ABSTRACT

Effects of Aniracetam, a cognition enhancer, in healthy subjects: A Placebo-Control, Double-Blind Investigation

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Aniracetam is a therapeutically useful cognition enhancer (nootropic) for alleviating anxiety and treating various neurodegenerative conditions. Aniracetam is shown to enhance both glutamatergic neurotransmission and long-term potentiation (LTP) formation. Previous studies of aniracetam have focused on acute administration in different models of disease and have demonstrated dramatic cognition-rescuing effects. We investigated if, given a daily oral regimen at a clinically established dose, cognitive performance could be elevated in healthy C57 Black 6 male mice. In a double-blind, placebo-control design, we investigated the performance-enhancing potential of aniracetam on a variety of aspects of cognitive behavior. This study is timely considering the growing community of laypeople self-administering nootropics. Our findings suggest that while aniracetam is clinically effective to reverse impairment it does not appear useful as a cognitive enhancer in unimpaired populations.

APPROVED BY DIRECTOR OF HONORS THESIS

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EFFECTS OF ANIRACETAM, A COGNITION ENHANCER, IN HEALTHY SUBJECTS: A PLACEBO-CONTROL, DOUBLE-BLIND INVESTIGATION

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By

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CHAPTER ONE

Introduction

Global interest in aniracetam (1-[(4-methoxyphenyl)]-2-pyrrolidinone), a prescription drug in most countries but unregulated in the USA, is spiking (see Fig. 1). Google Trends data correlates the surge in search interest for nootropics, "smart drugs," with the release of the March 2011 film Limitless (Burger [Director]). The film focuses on how a fictional drug "NZT," modeled after aniracetam, elevates the intelligence of a starving author and sweeps him from the depths of poverty to the heights of Wall Street. A Google search for the terms "aniracetam," "nootropics," or "smart drugs" now returns thousands of websites selling, reviewing, and praising the cognitive-enhancing efficacy of nootropic, cognition enhancing, drugs. A quick google search reveals that aniracetam is among the most popular in this class.

Pharmacologically, aniracetam, a piracetam analog, is an indirect dopaminergic, serotinergic, and acetylcholinergic agonist that exerts its agonistic effects as a positive allosteric modulator of Glutamate receptors GluA1-4 along with the AMPA and NMDA glutamate receptors (IUPHAR and Rao et al. 2001). Pharmacological studies confirm that post-synaptic nicotinic acetylcholine (nACh) receptors, dopamine D2 receptors, and Serotonin 2A receptors have elevated action (Nakamura & Kurwasa 2001) as a result of the NMDA glutamate receptor (Nakamura & Shirane, 2000). DA and 5-HT release is elevated by mediating the action of N-anisoyl-GABA, the principle metabolite of aniracetam, that targets not only somatodendritic nACh and NMDA receptors but also presynaptic nACh receptors (Shirane & Nakamura, 2001). Intracellular acetylcholine levels are indirectly elevated in the co-localized reticulothalamic glutaminergic/cholinergic pathway via enhanced group II metabotropic glutamate receptor activation. Stimulation of these neurons produce an intracellular increase of choline acetyltransferase (Nakamura & Shirane, 2000), yielding higher rates of formation and transmission of acetylcholine.

This pharmacological effect takes place in both limbic and higher cortical areas. Transmitter binding assays investigating acetylcholine, glutamate, and other transmitters by Nakamura (2004) found

elevated action of these transmitters in the amygdala, hippocampus, striatum and prefrontal cortex. These brain regions are traditionally associated with the various functions of learning and memory and, more broadly, the facilitation of consciousness and intelligence. Also of interest is the unchanged action of GABA, an inhibitory neurotransmitter. Taken together, the Japanese investigators show us that aniracetam produces a primarily excitatory effect (e.g. glutamate) which has secondary excitatory effects (e.g. the colocalized acetylcholine both synthesized and released as a result of the widespread glutamatergic actvity) without GABA suppression. Here then is the neurochemical basis for the above claim that cells that fire more wire more. Nakamura and Kawasawa's (2001) data indicates that at the very least the neurons are firing more.

Aniracetam has few reported side effects (Gouliaev and Senning 1994). There are several studies that provide evidence that Aniracetam can improve cognitive performance. Aniracetam improves visual recognition, motor performance, and general intellectual function in humans (Ingvar, Ambros-Ingerson et al., 1997). Another report found that aniracetam improves memory in humans that have cognitive impairment (Koliaki, Messini et al., 2012). Similar results supporting the cognitive enhancing benefits of aniracetam have been found using non-human animals (Cumin, Bandle et al., 1982; Spignoli and Pepeu 1987; Bartolini, Casamenti et al., 1996; Lu and Wehner 1997; Masuoka, Saito et al., 2008). In addition to the improvement in cognitive function, aniracetam reduces anxiety in mice (Nakamura and Kurasawa 2001). This study found that aniracetam reduced anxiety in three different anxiety tests and found similar results across three different strains of mice. The studies above are often referred to when aniracetam is marketed through various websites. There is a large and growing group of humans buying, selling, and self-administering nootropic substances on the basis of dated scientific information and no clear answer of whether or not nootropics are effective when used daily in healthy subjects. Our study is the first.

