Disruption of Orolingual Behavior in Rats Treated with Atypical Antipsychotic Drugs.

By

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Abstract

The atypical antipsychotics are a group of second generation drugs used for the treatment of schizophrenia, schizoaffective disorder, as well as some forms of bipolar and major depressive disorder. First generation or the "typical antipsychotics" are an older group of drugs whose first member, chlorpromazine was developed in the early 1950's. The typical antipsychotics have a higher propensity to induce extrapyramidal side effects (EPS), and elevate prolactin levels. The introduction to this thesis begins with the history of the typical antipsychotics; particularly the introduction of chlorpromazine, up to the introduction of clozapine a compound which revolutionized the treatment of schizophrenia. Then the five subsequently approved atypical antipsychotic drugs are discussed. The introduction of clozapine soon revealed agranuocytosis as a relatively frequent side effect, and made it clear that a need remains for antipsychotics that are equally or more efficacious without deadly leukocytopenic side effects. While the atypical antipsychotics are often lumped into one category, they are diverse compounds with different EPS liabilities, side effects, and pharmacodynamic properties. Thus, the pharmacology of the atypical antipsychotics and the most interesting set of side effects, the extrapyramidal side effects are reviewed. Extrapyramidal side effects include akathisia, parkinsonism, dystonia, and tardive dyskinesia, and are complex motor side effects with mental components. This set of troublesome side effects often result in compliance issues particularly with the typical antipsychotics. Dopamine D_2 receptor antagonism in the nigrostriatal pathways of the brain is believed to be the primary

cause of extrapyramidal side effects. Dopamine D_2 receptor antagonism in the mesolimbic dopamine pathway is thought to result in the antipsychotic affect, and compounds that target this pathway selectively are hypothesized to have lower EPS liabilities. Although the atypical antipsychotics are a diverse group of drugs they have some common features including lower extrapyramidal side effect liabilities, and minimal or no prolactin elevation. Within this context two major hypothesis' of atypicality will be reviewed, the fast-dissociation hypothesis and 5-HT_{2A}/D_2 affinity ratio hypothesis. Orolingual components of extrapyramidal side effects will be reviewed as well as neural control of the tongue by the hypoglossal nucleus, hypoglossal nucleus organization, and tongue anatomy, and physiology. Relevant preclinical behavioral research of both typical and atypical antipsychotics will be reviewed. The research presented here is concerned with both the acute and subchronic effects of the atypical antipsychotic on orolingual function in rats as a model of EPS. Licking behavior in rats is believed to be controlled by central pattern generators in the brainstem, and the rhythm (Hz) of licking, peak force, and the number of licks will be quantitatively analyzed and compared. Tolerance and sensitization will be assessed using a subchronic dosing regimen. These data will then be discussed in the context of past studies concerning licking dynamics with haloperidol and clozapine, and to a lesser extent risperidone.

Introduction

In the early 1950's the drug chlorpromazine was introduced and was originally used for preoperative anxiety (1). A series of animal studies and clinical observations built a foundation of knowledge about chlorpromazine, which lead to it being the first antipsychotic drug used for the treatment of schizophrenia. In 1950, Courvoisier and colleagues observed that chlorpromazine had pronounced effects on the central nervous system, including the prolongation of sleep induced by barbiturates in rodents, the prevention of apomorphine-induced emesis in canines, and the inhibition of the conditioned avoidance-escape behavioral response in mice (3). In 1951, Laborit and Huguenard observed that surgical patients could be induced into a state of "artificial hibernation" by a "lytic cocktail" of chlorpromazine, promethazine when administered in combination with an analgesic. It was also observed that patients treated with this cocktail required lower doses of anesthetisia and were more capable of coping with the stress of surgical trauma. (3,4,5). Based on these observations of the CNS effects of chlorpromazine, Laborit and Huguenard provided chlorpromazine to two groups of psychiatrists. Chlorpromazine was given to Hamon, Paraire, and Velluz at the Central Military Hospital, in Paris (5) and Delay, Deniker, and Harl at the psychiatric clinic of Sainte Anne Hospital in Paris (3,6,7) . The first reported chlorpromazine-treated case was a 57-year-old laborer who was admitted to the Central Military Hospital because of erratic, uncontrollable behavior (25, 26). Shortly after the administration of chlorpromazine his symptoms were improved,

chlorpromazine was recognized for its effects on the mentally ill, and a clinical breakthrough had occurred.

The clinical success of chlorpromazine stimulated research, which led to many other drugs that were marketed for the treatment of schizophrenia, such as thioridazine, fluphenazine, and haloperidol. However, in 1954, two years after chlorpromazine first came into clinical use, extrapyramidal side effects (EPS) including Parkinsonism, dystonias, and akathisia began to be described and recognized as side effects of chlorpromazine (8). A 1961 study reported the prevalence of EPS in patients treated with typical antipsychotic drugs was estimated at 38.9% (9). The majority of clinicians and pharmacologists became convinced that EPS was associated with the clinical effectiveness of antipsychotic drugs (10,11). This attitude was reinforced by introduction of haloperidol in 1958 by Haase and Janssen, a drug that is both effective in the treatment of schizophrenia but also frequently induced EPS (10,11).

In the early 1960's German psychiatrists working with G. Stille at Wander Pharmaceuticals in Bern, Switzerland, were able to refute the concept that EPS was required for the efficacy of antipsychotic drugs through the development of clozapine (12). Clozapine, a second generation antipsychotic is only minimally associated with EPS (13). Clozapine was briefly marketed and quickly withdrawn (13), because it lacked the propensity to induce EPS believed necessary for therapeutic efficacy. Clozapine use was further limited by the purchase of Wander Pharmaceuticals by Sandoz Pharmaceuticals (14) and, most significantly, reports from Finland that life-

threatening incidents of agranulocytosis associated with clozapine treatment (15). However, enthusiasm for the drug was maintained by a few clinical investigators, notably G. Honigfeld at Sandoz, who observed that clozapine was remarkably effective in treatment-resistant schizophrenic patients. This observation led to the landmark double-blind clinical study of clozapine using a well defined group of treatment-resistant patients whose blood cell counts were closely monitored during treatment, (16) and ultimately to its introduction to the US market in 1990.

Clozapines' success stimulated the development of other second generation antipsychotic drugs with similar efficacy with the idea of eliminating leukocytopenic side effects. The first of these, risperidone, was approved in 1994, (17) olanzapine followed in 1996, (18) quetiapine in 1997 (19), ziprasidone in 2001 (56), and aripiprazole in 2002 (55). All subsequently released atypical antipsychotic drugs have yet to prove that they are as effective as clozapine, which is still considered the most efficacious drug for the treatment of schizophrenia. (13).

Pharmacology of Antipsychotic Drugs

The antagonism of dopamine D_2 receptors is believed to be the most important pharmacodynamic attribute of both the typical and atypical antipsychotics. Antagonism of D_2 receptors affects three main dopaminerigic pathways in the brain: the mesolimbic, nigostriatal, and tuberoinfudibular (79). The mesolimbic tract originates in the A10 area and innervates both cortical and limbic structures.

Mesolimbic dopaminerigic projections appear to be important in arousal, memory, stimulus processing, locomotor activity and motivational behavior. The positive symptoms of schizophrenia (hallucinations and delusions) are believed to be associated with hyperdopaminergic activity in the limbic system, thus it is likely that D_2 antagonism in the mesolimbic tract alleviates theses symptoms by reducing its dopaminerigic tone (79).

The nigrostriatal pathway originates in area A9 (zona compacta) and projects through the basal ganglia, and the blockade of D_2 receptors in this pathway is believed to be primarily responsible for Parkinsonism and other extrapyramidal side effects. The basal ganglia of the nigrostriatal pathway are thought to be involved in motor learning and movement sequencing. The tuberoinfudibular tract has its cell bodies in the hypothalamus and projects to the pituitary gland where it regulates the release of prolactin. Lactotrophs of anterior pituitary posses D_2 receptors that when stimulated by dopaminerigic projections inhibit prolactin secretion. Antagonism of the D_2 receptors of pituitary lactotrophs interferes with the dopaminerigic stimulation from tuberoinfudibular tract neurons and disinhibits the regulation of lactrotrophs resulting in the elevation of prolactin secretions (79). Prolactin elevation is a prominent side effect of the typical antipsychotics and to a much lesser extent the atypical antipsychotics.

Antagonism of cortical 5-HT_2 receptors in the cortex is believed to release tonic inhibition of dopaminerigic neurons and improve the hypodopaminergic frontal cortex found in schizophrenic patients (79). This mechanism may contribute to the

improved negative and cognitive symptoms of schizophrenic patients using the atypical antipsychotics.

Theories of Atypicality

Dopamine D_2 receptor blockade is a pharmacodynamic property of all antipsychotics, both typical and atypical, with the exception of aripiprazole, which is a D_2 receptor partial agonist (27). While the definition of atypical antipsychotics has not been solidified and is generally used as a blanket term to describe second generation antipsychotics, there are differences that distinguish the typical from atypical. The most obvious include lower EPS liability, none to minimal prolactin elevation, and a higher affinity for 5-HT_{2A} receptors than D_2 receptors. However, these parameters vary greatly among the atypical antipsychotics and provide only a loose definition. Several individuals have attempted to define the atypical nature of these second generation antipsychotics, and this work has led to two major hypotheses.

The two hypotheses that have been put forth in attempt to define atypicality are the fast dissociation hypothesis and the 5-HT_{2A}/D_2 affinity ratio hypothesis. The fast dissociation hypothesis is based of the fact that all antipsychotics antagonize D_2 receptors but that the atypicals dissociate more quickly from the receptors than the typicals. This hypothesis states that rapid dissociation of atypicals from D_2 receptors is the property that results in lower EPS liabilities and minimal prolactin elevation

and is the key pharmacodynamic property of atypicality (57). Photon emission tomography studies have shown that the threshold for inducing EPS is at about 80% nigrostriatal D_2 receptor occupancy in the, while antipsychotic activity only requires around 65% D_2 occupancy (58). Faster dissociation would result in a lower occupancy percentage at any given moment, and a lower incidence of EPS, as well as lower percentage occupancy producing the desired therapeutic results. One of the major weaknesses of this hypothesis is that it only takes into account D_2 receptor interactions yet all the atypicals have complex binding profiles and affect multiple neurotransmitter systems, which may contribute to their low propensity to induce EPS. On the other hand, no successful antipsychotic drugs have been made that are effective with out either D_2 antagonism or partial agonism in the case of aripiprazole, suggesting that D_2 receptor interaction is most important for antipsychotic treatment.

An experiment comparing pKi values of 13 typical and 7 atypical antipsychotic showed that atypical could be distinguished from typical drugs based on a lower affinity for the D_2 receptor and higher affinity for 5-HT_2 receptors (59). That is typical and atypical drugs formed into group based off their 5-HT_2/D_2 affinity ratios. This 1989 study included clozapine, and several drugs that to this date are not approved for the treatment of schizophrenia in the United States, such as ritanserin, SCH 23390, and zotepine (59). Interestingly, all subsequently approved atypical antipsychotics have a higher affinity for 5-HT_{2A} than D_2 receptors. This hypothesis is based on a correlation found in this receptor affinity study, namely that compounds found to have higher affinities for the 5-HT_2 receptor than D_2 tend not to induce EPS

in humans and animals. To the best of my knowledge no direct and solid evidence has conclusively demonstrated an interaction of antipsychotics with serotonin receptors is the only property of the atypicals that reduces the likelihood of EPS. Furthermore, anticholinergics compounds, such as Trihexyphenidyl, have been used with some success to treat EPS suggesting that EPS involve multiple neurotransmitter systems. Clozapine and olanzapine have substantial muscarnic antagonist activity, yet research I have done has shown that olanzapine frequently induces catalepsy, which is a laboratory model of EPS. Since atypicals interact at a number of other receptor sites that may help reduce EPS liability, the definition of atypicality remains the term used to group clinically similar yet chemically diverse group of compounds. Since the initial publication of this report in 1989 (59), it has been discovered that there are several 5-HT_2 receptor subtypes, and isoforms of the D_2 receptor raising questions about the validity of the 1989 results, because of the discovery of increased neuro-complexity.

