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The Effects of Abilify on Memory, Coordination, and Stereotypy in CD-1 Mice

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Abstract

The atypical antipsychotic Abilify is the second most popular treatment for schizophrenia and other related disorders. Its popularity and wide use is due to its unique pharmacological profile, working differently at dopamine and serotonin receptors compared to other antipsychotics. Schizophrenia is an infamous mental dysfunction involving cognitive impairment, positive symptoms, and negative symptoms. The current study investigated Abilify's effect in a dose-dependent manner on memory, coordination and stereotypic movement. The sample consisted of 15 male and 15 female CD-1 mice. Memory was tested in a cognitive maze, the Rota-Rod series 8 model measured coordination, and the Coulbourn Tru-Scan Activity Box recorded stereotypic movements. Abilify did not significantly affect memory, it inhibited coordination at all doses for female mice and at the highest dose for male mice, and it induced stereotypic movements at the lowest dose and inhibited such movements at the highest dose for male and female mice.

Keywords: Abilify, aripiprazole, antipsychotic, schizophrenia, dopamine, serotonin, memory, cognitive maze, coordination, Rota-Rod, stereotypy, Tru-Scan, CD-1 mice

The Effect of Abilify on Memory, Coordination, and Stereotypy in CD-1 Mice

Abilify is the second most popular pharmacological treatment for schizophrenia and psychosis-related disorders, yet there is scarce scientific literature on patients' premorbid history prior to being placed on this highly influential drug. Additionally, there is very little literature pertaining to long-term use of Abilify in the clinical population; however, there is an abundance of novel information on the behavioral, molecular, and social aspects of schizophrenia. This thesis will explain the psychopathology of schizophrenia and the relevance of Abilify as a psychotropic medication and explain many of the side effects of various doses of this medication, as well as investigate its effects on memory, coordination, and stereotypic movement.

One of the most infamous and well-known mental dysfunctions, schizophrenia is both as fascinating a disorder as it is misunderstood. Schizophrenia involves three key dimensions: cognitive impairment, positive symptoms, and negative symptoms (Rajagopal, Massey, Huang, Oyamada & Meltzer, 2014). Positive symptoms include hallucinations (false perceptions), delusions (false beliefs), and deranged thoughts and behaviors, while negative symptoms include loss of motivation, affective flattening, restricted emotional experience, reduced hedonic capacity, and disorganized speech (saying things that do not make sense) (Topolov & Getova, 2016). It is not unusual for schizophrenic patients to suffer from abnormal perceptions (eyetracking problems, slight movement disturbances), strange inferential judgments that lead to extraordinary beliefs and delusions, restricted emotions, and widespread cognitive problems (Topolov & Getova, 2016).

Schizophrenia is a devastating disorder for both patients and families and the second leading cause of disease burden. The three phases of most schizophrenic patients include the

prodromal phase, the active phase, and the residual phase. The prodromal phase is more important to this thesis since it includes peculiar behaviors, such as pacing, movement disturbances and restlessness. Although there are 3 key symptoms, no single symptom or specific set of symptoms is characteristic of all schizophrenic patients. Since there are many different clinical manifestations and levels of severity, *The Diagnostic and Statistical Manual of Mental Disorders* now lists Schizophrenia under Spectrum Psychotic Disorders. This heading now includes schizophrenia, schizoaffective disorder, delusional disorder, brief psychotic disorder, and psychotic mania, all of which might be alleviated or attenuated by the drug Abilify. Although there is a commonality involving a break in reality with all of these disorders, the three dimensions are mostly uncorrelated and consequently respond differently to antipsychotic treatment (Rajagopal et al., 2014). Specifically, cognitive impairment prevents patients from learning and memorizing the skills that are necessary for social relationships, accounting for more poor functional outcomes such as occupational functioning, social attainment, and independent living, than the other two dimensions, and in schizophrenic patients this might become visible before other neurological impairments, affecting working memory, attention, and learning (Topolov & Getova, 2016). Typically, cognitive impairments present first, developing completely before the onset of psychotic episodes in schizophrenic patients, but not in schizoaffective, schizophreniform, or manic psychotic patients (Topolov & Getova, 2016). Therefore, it would be beneficial to understand the effects of Abilify on memory prior to administration as well as future long-term effects on cognition.

The psychotic episodes involve breaks in reality and includes disconnected ideas (using words in peculiar ways, loose associations, otherwise known as derailment (i.e., switching topics too abruptly), and perseveration (e.g., persistently repeating the same word or phrase over and

over). This can even develop into what is now known as thoughtblocking, where the patient's train of thought is interrupted before an idea has been completed. Disorganization is another commonly seen bizarre behavior which may include a catatonic-like psychosis, which involves immobility and marked muscular rigidity, or wild excitement and overactivity. This altered state of responsiveness is of primary importance to this thesis, since most antipsychotic medications will further sedate or suppress movement.

While systematic tests for schizophrenia were developed by the 1940s, most of what is known regarding cognitive impairment in psychotic related illnesses came from targeted research that began in the 1980s (Topolov & Getova, 2016). Schizophrenic patients have been found to perform worse than control patients in all twelve neurocognitive domains: general intellectual ability, verbal memory, nonverbal memory, recognition, executive functions, motor skills, working memory, language, attention, and processing speed (Topolov & Getova, 2016). In particular, schizophrenic patients' ability to work fulltime is impacted by cognitive functioning, even prior to drug administration. Readministration of IQ tests to individuals who developed schizophrenia showed little change, implying that most of the intellectual decline occurred prior to the onset of the first psychosis (Topolov & Getova, 2016). For schizophrenics, the severity of the cognitive impairment in their first-episode is equivalent to the cognitive impairment in individuals with chronically established schizophrenia, highlighting neurocognitive deficits as one of the more stable traits of schizophrenia (Topolov & Getova, 2016). Moreover, genetic relatives of schizophrenic patients have shown attenuated cognitive impairment, meaning that neurocognitive domains may be genetic markers for schizophrenia (Topolov & Getova, 2016).

The success of various pharmacological therapies in psychotic patients has led to the proposal of 3 models: the dopamine hypothesis, the glutamate hypothesis, and the serotonin

hypothesis. Each model attempts to explain both the neuroreceptor targets for antipsychotics and how various neuroreceptors influence specific symptoms and side effects (Topolov & Getova, 2016). Among the models, the dopamine hypothesis is the most studied. It states that schizophrenia is due to an abundance of dopamine in the brain. More precesily, it suggests that hyperactivity of the mesolimbic dopaminergic pathway mediates symptoms of psychosis, while the negative and cognitive symptoms are mediated by separate mesocorted hypoactive dopaminergic pathways (Topolov & Getova, 2016). However, we now have greater technology showing us that this hypothesis does not tell the whole story. There are other interactions as previously stated with GABA and glutamate, but also with serotonin pathways (explaining most of the negative symptoms). COMT (located on chromosome 22) has also been looked at. This is a specific gene that is involved in breaking down the neurotransmitter dopamine, which may contribute to the movement disorders that are linked to the dysfunction as well as the medications to the dysfunction. Extrapyramidal symptoms (EPS) are motor side effects due to long standing treatments with antipsychotics; however, literature is now showing that some of the tremors, restlessness, and muscular rigidity are due to the imbalance of dopamine in the brain and not due to medications alone. Although the glutamate hypothesis states that schizophrenia is the result of hypo-activity on NMDA receptors, these receptors rarely modulate memory or movement (Leite, Guimaraes, & Moreira, 2008). However, the glutamate and serotonin hypothesis are less important with this thesis since Abilify primarily affects the dopamine system, involved in active symptoms, cognition, and movement.

While patients with schizophrenia demonstrate deficits across many cognitive domains, the underlying cognitive and neural mechanisms of the disorder are unclear. For instance, the striatal dopamine system is involved in signaling prediction errors and integrating them over

trials, while the prefrontal cortex is involved in using working memory to test hypotheses and represent values of prospective outcomes to guide choice (Collins, Brown, Gold, Waltz & Frank, 2014). The contributions of two separable cognitive processes, working memory (WM) and reinforcement learning (RL), are confounded by most learning paradigms, leading Collins et al. to develop a model to separate WM from RL. While patients with schizophrenia do show failure in RL, Collins et al. found that learning impairment in schizophrenic patients was due to deficits in WM rather than deficits in RL (2014). More precisely, they found that patients with schizophrenia had reduced WM capacity and faster WM decay, while RL rates were normal. This suggests that the inability of patients with schizophrenia to learn from outcomes stems from WM deficits rather than RL deficits. WM contributes to other aspects of cognition, including fluid reasoning and language comprehension, meaning that WM impairments could contribute to many of the cognitive deficits seen in schizophrenia, and thus would be detrimental to put them on a medication that might increase their memory loss. While they hypothesized that negative symptom severity was related to WM parameters, no association was found, perhaps because the task they used more closely related to WM processes involving the lateral prefrontal cortex, while negative symptoms are hypothesized to be attributed to the limbic portions of the prefrontal cortex, such as the orbitofrontal cortex (Collins et al., 2014).

Neuroanatomical studies of the dopamine system are widely published; however, there are very little, if no studies, identifying the effect on neural communication, both pre- and post-medication. Interdependence of firing rates in dorsolateral prefrontal cortex and parietal cortex is vital for WM performance (Deserno, Sterzer, Wustenberg, Heinz, & Schlagenhauf, 2012). The dysconnectivity hypothesis of schizophrenia focuses on abnormal synaptic plasticity. WM-dependent modulation of effective connectivity from dorsal-lateral prefrontal cortex (dlPFC) to

parietal cortex was shows to be reduced in schizophrenic patients, showing that this dysconnectivity could be a specific mechanism underlying cognitive deficits seen in schizophrenia (Deserno et al., 2012).