Previous studies demonstrated that the systemic administration of aniracetam improved behavioral performance in both rat and primate (Schwann et al. 1985). Rao (et al., 2001) found that aniracetam produced a strong positive correlation between the magnitude of hippocampal dentate-gyrus

LTP and performance in the Y-maze avoidance learning task, evidencing that aniracetam improves performance on memory-measuring tasks. This correlates nicely with the elevated activity of acetylcholine Nakamura et al. observed above. In conjunction with modern thought on LTP, that behavioral learning performance is positively influenced by the induction of LTP, it appears that aniracetam has a constructive impact on the formation of LTP (Ishihara and Nakamura, 1997). The Chinese studies effectually linked intrahippocampal AMPA activation by aniracetam and the induction of behavioral LTP (Rao et al. 2001).

In light of these behavioral, electrophsysiological, and pharmacological studies, we explored whether this elevated firing-wiring produces global changes in behavior. We investigated amygdalar, cerebellar, and hippocampal dependent learning as well as measures of anxiety and repetitive behavior. Our results indicate that these improvements do not occur when chronically administering aniracetam to healthy subjects.

CHAPTER TWO

Materials and Methods

Ethics Statement

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Institution of Animal Care and Use Committee of Baylor University.

Animals

Thirty adult male C57BL/6J mice between the ages of 3 and 6 months old at the start of experimentation were used in these experiments. Subjects were bred at Baylor University in Waco, Texas. Subjects were trained and tested in two groups of 15, staggering testing of the groups by one week.

Subjects were handled for approximately 90 seconds per day for five days to acclimate them to handing. Animals were individually housed in typical mouse cages with wood shavings in a humidity and temperature-controlled vivarium adjacent to testing rooms. A 12 hour light/dark cycle was maintained.

Aniracetam Treatment

Aniracetam (1-(4-Methoxybenzoyl)-2-pyrrolidinone) was obtained from Shanghai Soyoung Biotechnologies Inc. (Shanghai, China). In order to simulate consumption of aniracetam that would be used by humans, aniracetam was administered orally at 50 mg/kg in a mixture of 0.72% sucrose and 3.00% gelatin (St. Louis, MO, USA) matrix; the placebo group received a .72% sucrose and 3.00% gelatin matrix with no aniracetam, and the naïve control group received only an empty weigh boat. Sucrose was used to ensure appetitive consumption of the gelatin matrix. The gelatin matrix was prepared

fresh twice each week and animals were weighed at the beginning of the week to account for weight changes and to maintain precise dosing throughout testing. Animals were dosed one hour before testing each day and at the same time each day. This regimen allowed the animals thirty minutes to consume the gelatin and then thirty minutes rest once transported from the vivarium to the testing rooms. The consumption of the gelatin was verified by the experimenter before testing. All testing was conducted within a three-hour therapeutic window of administration, as determined by a previous pharmacokinetic investigation of aniracetam (Zhang et al., 2007). We utilizing a single-blind design experiment. One individual created all doses for the animals. A different person administered the doses to the animals but was blinded to the group of the animal. The person (different person than two mentioned previously) who performed the testing of the animals was blinded to the treatment of the animals.

Behavior Training and Testing

All 30 mice were tested on four learning tasks, two anxiety tasks, and one measure of repetitive behavior. After the completion of each task, animals received one day of rest. Because each task required one-to-five days the entire testing schedule was completed in six weeks. With the exception of marble-burying, rotorod, and odor discrimination, the performance of all animals was video-recorded and analyzed with video tracking software. Different experimenters were responsible for training and testing each group of animals, with one beginning one week after the other. No experimenter was aware of animals' performance on other tasks until the entire battery of behavioral tests, for both testing cohorts, was completed. Before and after each testing session, apparatuses were cleaned with a 30% isopropanol solution. To ensure the time of day did not affect performance, all animals were tested during the middle seven hours of the light cycle and all procedures were conducted as temporally proximate as possible.

Open-Field Exploration. Animals were placed in the placed in the center of a 40X40X40 cm clear acrylic container box in a well-lit room with consistent lighting and noise. Their behavior was video-monitored via Noldus Ethovision XT (Noldus, The Netherlands) motion-tracking software for 10 minutes. The open-field test was used to measure locomotion and to measure anxiety. We examined the time the animal spent in the outer and inner zones.

Elevated-Plus Maze. The elevated plus maze was used to examine anxiety. The maze consists of four elevated arms (40 cm from floor). There were 2 sets of equidistant arms that are 65cm long and 5cm wide. Two of the opposing arms were open and two opposing arms were enclosed by 15 cm high walls. The apparatus was constructed out of white acrylic and located in a well-lit room with constant light and background noise. Mice were placed in one of the open arms near the center of the maze at the beginning of the trial. The animals' movement was monitored via motion-tracking software for 10 minutes. We used a separate video capturing system to record the videos to be scored at a later time. Time spent in the enclosed arms is associated with anxious behavior and time spent in the open arms is associated with non-anxious behavior.