Atypical Antipsychotics Pharmacology

Clozapine is the only drug that is currently approved for treatment resistant schizophrenia, yet it is not clear what pharmacological properties are responsible for its superior efficacy. Clozapine, a dibenzodiazepine, is a mixed guanine nucleotide binding protein coupled receptor (GPCR) antagonist that binds to many dopaminergic, serotonergic, adrenergic, and muscarinic acetylcholine receptors.

Clozapines' major metabolite N-desmethylclozapine is pharmacologically active with antipsychotic efficacy (80). Clozapine like all of the FDA approved atypical antipsychotics has a high $5HT_{2A}/D_2$ binding affinity ratio, but also binds a number of other receptors including $5HT_{2C}$, D_1, D_3, and D_4 receptors. The search for the reasons why clozapine has superior efficacy had led researchers to look at individual receptor pharmacology in attempt to pinpoint both therapeutic effects as well as side effects. Clozapine has a higher affinity for the D_1 receptor than D_2 receptors (28), D_1 receptors are the main dopamine receptors in the prefrontal cortex and antagonism of these receptors is believed to be involved in improving negative (29) and cognitive (30) symptoms of schizophrenia. Interestingly, other atypical antipsychotics lack an appreciable affinity for D_1 receptor, making D_1 receptor antagonism a possible source of clozapine's superior efficacy (31), yet selective D_1 receptor antagonists lack antipsychotic properties (32,33). Clozapine also has a higher affinity for D_4 receptors than D_2, and antagonism of D_4 receptors results in increased dopamine release in the basal ganglia and prefrontal cortex that may in part explain clozapine's low propensity to induce EPS and the improved capacity to treat cognitive symptoms (34). Interestingly, D_4 receptors are also overexpressed in the schizophrenia patient (35,36), yet selective D_4 antagonists have had had little success as antipsychotics (37).

Risperidone, a benisoxazole derivative was the first atypical antipsychotic marketed after clozapine. The major metabolite of risperidone is 9-OH- risperidone is an active metabolite with similar efficacy to the parent drug (79). Risperidone also

bind $5HT_{2A}$ and D_2 receptors with high affinity, but has very little cholinergic blockade (38,39). Risperidone also antagonizes α1 and α2 adrenergic receptors, but with low affinity (79). Risperidone is also a low affinity H_1 receptor antagonist (79).

Olanzapine, a thienobenzodiazepine, has a binding profile similar to clozapine although the affinities of olanzapine at its receptors sites are different from those of clozapine. For example, olazapine has a higher affinity at D_1 and D_2 receptors , while clozapine has a slightly higher affinity at D_4 receptors (20). Olanzapine has a high binding affinity at D_1, D_2, D_4, D_3, 5-HT_{2A}, $5HT_{2C}$, H_1, α_1 adrenergic, muscarinic M_{1-5} receptors, and low affinity at α_2, $GABA_A$, and $5HT_1$ and β adrenergic receptors (20). In placebo-controlled studies, clinically significant alanine transferase (ALT) elevation of greater than three times the upper limit of the normal range was observed in 2% of patients taking olanzapine. Also, during pre-marketing studies, the incidence of ALT elevations was 2%, but this was not associated with jaundice or other symptoms attributable to liver impairment. Transient increases may be seen but usually normalize with olanzapine continuation (23).

Ziprasidone has a binding affinity ratio of 11:1 for 5-HT_{2A}/D_2. Ziprasidone also binds with relatively high affinity for 5-HT_{2C}, 5-HT_{1D}, α_1 adrenergic and D_1 receptors (21). Ziprasidone has the highest 5-HT_{2A}/D_2 affinity ratio, and the highest 5-HT_{2C}/D_2 ratio of all of the atypicals (71). Due to high affinity for 5-HT_{2C} receptors low does of ziprasidone result in substantial 5-HT_{2C} antagonism without appreciable D_2 antagonism that can result in dysphoria, hypomania, and panic without antipsychotic action (71). These effects have been made apparent by the tendency of

clinicians to prescribe doses too small for effective treatment. Ziprasidone is also unique because it has the highest $5HT_{1A}/D_2$ affinity ratio, and occupies this receptor to a greater extent than any approved antipsychotic drug. Another unique feature of ziprasidone is its potent $5\text{-}HT_{1D}$ antagonism , and it has been suggested that this action helps improve the mood of the schizophrenic patient because blockade of this receptor disinhibts serotonin release. In addition ziprasidone is a reuptake inhibitor of serotonin, dopamine and norepinephrine. Ziprasidone lacks M_1, α_1, and H_1 activity seen in several other atypical antipsychotics such as clozapine and olanzapine.

Quetiapine is dibenzothiazepine derivative with very little tendency to induce EPS (93). Quetiapine has high affinity for $5\text{-}HT_{2A}$ receptors and lower affinity for D_2 and D_1 receptors. This drug has some affinity for α_1, α_2, and H_1 receptors, and very little for muscarinic receptors (22, 93). Quetiapines' EPS liability is similar to that of clozapine, but does have the tendency to induce tachycardia, orthostatic hypotension, and sedation. One reason why quetiapine has a low propensity to induce EPS is probably because it is selective for mesolimbic D_2 receptors rather than nigrostriatal D_2 receptors (93).

Aripiprazole was discovered in the early 1980s as an attempt to find an antipsychotic that would function both as an antagonist and an agonist at the D_2 receptor. Hence, aripiprazole is the first potent D_2 partial agonist for the treatment of schizophrenia. In a hyperdopaminergic state, aripiprazole functions as an antagonist, while under conditions of hypodopaminergic activity, it functions more like an agonist. This novel mechanism of aripiprazole has earned it the title of dopamine

stabilizer. Interestingly aripiprazole occupies 95% of striatal D_2-like receptors yet the incidence of EPS was no greater than placebo in clinical trials. Aripiprazole has a high affinity for D_2, D_3, and 5-HT_{2A} (Ki values of 0.34, 1.7 and 3.4 nM respectively), moderate affinity for D_4, 5-HT_{2C}, 5-HT_7, α_1, and H_1 receptors (Ki of 44, 15, 39,57 and 62 nM respectively) and also has a moderate affinity for the serotonin reuptake site. Aripiprazole also has high affinity for D_3 receptors. It is a partial agonist at 5-HT_{1A} receptors and an antagonist at 5-HT_{2A} receptors. Aripiprazole has a moderate affinity for $\alpha 1$ and H_1 receptors with no appreciable affinity for the M_1 receptor (23).

Extrapyramidal Side Effects (EPS)

Extrapyramidal side effects (EPS), including akathisia, dystonia, tardive dyskinesia, and pseudoparkinsonism, are the major adverse effects associated with traditional antipsychotic therapy, and are associated to a lesser extent with the second generation antipsychotics. These side effects are widely believed to be the result of dopamine antagonism in the nigrostriatal pathways. Akathisia is the most frequently occurring of these adverse effects. Approximately 50% of patients treated with traditional antipsychotics will experience a subjective feeling of mental restlessness accompanied by motor symptoms. Akathisia causes intense anxiety, an inability to relax, and motor symptoms such as pacing, rocking while sitting, marching in place, constant fidgeting, and purposeless stereotypic movements (53).

Dystonia is an early onset EPS that includes involuntary contractions in

opposing flexor and extensor muscles resulting in abnormal postures. Symptoms of dystonia include tongue protrusion, laryngeal-pharyngeal constriction, oculogyric crises, torticollis, and strange positioning of limb and torso (53). Of these tongue protrusion and laryngeal-pharyngeal constriction may have effects on the rhythmic licking behavior of rats, providing one reason that licking dynamics should be examined as a model of EPS.

Tardive dyskinesia (TD) is a movement disorder characterized by abnormal choreiform (observable, rapid, purposeless, irregular and spontaneous movement) and athetoid (slow and irregular) movements occurring late in onset in relation to initiation of antipsychotic therapy. This adverse effect usually develops over several months or years but usually requires at least three months of neuroleptic treatment. Patients showing symptoms of TD often display hyperkinetic movements of the limbs and trunks, and orofacial movement disorder. Orofacial motor side effects consist of repetitive hyperkinetic movements including chewing, protrusion of the tongue, vermicular movements of the tongue, side to side or rotary jaw movements, and lip smacking (54). Even though TD is a late onset symptom of antipsychotic treatment and atypicals rarely produce TD, sensitization to licking variables in rats may prove as a predictor of atypical induced TD, or other subtle late onset movement disorders not heretofore fully characterized. The estimated average prevalence is 20% with a range of 13-36%. The incidence of new cases per treatment year with conventional antipsychotics is approximately 5% (24).

Neuroleptic induced Parkinsonism is named as such because of its similarities

to Parkinson's disease. This is an early onset side effect that can occur shortly after administration. The motor symptoms of drug induced Parkinsonism include tremor, rigidity, and bradykinesia. Patients exhibiting Parkinsonism often have expression less face, decreased arm movements during walking, and impaired ability to initiate movement, and small handwriting.

Clozapine is associated with little to no EPS and quetiapine has been found to have no greater rates of EPS than placebo. Olanzapine and risperidone can cause EPS in a dose related fashion, but less frequently than traditional antipsychotics. Risperidone treatment is associated with Parkinsonism rates similar to placebo in doses under 6mg/day. Doses higher than 6mg/day are associated with EPS rates of 20% or greater. Parkinsonism with olanzapine is similar to placebo with doses up to 10 mg/day. At higher doses the rates increases to 20%. Akathisia with olanzapine is significantly higher than placebo at doses greater than 10mg/day.

The Hypoglossal Nucleus and Neural Control of the Tongue

Neural control of the tongue enables a range of oropharyngeal behaviors, including licking, mastication, swallowing, vocalization, breathing, and coughing. Several of these behaviors, including licking, are controlled by central pattern generators (CPG's) located in the medulla and pons of the brainstem. These pattern generators transform ascending and descending signals into rhythmic and patterned behaviors. Disease states such as SIDS, and sleep apnea, and drugs have motor effects

on the tongue, making the study of the hypoglossal nucleus and licking dynamics a clinically relevant pursuit.

