QI usage. However, collateral activity for DRLS-72 rats did not differ greatly between AMPH and non-AMPH sessions (Figure 4-10A).

A three-way RM ANOVA of AMPH condition X DRL Schedule X Quadrant confirmed a significant effect of quadrant [$F(3,81) = 52.81, p < 0.0001$]; all other main effects failed to reach significance, but a 3-way interaction was detected [$F(3,81) = 5.97, p < 0.001$]. DRL schedule significantly interacted with AMPH condition [$F(1,27) = 4.36, p < 0.05$], suggesting DRLS-72 quadrant usage was more resistant to AMPH-induced disruption. A significant interaction of DRL schedule X quadrant [$F(3,81) = 5.97, p < 0.001$] and also found, mirroring previous results for quadrant usage discussed in Chapters 3 and 4—chiefly, a tendency for DRLS-72 rats to spend more time waiting near the operandum than DRLU-72 rats, particularly under drug-free conditions (Figure 2-6).

4.3j Effects of ARZ + AMPH on quadrant usage

Figure 4-10B depicts the group mean percentage of time spent in each quadrant for all AMPH + ARZ treatment sessions. ARZ administration to AMPH-treated DRLU-72 rats resulted in minor changes in quadrant usage; failing to restore their spatial configuration to a non-AMPH pattern of quadrant usage. ARZ administration to AMPH-treated signaled rats had little effect

on their quadrant location. A 3-way RM ANOVA (DRL Schedule X ARZ Dose X Quadrant) detected a significant effect of quadrant [$F(3,39) = 24.78, p < 0.0001$] and a significant interaction of ARZ dose X DRL Schedule [$F(3,39) = 4.55, p < 0.01$]; all other effects and interactions were not significant.

To evaluate how ARZ differentially influenced quadrant usage in AMPH-treated DRLU-72 and DRLS-72 rats, post hoc analysis was carried out using two-way RM ANOVAs (ARZ dose X DRL Schedule) for each individual quadrant. For QI, a main effect of DRL schedule did not reach significance, but a significant effect of ARZ dose [$F(3,39) = 3.32, p < 0.05$] was confirmed with no interaction. The ANOVAs for quadrants II and III indicated a significant main effect of DRL schedule [for QII, $F(1,13) = 11.91, p < 0.01$; for QIII, $F(1,13) = 7.93, p < 0.05$], with no effect of ARZ dose or interaction. These statistical results are reflected in Figure 4-10B, where DRLU-72 rats spent substantially more time in QII and QIII than the DRLS-72 group. An ANOVA for quadrant IV was not significant for any effect or interaction. Regardless of DRL schedules, both AMPH-treated groups did not significantly differ in the amount of time spent near the operandum (QI) and lick disk (QIV), and spent the majority of the pre-reinforcement interval waiting within these two quadrants. However, DRLU-72 rats spent significantly more time in QII and QIII, away from operant conditioning fixtures, than DRLS-72 rats. These findings reiterate the observation noted in Section 5.3g: that ARZ is more effective at hastening recovery from FS (Figure 4-1) than attenuating locomotor activity (e.g., hyperlocomotion and altered spatial patterning) induced by AMPH.

4.4 DISCUSSION

4.4a Effects of AMPH Treatment on DRL-Mediated Behavior

In the present study, AMPH administration impaired DRLU-72 operant performance by significantly increasing non-burst response rates (Figure 4-7A) and shifting median IRTs to smaller values (Figure 4-5B) relative to non-AMPH treatment sessions. Our findings confirm previous reports that AMPH and other psychostimulants (cocaine, methamphetamine) impair DRLU operant behavior (i.e., increased non-burst responding and decreased reinforcement rates) and induce left-ward shifts on IRT frequency distributions (Fowler et al, 2009; Balcells-Olivero et al, 1997; Cheng & Liao et al, 2007, McClure & McMillian, 1997; Carey & Kritkauskys, 1972).

In contrast, DRLS-72 rats were less prone to AMPH-induced disruption of operant performance. AMPH treatment did not significantly alter median IRT values (Figure 4-5B) and reinforcement rates (Figure 4-7C), but significantly increased non-burst response rates (Figure 4-7A) relative to the non-AMPH treatment session. However, AMPH-treated DRLS-72 rats maintained low rates of unreinforced responding (11.99 ± 4.41) that were significantly and markedly lower than AMPH-treated DRLU-72 rats (84.67 ± 7.05 ; Figure 4-8). This suggests that AMPH-induced increases in non-burst responses did not greatly impair the ability of DRLS-72 rats to produce reinforced responses but substantially impaired DRLU-72 response efficiency. Altogether, these results indicate that once rats overcame FS and resumed operant responding, AMPH disrupted operant performance in DRLU-72 rats, but not DRLS-72 rats.

In agreement with our results, past studies have also reported that AMPH treatment did not significantly alter IRT frequency distributions or mean/median IRT values in DRLS rats; however, reports of AMPH-induced changes on DRLS reinforcement and non-burst response rates have been mixed (Wiley et al, 2000; Carey & Kritkauskay, 1972). Wiley and colleagues (2000) reported 3.0mg/kg AMPH decreased non-burst response and reinforcement rates in rats on a DRLS-15 schedule, while Carey & Kritkauskay (1972) found 1.0mg/kg AMPH did not significantly alter response or reinforcement rates relative to saline treatment in rats trained to a DRLS-22 schedule. Different findings for AMPH-induced changes in DRLS operant responding may be related to several methodological factors, such as different timing requirements, AMPH dosages, and prior sensitization to AMPH (Wiley et al, 2000; Carey & Kritkauskay, 1972). Despite these discrepancies, past work—including the current study—support the assertion that signaled DRL operant performance is less sensitive to AMPH-induced disruption than unsignaled DRL schedules (Wiley et al, 2000; Carey & Kritkauskay, 1972). The present data also supports the more general belief that internally controlled behaviors, such as interval timing, are more susceptible to AMPH-induced disruption than externally controlled behavioral processes (Latties & Weiss (1966); Heise & Lilie (1970); Carey & Kritkauskay, 1972; Wiley et al, 2000).

AMPH disrupted space usage in both DRL groups, but these effects were less pronounced in the signaled condition, where internal regulation of spatial positioning was not as crucial to operant performance as in DRLU-72 rats (Figure 4-10A). Consistent with our past study (Fowler et al, 2009), AMPH treatment disrupted collateral activity in DRLU-72 rats by significantly increasing time spent away from operant fixtures (QII and QIII; the “back half” of the hybrid

chamber) and significantly decreasing time spent in QVI (lick disk). AMPH treatment also increased the time DRLU-72 rats spent near the operandum (Q1), though this effect did not reach significance (Figure 4-10A). For DRLS-72 rats, AMPH treatment significantly increased time rats spent in quadrants II and III, but did not significantly alter time spent in the remaining quadrants relative to the non-AMPH ARZ-VECH session (Figure 4-10A). Thus, similar to operant performance, collateral activity associated with the presence of external cues (DRLS-72) was resistant to AMPH disruption compared to collateral activity regulated by internal behavioral processes (DRLU-72). It should be noted, however, that prior to AMPH administration, DRLS-72 collateral behaviors were less restrictive in their use of chamber space than the DRLU-72 group (Figure 3-6A).

While AMPH exerted differential effects on DRLS-72 and DRLU-72 operant performance (Figure 4-7) and collateral activity (Figure 4-10), it produced the same degree of unconditioned behavioral responses in rats engaged in either DRL schedule. Regardless of the type of training, AMPH-treated DRLU-72 and DRLS-72 rats engaged in FS stereotypy for the same duration of time (Figure 4-2), and exhibited similar levels of hyperlocomotion after FS subsided (Figure 4-3; Figure 4-9). However, AMPH-induced increases in locomotor activity was more likely to disrupt DRLU-72 operant performance than DRLS-72, because minimal levels of locomotion during the pre-72 s reinforcement interval appeared to be an essential aspect of successful DRL performance when reinforcement was unsignaled (Fowler et al, 2009).

4.4b Effects of AMPH + ARZ on DRL-Mediated Behavior

ARZ partially antagonized the effects of AMPH treatment on operant performance by increasing median IRT values (Figure 4-5) and reducing response rates in both DRL groups (4-7A). These effects were highly comparable to other antipsychotics on AMPH-induced deficits, which have been shown to reduce AMPH-induced increases in response rates and prolong IRTs on DRL operant schedules (McGuire & Seiden, 1980; Seiden et al, 1985). Similar to the previous experiment, when ARZ was administered alone (Chapter 3), ARZ had a tendency to decrease reinforcement rates in both AMPH-treated DRL groups (Figure 3-1C); however, post-hoc testing (Section 5.3f) suggested this effect was stronger in the DRLS-72 group, whereas DRLU-72 reinforcement rates were primarily influenced by AMPH rather than ARZ administration. Overall, ARZ acted similar to other dopamine antagonists on DRL schedules by dose-dependently decreasing response and reinforcement rates while shifting IRT distributions to the right (Fowler et al, 2008; McGuire & Seiden, 1980; Seiden et al, 1985).

