January 2015

Alpha1a Adrenergic Receptor Influences On Adult Neurogenesis, Cell Fate, Mood, Learning, Memory, Lifespan, And Cancer Incidence

Katie Collette

[How does access to this work benefit you? Let us know!](#)

Follow this and additional works at: https://commons.und.edu/theses

ALPHA1A ADRENERGIC RECEPTOR INFLUENCES ON ADULT NEUROGENESIS, CELL FATE, MOOD, LEARNING, MEMORY, LIFESPAN, AND CANCER INCIDENCE

by

Katie M. Collette

Bachelor of Science, University of North Dakota, 2010

A Dissertation

Submitted to the Graduate Faculty

of the

University of North Dakota

in partial fulfillment of the requirements
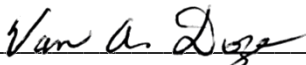

for the degree of

Doctor of Philosophy

Grand Forks, North Dakota

May
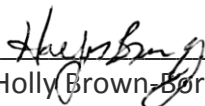
2015
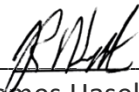
This thesis, submitted by Katie M. Collette in partial fulfillment of the requirements for the Degree of Doctor of Philosophy from the University of North Dakota, has been read by the Faculty Advisory Committee under whom the work has been done and is hereby approved.

_____
Chairperson, Van Doze

_____
Holly Brown-Borg

_____
Diane Darland

_____
James Haselton

_____
Joyce Ohm

This dissertation meets the standards for appearance, conforms to the style and format requirements of the Graduate School of the University of North Dakota, and is hereby approved.

_____
Dean of the Graduate School, Wayne Swisher

_____
April 23, 2015
Date

iii

PERMISSION

Title   Alpha$_{1A}$ Adrenergic Receptor Influences on Adult Neurogenesis, Cell Fate, Mood, Learning, Memory, Lifespan, and Cancer Incidence

Department   Pharmacology, Physiology, and Therapeutics
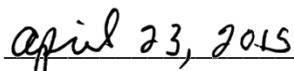
Degree   Doctor of Philosophy

       In presenting this dissertation in partial fulfillment of the requirements for a graduate degree from the University of North Dakota, I agree that the library of this University shall make it freely available for inspection. I further agree that permission for extensive copying for scholarly purposes maybe be granted by the professor who supervised my dissertation work or, in his absence, by the chairperson of the department or the dean of the Graduate School. It is understood that any copying or publication or other use of this dissertation or part thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of North Dakota in any scholarly use which may be made of any material in my dissertation.

Signature _____
            Katie Collette

Date _____
            April 21, 2015

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Acknowledgements

I would like to thank Dr. Van Doze and the Ronald E. McNair Postbaccalaureate Achievement Program for starting me in research in Van's lab. Performing research as an undergraduate confirmed in me that I want a career in research. Van allowed me to pursue my interests as a graduate student and provided me with opportunities to mentor undergraduate students throughout my graduate career. My faculty advisory committee members were so helpful at every stage, as advisors and mentors. I would also like to thank my son Kurt for understanding when I had to study in the evenings and work long hours during certain times of research. Also for being (or pretending to be) interested when I was so excited about a paper or my own results that I just had to share it with him. My family and friends, especially Susie Hensrud and my sister Leah Siewert, were always available to listen to me rant or rave about grad school or my research. I could not have done this without their support. Brianna Goldenstein and Elizabeth Sandquist were imperative in my success by discussing my hypotheses and results with me and supporting me as friends as well. "Knit" nights that rarely contained much knitting with Courtney Ali, Elizabeth, Brianna, Susie Hensrud, and Andrea Slusser helped keep me grounded socially. My classmates were also awesome. I will remember our happy hours and potlucks forever. Finally, the friends I interacted with on Twitter and networked with at conferences became additional mentors. They helped me grow as a scientist and made me feel part of a bigger whole. I look forward to keeping in touch and potentially collaborating with them in the future.

To Joshua David Collette

ABSTRACT

Norepinephrine is a catecholamine neurotransmitter that signals through the adrenergic receptors (ARs), α_1, α_2, and β, each with three subtypes. There is little research on the function of α_1AR subtypes, α_{1A}- and α_{1B}ARs, in the brain. By using α_1AR agonists, antagonists, and transgenic activation there appears to be a role for the subtypes in learning, memory, mood, adult neurogenesis, and cancer.

α_{1A}AR activation enhances learning and memory in the adult mouse. In the current study, we used α_{1A}- or α_{1B}AR knockout mice (α_{1A}AR or α_{1B}AR-KO mice) and behavioral testing to examine learning, memory, depression, and anxiety. We found that KO of each α_1AR subtype impairs novel object recognition, but not spatial memory. KO of either subtype did not reliably affect anxiety behavior. α_{1B}AR-KO mice had a significantly lower depression level than wild type mice.

Activation of the α_{1A}AR subtype increases proliferation of neural progenitor cells in the adult mouse dentate gyrus and *in vitro* work suggested a role in the differentiation stage. We used the α_{1A}AR agonist, cirazoline, BrdU incorporation, and immunohistochemistry to determine whether activation of the α_{1A}AR would direct cell fate toward an astrocytic phenotype in the adult mouse dentate gyrus. Cirazoline did not change the number or percent of new cells differentiating into neurons or astrocytes. The number of immature neurons was also not different.

The α_{1B}AR was proposed as a proto-oncogene based on *in vitro* and tumorigenesis studies. An age-related neurodegenerative disorder occurs when a constitutively active mutant form of the receptor is overexpressed in mice (CAM-α_{1B}AR mice). We tracked CAM-α_{1A}- and CAM-α_{1B}AR mouse lifespan, and performed gross pathology and histology to determine cancer occurrence. CAM-α_{1A}AR mice had an increased lifespan and decreased cancer incidence. CAM-α_{1B}AR mice had decreased lifespan but no change in cancer incidence.

Further research should pursue the mechanism of the α_1AR subtypes' role in mood and cancer incidence. The use of α_{1A}AR activation and of α_{1B}AR blocking should be examined in a mouse model of depression to determine the benefit as treatments. Heterodimerization of the receptors is also a potential mechanism.

CHAPTER 1

INTRODUCTION

The Noradrenergic System

Epinephrine, commonly known as adrenaline, and norepinephrine (noradrenaline) are signaling molecules synthesized and used in both the peripheral and central nervous systems. In the peripheral nervous system, synthesis occurs in the adrenal medulla where they are released into the bloodstream and act as hormones (von Euler, 1951; Outschoorn, 1952). In the central nervous system, the hindbrain houses the cell bodies that synthesize both molecules.

Adrenergic and noradrenergic neurons are located in the medulla and pons (Grzanna and Fritschy, 1991). The medullary nucleus includes both adrenergic and noradrenergic cell bodies that project to the spinal cord where they synapse onto preganglionic acetylcholine cells to regulate autonomic function (Swanson and Hartman, 1975). The medullary nucleus also has fibers that project to the limbic system. The locus coeruleus nuclei are located near the fourth ventricle in the dorsal pons and contain up to 50,000 neurons, including both left and right nuclei, in the human brain (Ohm et al., 1997).

Norepinephrine derives from the amino acid L-tyrosine in three steps requiring the enzymes tyrosine hydroxylase, DOPA decarboxylase, and dopamine β-hydroxylase (Figure 1). Intermediates in the process are L-DOPA and dopamine. Immunolabeling for dopamine

1
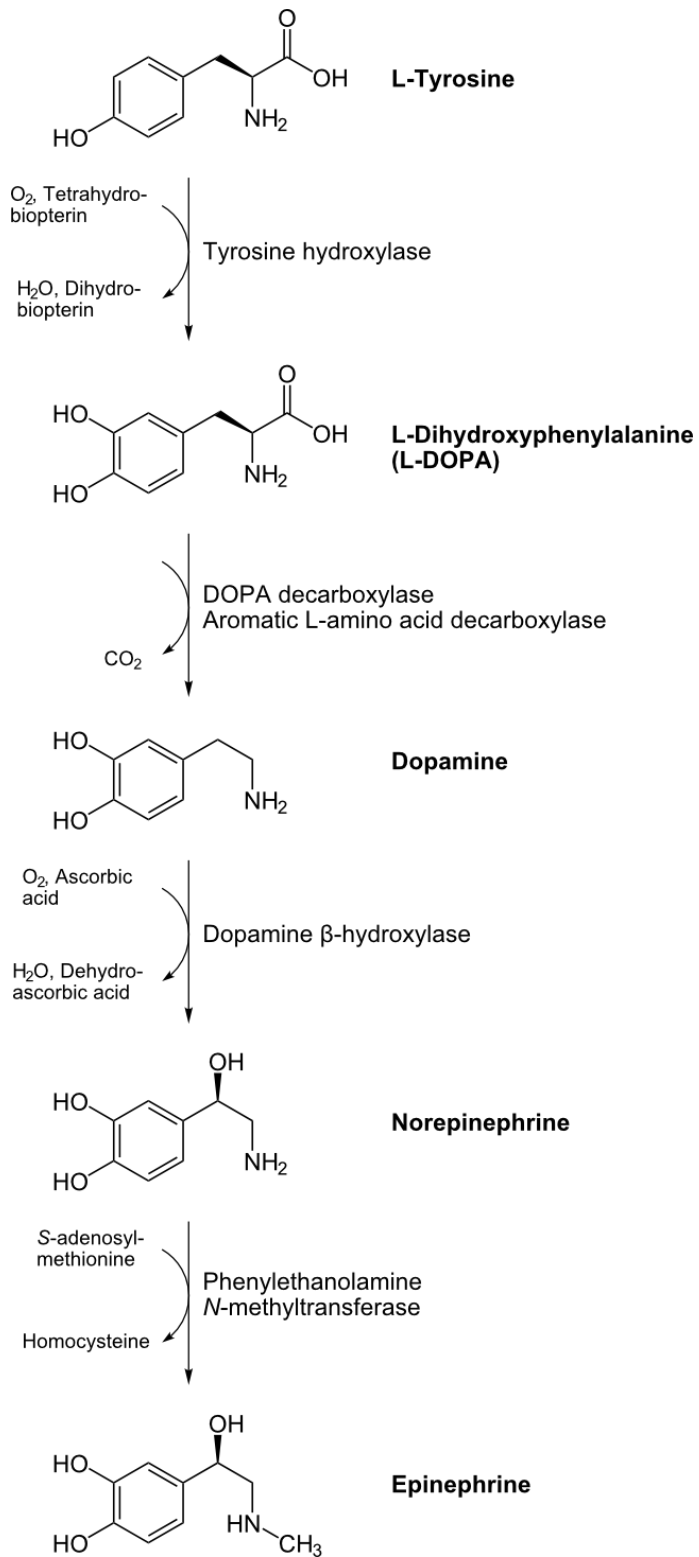
Figure 1. Synthesis of norepinephrine and epinephrine.

β-hydroxylase is one way to track noradrenergic projections in the brain. Almost every

region contains some level of noradrenergic input based on dopamine β-hydroxylase

studies (Swanson and Hartman, 1975). There are only a few areas of the brain that do not

contain dopamine β-hydroxylase. These include the caudate-putamen, nucleus accumbens,

globus pallidus, subthalamic nucleus, and substantia nigra (pars reticulata and compacta).

Noradrenergic projections have also been characterized using autoradiology and axonal

transport studies (Segal et al., 1973; Jones et al., 1977; Jones and Moore, 1977; Loughlin et

al., 1986). These studies showed that specific areas of the locus coeruleus project to

different brain regions. The rostral and medial portion of the locus coeruleus is comprised

entirely of noradrenergic cell bodies, which project to the hippocampus, hypothalamus, and

virtually all areas of the cortex (Loughlin et al., 1986) (Figure 2). The ventral area of the

locus coeruleus contains both noradrenergic and adrenergic cells that project descending

fibers to the spinal cord and cerebellum. Noradrenergic terminations in the spinal cord are

important for sympathetic nervous system functions such as heart rate. Noradrenergic cells

release norepinephrine, which bind to receptors for their effects.

Characterization of the Adrenergic Receptors

Pharmacological

The adrenergic receptors were characterized using both pharmacological and

genetic means, as better techniques became available. Prior to the advent of the term

"receptor", substances were applied to isolated tissues and the effects observed to

characterize their function. Using this technique, Raymond Ahlquist showed that
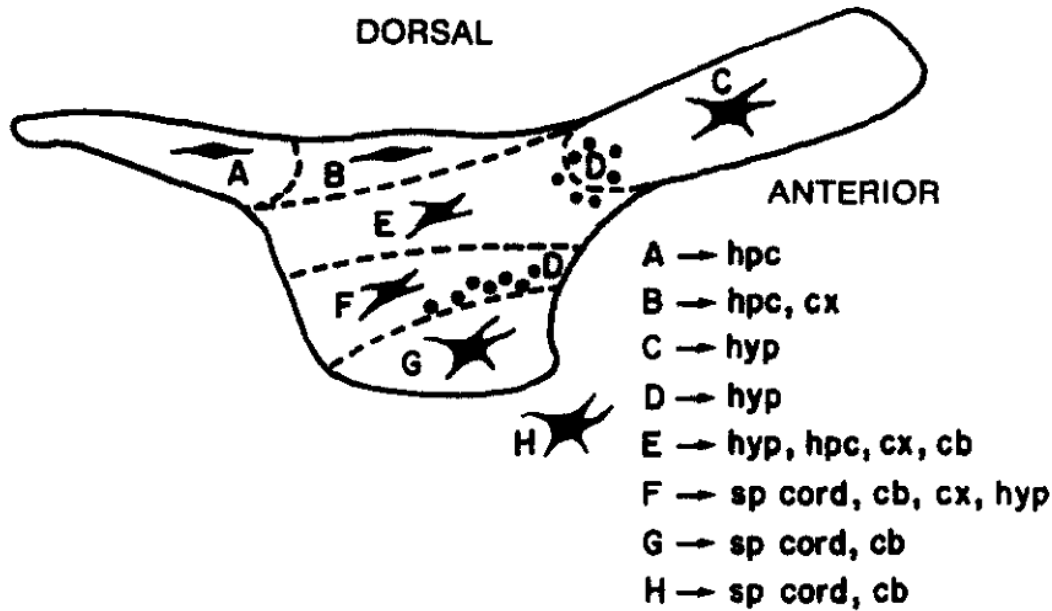
Figure 2. Locus coeruleus subregions and projections. "A schematic diagram of a sagittal view of locus coeruleus indicating proposed subdivisions, with the predominant cell type and efferents for each. Dorsal is up and anterior is to the right. Cb, cerebellum; cx, cortex; hpc, hippocampus; hyp, hypothalamus; sp cord, spinal cord." From Loughlin et al., 1986.

isoproterenol relaxed smooth muscle, norepinephrine contracted it, and epinephrine could do both (Ahlquist, 1948). In 1967, two types of receptors were proposed to bind the substances Ahlquist had been using in order to enact relaxation and contraction of smooth muscle (Furchgott, 1967). The two receptor types were characterized by rank order of agonist potencies.

For αARs, the potencies were: epinephrine = norepinephrine > isoproterenol. In contrast, βARs had an almost opposite profile: isoproterenol > epinephrine > norepinephrine (Lands et al., 1967). Radioligand binding studies using tritiated antagonists later confirmed the existence of at least two subtypes: α and βARs. In the early 1970s, the discovery of the receptors' downstream effects was only beginning.

Early work with hormone receptors showed there was an intermediate step between ligand binding and the downstream effect of adenylyl cyclase activation. Martin Rodbell showed this was due to guanine nucleotides (Pohl et al., 1971). Previously, researchers had been using preparations that were not as purified as they thought. They had inadvertently been adding nucleotides that masked the guanine-dependence of downstream effects. The first purified preparation of what came to be known as G-proteins was completed by Alfred G. Gilman's lab in 1976 (Northup et al., 1980; Maguire et al., 1976; Gilman, 1984). By this time, researchers knew the adrenergic receptors were coupled to G-proteins but they did not know how many subtypes there were.

In the late 1980s, the α_{1A}- and α_{1B}AR subtypes were proposed based on tritiated antagonist radioligand binding studies (Morrow and Creese, 1986; Minneman et al., 1988). Classification of the adrenergic receptors at this time only included two major families: α- and βARs. α_1ARs were further classified into the α_1- and α_2AR subtypes, with the α_1AR being postsynaptic and the α_2AR presynaptic (Figure 3A). David Bylund proposed, based on the pharmacological differences, differential coupling to G-proteins, and molecular cloning of the α_2AR subtypes, that the α_1- and α_2ARs were as distinct as each compared with βARs (Bylund, 1988). Thus, he proposed the classification should be α_1-,α_2-, and βARs (Figure 3B). After addition of these subtypes, Bylund's classification was officially adopted and modified as other receptor subtypes were discovered (Bylund et al., 1994; Hieble et al., 1995, Figure 3C).

A Traditional classification of receptors

adrenoceptors

- β-adrenoceptor
  - β1
  - β2
- α-adrenoceptor
  - α1
  - α2
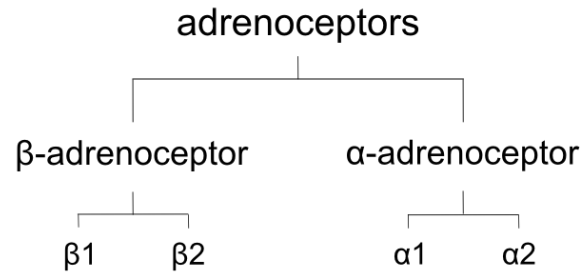
B Proposed classification in 1988

adrenoceptors

- β-adrenoceptor
  - β_1
  - β_2
- α2-adrenoceptor
  - α_{2A}
  - α_{2B}
  - α_{2C}
- α1-adrenoceptor
  - α_{1A}
  - α_{1B}
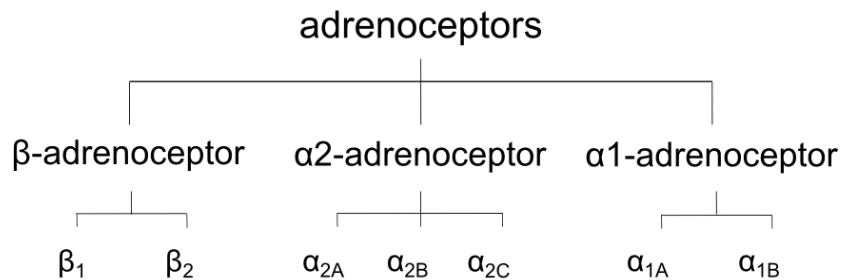
C Current classification

adrenoceptors

- β-adrenoceptor
  - β_1
  - β_2
  - β_3
- α_2-adrenoceptor
  - α_{2A}
  - α_{2B}
  - α_{2C}
- α_1-adrenoceptor
  - α_{1A}
  - α_{1B}
  - α_{1D}
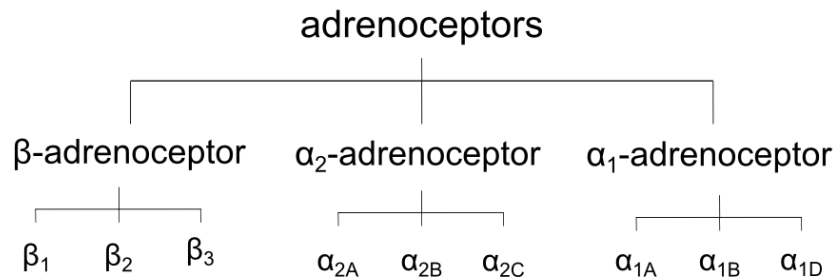
Figure 3. Classification of adrenergic receptors. A) traditional, B) proposed, and C) final classifications. Adapted from (Bylund, 1988; Bylund et al., 1994; Hieble et al., 1995).

Genetic

The advent of molecular cloning spurred a flurry of research to clone and confirm each adrenergic receptor subtype between 1986 and 1994. The α_{1A}- and α_{1B}AR were the first of the α_1AR subtypes to be cloned (Cottecchia et al., 1988; Schwinn et al., 1991). The only α_1AR subtype determined first by cloning rather than pharmacologically was the α_{1D}AR

6

(Perez et al., 1991). Soon after, the α_{1C}AR was found to be a variant of the α_{1A}AR (Perez et al., 1994). Thus, the final classification for the α_1ARs is α_{1A}, α_{1B}, and α_{1D} (Figure 3C). Another benefit of cloning the receptors' amino acid sequences was the ability to model the structure of the receptors.

<h3 style="text-align:center;">α_1 Adrenergic Receptor Structure and Signaling</h3>

Structure

The sequences determined by cloning were used to create potential structures of the receptors. The models confirmed that the adrenergic receptors were part of the GPCR family. GPCRs are a type of metabotropic receptor. GPCRs mediate their effects through conformational changes during ligand binding, which activates proteins within the cell. The n-terminus of GPCRs is located on the extracellular side of the membrane and the c-terminus on the intracellular side. All GPCRs have seven transmembrane helix domains and it is to the helices the ligand binds.

In the late 1980s, Robert Lefkowitz and Brian Kobilka isolated the β_2AR and deduced its possible structure from the amino acid sequence (Dixon et al., 1986). Kobilka's group confirmed the structure of the β_2AR bound to its G-protein using x-ray crystallography in 2007 (Cherezov et al., 2007). Later, Kobilka's group crystallized the β_2AR again, but bound to both a ligand and the G-protein subtype, Gs (Rasmussen et al., 2011). Because both the amino acid sequence and structure of the β_2AR is known, it is possible to model the potential structures of the other AR subtypes.

Pairwise sequence alignment of the amino acid sequences of the β_2- and α_{1A}AR, shows there are 120 identical, 86 conserved, and 30 semi-conserved residues (Collette,

2010). Computer modeling allows these domains to be placed on the β_2-AR structure to visualize where the differences are located (Figure 4A). Using the amino acid sequences and theoretical binding pockets, site-directed mutagenesis was used to determine the residues required for endogenous ligand binding (Strader et al., 1987). Norepinephrine, the common endogenous ligand in the brain, has three functional groups that participate in binding. These include the amine and alcohol groups, and the aromatic ring (with meta- and para-hydroxyl groups, Figure 4B, Easson and Stedman, 1933). For the α_{1A}AR, this includes residues on the third, fourth, and fifth transmembrane helices. The α_{1B}AR ligand-binding pocket does not include a residue on the fourth helix but does on the sixth. The residues for binding are the same for both subtypes on the amine and meta-hydroxyl groups. They differ by only four residue positions on the para-hydroxyl group. The largest differences are in the residues that bind to the catechol ring of the endogenous ligands.

