THE EFFECTS OF AN MGLUR7 AGONIST, AMN082,

ON CONDITIONED TASTE AVERSION

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by

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The undersigned, appointed by the Dean of the Graduate School, have examined the thesis entitled

THE EFFECTS OF AN MGLUR7 AGONIST, AMN082,
ON CONDITIONED TASTE AVERSION

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and hereby certify that, in their opinion, it is worthy of acceptance.

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DEDICATION

It is my honor to dedicate this thesis to all of the teachers and coaches who have encouraged me in my life. Thank you for helping me to grow as an individual, grow as a scholar, and grow as a member of the human race. I would like to say a special thanks to Mrs. Lynne Gronefeld. Thank you for believing in me. Without your guidance and compassion, I would not be where I am today.

I would also like to dedicate this thesis to the Johnson family and thank them for their everlasting encouragement and support. You have always treated me like family and have always been there for me. I am truly grateful.

Finally, I would like to dedicate this thesis to my wonderful soon-to-be husband, Brandon McDannald. Thank you for putting up with my rants about rebellious data points and my periods of grouchiness after long days of work. Thank you for loving me.

ILYKEN

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LIST OF ABBREVIATIONS

ACUC: Animal Care and Usage Committee

AMN082: N,N'-Dibenzyhydryl-Ethane-1,2-Diamine Dihydrochloride

AMPA: Alpha-Amino-3-Hydroxy-5-Methyl-4-Isoxazoleproprionic Acid

cAMP: Cyclic Adenosine Monophosphate

CNS: Central Nervous System

CTA: Conditioned Taste Aversion

DMSO: Dimethyl sulfoxide

FPS: Fear Potentiated Startle

GABA: Gamma-Aminobutyric Acid

HPA: Hypothalamic-Pituitary-Adrenal (Axis)

I.P.: Intraperitoneal

iGluR: Ionotropic Glutamate Receptor

LiCl: Lithium Chloride

mGluR: Metabotropic Glutamate Receptor

MSOP: (RS)-Alpha-Methylserine-O-Phosphate

MPEP: 2-methyl-6-(phenylethynyl)-pyridine

NMDA: N-Methyl-D-Aspartate

RNA: Ribonucleic Acid

THE EFFECTS OF AN MGLUR7 AGONIST, AMN082,

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ABSTRACT

Metabotropic glutamate receptors impact learning and memory. The current studies examined the effects of AMN082, a recently discovered selective metabotropic glutamate receptor 7 ($mGluR_7$) allosteric agonist, on the acquisition and extinction of conditioned taste aversion. It was shown that in larger doses, AMN082 inhibits the acquisition of conditioned taste aversion, and it also significantly attenuates extinction when administered after an initial extinction trial. An additional experiment demonstrated the ability of AMN082 to serve as an effective unconditioned stimulus in conditioned taste aversion, and this effect accounts for the attenuated extinction rate of the aversion. In sum, the results show that AMN082, an $mGluR_7$ agonist, attenuates taste aversion in learning when administered prior to the conditioning trial, but promotes taste aversion as a malaise-inducing agent when given after the trial.

INTRODUCTION

Glutamate serves as the primary excitatory neurotransmitter in the mammalian central nervous system (CNS) and is used by approximately 50% of the neurons in the brain (McGeer, Eccles, & McGeer, 1987; Pilc & Ossowska, 2007). Glutamate is involved in a variety of functions within the CNS including anxiety, fear, and learning and memory; it acts upon both ionotropic (iGluR) and metabotropic (mGluR) glutamate receptors. Although both ionotropic and metabotropic glutamate receptors play important roles within the CNS, they have different specialized functions. While iGluRs (NMDA, kainate, AMPA) exist as fast-acting ligand-gated ion channels (Hollmann & Heinemann, 1994), mGluRs are G-protein linked receptors that mediate slower, longer lasting effects through second-messenger systems and are responsible for other neuronal functions that are not typically controlled by iGluRs (Conn, 2003).

Eight different subtypes of mGluRs have been discovered via molecular cloning and have been divided into three distinct groups based on their pharmacological profile, sequence homology, and preferred signal transduction pathway (Cartmell & Schoepp, 2000). Group I mGluRs ($mGluR_1$ and $mGluR_5$) activate phospholipase C and phosphoinositide hydrolysis, while group II ($mGluR_2$ and $mGluR_3$) and group III ($mGluR_4$, $mGluR_6$, $mGluR_7$, and $mGluR_8$) mGluRs inhibit adenylyl cyclase via G_i proteins (Conn & Pin, 1997). Because the different subtypes of mGluRs have their own distinct pharmacological characteristics and distribution patterns, they also have different effects on the CNS.

Group I and group II mGluRs have been widely researched for use in the treatment of addiction, drug abuse, anxiety disorders, and neurological disorders such as stroke and Parkinson's disease (Conn & Pinn, 1997). They have received a lot of empirical attention recently due to their influence on learning, memory, and cognitive function, including roles in long-term potentiation and long-term depression (Balschun, Zuschratter, & Wetzel, 2006; Gravius, Pietraszek, Schafer, Schmidt, & Danysz, 2005; Schotanus & Chergui, 2008; Steckler et al., 2005; Wang et al., 2008; for a review see Riedel, Platt, & Micheau, 2003).