While ARZ significantly reduced the duration of FS, it did not antagonize AMPH-induced changes in operant-directed collateral activity. DRLU-72 space usage changed across ARZ dose, but these changes did not demonstrate a tendency to improve collateral activity and make spatial patterning more like drug-free conditions (Figure 4-10). Throughout ARZ and AMPH treatment sessions, DRLU-72 collateral activity was predominately influenced by AMPH (i.e., AMPH-induced increased use of non-operant-related quadrants II and III and decreased use of QI), and these AMPH-induced changes in DRLU-72 space usage could not be ameliorated by ARZ treatment. For DRLS-72 rats, space usage changed little throughout ARZ treatment sessions (Figure 4-10B), and was highly comparable to non-AMPH treatment sessions (Figure 4-10A).

In addition, ARZ significantly and dose-dependently decreased the duration of FS stereotypy (Figure 4-2) and AMPH-induced hyperlocomotion during the pre-reinforcement 72-s interval (Figure 4-9). This finding is in agreement with the literature, which has shown ARZ to antagonize the hyperlocomotive and stereotypy-inducing effects of dopamine agonists, such as amphetamine, metamphetamine, and apomorphine (Nordquist et al, 2008; Fowler et al, 2008; Fowler et al, 2011; Backstrom et al, 2011). In the current series of experiments, ARZ significantly decreased AMPH-induced hyperlocomotion (Figure 4-9) at doses that did not significantly decrease locomotor activity when ARZ was administered alone (Figure 4-4). This result is similar to a study by Backstrom et al (2011), which found ARZ dose-dependently decreased locomotion in AMPH-treated rats and had a non-significant tendency to decrease locomotion in saline-treated rats. These findings on locomotor activity may reflect ARZ's partial antagonistic effects: ARZ acted as a dopamine antagonist when co-administered with AMPH (Figure 4-9) but not when administered alone (Figure 4-4). However, several other studies have reported ARZ to dose-dependently decrease spontaneous locomotor activity, showing significant effects at doses as low as 1.0mg/kg (Feltenstein et al, 2007; Nordquist et al, 2008). Overall, ARZ's effects on AMPH-induced changes in locomotor behavior were in line with its dopamine partial agonist properties. However, the serotonergic effects of ARZ may have contributed to this effect, as 5-HT_{2a} antagonism has been shown to potentiate DA D₂ receptor blockade within the mesolimbic pathway (Akhondzadeh et al, 2008; Arora & Meltzer, 1991; Bortolozzi et al, 2010).

Statistical analysis and post-hoc testing for the current experiment suggested that ARZ had stronger effects on DRLS-72 operant performance and locomotor activity than for the

DRLU-72 group. Figure 4-7 shows clear, dose-dependent declines in DRLS-72 reinforcements, non-burst responses, and burst responses, while DRLU-72 operant responses were relatively more stable throughout ARZ treatment sessions (Figure 4-7). Likewise, while ARZ dose-dependently decreased locomotion, these effects were stronger for the DRLS-72 group (Figure 4-9). Considering the theory that externally controlled behaviors are less susceptible to AMPH disruption than internally controlled behaviors (Latties & Weiss, 1966; Heise & Lilie, 1970; Carey & Kritkauskay, 1972; Wiley et al, 2000), one possible explanation is that ARZ's effects were stronger in the DRLS-72 group because the AMPH-induced impairment was less severe than in the DRLU-72 group. Because AMPH produced greater impairments in the DRLU-72 contingency—where behaviors were governed by internal processes—the dopamine antagonistic effects of ARZ were less prominent in comparison to the DRLS-72 group, where rats were dependent on the ability to perceive and then act on an external stimulus. In other words, the effectiveness of ARZ on DRL-mediated behaviors (i.e., operant performance and locomotor activity) is inversely related to the severity of AMPH-induced impairment.

4.4c The Ability of ARZ to Reverse Deficits in an AMPH Model of Psychosis

The overall propose of the experiments presented here was to evaluate the ability of the partial dopamine agonist, ARZ, to attenuate a schizophrenic-like cognitive deficit in an animal model of psychosis. For this purpose, the DRL schedule operant procedure—which requires subjects to utilize interval timing and timing behaviors to estimate temporal durations—was used in the current set of experiments, as schizophrenic individuals have demonstrated impairments in temporal processing (Carroll et al, 2008, Elveag et al, 2008; Rammsayer, 1990). Furthermore, the effects of ARZ were evaluated in rats that were trained to

either signaled or unsignaled DRL-72 schedules. These DRL contingencies were chosen because DRLU-72 and DRLS-72 rats differed in their reliance on interval timing and timing behaviors to successfully perform the operant task, as DRLU-72 rats were more likely to be dependent on these cognitive and behavioral processes. In addition, temporal processing and interval timing are cognitive processes sensitive to the pharmacological modulation of dopamine (Meck, 2006; Balci t al, 2009); therefore, DRLU-72 and DRLS-72 rats also differed in their dependency on dopamine-related cognitive processes. Finally, DRLU-72 and DRLS-72 schedules differed in task complexity, with the unsignaled contingency being substantially more difficult than the signaled contingency, as illustrated by reinforcement rates and IRT distributions.

In the first study (Chapter 2), we evaluated intrinsic differences between signaled and unsignaled DRL schedules prior to ARZ or AMPH administration. DRLS-72 rats demonstrated superior temporal regulation and performed near optimal levels; they emitted significantly fewer unreinforced responses, earned more reinforcers, and produced longer IRTs than DRLU-72 rats (Table 2-1). In addition, DRLS-72 rats were less dependent on timing behaviors such as collateral activity due to the presence of an external signal for reward availability (Figure 2-6). These results confirmed that the DRLU-72 contingency was a more demanding and complex schedule than the DRLS-72 contingency, because DRLU-72 operant performance was solely dependent on internal processes such as interval timing. In addition, the results suggested that DRLS-72 contingency diminished the salience of temporal response-reward associations, as external cues overshadowed—rather than strengthened—the temporal properties of a response to the reward.

In the second experiment (Chapter 3), the effects of ARZ were evaluated on rats trained to signaled or unsignaled DRL schedules. When administered alone, ARZ improved temporal regulation in both DRL groups by reducing unreinforced responses, non-burst responses, and burst responses (Figure 3-1). In addition, ARZ dose-dependently shifted IRT distributions to the right (Figure 3-2) and prolonged median IRTs (Figure 3-3). As anticipated, right-ward shifts in median IRTs were greater in the DRLU-72 group, confirming that temporal processing in DRLU-72 rats was increasingly sensitive to dopaminergic modulation relative to the DRLS-72 group.

Though ARZ impaired DRLS-72 operant performance by dose-dependently decreasing reinforcement rates, DRLU-72 reinforcement rates significantly improved at the 1.0mg/kg ARZ dose and had a non-significant tendency to decrease at higher doses (Figure 3-1C). While this therapeutic-like effect is similar to those seen for antidepressants (McGuire & Seiden, 1980; Seiden et al, 1985; O'Donnell & Seiden; 1983), antidepressants tend to induce these effects in a dose-dependent manner (O'Donnell et al, 2005), whereas ARZ did not significantly effect reinforcement rates when administered at higher doses (Figure 3-1C). The effects of ARZ on DRLU-72 operant performance are in agreement with Pollard & Howard (1986), who reported low doses of chlorpromazine and haloperidol reduced non-burst response rates and increased reinforcement rates on a DRLU-72 schedule. Therefore, the low dose of ARZ had a therapeutic effect on DRLU-72 operant performance by improving temporal regulation and increasing reinforcement rates (3-1C). However, ARZ did not significantly alter space usage (Figure 3-5) or locomotor activity (Figure 3-4) during the pre-72 s interval. This suggests that the effects of ARZ were primarily seen in operant response measures, as it did not alter cognitive-related locomotor activity.

In the present study, AMPH was administered to induce a state of “psychosis” and produce schizophrenic-like timing deficits in rats performing signaled or unsignaled DRL schedules. While AMPH produced similar levels of hyperlocomotion and FS durations in both DRL groups (Figure 4-2; Figure 4-9), only DRLU-72 operant performance and timing behaviors were severely disrupted by AMPH. In contrast, AMPH treatment did not significantly alter reinforcement rates (Figure 4-7C), median IRT values (Figure 4-5), or collateral activity (Figure 4-10A) for the DRLS-72 group relative to the corresponding non-AMPH treatment session. Therefore, it appears AMPH administration only induced schizophrenic-like timing deficits in the DRLU-72 group, which was more reliant on dopamine-related cognitive processes and timing behaviors for successful operant performance than the DRLS-72 group.

