Effects of Atomoxetine and 7-NINA on Serotonin 1B-Induced Autism-like Non-Selective Attention Deficits in Mice: An Investigation of Novel Treatments

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Abstract

Autism spectrum disorder (ASD) is a developmental disorder characterized by impaired social interaction, language deficits, restricted interests, and stereotypic behavior. Autism is additionally associated with attention deficits, intellectual disabilities, and hyperactivity. Despite its recent increase in prevalence, the neural underpinnings of autism remain unclear. This research project investigates the neural substrates underlying attention deficits in a novel model of autism. Specifically, we measure non-selective attention (NSA) in 6-8 week old mice injected with a serotonin 1B agonist (RU24969) to induce autism-like behaviors. NSA is the spreading of attention across a visual field and is indexed by average rearing duration (ARD) in mice in an “open field chamber.” Mice are then injected with either atomoxetine, an approved treatment for ADHD, or a nitric oxide synthase inhibitor 23 7-NINA, a drug found to treat NSA deficits in animal studies. We hypothesized that RU24969 would decrease ARD and, thus, NSA. Similarly, we expected RU24969 to increase overall locomotion in the open field. We also hypothesized that atomoxetine and 7-NINA would reverse NSA deficits induced by RU24969. Our results confirmed our hypothesis that RU24969 decreases ARD and increases locomotion. Our results also indicated that neither atomoxetine nor 7-NINA increased ARD alone or following RU24969 treatments. Unexpectedly, atomoxetine significantly exacerbated the NSA deficits induced by RU24969. Our findings replicate the findings of autism-like behavior following RU24969 challenge, and suggest that the putative treatments atomoxetine and 7-NINA will not be effective for treating the NSA deficits in ASD.

Keywords: Autism Spectrum Disorder (ASD), non-selective attention (NSA) average rearing duration (ARD), total distance traveled (TDT)
Effects of Atomoxetine and 7-NINA on Serotonin 1B-Induced Autism-like Non-Selective Attention Deficits in Mice: An Investigation of Novel Treatments

Autism spectrum disorder (ASD) is a neurodevelopmental disorder that affects more than 3.5 million Americans (Buescher, Cidav, Knapp, & Mandell, 2014), approximately 1% of the world population (Kim et al., 2011), and is found in all racial, ethnic, and socioeconomic groups (Developmental, D. M. N. S. Y., & 2010 Principal Investigators, 2014). This disorder causes major individual and societal difficulties. For instance, a recent study found that only 34.5% of young adults with ASD attended college, and only 55.1% were employed during the first six years after high school (Shattuck et al., 2012). Moreover, autism services cost United States residents 236 to 262 billion dollars annually through medical costs and individual loss in productivity (Buescher, Cidav, Knapp, & Mandell, 2014).

ASD is characterized by social interaction and communication difficulties, repetitive behaviors, and restricted interests (APA, 2013). In addition, ASD is associated with attentional deficits, hyperactivity, intellectual disabilities, and seizures (APA, 2013; Goldstein & Schwebach, 2004; National Institute of Mental Health, 2011). To receive the ASD diagnosis, the core symptoms of ASD must have originated early in development and cause significant impairments in an individual’s social, academic, or professional life (American Psychiatric Association, 2013). In order to develop effective treatments for ASD, researchers must first comprehend the mechanisms that underlie ASD pathology. In particular, the neuronal mechanisms of the attentional deficits of ASD are not well understood.

Many patients with a primary diagnosis of ASD also exhibit core symptoms of attention-deficit/hyperactivity disorder (ADHD). Leyfer et al. (2006) showed that ADHD was the third most common secondary diagnosis in patients with ASD, just after specific phobias and
obsessive compulsive disorder. Specifically, 31% of children with autism in this study were also diagnosed with ADHD (Leyfer et al., 2006). Moreover, nearly 65% of those children were found to be diagnosed within the inattentive subtype, a subtype in which the patient fails to play close attention to detail, does not appear to listen, is easily distracted, and struggles to follow directions (American Psychiatric Association, 2013; Leyfer et al., 2006). Similarly, Sikora, Vora, Coury, and Rosenberg (2012) found that children with ASD and clinically significant ADHD symptoms had more health-related quality of life issues and impairment in adaptive functioning compared to children with ASD that exhibited fewer attention deficits symptoms. Together, these studies underscore the need for researchers to understand the neuronal mechanisms that underlie attention deficits in ASD.