Activation

All GPCRs have constitutive activity in the absence of an agonist. They spontaneously isomerize between the R (inactive) and R* (activate) states. Agonists bind with a higher affinity to the R* state and, when bound, stabilize the R* state. This is known as the ternary model of agonist binding. The extended ternary model states there is more than one active state, such as R1* and R2*, which can activate different G-proteins (Roberts and Waelbroeck, 2004). The idea that one receptor in unique states of activation can affect different G-proteins is called agonist trafficking. An example would be a ligand that binds to the α_{1B}AR as a full agonist and to the α_{1A}AR as a partial agonist. The extended ternary model theorizes activation of different G-proteins and thus different downstream effects. One

8
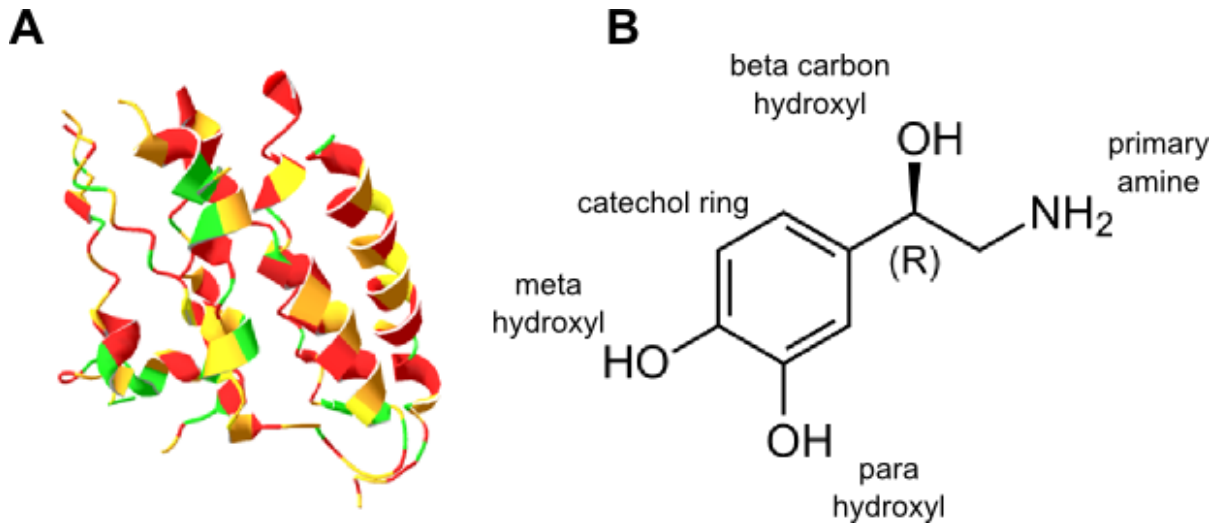
Figure 4. Receptor conservation and norepinephrine structure. A) β_2AR model with residues identical (red), conserved substitutions (orange), semi-conserved substitutions (yellow), and non-conserved residues (green), in the α_{1A}AR (Collette, 2010), B) Norepinephrine structure with labeled functional groups (Acdx, 2009).

effect may be to internalize the receptor while the other would activate the standard Gq pathway (Finch et al., 2006). Adrenergic receptor signaling depends on G-protein activation.

Signaling

There are many different types of G proteins. The most common are Gs (stimulates adenylyl cyclase), Gi (inhibits adenylyl cyclase), and Gq (mediates another pathway). Each G-protein is bound to the intracellular side of a receptor and consists of three subunits α, β, and γ. G-proteins are associated with guanosine di- and tri-phosphates (GDP and GTP), which provide energy for subunit activation. When a ligand binds the receptor, the transmembrane helices change conformation which exposes the phosphorylation area of the G-protein (Farrens et al., 1996). When phosphorylated, the protein is active and the α subunit, Gαq, dissociates from the β and γ subunits, G$\beta\gamma$, and slips along the membrane. The effects of the Gα subunit depend on the type of G-protein bound to the receptor.
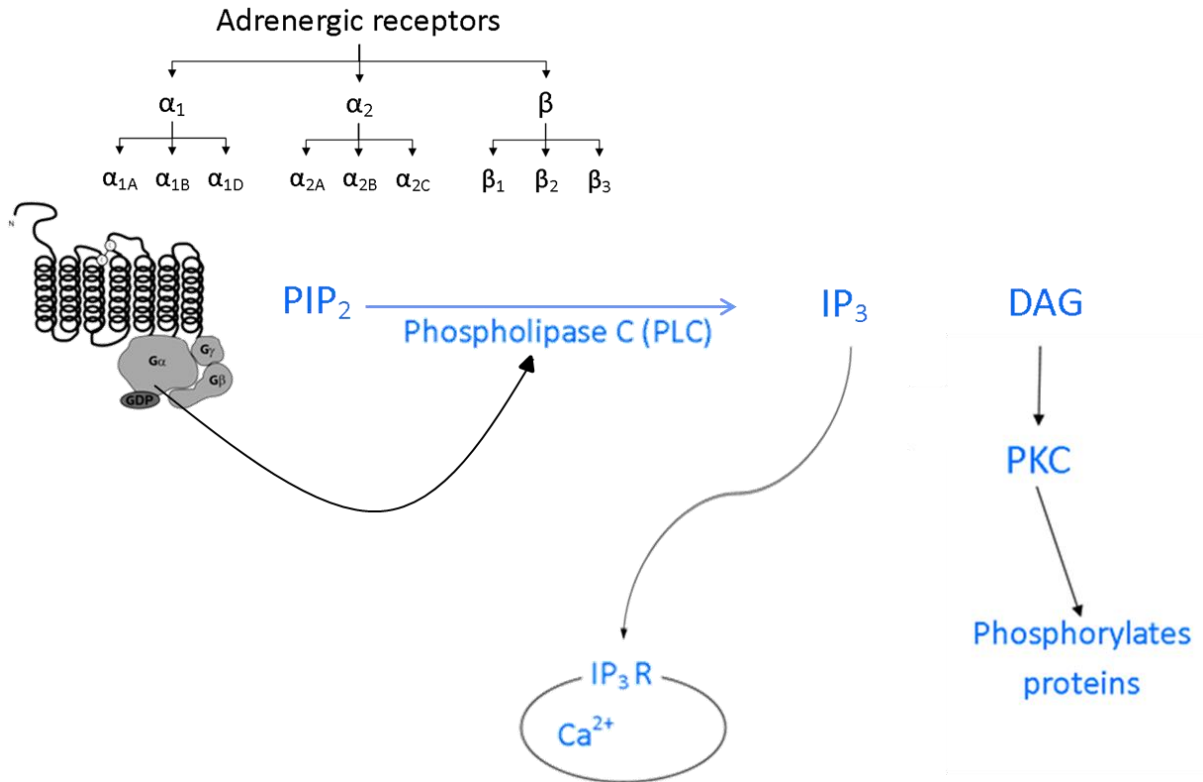
Figure 5. Signaling pathway of α_1ARs. The α_1ARs signal through the α subunit of the Gq protein. The downstream effects including increasing intracellular calcium and phosphorylation of proteins. (Figure created by Collette, 2015.)

The α_1ARs are coupled to Gαq, which activates a specific downstream cascade within the cell (García-Sáinz et al., 2000, Figure 5). The α designates the subunit mediating the main effect of G-protein activation. In some cases, the G$\beta\gamma$ subunits also activate downstream effectors. When a ligand binds to the α_1ARs, the Gαq moves along the membrane to a phospholipase C (PLC) protein. Gαq activates PLC, which cleaves phosphotidylinositol 4,5-bisphosphate (PIP2) into diacylglycerol (DAG) and inositol trisphosphate (IP3). DAG stays in the membrane and activates protein kinase C (PKC), which then phosphorylates other proteins. IP3 leaves the membrane and binds to IP3 receptors on

the endoplasmic reticulum. This releases free calcium ions into the cytosol, which mediate

many downstream effects. An important part of GPCR signaling is signal amplification.

Signal Amplification

The downstream effect of activating receptors must be amplified to cause

appreciable changes in cell function. Thousands of ligand molecules are released from the

pre-synaptic terminal into the synapse where they bind to receptors on the post-synaptic

cell. This is the first step of signal amplification. In the second step, one G-protein activates

many of its direct downstream targets. In the case of α_1ARs, the Gαq subunit can activate

many PLC molecules that, in turn, cleave more than one PIP2 molecule. Thus, the signal is

amplified at each step in the cascade.

Deactivation

Deactivation of the α_1ARs includes 1) desensitization, 2) sequestration and

internalization, and 3) downregulation (Finch et al., 2006). Desensitization is the quickest

method of deactivation, taking seconds to minutes to occur, and involves uncoupling of the

receptor from the G-protein. The α_{1B}AR undergoes desensitization through phosphorylation

by G-protein receptor kinases (GRKs) and PKC (Diviani et al., 1996). Once phosphorylated, β

arrestin uncouples the receptor from the G-protein. α_{1B}ARs are constitutively internalized

after ligand binding (Stanasila et al., 2008; Reviewed in Toews et al., 2003).

After uncoupling, β arrestin sequesters the receptor by targeting it to clathrin

adaptor complexes, such as AP2 (Stanasila et al., 2008). The receptor is internalized via

endocytosis in clathrin-coated pits, taking minutes to hours. α_{1A}ARs are rarely internalized

after ligand binding. When they are internalized, the process is slower than with α_{1B}ARs

(Chalothorn et al., 2002; Vázquez-Prado et al., 2000). The mechanism of α_{1A}AR internalization is still elusive. It appears that GRKs and PKC can phosphorylate α_{1A}ARs prior to internalization but while PKC is sufficient for this, it is not necessary (Price et al., 2002). Furthermore, the carboxy tail of the α_{1B}AR is the site of phosphorylation during internalization but PKC and GRK phosphorylation of α_{1A}ARs does not require the carboxy tail.

Downregulation is the third mechanism of deactivation and is due to a decrease in the number of surface receptors. Downregulation can occur through internalization, increased degradation via the endosomal system, or reduced transcription of mRNA (Lefkowitz, 1998).

The mechanisms of α_1AR deactivation were studied *in vitro*, usually in cells transfected with the receptor in question (Stanasila et al., 2008). The process could be different in endogenous tissues and be tissue-dependent. The α_1AR subtypes are expressed at different levels in tissues throughout the body.

<center>α_1 **Adrenergic Receptor Localization in the Brain**</center>

Regional Expression

Expression of the α_1AR subtypes in the brain differs in distribution and expression level. Early work using tritiated antagonists showed α_{1A}ARs bind highly in the hippocampus and α_{1B}AR mainly in the thalamus. Both subtypes were found in the cortex (Wilson and Minneman, 1989).

Using *in situ* hybridization with a rat probe, α_{1A}AR mRNA is expressed highly in the olfactory bulb, cortex, hypothalamus, and regions of the hindbrain (Domyancic and Morilak,

<center>12</center>

1997). The expression was moderate in the hippocampus but high in the granule cell layer of the dentate gyrus. The Allen Institute for Brain Science, also using *in situ* hybridization, showed α_{1A}AR RNA in the cortex and olfactory bulb but little to none in the hippocampus (Allen Institute for Brain Science, 2014). This is in contrast to earlier comparative studies. The Allen Institute for Brain Science has not yet published data for the α_{1B}AR subtype. However, another *in situ* experiment showed high expression in the cortex, reticular thalamic nucleus, and hypothalamus (Zuscik et al., 2000). The α_{1D}AR is highly expressed in many regions of the brain, particularly the CA1 region of the hippocampus (Day et al., 1997; McCune et al., 1993; Pieribone et al., 1994). Using *in situ* hybridization, the Allen Brain Institute showed α_{1D}AR expression high in the olfactory bulb, cerebellum, and mouse hippocampus (Allen Institute for Brain Science, 2014). *In situ* hybridization only shows that the gene is present in the cells and RNA expression is not well correlated with protein expression.

Autoradiographic studies better imply protein expression because the radioligands must bind to the receptors. Autoradiography shows very high expression of α_1ARs in human and primate hippocampus, especially the DG and CA3 regions, compared with rat brain (Palacios et al., 1987). The density of α_1ARs in neocortex and thalamus are similar in rat, guinea pig, human, and non-human primate. These findings were comparable to other autoradiographic studies (Jones et al., 1985). The α_{1A}AR mRNA is expressed in the dentate gyrus granule cell layer of the human hippocampus (Szot et al., 2005). Autoradiography is not helpful in α_{1D}AR protein expression because of the receptors' intracellular localization. *In situ* hybridization shows expression of α_{1D}AR mRNA in the CA1, CA2, and CA3 regions.

α_{1B}AR is not expressed in the human hippocampus. Currently, the most accurate way to determine localization is to use a promoter-based expression approach in transgenic mice.

Mice with enhanced green fluorescent protein (EGFP) expressed under a large portion of the α_{1A}AR endogenous promoter were created to localize protein expression in the brain (Papay et al., 2006). Promoter-specific mice show that the gene is being transcribed in the cell and the α_{1A}AR subtype predominates in almost all regions (Papay et al., 2006, Table 1). Transgenic mice expressing both the endogenous mouse gene and a human α_{1B}AR gene fused with EGFP were used to determine α_{1B}AR localization (Papay et al., 2004). These mice showed receptor protein expression in the cell rather than just transcribed RNA. α_{1B}AR-EGFP mice confirmed expression of α_{1B}ARs in the thalamus though, in contrast to *in situ* studies, the level of expression was not specifically higher in the reticular thalamic nucleus (Papay et al., 2004; Zuscik et al., 2000). The α_{1B}AR predominates in only the cerebral and piriform cortices and pontine nucleus of the hindbrain. In contrast to peripheral vessels, where the α_1ARs regulate vasoconstriction, the cerebral blood vessels do not express either receptor subtype.

Cellular Expression

Cell types with α_1AR subtype expression. The promoter-specific α_{1A}AR-EGFP and protein-specific α_{1B}AR-EGFP mice were used to determine co-labeling of each subtype with specific cell types. The α_{1A}AR is transcribed in the cortex in NG2 oligodendrocytes, GABAergic interneurons, and NR1-containing cells; NR1 is a subunit of the NMDA receptor (Papay et al., 2006). In the hippocampus, α_{1A}AR EGFP co-localizes with NR1 in the CA2 and CA3 The α_{1A}AR is transcribed in the cortex in NG2 oligodendrocytes, GABAergic

TABLE 1. Distribution of the α_{1A}- and α_{1B}ARs in the Brain (Adapted from Papay et al., 2006).

Tissue	α_{1A}-AR	α_{1B}-AR
Amygdaloid	+++	+++
Basal ganglia		
Caudate putamen	+	+
Claustrum	+	+
Cerebellum		
Granule cell layer	+	+
Molecular layer	++	+
Purkinje cell layer	+++	+
Cerebral cortex	++	+++
Piriform cortex	++	+++
Visual cortex	+++	
Corpus callosum	−	−
Hindbrain		
Cochlear nu	++	
Medullary reticular nu	++	
Dorsal raphe nuc.	++	+
Medial vestibular nu	++	+
Parvicellular reticular nu	++	+
Pontine nuc.	+++	+++
Periolivary region	+++	+
Raphe Pallidus nuclei	+++	+
Spinal trigeminal tract	++	+
Superior olive	++	
Inferior olive	+++	
Spinocerebellar tract	++	++
Tegmental nu	+++	
Hippocampus		
Dentate gyrus	+++	+
CA1 field	+++	+
CA3 field	+++	+
Hypothalamus		
Hypothalamic nu.	+++	++
Hypothalamic area	+++	++
Preoptic nu	++	++
Periventricular hypothalamic nu	++	++
Interpeduncular nu	+++	
Midbrain		
Inferior colliculus	++	++
Periaqueductal gray	++	++
Raphe cap	++	+
Substantia nigra	+	+
Superior colliculus	++	++
Olfactory	+++	++
Pituitary	++	++
Spinal cord	++	++
Thalamus	+	+

Plus and minus signs indicate various levels of relative expression based on the intensity of the label within each mouse model. There may be differences in intensity due to experimental parameters. α_{1B}-AR expression is shown for comparison (Papay et al., 2004).

Interneurons, and NR1-containing cells; NR1 is a subunit of the NMDA receptor (Papay et al., 2006). In the hippocampus, α_{1A}AR EGFP co-localizes with NR1 in the CA2 and CA3 regions and with GAD 65/67 in the dentate gyrus. They are also functionally expressed in somatostatin interneurons of the CA1 region (Hillman et al., 2007, 2005). The α_{1B}AR also co-localizes with NG2 positive oligodendrocytes in the cortex but is also expressed in neurons and CC1 cells (Papay et al., 2004). α_{1B}AR expression coincides with granular, Purkinje, and molecular layer cells of the cerebellum.

Determining localization of the receptors on the plasma membrane or internal was examined using co-immunoprecipitation. For *in vivo* confirmation, mice were created with a human α_{1A}AR and EGFP both under the endogenous promoter (Papay et al., 2006). However, the endogenous signal is quite faint and amplification with GFP antibodies has been difficult. Additional methods have been employed to determine α_{1}AR function.

Methods to Study α_{1} Adrenergic Receptor Function

Pharmacology

α_{1}ARs have been studied using pharmacological agents for decades. Several non-selective α_{1}AR agonists are used clinically. Phenylephrine is used to dilate pupils for eye exams and in some pathological conditions. Oxymetazoline is used in a nasal spray to reduce congestion by constricting the blood vessels in the sinuses. An adverse effect is that it causes rebound congestion due to receptor downregulation.

Prazosin and doxazosin are non-selective α_{1}AR antagonists used to reduce lower urinary tract symptoms in men with benign prostatic hyperplasia. Both are also sometimes used as a treatment for hypertension but only if the patient also suffers from lower urinary

tract symptoms. The adverse effects, mainly lightheadedness and faintness due to vasodilation preclude them from being front-line treatments for hypertension. Furthermore, non-selective α_1AR antagonists given to patients with heart failure had worse outcomes in a clinical trial, forcing patients to be removed from that arm of the trial (ALLHAT, 2000). To circumvent the adverse effects caused by $\alpha_{1B}AR$ blocking, selective $\alpha_{1A}AR$ antagonists were created. These include siludosin and tamulosin and are the first line treatment for benign prostatic hyperplasia and lower urinary tract symptoms. All of the currently available clinical $\alpha_{1A}AR$ agents are either too large or too hydrophilic to cross the blood brain barrier. Cirazoline is a full agonist at $\alpha_{1A}ARs$ and a partial agonist at α_{1B}- and $\alpha_{1D}ARs$ (Horie et al., 1995). This provides a 5-8-fold selectivity for the $\alpha_{1A}AR$ over the α_{1B}- and $\alpha_{1D}ARs$. However, cirazoline is not ideal because it also has α_2AR antagonist properties (Ruffolo et al., 1982). Researchers addressed the lack of subtype-selective research drugs by creating transgenic animals to study the subtypes' function.

Transgenic

Adrenergic receptor cloning allowed researchers to use cDNA to create mutant cells and transgenic animals, moving the field toward a better understanding of adrenergic receptor function. Transgenic models were created either without the receptor expressed (knockout, KO) or with a mutated form of the receptor holding it in its active conformation, mimicking an agonist effect.

Knock-out models. The knock-out mice for all α_1AR subtypes were originally created to study hypertension. The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT) showed that non-selective α_1AR treatment worsens heart

17

failure in hypertension (ALLHAT, 2000). Further work using the following transgenic mouse models is helping to clarify the differing roles for each subtype in the peripheral and central nervous systems.

The α_{1A}AR gene was cloned from a 129/SvJ cDNA library (Rokosh and Simpson, 2002). The *Escherichia coli* β-galactosidase gene, LacZ, and the neomycin-resistance gene replaced a large part of the gene's first exon and part of the intron. The resulting mice were bred separately onto FVB/N and C57BL/6 backgrounds. α_{1A}AR-KO have also mainly been used to study cardiac function and pathology (Reviewed in O'Connell et al., 2014 and Perez and Doze, 2011). Our lab is the only one to study central nervous system function using these mice. We showed α_{1A}AR-KO is detrimental to learning and memory in the Barnes maze (Doze et al., 2011).

α_{1B}AR-KO mice were established using 129/Sv and BALB-c genomic libraries with hamster cDNA as a probe (Cavalli et al., 1997). Exon I of the α_{1B}AR gene, derived from the 129/Sv library, was replaced with the neomycin resistance gene, which codes for the first six transmembrane helices. The mice were originally bred as a 129Sv/ C57BL/6 hybrid but have since been backcrossed onto the C57BL/6 line, the most commonly used strain. α_{1B}AR-KO mice have mainly been used to study cardiovascular and cardiac function (Reviewed in O'Connell et al., 2014 and Perez and Doze, 2011). One study thus far examined locomotion, learning and memory (Spreng et al., 2001).

α_{1D}AR-KO mice were generated using mouse cDNA (Tanoue et al., 2002). The α_{1D}AR gene was replaced with the neomycin resistance gene and the resulting mice were a 129Sv/ C57BL/6J hybrid. The mice have been used to clarify the role of the α_{1D}AR in

vasoconstriction and resulting effects on arterial blood pressure. Behavioral work shows that the α_{1D}AR impairs working memory and attention but not spatial memory (Mishima et al., 2004). In addition, MK-801, an NMDA receptor antagonist, induces deficits in the acoustic startle reflex but this effect was blocked in α_{1D}AR-KO mice.

Double $\alpha_{1A/B}$AR-KO mice were created by breeding α_{1A}AR-KO (FVB, 129Sv) mice with α_{1B}AR-KO mice (C57BL/6, 129Sv) (O'Connell et al., 2003). F1 heterozygotes were bred to produce WT and A/B-KO; breeding pairs from the lines were established. The double A/B-KO mice are used to study cardiac function including the role of the α_{1A} in cardiac apoptosis (Huang et al., 2007).

Double $\alpha_{1B/D}$AR-KO mice were produced by breeding of single α_{1B}- and α_{1D}AR-KO mice which were of mixed 129Sv and C57BL/6J backgrounds (Hosoda et al., 2005). These mice were also used to study blood pressure regulation.

Activation models. Transgenic models of α_1AR subtype activation were also established and utilized for the study of cardiac function. Activation models include low (2x) to high level (66x) overexpression with or without constitutive activity.

CAM-α_{1A}AR mice have a constitutively active mutant form of the rat α_{1A}AR under the control of the mouse α_{1A}AR promoter (Rorabaugh et al., 2005). Constitutive activity is conferred by two mutations: M292L and A271E, and results in 3.5-fold overactivity based on IP3 levels. CAM-α_{1A}AR mice have improved survival due to heart failure after myocardial infarction in addition to other cardiac benefits (Du et al., 2006). Previously, we showed that CAM-α_{1A}AR mice have lower levels of depression and anxiety, enhanced learning and memory, and live longer than WT mice (Doze et al., 2009, 2011).

An $\alpha_{1B}AR$ model of activation was achieved by expressing a constitutively active mutant form of the hamster α_{1B}-AR under the endogenous mouse $\alpha_{1B}AR$ promoter (Zuscik et al., 2000). Constitutive activity was achieved by mutating three residues: C128F/A204V/A293E. The triple mutant receptor spontaneously couples to Gq without an agonist present conferring a 4-fold overactivity based on IP3 levels. These mice were initially called T1 mice but later changed to CAM-$\alpha_{1B}AR$ mice to more easily identify the transgenic changes (Yun et al., 2003; Doze et al., 2009). In the cardiovascular system, CAM-$\alpha_{1B}AR$ mice have faster progression to heart failure with pressure overload and a pathological cardiac hypertrophy (Wang et al., 2000; Zuscik et al., 2001). Surprisingly, CAM-$\alpha_{1B}AR$ mice also have a synucleinopathy, progressive neurodegeneration, and locomotion impairments (Zuscik et al., 2000).