Morris Water Maze. The Morris Water Maze was used to measure hippocampal-dependent spatial learning. In this task, animals were placed in a round pool (diameter ≈ 130cm, height ≈60 cm) of opaque water from which they can escape onto a submerged, hidden platform. The pool was located in a dimly-lit room with constant light and noise and had extra-maze spatial landmarks (i.e. multicolored geometric shapes) placed in consistent locations on the walls. The round pool was filled to within 18cm of the top with water made opaque by the addition of non-toxic, water soluble paint. The maze was divided into four quadrants defined by the cardinal directions (i.e. North, East, South, West); a hidden platform was submerged 2 cm beneath the water's surface and consistently located in the same quadrant of the water maze. The animal had a maximum time of 1 minute to find the submerged platform. If the animal did not escape the maze via the hidden platform, then the animal was placed on the submerged platform for 10

seconds. If the animal successfully escaped the maze, it was allowed to remain on the platform for 10 seconds before being removed for the next trial. Each animal received 4 trials per block, two blocks per day, with four days of testing. On day five we conducted a visible platform trail. We placed a visible platform in two regions of the maze which had not previously contained the hidden platform. We then measured the latency to the visible platform for all animals. We conducted 2 trials per block across 2 blocks for a total of 4 trials. This visible platform test was conducted to determine motor deficits or visual deficits in the mice. All testing was analyzed via Noldus Ethovision XT motion-tracking software.

Rotorod. We used an accelerating rotorod test (Series 8 Rotorod; IITC Inc., Woodland Hills, CA, USA) in order to examine cerebellar motor memory. In this experiment, animals were placed on a rotating rod which gradually rotated from 5 to 40 rpm over the five minute trial. Animals underwent four days of testing with two trials per day with a 60 min ITI. The experimenter live-scored the quantity of time each animal was able to stay on the rotating rod before falling off.

Odor Discrimination. Adapting a three-day protocol developed previously (Sara et al., 1996), mice learn to navigate a square field in which unique odor-marked (e.g. coffee, almond, banana) food cups are located in three corners. We used a clear acrylic 40cm X 40cm X 40cm chamber. Three 7.5 X 5 cm (diameter, height) cups made of black aluminum mesh were placed in three corners of the field. Food reinforcers (pea-sized amounts of chocolate-flavored puffed rice) were placed in small weigh boats. The food in two of the weigh boats was covered by the black mesh cups so that it was not accessible to the animal; in the third cup (the "target" cup), the food was accessible and could be consumed. A cotton-tipped laboratory swab, attached to the corner-facing portion of each cup, was extended vertically 5cm from the surface of the cups. Odorants (McCormick flavor extract) were prepared fresh on test day at a 1:100 dilution. Immediately before each trial, swabs were dipped in the odorant solution to ensure strong olfactory stimuli for the animal. The coffee odor was always associated with the target food cup.

On day one, food was removed from the animals home cage. On day two (acclimation day) the food-deprived animals were placed in the test arena for 20 minutes with no food cups present. At the end of that day's light cycle, animals were introduced to the novel reinforcer in their home cage (10 pea-sized amounts of chocolate-flavored puffed rice). On day three (test day) animals underwent four trials in the field with the three food cups present. On the first trial, the reinforcer was available in a double-portion (two pea-sized amounts) to the animal in the cup marked by the coffee odor. This was to ensure that the food-deprived animal learned to associate the odor with the accessibility of food. The trial persisted until the animal retrieved and consumed the food from the cup. Once the animal completed the trial, it remained in the field for an additional 20 seconds and then returned to its home cage for a six minute inter-trial interval (ITI). The location of the food cups were rearranged on trials 2-4 but the baited "target" cup remained consistently marked by the coffee odor. Both the corner location of the coffee odor and its position relative to the other odors were changed each trial.

On each trial the time (latency) to retrieve and consume the food and number of errors, were live-recorded by the experimenter. An error was defined as any time that an animal made contact with an incorrect cup or attempted to poke inaccessible food with its nose. An error was also recorded when an animal sampled the "target" food but did not consume it. Errors served as the dependent analysis to circumvent the complication of differences in the speed of locomotion of each animal.

Marble Burying. In this measure of stereotyped, repetitive behavior clear plastic cages were filled with 4 cm of cage bedding. Twenty black glass marbles were placed in five columns of four rows at evenly spaced intervals. The animal was placed in the clear plastic cage with the evenly spaced marbles. Animals were allowed 30 minutes in the cage and the number of marbles buried at least two-thirds buried in bedding was recorded.

Classical Conditioning. Utilizing a two-day protocol, animals on the first day were placed in an operant-chamber housed within an isolation cubicle (Coulbourn Instruments, Allentown, PA, USA) which prevented external light or sound from entering the operant-chamber. The operant-chamber was illuminated by an interior light providing constant luminescence (2 lux) and contained a shock-grid floor and a speaker. On day one animals were placed in the chamber and five minutes of baseline activity was recorded. Then a conditioning stimulus (CS) (85dB white-noise tone) was presented for 20 seconds. At the end of the CS a two second unconditioned stimulus (US) (.75 milliamp mild foot shock) was presented. There were two CS-US pairings per animals the first day.

To assess this form of amygdala-dependent learning, we used a novel context minus foot shock on day two. In the novel context, a clear Plexiglas square was placed over the shock grid (proving a novel tactile context) and 20 ml of pure vanilla extract (Adam's Extracts) was placed in the chamber, beneath the floor (novel olfactory context). On day two we first recorded the animal's freezing in the new context for 3 minutes. We then presented the CS for 3 minutes. Throughout all testing the time spent freezing was recorded by the software.