Studies also indicate that 45 to 80% of patients with ASD have difficulty with disengagement of visual stimuli in a horizontal field, which is an aspect of non-selective attention (NSA) deficits (Landry & Bryson, 2004; Townsend et al., 1999). NSA is the active spreading of attention across visual space in order to process as many shapes and edges as possible in parallel while limiting distraction by any one object (Aspide, Fresiello, de Filippis, Gironi Carnevale, & Sadile, 2000). Aspide et al. (2000) explained that NSA involves scanning the environment and is a prerequisite to detecting and orienting to stimuli in the selective attention mode. Deficits in NSA are characterized by difficulties shifting and disengaging in visual attention from objects in a person’s visual space (Landry & Bryson, 2004). Landy and Bryson (2004) found that children with ASD have difficulty disengaging and shifting attention from one of two competing visual objects. In their experiment, children viewed three different computer monitors, two peripheral and one middle screen, that displayed bright, moving geometric shapes. In the “shifting attention” trial of Landy and Bryson’s study (2004), a colorful
shape in the middle screen disappeared as soon as the child looked at the screen. After a brief delay, another shape would appear on one of the peripheral screens. In contrast, during the “disengaging” trial the object did not disappear from the middle of the screen when the child looked at it. In both the “shifting attention” and “disengaging” trials, Landy and Bryson (2004) found that it took children with ASD a longer time to shift their attention from the middle screen to a peripheral screen than healthy children. Rather, ASD patients tend to fixate their attention on specific objects in their visual field and have difficulty attending to many objects at once (Landy & Bryson, 2004). Since deselecting and entering a non-selective attentional state is required for processing objects in parallel, certain ASD patients are thought to have deficits in NSA.

Other studies have found that ASD patients display an inability to quickly switch their visual attention between target stimuli (Allen & Courchene, 2001; Harris, Courchesne, Townsend, Carper, & Lord, 1999; Townsend et al., 1999). In a “target detection task” similar to Landry and Bryson’s (2004) “disengaging” trial, Townsend et al. (1999) displayed an asterisk, which was the target stimulus, to the left or right of a central box at varying delays after an initial central-box target was displayed on a computer screen. Participants’ task was to press a button when they detected the target while maintaining their fixation on a central cross (Townsend et al., 1999). A “spatial discrimination task” was also administered and was the same as the “target detection task” except that participants had to move a joystick in the orientation of a target stimulus, which was either oriented up, down, left, or right (Townsend et al., 2004). In both the “target detection” and “spatial discrimination” tasks, Townsend et al. (2004) measured response time, which was the duration until participants responded to the target, by either pushing a button in the first experiment or by moving the joystick, after the target was displayed. In both tasks, Townsend et al (2004) found that patients with ASD were slow to orient spatial attention, which
was indicated by their longer reaction times to the target stimulus compared to healthy individuals. ASD patients in both Landry and Bryson (2004) and Townsend et al.’s (1999) studies displayed NSA deficits as they struggled to attentionally deselect, actively spread their visual attention across visual space, and switch between attentional selections. Aspide et al. (2008) further suggested that NSA deficits may likely cause ASD patients to be less motivated to thoroughly explore novel environments and may contribute to both social deficits and perseverative behaviors associated with ASD.

In order to treat NSA deficits, researchers must understand the neural mechanisms that underlie NSA. Research suggests that the anterior attention system, which includes connections between the frontal cortex, anterior cingulate gyrus, and basal ganglia, is the underlying neural substrate of NSA (Aspide et al., 2000; Grammatikopoulos et al., 2002). Specifically, Posner and Dehaene (1994) found that the anterior attention system is involved in the recruitment and control of brain areas involved in performing complex cognitive tasks that require attention. The anterior attention system also assists in the scanning of all objects in the visual field when a task involves locating an object with specific properties (Posner & Dehaene, 1994). Selective attention, on the other hand, is thought to be mediated by the posterior attention system (Posner & Dehaene, 1994). This system, which is made up by the pulvinar nuclei, superior parietal cortex, and superior colliculus, is primarily engaged following the selection of one stimulus location among many (Posner & Dehaene, 1994).

However, more research is needed to explain the neural mechanisms that underlie both NSA and selective attention. Moreover, the role of the anterior attention system specifically in ASD patients remains unclear. In fact, no studies to our knowledge have investigated the neural substrates underlying NSA deficits in ASD. An improved understanding of the mechanisms that
underlie NSA may be necessary for the identification of novel medications for the NSA deficits in ASD.