CHAPTER 2:

ALPHA$_{1A}$ OR ALPHA$_{1B}$ ADRENERGIC RECEPTOR KNOCKOUT DOES NOT IMPAIR NOVEL OBJECT RECOGNITION, SPATIAL LEARNING AND MEMORY, OR PATTERN SEPARATION

Abstract

Norepinephrine, which binds to adrenergic receptors, has a clear role in arousal and vigilance, which can enhance performance on cognitive tasks. The role of the α_1 adrenergic receptor subtypes in learning and memory, however, is unclear as research shows conflicting results. Chronic activation of the α_{1A}AR subtype improves learning and memory performance in the Barnes and multi-t mazes. Knockout of the α_{1A}AR impairs performance in the Barnes maze. The α_{1B}AR subtype has rarely been studied in the context of cognition. In the current study, we examined the role of the α_1AR subtypes, α_{1A}- and α_{1B}AR, on novel object recognition, spontaneous alternation in the t-maze, and spatial learning, memory, and pattern separation in the Morris water maze. We found that α_{1A}- and α_{1B}AR-KO mice may have impaired object recognition memory; however, preference for one of the objects may have confounded the results. Unreliable performance by wild type mice precluded definitive conclusions for the role of the subtypes in spontaneous alternation. In the Morris water maze, α_{1A}AR-KO mice did not form an efficient path to the platform but were normal in latency and distance to reach the platform. All groups learned spatial strategies to solve the maze. Based on our previous work in α_{1A}AR activation and knockout mice, and the potential confounding variables in the present study, we suggest further research into the

role of the α_{1A}AR in cognition. Similarly, the role for the α_{1B}AR receptor was not clarified in the current study and should be further examined.

Introduction

Noradrenergic fibers project from the locus coeruleus to virtually every region of the brain, where they release the neurotransmitter norepinephrine. Norepinephrine is well known for its enhancing effects on arousal and vigilance (Reviewed in Sirviö and MacDonald, 1999). It has a role in plasticity, memory formation, consolidation, retrieval, and working memory (Izumi and Zorumski, 1999; Segal et al., 1991; Sara and Devauges, 1988; Murchison et al., 2004). However, the mechanisms are complex, partly due to multiple adrenergic receptors. Norepinephrine binds to three families of G-protein coupled adrenergic receptors (ARs), α_1, α_2, and β, each with three subtypes (Figure 6). The subtypes are expressed at different levels in various brain regions. Recent evidence suggests the involvement of norepinephrine in learning and memory is dependent on the receptor families and subtypes.

The α_1ARs are involved in learning and memory but whether the effect is an enhancement or detrimental is controversial. Norepinephrine, via α_1ARs inhibits memory consolidation in the chick at high doses of the α_1AR agonist methoxamine but enhances it at lower doses, potentially via astrocytic α_1ARs (Gibbs and Summers, 2001; Gibbs and Bowser, 2010). Arnsten's group found that α_1ARs impair performance on prefrontal cortex-dependent tasks while α_2ARs enhance it (Reviewed in Ramos and Arnsten, 2007). They also found impaired performance at a low dose of the α_{1A}AR agonist, cirazoline, and enhanced performance at a higher dose known to block imidazoline and α_2ARs (Arnsten and Jentsch,

22
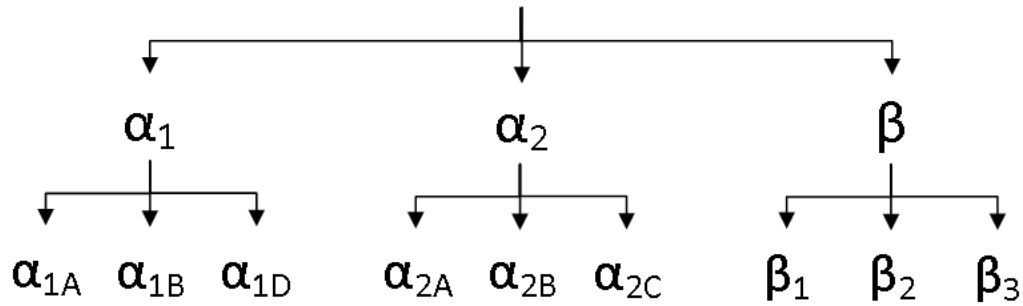
Figure 6. Adrenergic receptor subtypes.

1997). In contrast, microinjection of the α_1AR agonist phenylephrine into the CA1 region of the hippocampus improves acquisition in the Morris water maze; the α_1AR antagonist prazosin, an α_1AR antagonist, had the opposite effect (Torkaman-Boutorabi et al., 2014). Prazosin impaired performance in the Morris water maze in one study but had no effect in another (Puumala et al., 1998; Riekkinen et al., 1996). The drug ST-587 was originally thought to be selective for the α_1AR and improved learning in cognition-based experiments but was later found to be non-selective (Puumala et al., 1996; Puumala and Sirviö, 1997; Pussinen and Sirviö, 1998). Many studies examining the role of α_1ARs used acute administration of non-subtype selective drugs.

The generation of transgenic mice with α_1AR subtype activation or knockout (KO) properties greatly enhanced the ability to study the role of the receptor subtypes in behavior. The α_{1A}AR subtype, via transgenic or chronic pharmacological activation, enhances spatial learning and memory in the Barnes and multi-t mazes (Doze et al., 2011). Transgenic activation also enhances learning and memory in the Morris water maze and long-term potentiation in the CA1 region of the hippocampus. Knockout of the α_{1A}AR subtype impairs performance in the Barnes maze. The lack of learning and memory studies

using α_{1A}AR-KO mice is likely impaired by the incidence of seizures in these mice (Doze and Collette, unpublished observation). Therefore, seizure activity was taken into account in our study. Knockout of the α_{1B}AR subtype enhanced novel object recognition and impaired spatial memory in the Morris water maze but no other reports on the α_{1B}AR and learning and memory have been published (Spreng et al., 2001).

Based on the conflicting results for the role of α_1ARs in cognition, and the dearth of studies on the α_{1B}AR subtype in learning and memory, additional research was warranted. Based on our previous results of α_{1A}AR subtype activation, we hypothesized that α_{1A}AR-KO mice would have impaired learning and memory for hippocampal-specific learning and memory tasks. Based on the Spreng et al. study, we expected α_{1B}AR-KO to have enhanced object recognition memory and impaired spatial memory in the Morris water maze when compared with wild type mice.

Methods

Animals

Mice were bred and genotyped at the Cleveland Clinic Foundation then transferred to the University of North Dakota at 3-4 mo of age. Mice were provided standard chow with 5% fat (Teklad 22/5 Rodent Diet (W) 8640, Harlan, Indianapolis, IN) and water *ad libitum*, were maintained on a 12-hr light cycle (on at 0700), and provided veterinary care. α_{1A}- and α_{1B}AR-KO mice have been previously characterized (Rokosh and Simpson, 2002; Cavalli et al., 1997). Control animals (n = 13), were wild type (WT) littermates of the α_{1A}- (n = 22) and α_{1B}AR (n = 14) KO lines. α_{1A}AR-KO mice were of the F7-F9 generation while α_{1B}AR-KO mice were all of the F9 generation. There were approximately equal numbers of male and female

24

animals. All protocols conformed to the *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee at the University of North Dakota. The facilities at the Cleveland Clinic Foundation and the University of North Dakota are both accredited by the Association for Assessment and Accreditation of Laboratory Animal Care.

Seizure Incidence

The incidence of seizures was noted anytime the mice were observed, mainly prior to and during behavioral testing. Seizures were identified as a binary (yes or no) outcome (McKhann et al., 2003). Seizures were not classified by stage. However, informal observation indicated that seizures included Stages 1-3, immobility, rigidity, and head bobbing. No notes indicate that seizures progressed to Stages 4-6, intermittent or continuous rearing and falling or whole body convulsions.

Data for novel object recognition and t-maze were analyzed to determine if seizure occurrence affected performance in the tests. Handling of seizure occurrence during the Morris water maze is detailed in the Statistical Analysis portion of these Methods.

General Health and Neurological Assessments

The mice were assessed for general health and neurological reflexes as per a protocol provided by Dr. Maria Gulinello at Albert Einstein College of Medicine, which is based on the SHIRPA protocol (Rogers et al., 1997). General health was assessed by observing nesting and home cage activity, aggression, coat condition, piloerection, whisker quality, body weight, and body temperature. Most other parameters were assessed in a 82.0 x 51.8 x 42.4 cm (31 gal) container (Newell Rubbermaid, Atlanta, GA) with a grid of 8

squares drawn across the bottom. Activity/ arousal was assessed by how fast the animal moved from its initial position to cross the first gridline (transfer arousal); number of gridline crossings, rearings, and stretch attend postures; response to touch; positional passivity; and a gross measure of startle response which involved snapping a mouse trap above the container. Motor and autonomic function assessment involved abdominal elevation, paw position (width and pronation), gait, righting reflex, ataxia, and balance beam slips. The balance beam consisted of a wooden dowel with marks 7.6 cm apart secured lengthwise in a second 31 gal container. The number of balance beam slips within passing 10 marks on the dowel was used to assess motor skills and balance. Also in the second container, a 0.48 cm diameter rope was secured across the box lengthwise, upon which the animals were placed holding the rope with their front paws. The time before the mice lost their grip and fell was measured three times and averaged. Sensory reflex function was assessed by response to toe pinch, negative geotaxis, vision, pinna, whisker, and provoked biting.

Behavioral Testing

Behavioral testing of learning and memory occurred between 0800 and 1230. The experimental timeline includes the zero maze, which took place between 1200 and 1700, but the results were reported elsewhere (Figure 7). Testing for each mouse was set at least 24 hr apart. Mice were approximately 5 mo of age at the start of behavioral testing. Mice were acclimated to separate cages for 30 min prior to all tests except the general health and neurological assessment, which was performed directly from the home cage. Equipment was cleaned with 10 percent ethanol between each mouse.
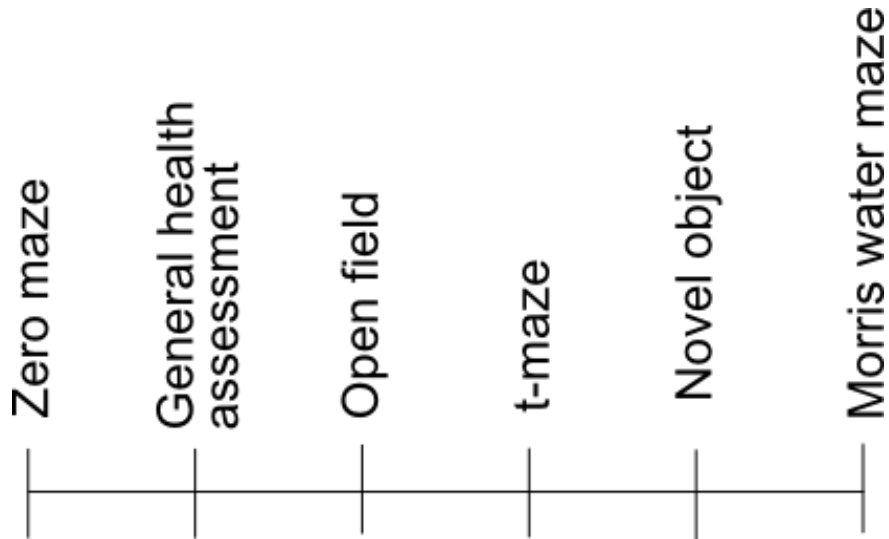
Figure 7. Behavioral testing timeline. The zero maze results were reported in a separate study. Testing began when mice were 4 mo of age.

Novel object recognition. In the first phase of the novel object recognition test, habituation, the animals were placed in an empty 40 x 40 x 35 cm open field box for 5 min (Stoelting, Wood Dale, IL). After one hour, the mice were returned to the open field for a 5 min familiarization phase. During this second phase, the open field contained two identical objects, which were counterbalanced to avoid object bias. The objects were 5.08 cm tall white pointed columns with a black stripe near the top or 4.13 cm black spheres (Figure 8, Lahud Craftsmen LLC, Fertile, MN; Stoelting, Wood Dale, IL). After another hour, the testing phase began. Mice were placed back in the open field with their respective original object (OO) and the novel object (NO). The time spent with each object was used to calculate the discrimination index and the percent of exploration time spent with the novel object. The discrimination index used the equation: [(time with OO - time with NO)/ (time with OO + time with NO)]. Habituation to the objects was determined by comparing the time spent exploring the original object during the familiarization phase and testing phase.

27
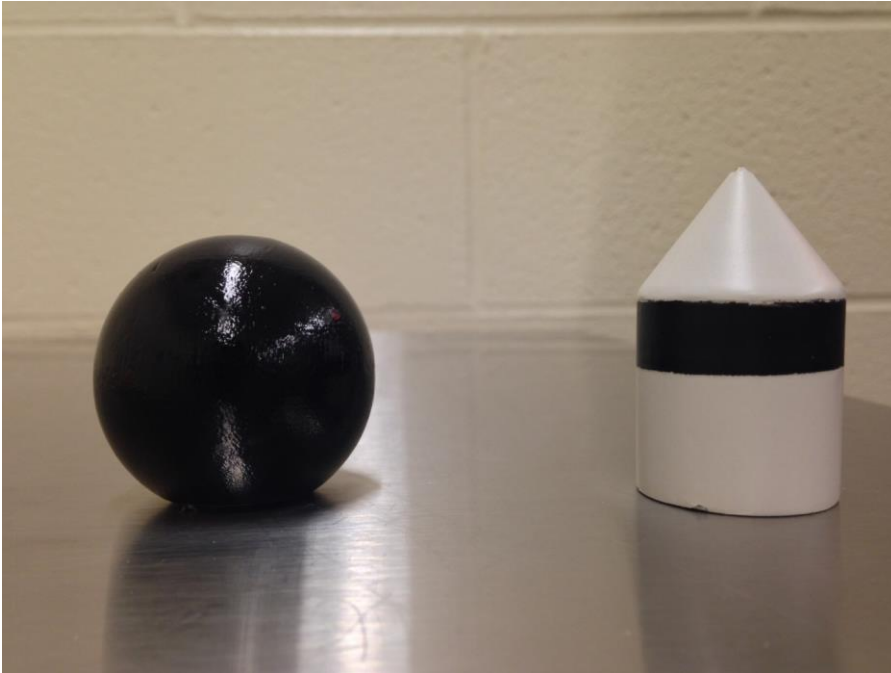
Figure 8. Novel object recognition objects. The objects used in the novel object recognition test included a black sphere and a white pointed column with a black horizontal stripe near the top.

T-maze. The t-maze was used to test for spontaneous alternation, a form of short-term memory. The t-maze apparatus was made with white melamine particle board with removable walls 12.7 cm in height (Figure 9). Mice were placed on the maze at the bottom of the T and were allowed to choose the left or right arm. The walls of the maze were lifted and the animal was placed back at the starting point. Each mouse received 5 trials and the percent of alternations was used for analysis.

Morris water maze. The Morris water maze was used to test spatial learning and pattern separation, via a reversal paradigm. The maze consisted of a circular tank 122 cm in diameter and 76 cm in depth (San Diego Instruments, San Diego, CA). The top of a 10 cm square platform was 25 cm from the top of the tank and room temperature water tinted
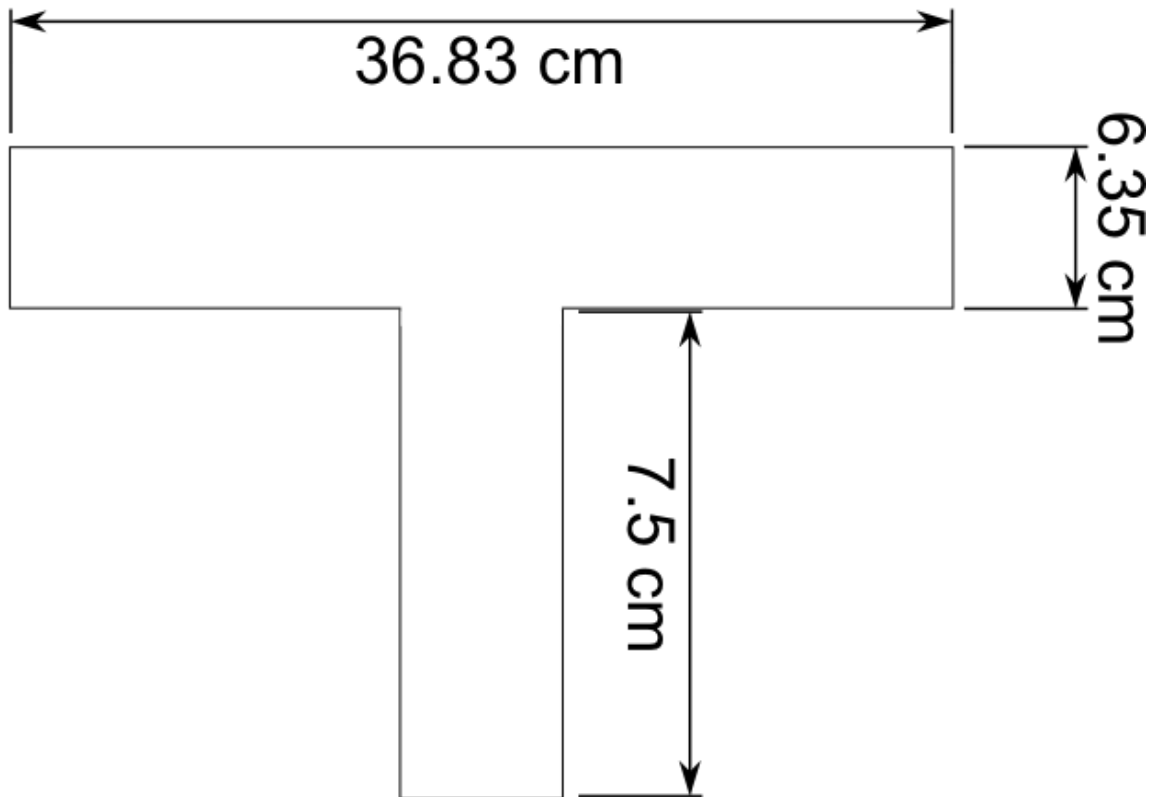
Figure 9. T-maze dimensions. The t-maze was used to test spontaneous alternation.

with Art Minds white non-toxic tempera paint covered the platform by 1 cm (Michaels

Stores Inc, Irving, TX). The tank was enclosed within a 152 x 152 cm grey tent (Ace Canopy,

Palmdale, CA). Distal spatial cues included a folded step ladder, a black cardboard circle 21.5

cm in diameter taped to a wall of the tent, a white lamp, and one side of the tent open to

the room. The experimenter was blocked from view of the mice during all trials. The search

area:target area ratio was 117:1, ideal for testing mice (Vorhees and Williams, 2006). The

platform location was counterbalanced equally within groups.

Mice were trained for 6 trials per day over 3 days. On Day 4, the platform location

was reversed and stayed in the reversal position on Day 5. Starting positions were chosen

using a pseudorandom algorithm and changed for each trial. AnyMaze software and a

Logitech web camera were used to digitally control and record the trials (Stoelting Co.,

Wood Dale, IL). The maximum allotted time for each trial was 120 s and mice were

considered by the software to have arrived on the platform if they were in the platform

zone for 2 s. They were allowed to remain on the platform for 30 s. If mice did not find the

platform, they were guided there using a plastic ruler and allowed to remain for 30 s.

Following each trial, mice were lightly dried with a towel and placed under a heat lamp until

the next trial, while other mice were tested. The inter-trial interval ranged from 5-10 min.

On Day 4, the platform location was reversed to test pattern separation. The platform

remained in this second location on Day 5. Qualitative strategies were analyzed using a

modified MATLAB script generously provided by Gerd Kempermann and Alexander Garthe

(Garthe et al., 2009).

Statistical Analysis

Statistical analyses were performed using GraphPad Prism 5.04 (GraphPad Software,

San Diego, CA). Normality was assessed using the Kolmogorov-Smirnov test. Variances were

assessed using the Bartlett's test and non-parametric tests were used if the variances were

significantly different. Significant outliers, found using the Grubb's test, were excluded. One

way ANOVA, with a Tukey post-hoc test, was used to assess differences between groups

even if the data were non-normal because ANOVA is robust for violations of normality. One-

sample t-tests were used to assess significant deviations from chance levels in the novel

object recognition and t-maze tasks. Sex differences were assessed with a 2 way ANOVA

with genotype X sex and a Bonferroni post-test for paired comparisons. Trial data missing

due to technological error or, in the case of α_{1A}AR-KO mice a seizure in the maze, were

interpolated by taking the mean of the previous and next trial. Two mice were excluded

from the study early due to a high number of seizures in the maze. Morris water maze data

were analyzed using 2 way ANOVAs of genotype X day, genotype X trial, and trial X

genotype, and day X genotype.

Results

General Health and Neurological Assessments

There were no group differences in any of the general health assessment

parameters, including body weight and temperature, transfer arousal, grid crossings,

rearings, or stretch attend postures. In another study, there were no differences in basal

locomotion in the open field test (Chapter 2).There were no obvious differences in the

qualitative assessment of neurological reflexes.

Novel Object Recognition

The variances between group means in the time spent with the NO differed

significantly; therefore, the non-parametric Kruskal-Wallis test was used for analysis. There

was a main effect of time spent with the NO ($\chi^2(2) = 8.74$, p = 0.01). A Dunn's post-hoc test

revealed that α_{1A}AR-KO mice spent less time with the novel object than α_{1B}AR-KO mice (p <

0.05) but not less than WT mice (p > 0.05).

When taking into account the total time spent exploring objects, there was no

significant difference between groups in the percentage of time spent with the NO [$F(2, 38)$

= 0.47, p = 0.62, Figure 10A]. There were no sex differences in the percent of time spent

with the NO [$F(1, 35) = 1.09$, p = 0.30]. There was also no difference between α_{1A}AR-KO

mice that did not have seizures (n = 7) and mice that did (n = 9), [$t(14) = 1.17$, p = 0.25].