In DRLU-72 rats, ARZ administration was more effective at antagonizing unconditioned behaviors, such as hyperlocomotion (Figure 4-9) and FS durations (Figure 4-2), then attenuating AMPH-induced impairment in learned behaviors, such as operant performance and collateral activity. Because psychostimulant-induced hyperlocomotion and stereotypes are related to enhanced dopaminergic transmission within mesolimbic and nigrostriatal structures, these unconditioned behaviors are thought to model positive-like symptoms in schizophrenia (Bubeníková-Valesová et al, 2008; Mavrikaki et al, 2010; Backstrom et al, 2010). In the current study, ARZ acted similar to other antipsychotics by dose-dependently antagonizing these positive-like behaviors; suggesting ARZ primarily acted as a dopamine antagonist (Backstrom et al, 2010; Nordquist et al, 2008; Fowler et al, 2008).

While antipsychotics are therapeutically effective at reducing positive symptoms, they lack efficacy in ameliorating schizophrenic cognitive impairments that are related to executive functioning (Andereasen et al, 1995). Atypical antipsychotics, including ARZ, are generally believed to be more efficacious in this regard (Leucht et al, 2003). Neurocognitive studies have found ARZ administration to improve some aspects of cognitive functioning, such as working memory and verbal learning, in schizophrenic individuals (Kern et al, 2006). Behavioral pharmacological studies have demonstrated ARZ to attenuate attentional deficits induced by AMPH (Carli et al, 2010; Nordquest et al, 2008) and impaired recognition memory induced by PCP (Nagai et al, 2009). In the present study, we sought to determine if ARZ could attenuate schizophrenic-like timing and cognitive deficits in AMPH-treated rats performing a DRL-72 schedule.

Our results indicate that ARZ partially attenuated AMPH-induced deficits in DRLU-72 operant performance by shifting IRT distributions to the right (Figure 4-5), prolonging median IRTs (Figure 4-6), and reducing non-burst responses (Figure 4-7A). However, ARZ did not attenuate AMPH-induced impairments in DRLU-72 collateral activity (Figure restore 4-10B), nor did it restore reinforcement rates (Figure 4-7C) or median IRT values (Figure 4-6B) to drug-free states. This suggests that ARZ was limited in its capacity to ameliorate cognitive and timing deficits induced by the AMPH model of schizophrenia.

4.5 FUTURE DIRECTIONS

4.5a Analysis of DRLU-72 and DRLS-72 behavior during extinction

Differences in operant responding have been previously described between cued and randomly-cued reinforcement schedules under conditions of disruption (e.g., extinction, satiation). In a study by Tarpy et al (1985), female Long-Evans rats were trained to a DRL-20 schedule where an external stimulus (i.e., flash of light) indicated reinforcement availability (DRLS-20) or occurred randomly and was independent of the operant schedule contingencies (DRLR-20). Upon satiation, DRLS-20 rats produced higher response rates relative to their baseline acquisition performance than DRLR-20 rats. These findings lead Tarpy and colleagues (1985) to conclude that reward-correlated cues strengthened the response-reward association, because satiated DRLS-20 rats demonstrated relatively higher response rates or “increased response strength” (Nevin, 1974; Tarpy et al, 1985) than satiated DRLR-20 rats. Similarly, studies have found rats trained to signaled VI schedules are increasingly resistant to extinction and satiation when compared to rats trained to randomly-cued VI schedules (Roberts et al, 1984, Hall, 1982). In fact, Roberts et al (1984) found that signaled VI rats produced relatively higher response rates than randomly-cued VI rats *regardless if the external stimulus was presented during satiation or not*. Thus, subjects trained to an operant schedule with food-correlated cues demonstrated an increased resistance to change operant behavior (persistence) under conditions of disruption in comparison to rats trained without food-correlated cues (Roberts et al, 1984; Podlesnik & Shahan, 2010)

The results of the current experiments (Chapter 2-4) support the notion that food-correlated cues, or Pavlovian stimulus-reinforcer relationships, can influence the strength and

salience of response-reward associations. However, the belief that stimulus-reward associations *enhance* the learning of response-reward associations (Roberts et al, 1984; Tarpy et al, 1985) was challenged by our findings in Chapter 2, where rats trained to the signaled DRL contingency were less likely to utilize interval timing and engage in timing behaviors (e.g., controlled collateral activity, minimal locomotor activity) than DRLU-72 rats. Thus, DRLS-72 rats did not engage in the usual timing behavior that normally accompanies or promotes strong temporal response-reward associations under unsignaled DRL schedules, suggesting that the stimulus-reward association overshadowed the response-reward association (Section 2.9). Our experiments, however, did not examine DRLS-72 and DRLU-72 rats under conditions of disruption, such as extinction or satiation.

To further assess the influence of response-reward and stimulus-reward associations on operant responding, DRLU-72 and DRLS-72 rats could be evaluated in hybrid chambers during extinction. Because hybrid chambers can measure the spatial positioning, locomotion, and response characteristics (e.g., peak force, response duration) of a single rat performing an operant schedule, our study could measure within-session or session-to-session degradation of DRL-mediated timing behaviors (e.g., collateral activity, locomotor activity) in signaled and unsignaled rats during extinction. Thus, our study would provide a more detailed analysis of the strength of response-reward associations in both signaled and unsignaled schedules than what currently exists in the literature (Roberts et al, 1984; Tarpy et al, 1985; Hall, 1982; Pearce & Hall, 1978; St. Claire-Smith, 1979), as well as examine the role of stimulus-reward associations in the persistence of operant responding under extinction schedules (Podlesnik & Shahan, 2010; Nevin & Grace, 2000).

In addition, the effects of AMPH on operant behavior during extinction could be assessed. Currently, little is known about the effects of AMPH in this context. A study by Olds (1970) trained drug-free albino rats to lever press for either a food reward or self-stimulation reward (electric shock in the posterior hypothalamus). Olds (1970) found rats pre-treated with 2.0mg/kg AMPH were increasingly resistant to extinction in comparison to saline-treated control subjects. However, the study lacked detailed information on the contingencies of the operant schedule, and did not evaluate the effects of other AMPH doses on extinction (Olds, 1970). Due to the lack of information available in the literature, hybrid chambers could be used to assess AMPH-induced deficits during extinction, and if these AMPH-induced impairments resembled schizophrenic-like cognitive deficits, such as impaired attentional performance and increased preservation behavior (Crumpton, 1963). Based off the findings from Olds (1970), it is likely that moderate to high doses of AMPH administration would significantly increase resistance to extinction in both DRLU-72 and DRLS-72 groups; however AMPH treatment may produce a greater impairment in DRLS-72 rats, because past studies have shown rats trained to signaled operant schedules are increasingly resistant to extinction than rats trained to randomly-cued schedules (Podlesnik & Shahan, 2010; Nevin & Grace, 2000; Tarpy et al, 1985). Therefore, AMPH administration would be expected to produce a more pronounced, schizophrenic-like cognitive deficit in DRLS-72 rats than their unsignaled counterparts. It should be noted, however, that low doses of AMPH administration may produce the opposite effect and decrease resistance to extinction. Past studies have shown low doses of AMPH in rats can enhance associative learning in conditioned taste aversion and conditioned avoidance tasks (Fenu and Di Chiara, 2003; Davies et al, 1974).

4.5b Analysis of DRLU-72 and DRLS-72 rats during training

For the current experiments, DRL-mediated behavior and operant performance were recorded for both signaled and unsignaled rats during training sessions. As described in the behavioral procedure section (Section 3.7c), rats were given either DRLS-1 or DRLU-2 schedules and were gradually trained to emit a response on the operandum. During training, it appeared that signaled rats required substantially more training sessions when learning to initiate a response on the operandum. Several signaled rats also required additional DRL training sessions of lower criterion temporal intervals (DRLS-1s to DRLS-8s) while gradually being trained to perform the DRLS-72 task. Because all of these sessions were recorded, differences in learning could be evaluated by calculating the number of sessions required by each rat to reach the criterion number of reinforcements for each DRL training phrase (e.g., the number of training sessions for a DRLS rat to earn 50 reinforcers for a 30-min DRLS-4s session). Investigation of these differences could determine if the DRLS contingency was a more difficult task to initially learn, despite being a less complex task to perform, than the DRLU contingency. If significant, this finding would also support the idea that rats performing signaled schedules develop weaker response-reward associations than rats performing unsignaled schedules, as it took DRLS rats longer to learn the task. This hypothesis could be further investigated by assessing operant performance measures, such as response and unreinforced response rates, for DRLS and DRLU rats at each DRL training phase.

In addition, the formation and development of collateral activity in DRLS and DRLU rats could be described at each DRL training phrase. This analysis could provide information on how collateral activity changed as the temporal criterion was progressively increased. It would also

reveal at what point in training DRLS and DRLU rats exhibited similar patterns of spatial usage and locomotion, as well as which training sessions DRLS and DRLU collateral activity diverged. Thus, analysis of signaled and unsignaled DRL rats during training would provide valuable insights on collateral activity as related to successful timing behavior.