Typical antipsychotics, such as Thorazine and mellaril, can reduce the activity of hyperactive dopaminergic pathways by inhibiting dopamine transmission, thus reducing positive symptoms that are related to hallucinations and delusions (Topolov & Getova, 2016). More precisely, typical antipsychotics work as full antagonists at dopamine D_2 receptors (Topolov & Getova, 2016). However, the dopamine inhibition causes unwanted side motor effects, but less dry mouth and memory disturbances. However, some psychotropic medications (Thioridazine, Haldol, and other piperadines) diminish levels of dopamine in pathways where dopamine excess was not a problem, such as the mesocortical, nigrostriatal, and tuberoinfundibular pathways, and thus induce extrapyramidal side effects (EPS) and possibly cause cognitive impairment (Topolov & Getova, 2016). Negative symptoms and cognitive symptoms often worsen on long-term psychotropic medications, since these behaviors are not mediated through typical dopamine receptor subtypes, but rather incorporate other systems, such as the serotonin and norepinephrine systems (Topolov & Getova, 2016).

Consequently, D_2 receptor antagonists are known to induce catalepsy and cause increased dopamine metabolism in the striatum, where their effects contribute to extrapyramidal motor side effects (EPMS), and in the limbic system, where their effects contribute to antipsychotic activity (Kirschbaum, Hiemke, & Schmitt, 2009). To provide an example of EPMS, D_2 receptors have been shown to play a role in central components of locomotion, such as time spent in motion, horizontal distance traveled, and initiating movement, meaning that D_2 receptor antagonists could inhibit locomotion (Kirschbaum et al., 2009). As demonstrated by Sanberg's 1980 study, a

typical antipsychotic such as haloperidol would induce catalepsy by blockade of postsynaptic striatal dopamine D_2 receptors (qtd. in Kirschbaum et al., 2009). Atypical antipsychotics, such as clozapine, Risperdal, and Abilify, work on serotonin receptors in addition to dopamine receptors, and are known for inducing less EPS than typical antipsychotics (Topolov & Getova, 2016). Additionally, atypical antipsychotics do not increase prolactin secretion and may improve cognitive impairment (Topolov & Getova, 2016). It has been shown that the preferable EPS effects are attributed to serotonin 5-$HT_{2A,C}$ receptor antagonism and/or 5-HT_{1A} partial agonism, while the cognitive enhancement is attributed to 5-HT_6 and 5-HT_7 receptor antagonism (Topolov & Getova, 2016). Moreover, the pathophysiologic mechanism of schizophrenia is thought to be based on too much dopamine activity in some regions and too little dopamine activity in other regions, which has led to research focusing on developing D_2 partial agonists. These could work as antagonists in areas with too much dopamine activity, and work as agonists in areas with low dopamine activity (Topolov & Getova, 2016). Thus, D_2 partial agonists could reduce positive symptoms while not inducing EPS or elevated prolactin levels.

Aripiprazole (brand name Abilify) is a psychotropic drug commonly used to treat many forms of psychosis. It is available as tablets, orally disintegrated tablets, oral solution, and injection (Topolov & Getova, 2016). Its chemical formula is 7-[4-[4-(2,3-dichlorophenyl)-1-piperazinyl]butoxy]-3,4-dihydrocarbostyril, while the empirical formula is $C_{23}H_{27}Cl_2N_3O_2$, and its molecular weight is 448.39 (Topolov & Getova, 2016). After oral administration, peak plasma concentrations occur after 5 to 7 hours, and the absolute oral bioavailability is 87% (Topolov & Getova, 2016). When administered intramuscularly, the peak time is between 1 and 3 hours and the absolute bioavailability is 100% (Topolov & Getova, 2016). There are 3 primary biotransformation pathways that metabolize aripiprazole: dehydrogenation, hydroxylation, and

N-dealkylation (Topolov & Getova, 2016). The elimination half-life is considered to fall within 75 hours to 146 hours. The active metabolite, dehydro-aripiprazole, is present for an additional 146 hours. The cytochrome P 450 enzymes CYP3A4 and CYP2D6 are responsible for aripiprazole metabolism.

Despite being an atypical antipsychotic, aripiprazole's mechanism of action differs from other atypical antipsychotics such as clozapine, olanzapine, and risperidone. Where other antipsychotics act as antagonists at D_2 receptors, aripiprazole acts as a partial agonist at D_2 receptors and at 5-HT_{1A} receptors (Topolov & Getova, 2016). Aripiprazole's profile leads to antagonistic activity in areas with dopaminergic hyperactivity and agonist activity in areas with dopaminergic hypoactivity, decreasing the risk of EPS and some catalepsy compared to some older antipsychotics and other new atypical antipsychotics (Kirschbaum et al., 2009). Two animal models for predicting EPS in humans are inducing catalepsy in rodents that reflects the substantial decrease in dopamine signaling at postsynaptic striatal D_2 receptors and the Rota-Rod test, which tests coordinated motor skills. In their 2009 study, Kirschbaum et al. investigated the effects of aripiprazole and other antipsychotics on motor impairment using the catalepsy and Rota-Rod tests. Three doses of aripiprazole were administered: 1mg/kg, which showed no motor impairment, and 5mg/kg and 10mg/kg, which caused significant motor impairments and also induced catalepsy (Kirschbaum et al., 2009). Aripiprazole induced less motor impairment than haloperidol or risperidone, as these drugs caused impairments even at low doses (Kirschbaum et al., 2009). Also, 5-HT_{1A} receptor activation increases dopamine release in a regionally selective manner in the prefrontal cortex, suggesting alleviation of the proposed deficiency in dopaminergic neurotransmission in this brain region in schizophrenics (Bardin, Kleven, Barret-Grevoz, Depoortere, & Newman-Tancredi, 2005). Activation of this receptor has also been

shown to improve negative symptoms as well as cognitive symptoms (Bardin et al., 2005). Like other antipsychotics, aripiprazole works as an antagonist at 5-HT_{2A} receptors and the 5-HT_7 receptor, and it also works as a partial agonist at the 5-HT_{2C} receptor (Topolov & Getova, 2016). Aripiprazole has been shown to enhance short-term memory and long-term memory on the inhibitory avoidance task (Nascimento et al., 2011**)**

Aripiprazole dampens the action of dopamine and serotonin in the brain, lowering abnormal levels of excitement, and inhibiting the more extreme moods associated with psychotic disorders. It is used as acute and maintenance treatment for manic or mixed episodes in bipolar I disorder as monotherapy, or as an adjunct to lithium or valproate. It is also used as an adjunct to antidepressants for major depressive disorder. While it is also used to treat autistic disorder, it has been associated with irritability.

Despite aripiprazole's preferable profile, it does not come without risks. It should not be used by elderly with dementia-related psychosis or those with a history of leukopenia/neutropenia. The drug presents an increased risk of death or cerebrovascular events, hypotension, aspiration pneumonia, seizures, and diabetes. Also, patients receiving aripiprazole need to be monitored for hyperglycemia, dyslipidemia, and weight gain. There is also an increased risk of suicidal thoughts and behavior in children, adolescents, and young adults. Other adverse reactions include drowsiness, headache, anxiety, insomnia, constipation, nausea, vomiting, somnolence, fatigue, sedation, dizziness, restlessness, akathisia, blurred vision, tremor, pyrexia, salivary hypersecretion, EPS, neuroleptic malignant syndrome, and tardive dyskinesia.

One challenge in antipsychotic research is that schizophrenia cannot be fully mimicked in healthy volunteers or laboratory animals. As such, psychotomimetics (drugs that can mimic some symptoms) are used to test the efficacy of antipsychotic drugs to prevent the psychotomimetic

drugs' effects. Amphetamine enhances the release of monoamines, thus increasing the levels of dopamine in meso-limbico-cortical and meso-striatal pathways, and may cause delusions and hallucinations in humans, mimicking positive symptoms of schizophrenia (Leite et al., 2008). Phencyclide, ketamine, and MK-801 act as antagonists at the glutamate NMDA receptor, mimicking some positive and negative symptoms such as social isolation and cognitive deficits (Leite et al., 2008). Recall that the glutamate hypothesis states that schizophrenia is the result of hypo-activity on NMDA receptors, thus explaining why these particular psychomimetics may be useful (Leite et al., 2008). In rodents, psychotomimetics induce behavioral changes such as motor hyperactivity, reduced social interaction, and impairment in the pre-pulse inhibition of the startle reflex (Leite et al., 2008). Aripiprazole has been shown to inhibit the stereotyped behavior and the impaired pre-pulse inhibition induced by apomorphine (dopamine agonist) in rats (Leite et al., 2008). While aripiprazole did not inhibit spontaneous locomotion, significantly less movement was observed (Leite et al., 2008). The drug inhibited amphetamine-induced locomotion and cocaine-induced motor hyperactivity, both drugs that enhance dopamine-mediated neurotransmission. Also, the highest dose of aripiprazole inhibited hyperlocomotion induced by MK-801 while the lowest dose of aripiprazole enhanced it. This study also showed that the highest dose of aripiprazole produced catalepsy, but to a lower extent than haloperidol (Leite et al., 2008). Antipsychotics' liability to induce catalepsy correlates well with D_2 receptor antagonism, whereas aripiprazole has been shown to not induce significant motor impairments, even in doses that induce 95% of receptor occupation. This is most likely due to its partial agonism at these receptors (Leite et al., 2008). Additionally, aripiprazole induces less EPS at higher doses than other antipsychotics, demonstrating its lack of aversive side effects (Leite et al., 2008).

Since previously mentioned studies show that working memory and retention are unstable in schizophrenic patients, it would be beneficial to know whether or not Abilify affects memory in normal mice, let alone schizophrenic animal models. Schizophrenic patients show deficits in spatial WM tasks by having significantly lower recognition rates for previously seen objects. They differ from "normal controls in regional cerebral blood flow changes in the pulvinar region of the right thalamus and the right or left prefrontal cortex during the recognition of new and previously seen objects" (Rajagopal et al., 2014). The hippocampus, the prefrontal cortex, the nucleus accumbens, and the dorsal striatum are implicated in the pathobiology of schizophrenia (Rajagopal et al., 2014). These neural systems require "closely modulated glutamatergic, GABAergic, cholinergic, dopaminergic, serotonergic, and nitric oxide-dependent neurotransmitters and growth factors for their action to achieve one-trial memory formation and retention for a limited period of time" (Rajagopal et al., 2014, p. 3).

The hypotheses of this thesis are that Abilify will significantly increase memory, that the lowest dose will induce stereotypy while the highest dose will inhibit stereotypy, and that the highest dose will inhibit coordination while the other doses will have little or no effect.