31
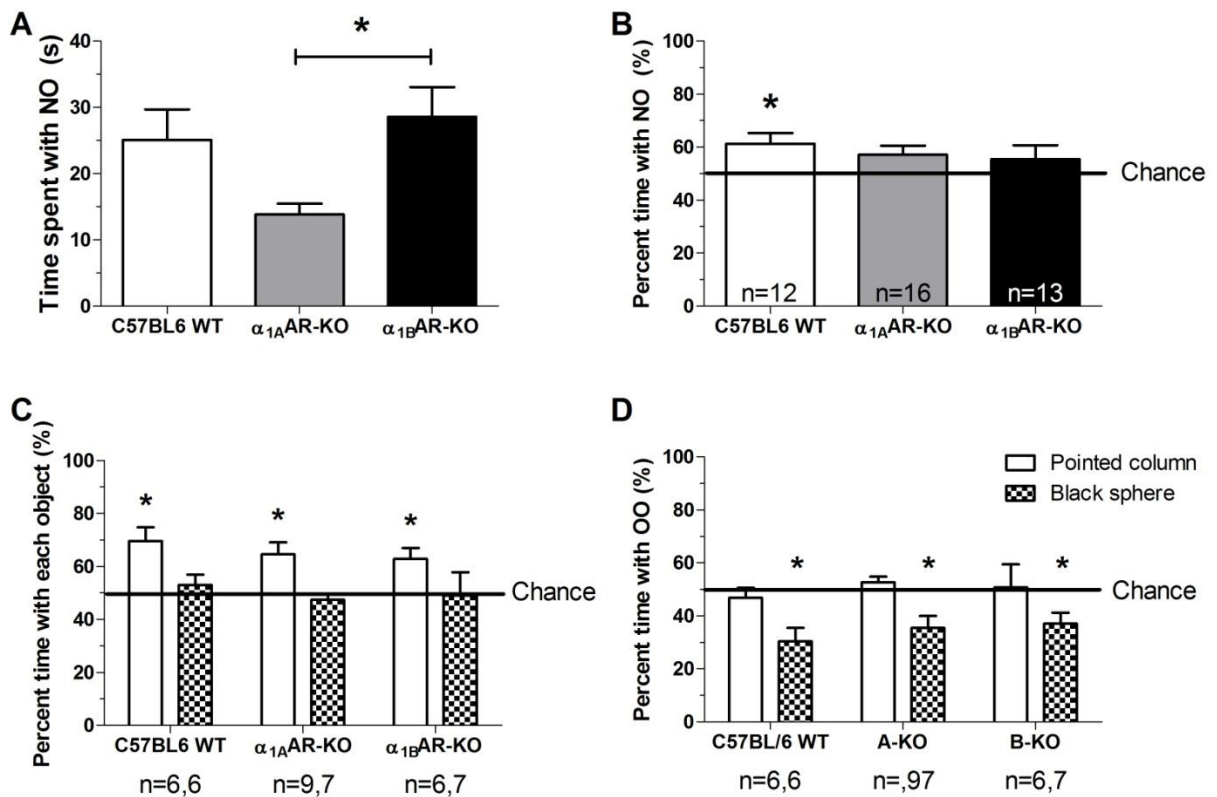
Figure 10. Novel object recognition. A) α_{1A}AR-KO mice spent less time with the NO then α_{1B}AR-KO (p < 0.05) but not WT mice (p > 0.05). B) WT mice spent a higher than chance level percentage of time with the NO (p < 0.05), while α_{1A}AR-KO and α_{1B}AR-KO mice did not. C) All groups spent a higher than chance level percentage of time with the pointed column. D) All groups habituated to the black sphere as an original object but not to the pointed column. *p < 0.05

A one-sample t-test showed that WT mice spent a higher than chance percentage of time with the NO (50 percent) (p = 0.01), while α_{1A}AR-KO (p = 0.05) and α_{1B}AR-KO (p = 0.32) mice did not (Figure 10B). This shows that WT mice remembered the NO while the KO mice did not.

Further analysis revealed that each group spent a higher than chance percentage of time with the pointed column (Figure 10 C). All groups habituated to the black sphere when it was the original object, WT (p = 0.01), α_{1A}AR (p = 0.01), and α_{1B}AR-KO (p = 0.03) (Figure 5D). However, when the pointed column was the original object, WT (p = 0.44), α_{1A}AR (p =

0.25), and α_{1B}AR-KO (p = 0.94) mice spent a chance level of time with it during the testing phase, suggesting no habituation (Figure 10D). This implies all groups preferred the pointed column during the testing phase, even when it had been the original object. Two way ANOVAs for this data showed no main effect of genotype, a main effect of object, but no post-hoc differences (Genotype: $F(2, 35) = 0.65$, p = 0.52, Object: $F(1, 35) = 13.29$, p <0.0009).

T-maze

In the t-maze for spontaneous alternation, there was no difference between groups for the percent of alternations [$F(2, 43) = 0.73$, p = 0.48, Figure 11]. The Wilcoxon signed rank test, for non-normally distributed data, showed that no group performed better than chance levels (50 percent correct). There were no differences in the percentage of alternations between mice that had seizures and mice that did not [$t(17) = 0.68$, p = 0.50]. Mice that had seizures and mice that did not have seizures performed at chance levels.

Morris Water Maze

Learning, Days 1-3. For latency to reach the platform, there was a main effect of training days [$F(4, 140) = 30.25$, p < 0.0001] but no effect of genotype [$F(2, 140) = 1.10$, p = 0.34, Figures 12A and 12B]. All three groups decreased the latency to reach the platform by Day 2: WT (p < 0.001), α_{1A}AR-KO (p < 0.0001), and α_{1B}AR-KO (p < 0.001).

Distance to reach the platform also showed a main effect of days, [$F(4, 140) = 29.00$, p < 0.0001] but no effect of genotype [$F(2, 140) = 2.78$, p = 0.07, Figures 12C and 12D]. All
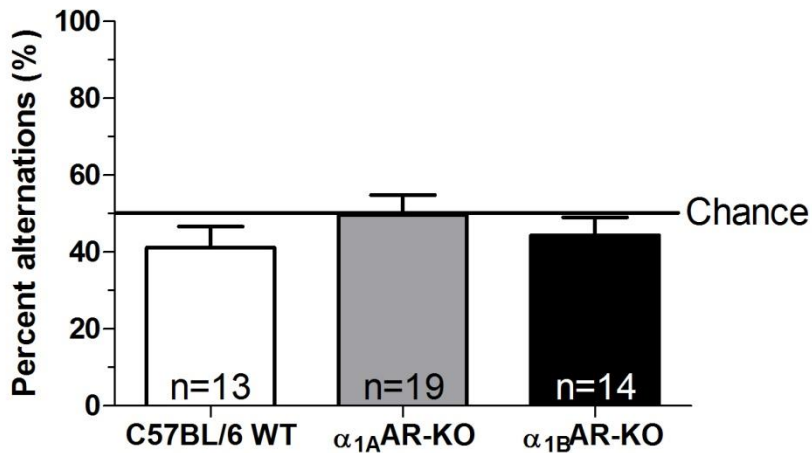
Figure 11. T-maze. In the t-maze, none of the groups alternated a higher than chance percentage of times ($p > 0.05$). There were no group differences in the percentage of alternations.

groups decreased the distance it took to reach the platform by Day 2: WT ($p < 0.001$), α_{1A}AR-KO ($p < 0.01$), and α_{1B}AR-KO ($p < 0.001$).

Path efficiency showed a main effect of training days [$F(4, 140) = 11.69$, $p < 0.0001$]; however, no effect of genotype was found [$F(2, 140) = 0.81$, $p = 0.44$, Figures 12E and 12F]. Post-hoc testing revealed that WT ($p < 0.05$) and α_{1B}AR-KO mice ($p < 0.01$) increased efficiency to the platform by Day 3. α_{1A}AR-KO mice did not significantly increase path efficiency until Day 5 ($p < 0.001$). For swim speed, there was no main effect of days [$F(4, 140) = 0.99$, $p = 0.41$]; however, there was an effect of genotype [$F(2, 140) = 3.97$, $p = 0.02$, Figures 12G and 12H]. Post-hoc testing showed that α_{1A}AR-KO mice swam slower when compared with WT mice on Day 1 ($p < 0.05$) and Day 2 ($p < 0.05$). However, there was also an interaction between genotype and day of testing [$F(8, 140) = 3.30$, $p = 0.001$], which makes this result difficult to interpret. The slight differences between swim speed (0.02 m/s

34

or 2 cm/s) likely were not relevant to interpretation of other factors based on speed, such as latency.

The combined results suggest that all groups learned the location of the platform by Day 2. However, α_{1A}AR-KO mice did not learn to use a more efficient path to the platform during the three-day training period.

Reversal learning, Days 4-5. The α_{1A}ARs are highly expressed in the dentate gyrus, an area of the hippocampus important for pattern separation, which can be tested in a reversal paradigm of the Morris water maze (Sahay et al., 2011; Aimone et al., 2011; Piatti et al., 2013; Garthe et al., 2009). In this protocol, the location of the platform is changed but the spatial cues remain the same. The mice must learn the new location of the platform by using spatial information learned in the first phase of training.

As reported in the previous section, there were no main effects of genotype on any of the days of testing for latency, distance traveled, or path efficiency to the platform. There was a main effect of swim speed, reported above; however, it was limited to Day 1 and Day 2 of testing between WT and α_{1A}AR-KO.

Overall, the reversal protocol did not significantly change pattern separation in any of the groups. Interestingly, the α_{1A}AR-KO mice improved path efficiency by Day 5. One reason the path efficiency may not have improved in this group could be due to the use of non-spatial search strategies.

Search strategies. Rodents use both non-spatial and spatial search strategies while learning the location of a platform in the Morris water maze. Initially, searching is through non-spatial means which tend to follow a pattern. Garthe, Behr, and Kempermann

35

Figure 12. Morris water maze. A) WT ($p < 0.001$), α_{1A}AR-KO ($p < 0.0001$), and α_{1B}AR-KO mice ($p < 0.001$) reduced the latency to reach the platform by Day 2 of training but B) there were no differences between genotype. C) WT ($p < 0.001$), α_{1A}AR-KO ($p < 0.01$), and α_{1B}AR-KO mice ($p < 0.001$) reduced the distance traveled to the platform by Day 2 of training but D) there were no differences between genotype. E) WT ($p < 0.05$) and α_{1B}AR-KO mice ($p < 0.01$) increased path efficiency to the platform by Day 3 while α_{1A}AR-KO did not even after 5 days ($p > 0.05$) and F) there were no between group differences. G/H) α_{1A}AR-KO mice swam slower than WT mice on Day 1 ($p < 0.05$) and Day 2 ($p < 0.05$). ↓ Platform reversal, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$), ****$p < 0.0001$
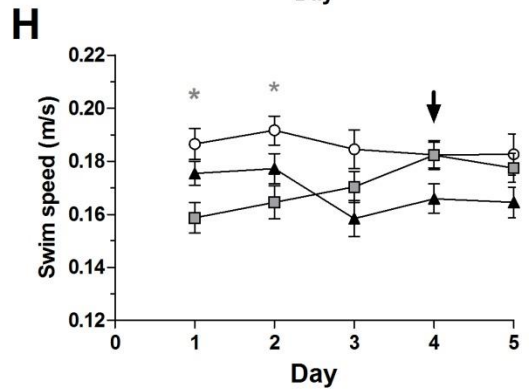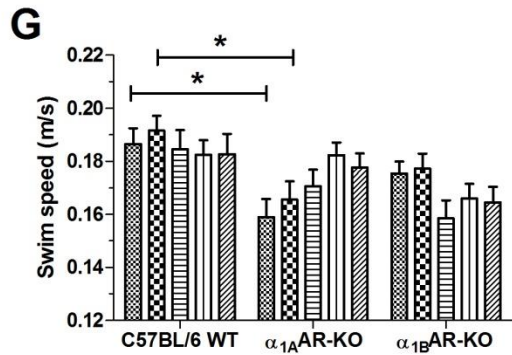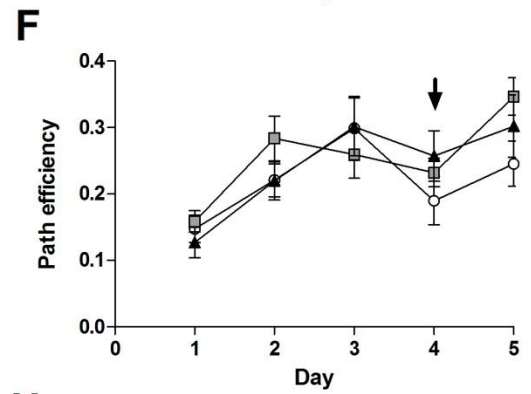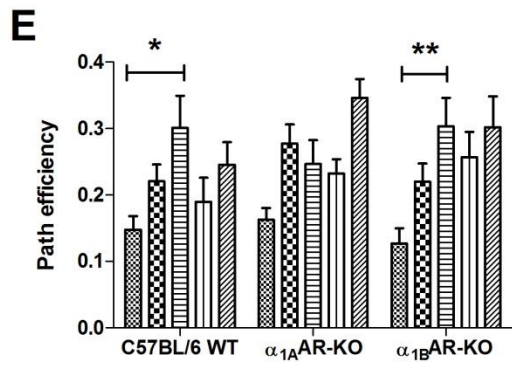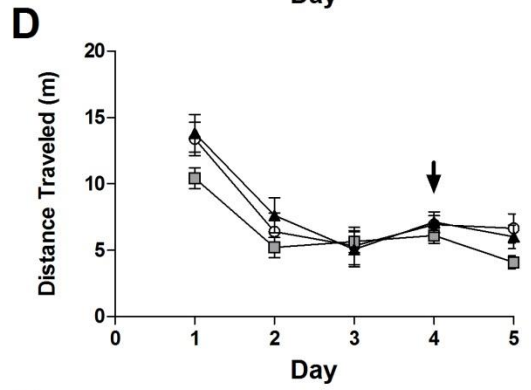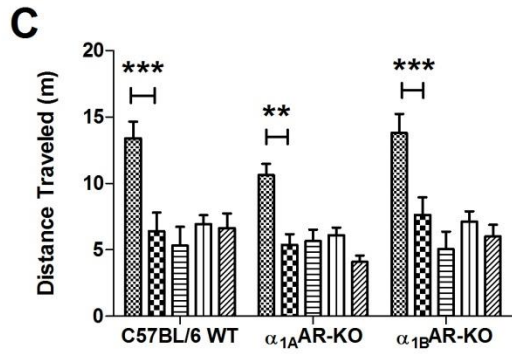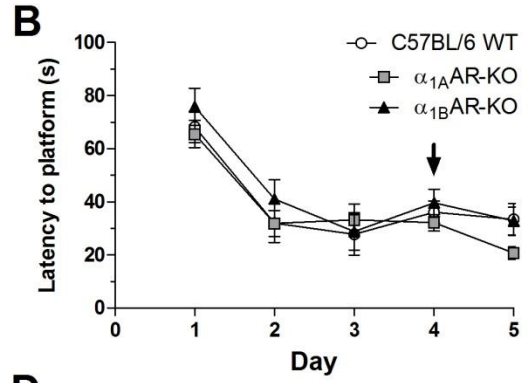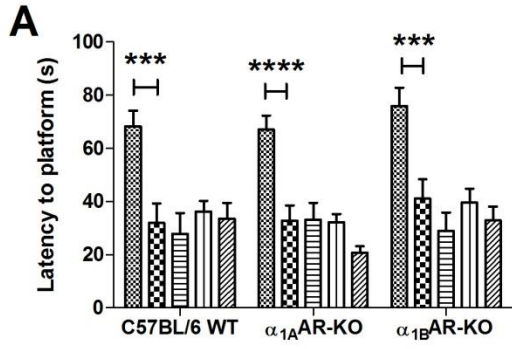
developed an algorithm to assess search strategies (Garthe et al., 2009, Figure 13). Mice

often begin searching by hugging the walls of the maze, a tactic called thigmotaxis. Mice

also search randomly then progress to searching in the general area of the platform. After

several trials, the strategies begin to follow spatially oriented patterns including chaining,

which is when the mouse swims at a distance from the wall equal to the diameter of the

platform location. In the last part of learning, mice use spatial approaches such as directed

search, focal search, and direct swimming to the platform. During a platform reversal

paradigm, mice will persevere at the original platform location then default to non-spatial

strategies but quickly switch back to spatial strategies by the second day of training. We

utilized a MATLAB script kindly provided by the Kempermann lab to assess search

strategies. Mice performed as expected, first using non-spatial strategies and gradually

switching to spatial strategies (Figure 14A).

There was a main effect of days [$F(4, 140) = 14.90$, $p < 0.0001$] but not genotype

[$F(2, 140) = 0.91$, $p = 0.41$] for the percent of spatial strategies used (Figure 14B). Post-hoc

testing showed that α_{1B}AR-KO mice significantly increased the use of spatial strategies by

Day 2, while WT and α_{1A}AR-KO mice did not do so until Day 3. As expected on Day 4 after

platform reversal, WT mice significantly decreased the use of spatial strategies ($p < 0.001$).

Surprisingly, α_{1A}AR-KO ($p > 0.05$) and α_{1B}AR-KO mice ($p > 0.05$) did not show a significant

decrease in spatial strategy use upon platform reversal. None of the groups showed a

Spatial strategies

■ Direct Swimming   ■ Focal Search   ■ Directed Search   ■ Chaining

Non-spatial strategies

■ Perseverance   ■ Scanning   ■ Thigmotaxis   ■ Random Search
(Days 4-5 only)
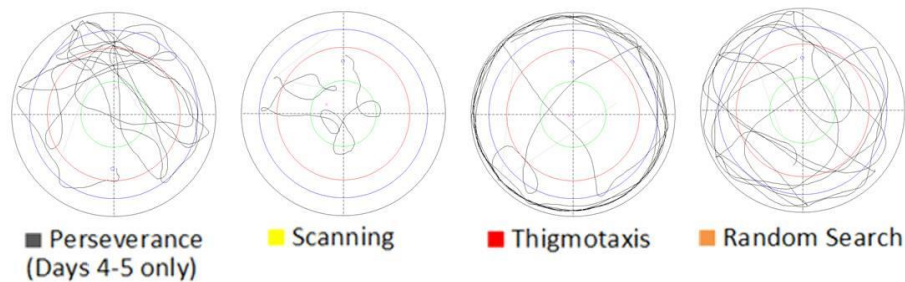
Figure 13. Search strategies in the Morris water maze. Adapted from (Garthe et al., 2009). A) The Morris water maze was divided into a wider wall zone closest to the wall of the maze, a closer wall zone, an annulus zone around the diameters of the inner and outer platform corners, and a center zone. A MATLAB algorithm was used to determine search strategies.

significant increase in the use of spatial strategies on Day 5. All groups showed

perseverance to the original platform location on Day 4 but there was not a significant

decrease in perseverance on Day 5 [$F(1, 35) = 3.95$, $p = 0.05$, Figure 14C]. In the cued trial on

Day 5, there were no differences in latency [$F(2, 31) = 8.71$, $p = 0.61$], distance traveled [$F(2,$

$32) = 0.79$, $p = 0.36$], or swim speed [$F(2, 34) = 0.002$, $p = 0.15$].

Overall, WT mice performed as expected in the Morris water maze with regards to

search strategies but there were no differences between genotypes in either search

strategy or perseverance.
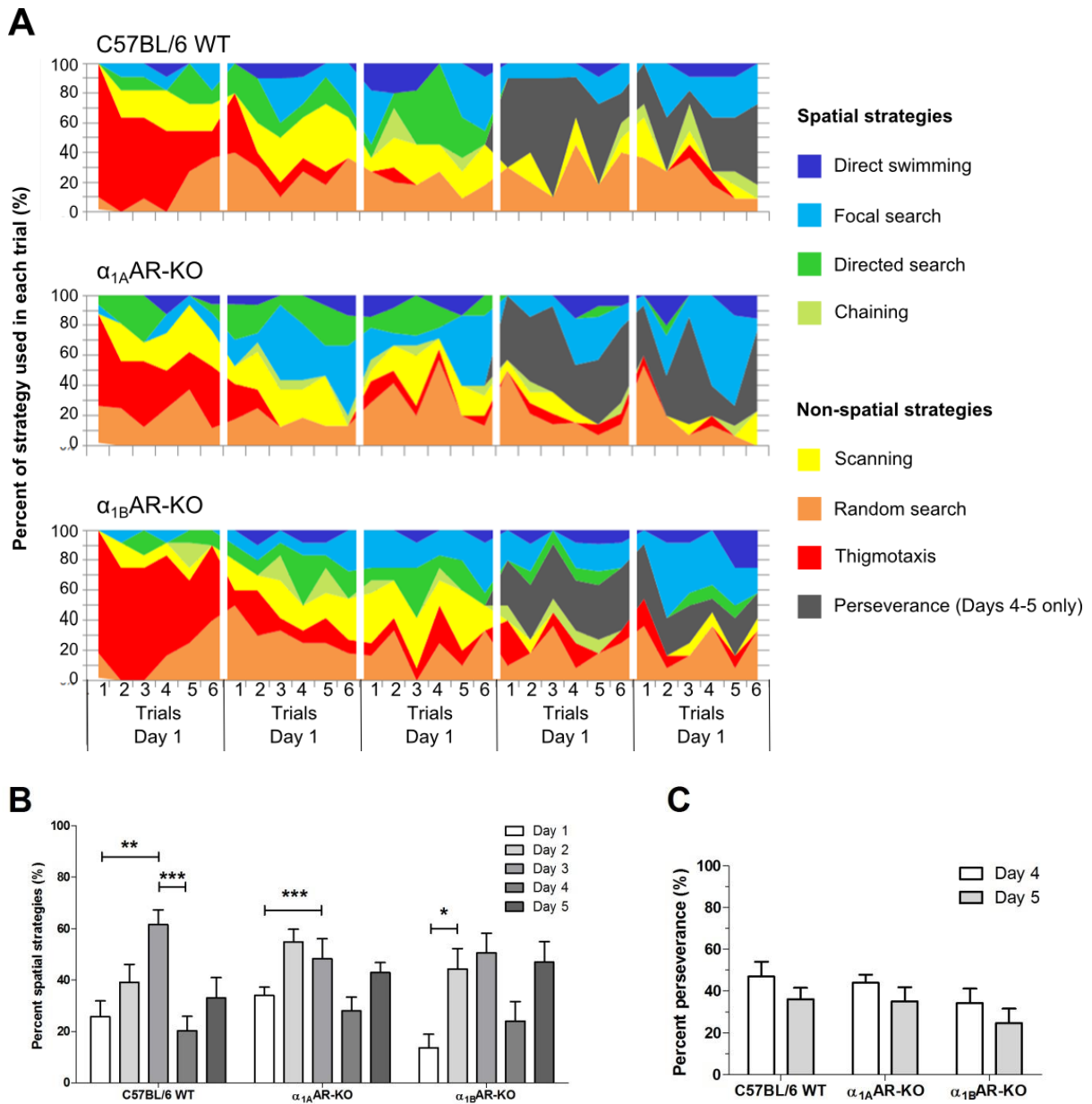
Figure 14. Spatial and non-spatial strategies in the Morris water maze. A) WT, α_{1A}AR-KO, and α_{1B}AR-KO mice followed a typical progression from non-spatial to spatial search strategies while learning the location of the platform. B) WT mice significantly increased the use of spatial strategies by Day 3 of training ($p < 0.01$) and decreased the use of spatial strategies on platform reversal Day 4 ($p < 0.001$). α_{1A}AR-KO mice increased the used of spatial strategies by Day 3 of training ($p < 0.001$) but did not significantly decrease their use upon platform reversal. α_{1B}AR-KO mice increased the use of spatial strategies by Day 2 of training ($p < 0.05$) but did not decrease their use on platform reversal Day 4. C) All groups persevered at the previous platform location on Day 4 but did not significantly decrease the amount of perseverance by Day 5.

Discussion

The current study found no difference in general health or neurological reflexes between groups. A previous experiment from our lab showed no difference in locomotion in the open field test (Chapter 2).

Previous studies of the α_1ARs found conflicting results in learning and memory tasks, likely due to differential effects of the α_{1A}AR and α_{1B}AR subtypes. Our lab showed that α_{1A}AR stimulation via pharmacological or transgenic means enhances spatial learning and memory (Doze et al., 2011). In the same study, α_{1A}AR-KO impaired both learning and memory in the Barnes maze. The only published research regarding the role of the α_{1B}AR subtype in cognition showed that transgenic mice lacking the α_{1B}AR have enhanced novel object recognition but impaired performance in the Morris water maze (Spreng et al., 2001).

