EFFECTS OF BDNF ANTAGONIST ANA-12 ON RATS' USE OF SPATIAL LEARNING STRATEGIES

by

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Abstract

Prior research has implicated brain-derived neurotropic factor (BDNF) in synaptic plasticity in the hippocampus and in forms of spatial learning and memory that depend on this brain system. Much of this evidence has been indirect, however, and relatively few behavioral studies have directly manipulated BDNF or the receptor that binds it, tropomyosin receptor kinase B (Trk B). The present study examined the role of BDNF in spatial learning by investigating the effects of ANA-12, a noncompetitive antagonist for the Trk B receptor, on spatial learning in a T-maze. Rats received 0.5 or 1.0 mg/kg ANA-12 or saline 4 hours before training on a T-maze task that could be solved using either a hippocampal-dependent place strategy or a striatum-dependent response strategy. After reaching criterion, the rats received a probe trial in which the two strategies were pitted against one another. Administration of ANA-12 did not impair rats' ability to learn or perform the T-maze task, but it did cause dose-dependent decreases in their use of place strategies on the probe trial. These results support the idea that BDNF and the Trk B receptor play an important role in spatial learning and memory.

Keywords: brain-derived neurotropic factor (BDNF), spatial learning strategies, ANA-12, response learning, place learning

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Chapter 1 - Introduction

Brain-derived neurotropic factor (BDNF) is a neurotrophin, a substance that helps to regulate development of the nervous system and maintain the nervous system's normal functioning in vertebrates (Huang & Reichardt, 2001). The first member of the family of neurotrophins discovered was nerve growth factor (Levi-Montalcini & Hamburger, 1951). BDNF was the second member of the family of neurotrophins to be discovered (Barde, Lindsay, Monard, & Thoenen, 1978). A later discovery of BDNF identified it as an important factor in the survival and outgrowth of developing neurons (Barde, Edgar, & Thoenen, 1982). Subsequent studies showed that BDNF plays a key role in the *in vivo* development of dorsal roots (Kalcheim, Barde, Thoenen, & Douarin, 1987), and that BDNF prevents *in vivo* apoptosis in both the central nervous system and the peripheral nervous system (Hofer & Barde, 1988). BDNF has also been shown to aid in the differentiation of function and the survival of a variety of neural cells in cultures, including retinal ganglion cells (Johnson, Barde, Schwab, & Thoenen, 1986) and septal cholinergic neurons (Alderson, Alterman, Barde, & Lindsay, 1990). Thus, BDNF plays several important roles in the normal development of the nervous system.

BDNF also appears to play an important role in normal functioning of the adult nervous system. Infusions of BDNF into the dentate gyrus of the adult rat hippocampus, for example, increased neurogenesis in that part of the brain (Scharfman et al., 2005). Similarly, Falkenberg and colleagues demonstrated that glutaminergic activation of entorhinal cortex enhanced the expression of mRNA for BDNF and its metabotropic receptor (tropomyosin receptor kinase B, Trk B) in the dentate gyrus (Falkenberg, Ernfors, Persson, & Lindefors, 1992; Falkenberg, Mohammed, Henriksson, Persson, Winblad, & Lindefors, 1992). Lindholm, Dechant, Heisenberg, and Thoenen (1993) also demonstrated that activation of glutamate receptors

increased mRNA expression of BDNF in cultured cerebellar granule cells, and that BDNF protected such cells against apoptosis caused by high glutamate concentrations. Behavioral experiences in adults can also affect expression of BDNF; Marmigère, Givalois, Arancibia, and Tapia-Arancibia (2003) demonstrated that exposing rats to immobilization stress caused a rapid increase in expression of mRNA for BDNF in the hippocampus. As is the case during development, the evidence suggests that BDNF plays multiple roles in the functioning of the adult nervous system.

BDNF has been shown to play an important role in the normal functioning of the hippocampus. Research has demonstrated that there are distinctly different types of learning and memory that depend on anatomically distinct neural systems (e.g., Nadel, 1992; Schacter & Tulving, 1994; White & MacDonald, 2002) and that the hippocampus is a critical part of a system that mediates spatial learning and cognitive mapping (O'Keefe & Nadel, 1978). BDNF has been shown to play an important role in recovery from stressors that impair hippocampal function. Ortiz et al., (2014) demonstrated that chronic restraint stress impaired spatial reference memory in a radial arm maze task when rats were tested shortly after the end of the stressor, but not after a 21-day recovery period. This recovery of spatial learning failed to happen if rats received treatments that decreased BDNF levels in dorsal hippocampus during the recovery period. They later extended this finding by showing that chronic stress decreased apical dendritic complexity of hippocampal CA3 neurons in dorsal hippocampus, and that decreasing BDNF levels in this region or administering ANA-12, an antagonist for the Trk B receptor, prevented the recovery in dendritic morphology and spatial learning (Ortiz et al., 2018). BDNF is important for the normal functioning of the hippocampus.