Methods

Subjects

Fifteen adult male (42-58g) and 15 adult female CD-1 mice (33-44g) were purchased from Charles River Laboratories Inc., (Portage MI) and housed in the Long Island University Animal Vivarium. Animals were housed in same sex groups (8-10 adult mice per cage) in a temperature-controlled room maintained on a 12-h light /12-h dark cycle, and food and water were available to the mice *ad libitum*. Mice were divided into 2 groups for both genders, (n = 10/experimental group and n=5/control group). Control group subjects were tested once while

experimental group subjects were tested at three doses, and studies were carried out in accordance with *The National Institutes of Health Guidelines* and regulations specified by the LIU-Post Animal Care & Use Committee.

Drug

Abilify (Aripiprazole) was purchased from Fisher Scientific (Union City, CA) and was dissolved in 0.9% saline and Tween 80. The drug was tested during their peak time of effect (Abilify's peak time of effect is 30 minutes). A vehicle of 0.9% saline solution was injected as a control in 5 males and 5 female mice and used as vehicle comparisons. All intraperitoneal injections were administered via 1 milliliter (ml) syringes with 28 gauge needles infused with either saline or the drug solution.

Apparatus

The Cognitive Maze (see Appendix A) consists of a 30cm x 30cm wooden box with a removable Plexiglas top. Spatial memory was tested within this box using a stopwatch (Sportline) and an error counter (Sportline) (a picture of the maze and the other lab machinery used in the study can be seen in the appendix).

The Rota-Rod series 8 model (see Appendix B) from the IITC-755 Life Sciences Company (Victory Blvd, Woodland Hills, CA 91367) consists of five elevated rods (diameter 1.25" each, which is optimal for mice to grip onto and run on), and 5 individual lanes enclosed via Plexiglas screens. Clear Plexi-Glass front panels are supplied for viewing during test. Test parameters are entered via the front panel keyboard. All test results are displayed on the front panel display and also sent out the rear panel printer port. The test ending results for each position are rpm's (revolutions per minute), test time, and distance traveled in meters. The user selects from 1 to 5 lanes at a time of testing to be included in the test. The Roto-Rod is designed

to enable the operator a more precise reading of equilibrium thresholds for coordination and endurance. This digitally controlled unit has a high degree of repeatability but all mice are acclimated on the machine prior to any baselines or testing. Specifically, mice are placed on textured drums to avoid slipping. Textured drums, which act like round-treadmills begin to turn slowly and the animal begins to walk on it. When the animal drops off of the textured drum (18.5 cm), it activates a sensing platform below. Elapsed time, distance, and rpm's can then be recorded.

The Coulbourn Tru-Scan Activity Box (see Appendix C) consists of two plexiglas, box-like arenas containing photobeam sensors (2mm part) along the ventral surface of the arena (E63-12 model). Specifically, the Activity Boxes can be used to measure and record various horizontal floor movements, stereotypical motions, locomotor activities, as well as other behaviors such as jumping and resting through floor and wall sensors. Sensor rings: Tru-Scan's photobeam sensor rings are separate from the station's processor. There is only one type of sensor ring. It senses in two dimensions. The same ring is used for FP (floor plane) sensing. The connector into which you plug the ring determines its recording function. The system logs coordinates instead of just a "yes or no." Tru-Scan's precision is 32 x 32cm, twice the cage's beam resolution; 0.5 inch (1.27 cm) for the beam spacing of the large cage and 0.3 inch (.76 cm) for spacing of the small cage. This is accomplished by calculating the spread between the lowest and the highest beam blocked and defining the coordinate as one half the distance between the two. To get readings, mice need to be placed on the gray, square plastic floor of the activity box arena which allows the experimenter to view the mice during the tests. The protocol set up for the activity boxes for each mouse was 10 minutes long. The following operational definitions were used for the activity variables. Total movements (TM) were defined for each movement as

a series of successive coordinate changes with no rest for at least 1 sample interval. Latency to First Movement (LFM) is defined as datum measuring the latency from run start to the first coordinate change and then to the 5th, 10th, 20th, 50th, and 100th unit (inches or centimeters) of distance traveled. Rest Time (RT) is operationally defined as the total session time less Total Movement Time (TMT) is defined as the sum total of elapsed time of all movements in the floor plane). Stereotypy (STPY) or stereotypical movements are measured as repetitive behaviors which do not contribute to large location changes progressively further from the starting point (e.g., grooming behavior, shaking, or a seizure etc.). Stereotypy Moves are defined as the total number of coordinate changes less than plus or minus 0.999 beam spaces in each floor plane (X and Y) dimension and back to the original point that do not exceed 2 seconds apart. Stereotypy Episodes are defined as the total number of episodes of stereotypic moves. Three such movements must be made before a stereotypy episode starts, and when it does, the qualifying 3 movements are included in the total number of moves. When the subject moves outside of the region of qualified coordinates, or fails to move within them for 2 seconds, the episode breaks and its current position becomes the new starting point.

Measurements of body weights were taken on a scale from Salter Housewares (Tonbridge, Kent) prior to and after the behavioral testing.

Procedure

The study was conducted in a scientific laboratory at a college campus. Baselines were taken on all apparati for all mice prior to the start of the study. Two mice at a time were injected with the desired dose (0.1mg/kg, 5mg/kg, 20mg/kg) with an injection volume of 0.2cc's. Twenty-seven minutes after the mice received their dose (which was the peak time of the drug's action), the mice were evaluated (2x each) for distance traveled (how long the mice ran before

they fell off the rod), rotations per minute (rpm), and time were recorded both times on the Roto-Rod. Immediately thereafter, each mouse was separately run through a maze that contained a food pellet at the end (this was recorded as elapsed time-to-goal). Time was recorded as the amount of seconds it took for a mouse to go from the start of the maze to the food pellets with 3 minutes as the cut-off time. Maze errors and elapsed time were recorded using a stopwatch and an error counter. An error was recorded every time a mouse went in a direction that did not lead to the pellets. The mice were then put in the Tru Scan Activity Arenas and activity was recorded. Overall activity was recorded using the Tru Scan for 10 minutes. 10 minutes ensured that the mice would be in the activity box during the peak time effect of the antipsychotic medication.

Data Analysis

Statistical significance ($p < .05$) was assessed for analgesia using GraphPad Prism Graphing and Statistical program using Student's t test when comparing 2 means or a 1-way Analysis of Variance (ANOVA) was used for the comparison of 3 or more means. This was followed by Tukey's Post-hoc Test to identify where (which gps) significance was located (San Diego, CA, USA).

Results

One-way ANOVA tests were conducted to test for significant effects. If significant effects were found, post-hoc comparison's using Tukey's post-hoc tests were done.

In a dose-dependent manner, Abilify (0.1 – 20 mg/kg) significantly increased latency-to-goal for female mice (n = 6-10) when challenged in a classic maze, $F(2, 22) = 5.50$, $p < 0.05$ (see Figure 1b). Tukey's post-hoc test showed that female mice spent significantly longer time to complete the maze at 20mg/kg compared to 0.1mg/kg and 5mg/kg. Abilify (0.1 – 20 mg/kg) did

not significantly affect latency to goals for male mice (n = 6-10) when challenged in a classic maze, $F(2, 22) = 0.77$, $p > 0.05$ (see Figure 1a).

Abilify (0.1 – 20 mg/kg) did not significantly affect errors in memory for male mice (n = 6-10) when challenged in a classic maze, $F(2, 22) = 0.75$, $p > 0.05$ (see Figure 2a). Abilify (0.1 – 20 mg/kg) did not significantly affect errors in memory when female mice (n = 6-10) were challenged in a classic maze, $F(2, 22) = 0.58$, $p > 0.05$ (see Figure 2b).

Catalepsy is a condition characterized by lack of response to external stimuli and by muscle rigidity, so that the limbs remain where they are positioned. Catalepsy is a symptom of certain nervous disorders such as Parkinson's disease and epilepsy, but is also a characteristic feature of catatonic schizophrenia. The mice in this study were all examined prior to testing for any signs of catalepsy, and no signs were found. Once drug administration was initiated, if any signs of catalepsy were seen, it was assumed that this was a result of the drug, not any prior CNS dysfunction in the mice. At 20mg, some female mice were cataleptic, eliciting less movement as shown by a decrease from the lowest dose. Abilify (0.1 – 20 mg/kg) did not significantly affect the total distance that male mice (n = 6-10) traveled on the Rota-Rod, $F(3, 110) = 1.24$, $p > 0.05$ (see Figure 3a). However, in a dose-dependent manner, Abilify (0.1 – 20 mg/kg) significantly affected the total distance that female mice (n = 6-10) traveled on the Rota-Rod, $F(3, 104) = 8.83$, $p < 0.001$ (see Figure 3b). Tukey's post-hoc test verified that all three doses significantly impacted total distance traveled in females, as compared to their baseline times.

Abilify (0.1 – 20 mg/kg) did not significantly affect the total time that male mice (n = 6-10) spent on the Rota-Rod, $F(3, 66) = 0.44$, $p > 0.05$ (see Figure 4a). In a dose-dependent manner, Abilify (0.1 – 20 mg/kg) significantly affected the total time that female mice (n = 6-10) spent on the Rota-Rod, $F(3, 64) = 13.27$, $p < 0.001$ (see Figure 4b). Tukey's post-hoc test

verified that all three doses significantly impacted total time spent on the Rota-Rod in females, as compared to their baseline times.

As dose increased, male mice's (n = 6-10) coordination was significantly inhibited, showing less moves at 20mg dose, $F(3, 31) = 11.90$, $p < 0.001$ (see Figure 5a). Tukey's post-hoc test showed the 20mg dose to inhibit coordination significantly compared to the 0.1mg/kg dose, the 5mg/kg dose, and baseline. As dose increased, female mice's (n = 6-10) coordination was significantly inhibited, showing less moves at 20mg dose, $F(3, 32) = 27.54$, $p < 0.001$ (see Figure 5b). Tukey's post-hoc test showed the 20mg dose to inhibit coordination significantly compared to the 0.1mg/kg dose, the 5mg/kg dose, and baseline.

