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Chronic α_{1A}-adrenergic receptor stimulation improves synaptic plasticity, cognitive function, mood, and longevity

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Running Title: *α_{1A}ARs in Synaptic Plasticity, Cognition, Mood, and Lifespan*

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Abbreviations: AD, Alzheimer's disease; AR, adrenergic receptor; CAM, constitutively active mutation; CSF, cerebrospinal fluid; DG, dentate gyrus; ERK, extracellular signal-regulated kinase; fEPSPs, field excitatory post synaptic potentials; KO, knock-out; LTD, long-term depression; LTP, long-term potentiation; NE, norepinephrine; OCD, obsessive-compulsive disorder; PPF, paired-pulse facilitation; SNRI, serotonin-norepinephrine reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor; TBS, theta burst stimulation; WT, wild type.

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Abstract

The role of α_1-adrenergic receptors (α_1ARs) in cognition and mood is controversial, likely due to past use of non-selective agents. α_{1A}AR activation was recently shown to increase neurogenesis, which is linked to cognition and mood. We studied the effects of chronic α_{1A}AR stimulation using transgenic mice engineered to express a constitutively active mutant (CAM) form of the α_{1A}AR. CAM-α_{1A}AR mice showed enhancements in several behavioral models of learning and memory. In contrast, mice that have the α_{1A}AR gene knocked-out (KO) displayed poor cognitive function. Hippocampal brain slices from CAM-α_{1A}AR mice demonstrated increased basal synaptic transmission, paired-pulse facilitation, and long-term potentiation compared to wild type (WT) mice. WT mice treated with the α_{1A}AR-selective agonist, cirazoline, also showed enhanced cognitive functions. In addition, CAM-α_{1A}AR mice exhibited antidepressant and less anxious phenotypes in several behavioral tests when compared to WT mice. Furthermore, the lifespan of CAM-α_{1A}AR mice was 10 percent longer than that of WT mice. Our results suggest that chronic α_{1A}AR stimulation improves synaptic plasticity, cognitive function, mood, and longevity. This may afford a potential therapeutic target for counteracting the decline in cognitive function and mood associated with aging and neurological disorders.

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Introduction

Norepinephrine (NE) has been shown to influence a variety of cognitive functions in the brain, from enhancing learning and memory to modulating mood (Sirviö and MacDonald, 1999). NE mediates its effects by selectively binding to and activating adrenergic receptors (ARs), a family of glycosylated integral membrane proteins. AR subtypes are defined according to their pharmacological properties, physiological characteristics, and primary structure, and are classified as α_1, α_2, and β. In the brain, α_1ARs are the least understood.

The function of α_1ARs in learning and memory is controversial and has not been clearly defined. Some studies have shown that α_1AR stimulation inhibits memory consolidation in chicks and impairs spatial memory in monkeys and rats (Sirviö and MacDonald, 1999). In contrast, other studies suggest that α_1AR activation facilitates learning and memory in rodents. Furthermore, α_1ARs can promote long-term potentiation (LTP) and long-term depression (LTD) in the hippocampus and may be important modulators of synaptic plasticity in the adult central nervous system (Sirviö and MacDonald, 1999). However, many of these previous studies used high doses of weakly selective α_1AR agents, possibly cross-activating other AR subtypes.

We recently showed that chronic stimulation of the α_{1A}AR increases neurogenesis (Gupta et al., 2009). Substantial evidence suggests that newly generated neurons contribute to learning and memory, particularly hippocampal-dependent tasks (Deng et al., 2010). Improved memory performance in aged rats correlates with higher numbers of newly generated neurons in the hippocampus. In addition to modulation of learning and memory, adult neurogenesis has been implicated in the enhancement of hippocampal synaptic plasticity. Increased synaptic plasticity is strongly associated with improved cognition and adult born hippocampal granule cells

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possess lower thresholds for the induction of LTP and are more sensitive to excitatory input (Schmidt-Hieber et al., 2004).

The role of α_1ARs in mood is also not well understood; however, we recently showed that chronic α_{1A}AR stimulation is associated with a decrease in depression and anxiety-like behavior in mice (Doze et al., 2009). Antidepressants that act through NE and/or serotonin increase neurogenesis, and in some instances, their effectiveness appears to be dependent on neurogenesis (Santarelli et al., 2003). In addition, the time for the clinical effect of antidepressants to occur correlates with the time required for newborn cell migration and functional integration (Malberg et al., 2000). Anxiety and stress are also common risk factors for depression. Chronic stress in rodents has been shown to decrease neurogenesis, which is reversed with antidepressants (Alonso et al., 2004).

The role of α_1-ARs or any mammalian G protein-coupled receptor in longevity has not been explored. Models of neurodegeneration have shown shortened lifespan in rodents (Ohsawa et al., 2008). Likewise, human life expectancy after diagnosis with Alzheimer's disease (AD) is approximately half as long as without the disease (Larson et al., 2004). Recent evidence suggests that the longevity gene, *sirt1*, is linked to the activity of neural stem cells (Libert et al., 2008), providing another association between neurogenesis and longevity.

Transgenic mice engineered to express a CAM-α_{1A}AR and normal mice treated with an α_{1A}AR-selective agonist, cirazoline, were studied to determine the effects of chronic α_{1A}AR stimulation on learning, memory, synaptic plasticity, depression, anxiety, and longevity. We found that long-term α_{1A}AR activation enhances learning and memory, promotes synaptic plasticity, improves mood, and increases lifespan. α_{1A}AR stimulation may offer a new strategy for treating the decline in cognition and mood associated with aging and neurological disorders.

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Materials and Methods

Transgenic mice

Animals used in this study included transgenic CAM-α_{1A}AR mice which were created on a B6CBA background, α_{1A}AR-KO mice created on a C57BL/6 background, and their respective WT controls. Male and female mice, 182 total, were used for the behavioral tests and 86 total for the longevity studies. Mice were bred and genotyped at the Cleveland Clinic Foundation and were housed and provided veterinary care in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited animal care facility. Large cohorts of mice were transferred to the University of North Dakota's AAALAC-accredited animal care facility. Mice were maintained on a 12 h light/dark cycle (lights on 0500-1700), housed in 17 x 28 x 13 cm translucent, polycarbonate boxes attached to an automatic watering system (Edstrom Industries, Inc, Waterford, WI, USA), and were provided *ad libitum* access to pelleted food with 5% fat (Teklad 22/5 Rodent Diet (W) 8640, Harlan, Indianapolis, IN, USA). Room air was 100% exchanged 12-40 times per h with no recirculation, the temperature was 22°C, and the humidity was 23-27%. Mice were identified by ear tags placed at the Cleveland Clinic. The experimental protocols employed in this study conform to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health and were approved by the Animal Care and Use Committee at both institutions.

Behavioral testing

Behavioral testing was completed when animals were aged 3-6 mo, except for mice treated long-term with cirazoline, which were aged 6-11 mo. Tests for learning and memory included the Barnes, Morris water, and multi-T mazes. The Barnes maze was performed between 0800 and 1200 h while the other cognitive tests took place between 1000 and 1200 h. Tests for

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depression and anxiety included the tail suspension test, marble burying test, elevated zero maze, and light/dark exploration. All mood tests were performed between 1200 and 1700 h. The Morris water and multi-T mazes were performed at the Cleveland Clinic Foundation; all other tests were completed at the University of North Dakota. Animals were acclimated in separate cages in the testing room for 30 min prior to Barnes maze testing and for 1 h prior to all other tests. Animals were deprived of food and water for the duration of the tests. Lighting was measured using a digital lux meter (Mastech, Fremont, CA, USA) and was held at 40 lux for depression and anxiety tests, with the exception of light/dark exploration which required high and no light. Testing equipment was cleaned between trials with alcohol. All testing was video captured, performed, and analyzed blind to mouse genotype.

Barnes maze

The Barnes maze was used to assess spatial learning and memory in mice with a modified protocol. The Barnes maze consisted of a white, flat, circular platform (120 cm in diameter) elevated 140 cm above ground (Med Associates, St. Albans, VT, USA). An escape box (21 x 5.5 x 5 cm; length x width x depth), not visible from the top of the maze, was located under one of 40 holes (5 cm in diameter) evenly spaced 3.5 cm apart around the perimeter. Three visual cues were placed on a black curtain surrounding the maze and their locations in relation to the escape box remained the same throughout the experiment. Two flood lights (1700 total lux) and four evenly spaced fans above the maze provided aversion.

The first four days consisted of four learning trials with 30 min intervals between trials. At the start of each trial, a mouse was placed in the center of the maze under a holding chamber for 30 s. When the chamber was lifted, timing of the trial began and the mouse was allowed up to

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300 s to enter the escape box. If a mouse failed to enter after the allowed time, it was gently placed into the hole containing the escape box for 30 s. Memory trials were conducted on days 1, 4, 5, and 8 (transgenic mice) and days 1, 4, and 6 (cirazoline-treated mice) after the four days of training. The procedure for the memory trials was the same as learning, except the mice were allowed only one attempt to solve the maze each day. Later analysis included the time to solve, number of errors made, and distance traveled on the maze. Errors were defined as when a mouse poked more than three-quarters of its head into any hole other than the appropriate escape hole. Distance traveled was measured using ANY-maze software v4.73 (Stoelting Co., Wood Dale, IL, USA).

Morris water maze

The Morris water maze was used to assess spatial learning and memory. The maze consisted of an oval tub (76 cm diameter) with 15 cm of water held at 26^{o}C. A stationary platform was placed below the surface near the middle of the tub to allow the mice an escape from the water. An identical free-floating platform was placed next to the stationary platform, which remained for the duration of training and testing. The free-floating platform did not provide an escape. The tub was aligned with visual cues, red stickers placed inside, which remained constant throughout the experiment. Each mouse was placed at one end of the tub and observed until it climbed onto the stationary platform, at which time they were removed. If a mouse did not find or remember the correct stationary platform in 300 s, it was guided to the platform and allowed to remain there for 30 s before escaping from the water. This procedure was repeated for six days to determine learning. The stationary platform and cues were then reversed and mice were tested for memory on day 9. The tests were digitally recorded and later

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analyzed for the time required to locate the stationary platform during learning and memory trials.

Multi-T maze

The multi-T maze (60 x 60 x 16 cm) is a test of spatial working memory. Visual cues were placed along the correct path of the maze, which led to a peanut butter reward and an escape box. Mice were trained five times per day on five consecutive days with the incorrect paths blocked, only allowing access to the correct solution. On day 5 of training, mice were timed while they solved the maze with paths unblocked to assess learning. To test memory, the mice were retested on days 1, 4, 5, and 8 after training. Activity was recorded and later analyzed for the time required to solve the maze and the number of errors made. Each instance the mouse turned down the wrong path was counted as an error.