Although a variety of studies provide evidence for the roles of mGluRs in learning and memory processes, until recently research examining $mGluR_7$, a group III mGluR, has been very limited due to a lack of pharmacological tools. Before the development of selective $mGluR_7$ ligands, one of the main methods for studying the effects of $mGluR_7$ in vivo was through the use of knockout mice. $MGluR_7$ knockout studies have demonstrated that an absence of $mGluR_7$ leads to an array of behavioral and anxiety-related abnormalities, including deficits in innate and learned fear responses, delays in the acquisition and extinction of complex stimulus associations, an increased susceptibility to seizures, impairments in short-term and spatial working memory, reduced anxiety, and reduced stress (Callaerts-Vegh et al., 2006; Cryan et al., 2003; Goddyn et al., 2008; Hölscher et al., 2004; Masugi et al., 1999; Sansig et al., 2001). $MGluR_7$ knockout mice also have disruptions in their hypothalamic-pituitary-adrenal (HPA) axis, an axis that plays a key role in the regulation of stress responses (Mitsukawa et al., 2006). These effects can be explained by the unique distribution pattern of $mGluR_7$ in the CNS. $MGluR_7$ is located presynaptically and can be found across a wide variety of

brain regions, especially the amygdala, hippocampus, insular cortex, and locus coeruleus -- all of which play a critical role in anxiety, depression, fear responses, and/or aversive learning (Cryan et al., 2003; Kinoshita, Shigemoto, Ohishi, van der Putten, & Mizuno, 1998; Yamamoto, Shimura, Sako, Yasoshima, & Sakai, 1994).

Past studies have demonstrated that $mGluR_7$ knockout mice fail to develop a strong conditioned response relative to control mice in a conditioned taste aversion (CTA) procedure (Masugi et al., 1999). In CTA, a novel flavor such as saccharin is presented for consumption and is followed by an injection of a malaise-inducing agent, usually LiCl. This pairing creates an association between the flavor and the aversive effects of LiCl, which leads to a decrease in the consumption of the saccharin on post-conditioning test trials. CTA enables an organism to avoid toxic substances in the future and, therefore, is an adaptive response to unpalatable or toxic solutions or foods (for reviews see Gaston, 1978; Reilly & Schachtman, 2009).

In contrast to the gene knockout technique, Fendt et al. (2008) used a short interfering RNA delivery technique and showed that the acquisition of a CTA was not affected in mice when $mGluR_7$ expression was downregulated, but that the extinction of the aversion was significantly disrupted. This contradiction between Masugi et al.'s findings and Fendt et al.'s findings using mice warrants the need for further investigations on the effects of $mGluR_7$ on conditioned taste aversion, including an examination in rats.

Another technique that can be used to assess the role of $mGluR_7$ on learning is to use a selective $mGluR_7$ agonist such as N,N'-dibenzyhydryl-ethane-1,2-diamine dihydrochloride (AMN082). As a selective allosteric agonist, AMN082 binds to $mGluR_7$

allosteric sites and causes receptor activation. In addition to this receptor activation, AMN082 has several other important effects on $mGluR_7$. For example, using two independent assays, Pelkey, Yuan, Lavezzari, Roche, and McBain (2007) demonstrated that AMN082 causes a rapid $mGluR_7$ internalization that was not counteracted by the presence of MSOP, a competitive group III mGluR antagonist. This property of AMN082 makes it an ideal tool for studying the physiological effects of $mGluR_7$ internalization and the secondary processes it triggers.

While investigating the role of $mGluR_7$ in the expression and extinction of fear potentiated startle (FPS), Fendt et al. (2008) discovered that when a systemic administration of AMN082 was given before conditioning in rats, it decreased the acquisition of fear. However, when given before an extinction trial, AMN082 facilitated the extinction of the previously conditioned fear, suggesting that $mGluR_7$ may affect acquisition and extinction differently in a FPS procedure; that is, the underlying mechanisms affecting the acquisition of FPS may be different from those affecting the extinction of FPS.

Although fear acquisition is attenuated with the systemic administration of AMN082 in the FPS paradigm, the acquisition of CTA remains unaffected by the administration of AMN082 prior to conditioning in mice (Fendt et al., 2008). In response to these differences in acquisition between the FPS and CTA procedures, the authors noted that the brain regions involved in the acquisition of a conditioned taste aversion are likely different from the brain regions involved in the acquisition of a conditioned fear. However, when AMN082 was administered thirty minutes prior to conditioning in the CTA experiment, the extinction of the CTA was rapid, similar to the results of the effects

of AMN082 on extinction in the FPS experiment. Although AMN082 enhanced extinction in both the CTA and FPS procedures, the unexpected differences between acquisition in the CTA experiment and acquisition in the FPS experiment validate the need for further research on the effects of AMN082 on aversive learning.

In order to further characterize the role of $mGluR_7$ in conditioned taste aversion, the current experiments tested the effects of systemic administrations of AMN082 on acquisition and extinction using rats. In Experiment 1, AMN082 was used to examine the role of $mGluR_7$ on extinction of CTA. In Experiment 2, AMN082 was administered during conditioning, specifically after the consumption of saccharin, in order to test its ability to induce CTA due to its potentially aversive properties. In Experiments 3, 4, and 5, AMN082 was administered just prior to conditioning to investigate the role of $mGluR_7$ on the acquisition of CTA. These experiments clarify the role of $mGluR_7$ in aversive learning, particularly in conditioned taste aversion learning.

EXPERIMENT 1

In Experiment 1, AMN082 was systemically administered immediately after an initial extinction trial in order to investigate the role of $mGluR_7$ on subsequent extinction test trials in a CTA procedure. Although Masugi et al. (1999) and Fendt et al. (2008) came to different conclusions about the effects of $mGluR_7$ on the conditioning of a taste aversion, Masugi et al. did not evaluate the effects of $mGluR_7$ on extinction. Masugi et al. conducted two test trials, but the data from both test trials were combined so it is not clear if an effect occurred during one particular extinction trial. However, Fendt et al. found that AMN082 administered at the time of conditioning enhanced the rate of extinction and suggested that $mGluR_7$ plays a role in extinction.

Materials and Methods

Subjects. Thirty male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 170-240 grams were used. The rats were individually housed in a colony room with a 16:8 light/dark cycle (time on at 6:00 AM). After arrival, the rats had food and water access *ad libitum* for five days while adjusting to the colony room. Before the experiment began, all rats were randomly assigned to groups based on their saccharin intake on the conditioning day. All procedures occurred during the light portion of the light/dark cycle, were carried out in accordance with federal animal usage guidelines, and were approved by the University of Missouri Animal Care and Use Committee (ACUC).