Currently, a variety of off-label medications are used to manage the attentional deficit symptoms in ASD. Moreover, behavioral, occupational, speech, and physical therapists work with ASD patients to improve attentional deficits. In their review of ASD pharmacological studies, Amen, Farmer, Holwar, and Arnold (2008) found that methylphenidate, atomoxetine, alpha-2 adrenergic agonists, and some atypical antipsychotics have therapeutic effects on impulsiveness, overactivity, and inattention in children with pervasive developmental disorders such as ASD. In their study, Harfterkamp et al. (2009) found that children with ASD that took atomoxetine, also known as Strattera, for 8 weeks had no serious side effects and had improved hyperactivity-impulsivity and attention-deficit clinician ratings compared to ASD children that took a placebo. Harfterkamp et al. (2009) suggested that atomoxetine is an effective medication to treat symptoms of attention-deficit/hyperactive disorder (ADHD). While these small studies provide the grounds to justify larger studies, they do not themselves allow for broad-sweeping conclusions about the effectiveness of atomoxetine in ASD as they lack external validity. Therefore, further studies examining the effectiveness of atomoxetine in ASD, including large double-blind placebo controlled clinical trials and animal studies, are needed.

Atomoxetine helps to attenuate attentional deficits associated in ADHD because it affects an area of the brain that is involved in attention. Specifically, atomoxetine works as a norepinephrine (NE) reuptake inhibitor, which increases norepinephrine in the brain (Bymaster et al., 2002). Neuronal cell bodies of NE are located in the locus coeruleus and project to many areas of the brain including the frontal cortex, limbic system, and cerebellum (Bymaster et al., 2002; Segal & Bloom, 1974.). Atomoxetine is thought to work because it increases NE
specifically in the prefrontal cortex (Bymaster et al., 2002), a region in the anterior attention center where NSA is processed (Aspide et al., 2000; Grammatikopoulos et al., 2002). Increased levels of NE have been shown to also increase alertness, concentration, and attention (Pliszka, McCracken, & Maas, 1996). The increase in NE cell bodies of the locus coeruleus is thought to be therapeutic by decreasing the pre-frontal cortex response (Pliszka et al., 1996). Further, atomoxetine seems to be an effective medication at improving attention in people with attention deficits (Harfterkamp et al., 2009). However, researchers have not tested how effective atomoxetine is at treating NSA deficits in patients with ASD. Thus, the mechanisms by which atomoxetine treats inattention in ASD remains unclear.

In addition to atomoxetine, nitric oxide synthase (NOS) inhibitor drugs show promise as medications for the attention deficits in ASD. Drugs that inhibit the NOS system are currently only at the preclinical stage of research, yet findings from animal model studies suggest they may be effective in humans. For instance, Grammatikopoulos et al. (2002) found that in a rat model of ADHD, injection of 7-nitroindazole (7-NINA), an NOS inhibitor, leads to increased rearing frequency and duration in the NSA test. These increased measures of NSA indicate that 7-NINA treated the NSA deficits in the ADHD-bred mice. Grammatikopoulos et al. (2002) explained that 7-NINA works by selectively inhibiting NO release at the neuronal level at the midbrain and forebrain, which may also decrease dopamine (DA) neurotransmission. Specifically, 7-NINA is thought to affect the mesocorticolimbic dopamine system (MCL) (Grammatikopoulos et al., 2002), a system that has been found to modulate NSA (Aspide et al., 2000) and to be hyperfunctioning in animal models of ADHD and in children with ADHD (Accili et al., 1996; Cabib, Puglisi-Allegra, & Ventura, 2002; Sadile, Sergeant, & Solanto, 2000). The MCL transmits DA from the ventral tegmental area to the limbic system, which is located in
the midbrain, and to the frontal cortex, an area in the anterior attention center (Aspide et al., 2000; Grammatikopoulos et al. 2002). Not only does 7-NINA affect DA levels in the brain, but Grammatikopoulos et al. (2002) explained that 7-NINA has been shown to affect neurotransmission of acetylcholine (ACH) and glutamate. The varying levels of DA, ACH, and glutamate, Grammatikopoulos et al. (2002) suggested, modify the operations in the attentional networks in the brain. Based on the results of Grammatikopoulos et al.’s (2002) study, 7-NINA may also treat NSA deficits in ASD. However, the efficacy of NOS inhibitors in treating attention deficits of ASD is unknown.