Locomotor activity (open field)

The open field test is used to measure spontaneous locomotor activity in rodents. The open field for these experiments was a 41 x 41 cm enclosure with infrared beams of light aimed to form two grids, one 2.5 cm near the floor and one 7.5 cm above the floor of the enclosure. The mice were placed in the open field and allowed to explore freely for 15 min. An Active8 Open Field Activity System (Harvard Apparatus, Holliston, MA, USA) was used to monitor total activity, distance traveled, and number of rearings. Locomotor activity (total number of beam breaks/min) was determined using a computer algorithm that calculated the distance traveled/min during horizontal ambulation.

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Tail suspension test

The tail suspension test is a learned helplessness model used as a measure of depression in rodents. Classically, the test was used only to assess antidepressant efficacy. Recently, it has gained popularity as a way to assess depression-like behavior in transgenic mice models. Increased mobility in transgenic models mimics the effect seen with antidepressant treatment and is a measure of decreased depression-like behaviors. More commonly, immobility time is reported to express depression-like behavior. The test apparatus was a box made of 1.25 cm white melamine-coated particleboard. Each mouse was suspended by its tail on a hook using 1.25 cm label tape, 26 cm from the bottom of the box, for 6 min. Each test was digitally recorded and later analyzed for the time spent immobile. Data was excluded if the mouse climbed their tail ≥ 20% of the time (72 s).

Marble burying test

The marble burying test is as a measure of obsessive-compulsive type anxiety in mice . The test was performed in 17 × 28 × 13 cm translucent, polycarbonate boxes containing bedding 5 cm deep with 20 marbles positioned in five rows of four. Each mouse was placed in a box for 30 min. Marbles buried, defined as at least two-thirds covered, were counted after the mouse's removal.

Elevated zero maze

The elevated zero maze is designed to assess anxiety in rodents. The maze was 61 cm in diameter. Aversive stimuli included the height of the maze (50 cm) and light (40 lux). Each mouse was placed next to and facing a closed quadrant and allowed to explore the maze for 10

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min. Activity was digitally recorded and later analyzed for time spent in and the number of entries to the open sections.

Light/dark exploration

Light/dark exploration is used as a measure of anxiety in rodents. The light/dark apparatus consisted of a 35 × 40 × 40 cm box with a partition creating a 35 × 40 × 14 cm dark compartment (0 lux) connected to the light side (400 lux) by a 7.5 × 7.5 cm opening. Each mouse was placed in the light side of the box facing away from the opening to the dark side. Activity was digitally recorded for 10 min and later analyzed for the time spent in the light side and transitions to the dark side.

Hippocampal slice preparation

Mice were weighed, deeply anesthetized with isoflurane, and then immediately decapitated. The brain was removed quickly and placed in ice-cold oxygenated choline chloride solution (110 mM choline chloride, 25 mM $NaHCO_3$, 25 mM dextrose, 11.6 mM sodium ascorbate, 7 mM $MgSO_4$, 3.1 mM sodium pyruvate, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 0.5 mM $CaCl_2$). While submerged, hippocampi were removed and placed on a tissue chopper. Coronal brain slices were cut 400 μm thick and immediately transferred to an oxygenated holding chamber filled with oxygenated artificial cerebrospinal fluid (CSF) solution (119 mM NaCl, 26.2 mM $NaHCO_3$, 11 mM dextrose, 5 mM KCl, 2.5 mM $CaCl_2$, 1.3 mM $MgSO_4$, 1 mM NaH_2PO_4) warmed to 33°C in a water bath for approximately 30 min. Slices were then removed from the water bath and allowed to cool to room temperature (22°C). After 15 min, the entorhinal cortex and CA3 region of each slice was quickly removed. Slices were then returned to the holding

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chamber and allowed an acclimation period of approximately 2 h (not including incubation time and time to remove the entorhinal cortex and CA3). Slices were transferred to recording chambers in preparation for electrophysiological recordings, where they were constantly perfused with oxygenated artificial CSF at a rate of 1.5 mL/min at 24°C. For these experiments, 22-24 mo old mice were used to examine the effects of long-term chronic α_{1A}AR stimulation on synaptic transmission and plasticity in aged mice.

Electrophysiology recordings

Glass micropipettes were backfilled with 3 M NaCl solution for the recording electrodes and subsequently placed in the stratum radiatum of the CA1 region. Evoked field excitatory post-synaptic potentials (fEPSPs) were recorded and measured using a BVC-700A Cornerstone amplifier (Dagan Corporation, Minneapolis, MN, USA) in current clamp mode with 100X gain. An ISO-flex stimulator (A.M.P.I., Jerusalem, Israel) paired with a 7.5 cm bipolar tungsten-stimulating electrode (World Precision Instruments, Sarasota, FL, USA) was used for presynaptic stimulation of the Schaffer collateral-commissural fibers in the stratum radiatum, between the CA3 region and the recording electrode. Signals were converted from analog to digital using an Axon Digidata1440A Data Acquisition System (Molecular Devices Inc., Sunnyvale, CA, USA), and electronic cycling and noise were filtered using a HumBug 50/60 Hz noise eliminator (Quest Scientific, Vancouver, BC, Canada). Recordings were made using Axon Instruments Clampex v10.2. Basal synaptic transmission was assessed by determining input-output (I/O) curves, generated by applying a stepwise 5 μA increase in stimulation intensity, with a range of 5-80 μA. Responses were elicited every 20 s with duration of 100 μs per pulse. For the subsequent experiments, the stimulus was set to approximately 50% of the

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maximal response. Short-term plasticity was investigated by assessing paired-pulse facilitation (PPF) by applying two pulses with inter-pulse intervals of 35, 50, 75, 100, 150, 200, and 300 ms. A baseline response of 30 min was recorded immediately after PPF, which was followed by theta burst stimulation (TBS = 10 trains, each train of 4 pulses at 100 Hz, inter-train interval of 200 ms, total train duration of 40 ms) given at 80% maximal response to induce LTP. fEPSPs were then recorded at 50% maximal response every 20 s for 90 min.

Cirazoline treatment

Normal, non-transgenic WT mice received bottled water containing cirazoline at 10 mg/L for 2 to 9 mo. The water was changed either weekly or biweekly as needed. Food was provided *ad libitum*. No adverse side effects were noted with this treatment or in the CAM-α_{1A}AR mice.

Longevity methods

Animals in the longevity aim of this study were observed daily, but only handled for cage changes, and the date of death logged accordingly. The body weight of a selection of the mice was measured at different ages using a pan electronic scale. Animals that appeared near death (not likely to survive for another 48 h) were euthanized with carbon dioxide; the date of euthanasia was then taken as the best estimate of date of natural death. Factors for this decision were based on clinical signs set forth by The Jackson Laboratory, Bar Harbor, ME, USA (Yuan et al., 2009). The signs included failure to drink or eat, extreme weight loss over a short period of time, severe weakness based on responsiveness to touch, serious locomotor impairments, or tumors which had ulcerated or were bleeding.

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Statistical analysis

All results were analyzed using Prism 5.03 (GraphPad Software, La Jolla, CA). Statistical comparisons were performed between the transgenic mice and their WT controls using a Student's unpaired t-test. Electrophysiological data was analyzed using Clampfit v10.2 and Prism 5.03. Analysis of I/O curves was done by finding the slope of each fEPSP from 5-80 μA, at 5 μA increments. PPF analysis compared the slope of the second elicited fEPSP and divided it by the first elicited fEPSP. Fiber volley amplitude was also analyzed in order to better assess basal synaptic transmission. Pre- and post-TBS baselines were analyzed by measuring fEPSP slope every 20 s, and comparing the average pre-TBS baseline slope to the average post-TBS baseline slope for both CAM-α_{1A}AR and WT mice. fEPSP slopes were expressed as a ratio of the pre-TBS baseline and normalized to the pre-TBS baseline. Data are presented as mean ± SEM. Significance levels were taken as * $p < 0.05$, ** $p < 0.01$, or *** $p < 0.001$.

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Results

Chronic stimulation of the α_{1A}AR enhances learning and memory

Learning and memory were assessed using the Barnes, Morris water, and multi-T mazes. The Barnes maze is widely accepted as a hippocampal-dependent task of spatial learning and memory. The time to solve, number of errors, and distance traveled are inversely correlated with learning and memory. As shown in Fig. 1, CAM-α_{1A}AR mice (n = 15) showed enhanced cognition when compared to WT mice (n = 17). During learning trials, CAM-α_{1A}AR mice took less time to solve the maze (56 ± 8.9 s) (inset, Fig. $1A_1$) when compared to WT mice (87 ± 9.8 s) [$t(30) = 2.3$, $p < 0.05$]. CAM-α_{1A}AR mice also made fewer errors during learning trials (9.7 ± 1.1) (inset, Fig. $1A_2$) than the WT mice (18 ± 2.0) [$t(30) = 3.5$, $p < 0.001$]. During memory trials, the CAM-α_{1A}AR mice better remembered the escape box's location, shown by a decreased mean solve time (21 ± 3.5 s) (inset, Fig. $1B_1$) when compared to the WT mice (38 ± 5.8 s) [$t(30) = 2.4$, $p < 0.05$]. CAM-α_{1A}AR mice also made fewer errors (5.2 ± 0.6) (inset, Fig. $1B_2$) than the WT mice (11 ± 1.3) [$t(30) = 3.6$, $p < 0.001$]. For both learning and memory trials, CAM-α_{1A}AR mice travelled a shorter distance than WT mice (4.3 ± 0.47 *vs.* 5.2 ± 0.51 m for learning, $p = 0.10$; 2.3 ± 0.29 *vs.* 2.6 ± 0.38 m for memory, $p = 0.29$) (data not shown).