Drugs. AMN082 (Ascent Scientific, Princeton, NJ) was dissolved in dimethyl sulfoxide (DMSO) and diluted to the desired dose with sterile saline. Control rats were

only administered the DMSO/saline (1:9) solution. A 0.1% (w/v) saccharin solution (Sigma-Aldrich, St. Louis, MO) was used as the taste solution. LiCl (Sigma-Aldrich, St. Louis, MO) was used as the malaise-inducing drug and was injected intraperitoneally at a dose of 0.15M and at an amount equal to 1.33% of each rat's body weight.

Procedure. After becoming acclimated to the colony room, the rats were water deprived for 24 hours. For four days, the animals were acclimated to drinking from the drinking tubes to obtain their daily water within a 15 min period in their home cages. On each of these days, the rats' water consumption was measured.

On Day 1, all rats were given access to the saccharin solution for 15 min (see Table 1 for the experimental design). All drinking tubes filled with the saccharin solution were weighed before and after consumption by the rats to assess how much saccharin was consumed. Immediately following consumption of the saccharin, the rats received an injection of LiCl. The rats were observed for symptoms of malaise (indicative body posture, decreased mobility) to ensure the effectiveness of the LiCl. The rats were then given access to their daily water for 15 min approximately 90 min after the LiCl injection. On Day 2, the rats were only given their usual *ad libitum* access to food and their daily 15 min of water. This Day 2 treatment allowed the rats to have a full recovery from the LiCl injection.

On Day 3, all rats received access to the saccharin solution for 15 min without exposure to LiCl (i.e., an extinction trial). Immediately following consumption of the saccharin solution, the rats were administered either an AMN082 solution or a vehicle solution. More specifically, rats received an i.p. injection of the DMSO/saline vehicle solution (n = 12), an i.p. injection of the 3 mg/kg AMN082 solution (n = 12), or an i.p.

injection of the 10 mg/kg AMN082 solution (n = 6). These drugs were administered after the extinction trial rather than prior to the trial in order to avoid the drugs potentially causing differential consumption in saccharin on that trial, thereby confounding subsequent performance. Approximately 90 min after the administration of the AMN082 and vehicle solutions, the rats were given access to water for 15 min.

On Days 4-7, all rats received access to the saccharin solution for 15 min per day (i.e., the second, third, fourth, and fifth extinction trials which served as critical test trials). Each day, the amount of consumption was calculated and served as the measure of extinction. Following the 15 min of access to the saccharin solution, all rats received their daily 15 min access to water.

Group	Conditioning (Day 1)	Extinction Trial/Drug Admin. (Day 3)	Test (Days 4-7)
Vehicle Control	Saccharin-LiCl Pairing	Saccharin followed by the vehicle solution	Saccharin alone
3 mg/kg dosage	Saccharin-LiCl Pairing	Saccharin followed by AMN082	Saccharin alone
10 mg/kg dosage	Saccharin-LiCl Pairing	Saccharin followed by AMN082	Saccharin alone

Table 1. Design for Experiment 1. AMN082 or the vehicle solution was administered immediately after the saccharin presentation.

Data Analysis. Repeated measures analysis of variance (ANOVA) procedures were used to analyze the data (SPSS 16, SPSS Inc., Chicago, USA). *Test day* (1, 2, 3, or 4) was used as the within-subject factor and *treatment group* (vehicle, 3 mg/kg AMN082, or 10 mg/kg AMN082) was used as the between-groups factor. Post-hoc analyses were performed using Fisher's LSD tests in order to investigate any specific differences among

test days or treatment groups. For all statistical procedures, the criterion for statistical significance was set at $p = 0.05$.

Results and Discussion

When administered immediately after an initial extinction trial, AMN082 attenuated the extinction of a conditioned taste aversion as revealed by a main effect of group across the four test days ($F(2, 27) = 10.76$, $p < 0.001$) (see Figure 1). Post-hoc analyses showed that while there was no significant difference between the vehicle group and the 3 mg/kg group on the four test trials ($p = 0.115$), there was a considerable significant difference between the vehicle group and the 10 mg/kg group ($p < 0.001$). There was also a significant interaction between *group* and *test day* ($F(6, 81) = 5.018$, $p < 0.001$), indicating that AMN082 had a significant impact on saccharin consumption (i.e., producing less consumption) over the course of extinction.

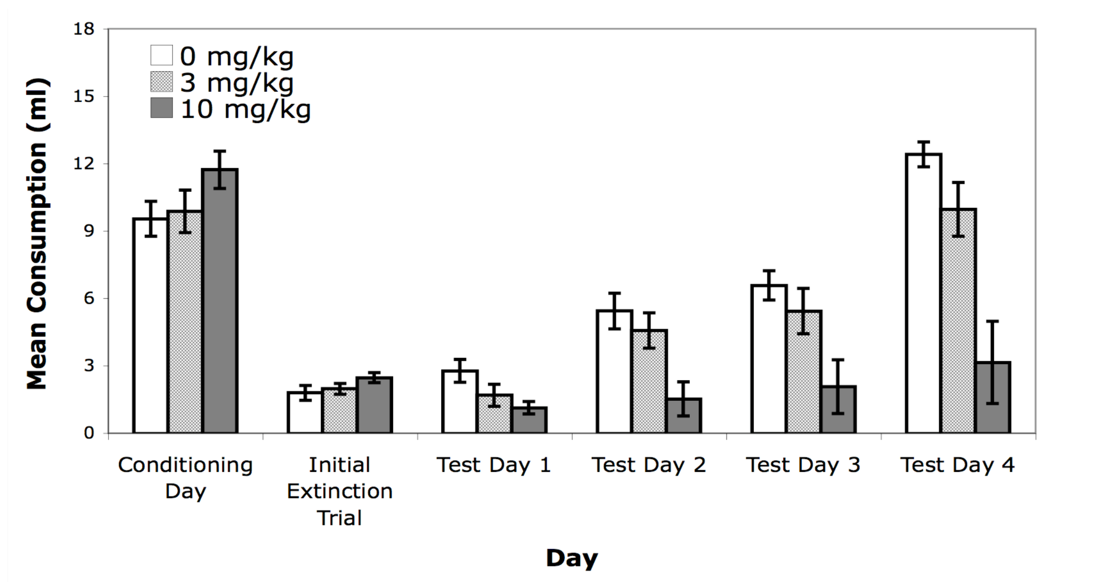

Figure 1. Mean saccharin consumption from Experiment 1. Extinction was attenuated in the 10 mg/kg AMN082 group. Error bars: +/- 1 SEM.