As dose increased, male mice's (n = 6-10) rest time significantly increased, $F(3, 31) = 34.29$, $p < 0.001$ (see Figure 6a). Tukey's post-hoc test showed the 20mg/kg dose to significantly increase rest time compared to the other doses and baseline, and the 5mg/kg dose significantly increased rest time compared to baseline. As dose increased, female mice's (n = 6-10) rest time significantly increased, $F(3, 32) = 68.67$, $p < 0.001$ (see Figure 6b). Tukey's post-hoc test showed all doses to be significant compared to each other and to baseline.

As dose increased, male mice's (n = 6-10) stereotypic movements significantly decreased, $F(3, 31) = 9.75$, $p < 0.001$ (see Figure 7a). Tukey's post-hoc test showed the 20mg/kg dose to cause less stereotypy moves compared to the other doses, and 0.1mg/kg to cause significantly more stereotypic movements compared to baseline. As dose decreased, female mice's (n = 6-10) stereotypic movements significantly increased, $F(3, 32) = 14.17$, $p < 0.001$ (see Figure 7b). Tukey's post-hoc test showed the 0.1mg/kg to cause significantly more stereotypic movements compared to the 5mg/kg dose, the 20mg/kg dose, and baseline.

In a dose-dependent manner, Abilify significantly impacted the number of stereotypic episodes in male mice (n = 6-10), $F(3, 31) = 10.16$, $p < 0.001$ (see Figure 8a). Tukey's post-hoc test showed that the 0.1mg/kg significantly increased stereotypy episodes compared to baseline, and that the 20mg/kg dose significantly reduced stereotypy episodes compared to the other doses. In a dose-dependent manner, Abilify significantly impacted the number of stereotypic episodes in female mice (n = 6-10), $F(3, 32) = 19.90$, $p < 0.001$ (see Figure 8b). Tukey's post-hoc test showed that the 0.1mg/kg significantly increased stereotypy episodes compared to the other doses and baseline, and that the 20mg/kg dose significantly reduced stereotypy episodes compared to the other doses and baseline.

In a dose-dependent manner, Abilify significantly impacted time spent engaging in stereotypy in male mice (n = 6-10), $F(3, 31) = 9.24$, $p < 0.001$ (see Figure 9a). Tukey's post-hoc test showed the 0.1mg/kg dose to significantly increase stereotypy time compared to baseline, and the 20mg/kg dose to significantly decrease stereotypy time compared to the other doses. As dose decreased, time spent engaging in stereotypy significantly increased in female mice (n = 6-10), $F(3, 32) = 9.20$, $p < 0.001$ (see Figure 9b). Tukey's post-hoc test showed the 0.1mg/kg dose to significantly increase stereotypy time compared to the other doses and baseline.

Discussion

Our hypothesis that Abilify would improve memory was not supported by our results. The cognitive enhancement of atypical antipsychotics is attributed to antagonist activity at 5-HT_6 and 5-HT_7 receptors, and since aripiprazole works as an antagonist at 5-HT_7 receptors, we expected to see Abilify enhance cognition in our study. However, neither the males nor the females showed cognitive improvement. The male mice committed the most errors at the 20mg/kg dose, the second most at the 0.1mg/kg dose, and the fewest at the 5mg/kg dose, while

the exact opposite pattern was true for the female mice. Such contradictory results are difficult to interpret. While both males and females spent more time in the maze at the highest dose, the effect was significant only for the females, which might have something to do with the involvement of prolactin in the females. Our findings regarding coordination showed that the female mice's coordination was more inhibited compared to the male mice, meaning that the female mice perhaps took longer in the maze at the highest dose due to greater inhibited coordination than the males, rather than any cognitive differences. While the results conflicted with our hypothesis, we could see gender differences at doses showing that most errors were committed by excessive time spent in maze. Again, this memory problem could be due to a prolactin change in females that was not seen in males.

We hypothesized that the highest dose would inhibit coordination, which was mostly supported by our findings. While the results were not significant for the male mice, all doses produced shorter distances traveled in the Rota-Rod compared to baseline for the female mice. Remarkably, even the lowest dose had a stronger impact on female mice than any of the doses had on the male mice. Also, the lowest and highest dose produced less distance travelled than the middle dose. The time spent by male mice on the Rota-Rod was not significant depending on dose, and the results were quite consistent across the doses and baseline. All doses significantly reduced time on the Rota-Rod compared to baselines for the female mice. The female mice seemed to experience inhibited coordination at all doses, whereas the males experienced no significant inhibition. Also, the middle dose was closest to the baselines for the females, once again. The highest dose caused significantly fewer moves and thus showed inhibited coordination among the male mice. The lowest and middle dose produced more moves than baseline, without being significant. The highest dose caused significantly fewer moves in the

female mice as well, indicating inhibited coordination. In males, the highest dose produced significant effects across all conditions, while the middle dose also produced significantly more rest time compared to baseline. All doses significantly increased rest time compared to baseline in female mice, and the rest time also increased significantly in a dose-dependent manner.

We hypothesized that the lowest dose would increase stereotypy and that the highest dose would inhibit stereotypy, which was generally supported by our findings. In male mice, the highest dose produced significantly fewer stereotypic moves compared to the other doses, and the lowest dose produced significantly more moves compared to baseline. In females, the lowest dose produced significantly more stereotypic moves compared to the other doses and baseline, while the highest dose produced less stereotypic moves in a non-significant manner, and the middle dose was comparable to baseline. In male mice, the lowest dose produced significantly more stereotypic episodes compared to the highest dose and baseline, and the highest dose produced significantly more episodes compared to the middle dose. In females, the lowest dose produced significantly more episodes compared to the other doses and baseline, and the highest dose produced significantly fewer episodes compared to the other doses and baseline. In male mice, there was a significant difference in stereotypy time between the highest and the lowest dose, while the lowest dose produced significantly longer time than baseline, and the highest dose produced significantly less time than the middle dose. In female mice, the lowest dose produced significantly more stereotypy time compared to the other doses and baseline.

At moderate to high doses of Abilify, catalepsy or a modified form of catalepsy may arise when individulas sustain their limbs in the same position for very long periods of time, which may lead to significant health problems, such as formation of the blood clots. Also, since high doses of Abilify is linked to slowing down of bodily functions a possible future study should

include evaluating respiratory depression. Also, we did note gender differences among the male and female mice, and these differences could be investigated by future studies.

Acknowledgements

This work was supported in part by an LIU-Post Research Monetary Grant and by the Psychology Department at LIU-Post. The author thanks Dr. Grace Rossi for assistance with these studies and for the resources provided to make this thesis research possible. The author would also like to thank Chris McAllister for his help with managing and taking care of the mice used in the study.

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Figure 1a

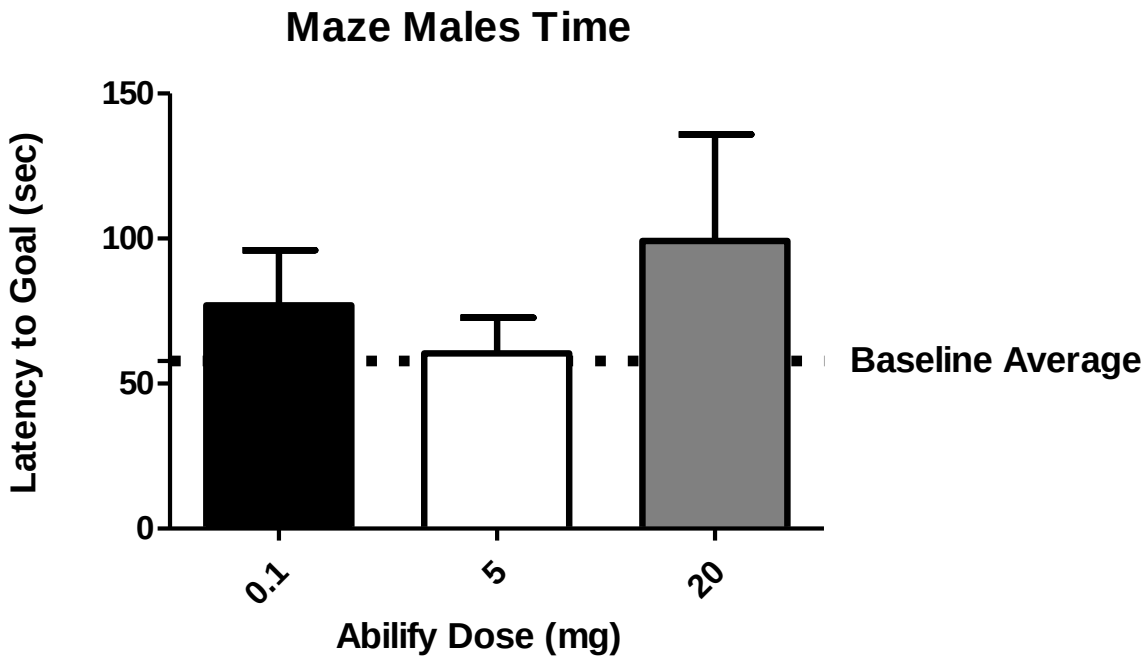

Figure 1a. Latency-to-goal in seconds for male mice in a cognitive maze at 0.1mg/kg, 5 mg/kg, and 20mg/kg doses of Abilify.