The Morris water and multi-T mazes were used as additional assessments of learning and memory. In the Morris water maze, CAM-α_{1A}AR mice (n = 11) solved the maze in less time (18 ± 4.6 s) than WT mice (n = 11) (50 ± 17 s) [$t(20) = 1.8$, $p < 0.05$] during the first day of learning (Fig. 2A). CAM-α_{1A}AR mice also took less time to find the platform (8.5 ± 1.3 s) than WT mice (18 ± 3.8 s) [$t(19) = 2.3$, $p < 0.05$] during the memory phase (Fig. 2B). In the multi-T maze (Fig. $3A_1$), CAM-α_{1A}AR mice (n = 9) took less time to solve the maze during learning

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trials (32 ± 2.1 s) (Fig. $3A_2$) than WT mice (n = 9) (88 ± 17 s) [$t(16) = 3.2$, $p < 0.01$]. CAM-α_{1A}AR mice also made fewer errors (2.3 ± 0.5) (Fig. $3A_3$) than the WT mice (12 ± 1.3) [$t(16) = 6.9$, $p < 0.001$]. During memory trials, CAM-α_{1A}AR mice solved the multi-T maze in less time (46 ± 7.7 s) (inset, Fig. $3B_1$) than WT mice (77 ± 7.9 s) [$t(16) = 2.8$, $p < 0.01$]. CAM-α_{1A}AR mice also made fewer errors (5.6 ± 1.6) (inset, Fig. $3B_2$) than WT mice (13 ± 1.8) [$t(16) = 3.2$, $p < 0.01$]. Taken together, the results suggest that the CAM-α_{1A}AR mice possess improved cognitive abilities.

Aged CAM-α_{1A}AR mice have enhanced synaptic plasticity

To assess whether the behavioral gains seen in CAM-α_{1A}AR mice correlated with enhanced hippocampal plasticity, several cellular properties were investigated using electrophysiology, including basal synaptic transmission, short-term plasticity as assessed by PPF, and long-term plasticity. Basal synaptic transmission was investigated by analyzing the fEPSP slope at various stimulus intensity intervals (10-80 μA) and plotting it against fiber volley amplitude. A significant difference was observed in the average input-output slopes of basal synaptic transmission between CAM-α_{1A}AR and WT mice (2.9 ± 0.48, n = 11 *vs.* 1.1 ± 0.07, n = 23; $p < 0.001$) (Fig. 4A, inset). These findings suggest that basal synaptic transmission is enhanced in CAM-α_{1A}AR mice compared to WT mice.

The slope ratio of fEPSP was calculated by taking the slope of the second elicited fEPSP and dividing it by the first elicited fEPSP in PPF experiments. Inter-pulse intervals were then set at 35, 50, 75, 100, 150, 200 or 300 ms. A significant difference was found between CAM-α_{1A}AR and WT mice at 35 ms (1.3 ± 0.037, n = 23 *vs.* 1.6 ± 0.052, n = 21, $p < 0.001$), 50 ms (1.3 ± 0.035, n = 23 *vs.* 1.6 ± 0.049, n = 21, $p < 0.001$), 75 ms (1.3 ± 0.043, n = 13 *vs.* 1.5 ±

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0.047, n = 17, $p < 0.001$), 100 ms (1.2 ± 0.027, n = 23 *vs.* 1.5 ± 0.037, n = 21, $p < 0.001$), 150 ms (1.2 ± 0.039, n = 13 *vs.* 1.4 ± 0.032, n = 19, $p < 0.001$) and 200 ms (1.1 ± 0.027, n = 10 *vs.* 1.3 ± 0.079, n = 4, $p < 0.05$). These results indicate that short-term plasticity is enhanced in the CAM-α_{1A}AR compared to WT mice.

Synaptic plasticity (particularly LTP) is thought to underlie learning and memory. We measured LTP in the apical dendrites of the hippocampal CA1 region of very old (age 22 to 24-mo) CAM-α_{1A}AR and WT mice induced by TBS (10 trains of 4 pulses at 100 Hz) of the Schaeffer collateral pathway (Fig. 4C_1). These recordings showed a significant enhancement of normalized LTP in CAM-α_{1A}AR compared to WT mice at 15 min (1.5 ± 0.073 *vs.* 1.2 ± 0.036, $p < 0.01$), 30 min (1.4 ± 0.053 *vs.* 1.2 ± 0.036, $p < 0.01$) and 60 min (1.3 ± 0.052 *vs.* 1.1 ± 0.041, $p < 0.01$) after TBS (Fig. 4C_2) (n = 9 animals for each comparison). These results demonstrate that CAM-α_{1A}AR mice have increased LTP relative to WT mice, suggesting that chronic α_{1A}AR stimulation enhances LTP. This finding is consistent with our observations of enhanced basal synaptic transmission and PPF in CAM-α_{1A}AR mice compared to WT.

Chronic treatment with an α_{1A}AR-selective agonist improves cognitive function

To determine whether endogenous stimulation mimics the cognitive effects observed in CAM-α_{1A}AR mice, we treated normal WT mice with the α_{1A}AR-selective agonist, cirazoline. We assessed learning and memory using the Barnes and Multi-T mazes (Fig. 5). During learning, cirazoline-treated mice solved the Barnes maze in less time (16 ± 3.4 s, n =14) (Fig. 5A_1) than control mice (49 ± 13 s, n = 11) [$t(23) = 2.1$, $p < 0.05$] while making fewer errors (2.9 ± 1.0) (Fig. 5A_2) than control mice (7.1 ± 2.0) [$t(23) = 1.7$, $p < 0.05$]. Similarly, during learning, cirazoline-treated mice solved the multi-T maze in less time (72 ± 24 s, n = 10) (Fig.

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$5B_1$) than control mice (130 ± 22 s, n = 9) [$t(17) = 1.8$, $p < .05$]. Cirazoline-treated mice also made fewer errors (15 ± 6.2) (Fig. $5B_2$) than the control mice (32 ± 5.9) [$t(17) = 1.9$, $p < 0.05$]. During memory trials for the Barnes maze (Fig. $5C_1$), cirazoline-treated mice solved the maze in less time (24 ± 3.6 s) (inset, Fig. $5C1_1$) than control mice (44 ± 7.3 s) [$t(23) = 2.2$, $p < 0.05$], while making fewer errors (3.0 ± 0.7) (inset, Fig. $5C_2$) than control (7.3 ± 1.7) [$t(23) = 2.1$, $p < 0.05$]. During multi-T maze learning trials, cirazoline-treated mice solved the maze in less time (110 ± 9.1 s) (inset, Fig. $5D_1$) compared to control mice (140 ± 8.3) [$t(17) = 2.3$, $p < 0.05$], while making fewer errors (25 ± 2.8) (inset, Fig. $5D_2$) than control mice (31 ± 1.7) [$t(17) = 1.8$, $p < 0.05$]. These results suggest that endogenous α_{1A}AR activation by a subtype selective agonist improves cognitive function in normal mice.

α_{1A}AR-KO mice display poor cognitive function

To further define the role of α_{1A}ARs in learning and memory, we next examined the effects of blocking α_{1A}ARs on cognitive function. Because α_1AR antagonists can cause sedation which would affect behavior testing, we studied α_{1A}AR-KO mice using the Barnes maze. We chose the Barnes maze because it creates a less stressful environment than the water maze and is safer for the α_{1A}AR-KO mice, which are prone to seizures. As shown in Fig. 6, α_{1A}AR-KO mice (n = 12) displayed impaired learning and memory when compared to WT mice (n = 10). During learning trials, α_{1A}AR-KO mice took more time to solve the maze (120 ± 27 s) (inset, Fig. $6A_1$) than control mice (54 ± 9.6 s) [$t(20) = 1.9$, $p < 0.05$]. α_{1A}AR-KO mice also made more errors (36 ± 9.8) (inset, Fig. $6A_2$) than control mice (16 ± 1.8) [$t(20) = 1.8$, $p < 0.05$]. During memory trials, the α_{1A}AR-KO mice displayed a poorer recollection of the escape box's location, indicated by an increased mean solve time (95 ± 36 s) (inset, Fig. $6B_1$) compared to WT mice

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(19 ± 4.8 s) [$t(20) = 1.9$, $p < 0.05$]. α_{1A}AR-KO mice also made more errors (35 ± 15) (inset, Fig. $6B_2$) than the WT mice (6.3 ± 1.0) [$t(20) = 3.6$, $p < 0.001$]. For both learning and memory trials, α_{1A}AR-KO mice travelled a longer distance than WT mice (9.6 ± 2.5 *vs.* 4.1 ± 0.6 m for learning, $p < 0.05$; 8.5 ± 3.2 *vs.* 1.9 ± 0.26 m for memory, $p < 0.05$) (data not shown). These results suggest that α_{1A}AR-KO mice have poor cognitive abilities and that the α_{1A}AR is directly involved in affecting cognitive behavior.

CAM-α_{1A}AR mice have normal levels of locomotion

To determine whether differences on behavioral tests (e.g., Barnes, Morris water, and multi-T mazes) were due to improvements or degradation of cognitive ability or to differences in motor function, the open field locomotion test was used to assess the spontaneous locomotor activity of the CAM-α_{1A}AR and WT mice. Total activity (or beam breaks/min) was assessed for 15 min. As shown in Fig. 7, the CAM-α_{1A}AR ($n = 12$) and WT mice ($n = 7$) exhibited an average total activity of 234 ± 15 and 236 ± 14 beam breaks/min, respectively, indicating there is no difference in motor activity between these mice. These results indicate that the differences observed in this study are not due to differences in locomotive ability.

Chronic α_{1A}AR activation improves mood

Since depression and neurogenesis may be linked, we used the tail suspension test to compare the level of depression in the CAM-α_{1A}AR mice to their WT counterparts. The time spent immobile while hanging by their tail is positively correlated with depression. As shown in Fig. 8A, CAM-α_{1A}AR mice spent less time immobile (52 ± 12 s, $n = 19$) than the WT mice

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(100 ± 8.2 s, n = 33) [$t(50) = 3.5$, $p < 0.001$]. These results suggest that chronic α_{1A}AR stimulation elicits antidepressant-like behavior.

Because depression and anxiety are often co-morbid, we next compared levels of anxiety in CAM-α_{1A}AR mice to their WT counterparts using a number of behavioral tests for anxiety including the marble burying test, elevated zero maze, and light/dark exploration. In the marble burying test, the number of marbles buried is positively correlated with increased obsessive compulsive-type anxiety. As shown in Fig. 8B, CAM-α_{1A}AR mice buried significantly fewer marbles (11 ± 0.9, n = 21) than the WT mice (14 ± 0.5, n = 36) [$t(55) = 2.5$, $p < .01$]. These results suggest that CAM-α_{1A}AR mice exhibit less obsessive compulsive-like behavior.

The elevated zero maze uses a simple paradigm of elevated walkways, two enclosed and two open, to determine anxiety levels in rodents. The amount of time spent in the open areas is positively correlated with lower anxiety. As shown in Fig. $8C_1$, CAM-α_{1A}AR mice spent significantly more time in the open sections (280 ± 14 s, n = 20) than WT mice (240 ± 11 s, n = 36) [$t(54) = 2.5$, $p < .01$]. As illustrated in Fig. $8C_2$, CAM-α_{1A}AR mice also made more entries to the open areas (35 ± 2.0) than WT mice (24 ± 1.6) [$t(49) = 4.2$, $p < .001$]. These results suggest that CAM-α_{1A}AR mice exhibit less anxiety-like behavior.