4.5c Further examination of ARZ's Ability to Attenuate Behavioral and Cognitive Deficits in the DRL procedure and other Operant Schedules

The results for the current experiments demonstrated that ARZ, when administered by itself, produced a therapeutic-like response in DRLU-72 operant performance at the 1.0mg/kg dose by significantly increasing reinforcement rates while decreasing non-burst and unreinforced response rates (Figure 3-1). Additionally, ARZ partially attenuated AMPH-induced cognitive impairments by significantly decreasing non-burst responses rates (Figure 4-7A) and prolonging median IRT values in both DRLU-72 and DRLS-72 groups (Figure 4-6A). These findings suggest that ARZ—although in a limited capacity—can attenuate some schizophrenic-like timing deficits on the DRL schedule.

For the current study, the doses used (1.0mg/kg-6.0mg/kg) may have been too large to detect the full range of ARZ-induced therapeutic effects for the second experiment (Chapter 3), when ARZ was administered alone. Lower doses of ARZ (0.10mg/kg, 0.30mg/kg) were initially examined in AMPH-treated rats; however, because these doses did not produce strong effects in AMPH-treated rats, it was decided to use higher doses of ARZ (1mg/kg-6.0mg/kg). It is possible that lower doses may have improved DRL-mediated behavior in the second experiment, particularly in DRLU-72 rats.

Additionally, locomotor activity for the entire 4-hour session (as opposed to the pre-reinforcement intervals) could not be assessed in the present studies. Because DRLS-72 rats consistently earned more reinforcements than DRLU-72 rats, differences in locomotor activity could be attributed to DRLS-72 rats making more trips between operant fixtures. Thus, analysis of locomotor activity was restricted to the 72 s preceding a reinforced response, where group means contained each individual subject's mean distance regardless of the number of reinforced responses made by each subject. The effects of ARZ on locomotor activity could be assessed in both drug-free and AMPH-treated rats using force-plate actometers, as these chambers do not contain operant fixtures. Therefore, ARZ-induced effects on locomotor activity could be assessed with high precision without an operant response component.

Future studies of ARZ on other timing schedules, such as the bi-section and peak interval procedure, may provide additional insights into the capacity of ARZ to improve temporal processing in drug-free subjects, as well as attenuate temporal processing defects in AMPH-treated subjects. In addition to defects in interval timing, other commonly occurring schizophrenic cognitive deficits, such as spatial learning, performing sequential tasks, and complex and sustained attention, can be assessed in challenging operant conditioning schedules using hybrid chambers.

Finally, DRL-mediated timing behaviors could be assessed in other animal models of psychosis besides the AMPH model. Because the AMPH model predominately caters to dopamine-related disease mechanisms and experimental therapies, its use may impede the discovery of novel, non-dopamine-based therapeutic drug targets (Lipska & Weinberger, 2000).

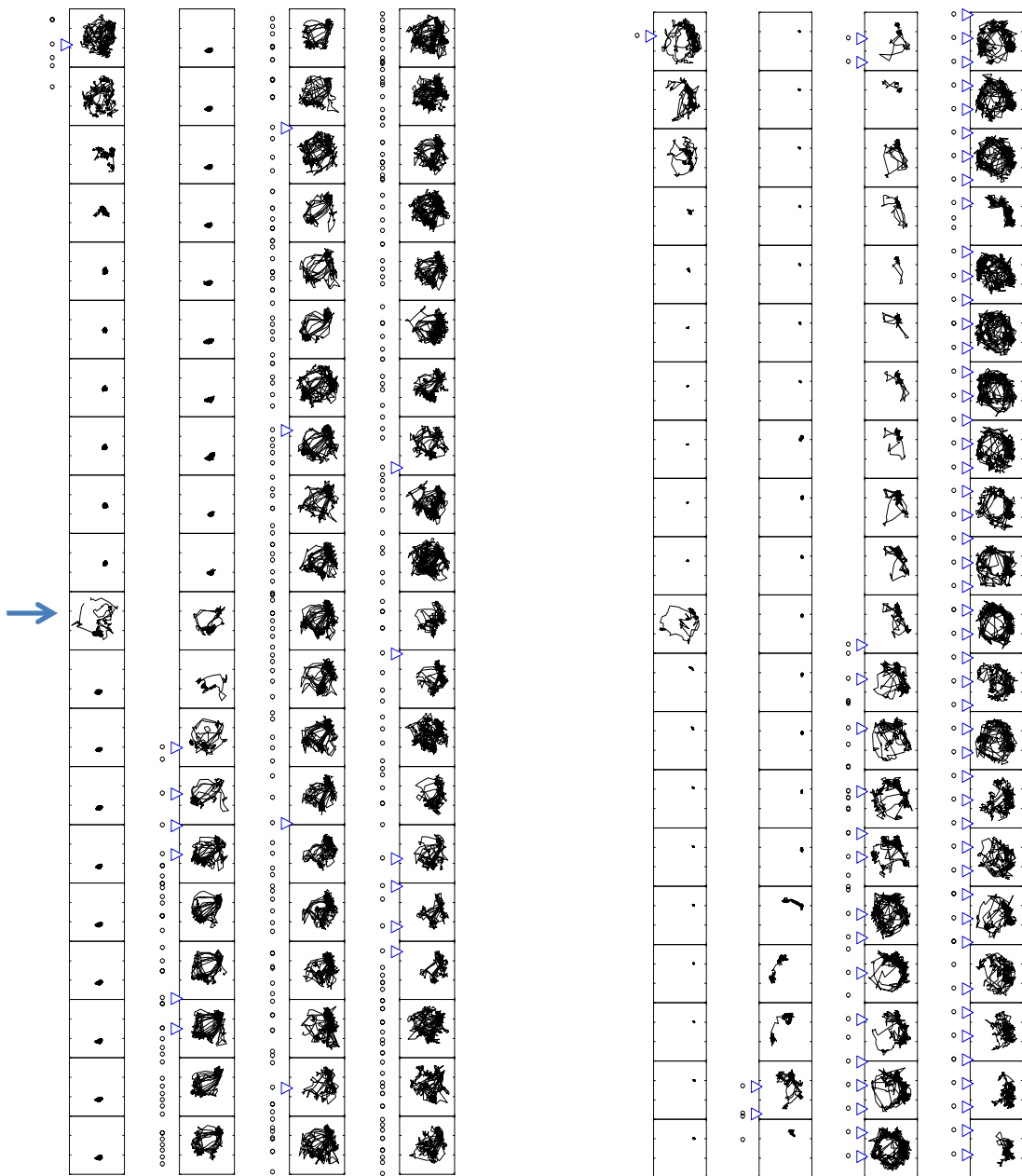
We have previously investigated chronic and acute phencyclidine (PCP) administration in rats trained to a DRLU-72 schedule (Latif et al, 2010; Latif et al, preparation). Our results indicated that chronic PCP administration did not induce long-lasting impairments in DRL-mediated behaviors, while the effects of acute PCP treatment on operant performance and locomotor activity were highly variable across subjects at moderate to high doses (4.0mg/kg – 8.0 mg/kg). However, it should be noted that acute PCP administration significantly impaired DRLU-72 operant performance by dose-dependently reducing reinforcement rates, increasing burst response rates, and flattening IRT distributions (Latif et al, 2010; Latif et al, preparation).

The evaluation of neurodevelopmental models of psychosis in the DRL-72 task may lead to unique insights into schizophrenic-like timing deficits and in turn provide additional support to the face validity of such models. Neurodevelopmental models are thought to have high etiological validity; their use stems from the hypothesis that complications and insults during prenatal/postnatal development produce schizophrenia later in life (Weinberger, 1996). Experiments in behavioral psychopharmacology have demonstrated that prenatal or postnatal exposure to NMDA receptor antagonists (e.g., MK-801, PCP) in rodents produces impairments in spatial learning and PPI tasks during adulthood (Wang et al, 2001; Stefani & Moghaddam, 2005). Similarly, the neonatal ventral hippocampus lesion (NVHL) model has produced schizophrenic-like impairments in spatial learning, social behaviors, and working memory-related tasks in both rats and monkeys (Sams-Dodd et al, 1997; Lipska & Weinberger, 2000; Lipska et al, 1993; Bachevalier et al, 1999). Thus, investigating schizophrenic-like timing deficits in models with “superior heuristic” validity (Lipska & Weinberger, 2000) over pharmacological models would further validate the use of timing behavior as a cognitive measure in behavioral

studies, as well as lead to the discovery of novel compounds that have high therapeutic efficacy in attenuating the cognitive impairments present in schizophrenia.