The tongue is composed of eight uncompartmentalized interdigitated muscles capable of meeting the motor demands of a multitude of complex orolingual behaviors. The tongue has four extrinsic muscles the syloglossus, hypoglossus, genioglossus, and geniohyoid, and four intrinsic muscles the longitudinal, transverse, vertical, and superior. The intrinsic muscles determine the shape of the tongue and have no bony attachments, and extrinsic muscle have bony attachments capable of directing tongue protrusion and retrusion. The genioglossus and the geniohyoid are tongue protrusor muscles, and the syloglossus and hypoglossus are the tongue retrusor muscles (43,44). However, current knowledge states that co-contraction of both intrinsic and extrinsic muscle simultaneously results in tongue movements (40,41,42). Tongue protrusion is predominantly mediated by the activity of the genioglossus in combination with the intrinsic vertical and transverse tongue muscles. Retrusion is mediated predominantly by contraction of the styloglossus and hypoglossus and intrinsic longitudinal muscles. The diversity of tongue movements required for the continuum of oropharyngeal behaviors is reflected by the myotopic organization of hypoglossal (XII) motor nucleus. The extrinsic and intrinsic tongue muscles are innervated by the medial branch of the XII nerve, and the somata of protrusor motor neurons are located in the ventral compartment of the hypoglossal nucleus. In contrast the extrinsic and intrinsic retrusors are innervated by the lateral branch of the XII nerve and the cell bodies are located in the dorsal compartment of

the nucleus. In addition, a small population of interneurons are intermingled among the XII motorneurons providing more complexity to the hypoglossal nucleus. These intrannuclear interneurons make up about 5% of the neurons of the hypoglossal nucleus, and are mainly located in the dorsolateral, lateral, and ventral margins. The genioglossus muscle is believed to control airway patency and is the main tongue protrusor muscle that is innervated by the motor neurons of the hypoglossal nucleus. The motorneurons here are believed to be stimulated at least in part by serotonin (81, 82) and norepinephrine (83,84). Receptor localization studies have shown the presence of high numbers of 5-HT_{2A} receptors on hypoglossal motor neurons (85,86,87), further supporting this receptors role in the control of hypoglossal motor neurons.

Therefore, I hypothesize that compounds with α_1, α_2, and 5-HT_{2A} antagonism will substantially reduce licking rhythm and D_2 antagonism will reduced motivational behavior (defined as engaging in licking). Furthermore, a neuroleptic influenced reduction in the number of licks is reflective potential catalepsy, a model of EPS, based of the fact that several olazapine injections induces catalepsy, an others resulted in two minute sessions with minimal number of licks.

Pre-Clinical Behavioral Analysis and Neuroleptic Drugs

Despite its tendency to induce agranulocytosis (75), clozapine is an effective drug for treatment-resistant schizophrenic patients (60); moreover, clozapine has a

low EPS liability (61, 62). The therapeutic success of clozapine has stimulated the search for clozapine-like drugs without leukocytopenic side effects. Preclinical behavioral research has had a prominent role in this effort (63, 64, 65, 66, 67, 68, 69, 70), and the identification of behavioral bio-markers can aid in the identification of clozapine-like drugs. Within this context it has been reported that the dominant rhythm of oscillations in rats' forelimb force was slowed by clozapine but not by haloperidol (76), a behavioral property distinguishing clozapine from the typical antipsychotic haloperidol. In a separate study, it was observed that another rhythmic behavior of rats, tongue movements made while licking water from a force sensing disc, was only slightly affected by haloperidol (77,78). Another study showed that only a chronic haloperidol regimen was capable of reducing licking rhythm (74). Other research has shown that risperidone also slows licking rhythm in a similar fashion to clozapine (73). On the other hand, acute clozapine treatment was previously shown to induce a dose dependent reduction in licking rhythm (72). The data presented in this thesis, in addition to previous studies, show that clozapine has significant effects on several variables of tongue dynamics including peak force, number of licks, and rhythm.

In addition to testing the above mentioned hypothesis this work had several other purposes, including comparison among the atypicals, and a comparison to similar research done with the typical neuroleptic haloperidol. One purpose of the present work was to compare the effects of clozapine on licking behavior to risperidone, olanzapine, aripiprazole, quetiapine fumarate, and olanzapine, and to

compare the effects of oral clozapine to previous reports. Accordingly rats were administered three or four acute doses of each of the atypical antipsychotic drugs (AADs) listed above, and the effects on licking behavior were characterized by measuring the rhythm of tongue (with Fourier methods), the peak force, and number of licks. A second purpose was to assess compare the potency of AAD induced motor effects and to compare them to previous data collected for haloperidol. A third purpose of this work was to search for putative behavioral markers of atypicality using clozapine as a standard on the basis that clozapine is a drug of choice in treatment resistance schizophrenic patients. A fourth purpose was to assess the tolerance and/or sensitivity to the behavioral effects using a subchronic dosing regimen in order to assess any tolerance or sensitization effects that could be used to distinguish among AADs. Tolerance to such motor side effects could suggest a more effective drug particularly with respect to patient compliance. Sensitization may be useful in predicting if a drug will be likely to induce worsening side effects.

Material and Methods

Acute regimen

Nine male, Sprauge-Dawley rats (Harlan, Indianapolis, Ind.) served as subjects. Rats were maintained on a water restriction regimen of 10-15 minutes access 30 minutes after the 2 minute experimental session. This water restriction regimen allowed for adequate hydration as rats steadily gained 5-6 grams per week. The purpose of the water restriction was to obtain maximal motivation to lick water

during the 2 minute test period. Measurement of licking dynamics occurred between 12 and 2 pm daily during the light portion of the light-dark cycle in the vivarium (lights on from 6 a.m. to 6 p.m.). At the time of experimental evaluation of the drugs effects on licking dynamics, rats averaged 225 grams in body weight and were about 3 months old.

Sub-chronic regimen

Thirty four male, Sprauge-Dawley (Harlan, Indianapolis, Ind.) rats served as subjects. Rats were maintained on a water restriction regimen of 10-15 minutes access 30 minutes after the 2 minute experimental session for the same reasons described above for the acute experiments. Recording times, light cycle were the same as in the acute experiments. At the time of the evaluation of the drugs effects on licking dynamics, rats averaged 247 grams and were about three and a half months old.

Apparatus

The licking recording chamber has been described elsewhere in detail (78). A rodent operant chamber was modified so that the panel in which the lever was mounted was replaced with another panel containing a 6 cm x 6 cm opening at flour level. Covering this opening from the outside was a 3 cm deep transparent plastic

enclosure with a 12 mm diameter hole on the bottom of its surface. Lick force-time wave plots were recorded by attaching an 18 mm diameter lick disc to the shaft of a force transducer (Sensotec model 31a) (Figure 1). The surface of the lick disc was placed 1mm below the 12 mm diameter hole through which the rat extended its tongue. The plastic material through which the hole was cut was 1 mm thick making the lick disc 2 mm from the rats' mouth. The larger the distance between the rats' mouth and the force transducer, the lower the number of licks, the peak force, as well as the lick rhythm (figure 2). The force transducer was calibrated to measure force in units of 0.2 gram equivalent weight. A Labmaster interface recorded the force-time wave data at a sample rate of 100 per second via a 386 based computer. Water (0.055 ml) was delivered onto the lick disc by 5 gauge stainless steel tubing when the computer activated peristaltic pump connecting the water reservoir and the tubing to the lick disc. The computer program measured the number of licks and continuous force transducer output in real time. The force threshold for lick detection was 1.0 gram, and the force criterion for programmed consequences was 4.0 g. The entire 2-min session was stored in RAM and transferred to a hard disc at the end of each session for Fourier analysis, and other analyses.

Procedure

The rat training procedure was previously described (78). Water (0.100 ml), was placed onto the lick disc and naive rats were placed into the chamber for two

minutes until rats licked at least 400 times in a two minuet session three days in a row. The emission of 12, 4 g licks resulted in the delivery of 0.055 ml of tap water onto the disk. Recording sessions lasted 120.32 seconds or about 2 min. Observation of the effects of acute oral clozapine (10.0 mg/kg, 5.0 mg/kg, 20.0 mg/kg), acute oral aripiprazole (6.0 mg/kg, 12.0 mg/kg, 18.0 mg/kg), acute oral risperidone (0.50 mg/kg 0.25 mg/kg, 1.0 mg/kg, 2.0 mg/kg), acute oral ziprasidone (1.0 mg/kg, 0.50 mg/kg, 2.0 mg/kg, 4,0 mg/kg) acute oral quetiapine fumarate (10.0 mg/kg, 5.0 mg/kg, 20.0 mg/kg), and acute oral olanzapine (2.0 mg/kg, 1.0 mg/kg, 4.0 mg/kg) were administered in the dose order indicated in the parenthesis three days apart. After two days of licking and no drug or vehicle treatment each rat served as its own control and received vehicle (Carboxymethylcellulose sodium salt) at 5 ml/kg the day before drug treatment. The additional doses of risperidone (2.0 mg/kg), and ziprasidone (4.0 mg/kg) were added because the effects of these drugs on licking dynamics were present but small in the series of lower doses.

In the subchronic studies 10 rats were given vehicle (5 ml/kg), 8 rats were given oral clozapine (20.0 mg/kg), 8 rats were given oral risperidone (2.0 mg/kg), and 8 rats were given oral olanzapine (4.0 mg/kg) twice a day 12 hours apart. Drugs were administered at 12 noon and 12 midnight seven days a week, and the experimental session was between 12-2 pm seven days a week.

Drugs

All drugs were obtained in tablet form from the pharmaceutical companies listed below and were crushed and placed in a constantly stirred suspension of carboxymethylcellulose sodium salt immediately prior to administration. Clozapine 25 mg (TEVA), aripiprazole 30 mg (Bristol-Myers Suibb), risperidone 2mg and 4 mg (Janssen), ziprasidone 20 mg (Pfizer), quetiapine fumarate 25 mg (AstraZeneca), and Olanzapine 10 mg (Eli Lilly). Vehicle and drug were administrated at approximately equal volumes. Oral administration of each drug or vehicle was given 45 min before the beginning of each 2 min session.

Dependent Variables

As rats lick the force transducing lick disc, force measurements are recorded in real time by a computer for subsequent quantitative analysis. Shown below is an example force-time wave plot (Figure-3) depicting variables generated by the lick-force-rhythm test for quantitative analysis. The number of licks is a behavioral variable that can provide insight into the effects of AADs on motivation that should be maximal due to the water restriction regimen. Peak force can reveal the effects of atypical antipsychotics on the neuromuscular control of tongue and the effects of AADs on hypoglossal motor neuron output. The lick rhythm reveals the effects of AADs on the established dominate rhythmic frequency of licking in Sprague Dawley rats of 6 Hz. Lick duration, inter-lick, and period are other variables available for analysis from the lick-force-rhythm test for analysis. The duration of the lick is the

width of the peak generated by the force of one lick and the inter-lick interval is the time elapsed between licks. The period is defined as the sum of the lick duration and inter-lick interval and provides complimentary data to the lick rhythm.

Quantitative Analysis

The effects of acute and subchronic doses were measured in terms of the number of licks in 2 minutes and the dominant rhythm of licking was measured in Hz (cycles/sec) by Fourier spectral analysis techniques described previously (77, 78). The dominant rhythm of lick oscillation was taken as the spectral peak in the 3.5-6.5-Hz region of the spectrum computed by Fourier methods for each rat each session. With these methods the dominant lick rhythm is a measure of periodic tendency of the licking behavior; therefore, the rhythm of the oscillatory process of licking can be largely independent of the number of licks. For example, two rats could have nearly the same dominant licking rhythms, even though one of them stops licking halfway through the session and thus emits 50% fewer licks than the other rat. Dose effect one-way analyses of variance (ANOVAs) were used for all acute dosing experiments and linear trend tests were used to analyze subchronic data. Data were expressed as a proportion of vehicle control, where the vehicle performance was for each subject an average of vehicle sessions.