CHAPTER THREE

Figures

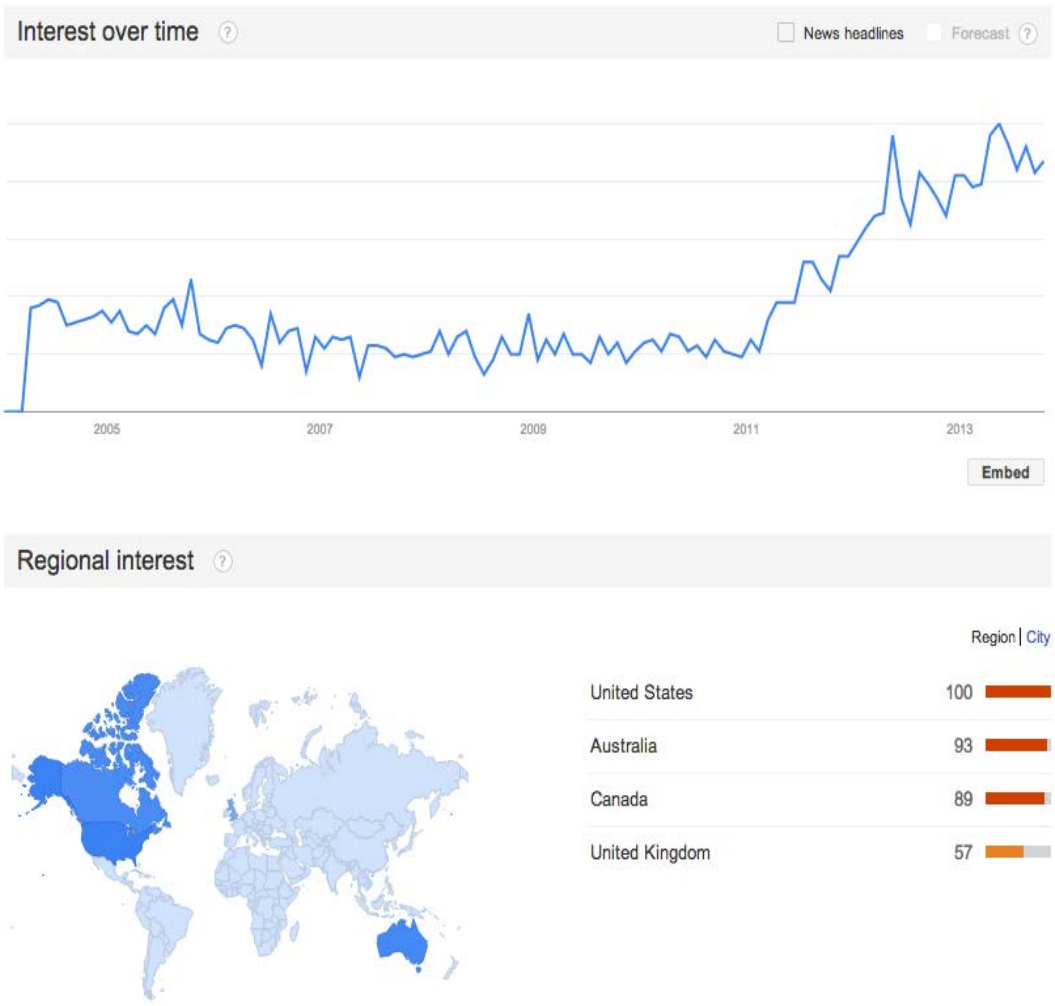


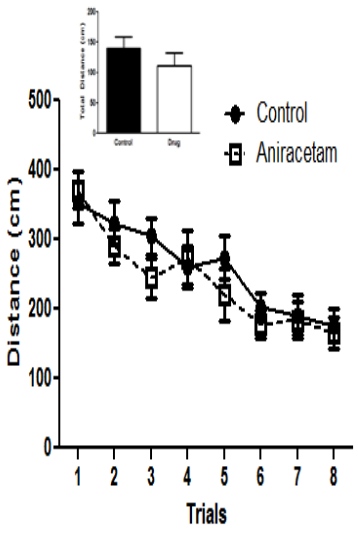
Figure 1.

October 2013 Google Trends data for the search term “nootropics”. The spike in early 2011 correlates with the release of the film Limitless which prominently probes the possibilities of a drug (“NZT”) which could unlock human cerebral potential. The sustained slope indicates a sustained interest by humans across the world.

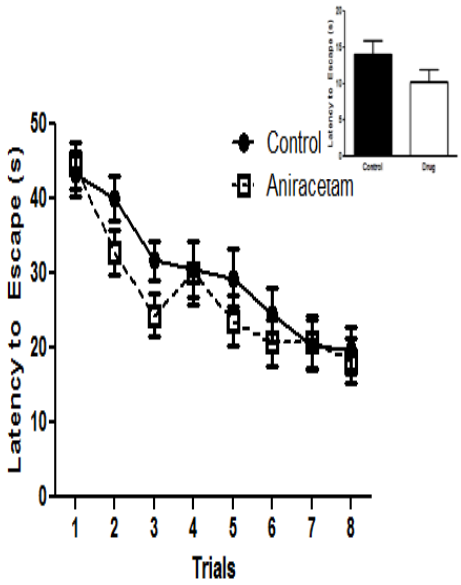
Aniracetam does not enhance hippocampus, amygdala, or cerebellum dependent learning and memory.

Morris Water Maze

A.