Although previous findings by Fendt et al. (2008) showed that AMN082 accelerated the extinction of CTA, the current results from Experiment 1 show the opposite effect in which AMN082 attenuated the extinction of CTA. The present results suggest that elevated levels of $mGlu_7$ receptor activation may interrupt extinction processes. However, it is also possible that the reduced saccharin consumption on the test trials was not due to an attenuation of extinction but was instead because of a strong taste aversion promoted by AMN082 per se. In this latter case, AMN082 would serve as a strong unconditioned stimulus and would result in the development of a taste aversion when paired with saccharin prior to extinction trials 2-5. Experiment 2 tested this alternative hypothesis.

EXPERIMENT 2

Experiment 2 tested the alternative hypothesis that AMN082 causes a conditioned taste aversion in the absence of any known malaise-inducing agent. In Experiment 2, AMN082 was paired with saccharin in the absence of LiCl as the unconditioned stimulus in a conditioned taste aversion study. The results from Experiment 1 could be explained by a mechanism in which AMN082 produces malaise in its own right rather than affecting the processing of the previously formed saccharin-LiCl association.

Materials and Methods

Subjects. Twenty-four male Sprague-Dawley rats (Harlan, Indianapolis, IN) were used. All other details concerning the housing, care, and maintenance of the rats were the same as in Experiment 1 except that the rats were put into different groups based on their average water intake.

Drugs. All drugs for Experiment 2, including AMN082, the DMSO/saline vehicle, and the saccharin solution, were prepared and administered in an identical manner to that of Experiment 1. Unlike Experiment 1, however, no LiCl was used.

Procedure. On Day 1, all rats were given access to the saccharin solution for 15 min (see Table 2 for the experimental design). Immediately following consumption of the saccharin solution, the rats were administered either an i.p. injection of the DMSO/saline vehicle solution (n = 9), an i.p. injection of the 3 mg/kg AMN082 solution

(n = 8), or an i.p. injection of the of the 10 mg/kg AMN082 solution (n = 7). Ninety min later, the rats were given access to their daily water for 15 min.

On Days 2 and 3, all rats received access to the saccharin solution for 15 min as test exposures. All other unspecified details of the experiment were the same as in Experiment 1.

Group	Conditioning (Day 1)	Test (Days 2-3)
Vehicle Control	Saccharin-Vehicle Pairing	Saccharin alone
3 mg/kg dosage	Saccharin-AMN082 Pairing	Saccharin alone
10 mg/kg dosage	Saccharin-AMN082 Pairing	Saccharin alone

Table 2. Design for Experiment 2. AMN082 was administered immediately after the saccharin presentation.

Data analysis. Repeated measures analysis of variance (ANOVA) procedures were used to analyze the data from Experiment 2 in a similar manner to that of Experiment 1.

Results and Discussion

When paired with a saccharin solution in the absence of any known malaise-inducing agent, the administration of AMN082 resulted in the development of CTA as shown by a main effect of group on the first test day ($F(2, 21) = 9.165$, $p = 0.001$) (see Figure 2). There were no significant differences among the groups on the conditioning day ($p = 0.301$). Similar to Experiment 1, post-hoc analyses showed that while there was

no significant difference in acquisition between the vehicle group and the 3 mg/kg AMN082 group ($p = 0.406$), there was a significant difference between the vehicle group and the 10 mg/kg AMN082 group ($p < 0.001$). There was also a significant effect of test day ($p < 0.001$).