Results

In Figure-4 is a sample of an actual force-time wave plots for the same rat when given saline vehicle (top two rows) and an acute oral dose of 20.0 mg/kg of clozapine (bottom two rows). All data discussed is either directly obtained from force-time wave plots or derived from force-time wave plots, in the case of lick rhythm by Fourier analysis. All of the six atypical antipsychotic drugs approved for the treatment of schizophrenia with the exception of aripiprazole had marked effects on licking dynamics when compared to vehicle controls. The effects of Aripiprazole on licking dynamics were either statistically insignificant or slight. This is probably due to Aripiprazole's partial agonism of D_2 receptors and its dopamine stabilizing activity, which is unique among the compounds studied. In the acute studies each rat served as its own control and was administered saline vehicle the day before drug administration. The data from the vehicle days were averaged for comparison to data from days in which drugs were administered, and all data was analyzed by one way ANOVA. All mean, SEM, and one way ANOVA data of acute oral AAD administration for the peak force of licking, the number of licks, and lick rhythm are presented in tables 1, 2, and 3. Clozapine dose dependently reduced the peak force, the number of licks, and lick rhythm in a two minute session. Aripiprazole showed no statistically significant effect on the average number of licks, but had slight statistically significant effects on the peak force, and lick rhythm. Aripiprazole was the only compound studied that had no significant effect on the number of licks.

Clozapine risperidone, ziprasidone, quetiapine fumarate, and olanzapine, all significantly reduced the number of licks in the two minute experimental session (figure-3, and figure -6, tables 1-3).

With respect to licking rhythm, all six AADs had a significant effect. Data collected on days when no vehicle or drug was administered showed that the dominant lick rhythm for Sprague Dawley rats is about 6.0 Hz with no statistically significant variation (data not shown), and all vehicle data are consistent with that finding. The 20.0 mg/kg dose of clozapine had the largest effect on lick rhythm of all compound and doses in this study. The effects of aripiprazole on lick rhythm were significant from vehicle but each increasing the dose had no additional significant decrease of rhythm. Risperidone was the most potent, affecting lick rhythm at doses substantially lower that all drugs studied. Risperidone effect on rhythm at 2.0 mg/kg was close to the effects of clozapine at 20.0 mg/kg. Ziprasidone also has a very potent effect on lick rhythm at low doses. Olanzapine at 4.0 mg/kg was comparable to 20.0 mg/kg of clozapine but was much more potent in causing an effect. Aripiprazole had only minor effects on licking rhythm, as each dose of aripiprazole was statistically significant from vehicle, but no dose (6.0 mg/kg, 12.0 mg/kg, or 18.0 mg/kg) was significant from each other. That is no dose response was observed, possibly due to its low affinity for the α_1 adrenergic receptor, and low occupancy at 5-HT_{2A} seen at therapeutically relevant doses of aripiprazole (23), antagonism of which is believed to slow hypoglossal motor neuron basal activity (86). Risperidone the most likely of the atypicals to induce extrapyramidal symptoms was most potent at all motor-behavioral

variables analyzed (89,90).

All six AADs had significant effects on the peak force of licking with varying degrees of potency. The highest dose of clozapine had a large effect on the peak force of licking but the potency of the effect was low at 20.0 mg/kg clozapine produced an effect similar to 2.0 mg/kg of risperidone, 4.0 mg/kg of ziprasidone, and 4.0 mg/kg of olanzapine. Again, the effects of aripiprazole were slight compared to most compounds tested. Risperidone was the most potent at reducing the peak force of licking producing a large effect at just 2.0 mg/kg. Ziprasidone also had a very potent effect on the peak force. Quetiapine had a significant but not very potent dose dependent reduction of the peak force. Olanzapine, risperidone, and ziprasidone making up the high potency group at all acute licking variables analyzed. Data for all six drugs are shown as proportion of control as well as in graphs not including the control values for better visualization (figure 3-6). In order to allow for potency comparison of the effects of all six AADs on licking dynamics all drugs were placed on the same graph by using a Log_{10} of dose scale.

Subchronic experiments were done using clozapine, risperidone, olanzapine in order to assess whether tolerance or sensitization to effects of AADs could be detected. Altered lick dynamics can be viewed from several different perspectives. It can be viewed as a model of EPS, a bio-behavioral marker for clozapine-like drugs or as a side effect of atypical antipsychotic drugs. Tolerance to the effects of AADs on licking dynamics can be viewed as a predictor to whether or not orolingual side effects will ease with time. The pharmacology of clozapine is so complicated that the

Lick-force-rhythm test could serve as an early screen for putative antipsychotic drugs. As a model of EPS it may be predictive of orolingual effects of neuroleptic Parkinsonism, or in the case of sensitization of orolingual motors effect associated with late onset EPS such as Tardive Dyskinesia.

Clozapine [$F(1,13)= 39.775$, $p< 0.0001$] significantly reduced the number of licks each of the ten days with no trend towards tolerance or sensitivity as determined by polynomial test of order 1 [$F(1,13)= 0.876$, $p<0.366$]. Risperidone also reduced the number of licks each of the ten days [$F(1,14)= 21.877$, $p< 0.0001$], again with no trend toward tolerance or sensitization [$F(1,14)= 0.717$, $p<0.411$]. Olanzapine also significantly reduced the number of licks [$F(1,10)= 45.438$, $p< 0.0001$] and displayed a sensitization effect [$F(1,10)= 4.112$, $p<0.0001$]. Olanzapine frequently induced a cataleptic state in which animals did not lick or move during their two minute session. Cataleptic episodes increased later in the ten day dosing regimen.

Clozapine reduced the lick rhythm each of the tend days [$F(1,13)= 8.886$, $p< 0.012$] with no trend toward tolerance or sensitization [$F(1,13)= 0.081$, $p<0.780$] Risperidone reduced the lick rhythm each of the ten day [$F(1,14)= 15.965$, $p< 0.0001$] with no trend towards tolerance or sensitization [$F(1,14)= 0.475$, $p<0.503$]. Olanzapine reduced the licking rhythm each of the ten days [$F(1,9)= 19.220$, $p< 0.002$] and showed no significant trend towards sensitization [$F(1,9)= .089$, $p< 0.769$].

Clozapine effected the peak force of licking each of the ten days [$F(1,13)=12.337$, $p<0.003$] with no significant trend toward tolerance or sensitization

[F(1,13)=0.321, p<0.579]. Risperidone has a significant effect on the peak force of licking [F(1,14)=10.558, p<0.005] with a significant trend towards sensitization [F(1,14)=5.334, p<0.034]. Olanzapine had a significant effect on the peak force of licking [F(1,9)=8.219, p<0.011] with no significant trend towards tolerance or sensitization [F(1,9)=2.424, p<0.139]. Risperidone displayed a trend towards sensitization of peak force of licking suggesting its potential for orofacial EPS that may worsen with a more chronic dosing regimen.

Conclusion and Discussion

Licking dynamics serves as a model for EPS induced by atypical antipsychotics. Both tardive dyskinesia, dystonias and neuroleptic induced Parkinsonism have orofacial and tongue motor side effects, thus irregularities in tongue movements can be used to model the likelihood of neuroleptic agents to induce EPS. Tardive dyskinesia is a late onset side effect, whereas dystonias, and Parkinsonism is an early onset side effect. The acute and subchronic alterations in licking dynamics described here may be more suggestive of Parkinsonism and early onset EPS than Tardive dyskinesia and later onset EPS.

Previous work with the effects of clozapine on licking dynamics showed similar dose dependent decreases in licking rhythm and number of licks with acute dosing regimens (72). Subchronic investigations revealed a tolerance to the disruptive effects on the number of licks but not to clozapines' rhythm slowing effects (72). The

current study differed in that no tolerance to clozapines' disruptive effects on number of licks was observed. This discrepancy could have been due to the fact that oral doses were administered in this study instead of the intraperitoneal route of administration in the previous study, or due to differences in doses. Subchronic intraperitoneal doses in the previous study were administered at 1.5, 4.5, and 6.0 mg/kg once a day, whereas subchronic doses in this experiment were given orally at 20.0 mg/kg twice daily and this may account for observed differences. Thus, it is possible that even after first pass metabolism rats in this study were exposed to more drug (particularly with the two doses per day), and tolerance to the disruption of the number of licks may have developed if the experiment had included more days. Both studies confirmed that no tolerance to the rhythm slowing effects of clozapine. Other work has demonstrated that risperidone also dose dependently reduced the lick rhythm in a similar fashion to clozapine (91).

Olanzapine has also been shown to have clozapine-like effect on the peak force, rhythm and number of licks (92). An in depth analysis and comparison of the effects of all six atypical antipsychotics on licking dynamics has not been done, and this research allowed a comparison of available AADs with clozapine. Clozapine is viewed as a reference compound in which to compare the rest of the AADs, because its effectiveness has yet to be surpassed and is the only AAD currently indicated for the treatment of refractory schizophrenic patients. Similarities with clozapine may predict which new agents will be clozapine-like, and effective antipsychotic compounds warranting future investigation. The high potency group contained the

most likely AADs (risperidone, olanzapine) to induce EPS, whereas the low potency group contained those least likely to induce EPS (clozapine, quetiapine, and aripiprazole) (89). In this way the lick-force-rhythm test appeared to identify clozapine like drugs with respect to the potential to induce EPS, and may be useful as a bio-behavioral screen for putative clozapine-like antipsychotics.

Upon comparison of the number of licks, peak force and rhythm of the six atypical antipsychotics after acute exposure, it becomes apparent that there are at least two potency groups. Risperidone, ziprasidone, and olanzapine make up an easily distinguishable high potency group in which low doses have large effects on all three variables. Clozapine, aripiprazole, and quetiapine fumarate make up a low potency group, but even at these higher doses only clozapine produced effects comparable to the maximal effects observed with risperidone, ziprasidone, and olanzapine. Aripiprazoles' effects were particularly slight with no effect on the number of licks and only a slight effect on the peak force and lick rhythm. Aripiprazole has dopamine stabilizing activity functioning as an agonist in a hypodopaminergic state and an antagonist in a hyperdopaminergic state (23), and this may explain its only slight effects on licking dynamics. While quetiapines' effects were statistically significant for all three variables, the effects were not large, which is consistent with its low propensity to induce EPS (93).

Subchronic dosing regimens were administered in order to determine if tolerance or sensitization to these disruptive effects on licking dynamics occurred over time. Clozapine, olanzapine, and risperidone were selected for subchronic

studies for several reasons. One reason is that risperidone has very high affinity for 5-HT_{2A} receptors, olanzapine has intermediate affinity, and clozapine has a lower affinity for this receptor. This would allow for the investigation of a range affinities for 5-HT_{2A} receptors which are hypothesized to slow hypoglossal motor neuron control of the genioglossus muscle. Also these drugs represented a low (clozapine), medium (olanzapine) and high potency (risperidone) sample of the six drugs tested acutely. Additionally, the maximum effects of these compounds on licking dynamics were large allowing lots of room for quantifiable tolerance even though no tolerance was observed. All three compounds had a significant effect on the number of licks, peak force, and lick rhythm with no trend towards tolerance or sensitization with two exceptions. First, olazapine showed sensitization to the disruptive effects to the number of licks. The number of lick diminished over ten days, in fact, several rats did not lick and appeared cataleptic. Upon experimentation with olanzapine it was observed that rats given higher doses of olanzapine assumed hunched postures, with an inability to control their front limbs enough for simple movement, and were generally flaccid when held. Furthermore, rats became unresponsive to stimuli and ceased engagement in the learned task of licking from the lick disc. This type of cataleptic behavior is suggestive of olanzapine induced EPS-like syndrome only observed with olanzapine in these studies. All olanzapine rats that did not lick at all were excluded from the data analysis to prevent skewing data concerning peak force and rhythm, and had they been included the trend towards sensitization to the disruptive effect to the number of licks would have been more marked. Second, the

peak force of licking for the rats administered risperidone showed sensitization, that is a trend over the ten days to a lower peak force of licking. Risperidone and olanzapine have been shown have a higher EPS liability than other AADs and these results towards sensitization agree with that.