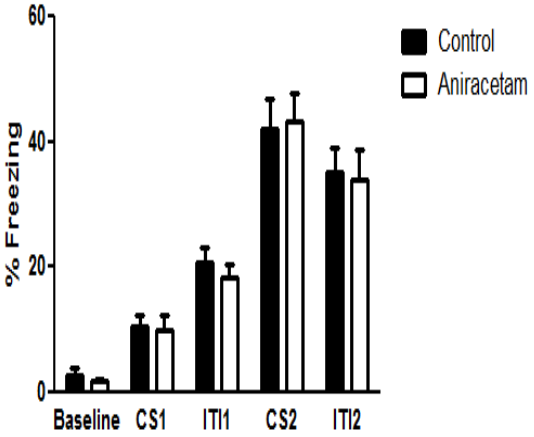


B.



Fear Conditioning

C.



Rotarod

D.

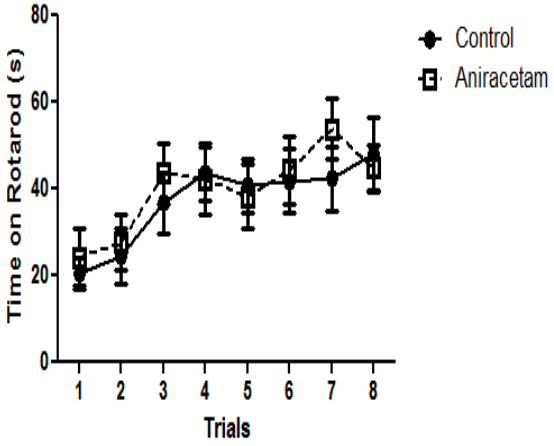


Figure 2.

To probe hippocampal dependent memory we used the Morris Water Maze. There was no significant difference in the learning curves between the groups in either the distance swam to the hidden platform (A) or the latency to escape the maze (B). We used two day, novel context fear conditioning to examine amygdala dependent learning and memory. Across all measures, there was no significant difference between control and drug groups (C). The rotorod was employed to investigate cerebellum dependent memory; no significant difference between the groups was found in the time the animals were able to stay on the rotating rod (D). Taken together, these results show that aniracetam does not enhance spatial, emotional, or motor memory systems despite enhancing the pharmacological profile associated with elevated activity in these brain regions (Nakamura and Kurasawa, 2001; Nakamura and Shirane, 2001; Yu and Cai, 2003).

Aniracetam does not reduce anxiety in healthy subjects

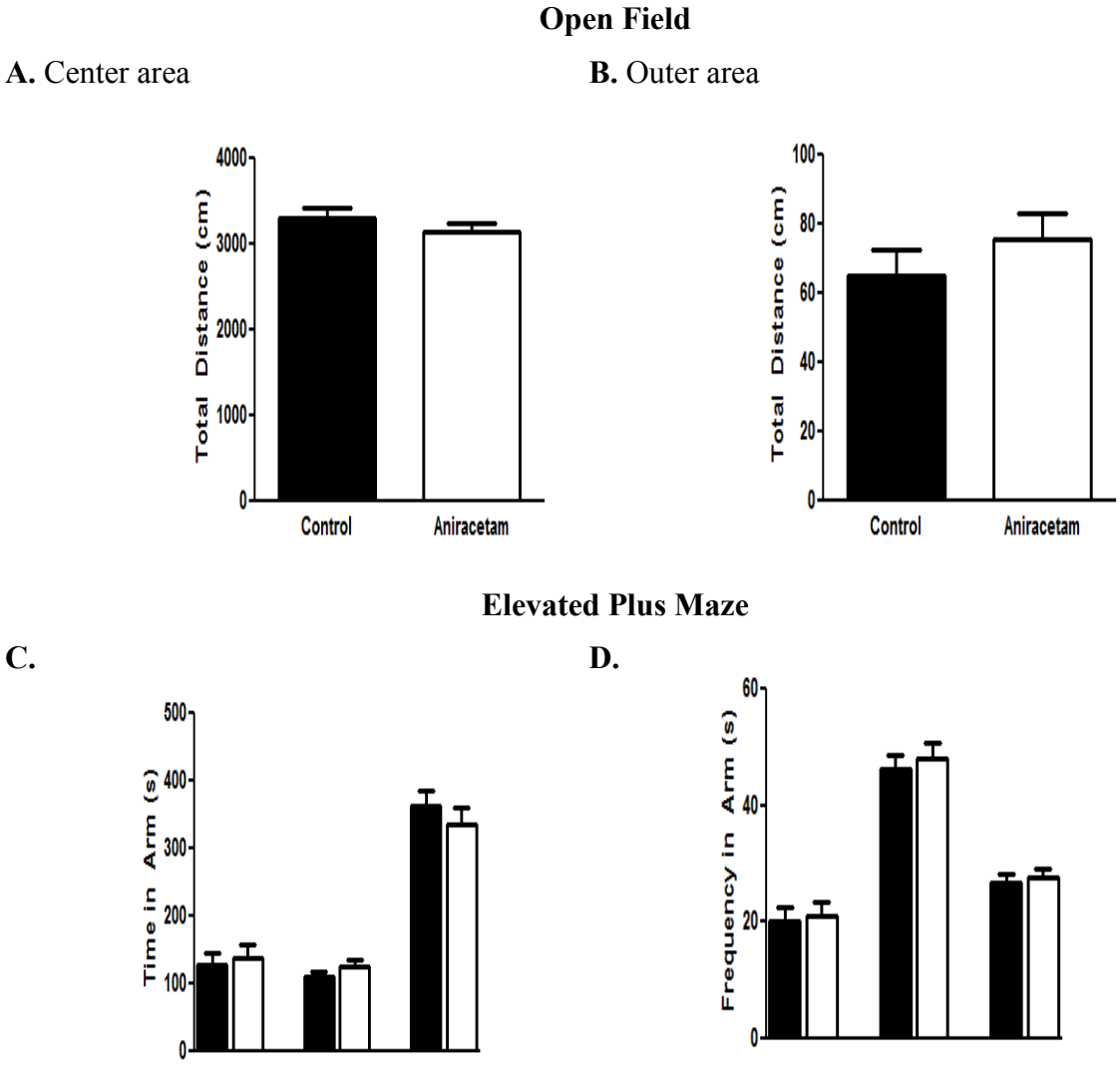


Figure 3.