In the novel object recognition test, α_{1A}AR-KO mice spent less time with the NO impaired when compared with α_{1B}AR-KO but not WT mice. WT mice were the only group to spend a greater than chance percentage of time with the novel object but there were no differences between groups. Comparing performance to chance levels is an indicator of whether the test worked because WT mice should do better than chance levels at recognizing the NO. That neither KO group did better than chance suggests impaired memory in both groups but the results could be confounded by the preference for a particular object. The objects used were counterbalanced but none of the groups habituated to the pointed column, suggesting they preferred it whether it was the original

or novel object. In order to avoid object bias, a greater number of animals or objects should be assessed with cohorts of WT mice prior to use in the novel object recognition test.

In the spontaneous alternation version of the t-maze, WT mice generally show greater than 80 percent alternation (Deacon and Rawlins, 2006). In the current study, WT mice did not alternate at a level higher than chance (50 percent). This could be due to the short (0 s) intra-trial interval, the amount of time mice were confined to the chosen arm prior to the next trial (Lalonde, 2002). The intra-trial interval is when memories are encoded; therefore, a longer interval may have enhanced the memory of the previously chosen arm. Typically, each trial should take less than 2 min; however, some mice took much longer. It is possible the short intra-trial interval increased anxiety leading to hesitancy to explore.

Previous research on the α_1AR showed that prazosin, a non-subtype selective α_1AR antagonist, can either impair or improve cognitive performance in the Morris water maze (Riekkinen et al., 1996). In the Morris water maze, all mice decreased the latency and the distance traveled to the platform by Day 2. WT and $\alpha_{1B}AR$-KO mice increased their path efficiency by Day 3 but $\alpha_{1A}AR$-KO did not, even by the final day of testing. The platform reversal did not significantly increase difficulty for mice to solve the maze on Day 4. However, WT mice did significantly reduce the use of spatial strategies on Day 4.

A high dose of prazosin can increase the time to complete a cued trial, suggesting a slower swimming speed (Riekkinen et al., 1996). In another study, $\alpha_{1B}AR$-KO mice had decreased swimming speed (Spreng et al., 2001). Surprisingly, in the current study $\alpha_{1A}AR$-KO but not $\alpha_{1B}AR$-KO showed a decreased swim speed during testing but all groups swam the

same speed during the cued trial. The decrease in swim speed during testing was significant but not large therefore it is not necessarily biologically relevant.

These results combined suggest there may be deficits in novel object recognition for both α_{1A}AR-KO and α_{1B}AR-KO mice but spatial memory impairment only in α_{1A}AR-KO mice based on a lack of increased path efficiency. Further research is important because α_{1A}AR-KO mice performed well on all other measures in the Morris water maze. Future testing should include another measure of spatial memory, such as the radial arm maze or a spatial cue version of the t-maze, both of which require food restriction. It appears that the α_{1A}AR is important for spatial memory based on our CAM-α_{1A}AR mouse studies so it is interesting but not wholly unexpected that knock-out did not cause severe impairments. Compensation in KO mice is a common limitation and can be avoided using the Cre lox system for conditional KO. Barring that, the use of an α_{1A}AR antagonist via a cannula could also help clarify the role of the α_{1A}AR in learning and memory.

CHAPTER 3
ALPHA$_{1B}$ ADRENERGIC RECEPTOR KNOCKOUT DECREASES DEPRESSION-LIKE BEHAVIOR WHILE KNOCKOUT OF EITHER THE ALPHA$_{1A}$AR OR ALPHA$_{1B}$AR DO NOT RELIABLY AFFECT ANXIETY

Abstract

Blocking neurotransmitter uptake via the norepinephrine transporter has long been a target of antidepressants. The role of the adrenergic receptors in the treatment or etiology of depression and anxiety has not been clearly defined. Previous research showed that chronic activation of the α_{1A}AR subtype leads to a lower level of depression-like behavior in the tail suspension test while activation of the α_{1B}AR subtype leads to a higher level. In the present study, we further investigated the involvement of the α_{1}AR subtypes, α_{1A}AR and α_{1B}AR, in depression and anxiety-like behavior in the adult mouse. We used two cohorts of subtype-specific receptor knockout (KO) mice. Group 1 mice began testing at 155 d and Group 2 at 90 d of age. Because α_{1A}AR-KO mice have seizures, we addressed seizure occurrence in the analysis. We found that α_{1A}AR-KO mice had higher levels of anxiety in Group 1 mice compared with wild type (WT), after accounting for seizure incidence. However, there was no change in Group 2 α_{1A}AR-KO mice in either anxiety or depression compared with WT mice. Lack of α_{1B}AR showed a lower depression-like phenotype compared with WT mice but had no change on anxiety in Group 1 mice and unclear results in Group 2 mice. We conclude that the role of the α_{1}AR subtypes in anxiety behavior is

44

complex. Combined with our previous results regarding α_1AR subtype activation, it is likely

the α_{1A}AR is involved in alleviating depression. Based on previous and the current results,

the α_{1B}AR subtype is clearly involved in depression-like behavior in the mouse. These results

suggest the α_1AR subtypes may be therapeutic targets for depression. A drug with α_{1A}AR

agonist and α_{1B}AR antagonist properties may be ideal.

Introduction

Major depressive disorder affects 16 percent of the United States population at least

once in their lifetime and has an economic burden of $80 billion per year (Kessler et al.,

2003; Wang et al., 2003). According to the 2012 National Survey on Drug Use and Health,

almost 7 percent of adults in the US had at least one depressive episode in the previous

year (Hedden et al., 2013). Yet currently available treatments are not ideal. Fifty percent of

patients with major depression have only a moderate response to currently available

antidepressants and just 30 percent find full remission (Trivedi et al., 2006). Norepinephrine

(NE) and serotonin (5-HT) were the first neurotransmitters found to alleviate depressive

symptoms and are still the leading targets for current antidepressants. Most treatments are

based on blocking reuptake of 5-HT and/or NE; however, many of these antidepressants

also have antagonist actions at 5-HT, α adrenergic, and dopamine receptors. Despite this,

the evidence for an antidepressant effect based on agonist activity at an α_1 adrenergic

receptor (α_1AR) subtype, the α_{1A}AR, has been mounting.

Many serotonin selective reuptake inhibitors (SSRIs) and serotonin norepinephrine

reuptake inhibitors (SNRIs) are used clinically for both depression and anxiety, which are

often comorbid (Kessler et al., 2003). Milnacipran, an SNRI, activates the α_1AR in rat

organotypic raphe slice cultures in addition to its blockade of NE and 5-HT reuptake

(Nagayasu et al., 2013). α_1AR antagonists can block anxiety and depressive behavior in

animal models (Kitada et al., 1983; Danysz et al., 1986; Cunha et al., 2013; Stone et al.,

2011; Kakui et al., 2009). The α_1AR inverse agonist prazosin reverses the anti-immobility

effect of paroxetine and citalopram, both SSRIs, in the forced swim test (Sugimoto et al.,

2011; Izumi et al., 1997). α_1- and α_2AR antagonists show a similar effect with the mood

stabilizer lamotrigine (Kaster et al., 2007). When the α_1AR agonist phenylephrine and

lamotrigine were given at sub-effective doses the antidepressant action was potentiated.

Prazosin also increases latency to enter the dark compartment in the passive avoidance task

suggesting α_1ARs are involved in anxiety as well (Puumala et al., 1996). A blockade of

antidepressant behavior was not seen in other studies using α_1AR antagonists with 7 d of

treatment with desipramine, a tricyclic antidepressant blocking reuptake of NE (Borsini et

al., 1984; Pulvirenti and Samanin, 1986). However, these studies used non-subtype selective

agonists and antagonists. Recent evidence from our lab suggests subtype selective

activation is critical for behavioral effects.

    The α_{1A}AR subtype is affected by chronic antidepressant treatment and

electroconvulsive therapy (Nalepa et al., 2002). Both increase the density and activity of

α_{1A}ARs in the frontal cortex and hippocampus without changing mRNA levels. Increased

density of receptors can cause increased sensitivity to the ligand translating into an

enhanced downstream effect. Desipramine increases the α_{1A}AR to α_{1B}AR ratio in rat

cerebral cortex (Hanft and Gross, 1990). Repeated electroconvulsive therapy also increases

binding to the α_{1B}AR in the cortex, thalamus, and amygdala; however, most of the binding

in the lateral amygdala was to α_{1A}ARs (Perry et al., 1990). Creation of transgenic mice either overexpressing or knocking out the α_{1A}AR and α_{1B}AR subtypes has provided the opportunity to further define the role of the subtypes in mood behavior.

Our lab found that activation of the α_{1A}AR and α_{1B}AR has differential effects on depressive-like behavior in the mouse (Doze et al., 2009). Mice with a constitutively active mutant form of the α_{1A}AR (CAM-α_{1A}AR) have increased agonist affinity and activation of the receptor. This provides an antidepressant and anxiolytic effect. However, CAM-α_{1B}AR mice have higher depression-like behavior but no change in anxiety (Doze et al., 2009, 2011).

We hypothesized that α_{1A}AR-KO mice would exhibit greater depression and anxiety-like behaviors than wild type (WT) mice. We also hypothesized that α_{1B}AR-KO mice would have a lower level of depression-like behavior but not anxiety behavior when compared with WT mice. To test this, we examined depression and anxiety behavior using the tail suspension test, elevated zero maze, marble burying test, and light dark exploration in Group 2 mice. Due to seizure activity appearing to be age-related, we also investigated anxiety behavior in Group 1 mice in the open field (center time) and the elevated zero maze.

The current study is the first to examine the effect of α_{1A}AR-KO and α_{1B}AR-KO on anxiety and depression-like behavior. Mice lacking each receptor subtype have been previously studied in the context of cardiac function, learning, and memory. In addition, seizure activity in the α_{1A}AR-KO mice has not been reported. The lack of behavioral studies is potentially due to this seizure activity, which makes analysis challenging (Doze et al.,

unpublished observation). Thus, we considered the incidence of seizures in our analysis and found interesting results.

Methods

Animals

All animals were bred and genotyped at the Cleveland Clinic Foundation and transferred to the University of North Dakota at 2 or 4 mo of age. Both facilities are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. The 4 mo old mice comprised Group 1 and included 15 WT, 17 α_{1A}AR-, and 11 α_{1B}AR-KO mice with approximately equal numbers of male and female animals. The 2 mo old mice comprised Group 2 and included 13 WT, 22 α_{1A}AR-, and 14 α_{1B}AR-KO mice also with approximately equal numbers of male and female mice. Mice received pelleted food with 5% fat (Teklad 22/5 Rodent Diet (W) 8640, Harlan, Indianapolis, IN) and water *ad libitum*, were maintained on a 12-hr light cycle (on at 0700 hrs), and provided veterinary care. All protocols conformed to the *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee at the University of North Dakota. The transgenic mice were created as previously described and littermates were used as controls (Cavalli et al., 1997; Rokosh and Simpson, 2002). Group 1 α_{1A}AR-KO mice were of the F7-8 generation and α_{1B}AR-KO mice of the F9 generation. Group 2 α_{1A}AR-KO mice were of the F3, F5, and F9 generation and α_{1B}AR-KO mice the F5 and F11 generation.

Seizure Incidence

The incidence of seizures was noted anytime the mice were observed, mainly prior to and during behavioral testing. Seizure occurrence was identified as "seizure" or "no

seizure" (McKhann et al., 2003). Seizures were not classified by stage. However, observation indicated that seizures included Stages 1-3, immobility, rigidity, and head bobbing. No notes indicate that seizures progressed to Stages 4-6, intermittent or continuous rearing and falling or whole body convulsions.

Behavioral Testing

Testing for Group 1 started at 160 d of age (Figure 15A). Group 2 testing began when mice were approximately 90 d of age (Figure 15B). Testing was completed between 1200 and 1700 hrs except open field which was performed between 0730 and 1200 hrs. Following locomotor and mood testing, Group 1 was involved in a learning and memory study not reported here. Mice were acclimated to individual cages in the testing room without food and water for 1 hr prior to testing, except for zero maze and open field when acclimation was 30 min. Lighting was adjusted using a LX1010B digital lux meter (Amazon.com, Seattle, WA). The tests and analysis were performed blind to genotype.

Open field test. The open field test was used to assess basal locomotor activity in Group 1 mice. Mice were placed in a 43.2 x 43.2 cm open field equipped with infrared beams every 2.54 cm above the floor of the field. Mice were allowed to roam freely for 20 min while activity was recorded. The data were analyzed using Activity Monitor 6.0 and Activity MDBtoExcel for distance traveled each min, total distance traveled, and time spent in the center zone (MedAssociates, St. Albans City, VT). The center zone was defined as a center square of 29.12 x 29.12 cm. Light was ambient room lighting at approximately 250 lux.

A

Zero maze

General health assessment

Open field

B

Zero maze

Tail suspension

Marble burying

Light dark
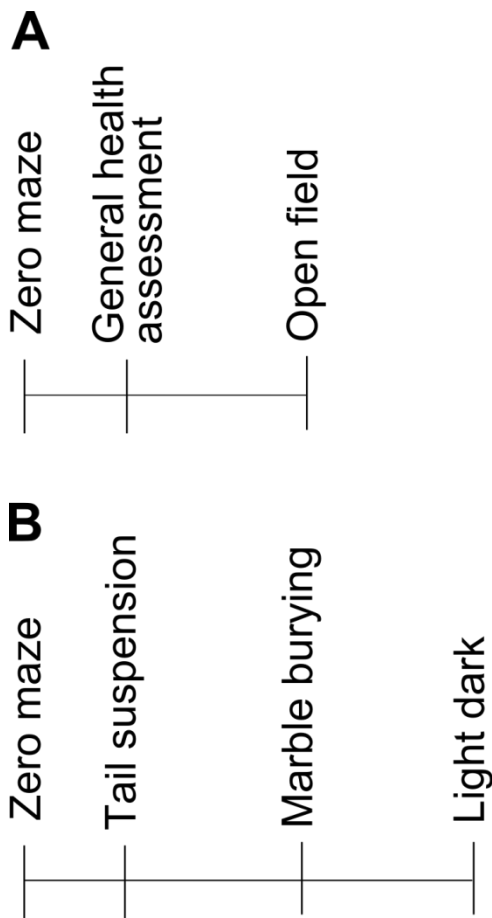
Figure 15. Behavioral timelines. A) Group 1 mice were tested for anxiety in the elevated zero maze, underwent a general health assessment, and were tested in the open field for locomotion (distance traveled) and anxiety (time spent in the center of the field). B) Group 2 mice were tested for anxiety in the elevated zero maze, marble burying test, and light dark exploration and for depression in the tail suspension test.

Zero maze. The zero maze was used to test anxiety (Stoelting, Wood Dale, IL). The maze consisted of an elevated circular walkway with a diameter of 59.7 cm and a lane width of 5 cm. Due to previous trials where mice jumped off the maze to the floor; the maze was placed on a table 72.4 cm in height. The maze was divided into 4 quadrants: two open and two with 15.4 cm high walls. Light was held constant across the maze at 40 lux to mimic a mouse's ethological environment. Mice were placed on the maze facing a closed area and were allowed to explore for 10 min. The time spent in each area, open or closed, was

determined when all four paws entered. The tests were recorded from an overhanging camera and used to analyze the time spent in and entries into the open areas.

Light Dark Exploration. Light dark exploration was used to test anxiety using a modified open field box (Stoelting, Wood Dale, IL). The 40 x 40 x 35 cm box was divided by an opaque plexiglass insert into a light side (40 x 26 x 35 cm) and dark side (40 x 14 x 35 cm) connected by a 7.5 x 7.5 cm opening. The dark side was kept at 0 lux by addition of an black opaque plexiglass lid. The light side was illuminated by overhead fluorescent lighting, approximately 220 lux within the box. Each mouse was placed in the light facing away from the opening to the dark section and allowed to explore for 10 min. A mouse was considered to have entered a side of the box when all four paws entered. The trials were digitally recorded and later analyzed for the latency to enter the dark side, time spent in the light side, and the number of entries into the light side.

Marble burying test. The marble burying test was used to assess obsessive compulsive-type anxiety behavior. A 17 x 28 x 13 cm polycarbonate box held 10 cm of sawdust bedding with 20 marbles placed on top. Lighting was held constant at 40 lux within each box. Each mouse was placed in a box and allowed to explore for 30 min then removed. Marbles were considered buried if more than two-thirds covered with sawdust bedding.

Tail suspension test. The tail suspension test was a 29.2 x 21.6 x 24.1 cm box made of 1.27 cm white melamine-coated particle board with a hook in center top of the box. A tape loop was placed on the tail of each mouse, which was then suspended on the hook for 6 minutes. Tail climbing was a frequent occurrence, as is common in the C57BL/6 strain (Mayorga and Lucki, 2001). Mice that tail climbed longer than 72 s (20 percent of the time)

were excluded. A subset of mice were tested a second time using a 3.8 cm long, 1.27cm in diameter hard plastic tube placed between the base of the tail and the tape loop to discourage tail climbing (Can et al., 2012).

Statistical Analysis

Statistical analyses were performed using GraphPad Prism 5.04 (GraphPad Software, San Diego, CA). The Kolmogorov Smirnov test was used to assess normality and the Bartlett's test was used to determine if variances were equal. ANOVAs were still used when a set of data were not normally distributed because ANOVA are robust for violations of normality and the equal variances assumption was met. When the variances differed, the Kruskal Wallis test was used with a Dunn's post-hoc test. Outliers were detected and excluded using the Grubb's test. Differences between groups were assessed with one-way ANOVA and a Tukey post-hoc test. Sex differences were detected using a two way ANOVA with genotype X sex and Bonferroni post-test for paired comparisons. The effect of the tail tube on tail climbing was determined using a Mann-Whitney test because the data were not normally distributed.

<div align="center">Results</div>

Seizure Incidence

α_{1A}AR-KO mice have an increased incidence of seizures. CAM-α_{1B}AR mice, with a mutation that keeps the receptor in its active conformation, have an age-related seizure disorder (Zuscik et al., 2000). Knock out the α_{1B}AR protects against seizures (Pizzanelli et al., 2009). Due to this, we did not expect nor observe seizures in the α_{1B}AR-KO mice. Similarly, no seizures were observed in WT mice. Group 1 α_{1A}AR-KO mice had a seizure incidence of

50 percent while Group 2 α_{1A}AR-KO mice had an incidence of 13 percent (2 mice). Seizure

incidence was roughly equal in male and female mice. Due to the high level of seizure

incidence, all behavioral tests for Group 1 were analyzed to determine if prior seizure

activity had an effect on the parameters of each test. If so, the results were analyzed again

separating α_{1A}AR-KO mice that did not have seizures and those that did.

Open Field

       α_{1A} or α_{1B}AR knockout did not affect basal locomotion in Group 1 mice. Changes in

basal locomotion make behavioral results challenging to interpret (Crawley and Paylor,

1997). Therefore, we examined basal locomotion in the open field test in Group 1. We

previously showed that activation of the α_{1A}AR does not change basal locomotion but

activation of the α_{1B}AR increases the total number of beam breaks in the open field

compared with WT mice (Doze et al., 2009). Knauber and Müller. found that α_{1B}AR-KO mice

traveled shorter distances (Knauber and Müller, 2000). In contrast, another study showed

that α_{1B}AR-KO mice traveled longer distances in the open field (Spreng et al., 2001). In the

current study, there were no differences in total distance traveled in the open field

between groups [$F(2, 44) = 2.32$, $p = 0.10$, Figure 16]. There was a main effect of sex in the

total distance traveled [$F(1, 41) = 7.421) = p = 0.009$] where female α_{1A}AR-KO and α_{1B}AR-KO

animals traveled less distance than female WT mice ($p < 0.05$). There was also an interaction

of sex [$F(2, 41) = 3.834$, $p = 0.03$]. There were no differences between α_{1A}AR-KO mice that

had seizures vs α_{1A}AR-KO mice without seizures [$t(20) = 0.03$, $p = 0.98$]. These results show

that in male mice locomotion was not affected by the lack of the receptor subtypes or by

seizure incidence. Female α_{1A}AR-KO and α_{1B}AR-KO have decreased locomotion.
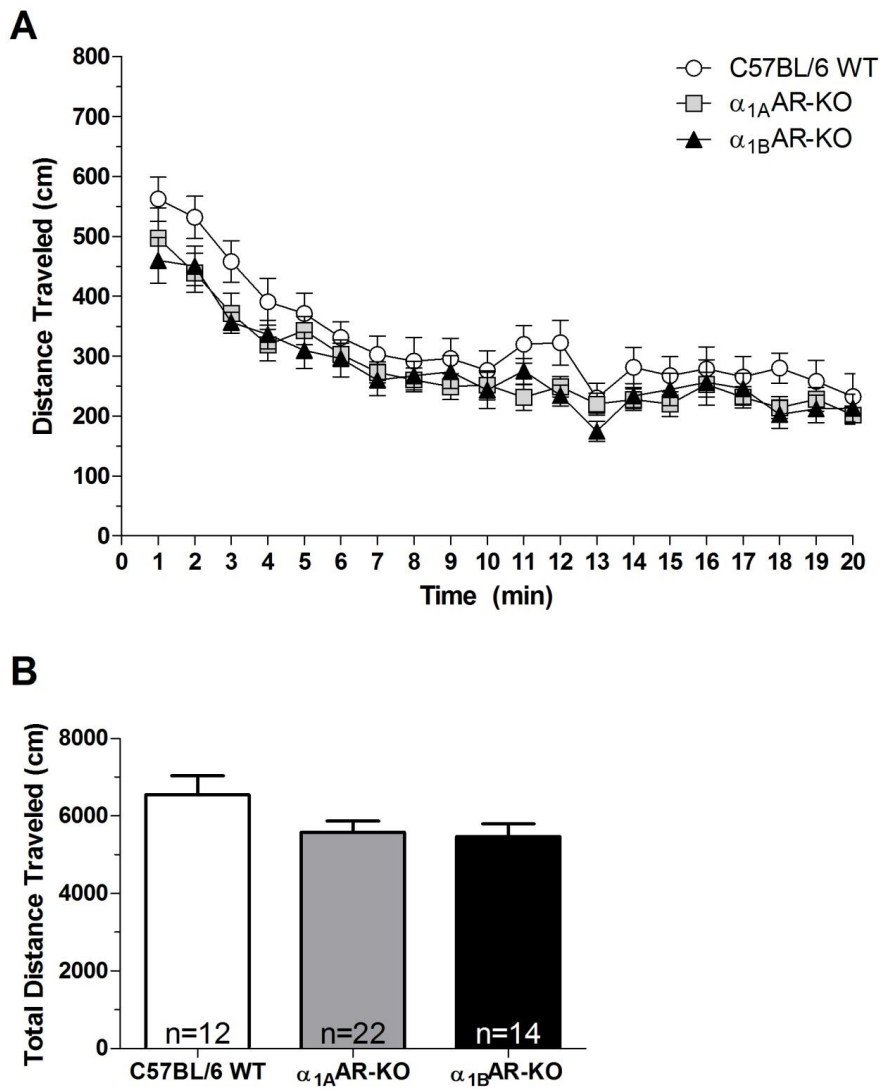
Figure 16. Open field test in Group 1. There was no difference between WT, α_{1A}AR-KO, and α_{1B}AR-KO in the total distance traveled. Shown as A) distance traveled over time and B) total distance traveled ($p = 0.10$).