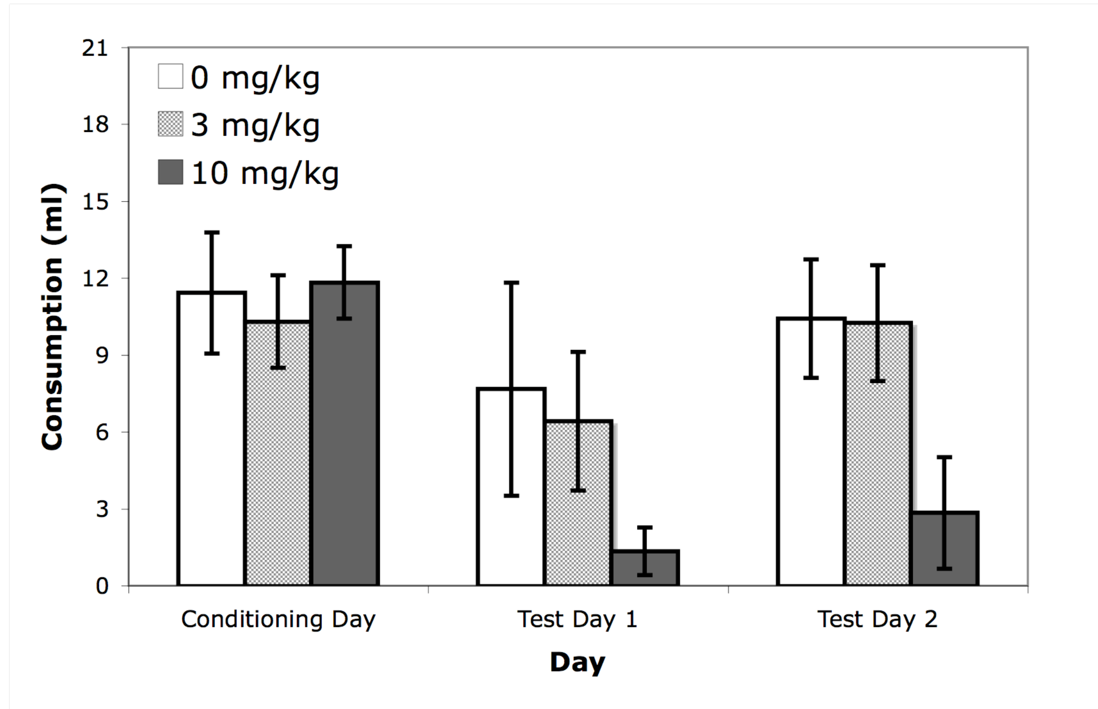

Figure 2. Mean saccharin consumption from Experiment 2. AMN082 at a dose of 10 mg/kg body weight caused a conditioned taste aversion. Error bars: +/- 1 SEM.

AMN082 served as an unconditioned stimulus and resulted in the development of CTA. This could have occurred either from elevated $mGluR_7$ activity or because some other property of the AMN082 compound allowed it to act as an aversive unconditioned stimulus. In either case, it is clear that when 10 mg/kg AMN082 is paired with saccharin in a CTA procedure, it causes a decrease in consumption of the saccharin on post-conditioning test trials.

EXPERIMENT 3

Bahar, Samuel, Hazvi, and Dudai (2003) used a double dissociation to show that different brain regions are involved in CTA acquisition and extinction, suggesting that $mGluR_7$ activation may differentially affect acquisition and extinction in CTA learning (see also Fendt et al., 2008). While Experiment 1 explored the effects of $mGluR_7$ on CTA extinction, Experiment 3 explored the effects of $mGluR_7$ on CTA acquisition by systemically administering AMN082 prior to conditioning. Although Fendt et al.'s research showed that the acquisition of a CTA was not affected by the administration of AMN082 prior to the conditioning trial in mice or in mice with a slight decrease in the overall expression of their $mGluR_7$ by siRNA injection, work by Masugi et al. (1999) showed that the absence of $mGluR_7$ in mice caused a deficit in the acquisition of CTA. Experiment 3 attempted to clarify the role of $mGluR_7$ in the acquisition of CTA.

Materials and Methods

Subjects. Nineteen male Sprague-Dawley rats (Harlan, Indianapolis, IN) were used and were put into different groups based on their average water intake. All other details concerning the housing, care, and maintenance of the rats were the same as in the previous experiments.

Drugs. All drugs for Experiment 3, including AMN082, the DMSO/saline vehicle solution, the LiCl, and the saccharin solution, were prepared and administered in an identical manner to that of Experiment 1. The only exception was that AMN082 at a dosage of 10 mg/kg body weight was not used in Experiment 3.

Procedure. The procedure for Experiment 3 was similar to the procedure used in Experiment 1 with the exception of the timing of the AMN082/vehicle solution administration. In order to investigate the role of $mGluR_7$ on acquisition of CTA, AMN082 was administered 25 minutes prior to the conditioning trial in Experiment 3.

On Day 1 of the experiment, the rats received an i.p. injection of the DMSO/saline vehicle solution (n = 10) or an i.p. injection of 3 mg/kg AMN082 (n = 9) (see Table 3 for the experimental design). Twenty-five minutes after the administration of the AMN082 or the vehicle solution, the rats were given access to the saccharin solution for 15 minutes. Immediately following consumption of the saccharin, the rats received an injection of LiCl. On Day 2, the rats were only given their daily 15 min of access to water. On each of Days 3-4, all rats received access to the saccharin solution for 15 min as test exposures. All other unspecified details of the procedure were the same as in Experiment 1.

Group	Conditioning/Drug Admin. (Day 1)	Test (Days 3-4)
Vehicle Control	Vehicle solution followed by a Saccharin-LiCl Pairing	Saccharin alone
3 mg/kg dosage	AMN082 followed by a Saccharin-LiCl Pairing	Saccharin alone

Table 3. Design for Experiment 3. Saccharin was presented 25 minutes after the AMN082 or vehicle injection.

Data analysis. Repeated measures analysis of variance (ANOVA) procedures were used to analyze the data from Experiment 3 in a similar manner to that of the previous experiments.

Results and Discussion

When administered 25 min prior to conditioning, AMN082 did not affect the acquisition of a conditioned taste aversion on the first test trial ($F(1, 17) = 1.691$, $p = 0.211$) (see Figure 3). There was a significant difference between the conditioning day and the first test day indicating the successful acquisition of a taste aversion ($p < 0.001$). Furthermore, there was a significant difference between the test days indicating the presence of extinction over time ($p < 0.001$). There was no significant interaction between *group* and *test day* ($p = 0.423$). However, a difference in saccharin consumption between the groups on the conditioning day was detected ($F(1, 17) = 4.807$, $p = 0.027$), as the 3 mg/kg group consumed more saccharin than the vehicle group. This greater consumption by the 3 mg/kg group makes it difficult to interpret the test performance by the two conditions.