Other evidence suggests that BDNF may play an even more direct role in mediating learning and memory that involves the hippocampus. A number of studies have shown that training on spatial or contextual learning tasks that involve the hippocampus also change levels of BDNF in this system (Hall, Thomas, & Everitt, 2000; Harvey et al., 2008; Kesslak, So, Choi, Cotman, & Gomez-Pinilla, 1998). Manipulations that impair spatial or contextual learning tasks that involve the hippocampus have also been shown to alter BDNF levels in this system. van Praag et al. (1997), for example, demonstrated that hippocampal lesions that impair spatial learning decreased BDNF levels in hippocampus. Chen, Kitanishi, Ikeda, Matsuki, and Yamada (2007) showed that contextual conditioning increased BDNF expression in CA1 neurons of hippocampus, and that administration of an N-methyl-D-aspartate (NMDA) receptor antagonist that blocked this contextual conditioning also blocked the increase in BDNF expression in CA1 neurons. Heldt, Stanek, Chhatwal, and Ressler (2007) provided more direct evidence that BDNF in hippocampus is involved in spatial learning by showing that selectively knocking out the BDNF gene in dorsal hippocampus of adult animals decreased BDNF levels in hippocampus and impaired place learning in the water maze.

The idea that BDNF is important for hippocampal learning and memory has been reinforced by studies showing that BDNF plays an important role in long-term potentiation (LTP) in the hippocampus. LTP refers to the observation that high frequency stimulation of inputs to many parts of hippocampus can cause a long-lasting increase in the strength of the synaptic connection between those inputs and their hippocampal targets (cf. Bliss & Lømo, 1973). LTP in hippocampus displays a number of properties (e.g., longevity, selectivity) that have made it a popular and widely studied cellular model of learning and memory, and much has been learned about the mechanisms that initiate LTP (Nicoll, 2017). A variety of evidence has

implicated BDNF in the changes that give rise to LTP. Patterson, Grover, Schwartzkroin, and Bothwell (1992), for example, demonstrated that stimulation that induced LTP in the CA1 region of the hippocampus also induced synaptic expression of BDNF in that area. More direct evidence for a role for BDNF in LTP has come from gene knockout studies in mice. Minichiello et al. (1999) demonstrated that rats lacking the TrkB receptor for BDNF in adult forebrain were deficient in LTP in hippocampus. Similarly, mice engineered to be deficient in BDNF production are deficient in LTP, but this deficit can be reversed by application of recombinant BDNF (Patterson et al., 1996). BDNF has also been shown to play a critical role in establishing corticostriatal LTP (Jia, Gall, & Lynch, 2010; Park, Popescu, & Poo, 2014). BDNF thus appears to be involved in synaptic plasticity in a variety of systems and appears to be important for both short- and long-lasting forms of LTP in the hippocampus (Lu, Christian, & Lu, 2008).

The goal of the present study was to provide more direct evidence of a role for BDNF in hippocampal learning and memory. The present report took advantage of the recent development of a non-competitive antagonist for the Trk B receptor, ANA-12 (Cazorla et al., 2011), to study the role of this receptor in spatial learning and memory. Rats were trained on a T-maze position discrimination task in which they were rewarded for choosing a specific goal arm on the maze. There were two obvious ways that the rats could learn to perform this task. The rat could learn to choose the correct goal arm by learning to approach the location on the maze that holds the food, a “place” strategy that involves the hippocampus or by learning to make a particular body turn when it leaves the start arm of the maze, a “response” strategy that involves the striatum (Packard & McGaugh, 1996; White & MacDonald, 2002). During initial training, these two strategies were redundant and either could be used to successfully perform the task. Thus, even if one form of learning was impaired by a treatment, the other strategy could be used to

successfully perform the task. Once the rat had reached criterion on the T-maze task, it received a probe trial that pit the two strategies against one another by rotating the maze 180°. This forced the rat to choose between returning to the same location on the maze (which requires it to make a different body turn), or making the same body turn as before and going to a different location on the maze. This made it possible to determine which of the two strategies the rat was using to guide its behavior on the probe trial.

Prior research using this paradigm has shown that NMDA antagonists that impair LTP selectively decreased rats' use of place strategies on the T-maze position discrimination task but did not affect their ability to learn the task (Mackes & Willner, 2006). The aim of the present study was to investigate the role BDNF played in different types of learning, specifically the role BDNF plays in different types of spatial learning. The experiment used a between-subjects design that consisted of three conditions of drug dose: vehicle injection of saline, a moderate dose of ANA-12 (0.5 ml/kg) in a saline solution, and a high dose of ANA-12 (1 ml/kg) in a saline solution. If BDNF is involved in the changes that give rise to spatial learning and memory, blocking the receptor activated by BDNF should decrease rats' use of place strategies in a dose-dependent fashion without impairing their ability to learn or perform the position discrimination task. The study primarily investigated three dependent variables: the spatial strategy used at a probe trial, the latency for the rat to choose an arm, and the overall number of training trials needed to reach criterion. We expect that the rats' ability to learn or perform the T-maze task would not be impaired but may decrease the likelihood that rats would use a place strategy to solve the T-maze task. The strategy each rat utilized to remember and choose a goal arm may differ such that rats administered different doses of ANA-12 would display dose-dependent decreases of utilization of place strategy while rats administered vehicle would display utilization

of a place strategy. The decreased likelihood of use of place strategy would provide evidence that BDNF plays a role in the process of spatial learning in rats.

All procedures were approved by the Radford University's Institutional Animal Care and Use Committee and abide by the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011).