5.6 FIGURES AND TABLES

Figure 4-1. Movement trajectories and operant responses of a single (A) AMPH -treated DRLU-72 and (B) AMPH-treated DRLS-72 rats in the operant chamber. Each square represents a 3-min time frame in the session, with time progressing in a downward direction. Each column represents one hour. Following AMPH administration ($t = 0$), rats engaged in focused stereotypy (FS) during the first half of the session, followed by hyperactivity and operant-directed behavior. Note that the movement trajectories in the 10th frame are due to handling rats to administer ARZ-VECH ($t = 30$ min). (C) The schematic diagram describes the location of the operandum and lick disk. Operant responses are depicted as open circles, and the reinforced responses are indicated by blue triangles.



C.

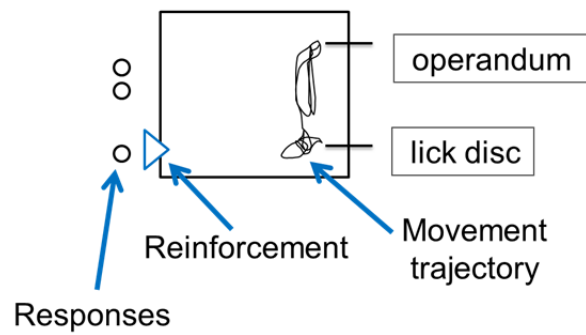


Figure 4-2. Group mean (+/- SEM) duration of FS expression within the 4-hr session. The plot below shows the DRLU-72 and DRLS-72 group mean (+/-SEM) time when FS subsided in rats treated with AMPH (AMPH represented by “A”). Post hoc analyses were conducted with one-way ANOVAs at each dose to determine a significant effect of schedule (#). One-way RM ANOVAs were performed to indicate a significant difference of the drug dose value from the vehicle dose (*). Post-hoc test significance levels are as follows: # = $p < 0.01$, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

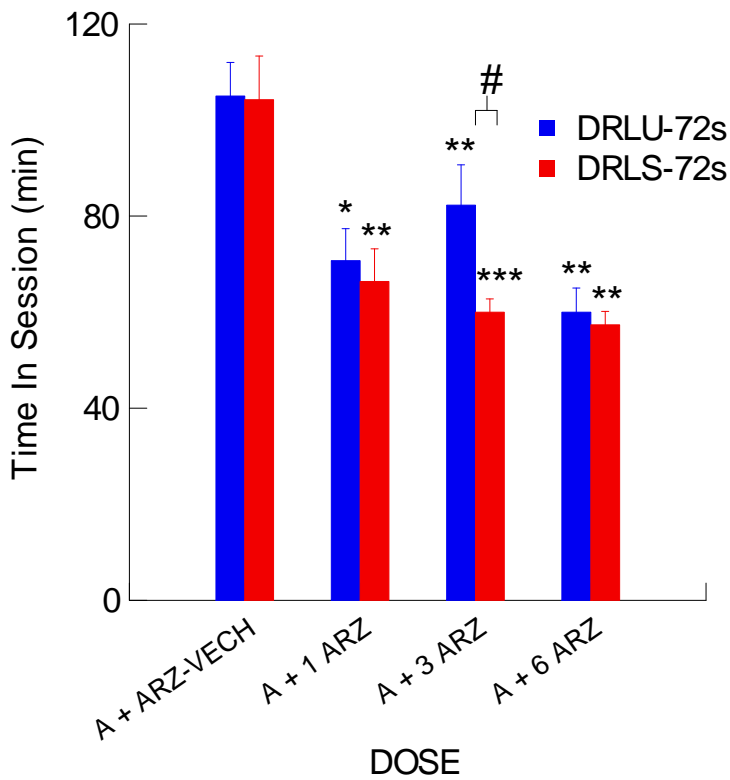


Figure 4-3. Group mean floor sector usage in d-amphetamine-treated rats. The number of sectors (1.72cm^2) occupied across 3-min time blocks are plotted below. The dashed line indicated the time in session ($t = 30$ min) when d-AMPH treated rats received ARZ-VECH or ARZ. The spike in sectors used during this time period is associated with the ARZ injection and being handled by the experimenter. The *blue line* indicates the effect of ARZ-VECH dose when administered to AMPH-treated rats. The *red line* represents the combined effects of AMPH and (A) 1.0mg/kg ARZ, (B) 3.0mg/kg ARZ, and (C) 6.0mg/kg ARZ dose. Relative to the AMPH + ARZ-VECH sessions, ARZ dose-dependently restored locomotion to AMPH-treated rats earlier in the session.

DRLU-72s

DRLS-72s

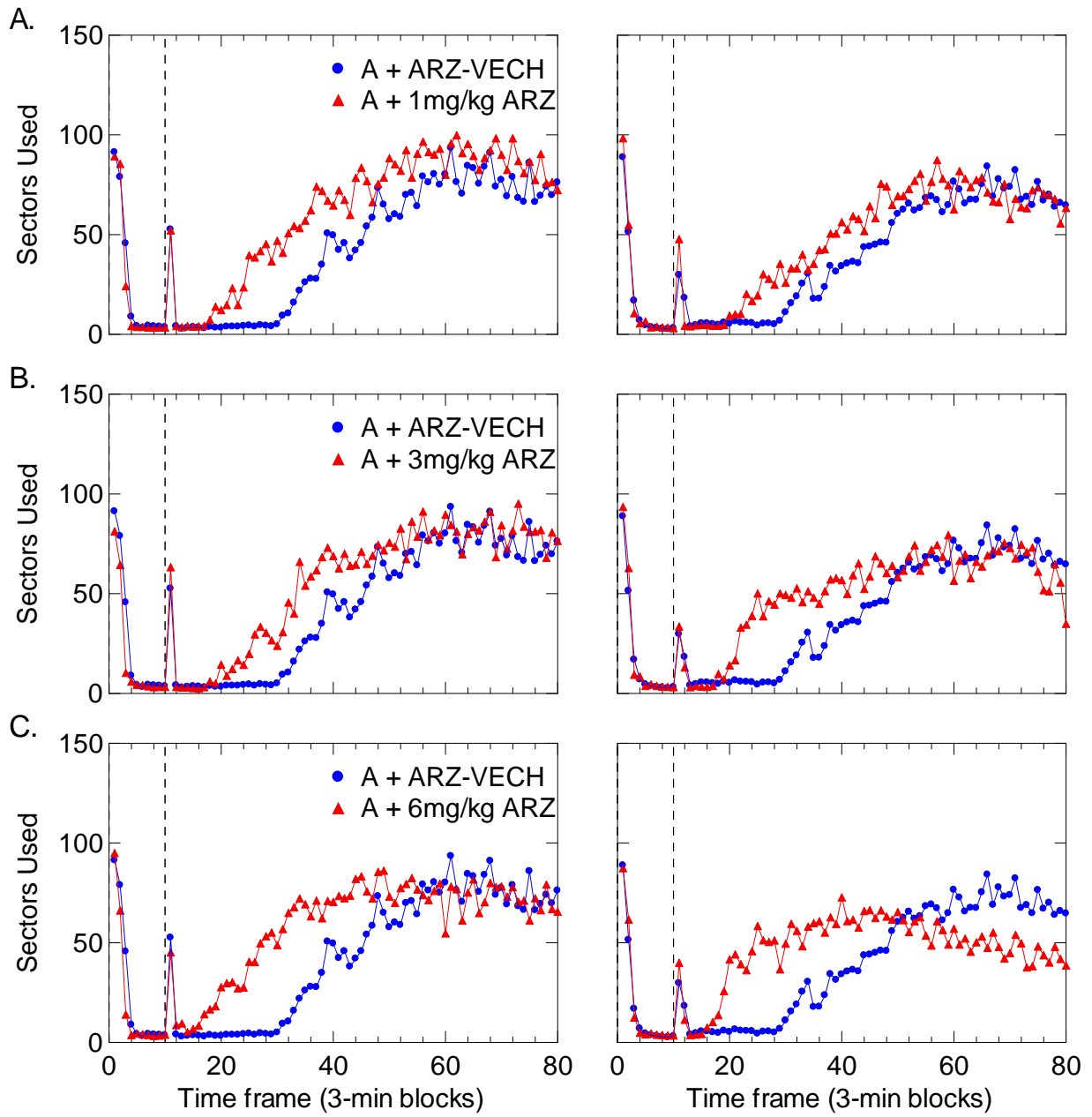
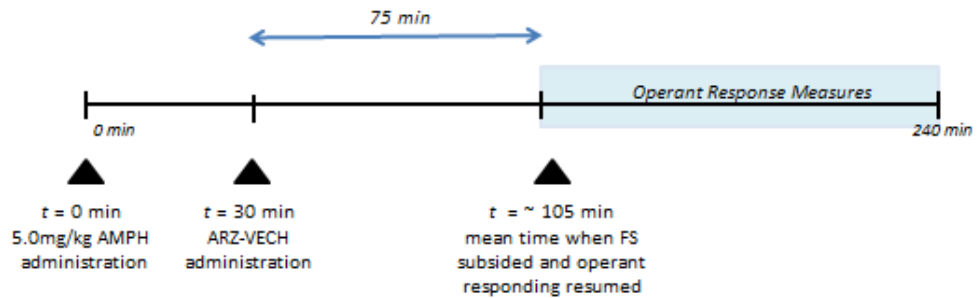


Figure 4-4. Comparison of operant response measures between AMPH and non-AMPH

treatment sessions. The following diagram depicts how time periods within the 4-hr AMPH and non-AMPH sessions were selected for analysis of operant performance. Specifically, this illustration shows how time periods for analysis were selected for DRLU AMPH and non-AMPH sessions when ARZ-VECH was administered. (A) For the current study, operant response measures used for analysis (*blue box*) were collected after FS subsided and operant responding resumed ($t = \sim 105 \text{ min}$). This time period occurred approximately 75 min after ARZ-VECH was administered. (B) To compare the operant response measures from the corresponding non-AMPH session (Chapter 4), operant response measures were collected 75 min after ARZ-VECH was administered.