Animal model studies will be essential to understanding the effectiveness and mechanisms of novel treatments, such as atomoxetine and NOS inhibitors, for attention deficits in ASD. Recently, a novel model of autism-like behavior in mice was produced which researchers can use to measure the effect of treatments for autism (Lawson, Grey, & Woehrle, 2014). Lawson et al.’s (2014) serotonin 1B agonist model of ASD exhibits two of the three core features of ASD as well as the associated feature of attentional deficits. Lawson et al. (2014) showed that pharmacological activation of serotonin 1B receptors in mice induces social interaction deficits, preservative hyperactivity, and NSA deficits. In this model, NSA deficits were measured using the open field test (Aspide et al., 1998). Specifically, mice injected with a serotonin 1B agonist exhibited decreased average rearing duration, ARD, compared to mice injected with vehicle, which was used as the placebo (Lawson et al., 2014). Serotonin 1B agonist injections also induce autism-like perseveration and hyperactivity, such as repeated circling in an open field and increased total distance traveled. (Lawson et al., 2014; Cheeth & Heal, 1993). Lawson et al. (2014) chose to use the serotonin 1B agonist for various scientific reasons. For instance, serotonin 1B receptor agonist sumatriptan has been found to exacerbate symptoms in patients
with ASD (Hollander et al., 2000). Similarly, serotonin reuptake inhibitors (SRIs), effective treatments for ASD, reduce the highly perseverative forms of locomotion and sensorimotor gating deficits in mice induced by serotonin 1B agonists (Cheetham & Heal, 1993; Shanahan et al., 2009; Shanahan, Velez, Masten, & Dulawa, 2011). Overall, the social interaction deficits, preservative hyperactivity, and NSA deficits induced by Lawson et al.’s (2014) RU24969 pharmacological model indicates that it is an effective model to induce ASD acutely in mice.

Serotonin 1B agonists work by activating serotonin 1B receptors, which are the human serotonin 5-HT1Dβ and rodent 5HT1β receptors (Sari, 2004). These are terminal receptors that reside at the end of a neuron and inhibit the release of specific neurotransmitters made by that neuron (Sari, 2004). Serotonin 1B receptors have been found on many types of neurons, including gamma-aminobutyric acid (GABA), glutamate, and serotonin neurons in the central nervous system (Sari, 2004). Serotonin 1B receptors are found in the pallidum, globus pallidus, substantia nigra, dorsal subiculum, and are expressed in the cerebral cortex, frontal cortex, hippocampus, and entopeduncular nucleus, superficial gray layer of the superior colliculus, caudate putamen, and deep nuclei of the cerebellum (Posner & Dehaene, 1994; Sari, 2004). Specifically, the serotonin 1B receptors in the frontal cortex are located in areas thought to mediate NSA (Posner & Dehaene, 1994; Sari, 2004). However, there has been no research that investigates the action of specific receptors and neurotransmitters involved in the anterior attention center that mediates NSA. Sari et al. (2004), though, found that the serotonin 1B agonist deactivates the prefrontal cortex, an area that makes up the anterior attention center, by inhibiting the release of serotonin and glutamate (Sari, 2004). Further, Lawson et al.’s (2014) finding that 1B agonists affect NSA is particularly important for researchers investigating effective medications to treat NSA deficits in ASD.
In order to measure NSA deficits in mice, one must be able to accurately measure NSA. In rodent studies, average rearing duration is thought to provide an index of NSA in rodents (Aspide et al., 1998; Aspide et al., 2000, DeLorey, Sahbaie, Hashemi, Homanics, & Clark, 2008). In a spatially novel situation, rearing episodes on the hind limbs of animals is a behavioral trait evident across the entire animal kingdom, and has been shown to precede visual scanning behavior (Aspide et al., 2000). Aspide et al. (2000) explains that non-selectively attending to an open field is beneficial for mice to identify and avoid threats. In order to identify threats, the animal must spread their attention on many visual objects at once without fixating on a single object (Aspide et al., 2000). Thus, in our study, we will measure NSA in mice using rearing behavior.

Further, we will assess the ability the norepinephrine reuptake inhibitor atomoxetine or the NOS inhibitor 7-NINA to block autism-like NSA deficits induced by serotonin 1B agonist challenge in mice. NSA will be assessed by calculating average rearing duration (ARD) from open field test data. We hypothesize that the serotonin 1B agonist RU24969 will decrease ARD in mice compared to mice that receive vehicle treatment. We further hypothesize that atomoxetine and 7-NINA will attenuate these serotonin 1B agonist-induced NSA deficits. Finally, we hypothesize that RU24969 will induce hyperactivity, which is measured by total distance traveled in the open field, and that neither atomoxetine nor 7-NINA will affect this autism-like behavior.

**Method**

**Animals**

Male and female C57BL/6J breading harems were purchased from Jackson Laboratories (Bar Harbor, Maine) in order to generate experimental mice. The mice were housed in a
controlled-temperature colony room with a 12 hour dark-light cycle with food pellets and water present at all times. Mice were weaned at 3 weeks of age and housed with same-sex siblings. All behavioral testing occurred during the light phase and in mice 7-8 weeks of age. All procedures followed the National Institutes of Health laboratory animal care guidelines and were approved by Wittenberg University’s Institutional Animal Care and Use Committee. Forty-two mice total were tested, however, fourteen mice were excluded from the statistical analysis due to accidental opposite-sex housing during the post-wean period. Therefore, a total of 28 mice, 12 males and 16 females, were included in the analysis.