Previous work investigating the effects of haloperidol on licking dynamics showed that acute haloperidol did not have a significant effect on lick rhythm, but did have disruptive effects on the number of licks and peak force. Therefore, it seems likely that compounds with α_1, α_2, and 5-HT_{2A} antagonism will substantially reduce licking rhythm, and compounds with D_2 antagonism will reduced the motivational behavior to lick. The alteration of licking dynamics may serve as a useful model in the screening of clozapine-like drugs that could be potentially used as antipsychotic drugs. Furthermore, a neuroleptic influenced reduction in the number of licks is reflective of potential catalepsy, a model of EPS, based on the fact that several olazapine injections induced catalepsy, an other olanzapine injections resulted in two minute sessions with minimal number of licks (77, 92). Thus, licking dynamics may be predictive of EPS liabilities of potential antipsychotic compounds.

The Effects of Acute AADS on the Peak Force of Licking (0.2 g)

Drug	Vehicle	Dose 1	Dose 2	Dose 3	Dose 4	One Way ANOVA
Clozapine	51.500 ± 1.829	5.0 mg/kg	10.0 mg/kg	20.0 mg/kg		[F(3,24)=16.838, p<0.001]
		53.778 ± 4.421	41.883 ± 3.745	27.889 ± 2.220		
Aripiprazole	47.630 ± 2.975	6.0 mg/kg	12.0 mg/kg	18.0 mg/kg		[F(3,24)=4.183, p<0.016]
		43.000 ± 3.636	40.111 ± 3.745	39.667 ± 3.100		
Risperidone	47.056 ± 3.666	0.25 mg/kg	0.5 mg/kg	1.0 mg/kg	2.0 mg/kg	[F(4,32)=19.132, p<0.0001]
		43.278 ± 4.242	43.000 ± 2.769	36.222 ± 3.459	28.444 ± 2.698	
Ziprasidone	47.569 ± 3.836	0.5 mg/kg	1.0 mg/kg	2.0 mg/kg	4.0 mg/kg	[F(4,32)=33.649, p<0.0001]
		43.889 ± 2.685	39.889 ± 3.490	33.778 ± 2.886	28.444 ± 2.352	
Quetiapine	42.185± 4.716	5.0 mg/kg	10.0 mg/kg	20.0 mg/kg		[F(3,24)=6.359, p<0.003]
		39.111 ± 4.008	38.889 ± 4.560	32.333± 1.929		
Olanzapine	43.019± 3.343	1.0 mg/kg	2.0 mg/kg	4.0 mg/kg		[F(3,24)=27.117, p<0.0001]
		39.444 ± 3.583	32.556 ± 2.678	28.667 ± 2.427		

Table 1- Mean and standard error of the mean values (SEM) of the peak force of licking for acute oral dosing of clozapine, aripiprazole, risperidone, ziprasidone, quetiapine, and olanzapine.

The Effects of Acute AADS on the Number of Licks

Drug	Vehicle	Dose 1	Dose 2	Dose 3	Dose 4	One Way ANOVA
Clozapine	647.185 ± 22.473	5.0 mg/kg	10.0 mg/kg	20.0 mg/kg		[F(3,24)=6.715, p<0.002]
		667.111 ± 15.515	498.667 ± 64.477	438.000 ± 40.744		
Aripiprazole	654.926 ± 19.222	6.0 mg/kg	12.0 mg/kg	18.0 mg/kg		[F(3,24)=1.954, p<0.148]
		667.111 ± 15.515	626.778 ± 22.515	608.333 ± 17.494		
Risperidone	710.194 ± 13.648	0.25 mg/kg	0.5 mg/kg	1.0 mg/kg	2.0 mg/kg	[F(4,32)=19.860, p<0.0001]
		692.333 ± 10.919	683.000 ± 17.912	656.333 ± 18.575	553.778 ± 21.216	
Ziprasidone	714.278 ± 13.133	0.5 mg/kg	1.0 mg/kg	2.0 mg/kg	4.0 mg/kg	[F=(4,32)21.146, p<0.0001]
		696.556 ± 21.868	688.111 ± 16.248	661.889 ± 25.313	559.778 ± 28.638	
Quetiapine	726.185± 13.800	5.0 mg/kg	10.0 mg/kg	20.0 mg/kg		[F=(3,24)14.204, p<0.0001]
		711.222 ± 14.016	704.556 ± 14.998	667.444 ± 22.703		
Olanzapine	715.185 ± 14.068	1.0 mg/kg	2.0 mg/kg	4.0 mg/kg		[F(3,24)=14.286, p<0.0001]
		712.889 ± 13.752	664.667 ± 16.686	437.556 ± 64.437		

Table 2- Mean and standard error of the mean values (SEM) of the number of licks for acute oral dosing of clozapine, aripiprazole, risperidone, ziprasidone, quetiapine, and olanzapine.

The Effects of Acute AADS on the Lick Rhythm (Hz)

Drug	Vehicle	Dose 1	Dose 2	Dose 3	Dose 4	One Way ANOVA
Clozapine	5.925 ± 0.078	5.0 mg/kg	10.0 mg/kg	20.0 mg/kg		[F(3,24)=15.492, p<0.0001]
		5.815 ± 0.090	5.632 ± 0.119	5.135 ± 0.096		
Aripiprazole	6.041 ± 0.084	6.0 mg/kg	12.0 mg/kg	18.0 mg/kg		[F(3.24)=12.664, p<0.0001]
		5.827 ± 0.054	5.689 ± 0.109	5.800 ± 0.096		
Risperidone	6.086 ± 0.087	0.25 mg/kg	0.5 mg/kg	1.0 mg/kg	2.0 mg/kg	[F(4,32)=32.643, p<0.0001]
		5.962 ±0.089	5.787 ±0.111	5.735 ± 0.124	5.418 ± 0.108	
Ziprasidone	6.116 ± 0.086	0.5 mg/kg	1.0 mg/kg	2.0 mg/kg	4.0 mg/kg	[F(4,32)=12.470, p<0.0001]
		5.972 ± 0.098	5.895 ± 0.098	5.871 ± 0.116	5.635 ± 0.125	
Quetiapine	6.191± 0.084	5.0 mg/kg	10.0 mg/kg	20.0 mg/kg		[F(3,24)=10.403, p<0.0001]
		6.072± 0.087	6.079 ± 0.080	5.832± 0.144		
Olanzapine	6.136± 0.086	1.0 mg/kg	2.0 mg/kg	4.0 mg/kg		[F(3,24)=41.494, p<0.001]
		6.048± .087	5.95± 0.093	5.337 ± 0.078		

Table 3- Mean and standard error of the mean values (SEM) of the lick rhythm for acute oral dosing of clozapine, aripiprazole, risperidone, ziprasidone, quetiapine, and olanzapine.

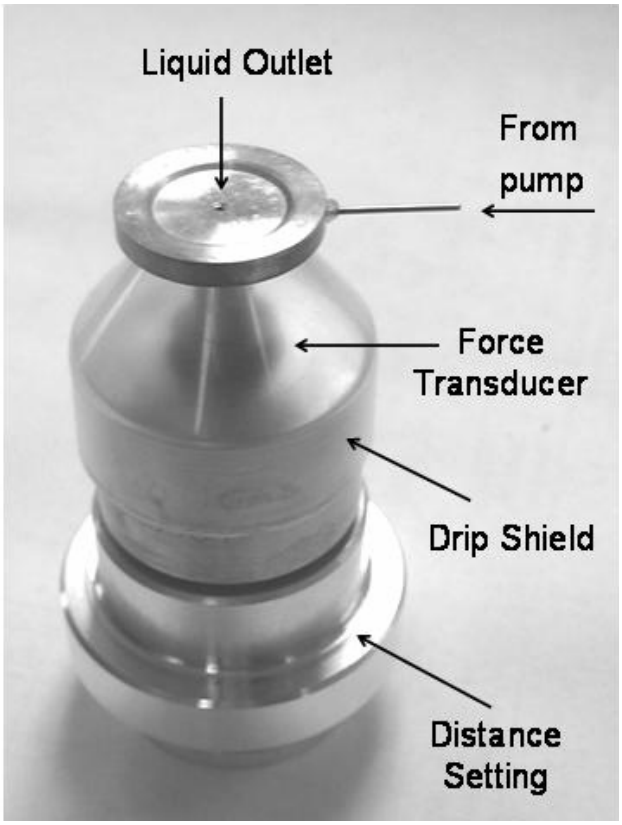

Figure 1. Photograph of a hardware ensemble for measuring tongue force during licking of liquids by rats or mice. Liquids to be ingested are carried to the lick disk by a computer-controlled peristaltic pump. The lick disk through which the liquid emerges for consumption is 18 mm in diameter. Rats or mice access the disk by protruding their tongues through a 12-mm-diameter hole (88).

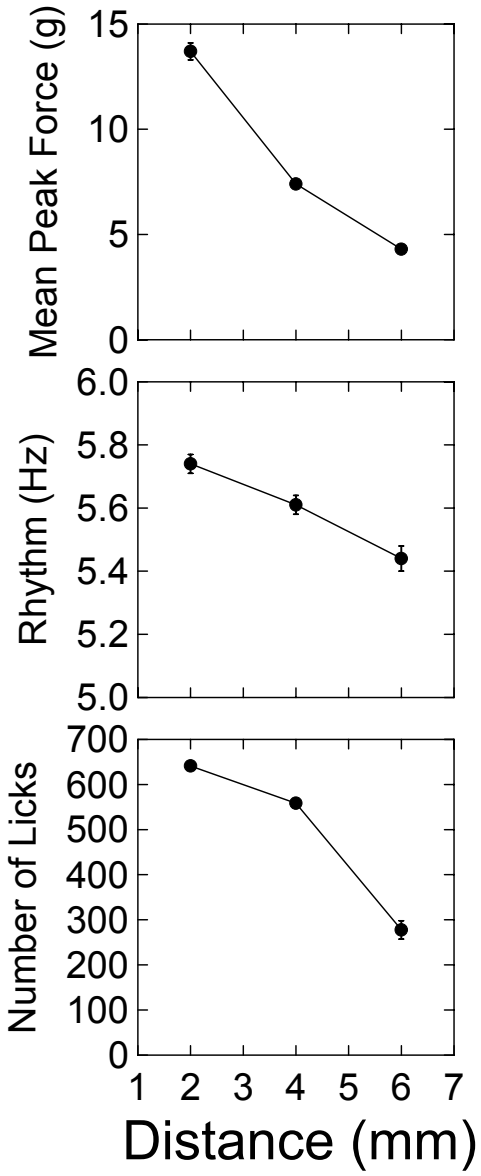

Figure 2. Effects on lick measures for three different distances between the rats' muzzles and the liquid orifice. Mean peak force (expressed in gram-equivalent forces), lick rhythm (Hz), and number of licks each significantly decreased as a function distance. Data were taken from Table 2, pg 83 in Fowler, S.C., McKerchar, T.L., Zarcone, T.J. (2005). Response dynamics: Measurement of the force and rhythm of motor responses in laboratory animals. In M. LeDoux (Ed.) Animal models of movement disorders. San Diego, CA: Academic Press, 73-100.