Chapter 2 - Methods

Subjects

A minimum of 48 male, Long-Evans rats obtained from Charles River Laboratories or the Radford University Psychology Behavioral and Cognitive Neuroscience (BACoN) vivarium breeding colony served as subjects for the study. Rats obtained from Charles River Laboratories were delivered to the BACoN vivarium at approximately postnatal day (PND) 50 and housed in same-sex group housing in an isolation room until PND 65. Rats bred in the BACoN vivarium were weaned from their mothers at PND 22, sexed at approximately PND 34, and housed in same-sex group housing until PND 65. All rats were handled and weighed daily during PND 55-65. Beginning on PND 66, rats were single housed in barrier cages with an experimental partner for the duration of the experiment. The rats were weighed daily during the duration of the experiment and always had free access to water. The housing room was maintained at a relatively constant temperature (ave. 70°F +/- 10°) and humidity (between 30% and 80%) on a 12-hour light/dark cycle (08:00/20:00). All training and testing occurred during the light portion of the light/dark cycle. Behavior testing began shortly after the rats had reached adulthood, with rats tested between PND 75 and 105.

Apparatus

Training and testing occurred on a wooden radial-arm maze painted flat white, with three arms arranged in a “T” configuration around a central platform 30 cm in diameter. All three wooden arms (62 cm long x 12 cm wide) contained a recessed food well (2.5 cm diameter, 1 cm depth), located 2.5 cm from the end of the arm. The arms and the central platform of the maze were surrounded by a 4 cm high wood wall. The surface of the maze stood 53 cm above a wooden platform (145 x 145 x 28 cm) on which it was placed. The maze was located in a

windowless room (3.5 x 3.4 m) that contained a variety of cues surrounding the maze, including a chair for the experimenter, a table with extra food, a white noise machine, vinegar/water cleaning solution, wall posters, a shelving unit holding miscellaneous objects, a cart and a clear holding cage containing corncob bedding used to transport the rat to and from the testing room, various metal switch plates on the walls, a door, and a video camera on the ceiling above the maze.

Procedure

Drug Preparation and Administration. ANA-12 was prepared by suspending appropriate amounts of the drug in 0.9% saline (Ortiz et al., 2018) to create a 1 mg/ml solution. The solution was sonicated for one hour and then aliquoted into vials and frozen at -20° C until used. The 0.5 mg/kg dose of ANA 12 was prepared by creating aliquots containing 0.5 mg ANA-12 in .5 ml saline, and adding .5 ml saline to the aliquot prior to use. Drug and saline solutions were vortexed immediately before each injection to ensure even suspension of the drug in the solution. All injections were made at a constant volume of 1 ml/kg, with rats administered intraperitoneal injections of 0.5 mg/kg, 1 mg/kg ANA-12, or 0.9% saline 4 hours before the start of all exposure and training sessions on the maze. This time interval was chosen based on values from previous studies that have used this drug (Cazorla et al., 2011; Leggio et al., 2014).

Behavioral Testing. The experiment was conducted as a series of replications, with an equal number of rats from each of the three conditions tested in each replication. The rats were handled and weighed daily for a minimum of 10 days before being gradually reduced to 85-90% of their free-feeding weights. One to 2 days prior to first exposure to the maze, rats received 1 gram of Froot Loops in their home cages to habituate to the Froot Loops. Rats were randomly assigned to one of three groups that differ in the dose of drug (Vehicle, 0.5 mg/kg ANA-12, or

1.0 mg/kg ANA-12) the day before their first exposure to the maze. After the first initial cohorts, rats were semi-randomly assigned to groups to counterbalance weight differences. For rats bred in the vivarium, litter mates were semi-randomly assigned to the three conditions of the study. Rats experienced 3 to 6 days of maze exposure (2 days of habituation and 1-4 days of training). Intraperitoneal injections were administered 4 hours prior to all maze exposure, training, and testing sessions, for a total of three to six injections, depending upon which day the rat achieved criterion.

Rats initially received 2 days of exposure to the maze. Each day, the rat was placed on the maze and allowed to explore the maze for two, 10-minute sessions, with a 30-minute interval between sessions. The rats were adapted to eat on the maze by placing pieces of Froot Loop cereal (cut into thirds) on the two arms that will be used as goal arms during training. For both sessions on Day 1, one reinforcer (Froot Loop third) was placed in the food well of each goal arm, with five additional reinforcers lining the arm, spaced 10 cm apart. Three additional reinforcers were evenly distributed on the center platform leading to the goal arms. On Day 2, both goal arms contained three reinforcers spaced 20 cm apart and a reinforcer placed in the food well. The time a rat spent in each goal arm during each of the four sessions across the 2 days of exposure and the number of reinforcers consumed was recorded, as were the number of boli left on the maze. All exposure sessions were recorded in AnyMaze video tracking software (Stoelting Co, Wood Dale, IL) for analysis, and were also recorded to DVR.

The amount of time a rat spent in each of the goal arms was used to determine that rat's arm preference. A rat was considered to have an arm preference if the total time spent in one goal arm across the four exposure sessions exceeded the time spent in the other goal arm by 60 seconds. If a rat was identified as having an arm preference, the rat was assigned the arm

opposite its initial preference as its goal arm for training. Goal arms were randomly assigned for rats that did not show a side preference, with the stipulation that arm assignments were counterbalanced across rats that did not show an arm preference within a condition.