In light/dark exploration, the amount of time spent in the light side and the number of transitions to the dark side are positively correlated with a reduced level of anxiety. As shown in Fig. $8D_1$, CAM-α_{1A}AR mice spent significantly more time in the light area (350 ± 13 s, n = 21) when compared to WT mice (310 ± 8.9 s, n = 34) [$t(53) = 2.6$, $p < 0.01$]. As shown in Fig. $8D_2$, CAM-α_{1A}AR mice also made significantly more transitions to the dark side (29 ± 1.9) than WT mice (22 ± 1.3) [$t(53) = 3.5$, $p < 0.001$]. These results are consistent with our hypothesis that CAM-α_{1A}AR mice display less anxiety-like behavior. Taken together, the results from this

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series of behavior tests evaluating depression and anxiety suggest that chronic α_{1A}AR stimulation improves mood and reduces anxiety.

Body weight was unaffected by chronic α_{1A}AR activation

Weight was compared in mature adult (6-8 mo), middle-aged (10-14 mo), old (16-20 mo), and very old (22-24 mo) WT and CAM-α_{1A}AR mice. No significant differences were observed between WT and CAM-α_{1A}AR mice classified as mature adult (43 ± 1.1 g, n = 8 *vs.* 42 ± 1.7 g, n = 20), middle-aged (42 ± 1.0 g, n = 58 *vs.* 44 ± 0.9 g, n = 34), old (41 ± 1.4 g, n = 18 *vs.* 44 ± 0.9 g, n = 34) or very old (38 ± 1.1 g, n = 20 *vs.* 38 ± 1.4 g, n = 19). This data suggests that potential differences in caloric intake and/or metabolic rate do not account for the differences observed in this study between WT and CAM-α_{1A}AR mice.

CAM-α_{1A}AR mice have increased longevity

Since increased neurogenesis could positively effect aging, log-rank testing was used to evaluate lifespan differences for WT and CAM-α_{1A}AR mice. With data from both sexes, median lifespan in CAM-α_{1A}AR mice was significantly increased by 72 days (from 747 to 819) or 10% relative to that of WT mice (χ^2 = 7.2, p < 0.01) (Fig. 9, Table 1). Analysis of each sex showed increased longevity. The median lifespan in female CAM-α_{1A}AR mice was increased, but not significant, by 107 days (from 711 to 818 days) or 15% relative to that of WT mice (χ^2 = 0.23, p = *ns*). However, the median lifespan in male CAM-α_{1A}AR mice was significantly increased, by 62 days (from 760 to 822 days) or 8% relative to that of WT mice (χ^2 = 8.2, p < *0.01*). As shown in Table 1, the 90th percentile age for CAM-α_{1A}AR mice was also increased by 73 days or 8% compared to WT mice (from 909 to 982 days), suggesting that chronic α_{1A}AR

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stimulation may also increase maximal lifespan. The results from this study support our hypothesis that chronic α_{1A}AR stimulation enhances learning and memory, promotes synaptic plasticity, improves mood, and extends lifespan in this mouse strain.

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Discussion

Although ARs are typically associated with peripheral modulation of the sympathetic nervous system, all three AR families (α_1, α_2, β) are highly expressed in the brain and regulate synaptic transmission. The NE system modulates cognitive function such as arousal, attention, learning and memory, as well as behavioral responses to stress, such as depression and anxiety disorders (Sirviö and Macdonald, 1999). In humans, α_1-AR ligands are currently used or are in clinical trials to treat orthostatic hypotension, seizures, alcohol dependence, cocaine addiction and post-traumatic stress disorders. Due to a lack of highly avid antibodies, localization studies in the brain were mainly performed in the past using autoradiography, but the signal intensity was poor due to low expression of α_1ARs and the lack of selective radioligands. We circumvented this problem by using mice expressing EGFP under the same α_{1A}AR promoter as in this study and compared the expression in the brain with α_{1A}AR-KO mice, where the α_{1A}AR gene was replaced with β-galactosidase. We found that the α_{1A}AR subtype is highly expressed in cognitive centers, such as the prefrontal cortex, entorhinal cortex, hippocampal CA1-3, and dentate gyrus. Other areas of the limbic system that also highly expressed the α_{1A}AR are centers for depression and anxiety, such as the amygdala (Papay et al., 2006).

Previous studies exploring the role of α_1ARs in cognitive and behavioral responses are inconsistent, with activation of α_1ARs in some studies facilitating cognition and other studies showing decreased function. Some studies suggest that α_1ARs inhibit memory consolidation in chicks (Gibbs and Summers, 2001) and impair spatial memory after infusion of α_1AR agonists in monkeys and rats (Arnsten et al., 1999; Mao et al., 1999). In contrast, other studies suggest that α_1ARs facilitated spatial and intermediate-term memory in rats (Pussinen et al., 1997; Puumala et al., 1998). These discrepancies could be due to species-specific functions of α_1ARs,

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the use of high doses of non-selective ligands, differing modes of administration, and improper pharmacological technique. For example, in a study claiming that α_1AR stimulation impairs spatial working memory in rhesus monkeys (Mao et al., 1999), low numbers of animals were used. In addition, α_1AR and α_2AR agonists were used at the same concentration, but they did not account for the dramatically different half-lives or receptor occupancy of these agonists. This makes any conclusions about α_1AR involvement doubtful. In Arnsten et al., 1999, muscular administration of cirazoline and the short delay before behavioral testing make it difficult to ascertain cirazoline's effects on the brain. Acute treatment with α_1AR ligands may not alter behavioral functions, which may be dependent upon reaching tonic levels of elevated signaling and/or be dependent upon increased synaptic plasticity (Nakadate et al., 2006) or neurogenesis, a recently discovered function of the α_{1A}AR (Gupta et al., 2009).

In our study utilizing a transgenic mouse model and several types of behavioral cognitive tests (Figs. 1-4), we found that chronic stimulation of the α_{1A}AR improved learning and memory. Cognitive enhancement is not due to changes in blood flow or blood pressure, as CAM-α_{1A}AR mice have normal resting and stimulated blood pressure (data not shown). The lack of effect on blood pressure in CAM-α_{1A}AR mice is likely due to the low receptor over expression and/or activity in the transgenic mice because of the use of the endogenous housekeeping promoter and possible compensation on blood pressure. Knock-out of the α_{1A}AR subtype has minimal effects (8-12%) on resting blood pressure and still retains 85% of the pressor response to PE (Rokosh and Simpson, 2002). Similarly, chronic low-doses of NE stimulate adaptive cardiac hypertrophy without increases in blood pressure (Jensen et al., 2010).

Numerous reports have suggested correlations between adrenergic function and age- or disease-associated changes in memory function (Szot et al., 2007). Abnormalities in α_1ARs are

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implicated in the cognitive deficits of AD. Furthermore, polymorphisms in ARs are associated with AD susceptibility (Bullido et al., 2004). Transgenic Tg2576 mice with Alzheimer plaque pathology display increased α_1AR binding (Klingner et al., 2003). In humans with AD, the expression of the α_{1A}AR mRNA subtype is significantly reduced in specific layers of the prefrontal cortex (Szot et al., 2007), suggesting that increased activity of the α_{1A}AR in these neurons may delay or prevent AD severity.

Synaptic plasticity is widely held as an essential component of learning, memory, and cognitive function, all of which have been shown to decline with age and neurodegenerative disorders. The hippocampus is a critical structure with respect to learning, memory and synaptic plasticity. Within the adult hippocampus, synaptic plasticity occurs primarily in two areas: the perforant path and the Schaffer collaterals. Both areas show susceptibility to age-related declines in LTP (Landfield and Lynch, 1977). Conversely, LTD occurs more readily in aged mice, suggestive of an age-related increase in the susceptibility to depression in the synaptic strength. Moreover, hippocampal CA1 pyramidal neurons show age-related deficits in PPF (Landfield and Lynch, 1977), a form of short-term plasticity related to the amplitude of synaptic responses. Each of these alterations in synaptic plasticity (LTP, LTD, PPF) correlates to age-related deficits in cognitive performance in the murine brain (Bach et al., 1999). AD mouse models also show similar reductions in LTP as in aged mice (Bach et al., 1999). The AD phenotype is not expressed until later in life, coinciding with considerable neuronal death. In contrast, the present study shows that aged CAM α_{1A}AR mice possess markedly improved basal synaptic transmission, PPF, and LTP at the CA3-CA1 synapses (Fig. 4A-C). Taken together, these results suggest that chronic α_{1A}AR activation improves synaptic efficiency throughout senescence compared to WT mice.

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Deficits in cognitive functioning seen with normal aging may be attributed to loss of neurons, as well as synaptic integrity. It is well established that young neurons near the proliferative zone in the DG have a lower threshold for LTP than mature neurons (Schmidt-Hieber et al., 2004) and that reduction of DG stem cell proliferation selectively inhibits LTP (Snyder et al., 2001), suggesting a relationship between the birth of new neurons and LTP. Neurogenesis induced by α_{1A}AR activation may play an important role in the maintenance of LTP and cognitive functioning, providing a potential therapeutic target and defense against aging and neurodegenerative diseases such as AD.

Recently, it was found that antidepressants, including those targeting NE, increase neurogenesis in the hippocampus (Santarelli et al., 2003). Anxiety is commonly co-morbid with depression and is often relieved upon treatment with antidepressants. The length of time for antidepressant action to relieve symptoms is often several weeks, which is similar to the length of time for new cells to be created and integrated into the neural network. These factors indicate that treatment to increase NE may incidentally enhance neurogenesis, which could play a role in the attenuation of symptoms of depression and anxiety.

Selective serotonin reuptake inhibitors (SSRIs) are often a first-line treatment for major depressive disorder but have detrimental side effects. Treatments that activate other neurotransmitter systems like NE and dopamine are important for patients who do not respond well to SSRIs (de Montigny et al., 1999). Many antidepressants have been shown to increase neurogenesis, including antidepressants such as venlafaxine, which increases responsiveness of α_1ARs (Maj and Rogóz, 1999). Our research shows that α_{1A}AR stimulation leads to a significant reduction in depressive-like behavior compared to WT mice (Fig. 8), suggesting that treatments increasing neurogenesis or α_{1A}AR activation may improve depression symptoms.