A. AMPH SESSION



B. NON-AMPH SESSION

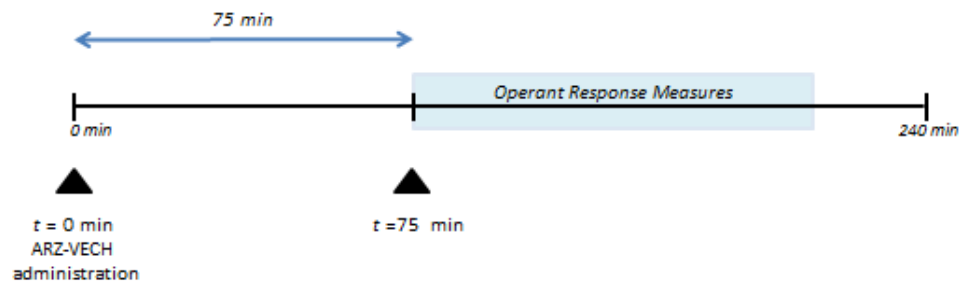


Figure 4-5. IRT Frequency distributions for 5.0mg/kg d-amphetamine sessions. The plots above show the group mean percentage of responses made at each 2-sec bin IRT for the 5.0mg/kg d-amphetamine (A) and aripiprazole (ARZ) sessions. The vertical dotted line indicates the 72-sec temporal requirement. The y-axis was log10 transformed in order to emphasize differences in IRT response frequency at each bin.

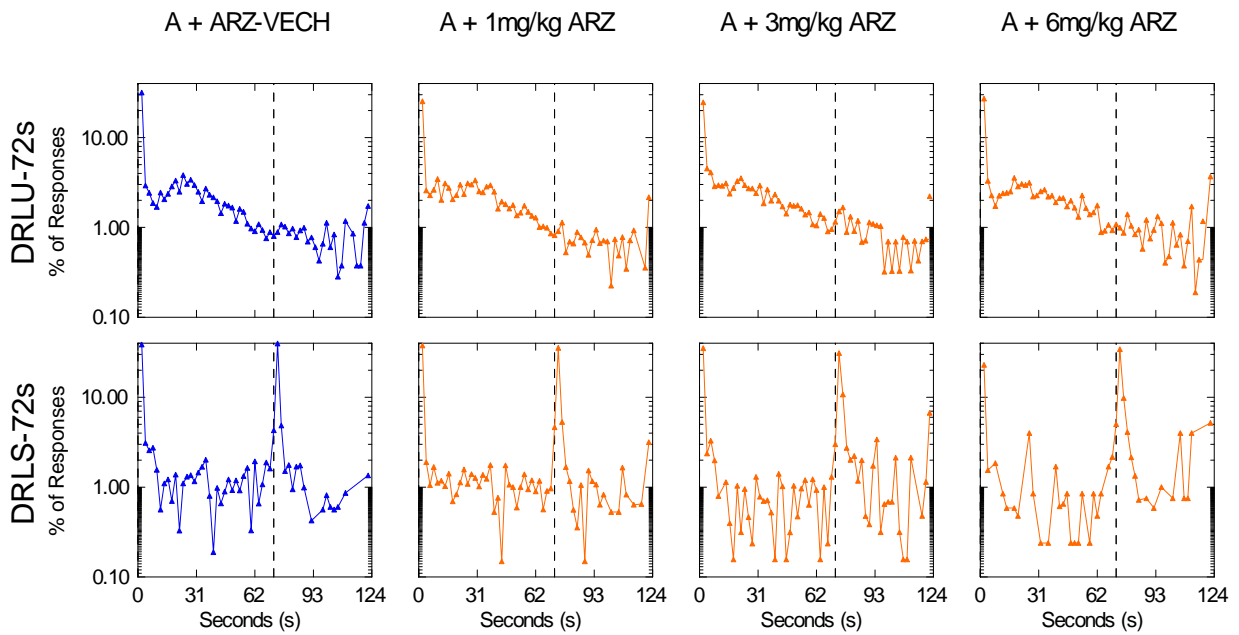
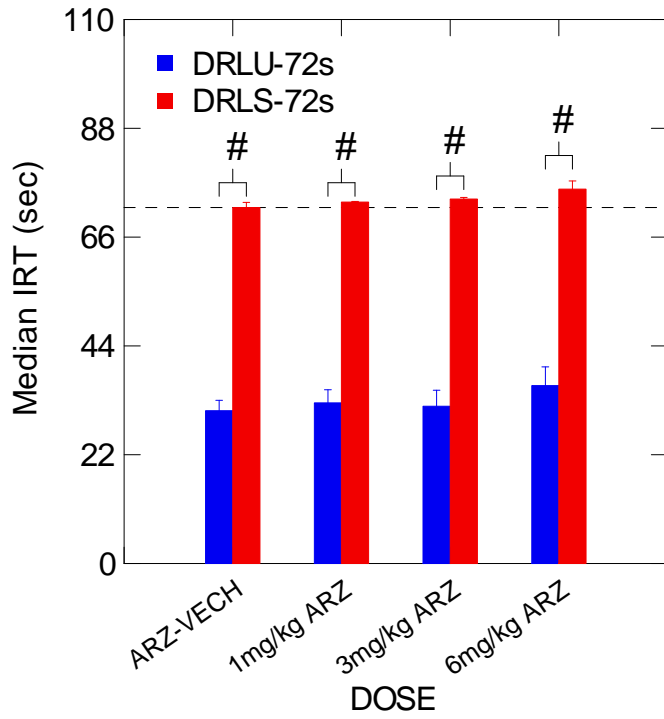


Figure 4-6. Median IRT values for AMPH and ARZ treatment sessions. DRLU-72 and DRLS-72 median IRT values are depicted below. The dotted line indicates the 72 sec criterion. (A) The DRLU-72 (*blue*) and DRLS-72 (*red*) group means (+/- SEM) for median IRTs are plotted for each drug dose. ARZ administration dose-dependently shifted median IRTs to the right [$F(3,39) = 3.22, p < 0.05$], though post hoc testing using one-way RM ANOVAs were not significant for any ARZ dose. Post hoc analyses were conducted with one-way ANOVAs at each dose to determine a significant effect of schedule (DRLU-72 vs DRLS-72; #). Post hoc test significance levels are as follows: # = $p < 0.001$. (B) The median IRT values for both non-amphetamine (*cyan*) and amphetamine (*orange*) sessions for both DRL groups are shown for each ARZ drug dose. Both AMPH and ARZ administration greatly altered median IRTs for the DRLU-72 group, but produced mild changes in the DRLS-72 group. These findings suggest that rats given external cues to guide temporal responding are more resistant to drug disruption than rats without external signals.

A.



B.