To examine whether aniracetam produces alterations in anxiety we examined male mouse behavior in the automated open field test and in the elevated plus maze test. In the open field there was no significant difference between the groups in either the time spent in the center (A) or the surrounding area (B). In the elevated plus maze there was no significant difference between the groups in either the time spent in the open arms (C) or the close arms (D). These results are among the most surprising in light of Nakamura and Kurasawa (2001) which demonstrated robust anxiolytic effects with aniracetam in the elevated plus maze and other measures. However, their study focused on impaired subjects.

Aniracetam does not affect executive function or stereotyped behavior.

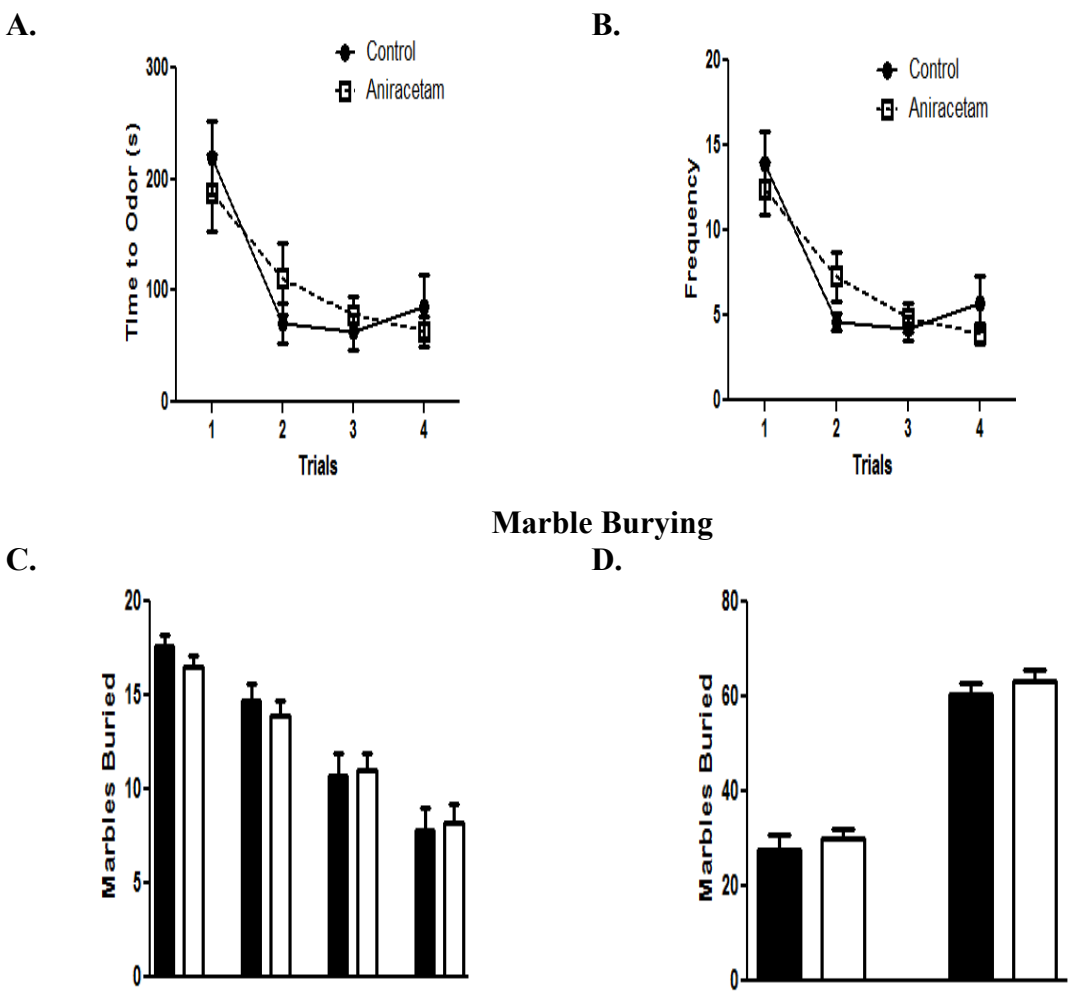


Figure 4.

We used odor discrimination to probe executive function. Adapting a three-day protocol developed by Sara (Sara et al., 1996), mice rapidly learn to navigate a square field in which unique odor-marked (e.g. coffee, almond, banana) food cups are located in three corners. Despite the food being available in all three cups, the food is accessible in only one cup (i.e. the one marked with the coffee odor). Over a course of four trials on a single test day, a food-deprived animal is placed in the empty corner and will explore the field until it retrieves the single piece of available food. The dependent measures were the time to target odor and the frequency of errors (errors were defined as interacting with the wrong cup and encountering but not eating from the target cup. There was no significant difference between the learning curves of the control and drug groups (A and B). Because aniracetam is a stimulant, we used marble burying to investigate any effects on stereotyped behavior. In this measure of stereotyped, repetitive behavior clear plastic cages were filled 75% with sawdust. Twenty black glass marbles were placed in five columns of four rows at evenly spaced intervals. The animal was placed in the clear plastic cage with the evenly spaced marbles. Animals were allowed 15 minutes in the cage and at the end of the 15 min trial the number of marbles at least two-thirds buried in sawdust was recorded. There was no significant difference between the groups.