**Drugs**

5-methoxy-3 (1,2,3,6) tetrahydropyridin-4-yl-1H-indole (RU24969), neuronal isoform of nitric oxide synthase (n-NOS) 7-NINA, and atomoxetine were purchased from Tocris Bioscience (Minneapolis, MN). Drugs were dissolved in 0.9% saline and injected intraperitoneally (IP) at a volume of 5 mL/kg bodyweight with 1-cc syringes and 27 gauge needles. The vehicle consisted of a 0.9% saline solution. RU24969 was administered at 10mg/kg, 7-NINA was administered at 1mg/kg, and atomoxetine was administered at 2 mg/kg. Drug dose selection was based on the results of previous dose response studies of RU24969 (Dulawa & Geyer, 2000; Dulawa, Holick, Gundersen, & Hen, 2004), 7-NINA (Grammatikopoulos et al., 2002), and atomoxetine (Gould, Rukstalis, & Lewis, 2005).

**Apparatus**

**Open field test.** A 42 cm long x 42 cm wide x 30 cm high Plexiglas box (Omnitech Electronics, Columbus, OH) was used to measure the total distance traveled, duration, and number of rearing episodes. This apparatus contained infrared beams that automatically recorded the animal’s position in space on an x, y, and z axes. The Plexiglas box was covered by a
removable Plexiglas lid with air holes and was illuminated by a lamp a few feet above the apparatus. A removable white board covered the front side of the box, while two dark walls and a computer hard drive encased the other three sides of the box.

Procedure

After a weight measurement, mice were injected with vehicle or a putative ASD treatment (atomoxetine or 7-NINA) 30 minutes prior to behavioral testing and were then placed in a holding cage with clean bedding. Mice were then injected with vehicle or the autism-inducing treatment (RU24969) 5-10 minutes prior to testing, and were then placed back into their holding cages. Thus, each mouse received two injections on test day: a putative ASD treatment followed by the ASD-inducing treatment (Figure 1A). All injections were completed in a prep room separate from both the colony and behavioral testing rooms. Thirty minutes after the first injection, mice were individually placed in the open field for 10 minutes. After 10 minutes, the mouse was taken out of the open field and the apparatus was cleaned with a 10% ethanol solution. Mice were tested one at a time at a random order. After each testing period, the mice were placed back into their home cages in the colony room.

Each mouse was tested on 2 separate testing days with at least 48 hours between the tests to allow the drugs to washout of the animal’s system (Figure 1A). Drug conditions were counterbalanced. Drug groups were between-subjects for putative ASD treatments (vehicle, atomoxetine, or 7-NINA) and within-subject for ASD-inducing treatment (vehicle or RU24969).

Statistical methods

A three-way ANOVA test was used with the putative ASD treatment (vehicle, atomoxetine, or 7-NINA) as a between subjects independent variable, the ASD-inducing treatment (vehicle or RU24969) as a within-subjects independent variable, and sex as a between-
subjects variable. The dependent variables were average rearing duration (ARD) and total distance traveled (TDT). ARD was calculated by dividing total vertical time by the number of vertical episodes in the open field. Post hoc ANOVAs (with Bonferroni corrections) and Fisher’s post hoc tests were used to resolve significant interactions.

**Results**

Before the open field test, mice received a “putative treatment” injection (30 minutes before testing) of vehicle, 7-NINA, or atomoxetine and a “pretreatment” injection (5-10 minutes before testing) of vehicle or RU24969. Therefore, mice in this experiment belonged to one of six conditions: vehicle-putative treatment/vehicle-pretreatment (V/V), vehicle-putative treatment/RU24969-pretreatment (V/R), 7-NINA-putative treatment/vehicle-pretreatment (N/V), 7-NINA-putative treatment/RU24969-pretreatment (N/R), atomoxetine-putative treatment/vehicle-pretreatment (A/V), atomoxetine-putative treatment/RU24969-pretreatment (A/R).