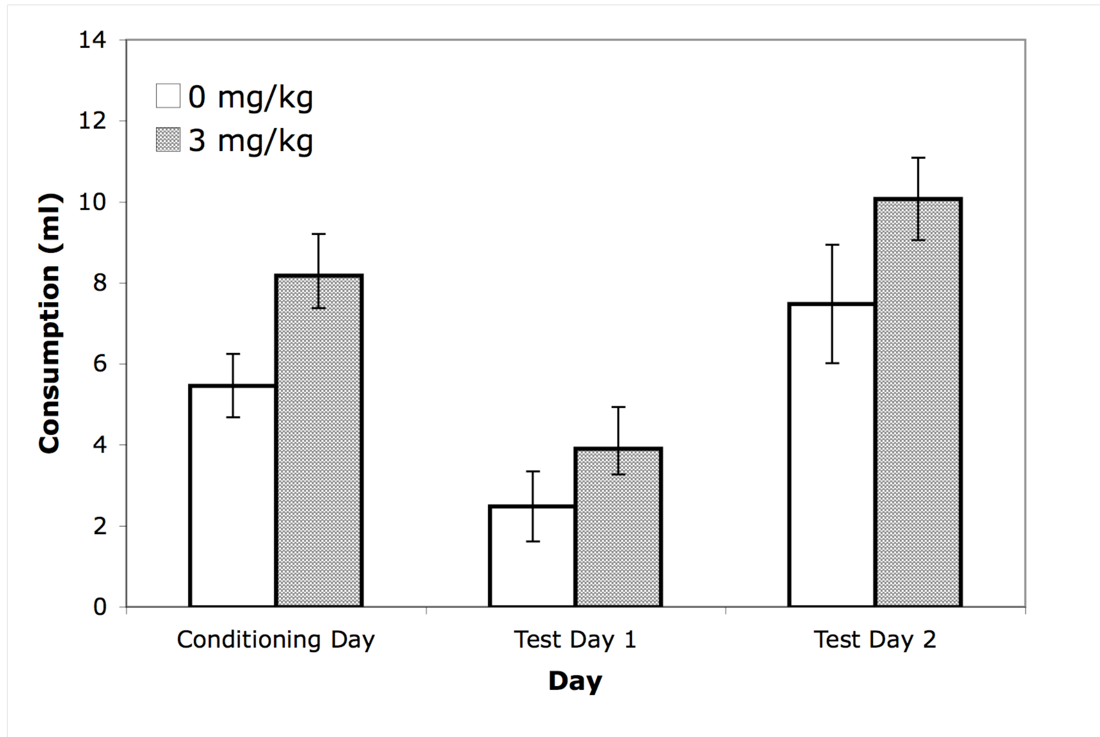

Figure 3. Mean saccharin consumption from Experiment 3. No significant differences in acquisition were found between the control group and the 3 mg/kg AMN082 group. There was a significant difference between the groups on the conditioning day. Error bars: +/- 1 SEM.

Consistent with the results of Fendt et al. (2008), AMN082 at the time of conditioning did not affect the acquisition of a conditioned taste aversion. However, consumption differences on the conditioning trial potentially confounded the interpretation of the test results. This consumption difference may be due to AMN082 causing the rats to be less reluctant to consume a novel flavor.

EXPERIMENT 4

Experiment 4 continued to investigate the effect of AMN082 on the acquisition of a conditioned taste aversion. This experiment was performed such that the rats only received access to a fixed amount of saccharin on the conditioning day in order to prevent the potentially confounding variable of differential consumption on the conditioning trial. The rats in Experiment 4 received access to 8 ml of saccharin on the conditioning day.

Materials and Methods

Subjects. Fourteen male Sprague-Dawley rats (Harlan, Indianapolis, IN) were used. All other details concerning the housing, care, and maintenance of the rats were the same as in the previous experiments.

Drugs. All drugs for Experiment 4, including AMN082, the DMSO/saline vehicle solution, the LiCl, and the saccharin solution, were prepared and administered in an identical manner to that of Experiment 3.

Procedure. The procedure for Experiment 4 was identical to the procedure for Experiment 3, with the exception that the rats were only given access to 8 ml of saccharin on the conditioning day instead of access to the much larger amount available in Experiment 3.

On Day 1 of the experiment, the rats received an i.p. injection of the DMSO/saline vehicle solution (n = 7) or an i.p. injection of 3 mg/kg AMN082 (n = 7). Twenty-five minutes after the administration of the AMN082 or the vehicle solution, the

rats were given access to 8 ml of the saccharin solution for 15 min. Immediately following consumption of the saccharin, the rats received an injection of LiCl. Ninety min later, the rats were given access to their daily water for 15 min. On Day 2, the rats were only given access to their daily 15 min of water. On each of Days 3-4, all rats received access to the saccharin solution for 15 min. All other unspecified details of the procedure were the same as in Experiment 3.

Data Analysis. Repeated measures analysis of variance (ANOVA) procedures were used to analyze the data from Experiment 4 in an identical manner to that of Experiment 3.

Results and Discussion

AMN082 (3 mg/kg) did not affect the acquisition of a conditioned taste aversion compared to control rats as revealed by an absence of a main effect of group on the first test trial ($F(1, 12) = 2.46$, $p = 0.143$) (see Figure 4). Unlike Experiment 3, there was no difference in saccharin consumption between the groups on the conditioning day, as expected ($F(1, 12) = 0.286$, $p = 0.603$). There was a main effect of test day ($p < 0.001$), showing that the rats extinguished the CR during these trials. Also, a significant difference was present between the conditioning day and the first test day indicating the successful acquisition of a taste aversion ($p = 0.023$).