CHAPTER FOUR

Results

Aniracetam does not alter activity levels or anxiety in the open field test. There were no differences in locomotion or anxiety between the control group and aniracetam-treated group in the open field test. An independent t-test found no difference between the group in total distance moved in 10 minutes $t(1,28) = 1.05$, $p = 0.3$. An independent t-test found no difference between the groups in total distance moved in the center of the open field test in the ten minute test $t(1,28) = 0.99$, $p = 0.33$. There was no difference in number of feces produced in the open field test with 1.73 ± 0.43 (mean ± standard error of the mean) for the control group and 1.8 ± 0.34 for the experimental group.

We then examined whether Aniracetam treatment altered levels of anxiety. It had previously been reported that aniracetam treatment altered anxiety in three strains of mice (Nakamura and Kurasawa, 2001). We found no difference in time or frequency in the open arms, center, or closed arms. An increase in time in the open arms would have suggested that there was a decrease in anxiety. An independent measures t-test found no difference in time in the open arm $t(1,28) = 0.44$, $p = 0.66$; center arm $t(1,28) = 1.23$, $p = 0.23$; or closed arms $t(1,28) = 0.86$, $p = 0.40$. We found similar results in the frequency in each arm with no difference in time in the open arm $t(1,28) = 0.22$, $p = 0.83$; center arm $t(1,28) = 0.44$, $p = 0.66$; or closed arms $t(1,28) = 0.43$, $p = 0.67$. There was no difference in total distance moved in the plus maze test $t(1,28) = 1.06$, $p = 0.30$. One interesting finding is that there was a difference in the number of feces produced in the ten minute plus maze $t(1,28) = 2.08$, $p < 0.05$. The number of feces produced by

the two groups was 1.28 ± 0.48 (mean ± standard error of the mean) for the control group and 0.73 ± 0.31 for the experimental group. However, we did not find this difference in the open field test.

Aniracetam does not alter motor learning. We then examined whether aniracetam-treatment altered cerebellar learning. We used an accelerating rotorod test to examine differences in motor learning. We used a mixed-design ANOVA to examine differences between the two groups over the 8 trials. There were 2 trials per day over 4 days with an hour between the daily trials. We did not find a difference between the groups $F(1, 28) = 0.10$, $p = 0.75$ (Figure 3). The within-subjects analysis did not reveal a group x trial interaction $F(7,196) = 0.69$, $p = 0.68$. However, there was a main effect of trial $F(1,7) = 9.69$, $p < 0.001$. Therefore both groups did demonstrate improvement in their ability to stay on the accelerating rotorod across the eight trials, but there was no difference between the two groups over the 8 trials.

Aniracetam does not alter spatial learning and memory. We then examined Aniracetam treatment altered hippocampus-dependent learning. The Morris Water Maze is a classic test to measure spatial learning and memory. We have previously published results using this protocol to investigate learning and memory changes in mice (Lugo, Brewster et al., 2012). We did not observe any change in learning between the control and drug treatment across the learning trials or in the probe test. There was no difference in distance traveled between the control and drug treated group $F(1,28) = 0.89$, $p = 0.35$ (Figure 4A). There was no interaction between group and trial $F(7,196) = 0.59$, $p = 0.76$. However, both groups showed improvement in their ability to find the hidden platform across the eight trials $F(7,196) = 2.64$, $p < 0.001$. We found similar

results when examined the latency to find the hidden platform across the 8 trials. There was no difference between the groups $F(1,28) = 1.21$, $p = 0.28$ (Figure 4A). There was no interaction between group and trial $F(7,196) = 0.74$, $p = 0.63$. However, both groups showed improvement in their ability to find the hidden platform across the eight trials $F(7,196) = 3.93$, $p < 0.001$. The results from the distance to find the hidden platform and latency to find hidden platform suggest that aniracetam -treatment does not improve spatial learning in mice. We examined whether there were difference in the ability to find a visible platform. There were no differences between the group in terms of distance to reach the visible platform $t(1,28) = 1.0$, $p = 0.32$; or in the latency to find the hidden platform $t(1,28) = 1.45$, $p = 0.15$.

Aniracetam does not alter repetitive behavior. We then examined whether treatment with Aniracetam altered repetitive behavior in mice. We examined repetitive behavior through a 30 minute marble burying test. We did not observe any statistical differences between the groups across the different measures of marbles buried. There was no difference in burying the marble when measured at: 50% $t(1,28) = 1.2$, $p = 0.24$; 75% $t(1,28) = 0.57$, $p = 0.57$; 100% $t(1,28) = 0.17$, $p = 0.86$; or at the level of completely burying them so that they were not visible $t(1,28) = 0.25$, $p = 0.80$. It does not appear that treatment with aniracetam alters repetitive behavior in mice.