Figure 1b

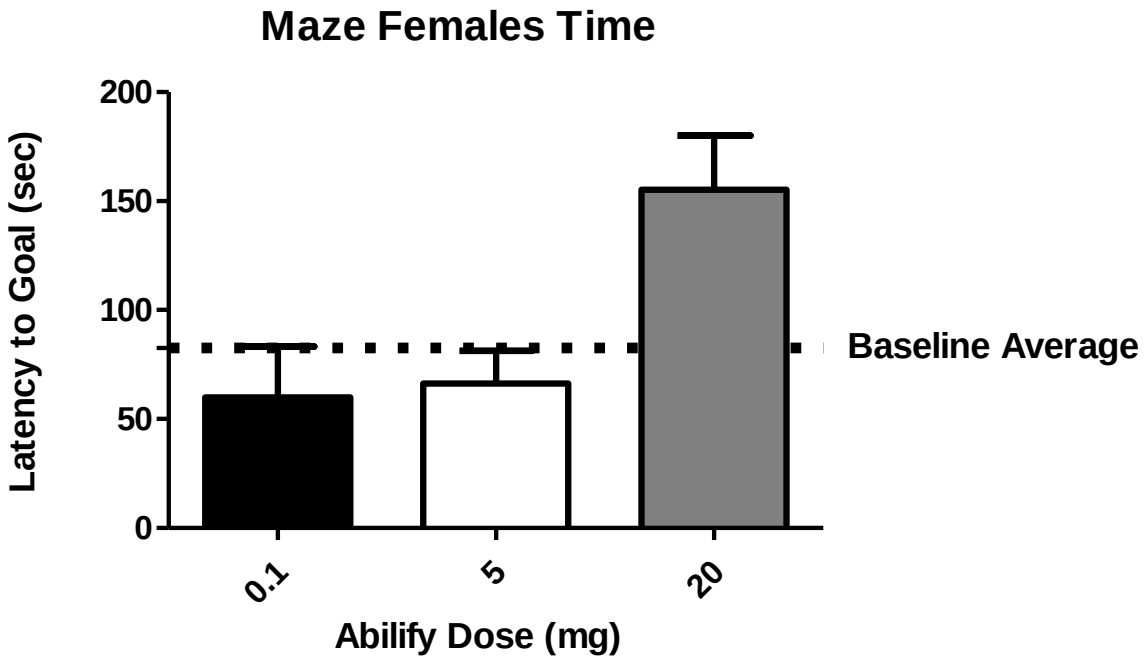

Figure 1b. Latency-to-goal in seconds for female mice in a cognitive maze at 0.1mg/kg, 5 mg/kg, and 20mg/kg doses of Abilify.

Figure 2a

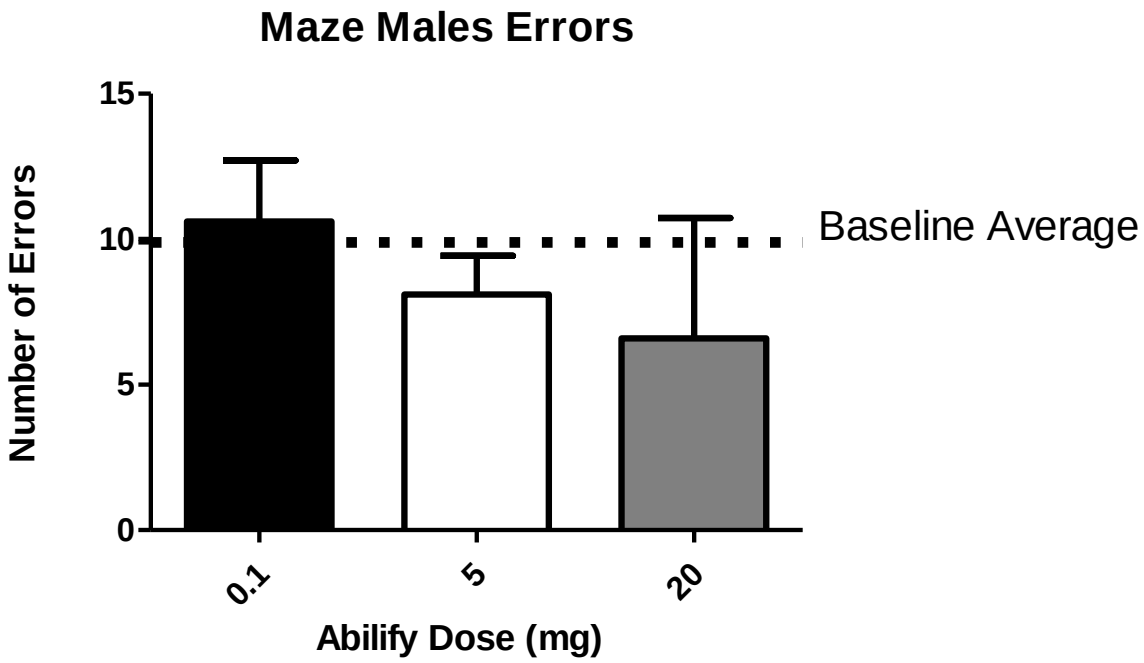

Figure 2a. Number of errors made by male mice in a cognitive maze at 0.1mg/kg, 5 mg/kg, and 20mg/kg doses of Abilify.

Figure 2b

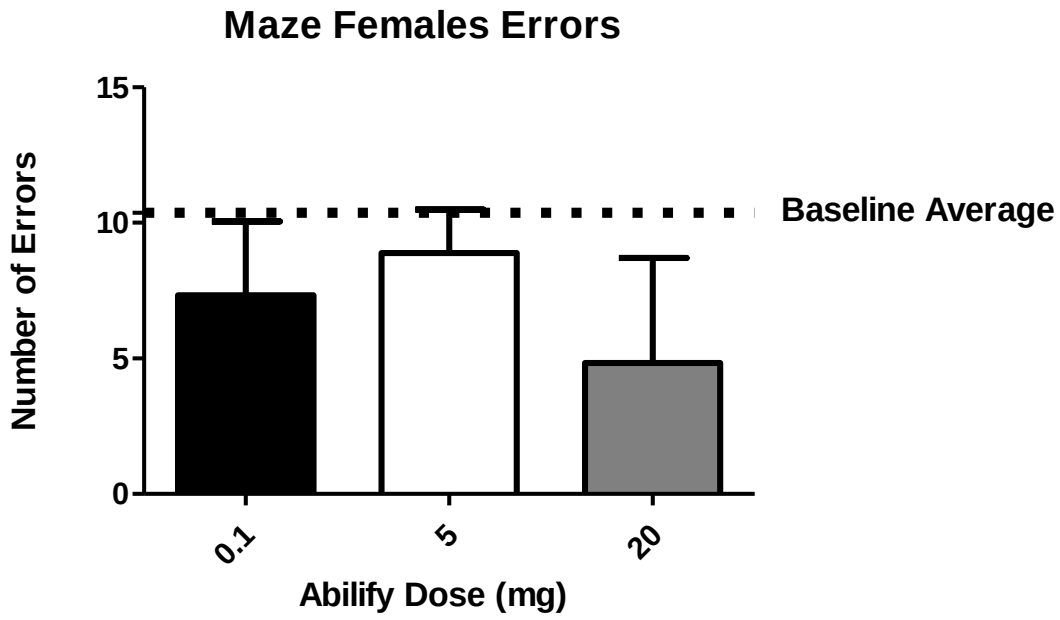

Figure 2b. Number of errors made by female mice in a cognitive maze at 0.1mg/kg, 5 mg/kg, and 20mg/kg doses of Abilify.

Figure 3a

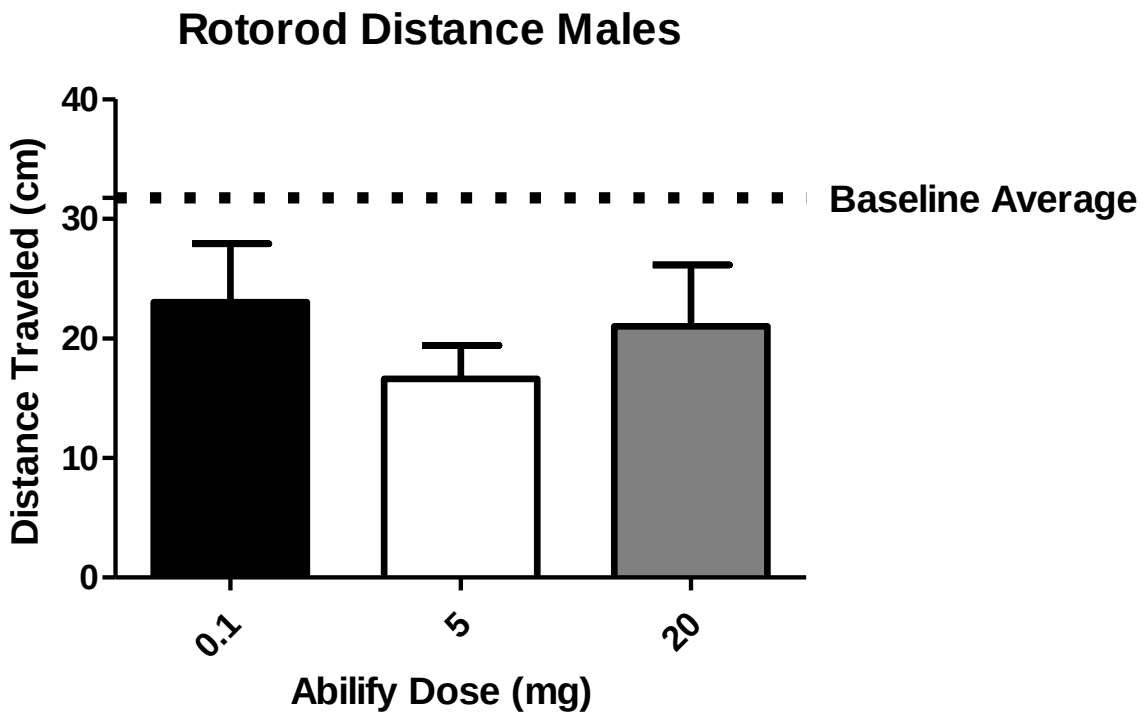

Figure 3a. Distance traveled in cm by male mice on the Rota-Rod at 0.1mg/kg, 5 mg/kg, and 20mg/kg doses of Abilify.

Figure 3b

Figure 3b. Distance traveled in cm by female mice on the Rota-Rod at 0.1mg/kg, 5 mg/kg, and 20mg/kg doses of Abilify.

Figure 4a

Figure 4a. Time in seconds spent by male mice on the Rota-Rod at 0.1mg/kg, 5 mg/kg, and 20mg/kg doses of Abilify.