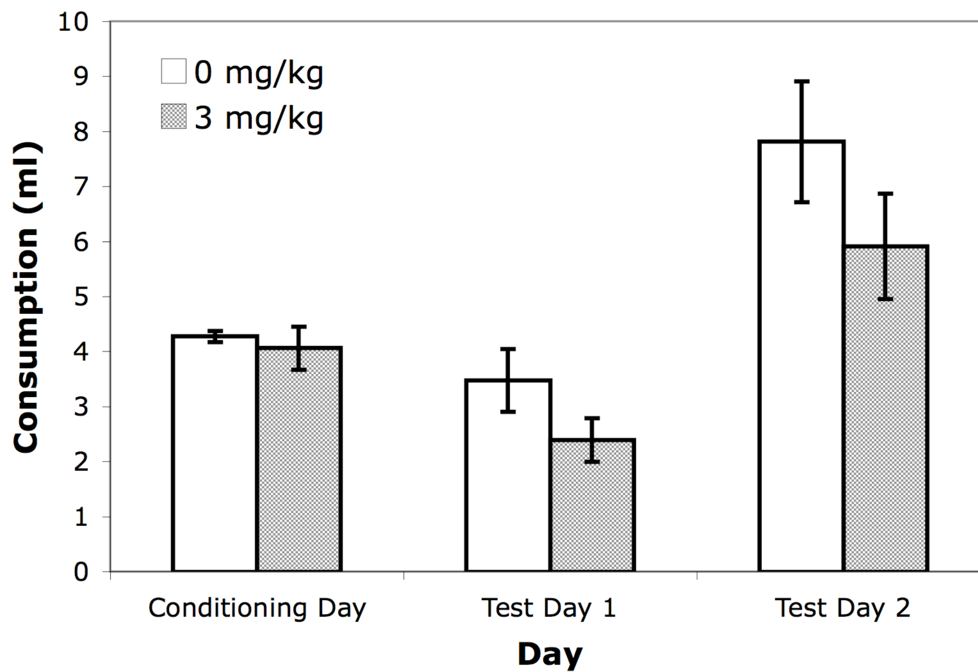

Figure 4. Mean saccharin consumption from Experiment 4. Controlled access to saccharin was used during conditioning. No significant differences in acquisition were found between the control group and the 3 mg/kg AMN082 group. Error bars: +/- 1 SEM.

Consistent with the results of Experiment 3 and Fendt et al. (2008), AMN082 at a dose of 3 mg/kg body weight did not affect the acquisition of CTA. This evidence suggests that $mGluR_7$ activation elicited by such a dose does not significantly impair or enhance the acquisition of a taste aversion.

EXPERIMENT 5

Although Experiment 4 showed that a low dose of AMN082 does not affect the acquisition of CTA, it is still possible that a higher dose of AMN082 could influence acquisition. For example, in Experiments 1 and 2, the 3 mg/kg dose of AMN082 did not affect extinction or result in CTA acquisition, but the higher dose of 10 mg/kg significantly attenuated extinction and served as a powerful US. Experiment 5 investigated whether AMN082 at a moderate or a high dose affects CTA acquisition.

Materials and Methods

Subjects. Thirty-two male Sprague-Dawley rats (Harlan, Indianapolis, IN) were used. All other details concerning the housing, care, and maintenance of the rats were the same as in the previous experiments.

Drugs. All drugs for Experiment 5, including AMN082, the DMSO/saline vehicle solution, the LiCl, and the saccharin solution, were prepared and administered in a similar manner to that of Experiments 3 and 4. The only exception was that AMN082 was administered at 5 mg/kg and 10 mg/kg body weight in Experiment 5.

Procedure. The procedure for Experiment 5 was identical to the procedure for Experiments 3 and 4. On Day 1 of the experiment, the rats received an i.p. injection of the DMSO/saline vehicle solution (n = 12), an i.p. injection of 5 mg/kg AMN082 (n = 9), or an i.p. injection of 10 mg/kg AMN082 (n = 11). Twenty-five minutes after the administration of the AMN082 or the vehicle solution, the rats were given access to the

saccharin solution for 15 minutes. Immediately following consumption of the saccharin, the rats received an injection of LiCl. On Day 2, the rats were only given access to their daily 15 min of water. On each of Days 3-4, all rats received access to the saccharin solution for 15 min as test exposures. All other unspecified details of the procedure were the same as in Experiments 3 and 4.

Data Analysis. Repeated measures analysis of variance (ANOVA) procedures were used to analyze the data from Experiment 5 in a manner similar to that of the previous experiments.

Results and Discussion

In elevated doses, AMN082 significantly attenuated the acquisition of a conditioned taste aversion as shown by the presence of a main effect of group on the first test day ($F(2, 29) = 3.826$, $p = 0.034$) (see Figure 5). The vehicle group differed from the 10 mg/kg group ($p = 0.017$) and the 5 mg/kg group ($p = 0.04$), but the two AMN082 groups were not statistically different from each other ($p = 0.817$). Unlike Experiment 3, there was no difference in saccharin consumption for the groups on the conditioning day ($F(2, 29) = 0.491$, $p = 0.617$). A comparison of the conditioning day and the first test day indicated the acquisition of a taste aversion for the vehicle group ($p = 0.003$), but not for the 5 mg/kg AMN082 group ($p = 0.188$) or the 10 mg/kg AMN082 group ($p = 0.745$).