Aniracetam does not alter associative conditioning. In addition to examine whether Aniracetam treatment altered motor behavior (rotarod) or spatial learning and memory (Morris water maze) we also examined whether anircetam altered the ability of the animal to condition to a tone when presented with an aversive stimulus. We examined control and Aniracetam-treated mice in delay

fear conditioning. In this test the animal is presented with a tone which is then paired with an aversive stimulus (shock). Within two trials the mice will form this association and when presented with the conditioned stimulus (tone) they will show an increase in freezing. We did not observe any difference of the mice to learn the tone on day one of conditioning (Figure 5A). There was no main effect of group across the difference conditioning trials $F(1,28) = 0.08$, $p = 0.77$. There was no interaction between group over the different testing parameters $F(4,112) = 0.11$, $p = .98$. There was a main effect of learning over the trials $F(28, 112) = 2.14$, $p < 0.01$. Therefore both groups do show an increase in freezing over the different aspects of the first conditioning day. We then examined their freezing levels when presented in a novel context for three minutes and found no difference in freezing $t(1,28) = 0.64$, $p = 0.52$. When we presented the conditioned stimulus in the new context both groups showed an increase in freezing to the tone. However, there was no difference between the groups in the amount of freezing when presented with the tone $t(1,28) = 0.86$, $p = 0.39$. These results demonstrate that treatment with Aniracetam did not enhance associative conditioning.

Aniracetam does not alter odor discrimination learning. We used a two-way ANOVA and found no main effect of group $F(1,27) = 0.001$, $p = 0.98$ for the effect over time. There was a main effect of trial $F(3,81) = 14.12$, $p < 0.001$. There was no group x trial effect $F(3,81) = 0.98$, $p = 0.41$. Similar results were found when investigating frequency of errors over time. We used a two-way ANOVA and found no main effect of group $F(1,27) = 0.001$, $p = 0.98$ for the effect over time. There was a main effect of trial $F(3,81) = 21.55$, $p < 0.001$. There was no group x trial effect $F(3,81) = 1.4$, $p = 0.25$.

CHAPTER FIVE

Discussion

When designing the experiment we wanted certain degrees of similarity to and difference from the above noted investigations. The similarities, dosing levels, route of administration, and several behavioral tasks (open field and elevated plus maze), were implemented as proverbial "meter sticks" so as to provide a head-to-head comparison of the differences. The differences, everything else, were chosen to probe the wider swath of behavior previously uninvestigated.

We demonstrated that aniracetam does not enhance cognitive performance in healthy subjects across a variety of behavioral tasks measuring spatial and motor learning and memory, anxiety, repetitive behavior, and executive function (Morris water maze, rota-rod, open field, elevated plus-maze, marble burying, odor discrimination) when chronically administered (PO) a clinically established dose (50mg/kg) suspended in gelatin. Aniracetam is shown to positively impact the pharmacological profile associated with learning and memory, elevating hippocampal acetylcholine, serotonin, glutamate, and dopamine levels (Nakamura and Kurasawa, 2001; Nakamura and Shirane, 2001; Yu and Cai, 2003). Given that aniracetam has been elsewhere demonstrated to significantly facilitate LTP formation in the hippocampus (Rao et al., 2001), reverse memory loss (Martin et al., 1994), reduce anxiety (Nakamura and Kurasawa, 2001), reverse ethanol-induced brain damage (Wijayawardhane et al., 2008), increase the rate of pharmacodynamic tolerance (Rial et al., 2009), significantly improve psychobehavioral measures in human Alzheimer's disease patients (Senin et al., 1991) and is effectively blocked by haloperidol (Nakamura and Shirane, 2001) our results are surprising. An important caveat to the above behavioral studies is that they were all performed in rodent models of disease and did not

explicitly address impacts in healthy subjects. Our investigation was the first clear and comprehensive study of the effects of aniracetam across a variety of behaviors in healthy subjects.

The evidence is clear that aniracetam is effective for enhancing cognitive performance in impaired (i.e. brain damaged) subjects but ineffective in healthy subjects. We will now consider potential sources of the variability between diseased and healthy subjects. Because aniracetam is agonistic primarily for glutamatergic receptors, one implication is that in diseased subjects the glutaminergic transmitter system is deficient and in healthy subjects it is not. This suggests that healthy subjects enjoy a glutaminergic-pharmacological homeostasis which acts as a sort of therapeutic threshold, implying that drugs targeting this system are limited in efficacy to the degree to which the receptor system is occupied. Aniracetam will occupy receptors in the deficit model because there are receptors available but cannot occupy them in the healthy model because in a healthy subject the glutaminergic receptors are already fully occupied at some pharmacodynamic homeostasis: aniracetam is able to neither allosterically bind nor exert an effect. Considering the extremely high resting levels of glutamate in the mammalian CNS, 5-10mmol/kg (Butcher & Hamberger, 1987), about a thousand times higher than other important neurotransmitters such as dopamine, norepinephrine, and serotonin (Sheldon & Robinson, 2007), and that excessive, persistent activation of glutamate receptors often kills neurons expressing these receptors (Meldrum & Garthwaite, 1990), this pharmacodynamic homeostatic threshold hypothesis has some empirical basis. Because glutamate is concomitantly ubiquitous and toxic it follows that the activation, endogenously or otherwise, of those receptors would be closely regulated in healthy subjects and dsyregulated in impaired subjects. Future investigations might probe other aspects of the pharmacodynamic homeostasis hypothesis epigenetically by relating

rates of experience-dependent gene expression to rates of pharmacodynamic trafficking and other sub-surface to surface related rates of change.

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