Figure 4b

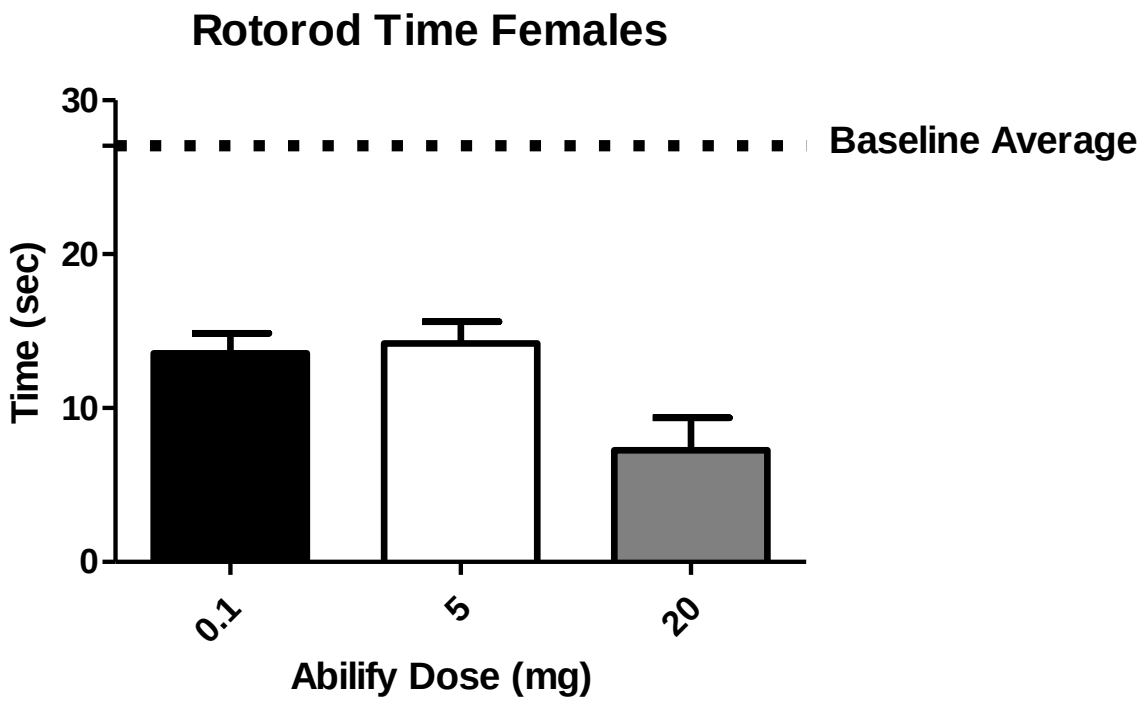

Figure 4b. Time in seconds spent by female mice on the Rota-Rod at 0.1mg/kg, 5 mg/kg, and 20mg/kg doses of Abilify.

Figure 5a

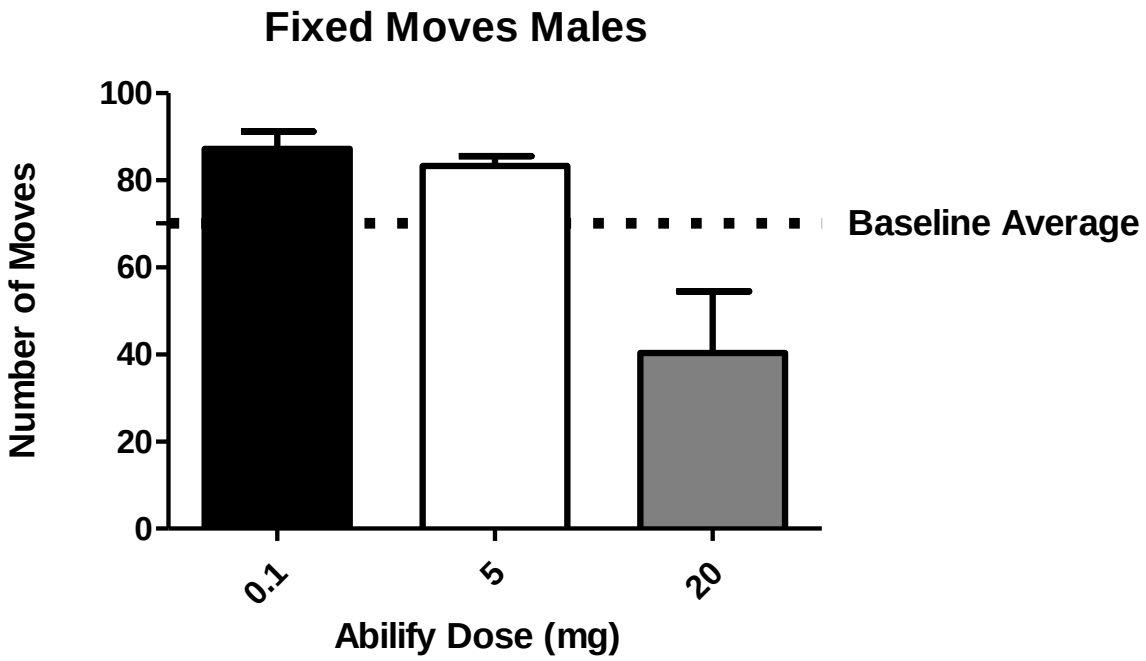

Figure 5a. Number of fixed moves made by male mice in the Tru-Scan at 0.1mg/kg, 5 mg/kg, and 20mg/kg doses of Abilify.

Figure 5b

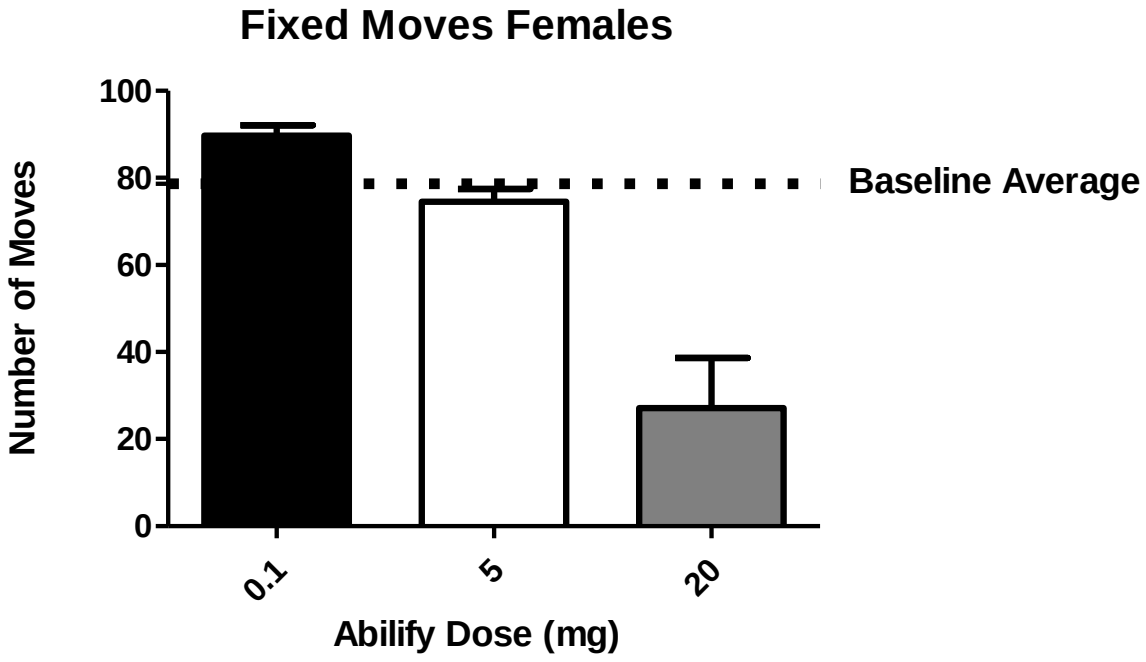

Figure 5b. Number of fixed moves made by female mice in the Tru-Scan at 0.1mg/kg, 5 mg/kg, and 20mg/kg doses of Abilify.

Figure 6a

Figure 6a. Rest time in seconds spent by male mice in the Tru-Scan at 0.1mg/kg, 5 mg/kg, and 20mg/kg doses of Abilify.

Figure 6b

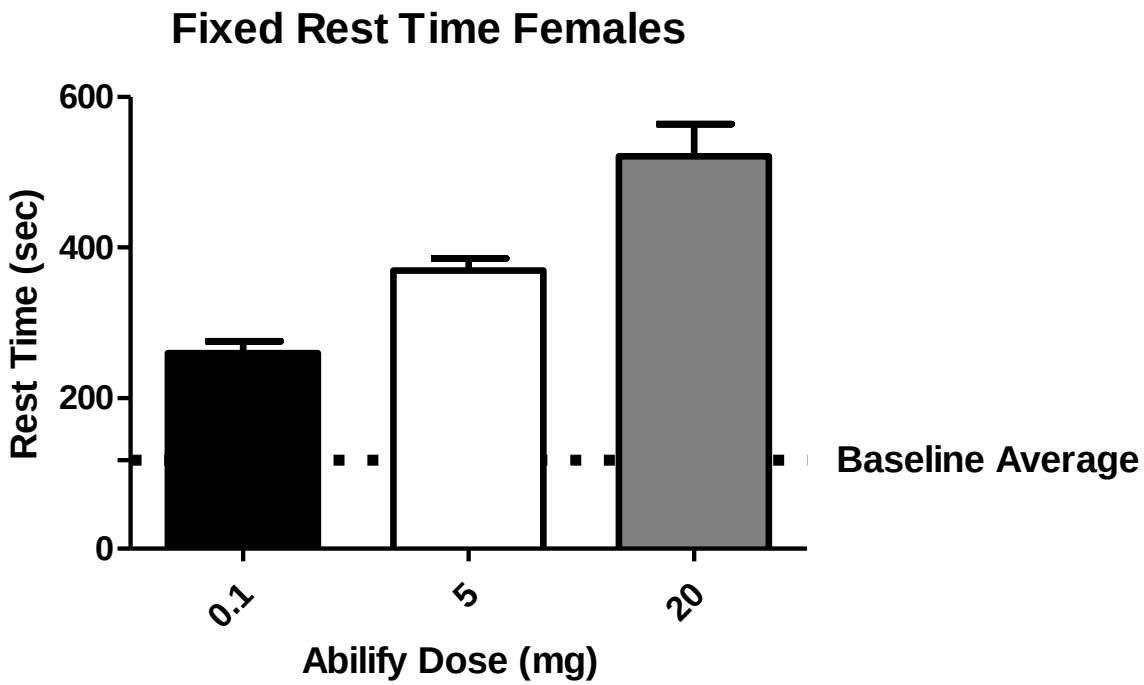

Figure 6b. Rest time in seconds spent by female mice in the Tru-Scan at 0.1mg/kg, 5 mg/kg, and 20mg/kg doses of Abilify.