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Since antidepressants are successfully used to treat the symptoms of obsessive-compulsive disorder (OCD), we hypothesized that stimulating the α_{1A}AR may decrease OCD symptoms in mice. Acute treatment with the serotonin-NE reuptake inhibitor (SNRI) milnacipran reduces OCD activities in mice as assessed by the marble burying test (Sugimoto et al., 2007). SNRIs have been used successfully to treat patients who are unresponsive to SSRI treatment (Hollander et al., 2003). Our results (Fig. 8) suggest that α_{1A}AR activation reduces anxiety in mice and may provide a better treatment for the 40% of patients who do not respond to traditional SSRI treatment for OCD.

Besides having enhanced learning, memory, LTP and decreased anxiety and depression, CAM-α_{1A}AR mice also live longer (Fig. 9, Table 1), suggesting that chronic α_{1A}AR stimulation can extend lifespan. The mechanism for this effect is unknown; it may be due to the cardioprotective (Huang et al., 2007; Rorabaugh et al., 2005) and neuroprotective (Goldenstein et al., 2008) effects of the α_{1A}AR. CAM-α_{1A}AR mice show an increase in both median and maximal lifespan that is consistent with delayed aging, yet this does not confirm that aging is slowed in these animals. In order to determine whether aging is altered in these mice, a comparison of the CAM-α_{1A}AR mouse model to other models of longevity, an assessment of telomerase activity, and examination of other age-dependent changes in molecular and cellular processes in these mice are necessary.

Mechanistically, cognitive enhancements and anti-depressive behavior seen in CAM-α_{1A}AR mice may be due to the ability of the α_{1A}AR to increase neurogenesis (Gupta et al., 2009), protective signals, and/or neuronal cell survival similar to the cardiomyocyte (Huang et al., 2007). Here, the signal transduction pathway involves ERK phosphorylation, which also has neuroprotective effects in the brain. It has been shown that both Akt and ERK have a role in

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regulating hippocampal neurogenesis, while reductions in ERK levels in hippocampal neurons may lead to memory deficits (Yan et al., 2007).

The use of cirazoline or other types of imidazoline-like α_{1A}AR agonists may have the best therapeutic potential. While most imidazolines have better selectivity for the α_2ARs, cirazoline is a notable exception as a strong partial agonist that has 10 to 30-fold selectivity for α_{1A}ARs over other α_1AR subtypes and 100-fold over α_2AR subtypes where it displays mild antagonist properties (Ruffolo and Waddell, 1982; Minneman et al., 1994). The imidazoline backbone in addition to partial agonism is thought to decrease the ability of imidazolines to raise blood pressure, which is a common disadvantage to using phenethylamine-type agonists, such as phenylephrine (Blue et al., 2004). Imidazoline is a nitrogen-containing heterocycle derived from imidazole, which dissipates the charged nitrogen over the ring, and therefore, can cross the blood brain barrier unlike phenylephrine (Guo et al., 1991; Davies and Wellman, 1992). While the half-life of cirazoline in the blood has not been determined, it's half-life should be longer than phenylephrine (2 h) due to its lack of breakdown by monoamine oxidase and similar to that of the other typical imidazolines (oxymetazoline = 6 h; clonidine = 12 h).

In summary, using a constitutively activated mutant α_{1A}AR mouse model and long-term administration of an α_{1A}AR agonist in normal mice, we demonstrated that activation of the α_{1A}AR subtype enhances learning and memory, and has antidepressant and anti-anxiety effects. Furthermore, chronic stimulation of the α_{1A}AR does not appear to be physiologically damaging, but has cardio- and neuroprotective effects, while also enhancing longevity. Therefore, α_{1A}AR agonists may offer a potential new strategy for treating the decline in cognition and mood associated with aging and many neurological disorders.

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Authorship Contributions

Participated in research design: Doze, Perez, Gupta, Goldenstein, Collette, Nelson, Lyons, Davis, Luger, Woods, Haselton, and Papay.

Conducted Experiments: Goldenstein, Collette, Nelson, Lyons, Davis, Luger, Woods, and Papay.

Contributed new reagents or tools: Simpson

Performed data Analysis: Doze, Perez, Goldenstein, Collette, Nelson, Lyons, Davis, Luger, and Woods.

Wrote or contributed to the writing of the manuscript: Doze, Perez, Goldenstein, Collette, Nelson, Lyons, and Haselton.

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Footnotes

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Legends for Figures

Fig 1. Chronic α_{1A}AR stimulation improves cognitive performance in the Barnes maze. During learning trials, CAM-α_{1A}AR mice (n = 17) took less time to solve the maze (**A_1**) and made fewer errors (**A_2**) when compared to the WT mice (n = 15). During memory trials, CAM-α_{1A}AR mice took less time to solve the maze (**B_1**) and made fewer errors (**B_2**) compared to WT mice. Schematic drawings represent paths traveled during learning (**C_1**) and memory (**C_2**) trials of the WT and CAM-α_{1A}AR mice. The bar graph insets show the mean solve time and errors during learning and memory trials. Statistically significant at * $p < 0.05$, ** $p < 0.01$ or *** $p < 0.001$.

Fig 2. Learning and memory in the Morris water maze is enhanced with chronic α_{1A}AR activation. (**A**) CAM-α_{1A}AR mice (n = 11) took less time than the WT mice (n = 11) to reach the correct platform on day 1 of the learning phase. (**B**) When the platform and spatial clues were reversed on day 9 to test memory, CAM-α_{1A}AR mice completed the maze in less time than WT mice. Statistically significant at * $p < 0.05$.

Fig 3. Long-term α_{1A}AR stimulation increases spatial working memory in the multi-T maze. Schematic diagram of Multi-T maze (**A_1**). During the learning phase, CAM-α_{1A}AR mice (n = 9) took less time to solve the maze (**A_2**) and made fewer errors (**A_3**) than the WT mice (n = 11). During memory trials, CAM-α_{1A}AR mice took less time to solve (**B_1**) and made fewer errors (**B_2**) than the WT mice. The bar graph insets show the mean solve time and errors during memory testing. Statistically significant at ** $p < 0.01$ or ***$p < 0.001$.

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Fig. 4. Hippocampal synaptic plasticity is enhanced with chronic α_{1A}AR activation. (**A**) Basal synaptic transmission, as determined by the input-output relation between fiber volley amplitude and fEPSP slope, is increased in CAM-α_{1A}AR (*n* = 11 slices from 3 animals) compared to WT mice (*n* = 23 slices from 7 animals). The bar graph inset shows the mean in-output slopes. (**B**) Frequency facilitation (PPF) is enhanced in the CAM-α_{1A}AR mice (*n* = 21 slices from 3 animals) compared to the WT mice (*n* = 23 slices from 7 animals). The facilitation was plotted as a function of interpulse interval of 35, 50, 75, 100, 150, 200, and 300 ms. Superimposed representative fEPSPs were recorded at 150 ms interval. (**C_1**) Chronic α_{1A}AR activation enhances LTP in the hippocampal CA1 region, shown by cumulative data of the normalized changes in field potential slope in CAM-α_{1A}AR mice (*n* = 23 slices from 9 animals) and WT mice (*n* = 27 slices from 9 animals). Superimposed representative fEPSPs were recorded 15 min before and 60 min after LTP induction. (**C_2**) Multiple LTP recordings for each mouse were grouped and averaged, giving a single fEPSP slope ratio per animal at different time points before or after TBS (-15, 15, 30, 60 min). CAM-α_{1A}AR mice (*n* = 9) showed enhanced mean LTP when compared to WT mice (*n* = 9) at each post-TBS time point. Statistically significant at * $p < 0.05$, * $p < 0.01$ or *** $p < 0.001$.

Fig 5. Chronic treatment with an α_{1A}AR-selective agonist improves cognitive function. Normal WT mice treated for 9 mo with the α_{1A}AR-selective agonist cirazoline (*n* = 11) solved the Barnes maze in less time (**A_1**) and made fewer errors (**A_2**) than the control WT mice (*n* = 14) on the last day (day 4) of learning trials. Similarly, WT mice treated with cirazoline for 2 mo (*n* = 10) solved the multi-T maze in less time (**B_1**) and made fewer errors (**B_2**) than the control WT

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mice (n = 11) on the last day (day 5) of learning trials. During memory trials, cirazoline-treated WT mice exhibited took less time to solve (**C_1**, **D_1**) and made fewer errors (**C_2**, **D_2**) than the control WT mice in both the Barnes and multi-T mazes. The bar graph insets show the mean solve time and errors during memory testing. Statistically significant at * $p < 0.05$ or ** $p < 0.01$.

Fig 6. Cognitive performance in the Barnes maze is reduced in mice lacking α_{1A}ARs. During learning trials, α_{1A}AR-KO mice (n = 12) took more time to solve the maze (**A_1**) and made more errors (**A_2**) when compared to the WT mice (n = 10). During memory trials, α_{1A}AR-KO mice took more time to solve the maze (**B_1**) and made more errors (**B_2**) compared to WT mice. Schematic drawings represent paths traveled during learning (**C_1**) and memory (**C_2**) trials of the WT and α_{1A}AR-KO mice. The bar graph insets show the mean solve time and errors during learning and memory trials. Statistically significant at * $p < 0.05$.

Fig 7. Chronic α_{1A}AR stimulation does not alter locomotor activity. An open field locomotion test revealed no difference in locomotor activity between the CAM-α_{1A}AR (n = 12) and WT mice (n = 7).

Fig 8. Long-term α_{1A}AR activation improves mood. Rodent tests of anxiety and depression show that α_{1A}AR stimulation increases antidepressant-like behavior and decreases anxiety-related behaviors in mice. (**A**) α_{1A}AR activation elicits antidepressant-like behaviors in the tail suspension test. CAM-α_{1A}AR mice (n = 19) spent less time immobile than the WT mice (n = 33). (**B**) α_{1A}AR stimulation reduces obsessive-compulsive type anxiety in the marble burying

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test. CAM-α_{1A}AR mice (n = 21) buried fewer marbles than WT mice (n = 36). α_{1A}AR activation decreases anxiety-like behaviors in the elevated zero maze, as CAM-α_{1A}AR mice (n = 20) spent more time in (**C_1**) and made more entries into (**C_2**) the open areas than WT mice (n = 36). α_{1A}AR stimulation also reduces anxiety-like behaviors in the light/dark exploration. CAM-α_{1A}AR mice (n = 21) spent more time in the light side of the box (**D_1**) and made more transitions to the dark side (**D_2**) than WT mice (n = 34). Statistically significant at ** $p < 0.01$ or *** $p < 0.001$.