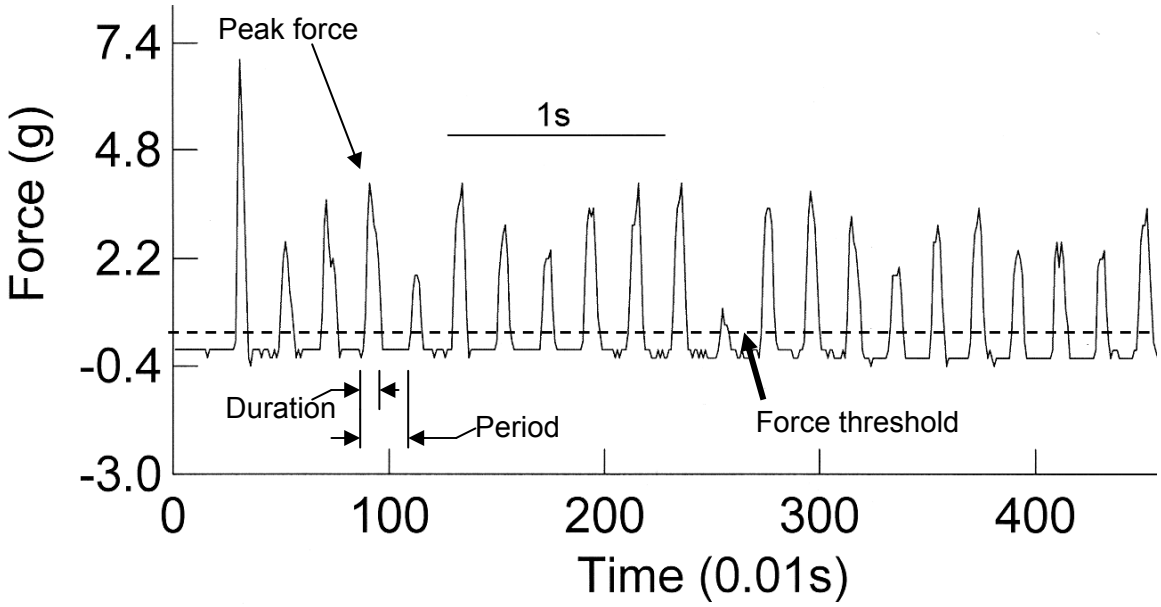

Figure 3 Force Time wave plot- Each lick is represented by a peak whose peak force is its apex, the duration of the lick is the base of the peak for any given lick. The inter lick interval is the time between the licking peaks. The period is defined as the inter-lick interval summed with the duration of the lick. Using Fourier methods licking rhythm (Hz) can be calculated. The dominant licking rhythm of a Sprague Dawley rat either untreated or treated with vehicle is approximately 6 Hz.

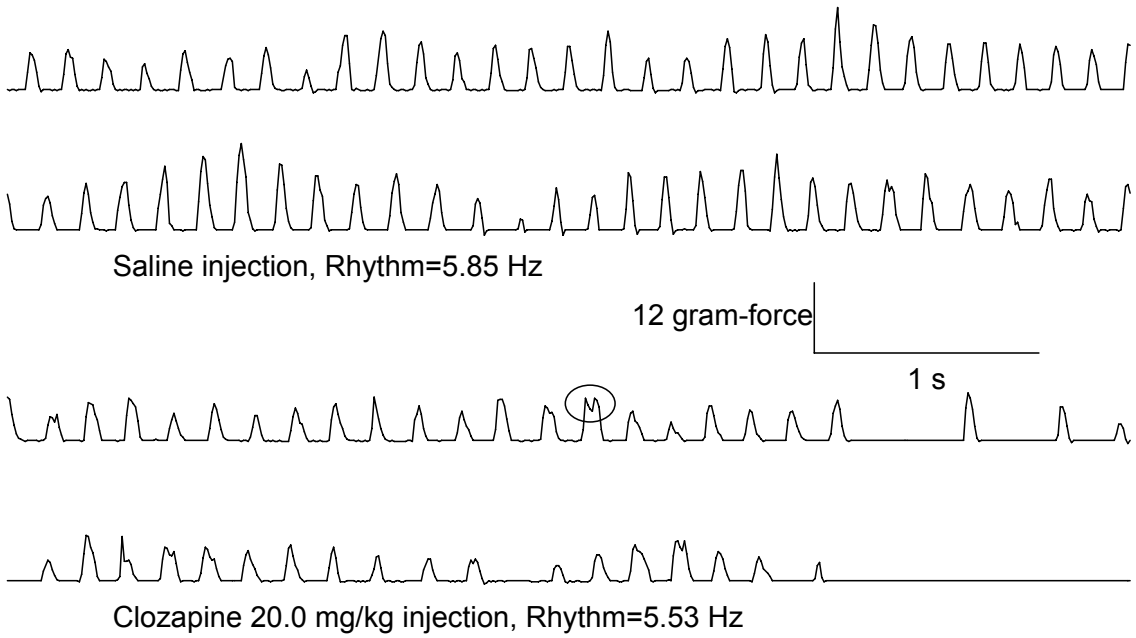

Figure 4 Effects of saline (top two rows) and the atypical antipsychotic drug, clozapine (bottom two rows), on lick-force-time waveforms generated by a rat licking water from the disk shown in Fig. 1 Note the diminution in peak force in the lower set of graphs, as well as the appearance of long inter-lick intervals. An illustrative example of a "notched" lick waveform induced by 20 mg/kg clozapine is indicated by the ellipse. The notched lick wave form represents a lack of control of the tongue under the influence of clozapine.

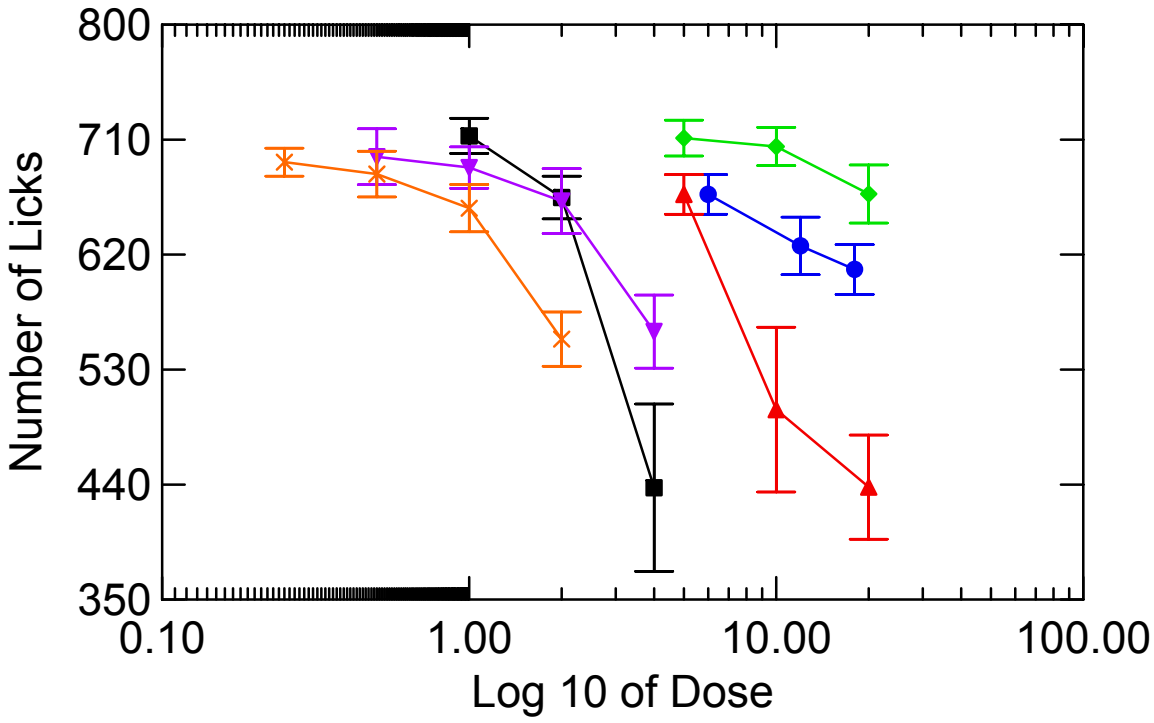

Figure 5- Shows the acute effects of AADs on the number of licks in a two min session. Risperidone in orange was the most potent at affecting the number of licks at the lowest doses (0.25 mg/kg, 0.5 mg/kg, 1.0 mg/kg, and 2.0 mg/kg) [$F(4,32)=19.860$, $p<0.0001$]., and ziprasidone (purple, 0.5 mg/kg, 1.0 mg/kg, 2.0 mg/kg, and 4.0 mg/kg) [$F=(4,32)21.146$, $p<0.0001$], and olanzapine (black, 1.0 mg/kg, 2.0 mg/kg, and 4.0 mg/kg) [$F(3,24)=14.286$, $p<0.0001$], also showed high potency effects. The lower potency group consisting of clozapine (red 5.0 mg/kg, 10.0 mg/kg, 20.0 mg/kg) [$F(3,24)=6.715$, $p<0.002$], aripiprazole (blue, 6.0 mg/kg, 12.0 mg/kg, and 18.0 mg/kg), which has no significant effects on the number of licks [$F(3,24)=1.954$, $p<0.148$], and quetiapine fumarate (green, 5.0 mg/kg, 10.0 mg/kg, 20.0 mg/kg) [$F=(3,24)14.204$, $p<0.0001$], displayed minimal effects or effects at much higher doses doses that risperidone, ziprasidone, and olanzapine. Higher doses, particularly of olanzapine and clozapine show the most variability from rat to rat as indicated by the SEM bars.

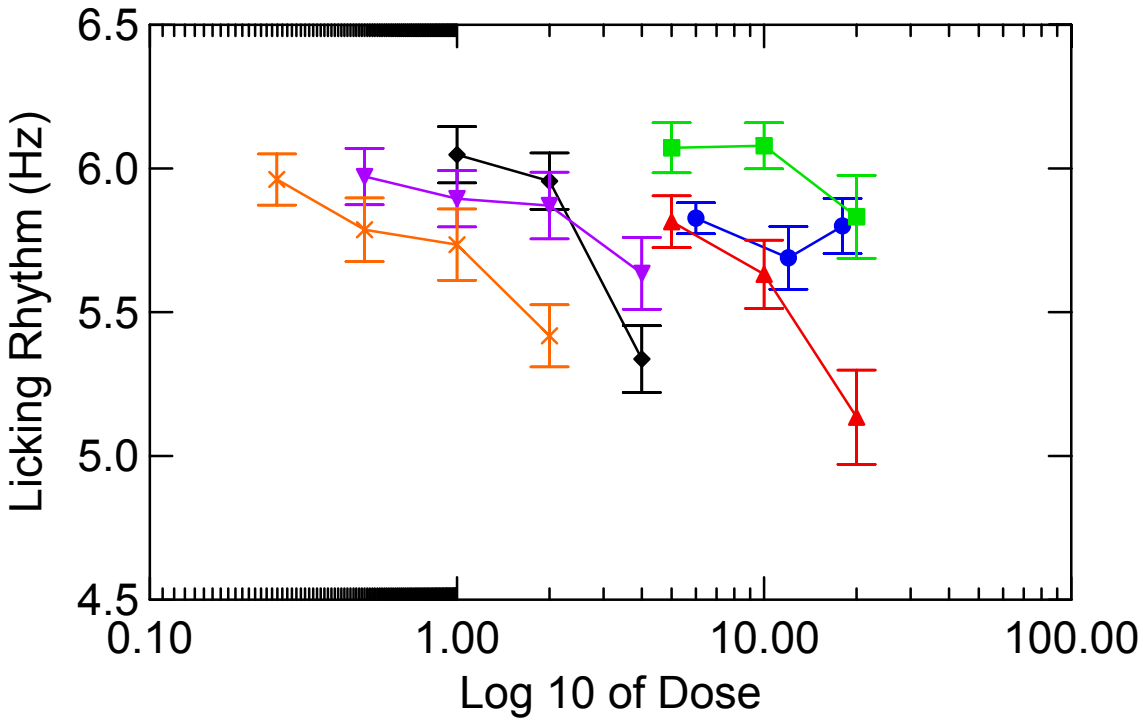

Figure 6- Shows the acute effects of AADs on lick rhythm during the two minute sessions. Risperidone again was the most potent at affecting licking rhythm. Lick rhythm was also more or less divided into low and high potency groups, once again risperidone (orange) [$F(4,32)=32.643$, $p<0.0001$] was the most potent at the slowing of the lick rhythm. Also in the high potency group was ziprasidone (purple) [$F(4,32)=12.470$, $p<0.0001$], and olanzapine (black) [$F(3,24)=41.494$, $p<0.001$]. The lower potency group is comprised of clozapine (red) [$F(3,24)=15.492$, $p<0.0001$]., Aripiprazole (blue) [$F(3.24)=12.664$, $p<0.0001$] , and quetiapine (in green) [$F(3,24)=10.403$, $p<0.0001$]. Compared to the rest of the compounds the effects of aripiprazole and quetiapine are small and not very potent.