Individual T-maze training for each rat began the day after the second day of exposure sessions. Before each training session, each rat was brought into the training room in a transparent plastic holding cage containing corn-cob bedding and allowed to acclimate to the room for 5 minutes. Each rat received a series of 20 trials per day in which it was rewarded with a third of a Froot Loop for choosing its assigned goal arm. On each trial, the rat was placed on the start arm, facing away from the center platform, and allowed 60 seconds to choose and enter a goal arm (defined as all four paws in a goal arm). The rat's latency to enter an arm and its arm choice were recorded on each trial. If the rat chose the correct goal arm, it was allowed to eat the reinforcer in the food well at the end of the arm before being returned to its holding cage for a 60-second intertrial interval (ITI). If the rat chose the incorrect goal arm during the first six trials, it was gently picked up and placed in front of the food well in the correct arm and allowed to eat the Froot Loop. After the first six trials, if the rat chose the incorrect goal arm, a non-correction procedure was used. The rat was removed from the goal arm after it reached the end of the arm and returned to its cage for a 60-sec ITI. The rat was immediately removed from the maze if it entered the incorrect goal arm and then tried to exit the arm and enter the correct goal arm. If the rat did not enter a goal arm within 60 seconds, the rat was removed from the maze, an entry of "no choice" was recorded, and the trial counted as an error. Between trials, the maze was cleaned with a sponge soaked in 10% vinegar/water solution, and the goal arm was rebaited.

Rats were trained until they met the criterion of seven correct choices in a set of eight consecutive trials. Each day's training ended after 20 trials, unless the rat was in the midst of a

potential criterion run, in which case it was given the opportunity to reach criterion. Therefore, if a rat started a criterion run on the 20th trial, the maximum number of trials that rat was able to run that day was 27 trials with a possible 28th trial, if seven of those eight trials were correct. Rats were given a maximum of 4 days of training and 80 trials to reach criterion. If rats did not achieve criterion by the end of the fourth day of training, the rat was discarded from the study, and the rat's data was excluded from analysis.

Once a rat had reached criterion, the rat was placed in the holding cage for a 60 sec ITI. The rat then received a probe trial with the maze rotated 180° relative to the maze's original orientation. The rat therefore started the probe trial 180° away from its original starting location in the room. All cues in the room, including the experimenter, remained in their original locations within the room. The rat was allowed 180 seconds to choose and enter a goal arm and eat the reinforcer at the end of the arm on the probe trial (both goal arms were baited). Based on the rat's initial choice of arm, it was classified as having used a place strategy if it made a different body turn and went to the original location of the reward in the room. The rat's choice was classified as a response strategy choice if it made the same body turn as it did during training and went to a different location in the room. Rats receiving ANA-12 during training sessions were under the influence of the drug at the time of the probe trial.

Tissue Collection. Animals were sacrificed by transcardial perfusions or overdose of sodium pentobarbital, and perfused animals' brains were saved for future use by other researchers. However, analysis of the brains was not a part of the current thesis project.

Statistical Analyses

Depending on the nature of the dependent variable, data were analyzed using repeated-measures analysis of variance (ANOVA), chi-square test of independence, one-way ANOVA, or

logistic regression. The number of animals to be included in the study was determined by a power analysis for a chi-square test of independence with three conditions. Sixteen rats per condition was determined to detect a large effect with a predicted power of .67. All analyses used an alpha of .05.

Chapter 3 - Results

A total of three rats (two from the vehicle condition and one from the 0.5 mg/kg condition) were excluded from the study for failing to reach the learning criterion after 80+ trials of training across the 4 days of training on the maze. The exclusion of the three rats led to a final sample size of 54 rats in the study, with 17 rats in the vehicle condition, 18 rats in the 0.5 mg/kg ANA-12 condition, and 19 rats in the 1.0 mg/kg ANA-12 condition.

Maze Habituation

Figure 1 illustrates the average number of boli left on the T-maze by each group across the four maze habituation sessions. As shown in the figure, the ANA-12 1.0 condition's average number of boli decreased from the first to second session on each of the 2 days. Conversely, the vehicle group's average number of boli increased from the first to second session on both days. For the ANA-12 0.5 condition, their average number of boli increased from the first to second session on Day 1 but decreased from the first to second session on Day 2. A repeated-measures ANOVA with Drug Condition, Day, and Session as factors failed to reveal main effects of Drug Condition, $F(2,51) = 0.606$, $p = .549$, Day, $F(2,51) = 0.223$, $p = .639$, or Session, $F(2,51) = 0.475$, $p = .494$ for number of boli left on the maze. There was, however, a significant Drug Condition x Session interaction, $F(2,51) = 5.504$, $p < .01$, and the Day x Session interaction also approached significance, $F(2,51) = 3.159$, $p = 0.081$. Neither the Drug Condition x Day interaction ($F(2,51) = 1.337$, $p = .272$) nor the Drug Condition x Day x Session interaction ($F(2,51) = 1.57$, $p = .216$) was significant. The Drug Condition x Session interaction was further analyzed by calculating separate repeated-measures ANOVAs for each drug group across the sessions, using a common error term. These analyses revealed that the rats in the vehicle condition left significantly more boli on the maze in Session 2 than they did in Session 1 [F(1,51)

= 4.159, p = .046], rats in the ANA-12 .05 group left similar numbers of boli on the maze during the two sessions [F(1, 51) = 1.100, p = .299], while rats in the ANA-12 1.0 condition significantly decreased the number of boli left on the maze between Session 1 and 2 [F(1,51) = 4.40, p = .040]. If the number of boli left on the maze is viewed as a measure of emotionality, these data suggest that ANA-12 may alter temporal patterns of emotionality during maze exploration.