In Group 1, knockout of either subtype did not affect anxiety in the open field; α_{1A}AR-KO mice had higher levels of anxiety in the elevated zero maze after accounting for seizure activity; α_{1B}AR-KO did not affect anxiety. α_{1A}AR activation decreases anxiety behavior while α_{1B}AR activation has no effect on anxiety (Doze et al., 2009). To further study
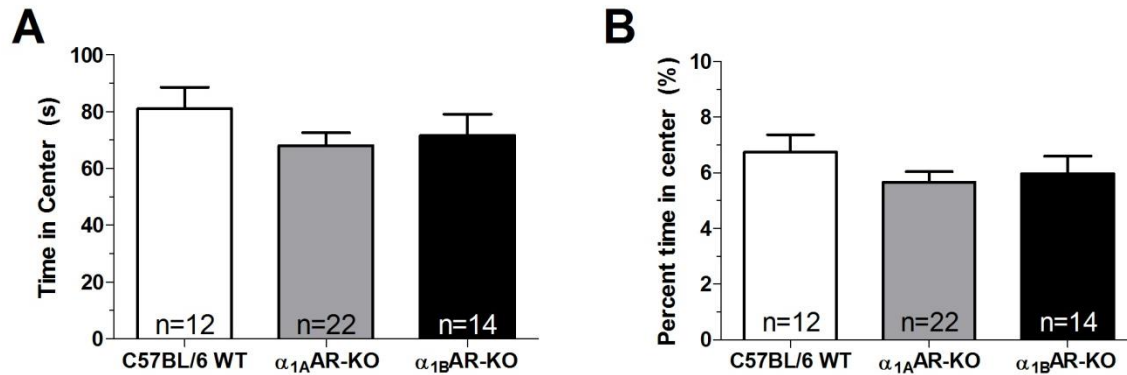
Figure 17. Anxiety in Group 1, Open field time in center. There were no main effects of A) time (p = 0.35) or B) percentage of time (p = 0.35) spent in the center of the open field, a measure of anxiety.

the effects of α_1AR subtype function on anxiety behavior, the open field data from Group 1 was analyzed for the time spent in the center zone. In addition, Group 1 mice were tested in the elevated zero maze. We hypothesized that α_{1A}AR-KO would result in more anxiety-like behavior while α_{1B}AR-KO would not affect anxiety.

Open Field Time Spent in the Center Zone

Time spent in the center of an open field is often used as a preliminary anxiety test (Crawley and Paylor, 1997). In Group 1 mice, there were no significant differences in the time spent in the center of the field [$F(2, 44) = 1.07$, $p = 0.35$] or in the percentage of time spent in the center [$F(2, 44) = 1.04$, $p = 0.35$] (Figure 17A and 17B). There was no effect of seizure incidence in the α_{1A}AR-KO mice on the time spent in the center of the field [$t(20) = 0.066$, $p = 0.94$] or the percentage of time spent in the center [$t(20) = 0.04$, $p = 0.96$]. There was no effect of sex in the time spent in the center of the open field [$F(1, 41) = 0.43$, $p = 0.51$]. There was an interaction between sex and genotype [$F(2, 41) = 5.36$, $p = 0.008$] such that female α_{1B}AR-KO mice spent less time in the center than female WT mice ($p < 0.05$).

Zero Maze, Group 1

There were no significant differences in the time spent in the open areas of the elevated zero maze when seizure activity was not accounted for [$F(2, 46) = 2.363$, $p = 0.10$] (Figure 18A). There was a main effect of genotype in the number of entries made into the open areas [$F(2, 45) = 9.58$, $p = 0.003$] (Figure 18B). A Tukey post-hoc test showed that α_{1A}AR-KO mice made fewer entries into the open areas compared with both WT ($p < 0.001$) and α_{1B}AR-KO mice ($p < 0.05$). There was no effect of sex in the time spent in the open areas [$F(1, 43) = 0.49$, $p = 0.48$] or entries [$F(1, 43) = 0.66$, $p = 0.41$].

Reanalysis of the data after separating α_{1A}AR-KO mice that had seizures and mice that did not, revealed a main effect in the time spent in the open areas [$F(3, 35) = 4.746$, $p = 0.0058$] and confirmed the main effect on the number of entries into the open areas [$F(3, 35) = 5.343$, $p = 0.0031$] (Figures 18C and 18D). Post-hoc testing showed that α_{1A}AR-KO mice that did not have seizures spent less time in the open areas when compared with both WT ($p < 0.01$) and α_{1B}AR-KO mice ($p < 0.05$). α_{1A}AR-KO mice that did not have seizures also made fewer entries into the open areas when compared with both WT ($p < 0.001$) and α_{1B}AR-KO mice ($p < 0.01$). These results show that Group 1 α_{1A}AR-KO mice that did not have seizures have a higher level of anxiety in the elevated zero maze while α_{1A}AR-KO mice that did have seizures and α_{1B}AR-KO mice have anxiety levels similar to WT animals.

Zero Maze, Group 2

In Group 2, α_{1A}AR-KO Mice had anxiety levels similar to WT; Lack of α_{1B}AR subtype activity did not reliably affect anxiety behavior. For additional study on the effect of α_1AR subtype function on anxiety behavior, Group 2 mice were also assessed in the elevated zero
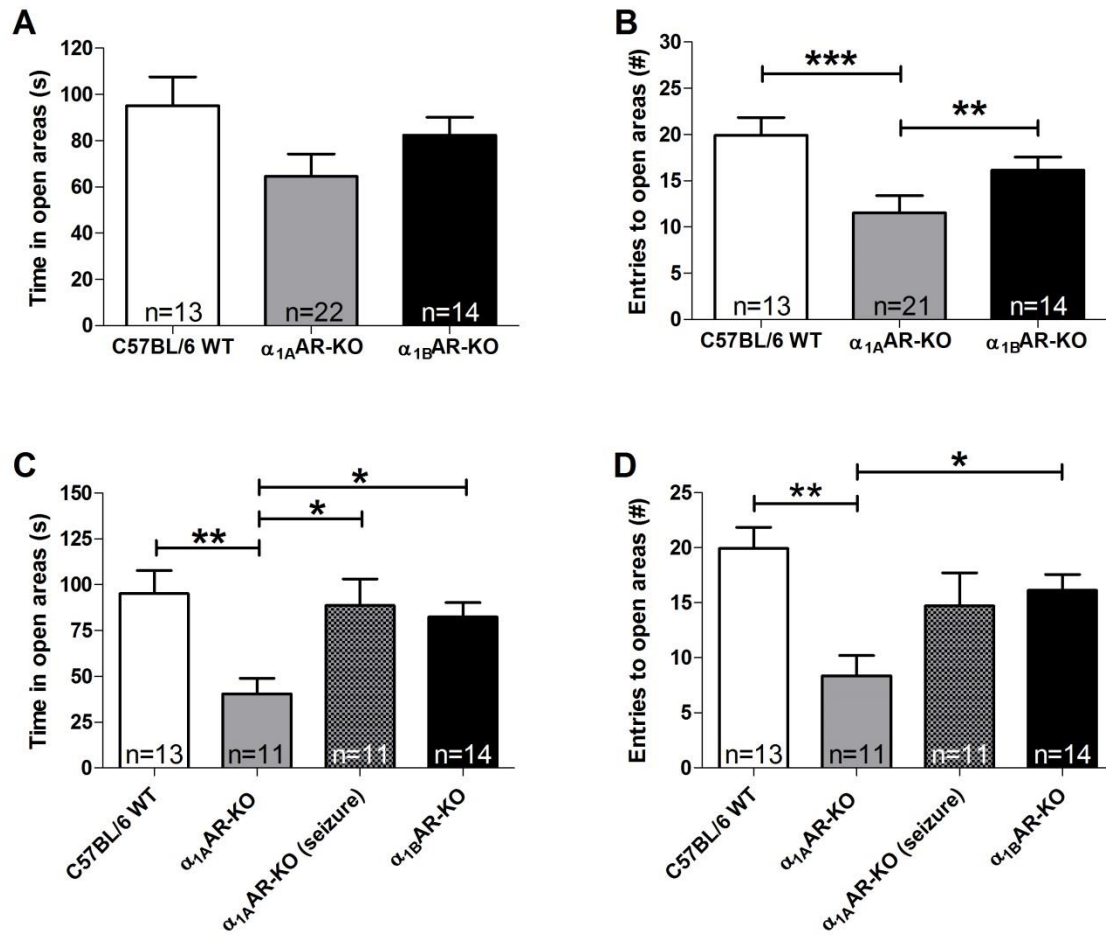
Figure 18: Anxiety in Group 1, Zero maze. In the zero maze: there was no effect of genotype for the A) time spent in the open areas. There was a main effect of genotype in the B) number of entries into the open arms. A Tukey post-hoc test showed that the α_{1A}AR-KO mice made fewer entries into the open arms when compared with WT ($p < 0.001$) and α_{1B}AR-KO mice ($p < 0.01$). When mice with a history of seizures were separated from those that did not, a main effect was found for the C) time spent in the open areas. Post-hoc analysis showed that α_{1A}AR-KO mice that did not have seizures spent less time in the open area when compared with WT ($p < 0.01$), α_{1A}AR-KO (seizure) ($p < 0.05$), and α_{1B}AR-KO mice ($p < 0.05$). There was also a main effect on the number of D) entries to the open areas of the zero maze. A Tukey post-hoc test revealed that α_{1A}AR-KO mice that did not have seizures made significantly fewer entries into the open areas when compared with WT ($p < 0.01$) and α_{1B}AR-KO mice ($p < 0.05$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$

maze as well as the light dark exploration and marble burying tests. The low number of

Group 2 α_{1A}AR-KO mice that had seizures (n = 2) precluded testing for the effect of seizure

incidence in this group. Our hypothesis was the same as for Group 1 mice: that α_{1A}AR-KO

would result in a higher level of anxiety-like behavior while α_{1B}AR-KO would not affect anxiety.

In the zero maze, there were no significant differences in the amount of time spent in the open areas [$F(2, 40) = 2.81$, $p = 0.07$] (Figure 19A). There was a statistically significant difference between groups in the number of entries into the open areas of the zero maze [$F(2, 40) = 4.32$, $p = 0.02$] (Figure 19B). Post-hoc testing showed that α_{1B}AR-KO mice made fewer entries into the open areas compared with WT mice ($p < 0.05$). There were no sex differences in either the time spent in [$F(1, 36) = 3.25$, $p = 0.07$] or entries into the open areas [$F(1, 37) = 0.44$, $p = 0.51$]. The results show a possibility that α_{1B}AR-KO mice have a higher level of anxiety than WT mice based on the reduced entries into the open areas of the elevated zero maze. However, this was not confirmed in the time spent in the open areas of the maze.

Light Dark Exploration

In an earlier study, we saw no difference in anxiety behavior in the light dark exploration after transgenic or pharmacological activation of the α_{1A}AR or α_{1B}AR (Doze et al., 2009). In a more recent study, α_{1A}AR activation significantly increased the time spent in the light side of the light dark exploration test (Doze et al., 2011). We hypothesized that KO of the α_{1A}AR would lead to a higher level of anxiety in the light dark exploration test.

In the current study, there were no statistically significant differences in the light dark exploration for the time spent in the light side [$F(2, 39) = 1.34$, $p = 0.27$] (Figure 19C), entries to the light side [$F(2, 40) = 1.21$, $p = 0.30$] (Figure 19D), or latency to enter the dark side [$F(2, 39) = 1.04$, $p = 0.36$] (Figure 19E). There was a main effect of sex in the time spent

Figure 19. Anxiety in Group 2: Zero maze, light dark exploration, and marble burying test. Lack of α_{1A}AR or α_{1B}AR function did not reliably affect anxiety behavior. In the zero maze, there were no significant differences between WT, α_{1A}AR-KO, or α_{1B}AR-KO for the A) time spent in the open areas ($p = 0.054$). There was a main effect of genotype in the B) entries made into the open areas of the zero maze ($p = 0.015$). Tukey's post-hoc test revealed that α_{1B}AR-KO mice made fewer entries into the open areas when compared with WT and α_{1A}AR-KO mice ($p < 0.05$ for each comparison). In the light dark exploration, there was no effect of genotype in the C) time spent in the light side ($p = 0.272$), D) entries to the light side ($p = 0.308$), or E) latency to enter the dark side of the maze ($p = 0.362$). In the marble burying test, there was a significant difference in the number of marbles buried between groups ($p = 0.005$). A Dunn's post-hoc test showed that α_{1B}AR-KO mice buried significantly more marbles than α_{1A}AR-KO mice but not WT mice. * $p < 0.05$, ** $p < 0.01$
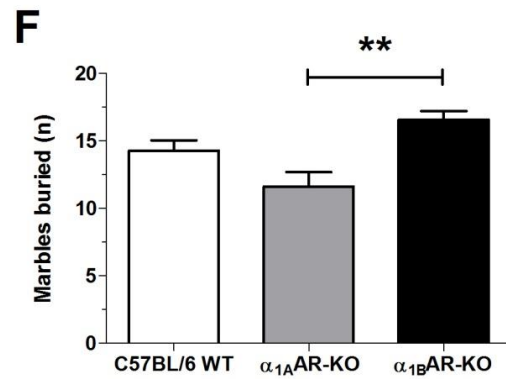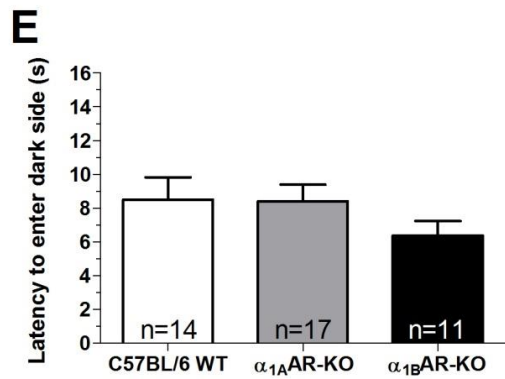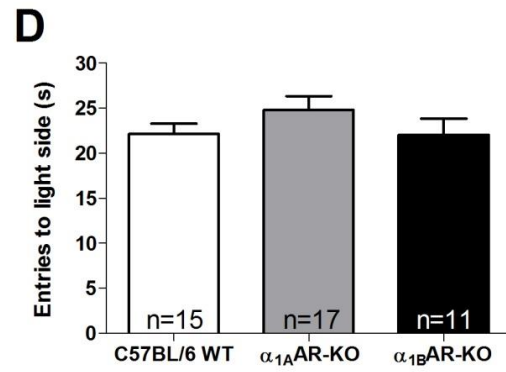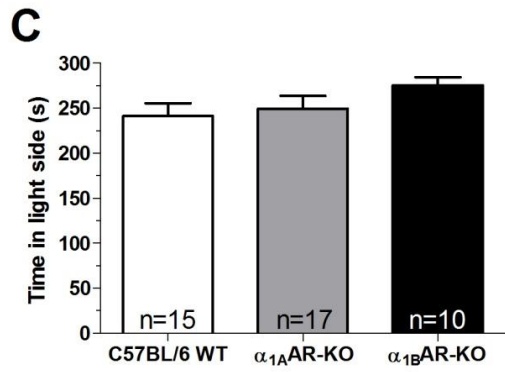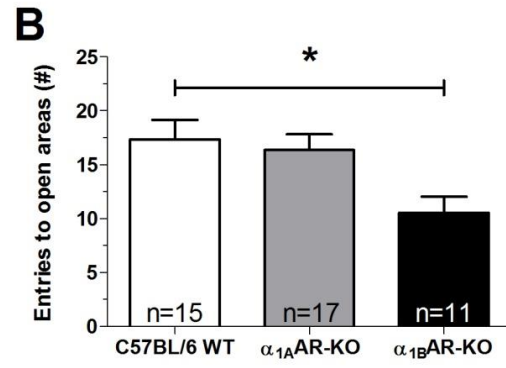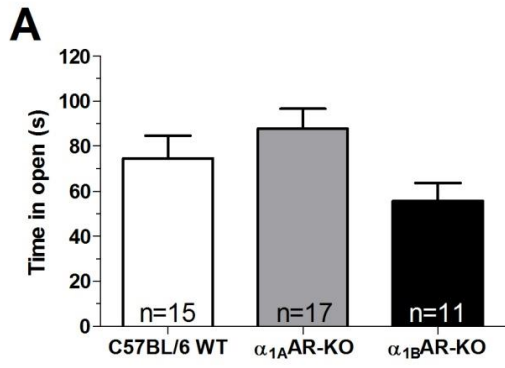
in the light side [$F(1, 36) = 7.12$, $p = 0.011$]; however, no post-hoc differences were seen. The results suggest there are no differences in anxiety between genotypes in light dark exploration.

Marble Burying Test

We have previously shown that transgenic or pharmacological α_{1A}AR activation decreases the number of marbles buried in the marble burying test, showing a lower level of anxiety than in WT or control mice (Doze et al., 2009). Activation of the α_{1B}AR did not have an effect on the number of marbles buried. Therefore, we hypothesized that KO of the α_{1A}AR would increase anxiety behavior in the marble burying test compared with WT mice but α_{1B}AR-KO would have no effect.

There was a statistically significant difference in the number of marbles buried [$\chi^2(3) = 10.54$, $p = 0.005$] (Figure 19F). A Dunn's post-hoc test showed that α_{1B}AR-KO mice buried more marbles than α_{1A}AR-KO mice ($p < 0.01$) but not more than WT mice ($p > 0.05$). There was no main effect of sex in the marble burying test [$F(1, 37) = 0.75$, $p = 0.39$]. The results of the marble burying test suggest there are no differences in obsessive-compulsive type anxiety between the α_{1B}AR-KO and WT but that α_{1B}AR-KO mice have a higher level of anxiety compared with α_{1A}AR-KO mice.

Tail Suspension Test

Loss of α_{1B}AR function decreased immobility when tail climbing was reduced. Activation of the α_{1A}AR, either using transgenic mice or pharmacological means, previously showed a robust decrease in depression-like behavior in the tail suspension test which was

blocked by prazosin, an $\alpha_{1A}AR$ antagonist (Doze et al., 2009, 2011). Transgenic $\alpha_{1B}AR$ activation led to a higher level depression-like behavior in the tail suspension test.

In the current study, there were no significant differences in the time spent immobile in the tail suspension test [$F(2, 30) = 0.50$, p = 0.61] (Figure 20A). However, two mice per group had to be excluded for tail climbing greater than 20 percent of the time. Other mice spent time tail climbing that they otherwise may have spent immobile; however, tail climbing in these mice was not present for greater than 20 percent of test time. In order to detect potential differences, a hard plastic tail tube was used to prevent mice from climbing their tail (Can et al., 2012). To assess the effect of the plastic tube as a barrier to tail climbing, all data were pooled. The reduction in tail climbing was statistically significant ($U = 236.5$, p < 0.0001) (Figure 20B). Under these conditions, there was a difference in the time spent immobile between groups [$F(2, 28) = 10.80$, p = 0.0003]. A Tukey post-hoc test showed that $\alpha_{1B}AR$-KO mice spent less time immobile than both WT (p <0.001) and $\alpha_{1A}AR$-KO mice (p < 0.01) (Figure 20C). There were no sex differences in the no-tail-tube [$F(1, 27) = 0.60$, p = 0.44] or tail tube version of the tail suspension test [$F(1, 25) = $ x2.01, p = 0.16]. These results suggest that $\alpha_{1B}AR$-KO results in lower levels of depression-like behavior.

Discussion

Locomotion was not different between groups overall, similar to another study using only male $\alpha_{1B}AR$-KO mice (Spreng et al., 2001). However, another report using male $\alpha_{1B}AR$-KO mice showed a reduction in open field locomotion. In the current study, both α_{1A}- and $\alpha_{1B}AR$-KO female mice traveled less distance in the open field but an interaction of
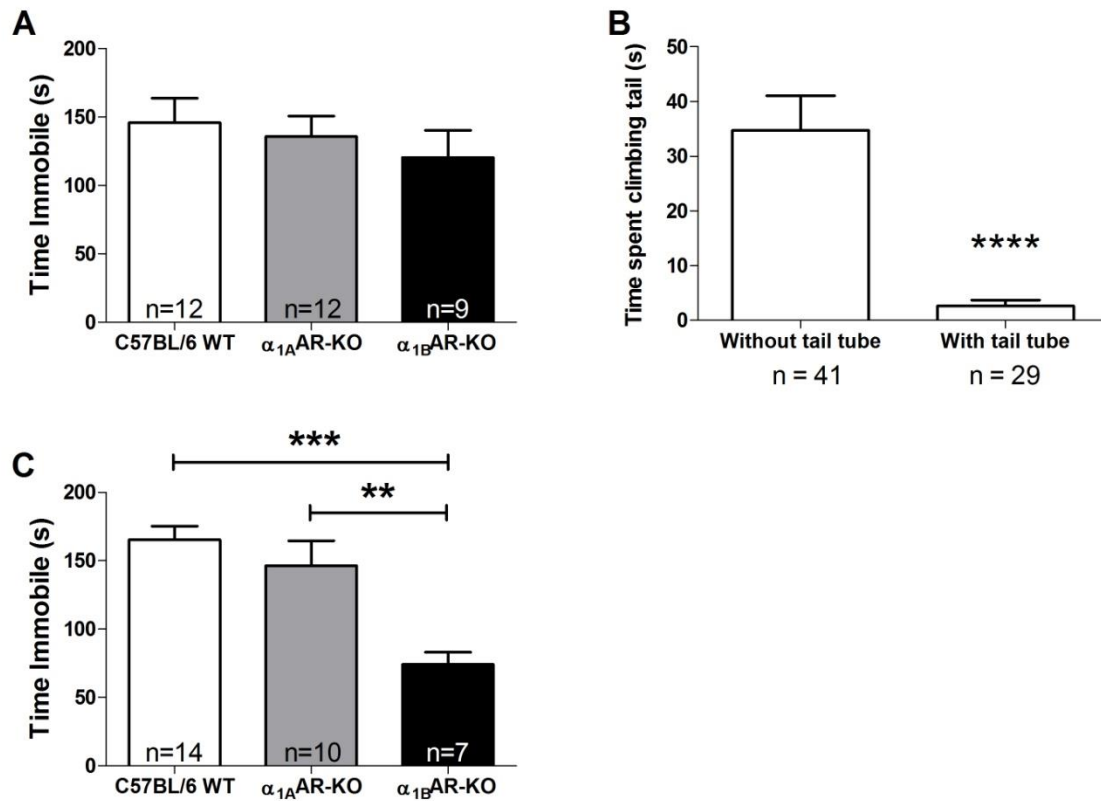
Figure 20. Depression-like behavior in Group 2, Tail suspension test. In the tail suspension test, there was no effect of genotype on A) the time spent immobile when mice were tested without an obstruction to tail climbing (p = 0.610). Using a hard plastic tube to impede tail climbing there was B) a significant reduction in the amount of time spent tail climbing (p < 0.0001). Retesting with a tail tube revealed C) a main effect of genotype on the time spent immobile (p = 0.0003). A Tukey post-hoc test showed that α_{1B}AR-KO mice spent less time immobile when compared with both WT (p < 0.001) and α_{1A}AR-KO (p < 0.01) mice. * p < 0.05, ** p < 0.01, *** p < 0.0001

genotype and sex makes the result difficult to interpret. Locomotion was not likely a

confounding factor as no sex differences were found in any behavioral tests. This is the first

study to examine anxiety and depression-like behavior after KO of the α_{1A}AR- and α_{1B}AR

(Spreng et al., 2001). Our results show that the α_{1A}AR subtypes are involved in anxiety

behavior in 4-month old mice lacking the α_{1A}AR only in those mice that did not have

observed seizures. Conversely, mice lacking the α_{1B}AR receptor subtype exhibited less

depression-like behavior and unclear effects on anxiety.