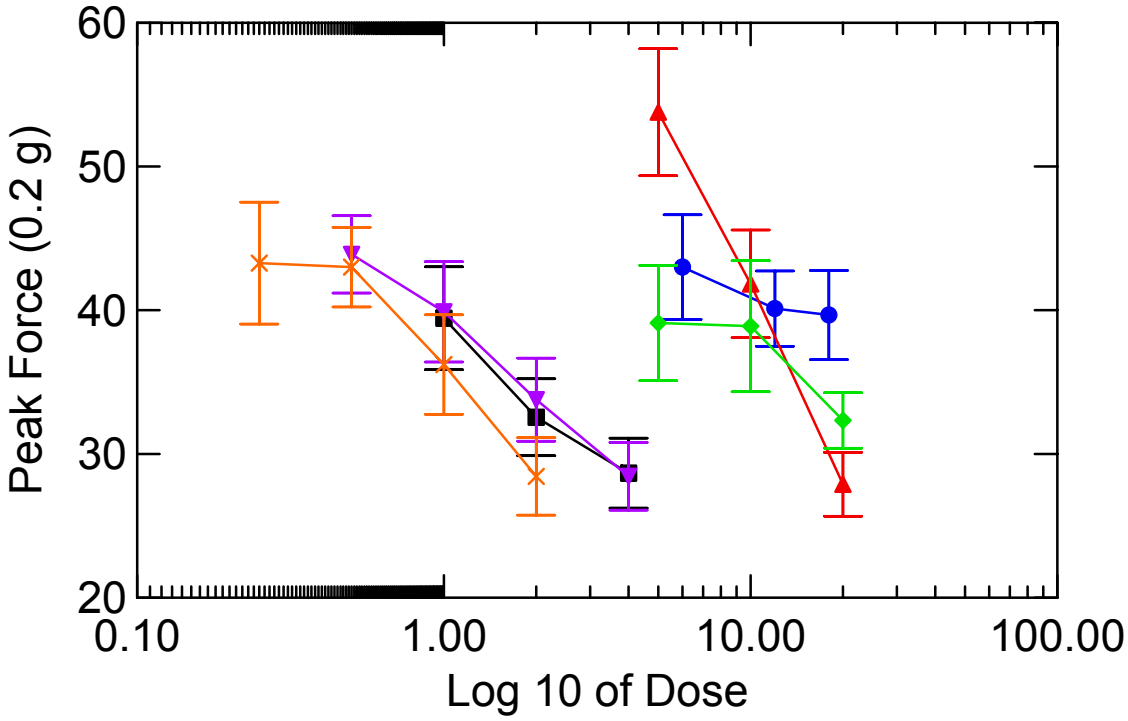

Figure 7-Shows the effects of acute AAD doses on the peak force of licking. Potency was again divided into two groups, a high potency group consisting of risperidone (orange) [$F(4,32)=19.132$, $p<0.0001$], ziprasidone (purple) [$F(4,32)=33.649$, $p<0.0001$], and olanzapine (black) [$F(3,24)=27.117$, $p<0.0001$]. The low potency group consisted of clozapine (red) [$F(3,24)=16.838$, $p<0.001$], aripiprazole (blue) [$F(3,24)=4.183$, $p<0.016$], and quetiapine (green) [$F(3,24)=6.359$, $p<0.003$].

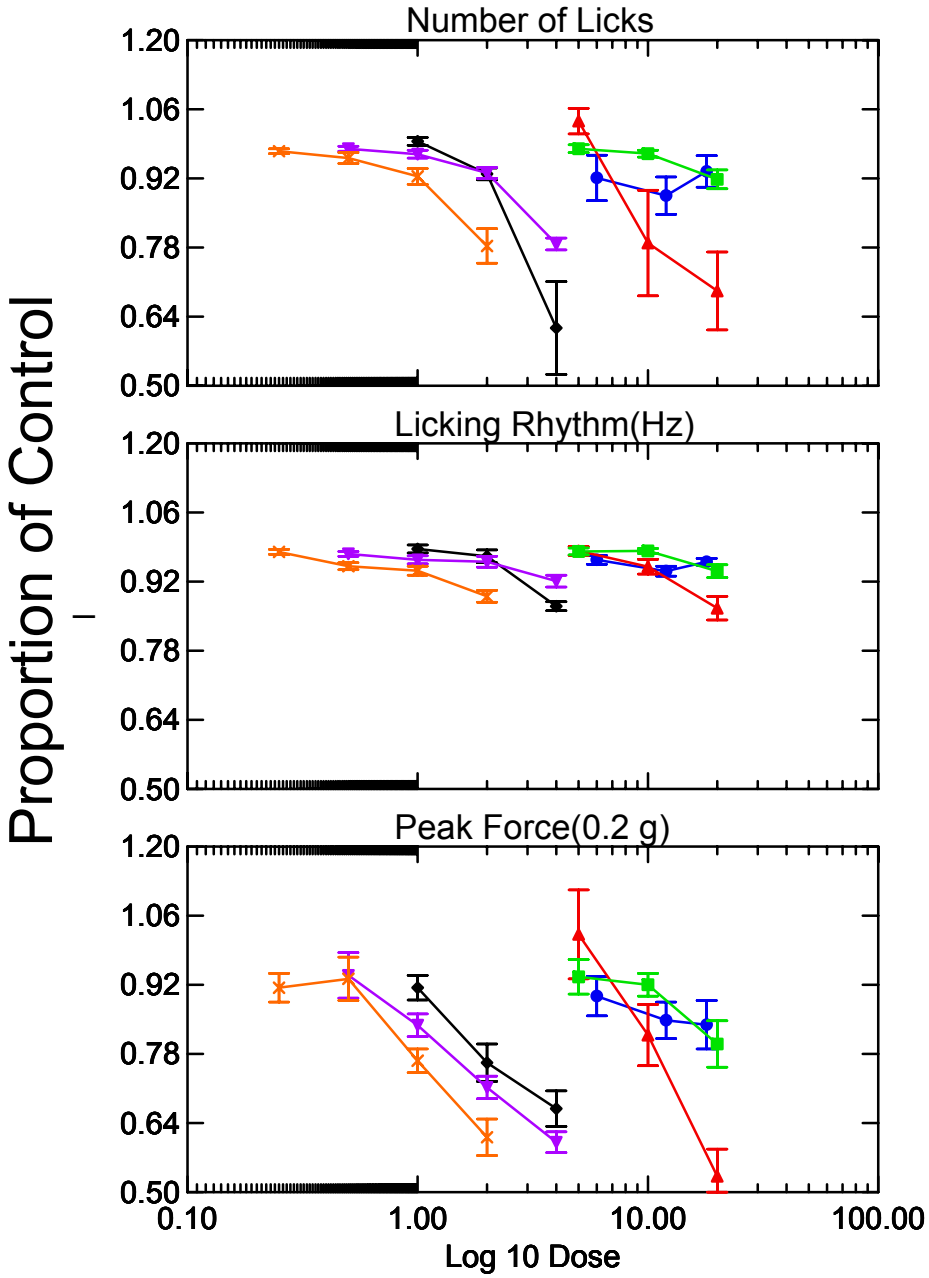

Figure 8- The Acute effect of AADs on the number of licks, licking rhythm and peak force of licking represented as a proportion of control. Risperidone (orange), ziprasidone (purple), olanzapine (black), clozapine (red), aripiprazole (blue), and quetiapine (green).

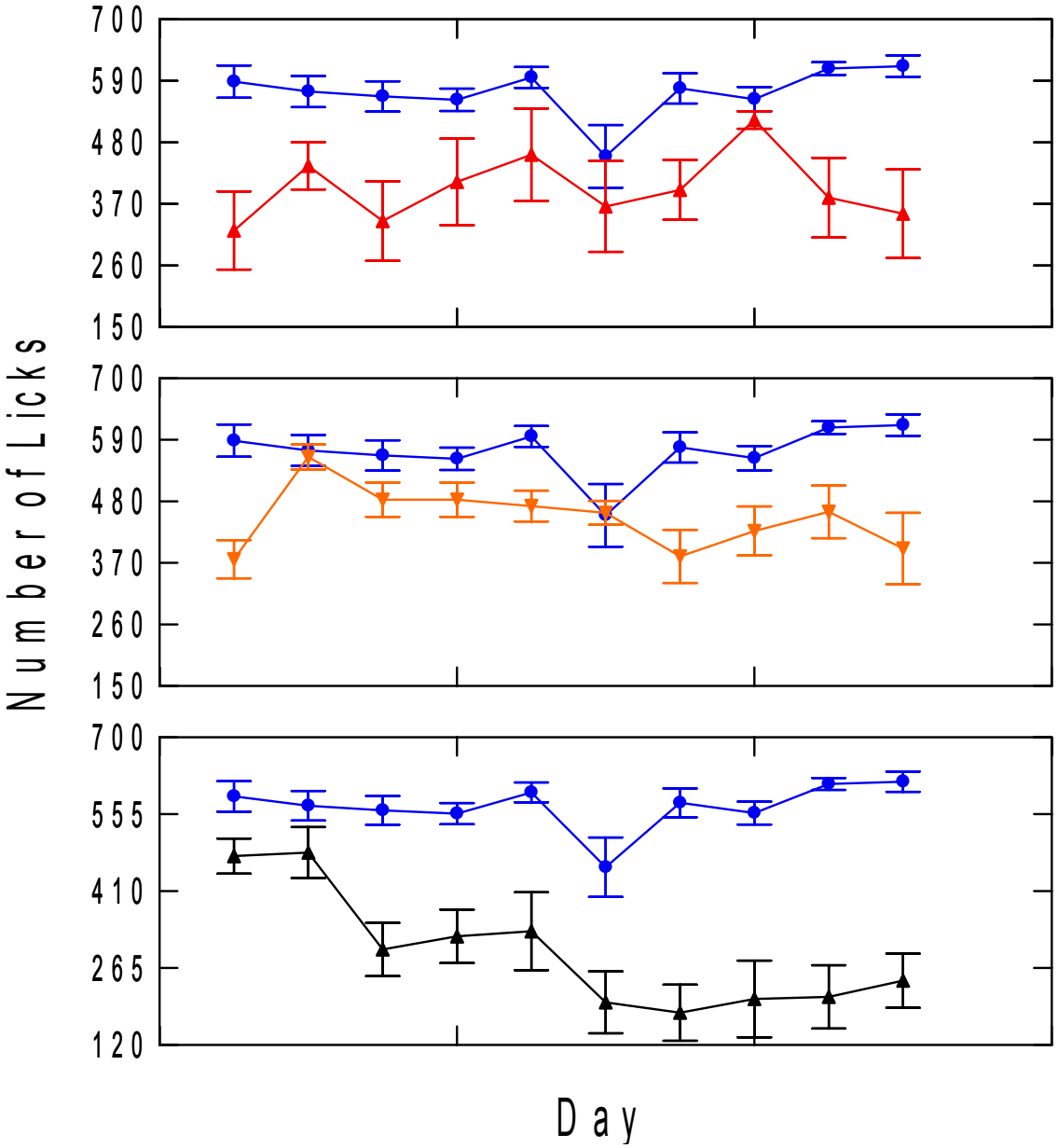

Figure-9 The effects of subchronic clozapine, risperidone, and olanzapine on the number of licks. Clozapine (red triangles, upper graph) given at 20.0 mg/kg twice daily had a significant effect on the number of licks each of the ten days [$F(1,13)= 39.775$, $p< 0.0001$], and displayed no trend towards tolerance or sensitization [$F(1,13)= 0.876$, $p<0.366$] (vehicle controls blue circles, upper graph). Risperidone (orange triangles, middle graph) also had a significant effect on the number of lick each of the ten days [$F(1,14)= 21.877$, $p< 0.0001$], with no significant trend towards