Figure 2 illustrates the average percentage of Froot Loops each group consumed across the four habituation sessions. The graph plots percentage of Froot Loops consumed because different numbers of Froot Loops were placed on the T-maze on Days 1 and 2. As is apparent from the figure, there was no difference in the number of Froot Loops that rats in the different conditions consumed across sessions, though there is a suggestion that both groups given ANA-12 may have been a little less likely to consume all the Froot Loops on their first exposure to the maze. A repeated-measures ANOVA with Drug Condition, Day, and Session as factors failed to reveal main effects of Drug Condition, F(2,51) = 1.276, p = .288, or Day, F(2,51) = .998, p = .323 for percentage of Froot Loops consumed on the maze. The main effect of Session approached significance, F(2,51) = 3.857, p = .055, but none of the interactions involving drug condition approached significance, Day x Condition, F(2,51) = 0.682, p = .510, Session x Condition, F(2,51) = 0.270, p = .765, or Day x Session x Condition, F(2,51) = 0.480, p = .621. There was a significant Day x Session interaction, however, F(2,51) = 5.131, p = .028. Inspection of Figure 2 suggests that the interaction was due to slightly lower levels of decreased consumption by all three groups on their first exposure to the maze. However, drug treatment did not have any obvious effect on rats' readiness to eat on the maze.

Most of the rats in the study exhibited a side preference for the left arm of the T-maze, which was the arm farthest from the entrance to the testing room. The percentage of rats in each group who exhibited a left-side preference was similar across the drug conditions with 82.4% of rats in the vehicle group, 77.8% of rats in the ANA-12 0.5 group, and 84.2% of the rats in the ANA-12 1.0 group preferring the left side. Chi-square test of independence revealed no significant difference among the groups in their side preference, X^2 (2, N = 54) = 3.130, $p <$ .536. Overall, it appears that ANA-12 had little or no effect on rats' side preferences on the maze.

T-maze Acquisition

Figure 3a presents each condition's average latency to choose an arm across the first seven training trials (the minimum number of trials required to reach criterion), while Figure 3b presents average choice latency across all training trials for the rats in each group. Although the figures suggest that drug administration increased rats' arm choice latencies, the differences among the groups were relatively small. A one-way ANOVA on average choice latency across the first seven training trials failed to reveal a significant effect of drug condition, $F(2,51) = 0.413$, $p = .664$. Similarly, a one-way ANOVA on choice latencies across all trials also failed to reveal an effect of drug treatment on arm choice latencies, $F(2,51) = 0.247$, $p = .782$. Drug administration did not have any obvious effects on how long it took rats to choose a goal arm during training.

Figure 4 shows the mean number of trials it took each condition to reach the learning criterion of seven correct choices in a set of eight trials. Although it looks like the ANA-12 0.5 condition took slightly more trials to reach criterion than the other conditions, the within-groups variability was large, and the average difference between groups was not significant. A one-way

ANOVA failed to demonstrate any significant between-group differences in number of trials to criterion, $F(2,51) = 0.796$, $p = .457$. ANA-12 therefore did not affect how long it took rats to make arm choices during training, or how many trials they needed to reach a learning criterion. ANA-12 does not appear to negatively impact rats' ability to learn the T-maze position discrimination task.

Probe Trial

Figure 5 shows each condition's average latency to choose a goal arm on the probe trial. The figure suggests that rats in the ANA-12 1.0 condition took somewhat longer to choose a goal arm during the probe trial. A one-way ANOVA on the latency data showed that this between-groups difference in probe trial latencies approached, but did not reach conventional level of significance, $F(2,51) = 2.512$, $p = .091$.

Figure 6 illustrates the percentage of animals in each condition that was classified as using a place learning strategy on the probe trial. As is apparent by looking at the graph, the percentage of rats that used a place strategy on the probe trial decreased as a function of drug dose. A logistic regression was conducted to investigate the effect of drug dose on the likelihood that the rats would exhibit place or response learning. The effect of drug dose was indeed significant, X^2 (1, N = 54) = 5.877, $p = .015$. ANA-12 thus caused a dose-dependent decrease in rats' utilization of place learning strategies on the T-maze position discrimination task.

Chapter 4 - Discussion

A variety of evidence has implicated BDNF and its associated Trk B receptor in learning and memory processes. Electrophysiological studies have shown that BDNF plays a role in long-term potentiation (LTP) in hippocampus, a widely studied cellular model of learning and memory. Stimulation that induces LTP in CA1, for example, also increases expression of BDNF in that region (Patterson et al., 1992). Manipulations that decrease BDNF or the number of Trk B receptors in hippocampus, on the other hand, impair LTP in hippocampus (Minichiello et al., 1999; Patterson et al., 1996). More recent studies have begun to delineate the role played by BDNF in development and maintenance of LTP. Researchers now distinguish two different forms of LTP, an early LTP (E-LTP) that lasts 1-2 hours and does not require new protein synthesis, and a late-LTP (L-LTP) that is longer lasting (8 hr+) and that does require new protein synthesis (Lu et al., 2008). Studies have implicated BDNF in both forms of LTP, but it appears to be particularly important in regulating the biochemical processes that give rise to L-LTP (Pang & Lu, 2004; Lu et al., 2008). Indeed, application of BDNF to the dentate gyrus *in vivo* can induce a form of LTP that closely resembles L-LTP (Messaoudi, Ying, Croll, & Bramham, 2002). Activation of the Trk B receptor induces multiple biochemical changes in cells, so there is still much to be learned about which of these changes is critical for producing L-LTP in hippocampus (Panja & Bramham, 2014).