RU24969 decreased average rearing duration (ARD) in mice across all putative drug treatments (vehicle, atomoxetine, and 7-NINA) \[F(2, 28)=4.42; p<0.001\] (Figure 2A). In the vehicle-putative treatment condition, mice injected with RU24969 (V/R mice) had reduced ARD compared in mice injected with vehicle (V/V mice) \[F(1,10)=97.50; p<0.001\] (Figure 2A). In the 7-NINA putative treatment conditions, mice injected with RU24969 (N/R mice) had reduced ARD compared to mice injected with vehicle (N/V mice) \[F(1,10)=49.83; p<0.001\] (Figure 2A). In the atomoxetine putative treatment condition, mice injected with RU24969 (A/R mice) had reduced ARD compared to mice injected with vehicle (A/V mice) \[F(1,8)=38.63; p<0.001\] (Figure 2A). Across all putative treatment conditions, there was no effect of sex on ARD \[F(1, 28)=0.10; p=ns\].
There was a significant main effect of putative treatment on ARD \([F(2,28)=4.42; p< 0.05]\) (Figure A2). Fisher’s post hoc tests reveals that in the vehicle pretreatment condition, mice injected with 7-NINA (N/V mice) did not exhibit ARD levels significantly different from the ARD in mice injected with vehicle (V/V mice), \([p = ns]\) (Figure 2A). Moreover, in the vehicle pre-treatment condition, Fisher’s post hoc tests revealed that mice injected with atomoxetine (A/V mice) were not significantly different than the ARD in mice injected with vehicle (V/V mice) \([p = ns]\) (Figure 2A). Similarly, in the vehicle pre-treatment condition, Fisher’s post hoc tests revealed that mice injected with 7-NINA (N/V mice) were not significantly different than the ARD in mice injected with vehicle (V/V mice) \([p = ns]\) (Figure 2A). In mice treated with the pretreatment RU24969, 7-NINA (N/R mice) did not exhibit significantly different ARD than mice injected with vehicle (V/R mice) \([p = ns]\) (Figure 2A). Fisher’s post hoc tests revealed that in the RU24969 pre-treatment condition, the mice injected with atomoxetine (A/R mice) had reduced ARD compared to mice injected with a vehicle (V/R mice) \([p< 0.05]\) (Figure 2A).

Across all putative treatment conditions, mice injected with RU24969 (V/R, N/R, and A/R mice) exhibited increased total distance traveled (TDT) compared to mice injected with vehicle (V/V, N/V, and A/V mice) \([F(1,28)=97.60; p< 0.001]\) (Figure 3A). No interaction of putative treatments (vehicle, 7-NINA, or atomoxetine) and pretreatment injection (vehicle or RU24969) were found with regard to TDT \([F(2,28)=0.66; p = ns]\) (Figure 3A). There was no significant interaction of sex and pretreatment injection on TDT \([F(2,28)=.05; p=ns]\).

**Discussion**

In this study, we found that serotonin 1B agonist treatment significantly reduced average rearing durations (ARD) in mice. These results suggest that the activation of serotonin 1B receptors induces non-selective attention (NSA) deficits. We also found that serotonin 1B
agonist treatment induced hyperactivity in mice. These findings replicate Lawson et al.’s (2014) findings that serotonin 1B agonist challenge induces NSA deficits and hyperactivity in mice. Serotonin 1B agonist challenge has previously been shown to induce core features of autism including social deficits and perseveration (Shanahan et al., 2011). These findings along with ours show that serotonin 1B receptor activation leads to both core and associated features of autism, and thus provide support for the validity of serotonin 1B challenge as a mouse pharmacological model of aspects of autism. Similarly, our research suggests that the serotonin 1B receptor may be involved in the anterior attention center because the activation of the serotonin 1B receptor affected NSA. Serotonin 1B agonist inhibits the release of serotonin and glutamate, which deactivates the frontal cortex (Sari, 2004), an area involved in the anterior attention center (Aspide et al., 2000; Grammatikopoulos et al., 2002). Therefore, the serotonin 1B agonist may work by deactivating the anterior attention center through the inhibition of serotonin and glutamate in the frontal cortex. However, more research is needed to indeed support that serotonin 1B agonist affects the anterior attention center.

In this study, we also showed that 7-NINA, a drug found to reverse NSA deficits in animal model of ADHD, did not attenuate NSA deficits in mice exhibiting serotonin 1B agonist-induced autism-like NSA deficits. The 7-NINA drug is currently only being tested pre-clinically, and our findings suggest that 7-NINA treatment may not be effective in treating attention deficits in ASD. Grammatikopoulos et al. (2002) explains that the decrease in nitric oxide (NO) levels induced by the selective inhibition of 7-NINA at the neuronal level might decrease dopamine (DA) neurotransmission in the midbrain and at target sites in the forebrain. However, dopamine agonists, which are psychostimulants, provide first-line treatment for ADHD. Thus, 7-NINA may reverse attentional deficits in ADHD mouse models by a mechanism independent of
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dopamine, such as the modulation of acetylcholine and glutamate (Grammatikopoulos et al., 2002). Our results show that the activation of the forebrain by 7-NINA did not reverse serotonin 1B agonist-induced NSA deficits in a mouse model of ASD. Thus, the neural substrates underlying attention deficits in ADHD and ASD may be distinct. The NO system may not be impaired in the ASD mouse model whereas it is impaired in ADHD mouse models. Indeed the NO system has been shown to be disordered in children with ADHD (Aspide et al., 1998) whereas more studies investigating the effect of the NO system in ASD are needed.