Fig 9. Chronic α_{1A}AR stimulation improves murine longevity. Kaplan-Meier survival plots of the CAM-α_{1A}AR (n = 32) and WT (n = 54) mice show that α_{1A}AR stimulation extends the average murine lifespan. *P*-values were calculated using the Mantel-Cox log-rank test and each symbol represents one mouse. CAM-α_{1A}AR mice had an increased lifespan compared to the WT mice ($\chi^2 = 7.2$; df 1; $p < 0.01$). See also Table 1.

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TABLE 1.

Median lifespan, mean and 95% confidence intervals (CI) in days for genotypes

Genotype (n)	Median	Mean	SEM	95 % CI	90th Percentile	Deaths
CAM-α_{1A}AR	819	806	24	757 – 856	982	32
Wild type	747	724	22	679 – 768	909	54

The median lifespan was the point at which the fractional survival of each curve equaled 50%.

Each group consisted of an equal number of male and female mice.

Fig. 1

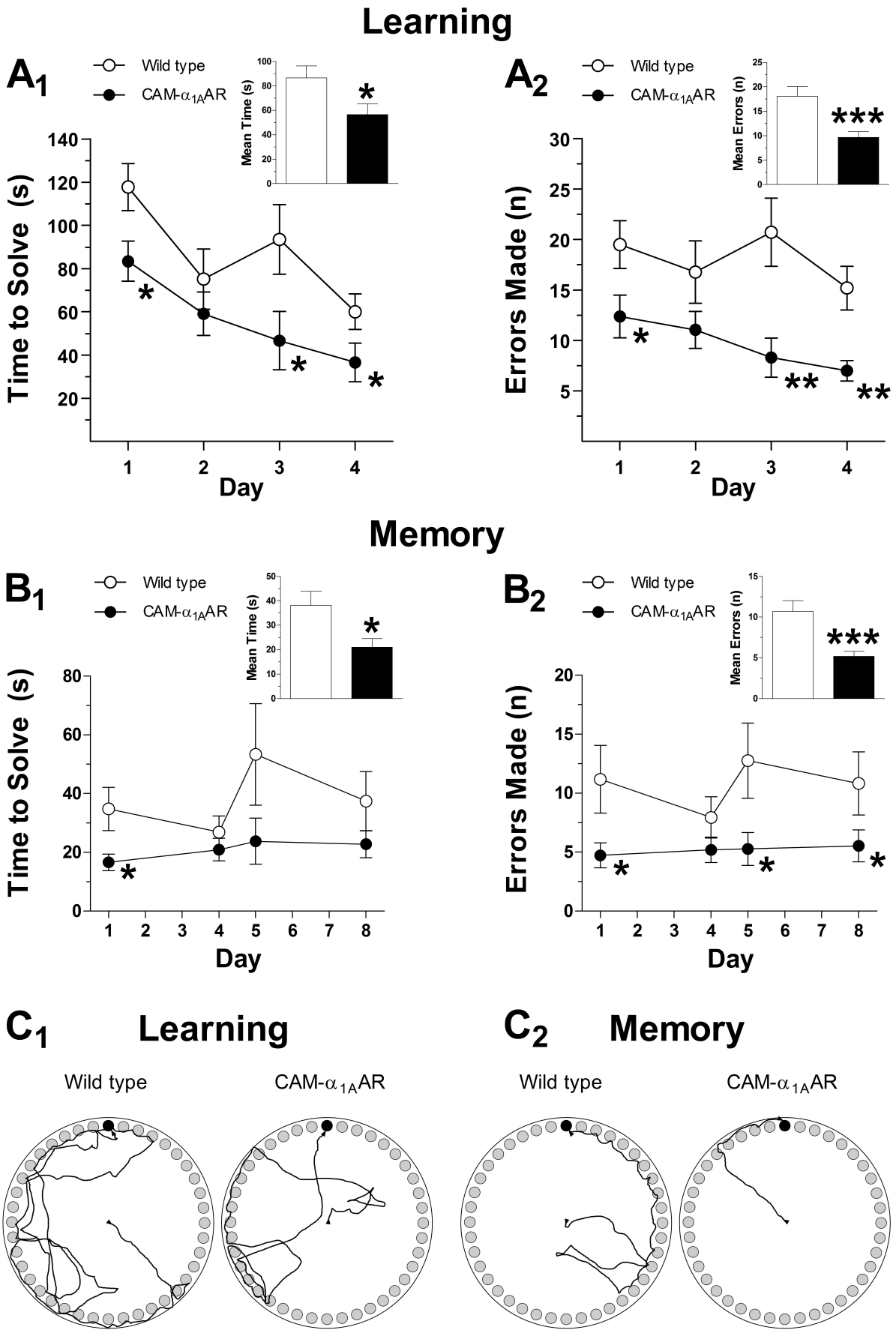

Learning
A1
Wild type
CAM-α1AAR
Time to Solve (s)
Day
Mean Time (s)
A2
Errors Made (n)
Mean Errors (n)
Memory
B1
B2
C1 Learning
C2 Memory
Wild type
CAM-α1AAR

Fig. 2

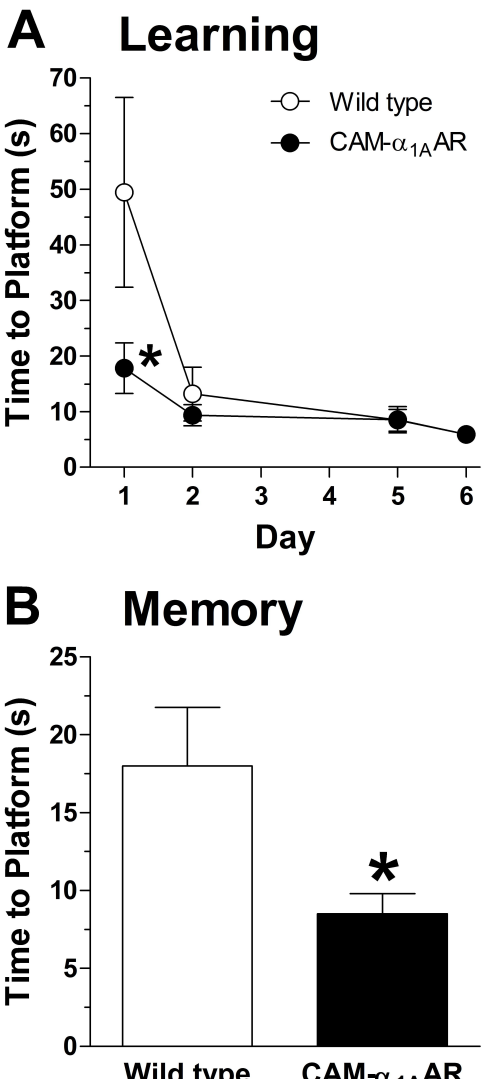
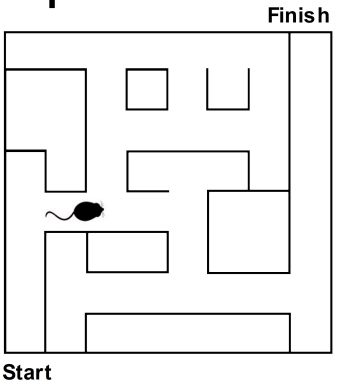

A
Learning
Wild type
CAM-α1AAR
Time to Platform (s)
70
60
50
40
30
20
10
0
*
1
2
3
4
5
6
Day
B
Memory
Time to Platform (s)
25
20
15
10
5
0
*
Wild type
CAM-α1AAR