In addition to its role in LTP, behavioral studies have also supported a role for BDNF in learning and memory. Correlational studies have shown that training on spatial or contextual learning tasks that involve the hippocampus is associated with hippocampal-specific increases in levels of BDNF (Hall et al., 2000; Harvey et al., 2008; Kesslak et al., 1998). Other studies have shown that lowering levels of BDNF in hippocampus through lesions (van Praag et al., 2007) or

gene deletion techniques (Heldt et al., 2007) will impair spatial learning in the Morris water maze. Although these data are consistent with the idea that BDNF is important for hippocampal-dependent learning processes, it should be noted that most of these studies are correlational and do not demonstrate a causal role for BDNF in hippocampal learning. Relatively few behavioral studies have attempted to directly manipulate BDNF or the Trk B receptor.

The present results provide more direct evidence in support of the idea that BDNF and the Trk B receptor are important for spatial learning and memory. Here, rats were given the Trk B receptor antagonist ANA-12 or saline 4 hours prior to training on a T-maze position discrimination task that could be solved using either place or response strategies. Rats were trained to a criterion of seven correct in a set of eight trials, and then given a probe trial that pitted the two strategies against one another. Administration of ANA-12 before training sessions caused dose-dependent decreases in rats' use of place strategies on the probe trial administered at the end of training. However, rats given ANA-12 prior to training did not differ from controls in how long it took them to learn the task. Interference with the functioning of the Trk B receptor decreased rats' use of place strategies even when it did not impair their ability to learn a T-maze position discrimination.

Before concluding that the decreased use of place strategies seen in the present study is due to a deficit in place learning, it is important to consider some potential alternative explanations for the results that were obtained. It seems unlikely that the decreased use of place strategies seen in rats given ANA-12 can be explained by changes in motivation or gross changes in sensory or motor functioning. If changes in motivation or deficits in sensory or motor functioning were responsible for the decrease in rats' use of place strategies, it is reasonable to

expect that such changes would also have impaired their ability to learn or perform the basic T-maze task. This clearly was not the case.

Another possibility is that the decreased use of place strategies by rats given ANA-12 may be due to anxiolytic properties of the drug. Cazorla et al. (2011) reported that animals given ANA-12 spent more time in the open arms of an elevated plus maze than controls did, suggesting that ANA-12 has anxiolytic properties (also, see Azogu, Liang, & Playmondon, 2018). Although ANA-12 may have anxiolytic properties, we did not see much evidence of this in the current study. There was no overall difference among the drug conditions in the number of boli left on the maze during maze exposure. Although the Session x Drug interaction for boli was significant, the interaction appears to reflect changes in the pattern of release among the groups, and not changes in the number of boli released by the different groups. Similarly, the groups did not differ in Froot Loop consumption during the maze exposure sessions, suggesting roughly equivalent levels of anxiety among the groups. This makes it rather unlikely that the dose-dependent decrease in rats' use of place strategies seen here can be reduced in any simple fashion to differences among the groups in levels of anxiety.

Although the acquisition data from the current study argue against gross deficits in sensory or motor functioning as an explanation for rats' decreased use of place strategies after receiving ANA-12, it does not rule out more subtle deficits in sensory processing as a basis for the results reported here. One possibility is that ANA-12 impaired rats' ability to perceive or discriminate cues distal to the maze. Restle (1957) argued that cues distal to a maze were especially important for place learning, whereas cues proximate to a maze were more important for response learning. In this way, what appears to be a selective deficit in place learning in the present study could be explained as a selective deficit in perception of distal cues. Although such

an account cannot be ruled out by our data, this alternative account does have the virtue of being testable and could be examined by looking at the effect of ANA-12 on rats' visual acuity and ability to discriminate distal cues.

In addition to ruling out this alternative interpretation of the present results, there are a number of other questions that remain to be answered about the effects of ANA-12 on brain and behavior. More pharmacodynamic studies, for example, are needed to establish a proper dose-response curve for the effects of ANA-12 on LTP and spatial learning, and to establish the time frame over which ANA-12 acts to affect the brain. Similarly, microinjection studies in which ANA-12 is localized to particular brain structures are needed to establish where in the brain ANA-12 is acting to produce its effects on spatial learning. Visual acuity should also be investigated as well as distal cue discrimination.

One final issue that needs to be pursued is to investigate the effects of ANA-12 on spatial learning in females. The present study did not include any females, and there are grounds for thinking that ANA-12 might have different effects in males and females. Gaulin and Fitzgerald (1986, 1989) showed sex differences among male and female voles dependent on the geographical range needed for the mating type the voles employed. Additional research should be conducted to examine the impact of BDNF on spatial learning in females as previous research has shown that ovariectomized female rats perform better than ovary-intact females on place learning in the Morris water maze task where place learning is the objective (Daniel, Roberts, & Dohanich, 1999). This suggests that ovarian hormones may play a role in spatial learning. Further research should explore the interaction between ovarian hormones and BDNF on utilization of spatial learning strategies.

In conclusion, findings from the present study demonstrated that administration of ANA-12 to male rats decreased their use of place strategies on a T-maze position discrimination task without impairing their acquisition of the task. These results support the idea that BDNF and its Trk B receptor play an important role in synaptic plasticity and are important for spatial learning and memory.