We also showed that atomoxetine, the first non-psychostimulant medication approved for treatment of ADHD, further decreased NSA in serotonin 1B agonist treated mice. This finding suggests that NSA deficits in ASD might be exacerbated by atomoxetine, and that atomoxetine would thus not be an effective treatment for the attentional deficits in ASD. This finding further suggests that atomoxetine influences serotonin 1B receptor activity, albeit not in a way that would be helpful to ASD patients. Atomoxetine is a norepinephrine (NE) reuptake inhibitor that increases extracellular NE levels throughout the brain. The cell bodies of NE neurons reside in the locus coruleus, and project all over the brain. In particular, NE neurons target the prefrontal cortex (Bymaster et al., 2002), an area of the brain’s anterior attention system and thus thought to be involved in NSA. Serotonin 1B receptors are also highly expressed in the frontal cortex so the increase in NE by atomoxetine could have further increased serotonin 1B receptor activity (Sari, 2004) and, in turn, caused greater NSA deficits. However, by itself, atomoxetine does not cause NSA deficits. Thus, the increase in NE levels only has an effect on NSA when it is coupled with the decrease in serotonin levels provoked by the serotonin 1B agonist. This complex pharmacological interaction will require further study to decipher. However, even though Atomoxetine did not treat the attentional deficits induced by serotonin 1B agonist in this
experiment does not mean it invalidated this model. Atomoxetine has not been approved as an affective medication by the FDA to treat attentional deficits in ASD patients without ADHD. Further studies are needed to determine the effectiveness of this medication in treating ASD.

As with 7-NINA, atomoxetine may not have worked in our animal model of autism because the neural substrates underlying attention deficits in animal models of ADHD may be significantly different than the nervous system in the serotonin 1B agonist animal model of ASD. However, Harfterkamp et al. (2009) found that atomoxetine treats attention deficits in children with ASD. Harfterkamp et al. (2009) found that children with ASD that took atomoxetine had improved hyperactivity-impulsivity and attention-deficit ratings compared to ASD children that took a placebo. It seems that atomoxetine may be an effective medication to treat symptoms of attentional deficits in ASD patients, but not in serotonin 1B agonist autism-like mice. Although much larger studies than this one are needed to establish atomoxetine’s efficacy in ASD, our model of the attentional deficits in ASD does not mimic this finding from the human literature.

Atomoxetine may have also exacerbated NSA attention deficits in serotonin 1B mice because both drugs decrease activation in the frontal cortex (Pliszka et al., 1996; Sari, 2004). For instance, NE reuptake inhibitors, such as atomoxetine, have been found to decrease the prefrontal cortex response (Pliszka et al., 1996). Similarly, the serotonin 1B agonist used in this experiment is found to decrease frontal cortex activity because it reduces the release of serotonin and glutamate (Sari, 2004). Yu-Feng et al. (2007) found that children with ADHD have decreased fMRI activation in the frontal cortex, which is an area in the anterior attention center (Aspide et al., 2000; Grammatikopoulos et al., 2002). Therefore, the deactivation of the frontal cortex by both atomoxetine and serotonin 1B agonist may have induced the NSA deficits.
Neither atomoxetine nor 7-NINA significantly affected baseline locomotion in healthy mice, which supports our hypothesis. Moreover, neither drug influenced serotonin 1B agonist-induced hyperactivity. This indicates that neither atomoxetine nor 7-NINA treated hyperactivity induced by the serotonin 1B agonist. The attention and hyperactivity deficits in ASD are not thought to be mediated by the same neural systems, and thus these results are perhaps not surprising. For example, currently medications exist for the treatment of perseverative hyperactivity in ASD, such as methylphenidate, amphetamine, and dextroamphetamine (Cheetham & Heal, 1993; Shanahan et al., 2009; Shanahan, Velez, Masten, & Dulawa, 2011); however, these treatments do not reverse the NSA deficits in ASD patients.

Atomoxetine and 7-NINA may have been effective treatments of attentional deficits in animal models of ADHD rather than in this model because they may be mediated by different neural pathways. The potentially different neural pathways that mediate attention may be a reflection of the inherently dissimilar attentional deficits ADHD and ASD patients exhibit. For instance, patients with ADHD tend to have deficits in sustained and selective attention (Tsal, Shalev, & Mevorach, 2005). Sustained attention deficits cause individuals with ADHD to have a gradual decline in attention to a task over time; while selective attention deficits cause difficulty focusing on the important stimuli and ignoring the insignificant stimuli (Tsal et al., 2005). On the contrary, Landry & Bryson (2004) and Townsend et al.’s (1999) showed that ASD patients do not necessarily become easily distracted like patients with ADHD. Rather, patients with ASD tend to hyper-fixate their attention on specific objects in their visual field, a deficit in NSA, which then causes them to have difficulty attending to many objects at once (Landy & Bryson, 2004; Townsend et al., 1999). Overall, the attentional deficits of ADHD and ASD seem different, and thus, may be a reason why atomoxetine and 7-NINA are effective at treating attentional
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deficits in animal models of ADHD rather than in animal models of ASD. However, further
studies are needed to determine the anatomical brain differences that underlie attention in ADHD
and ASD.