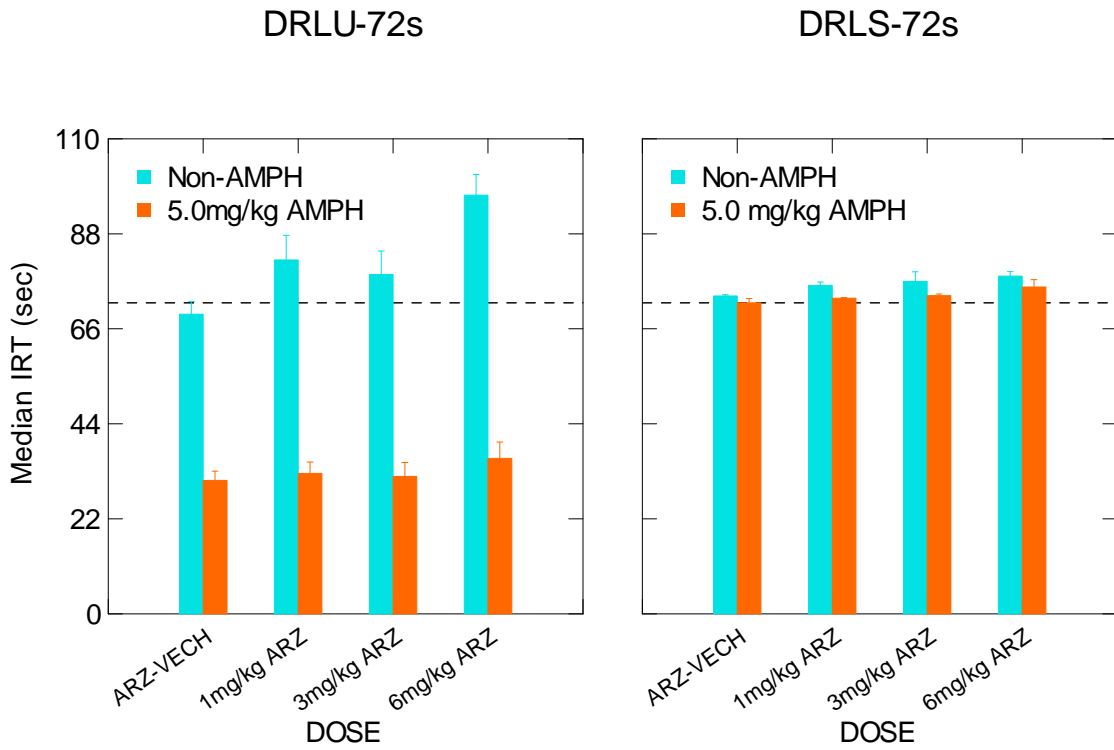


Figure 4-7. Response and reinforcement rates for AMPH treatment sessions. Plots depicted on the left and right describe operant response measures for DRLU-72 and DRLS-72 groups, respectively. The graphs for each row depicted the group mean (\pm SEM) values for (A) non-burst responses, (B) burst responses, and (C) reinforcements for all treatment conditions. Operant response measures *within of the gray box* indicate measurements for non-amphetamine sessions. Post hoc significance levels are as follows: # = $p < 0.01$, * = $p < 0.05$, ** = $p < 0.01$.

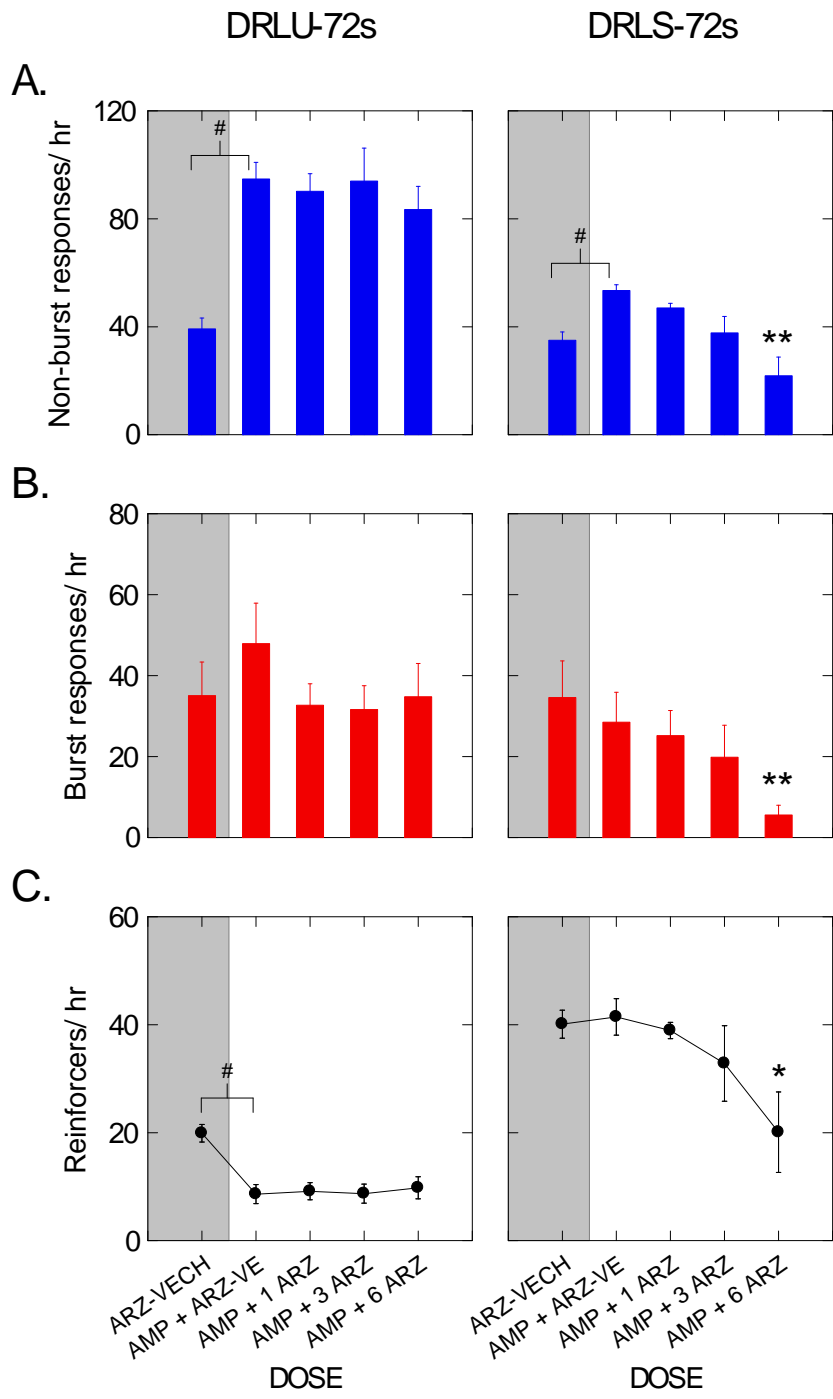


Figure 4-8. Group mean (+/- SEM) unreinforced response rates for AMPH and non-AMPH

sessions. Plotted below are the unreinforced ($3\text{ s} < \text{IRT} < 72\text{ s}$) response rates for each DRL-72 group. The *blue* and *red* lines represents the non-AMPH (ARZ-VECH session) and AMPH (A + ARZ-VECH) sessions, respectively. While AMPH treatment significantly increased unreinforced response rates for both DRLU-72 and DRLS-72 groups, the AMPH-treated DRLS-72 group still maintained relatively low unreinforced response rates when compared to the DRLU-72 group. Letters *a – d* were used to indicate different post hoc comparisons. Post hoc comparisons for a-b: $F(1,13) = 25.94$ $p < 0.0001$; a-c: $F(1,13) = 61.43$ $p < 0.0001$; c-d: $F(1,13) = 80.79$ $p < 0.0001$; b-d: $F(1,14) = 4.91$ $p < 0.05$

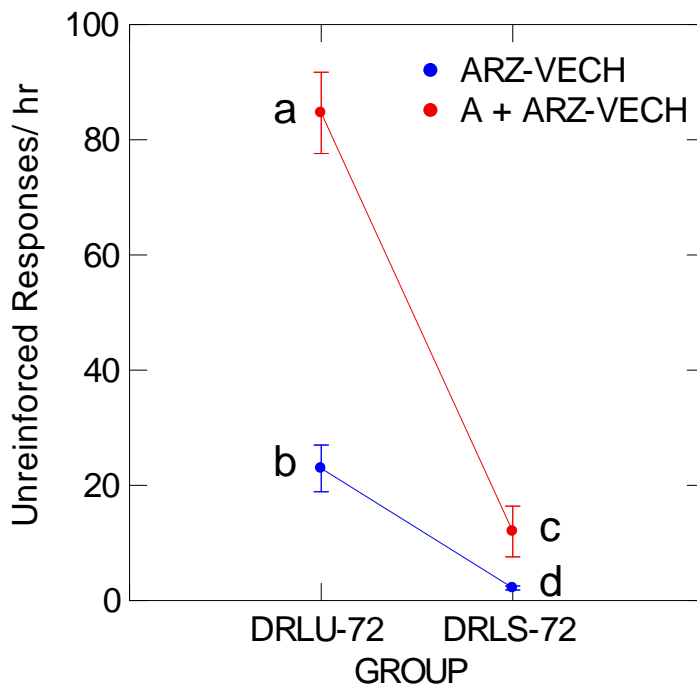
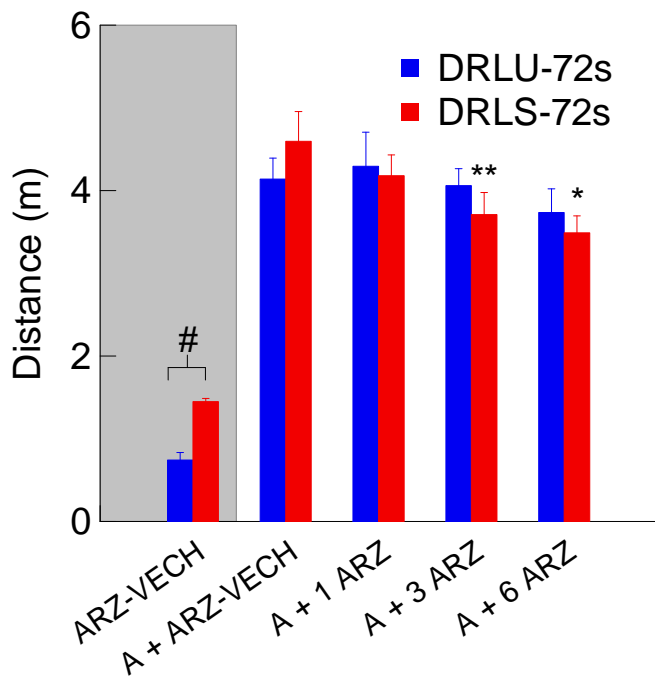


Figure 4-9. Distance traveled during pre-reinforcement interval for AMPH treatment sessions.