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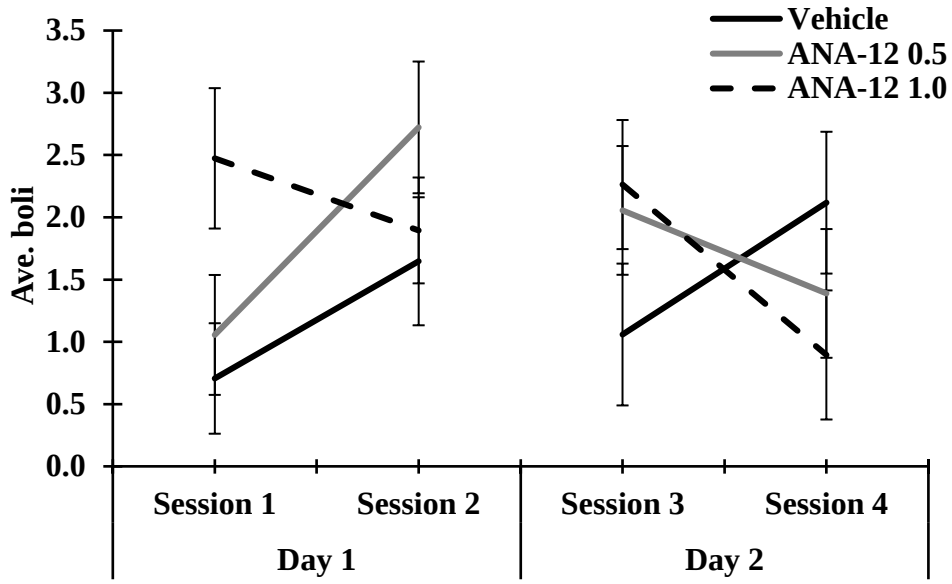

Figure 1. Average number of boli (+ s.e.m.) left on the T-maze during each of the four habituation trials for each drug condition.

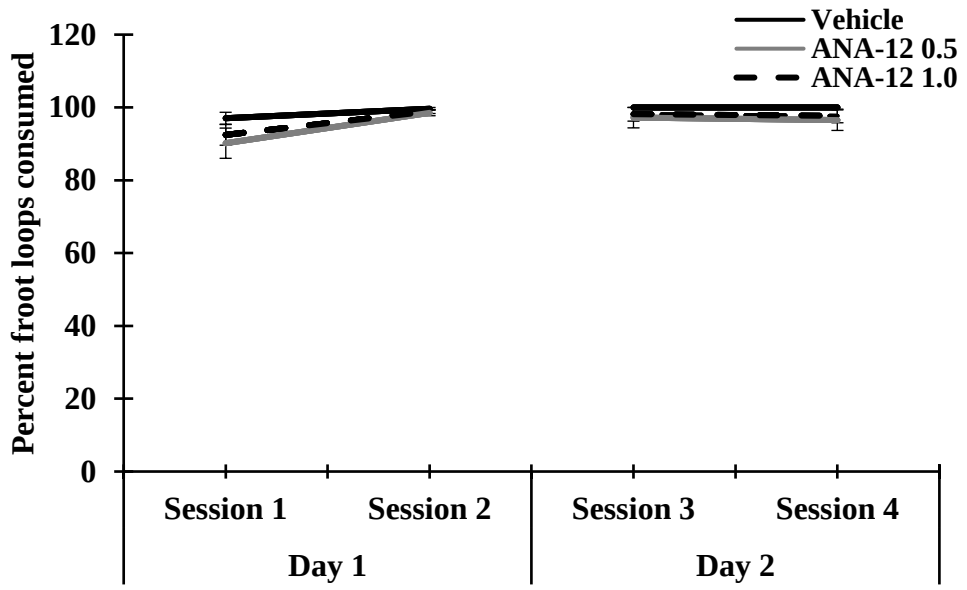

Figure 2. The average percentage of Froot Loops (+ s.e.m.) consumed on the T-maze during each of the four habituation trials for each drug condition.

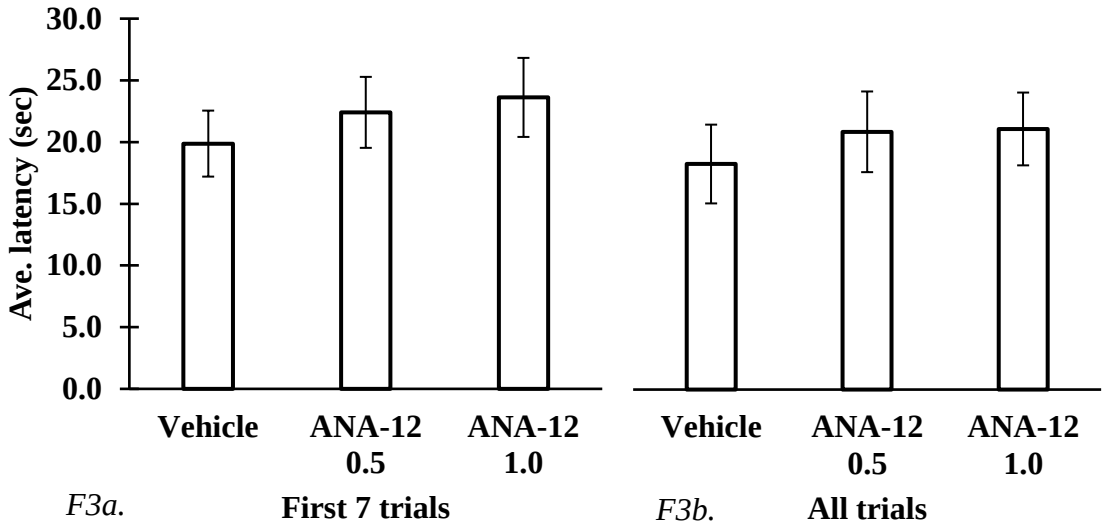

Figure 3. The average latency for the rat to choose an arm (+ s.e.m.) during acquisition for all drug conditions. *Figure 3a*. The left-hand panel shows each drug condition's average latency to make an arm choice for the first seven trials of acquisition. *Figure 3b.* The right-hand panel shows each drug condition's average latency to make an arm choice across all trials of acquisition.