Therapeutics for depression often alleviate symptoms of both depression and anxiety, which is a common comorbid finding (Kessler et al., 2003). Previous research has shown conflicting results for the α_1AR's role in depression and anxiety, likely due to the lack of highly selective agonists and antagonists able to cross the blood brain barrier (Cunha et al., 2013; Danysz et al., 1986; Borsini et al., 1984; Pulvirenti and Samanin, 1986). The current study furthers our understanding of α_1AR function in the central nervous system and the behaviors elicited. Early behavioral work from our lab showed that activation of the α_{1A}AR led to a lower level of depression and marble burying but not anxiety behavior in the elevated plus maze or light dark exploration (Doze et al., 2009, Figure 21). In a subsequent study, we utilized littermate controls and lowered the lighting for the elevated zero maze to better mimic the natural mouse habitat. Our findings were that α_{1A}AR activation also led to a lower level of anxiety in the elevated zero maze and light dark exploration tests (Doze et al., 2011). In corroboration with these studies, in the current investigation α_{1B}AR-KO mice had a lower level of depression compared with WT demonstrating a role for this subtype in depression. Both transgenic and pharmacological activation studies suggested a role for the α_{1A}AR in depression. Surprisingly, α_{1A}AR-KO mice did not show more depression-like behavior even after removing the opportunity for tail climbing. The role of the α_1AR subtypes in depression and anxiety appears to be more complex than anticipated and additional factors are likely involved.

Activation   Knock-out

$\alpha_{1A}AR$

↓ Depression   — Depression
↓ Anxiety   ↑ Anxiety (Group 1, without seizures)
                                — Anxiety (Group 2)
↑ Seizure threshold   50% incidence of seizures (Group 1)
                                  13% incidence of seizures (Group 2)

$\alpha_{1B}AR$

↑ Depression   ↓ Depression
— Anxiety   — Anxiety (Group 1 mice)
                                ↑ — Anxiety
↑ Age-dependent seizures   Protects against seizures

Figure 21. Overview of activation and KO of α_1AR subtype effects on behavior. Previous studies showed that transgenic activation of the α_{1A}AR reduces depression and anxiety-like behaviors and increases the seizure threshold, showing anti-epileptic properties (Doze et al., 2009, 2011, Doze, Jurgens, Nelson, and Goldenstein, unpublished data). Pharmacological activation of the α_{1A}AR also reduced depression levels (Doze et al., 2009). Activation of the α_{1B}AR increased depression-like behavior but had no effect on anxiety. In the current study, α_{1A}AR-KO did not affect depression-like behavior but had a potentially age-related effect on seizure activity and anxiety. Older mice, Group 1, mice had a higher occurrence of seizures and mice in Group 1 that did not have seizures had increased anxiety. The younger mice, Group 2, had a lower occurrence of seizures and no change in anxiety levels. α_{1B}AR-KO mice had lower depression-like behavior but no change in anxiety in Group 1 mice and unclear results on anxiety in Group 2 mice.

There are many potential explanations for the unclear results; one is that the

receptor subtypes require heterodimerization for some behavioral effects. GPCRs are

known to heterodimerize *in vitro* and *in vivo*, with some heterodimers required for or

changing downstream effects of the receptors alone. There is also *in vivo* evidence that

GPCR heterodimerization is involved in neuropsychiatric disorders. The D1-D2 heterodimer

is increased in human post-mortem brain and blocking formation of the heterodimer in rats

led to a reduction in immobility in the forced swim test (Pei et al., 2010). The α_1AR subtypes

can form heterodimers *in vitro,* which could potentially confer functional changes (Uberti et

al., 2003). Heterodimers of the α_{1A}AR and α_{1B}AR subtypes increase binding site density and protein expression of the α_{1A}AR with no change in the α_{1B}AR (Uberti et al., 2003). Increased density and protein expression suggest sensitization to the ligand and could increase downstream activity *in vivo*. The ratio of receptor subtypes may also be important.

In the CAM-α_{1B}AR mice, the α_{1A}AR is still present though at lower levels and activity than the α_{1B}AR, likely even after accounting for increased α_{1A}AR expression due to potential heterodimerization. The increased ratio of α_{1B}ARs and their constitutive activity appears sufficient to induce depression (Doze et al., 2009). In the current study, α_{1B}AR-KO reduces depression-like behavior but it is unknown if the lack of α_{1B}AR signaling is sufficient for this reduction or if the still present α_{1A}AR is necessary. α_{1A}AR-KO mice show no change in depressive activity, which suggests that lack of α_{1A}AR activity alone is not sufficient to induce depression. It is interesting to speculate that heterodimerization to increase α_{1A}AR density and expression may be needed. In CAM-α_{1A}AR mice, with both constitutive α_{1A}AR activity and increased surface expression of the α_{1A}AR due to hetero-dimerization, depression is reduced. But it is also reduced when the endogenous α_{1A}AR is activated via an α_{1A}AR agonist, cirazoline, and returns to control levels when CAM-α_{1A}AR mice are treated with an α_1AR antagonist, prazosin (Doze et al., 2009).

To add to the complexity, the α_1AR subtypes are not expressed at the same levels in all brain areas and there is evidence that the expression level affects whether receptors homo- or heterodimerize. Heterodimerization can also occur between different classes of receptors, such as with the D2 and SST receptors (Molchan et al., 1991). Clearly, more work is needed to clarify the role of each receptor subtype in depression behavior. Using α_1AR

selective agonists or antagonists in CAM-α_{1B}AR, α_{1A}AR-KO, and α_{1B}AR-KO mice would be a logical next step. Future research in this area should also explore α_{1A}AR activation and α_{1B}AR blocking in a mouse model of depression. Do these actions change only basal levels of depression, prevent depression, or relieve depression once it has begun?

The remaining question about anxiety behavior is also complex. There was no clear outcome for knockout of either subtype in other measures of anxiety. Possibly, the expression of both subtypes is necessary for changes in anxiety. Future work could utilize double-KOs of the α_{1A}AR and α_{1B}AR subtypes and combinations of single gene knockouts and α_1AR ligands.

In summary, we showed that α_{1B}AR-KO reduced depression-like behavior in the tail suspension test while α_{1A}AR-KO affected anxiety in Group 1 animals that did not have seizures. These results provide evidence for a negative role of the α_{1B}AR in depression and confirms a positive role for the α_{1A}AR, which could be taken advantage of when designing therapeutics.

CHAPTER 4
TREATMENT WITH CIRAZOLINE, AN ALPHA$_{1A}$AR AGONIST, DOES NOT INFLUENCE FINAL CELL FATE IN THE ADULT MOUSE DENTATE GYRUS DURING ADULT NEUROGENESIS

Abstract

Adrenergic receptors (ARs), α_1, α_2, and β bind to the endogenous ligand norepinephrine. Each family of AR has three subtypes and the present study examined the α_{1A}AR subtype's role in adult neurogenesis. Adult neurogenesis occurs in two areas of the adult brain: the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus. α_{1A}AR activation, via transgenic manipulation using a constitutively active mutant form of the receptor (CAM-α_{1A}AR mice), increases proliferation of neural progenitor cells in both the SVZ and SGZ. *In vitro* work using adult-derived neurospheres from the SVZ of wild type and CAM-α_{1A}AR animals suggested a role for these receptors in the differentiation stage of adult neurogenesis as well. In the present study, we treated C57BL/6 mice with the α_{1A}AR agonist cirazoline for 8 wks and injected the S phase marker BrdU after 4 wks of treatment. We then performed double-labeling immunohistochemistry to examine the fate of the newly created cells at the 4 wk timepoint. We found that cirazoline treatment did not change the number or percent of cells that became NeuN+ mature neurons or GFAP+ mature astrocytes. There was a large effect size, but no statistical significance, suggesting a decrease in the number of DCX$^+$ immature

neurons in the cirazoline treated group. The results suggest that the role of the α_{1A}ARs is complex and may be related only to the early stages of proliferation and differentiation.

Introduction

The catecholamine neurotransmitter norepinephrine signals through the α_1-, α_2-, and β adrenergic receptors (ARs). Each type of AR has three subtypes and the present study examined the role of the α_{1A}AR subtype in adult neurogenesis. Adult neurogenesis is the process whereby new brain cells are generated in the adult brain (Figure 22). It occurs in two discrete areas, the subgranular zone (SGZ) of the hippocampal dentate gyrus and the subventricular zone (SVZ) of the lateral ventricles. The process includes several steps: proliferation, migration/ differentiation, and survival/ integration. It is mediated by growth factors, synaptic input, and neurotransmitters among others.

The role of norepinephrine, and the α_1AR subtypes, in the process of adult neurogenesis is not well understood. *Xenopus laevis* embryos and cell culture studies suggest a role for norepinephrine and the αARs in differentiation. For example, norepinephrine increases the number of embryonic cells that differentiate into neurons via α_1ARs (Rowe et al., 1993; Messenger et al., 1999). However, not all regulators of embryonic neurogenesis are involved in adult neurogenesis. In adult mice, we previously showed that α_{1A}AR activation, via transgenic or pharmacological methods, increases proliferation in the SGZ and the SVZ (Gupta et al., 2009). Our work with adult neurospheres cultured from mouse lateral ventricles shows that α_{1A}AR stimulation affects differentiation and survival of new brain cells.
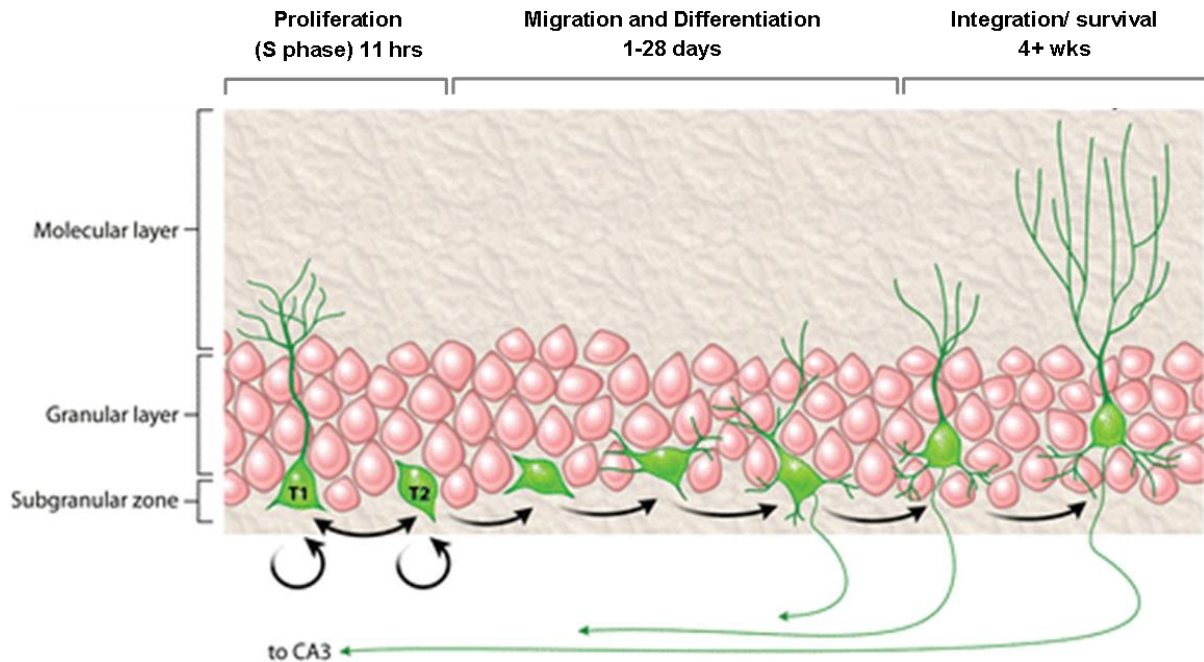
Figure 22. Adult neurogenic process. Proliferation: A) Neural stem cell (T1) dividing symmetrically or asymmetrically, B) Neural progenitor cell (T2) dividing symmetrically or asymmetrically, Migration and Differentiation: C) differentiating cells migrating into the granule cell layer, and Integration/ survival: D) integrating into the local circuitry. Adapted from Suh et al., 2009.

In neonatal neurospheres derived from normal mice or transgenic mice that express a constitutively active mutant form of the α_{1A}AR (CAM-α_{1A}AR mice), stimulation with phenylephrine, an α_1AR agonist, increases differentiation and migration and promotes survival of new cells (Gupta et al., 2009). In α_{1A}AR-knockout mouse-derived neurospheres, the cells revert to or maintain an undifferentiated state, as assessed by an increase in nestin RNA expression and lack of changes in differentiation-related transcription factors. α_{1A}AR signaling also promotes the survival of cultured neural progenitor cells by reducing stress-induced apoptosis (Ohashi et al., 2007). *In vivo*, only 60 percent of adult-generated cells in the SGZ survive more than two weeks (Kempermann and Gage, 2002). Those that

do survive are mostly of the neuronal phenotype and survive for at least eleven months (Kempermann et al., 2003).

Approximately 50-60 percent of the new cells in the SGZ are neurons (Reviewed in Abrous et al., 2005; Steiner et al., 2004). Approximately 10 percent are astrocytes and 25 percent of new cells are of an unknown phenotype. Astrocytes are important in the process of adult neurogenesis, releasing paracrine factors and balancing inflammatory mediators (Ashton et al., 2012; Lie et al., 2005). Astrocytes also promote the differentiation step of adult neurogenesis (Song et al., 2002; Oh et al., 2010). We previously showed that neurospheres derived from adult mouse SVZ and treated with phenylephrine increases glial markers (Gupta et al., 2009).

In the current study, we hypothesized that α_{1A}AR activation, via the selective agonist cirazoline, would promote survival of adult-born cells in the SGZ. Based on our adult-derived neurosphere evidence, we also hypothesized that a higher number of astrocytes would be generated when compared with wild type.

Methods

Animals

Animals were C57BL/6 wild type (WT) mice kindly donated by Dr. Colin Combs and Dr. Kendra Puig at the University of North Dakota. All animals were provided veterinary care at the University of North Dakota, an American Association for Laboratory Animal Science accredited institution. The Institutional Animal Care and Use Committee approved all protocols. Eight mice/ sex/ group were 10 wks old at the start of the study. Standard acidified water, provided by the Center for Biomedical Research at UND was provided in

250 ml glass bottles with intake monitored weekly. Harlan Teklad chow with 5 percent fat was provided *ad libitum* and intake monitored on a weekly basis.

Drug Treatment and BrdU Administration

Cirazoline hydrochloride was diluted to 40 µM in water provided by the Center for Biomedical Research animal facility (Tocris Bioscience, Minneapolis, MN). Control mice received the same water without drug. Treatment began when mice were 10 wks of age and continued until cardiac perfusion at 18 wks of age (Figure 23). After 4 wks of cirazoline treatment, BrdU was injected to label S phase dividing cells. BrdU was diluted to 10 mg/ml in saline then warmed and mixed by vortex for several min. BrdU was administered IP at 50 mg/kg twice per day, 12 hrs apart, for 5 d.

Tissue Preparation

Eight weeks after the start of cirazoline or control water administration, mice were anesthetized with a terminal dose of pentobarbital (150 mg/ml) and perfused. Using gravity perfusion equipment, 10 ml of ice-cold heparinized saline (0.02 mg/ml in 0.9 percent saline) was infused through the left ventricle using a 22G short bevel needle. This was followed by 30 ml of 4 percent paraformaldehyde (Fisher Scientific, Hanover Park, IL). Brains were removed and post-fixed for 2 d in 4 percent paraformaldehyde then transferred to 0.1 M phosphate buffered saline with 0.01 percent sodium azide (Fisher Scientific, Hanover Park, IL). Brains embedded in 3 percent agarose were sectioned at 40 µm on a vibrating microtome (MICROM, Thermoscientific, Watham, MA) in an ice cold PBS bath. Sections were collected and placed in PBS, ethylene glycol, and glycerol-containing cryoprotectant solution in 96 well plates. Approximately 60-70 sections per brain were kept with one
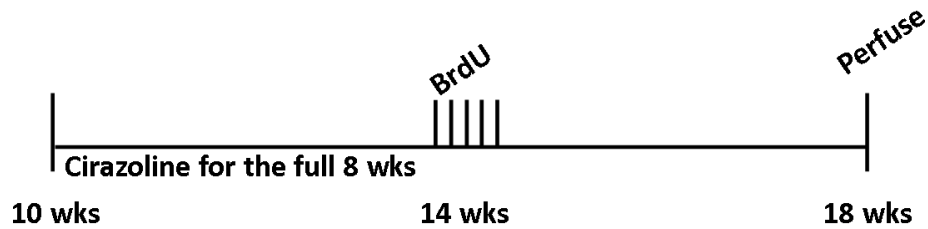
Figure 23. Treatment timeline. Mice were 10 wks old at the start of treatment. Cirazoline treatment lasted for 8 wks. BrdU was injected IP 2x/ d for 5 days after 4 wks of cirazoline treatment.

section per well and one brain per plate. Sections in well plates were stored at -20 C until used for immunohistochemistry.

Immunohistochemistry

Brains used for immunohistochemistry were pseudorandomly chosen from each group. For each labeling experiment, a 1 in 6 series of sections was used, with the starting section determined by the roll of a 6-sided die. Free-floating labeling took place in 12 well plates, each well containing the sections from one mouse. Negative controls, for specificity of the secondary antibody, included a well without primary antibody and one well containing primary antibody host IgG at the same concentration as the antibody (Jackson Immunoresearch, West Grove, PA). Positive controls for BrdU and DCX were from a 4 wk old mouse injected at a 2 hr BrdU timepoint. Positive controls for astrocytes (GFAP) were cells in the hilus and molecular layer of the dentate gyrus. All sections were double-labeled with Rat x BrdU and one of the following primary antibodies: Mouse x NeuN, Goat x DCX, or Mouse x GFAP (Table 2). AlexaFluor (AF) secondary antibodies included Goat x Rat AF488, Goat x Mouse AF568, and Donkey x Goat AF568. Either DAPI or TOPRO-3 was used as a counterstain.

73

Table 2

Antigen	Company, Catalog #	Host species	Clonality	Dilution/ concentration	Specificity	Negative control
BrdU, Clone [BU1/75 (ICR1)]	Abcam, ab6326, 662909	Rat	Monoclonal	1° 1:800 (1.25 µg/ml) 2° 1:500	Single stranded DNA with BrdU incorporated	No BrdU injection Rat IgG at 13.75 µg/ml
DCX (Clone 18)	Santa Cruz, sc-8066, L3113	Goat	Polyclonal	1° 1:100 (2 µg/ml) 2° 1:500	C-18 of human origin, Western blog detected one band at 40 kDa in 3T3-L1 whole cell lysate	Goat IgG at 117 µg/ml
NeuN, Clone A60	Millipore, MAB377, LV1634819	Mouse	Monoclonal	1° 1:100 (10 µg/ml) 2° 1:200	2-3 bands in the 46-48 kDa range	Mouse IgG at 55 µg/ml
GFAP, Clone GA5	Millipore, MAB360, LV1634946	Mouse	Monoclonal	1° 1:500 (2 µg/ml) 2° 1:500	Band from human glioma cell line (U33CG/343MG) at 51 kDa (Debus, 1983)	Mouse IgG at 11 µg/ml

Mature neurons: BrdU + NeuN. Sections were washed with 1X (0.1 M) phosphate buffered saline, incubated with 2N HCl for 30 min at 37 C, then rinsed with 0.1 M borate buffer. Sections labeled for Ms x NeuN were blocked using a Ms x Ms blocking buffer (Scytek, Cat. #MTM008, Logan, UT). NeuN sections were also blocked with 10 percent bovine serum albumin diluted in 5 percent normal serum from the secondary antibody host species in 1X PBS. Primary antibody incubation lasted 24 hr. Sections were washed then incubated with corresponding secondary antibodies for 2 hr. Sections were protected from light during secondary antibody incubation and thereafter. After the secondary antibody, the sections were washed, and incubated with 10 mM $CuSO_4$ solution in a 50 mM Ch_3COONH_4 buffer for 1 hr. After rinsing, sections were counterstained with DAPI at 1 µg/ml and mounted on SuperFrost Plus slides in a 0.1M phosphate buffer, coverslipped with a PVA/ DABCO mounting medium (cite Peterson's chapter here). Slides were left to dry overnight and then stored at 4°C until imaging.

Immature neurons and mature astrocytes: BrdU + DCX and BrdU + GFAP. Sections were washed in 10X Tris buffered saline, incubated in 10N HCl at 37C for 10 min, then rinsed in 0.1 M borate buffer. Sections were blocked with Ms x Ms blocking buffer, if needed, then blocked with 5 percent normal serum of the secondary antibody host species diluted in 10X TBS. Primary antibody incubation was 72 hr (GFAP and DCX) at 4°C. Sections were rinsed with blocking buffer containing 5 percent normal serum two times, 1 hr each. While protected from light, sections were incubated with secondary antibodies for 2 hr then rinsed and incubated with $CuSO_4$/ Ch_3COONH_4 solution, mounted, cover-slipped, and stored as above until counting.

Imaging and Cell Counting

BrdU + NeuN labeled samples were imaged using a 5X51WI Olympus DSU microscope at the Center for Stem Cell and Regenerative Medicine Confocal Stereology Research Laboratory at Rosalind Franklin University (Olympus Scientific Solutions, Waltham, MA). StereoInvestigator 11.0 and the Virtual Tissue feature were used to set up the contours and imaging continued overnight (MBF Bioscience, Williston, VT). Image files were provided to the University of North Dakota via Dropbox (Dropbox, Inc., San Francisco, CA). One hemisphere was counted. Cells labeled with BrdU and double-labeled with NeuN were counted exhaustively in the 1 in 6 series of sections using StereoInvestigator 10.0, excluding any cells where the top of the cell was out of focus. The total number of $BrdU^+$ and $BrdU^+NeuN^+$ cells were determined by multiplying the number of counted cells by 2 (for the other hemisphere) and then by 6 for the 1 in 6 series. The percentage of double-labeled cells was determined using the total counts of $BrdU^+$ and $BrdU^+NeuN^+$ cells. All other images were taken on an Olympus Fluoview 1000 at the University of North Dakota.

BrdU + DCX and BrdU + GFAP cell counting. Double-labeling experiments for BrdU and either DCX or GFAP were counted on an Olympus Fluoview 300 microscope using epifluorescence (Olympus Scientific Solutions, Waltham, MA). BrdU and double-labeled cells were counted exhaustively in the 1 in 6 series of sections. DCX only cells were counted in a 1 in 12 series of the same samples. Missing sections were accounted for by adding the average number of cells in a brain to the final counts.