(A) The group mean (\pm SEM) for distance traveled during the pre-72 s reinforcement interval are plotted for each drug dose. Bars that appear *within the gray box* indicate measurements for non-amphetamine sessions. Post hoc analyses were conducted with one-way ANOVAs at each dose to determine a significant effect of schedule (DRLU-72 vs DRLS-72; #). In addition, one-way RM ANOVAs were performed to indicate a significant difference of the ARZ drug dose value from the A + ARZ-VECH dose(*). Post-hoc test significance levels are as follows: * = $p < 0.05$; ** = $p < 0.01$, # = $p < 0.001$. (B) Locomotor distance for both non-amphetamine (*cyan*) and amphetamine (*orange*) sessions for DRLS-72 and DRLU-72 rats are shown for each ARZ drug dose. The administration of AMPH eliminated differences in locomotor activity previously seen between DRL groups.

A.



B.

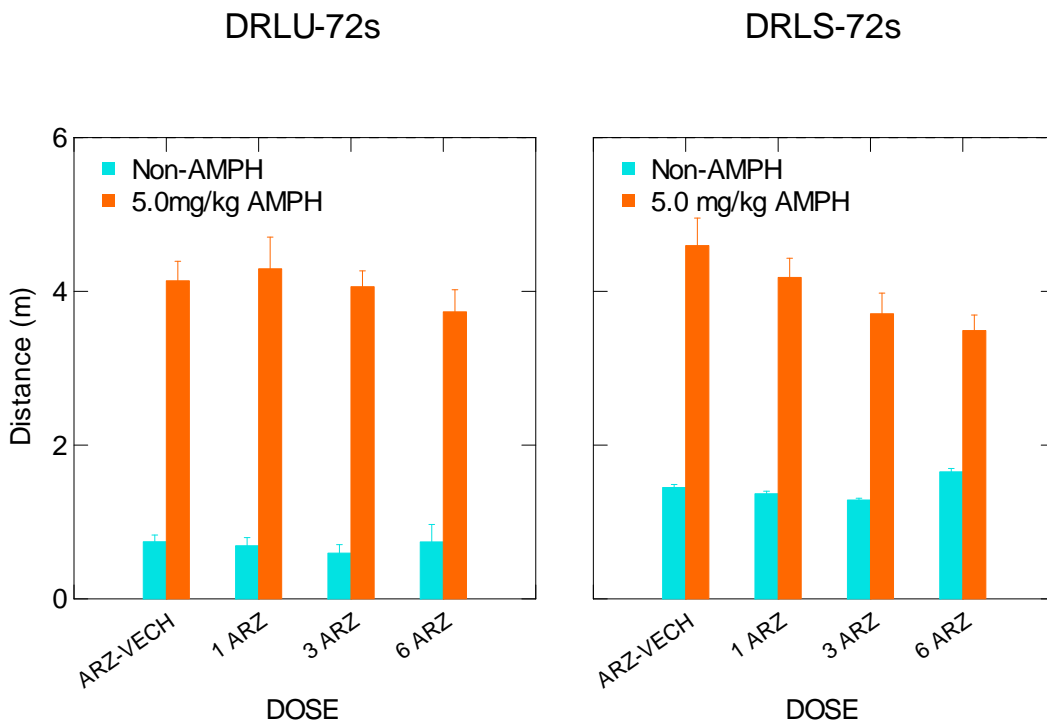
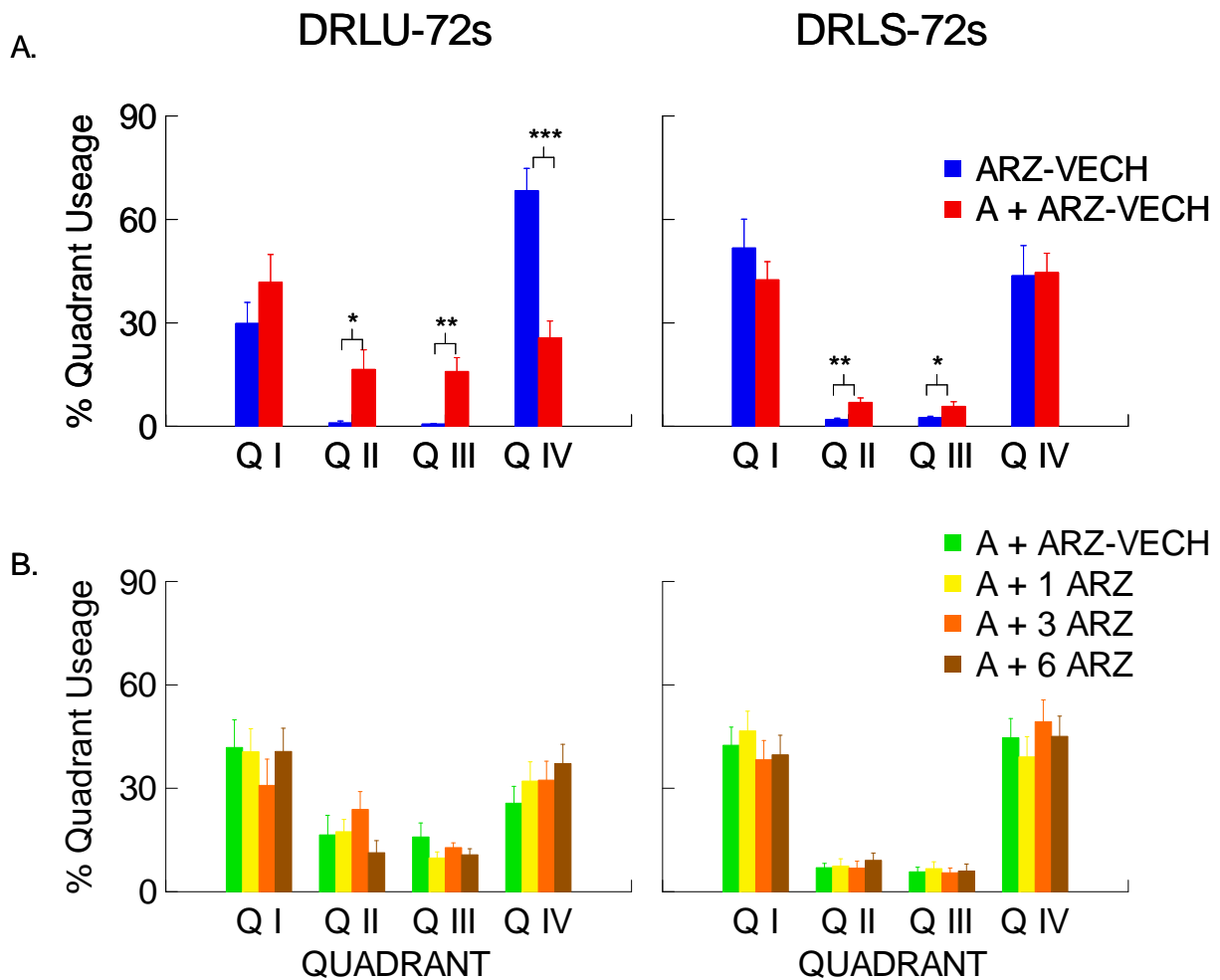


Figure 4-10. Quadrant usage during the pre-reinforcement interval for AMPH and ARZ treatment sessions.

The group mean (\pm SEM) percentage of time spent in each quadrant during the pre-72 sec reinforcement interval are shown for each DRL group. (A) Quadrant usage for ARZ-VECH sessions in AMPH-treated (*red*) and non-AMPH treated (*blue*) rats are described for DRLU-72 and DRLS-72 rats. (B) Quadrant usage for all AMPH treatment sessions. Quadrant location was measured in hundredths of a second.



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