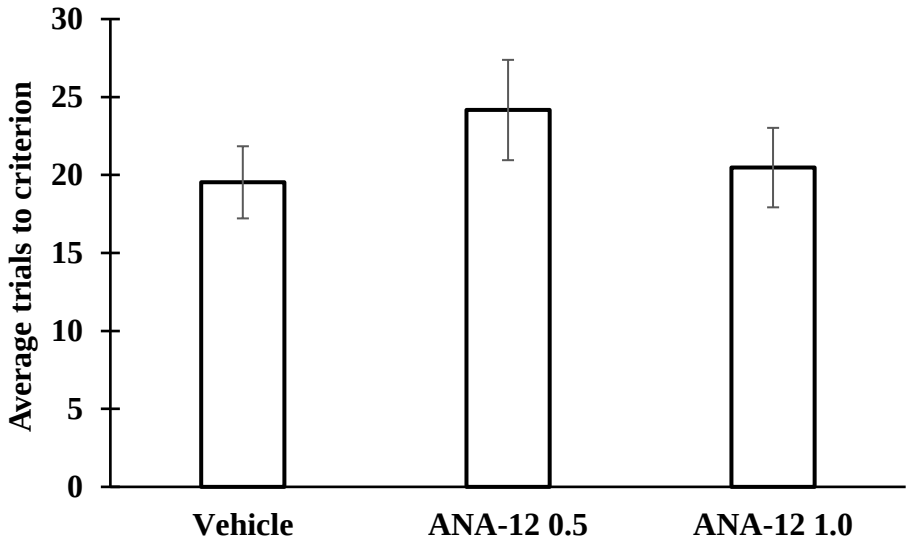

Figure 4. Average number of trials (+ s.e.m.) to reach criterion for each drug condition.

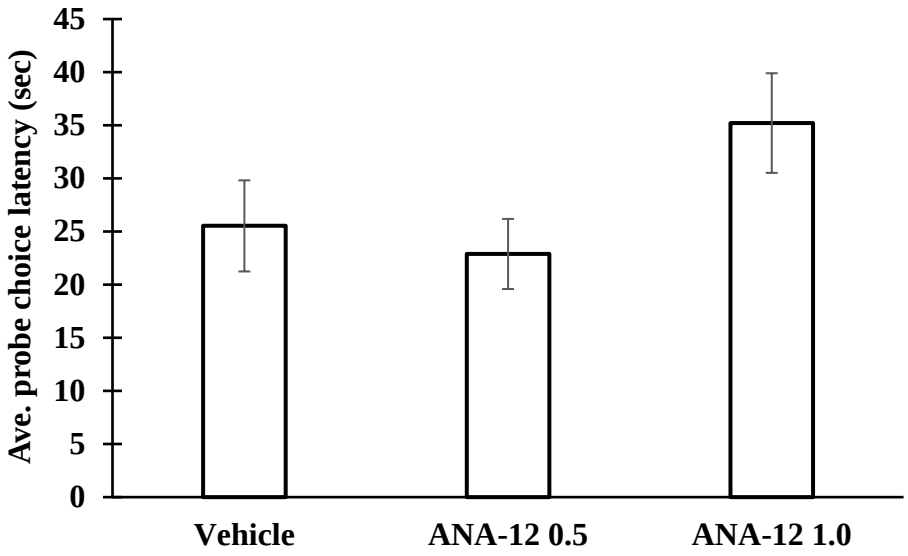

Figure 5. Average latency of rats to choose a goal arm during the probe trial (± s.e.m.) for each drug condition.

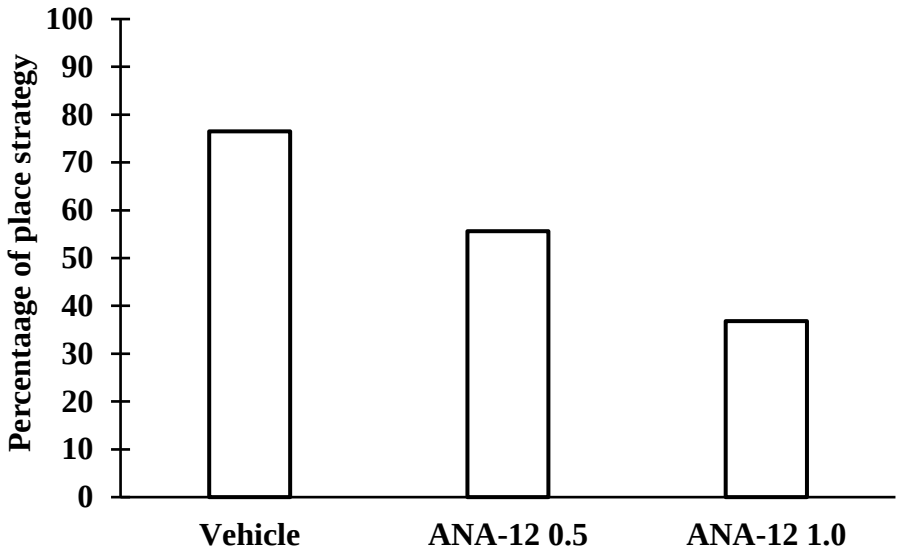

Figure 6. Percentage of rats in each condition that utilized a place strategy on the probe trial.