Figure 7a

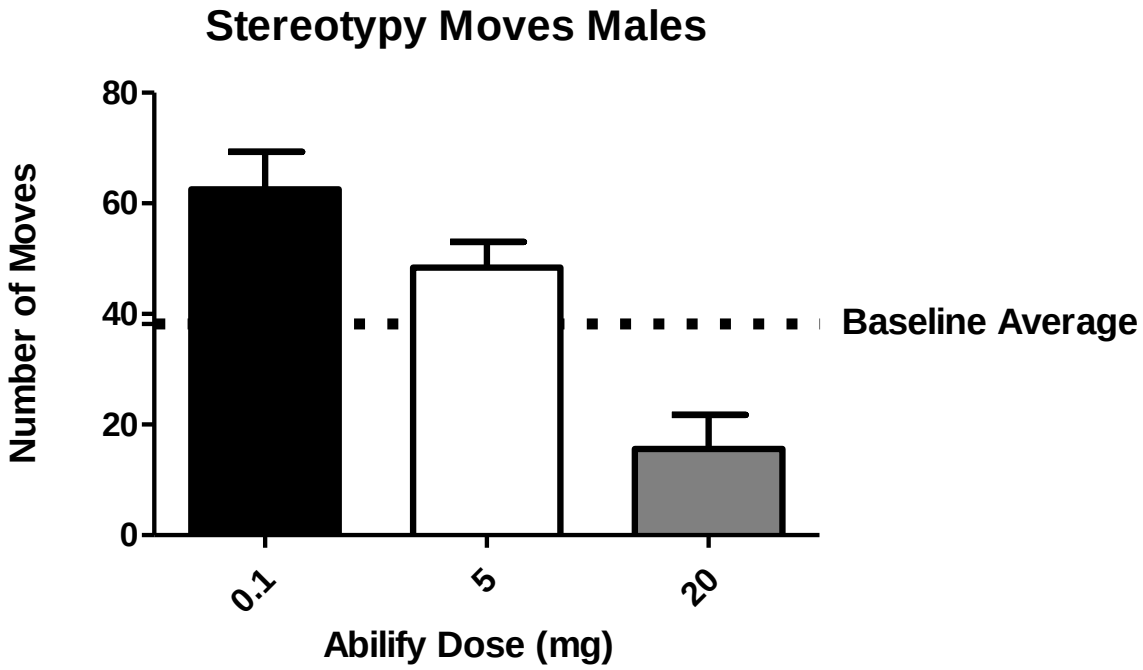

Figure 7a. Number of stereotypic moves made by male mice in the Tru-Scan at 0.1mg/kg, 5 mg/kg, and 20mg/kg doses of Abilify.

Figure 7b

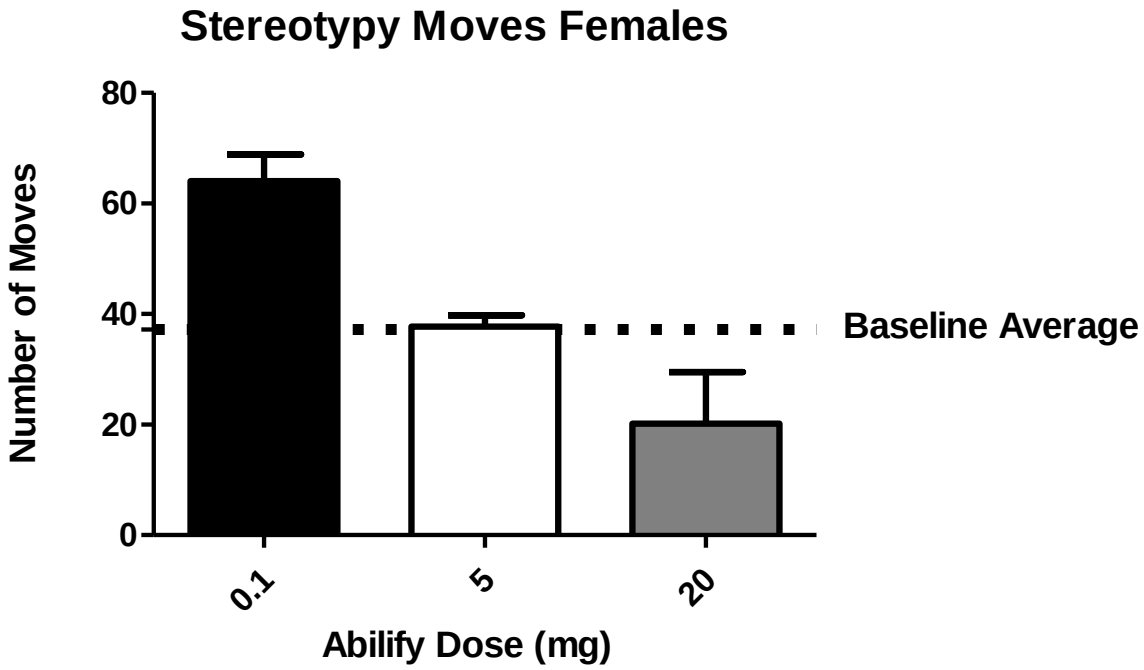

Figure 7b. Number of stereotypic moves made by female mice in the Tru-Scan at 0.1mg/kg, 5 mg/kg, and 20mg/kg doses of Abilify.

Figure 8a

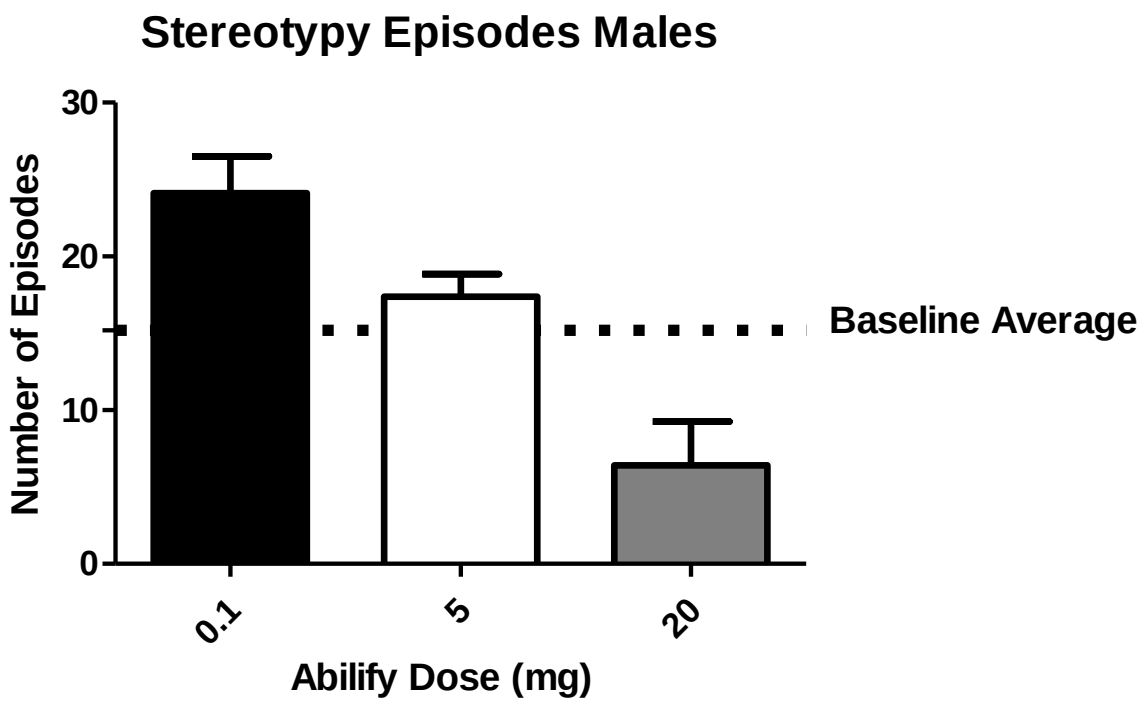

Figure 8a. Number of stereotypic episodes made by male mice in the Tru-Scan at 0.1mg/kg, 5 mg/kg, and 20mg/kg doses of Abilify.

Figure 8b

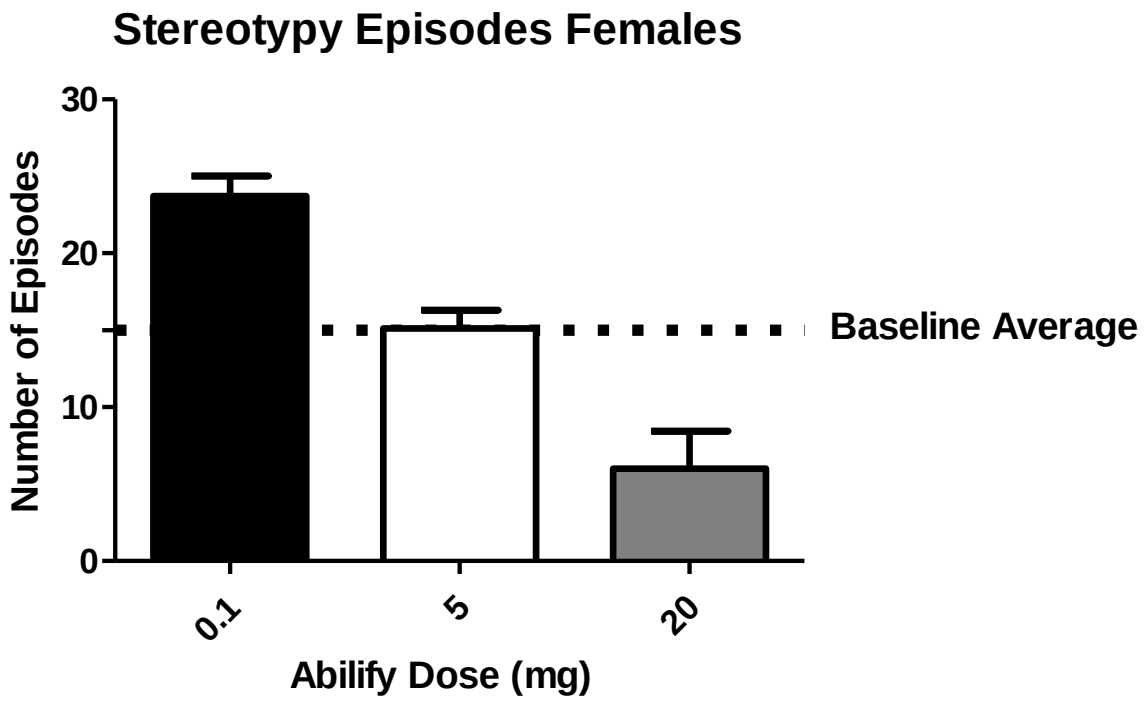

Figure 8b. Number of stereotypic episodes made by female mice in the Tru-Scan at 0.1mg/kg, 5 mg/kg, and 20mg/kg doses of Abilify.