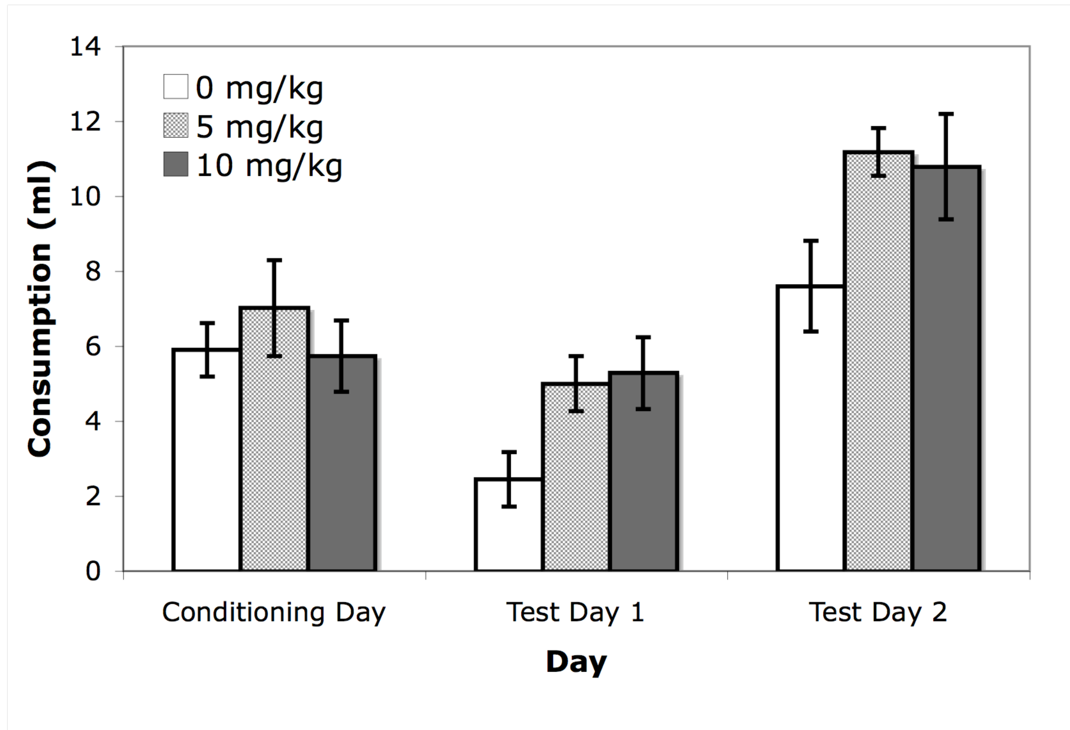

Figure 5. Mean saccharin consumption from Experiment 5. A significant attenuation of CTA acquisition was found for both the 5 mg/kg group and the 10 mg/kg group. Error bars: +/- 1 SEM.

When administered in the doses of 5 mg/kg and 10 mg/kg, AMN082 attenuated the acquisition of CTA as demonstrated by an increase in saccharin consumption on the first test day. This evidence suggests that $mGluR_7$ plays a role in the acquisition of aversive associations; more specifically, it suggests that higher levels of $mGluR_7$ activation can impair taste aversion learning.

GENERAL DISCUSSION

MGluR-based therapies offer a promising avenue for the treatment of learning, memory, and anxiety disorders. A full biological and behavioral understanding of the effects of specific mGluRs is needed in order to develop such treatments. Until recently, research with $mGluR_7$ has been limited due to a lack of pharmacological tools. With the advent of AMN082 as an $mGluR_7$ agonist, the functions of $mGluR_7$ in the CNS are beginning to be discovered.

Our findings show that, when given prior to the conditioning trial, systemic injections of 5 mg/kg and 10 mg/kg AMN082 inhibit the acquisition of CTA, and when given after an initial extinction trial, 10 mg/kg AMN082 attenuates CTA extinction. In the case of acquisition, the learning of the CS-US association was impaired. In the case of extinction, AMN082 produced an aversion in its own right. That is, Experiment 2 showed that the attenuation of extinction in Experiment 1 was due to AMN082 acting as an aversive stimulus itself (i.e. an unconditioned stimulus). Similar results are known to occur with a variety of other drugs including NMDA receptor antagonists (Aguado, San Antonio, Perez, del Valle, & Gomez, 1994; Etscorn & Parson, 1979; Jackson & Sanger, 1989; Myhal & Fleming, 1990; Welzl, Alessandri, & Battig, 1990) and, in elevated doses, the NMDA receptor agonist D-cycloserine (Nunnink, Davenport, Ortega, & Houpt, 2007). Our results suggest that AMN082 served as the US and promoted the knowledge from the previous saccharin-LiCl association that saccharin should be avoided. This resulted in a very strong conditioning memory that expressed itself via an attenuated rate of extinction.

A smaller dose of AMN082 (3 mg/kg) did not serve as an effective unconditioned stimulus for CTA. This finding is consistent with the results from Experiment 1 in which the same small dose of AMN082 did not affect extinction. Furthermore, since AMN082 at a lower dose of 3 mg/kg did not result in CTA per se in Experiment 2, the findings from Experiment 1 that used such a dose can be interpreted in terms of $mGluR_7$ activation. Thus, the stimulation of $mGluR_7$ using an administration of 3 mg/kg AMN082, at least to the extent that one can interpret a null finding, does not significantly affect the extinction of a previously learned taste aversion.

Given that glutamate release is necessary for CTA learning (Bielavska, Miksik, & Krivanek, 2000), the results from Experiments 3-5 suggest that higher levels of $mGluR_7$ activity, that is, doses of 5 mg/kg and 10 mg/kg AMN082, impair CTA acquisition while lower doses, such as 3 mg/kg AMN082, do not affect acquisition. $MGluR_7$ is not the first mGluR shown to affect CTA. Other mGluRs, especially $mGluR_5$, are also involved in CTA (Bills et al., 2005; Simonyi, Serfozo, Parker, Ramsey, & Schachtman, 2009; Yasoshima, Morimoto, & Yamamoto, 2000). Systemic injections of the $mGluR_5$ antagonist MPEP have been shown to attenuate CTA (Schachtman et al., 2003), analogous to the results of Experiment 5 in which AMN082 administered prior to conditioning also attenuated CTA.

Since AMN082 possesses aversive properties, it should be used with extreme caution in research. In addition to its effects on CTA, AMN082 also produces involuntary movements, which are another unwanted side effect capable of potentially confounding results. Mareš (2008) found that AMN082 in doses as small as 2 mg/kg elicited involuntary head and forelimb tremors. In addition to these effects, the

previously discussed results by Pelkey et al. (2007) in which endocytosis was triggered by AMN082 suggest that AMN082 may have produced unwanted side effects on the $mGlu_7$ receptor. Although other agonists have been known to induce receptor endocytosis, the decrease in $mGluR_7$ caused by the endocytosis may lead AMN082 to have effects similar to that of an antagonist.

In any case, the discovery of $mGluR_7$ specific agonists and antagonists with minimal unwanted side effects is needed in order to ascertain whether certain drug results are due to changes in $mGluR_7$ activity or whether the results are due to confounding effects of the drug compound itself—completely separate from the effects of $mGluR_7$ activity on learning processes per se. The discovery of such a drug would allow for an additional plethora of well-founded $mGluR_7$ research, resulting in the potential development of treatments for a variety of cognitive disorders.

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