One main limitation in this study was that sex experimental mice were mistakenly housed
together at sexual maturity. All mice in opposite-sex cages after weaning (10 female and 4 male
mice) were removed from the study. As a result, we lost power to investigate the effect of sex in
each condition. This would have been an important facet to investigate since ASD is more
common in males. In further studies, we would be sure all lab techs correctly sex all mice. In
addition, to improve this study, we could have tested in perfectly quiet rooms. Just after
experimentation, mice were placed in the same room as the open-field test. The noises and
smells of these mice as well as various sounds made by the experimenter may have impacted this
test. Similarly, approximately 4 mice were tested 30 minutes past the start of their dark cycle,
which may have affected the wakefulness and attention of those mice. Despite these limitations,
these results replicated previous findings from Lawson et al. (2014) and Shanahan et al.’s (2011)
studies. Thus, it appears sufficient care was taken to prevent confounding variables from
influencing outcomes during behavioral tests.

Future studies should investigate the usefulness of other ADHD medications and other
drugs that affect attention and mobility in mice, such as I-NAME. Aspide et al. (2000) found that
I-NAME significantly increased NSA in ADHD-bred mice, which shows that it could potentially
treat NSA deficits in animal models of ASD. Moreover, we aim to replicate the results of this
study to confirm that atomoxetine indeed reduces NSA in autistic-like mice. Future studies
should also examine the effect of 7-NINA and atomoxetine in other autism animal models to
determine the effectiveness of their ability to treat NSA. Finally, larger clinical trials are needed
to investigate the effectiveness of atomoxetine in treating ASD attentional deficits. Larger
double-blind placebo-controlled clinical trials, similar to Harfterkamp et al.’s (2009) study, are
needed. Currently, Harfterkamp et al.’s (2009) study is the only one that shows that atomoxetine
improved attentional deficits in patients with ASD. Finding effective treatments for the
attentional deficits of ASD is vital because 45-80% of patients with ASD have difficulty
disengaging from visual stimuli across a horizontal field (Landry & Bryson, 2004; Townsend et
al., 1999). A novel medication may allow ASD patients to switch their attention between visual
objects more easily. Improved attention would likely assist ASD patients to simply complete
daily tasks that they typically struggle with, such as paying close attention to detail, maintaining
focus, and following directions (American Psychiatric Association, 2013; Leyfer et al., 2006).
Once an effective putative treatment is identified through animal models, researchers could then
test the drug in Landy and Bryson’s (2004) shifting attention and disengaging study and
Townsend et al. (1999)’s attention task.

Overall, in this study we found that serotonin 1B agonist is an effective way at inducing
NSA and increasing hyperactivity in mice. We also found that atomoxetine and 7-NINA did not
treat NSA deficits in autism-like serotonin 1B agonist mice. Unexpectedly, atomoxetine caused
an even greater deficit in NSA, which shows that it also activated the serotonin 1B receptor, most
likely in the frontal cortex. 7-NINA may have not been effective because the neural substrates
that underlie NSA deficits in animal models of ADHD may be significantly unlike the neural
substrates in the serotonin 1B agonist animal model of ASD. Further, atomoxetine may have not
been effective because it, as well as serotonin 1B agonist, deactivate the frontal cortex to the
point that it causes NSA deficits. Our findings suggest that atomoxetine and 7-NINA will not be
effective treatments for ASD, but that the serotonin1b-induced animal model will be useful in identifying novel treatments for ASD.
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Appendix

**Figure 1A.** Procedure and timing of injections and testing during 2 days of testing

**Putative Treatments Injection**
- 0 minutes

**Pretreatment Injection**
- 20 minutes

**Open Field Test**
- 30 minutes

- **Inject Vehicle, Atomoxetine, or 7-NINA**
  - *Day 2: Receive same injection*

- **Inject vehicle or RU24969**
  - *Day 2: Receive opposite injection*

- **A 10 minute test to measure ARD & TDT**
Figure 2A. The effect of pretreatment injection on average rearing time in the open field test; the effect of putative treatments on rearing time in the open field test, *$P<0.05$.

Figure 3A. The effect of pretreatment injection on total distance traveled in the open field test; the effect of putative treatments on total distance traveled in the open field test, *$P<0.05$.