Figure 9a

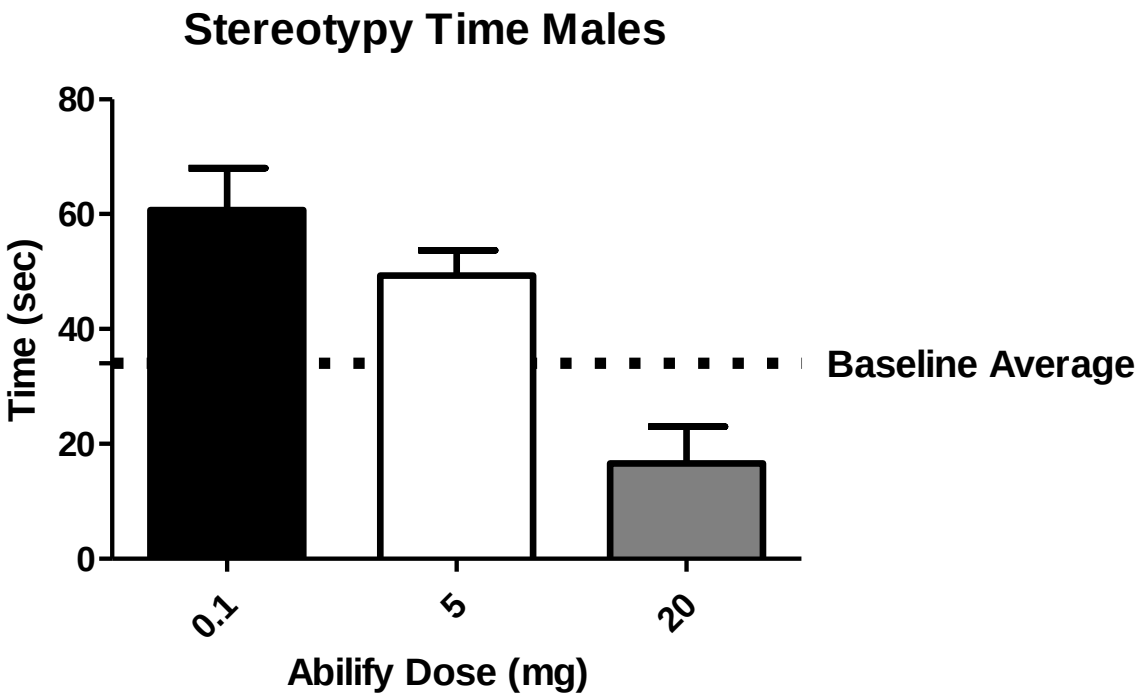

Figure 9a. Time in seconds spent by male mice making stereotypic movements in the Tru-Scan at 0.1mg/kg, 5 mg/kg, and 20mg/kg doses of Abilify.

Figure 9b

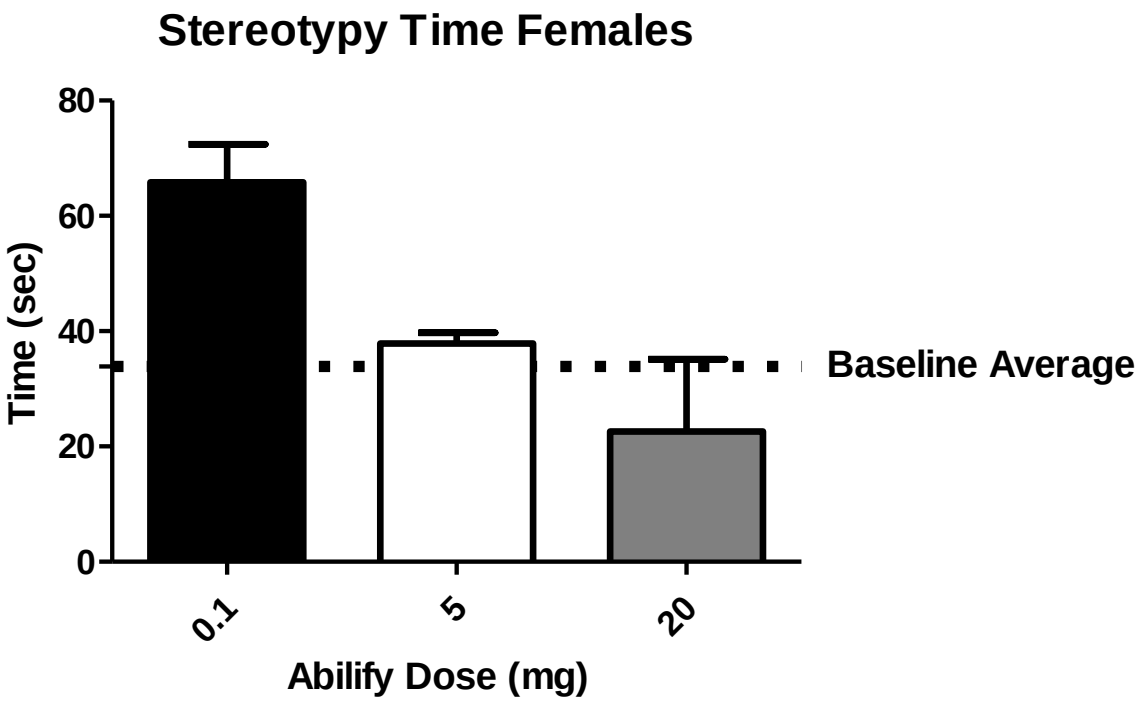

Figure 9b. Time in seconds spent by female mice making stereotypic movements in the Tru-Scan at 0.1mg/kg, 5 mg/kg, and 20mg/kg doses of Abilify.

Table 1. Statistical analysis of Abilify's effect on coordination in males.

Parameter					
Table Analyzed	Rotorod Time Males				
One-way analysis of variance					
P value	0.7192				
P value summary	ns				
Are means signif. different? (P < 0.05)	No				
Number of groups	4				
F	0.4484				
R squared	0.01998				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	3.134				
P value	0.3715				
P value summary	ns				
Do the variances differ signif. (P < 0.05)	No				
ANOVA Table	SS	df	MS		
Treatment (between columns)	36.44	3	12.15		
Residual (within columns)	1788	66	27.09		
Total	1824	69			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
0.1 vs 5	1.850	1.590	No	ns	-2.493 to 6.193
0.1 vs 20	0.4500	0.3157	No	ns	-4.869 to 5.769
0.1 vs Baseline	1.000	0.8592	No	ns	-3.343 to 5.343
5 vs 20	-1.400	0.9822	No	ns	-6.719 to 3.919
5 vs Baseline	-0.8500	0.7304	No	ns	-5.193 to 3.493
20 vs Baseline	0.5500	0.3859	No	ns	-4.769 to 5.869

Table 2. Statistical analysis of Abilify's effect on coordination in females.

Parameter					
	Rotorod Time				
Table Analyzed	Females				
One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	4				
F	13.27				
R squared	0.3835				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	18.69				
P value	0.0003				
P value summary	***				
Do the variances differ signif. (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	3175	3	1058		
Residual (within columns)	5105	64	79.76		
Total	8280	67			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
0.1 vs 5	-0.6500	0.3255	No	ns	-8.106 to 6.806
0.1 vs 20	6.300	2.385	No	ns	-3.563 to 16.16
0.1 vs Baseline	-13.50	6.760	Yes	***	-20.96 to -6.044
5 vs 20	6.950	2.631	No	ns	-2.913 to 16.81
5 vs Baseline	-12.85	6.435	Yes	***	-20.31 to -5.394
20 vs Baseline	-19.80	7.495	Yes	***	-29.66 to -9.937

Table 3. Statistical analysis of Abilify's effect on endurance in males.

Parameter					
	Rotorod Distance				
Table Analyzed	Males				
One-way analysis of variance					
P value	0.2994				
P value summary	ns				
Are means signif. different? (P < 0.05)	No				
Number of groups	4				
F	1.238				
R squared	0.03266				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	39.10				
P value	< 0.0001				
P value summary	***				
Do the variances differ signif. (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	4260	3	1420		
Residual (within columns)	126200	110	1147		
Total	130400	113			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
0.1 vs 5	6.400	0.8451	No	ns	-21.57 to 34.37
0.1 vs 20	2.000	0.2156	No	ns	-32.26 to 36.26
0.1 vs Baseline	-8.734	1.424	No	ns	-31.39 to 13.92
5 vs 20	-4.400	0.4744	No	ns	-38.66 to 29.86
5 vs Baseline	-15.13	2.467	No	ns	-37.79 to 7.525
20 vs Baseline	-10.73	1.318	No	ns	-40.81 to 19.34

Table 4. Statistical analysis of Abilify's effect on endurance in females.

Parameter					
Table Analyzed	Rotorod Distance Females				
One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	4				
F	8.831				
R squared	0.2030				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	48.65				
P value	< 0.0001				
P value summary	***				
Do the variances differ signif. (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	49540	3	16510		
Residual (within columns)	194500	104	1870		
Total	244000	107			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
0.1 vs 5	-2.950	0.3051	No	ns	-38.72 to 32.82
0.1 vs 20	13.13	1.026	No	ns	-34.19 to 60.44
0.1 vs Baseline Females	-41.48	5.254	Yes	**	-70.69 to -12.28
5 vs 20	16.08	1.257	No	ns	-31.24 to 63.39
5 vs Baseline Females	-38.53	4.881	Yes	**	-67.74 to -9.330
20 vs Baseline Females	-54.61	4.745	Yes	**	-97.18 to -12.04

Appendix A

Cognitive Maze

Appendix B

Rota-Rod series 8 model

Appendix C

The Coulbourn Tru-Scan Activity Box