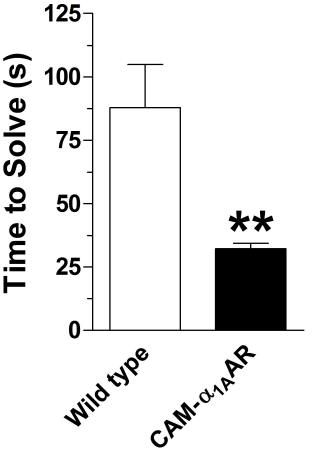
Fig. 3

Learning

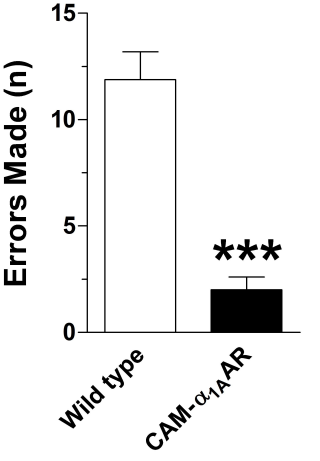
A_1 Multi-T Maze



A_2

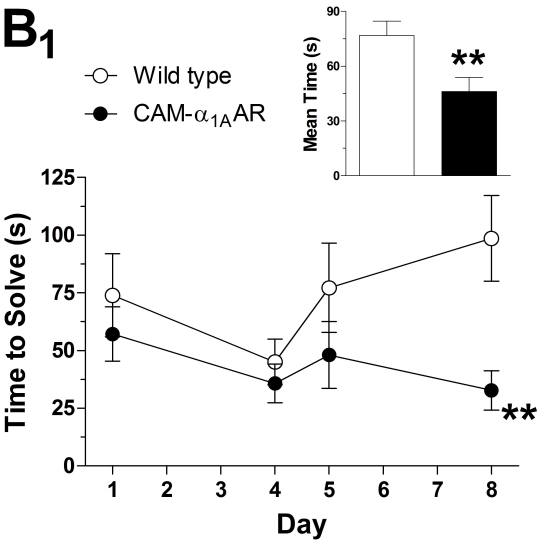


A_3



Memory

B_1



B_2

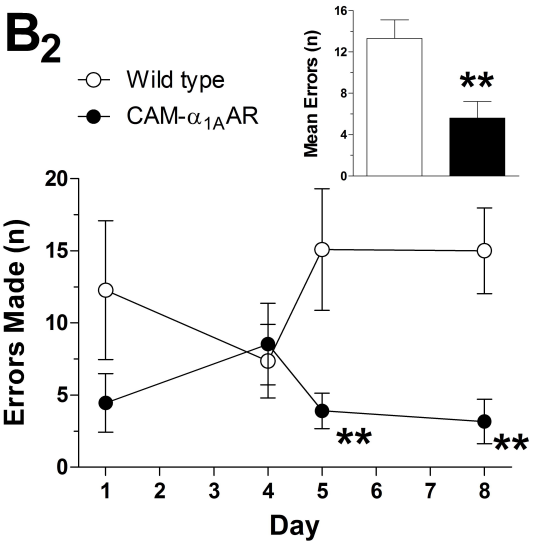
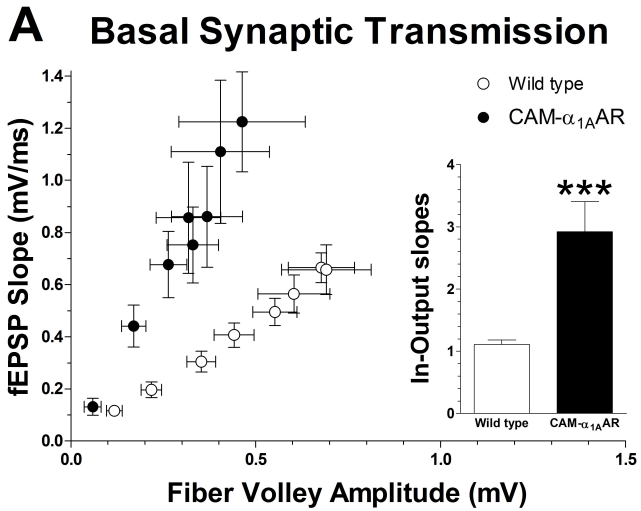
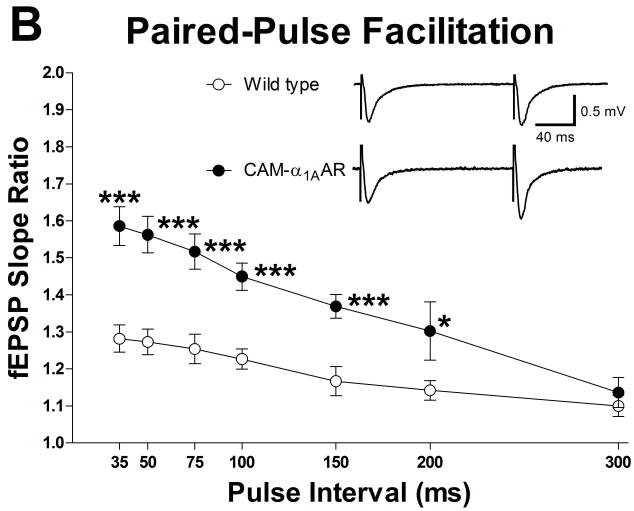
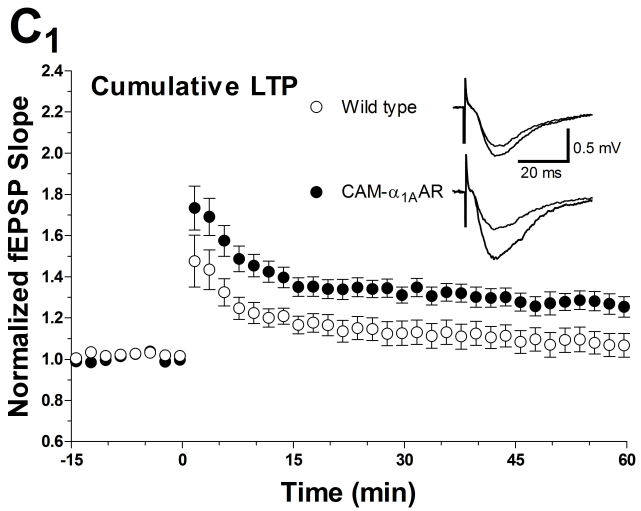


Fig. 4





Long-Term Potentiation



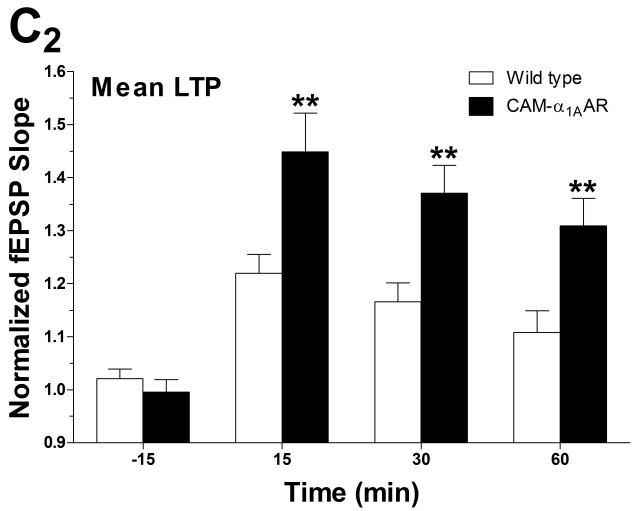


Fig. 5

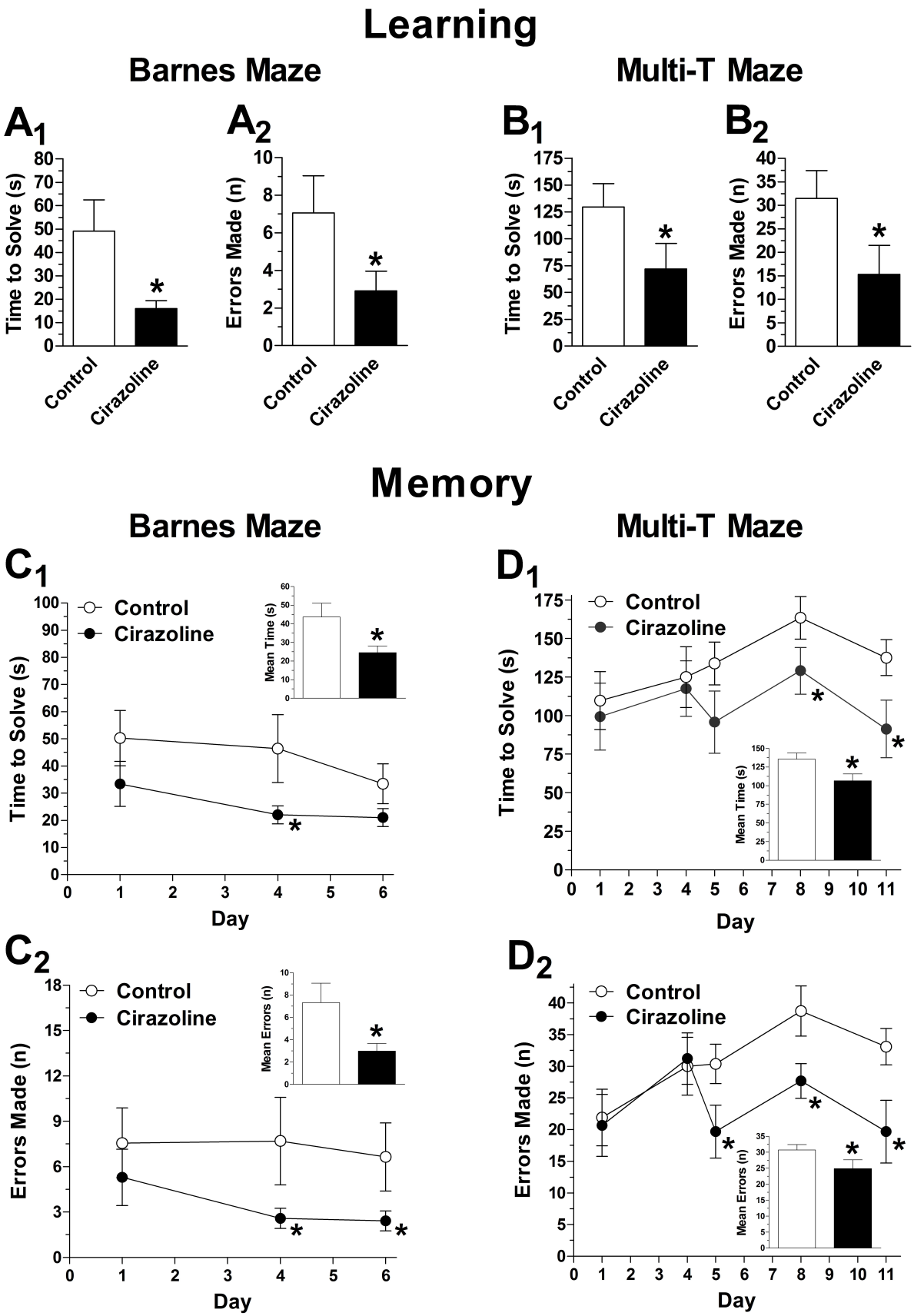

Learning
Barnes Maze
Multi-T Maze
A1
A2
B1
B2
Time to Solve (s)
Errors Made (n)
Control
Cirazoline
Memory
Barnes Maze
Multi-T Maze
C1
C2
D1
D2
Day
Mean Time (s)
Mean Errors (n)