tolerance or sensitization [$F(1,14)= 0.717$, $p<0.411$] (vehicle controls blue circles, middle graph). Olanzapine (black triangles, lower graph) had a significant effect on the number of licks each of the ten days[$F(1,10)= 45.438$, $p< 0.0001$], and resulted in sensitization or worsening of the disruption of licking induced by olanzapine [$F(1,10)= 4.112$, $p<0.0001$] (vehicle controls blue circles, lower graph).

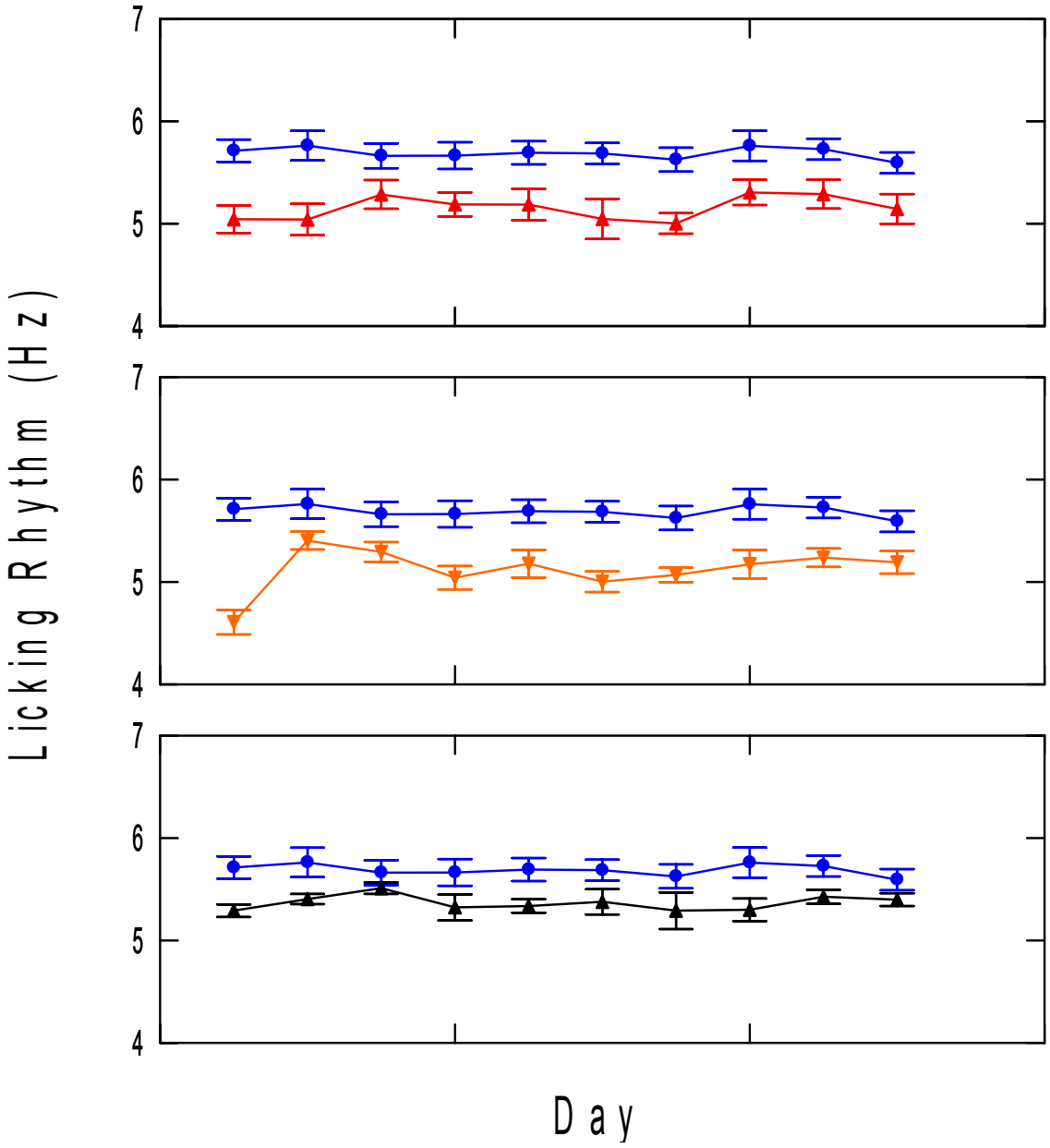

Figure-10 The effects of subchronic clozapine (red triangles, upper graph), risperidone (red triangles, middle graph), and olanzapine (black triangles, lower graph) on lick rhythm. All three compounds had a significant effect on lick rhythm each of the ten days, clozapine [$F(1,13)= 8.886$, $p< 0.012$], risperidone [$F(1,14)= 15.965$, $p< 0.0001$], and olanzapine [$F(1,9)= 19.220$, $p< 0.002$]. There was no trend towards tolerance or sensitization for clozapine [$F(1,13)= 0.081$, $p<0.780$], risperidone [$F(1,14)= 0.475$, $p<0.503$], or olanzapine [$F(1,9)= .089$, $p< 0.769$] (vehicle controls for each are blue circles).

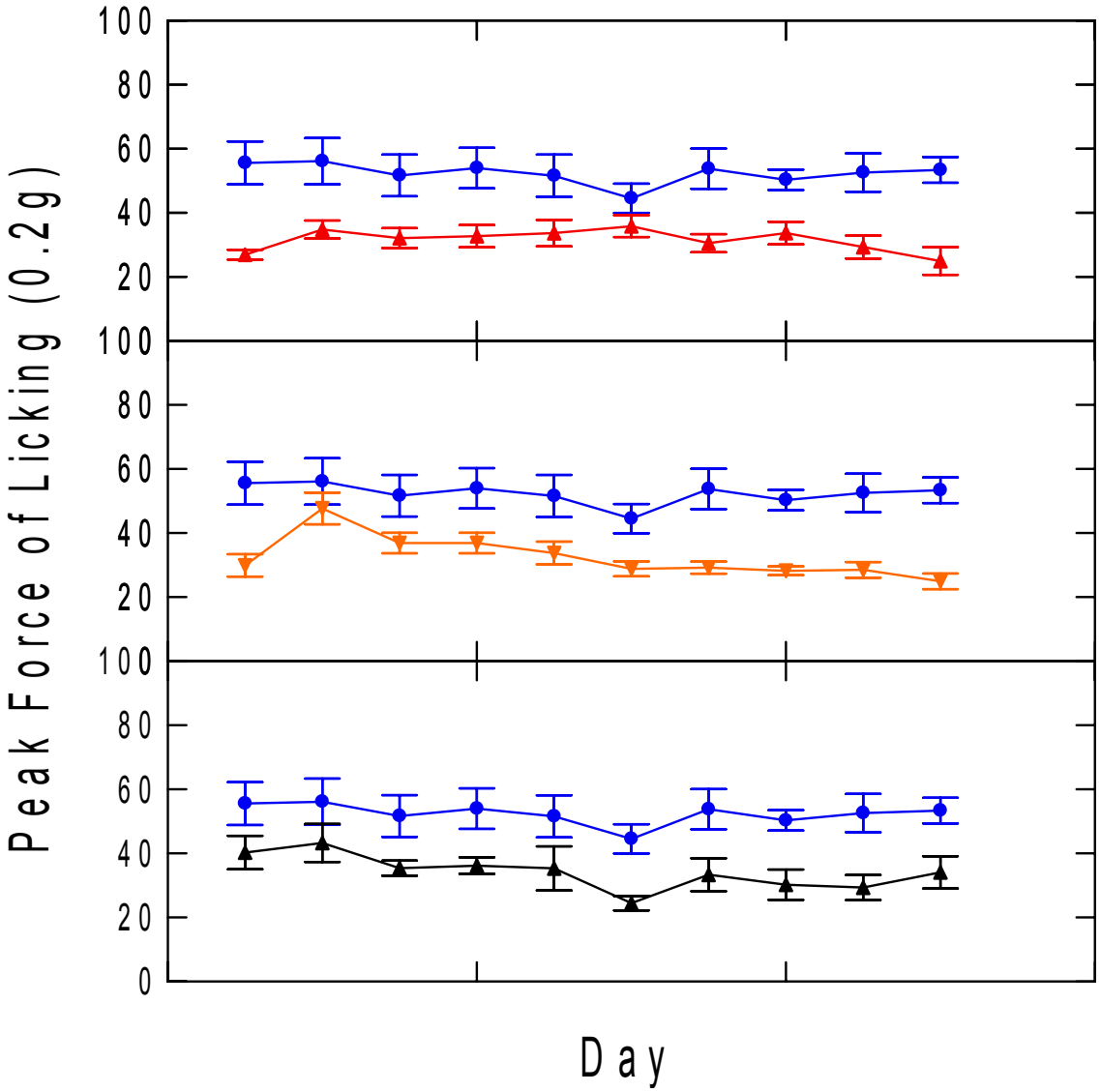

Figure-11 The effects of subchronic clozapine, risperidone, and olanzapine on the peak force of licking. Clozapine (red triangles, upper graph) was administered at 20.0 mg/kg twice daily once 45 minutes before the 3 minute licking session, and once twelve hours later. Clozapine significantly effected the peak force of licking each of the ten days [$F(1,13)=12.337$, $p<0.003$], with no trend towards tolerance or sensitization[$F(1,13)=0.321$, $p<0.579$], compared to vehicle controls (blue circles, upper graph). Risperidone (orange triangles, middle graph) was administered at 2.0 mg/kg in the same time schedule as clozapine. Risperidone significantly affected the peak force of licking each of the ten days [$F(1,14)=10.558$, $p<0.005$] and displayed a trend towards sensitization [$F(1,14)=5.334$, $p<0.034$] (vehicle controls are blue circles in the middle graph). Olanzapine (black triangles lower graph) was given at 4.0 mg/kg twice daily in the same time schedule for clozapine and risperidone. Olanzapine significantly effected the peak force of licking each of the ten days

[F(1,9)=8.219, p<0.011], with no trend towards towards tolerance and sensitization [F(1,9)=2.424, p<0.139] (vehicle controls are blue circles in the lower graph).

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