Fig. 6

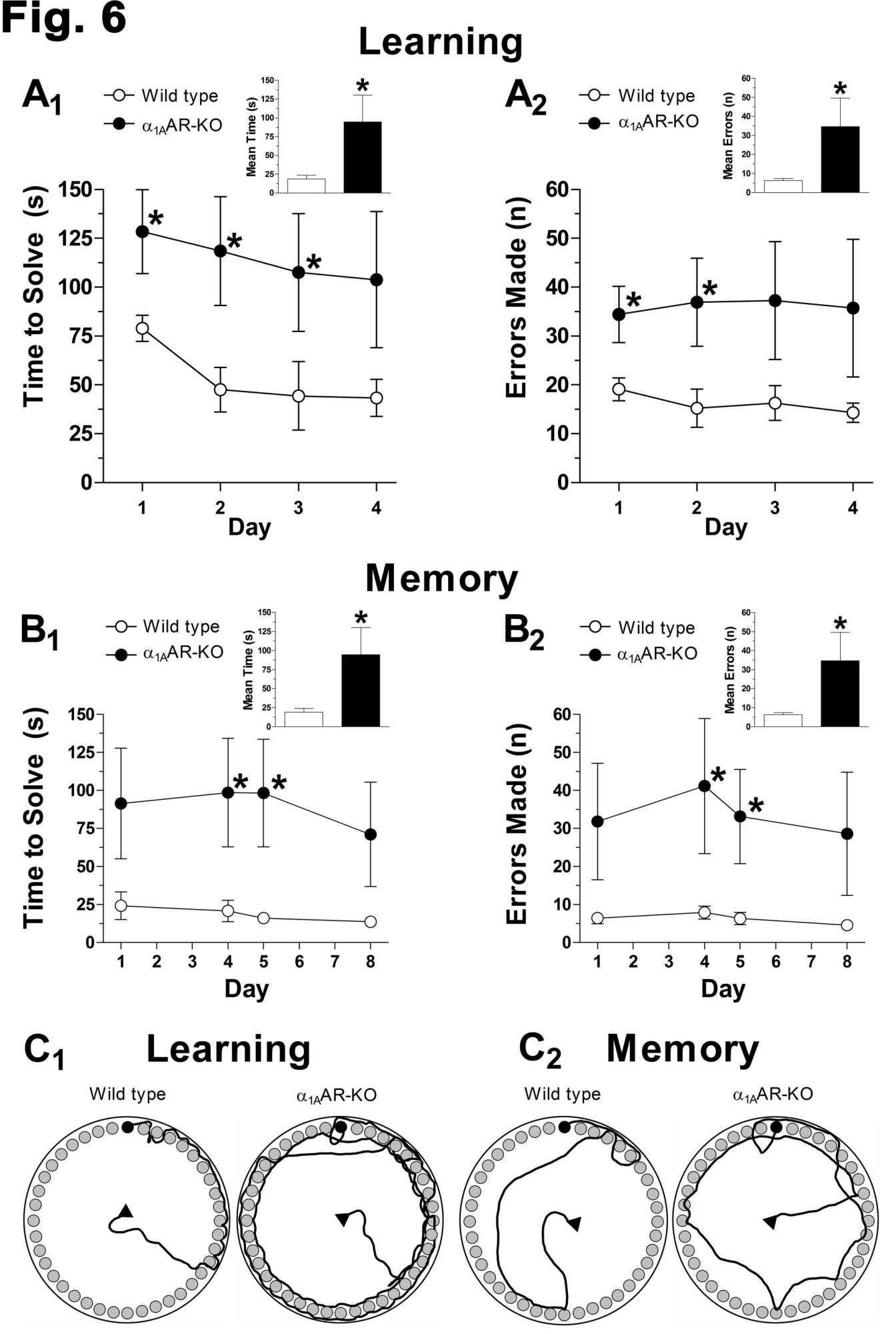

Learning
A1
Wild type
α1AAR-KO
Mean Time (s)
Time to Solve (s)
Day
A2
Mean Errors (n)
Errors Made (n)
Memory
B1
B2
C1 Learning
C2 Memory
Wild type
α1AAR-KO

Fig. 7

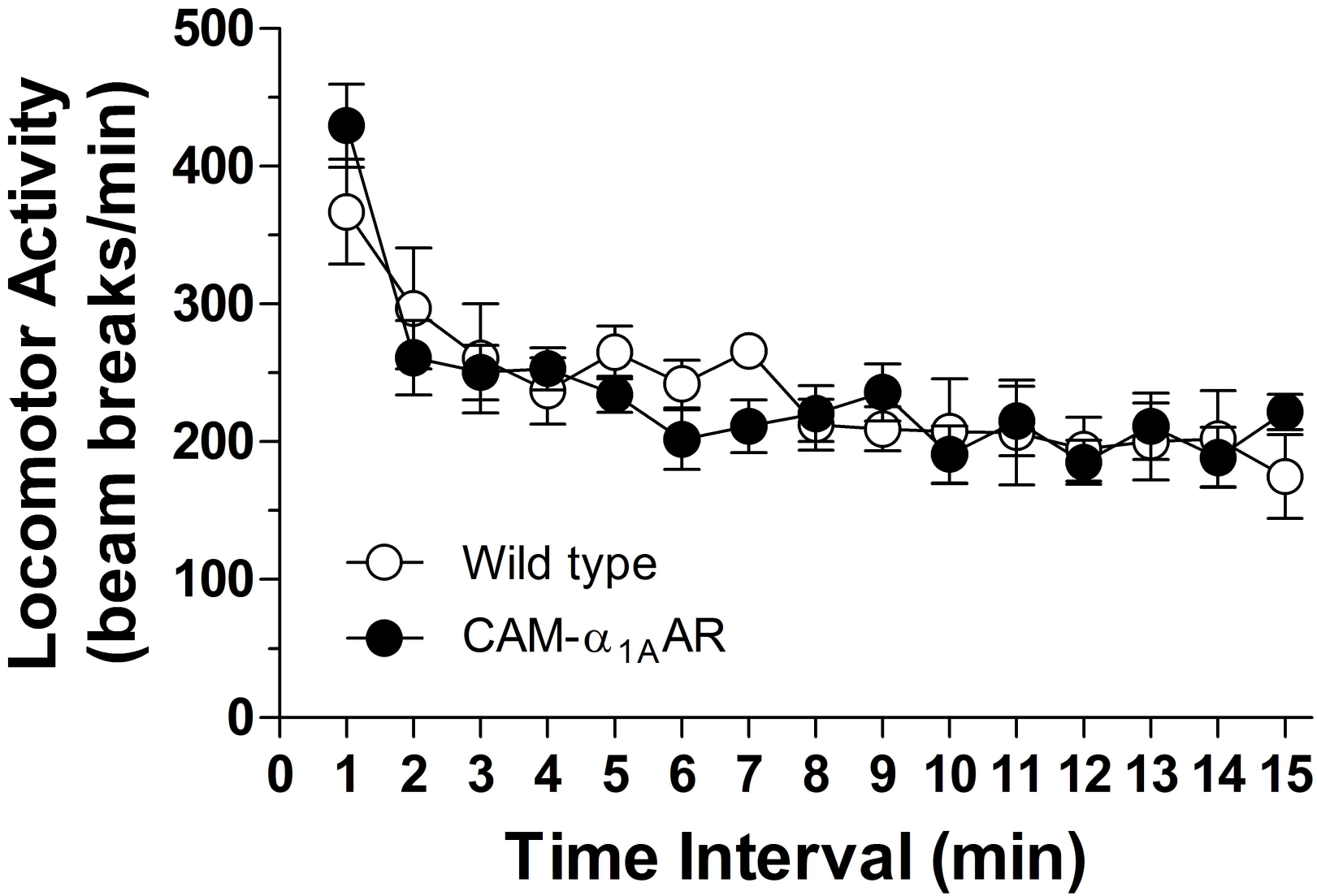
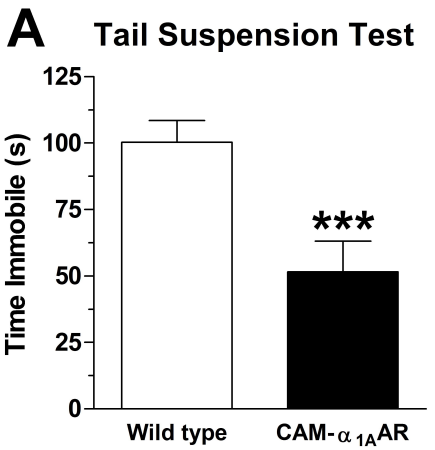
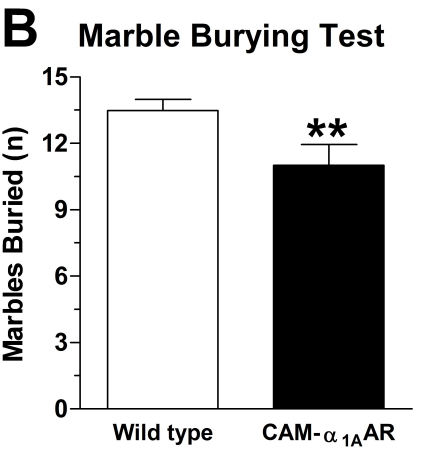
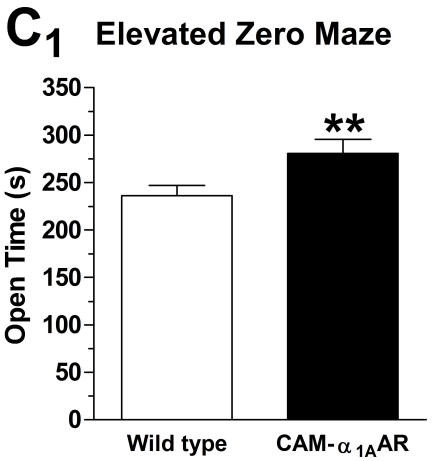
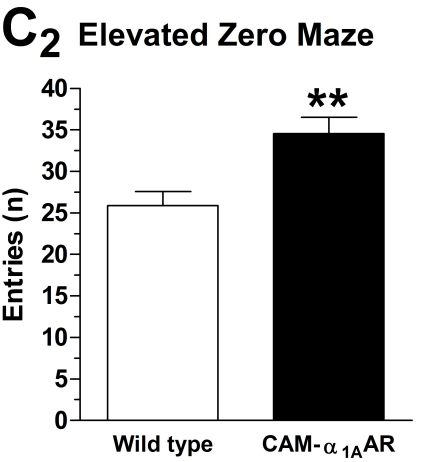

Locomotor Activity
(beam breaks/min)
500
400
300
200
100
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Time Interval (min)
Wild type
CAM-α1AAR

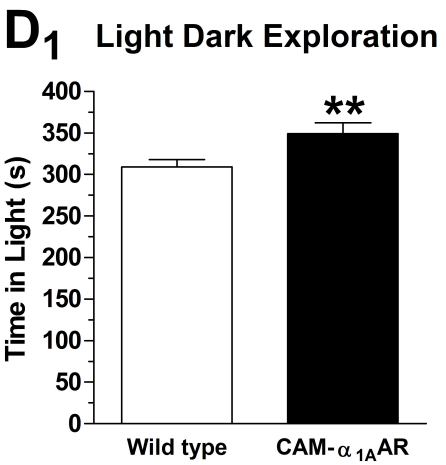
Fig. 8


A
Tail Suspension Test
Time Immobile (s)
125
100
75
50
25
0
***
Wild type
CAM-α1AAR


B
Marble Burying Test
Marbles Buried (n)
15
12
9
6
3
0
**
Wild type
CAM-α1AAR


C1
Elevated Zero Maze
Open Time (s)
350
300
250
200
150
100
50
0
**
Wild type
CAM-α1AAR


C2
Elevated Zero Maze
Entries (n)
40
35
30
25
20
15
10
5
0
**
Wild type
CAM-α1AAR


D1
Light Dark Exploration
Time in Light (s)
400
350
300
250
200
150
100
50
0
**
Wild type
CAM-α1AAR

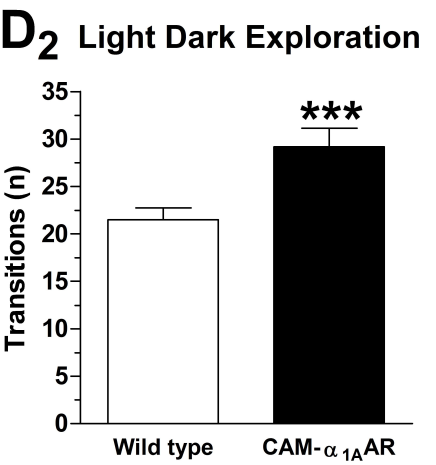

D2
Light Dark Exploration
Transitions (n)
35
30
25
20
15
10
5
0
***
Wild type
CAM-α1AAR

Fig. 9 **Mice survival proportions**