Statistics

Data were analyzed using GraphPad Prism 5.04. Kolmogorov-Smirnov test was used to assess normality and the Bartlett's test for equal variances. Unpaired t-tests were used for normally distributed data when the means were equal. If the assumption of normality was not met, a non-parametric Mann-Whitney test was used. Data with unequal variances was assessed using a t-test with Welch's correction. Effect sizes were analyzed using Cohen's *d* test.

Results

Body Weight and Water Intake

Cirazoline decreases food intake, without a change in water intake, when given acutely (Wellman and Davies, 1992; Davies and Wellman, 1992). Caloric restriction can affect the proliferation stage of neurogenesis. Therefore, we analyzed body weight and water intake to ensure cirazoline did not induce caloric restriction. There were no statistical differences in body weight between control and cirazoline-treated mice [$F_{(1, 287)} = 0.10$, p = 0.74]. There was a difference in weight over time but that was expected based on the age of the mice [$F_{(7, 287)} = 105.6$, $p < 0.0001$]. That there was no difference in body weight between groups suggests there was no difference in food intake. In a separate study, there was no difference in food intake between control and cirazoline-treated mice over 20 wks of treatment (Collette and Doze, unpublished).

A two-way ANOVA showed a difference in water intake between groups [$F_{(1, 273)} = 12.84$, $p < 0.0009$] and over time [$F_{(7, 273)} = 13.97$, $p < 0.0001$]. Post-hoc testing revealed that cirazoline-treated mice drank less water on Weeks 2-8 (Figure 24). The estimated daily

dose for cirazoline, based on water intake, was 0.4 mg/day. There was a main effect of treatment on the amount of water drank when analyzed for sex differences [$F(1, 37) = 1.44$, $p = 0.03$]. There was not a main effect of sex [$F(1, 37) = 1.44$, $p = 0.23$] and there was an interaction between treatment and sex [$F(1, 37) = 19.03$, $p < 0.0001$]. The interaction makes the results difficult to interpret. However, a Bonferroni post-hoc test showed that cirazoline-treated female mice drank less water than female control mice ($p < 0.0001$).

Survival

The total number of BrdU$^+$ cells surviving after four weeks was not significantly different between groups [$U(70, 100) = 22.50$, $p = 0.22$, Figure 25A]. This suggests that α_{1A}AR activation does not affect cell survival.

Differentiation

Immature neurons. The total number of DCX$^+$ cells was not significantly different between control and cirazoline-treated groups [$t(5) = 2.11$, $p = 0.08$, Figure 25B]. However, the effect size was very large (Cohen's $d = 1.22$). The variability in the control group was much higher (95% confidence intervals: control 14,065 - 46,701 vs cirazoline: 10,731 - 21,887). The high variability could likely be resolved with a higher number of animals, clarifying the results.

BrdU$^+$DCX$^+$ cells, adult-created cells in the immature neuron stage four weeks post-BrdU, was not statistically different [$t(5) = 1.94$, $p = 0.10$, Figure 25C]. Again, the effect size was large (Cohen's $d = 1.12$) and confidence interval was larger in the control group (301 - 730) than the treated group (304 - 396). The percentage of double-labeled cells was also not different [$U(44, 34) = 13.00$, $p = 0.46$, Figure 25D].
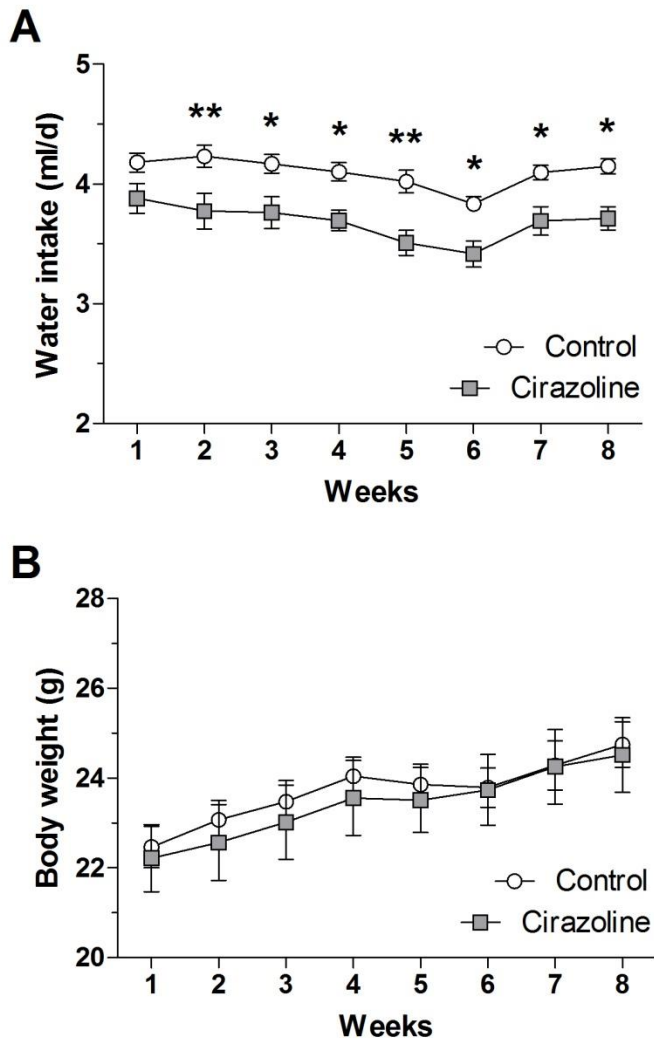
Figure 24. Water intake and body weight. A) Cirazoline-treated mice drank significantly less water than control mice after Week 1 but B) body weight did not differ between the groups (p = 0.74). *p < 0.05, **p < 0.01

Mature neurons. There was no difference between control and cirazoline-treated mice in the total number [$U(43.5, 34.5) = 13.50$, p = 0.52, Figure 26A] or percent [$U(47.5, 30.5) = 9.50$, p = 0.19, Figure 26B] of BrdU$^+$NeuN$^+$ mature neurons after 4 wk. The percentage of new cells differentiating into neurons was similar to previous research (Steiner et al., 2004; Kempermann and Gage, 2002). These results suggest that cirazoline

Figure 25. Immature neurons. A) There was no difference in the total number of BrdU$^+$ cells (p = 0.22). B) There was also no statistically significant difference between groups in the total number of DCX$^+$ cells (p = 0.08). However, C) there was a large effect size, but no significant difference in the number of double-labeled BrdU$^+$DCX$^+$ cells in the cirazoline group (p = 0.04) and D) the percent of BrdU$^+$DCX$^+$ cells was not different (0.46). Representative images are shown for E) control and F) cirazoline-treated animals. Arrowheads show double-labeled cells, arrows show single-labeled BrdU cells.
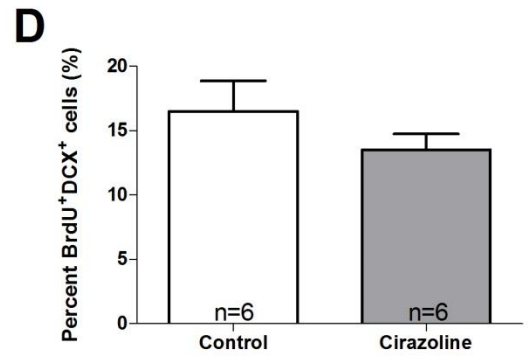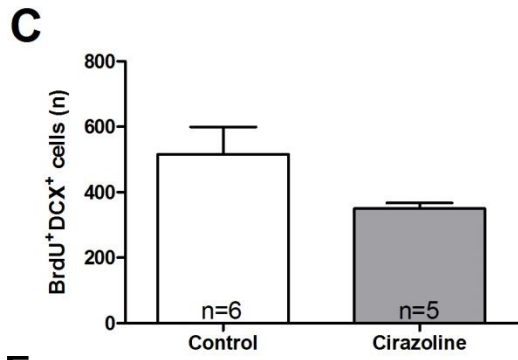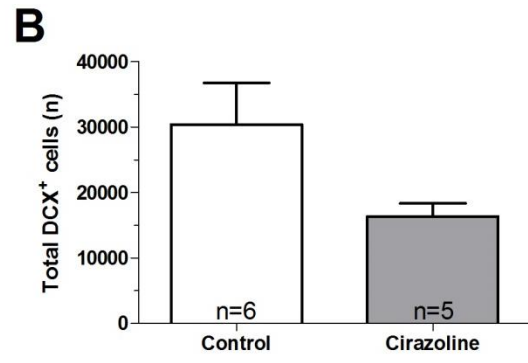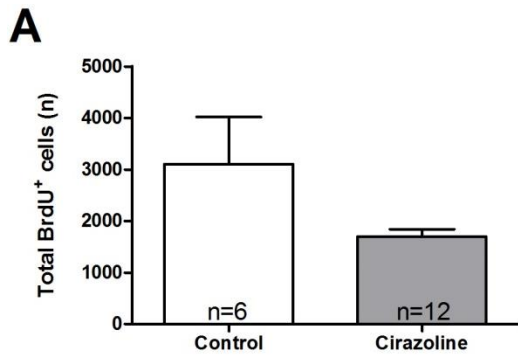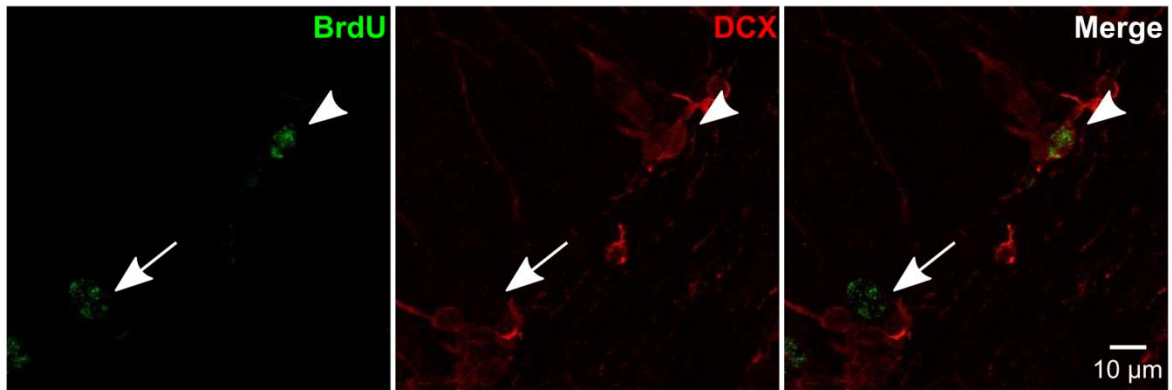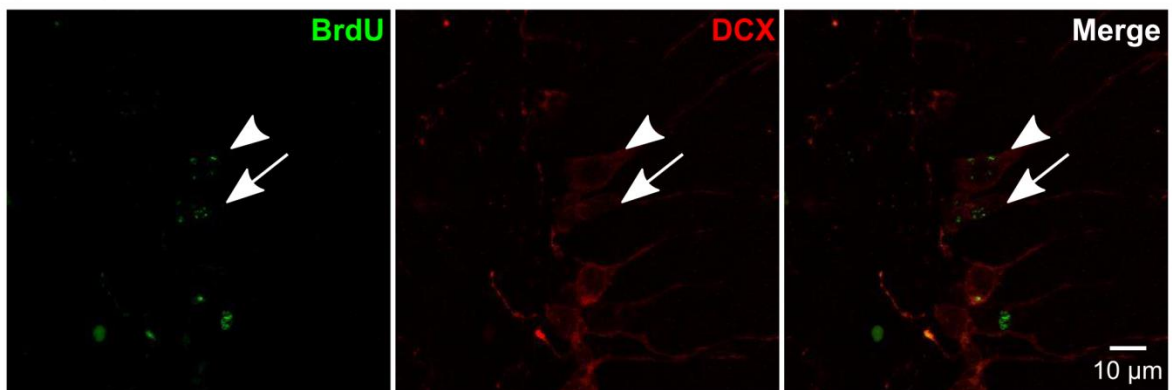
Figure 26. Mature neurons. There was no difference between control and cirazoline-treated mice in the A) total number (p = 0.52) or B) percent (p = 0.19) of BrdU$^+$NeuN$^+$ cells after 4 wk. Representative images are shown for C) control and D) cirazoline groups. Arrowhead shows double-labeled cells, arrow shows single-labeled BrdU cells.

A

B

C Control

BrdU   NeuN   Merge

10 µm

D Cirazoline

BrdU   NeuN   Merge

10 µm
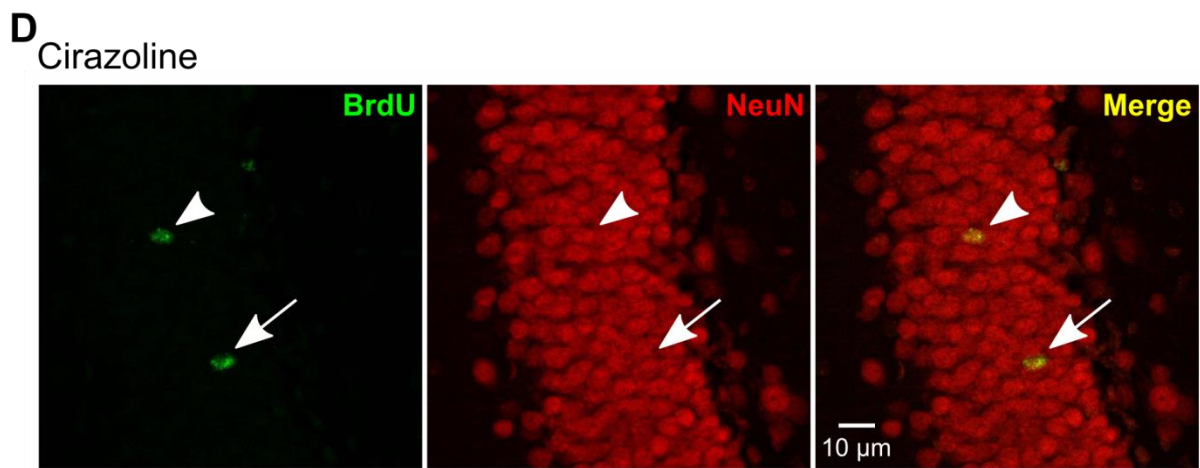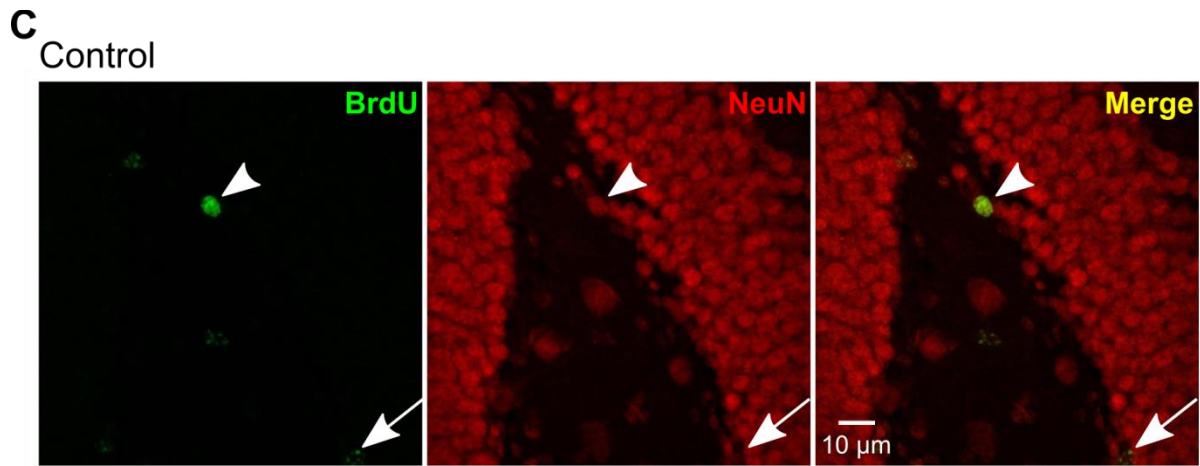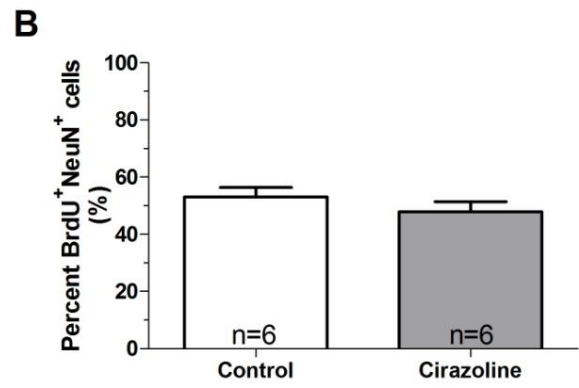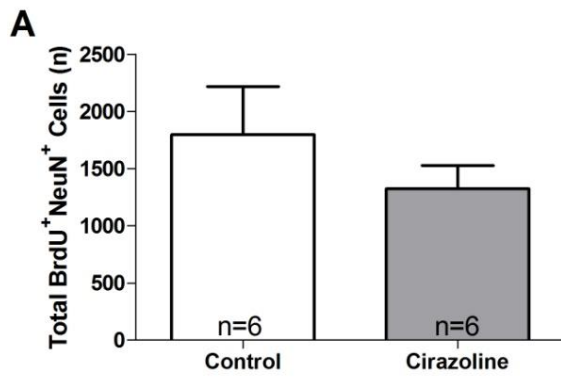
treatment does not change the number or percent of new cells that differentiate into mature neurons.

Astrocytes

Chromogen. There was no statistical difference between control and cirazoline-treated groups in the total number of BrdU$^+$GFAP$^+$ cells 4 wks post-BrdU [U(54, 99) = 33.00, p = 0.95, Figure 27A]. There was also no difference in the percent of double-labeled cells [U(56, 80) = 25.00, p = 0.63, Figure 27B].

Fluorescence. There was no difference in the number of BrdU$^+$GFAP$^+$ cells when counted in fluorescence [t(10) = 7.13, p = 0.49, Figure 27C]. There was also no difference in the percentage of new astrocytes [U(38, 40) = 17.00, p = 0.93, Figure 27D]. The average percent of new GFAP$^+$ SGZ cells is commonly 10-12 percent (Steiner et al., 2004). In both control and cirazoline-treated mice in chromogen and fluorescence, we found only 4-5 percent of new cells 4 wks after BrdU were GFAP$^+$ astrocytes. These results suggest that chronic α_{1A}AR activation via cirazoline does not change the number or percent of new cells that differentiate into mature astrocytes.

Discussion

Our results show there is no change in survival or differentiation of new cells in the adult mouse dentate gyrus after treatment with the α_{1A}AR agonist cirazoline. Previously, *in vitro* culture with adult-derived neurospheres from the SVZ induced cells to differentiate into glial cells (Gupta et al., 2009). SVZ-derived and SGZ-derived neurospheres, theoretically, should both be the same type of neural stem cell therefore culturing and reacting to stimuli

Figure 27. Mature astrocytes. With chromogen labeling and counting in brightfield with stereology, there was no statistically significant difference between control and cirazoline-treated groups in the A) total number of BrdU$^+$GFAP$^+$ cells (p = 0.95) or the B) percent of cells double-labeled 4 wks after BrdU injection (p = 0.63). Similarly, there was no difference between groups in the C) number (p = 0.49) or D) percent (p = 0.93) of matured astrocytes when counted in epifluorescence without stereology. Representative images are shown for E) control and F) cirazoline mice. Arrowhead shows double-labeled cells, arrow shows single-labeled BrdU cells.

A

B

C

D
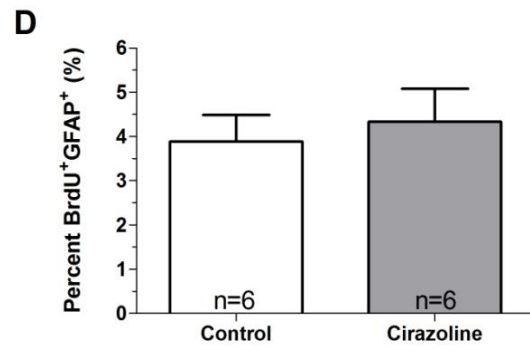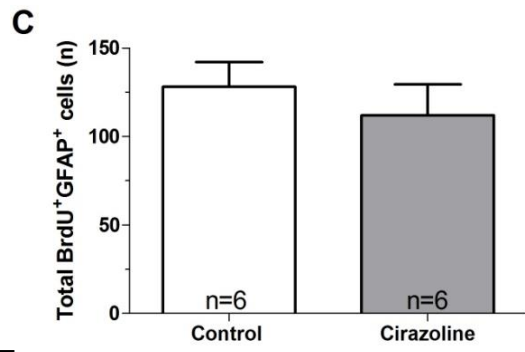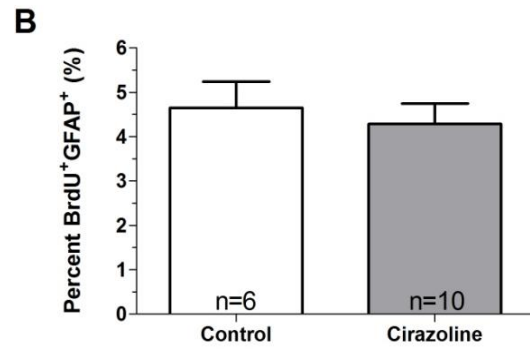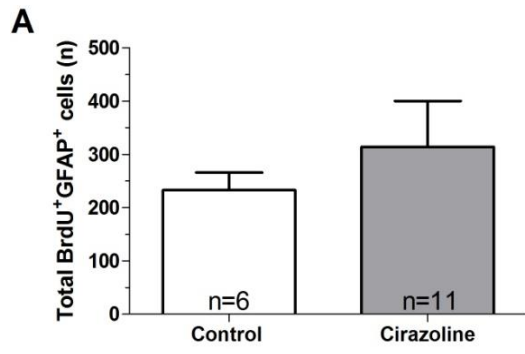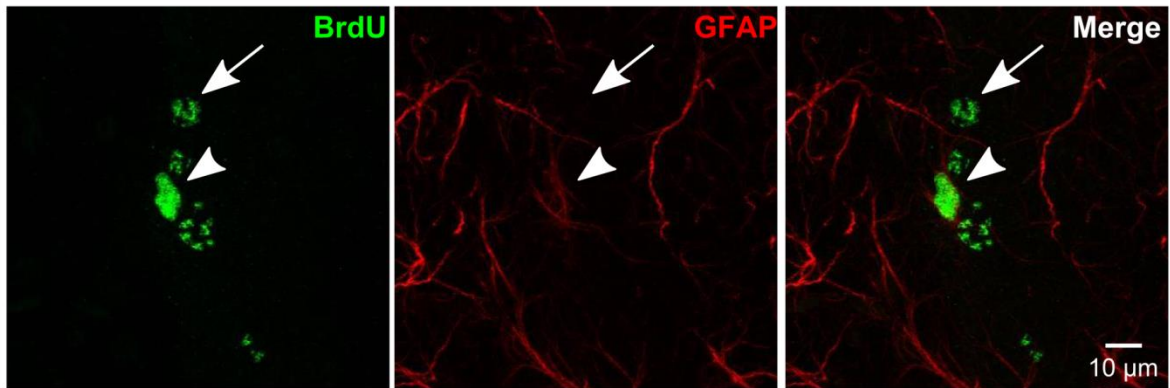
E Control

F Cirazoline
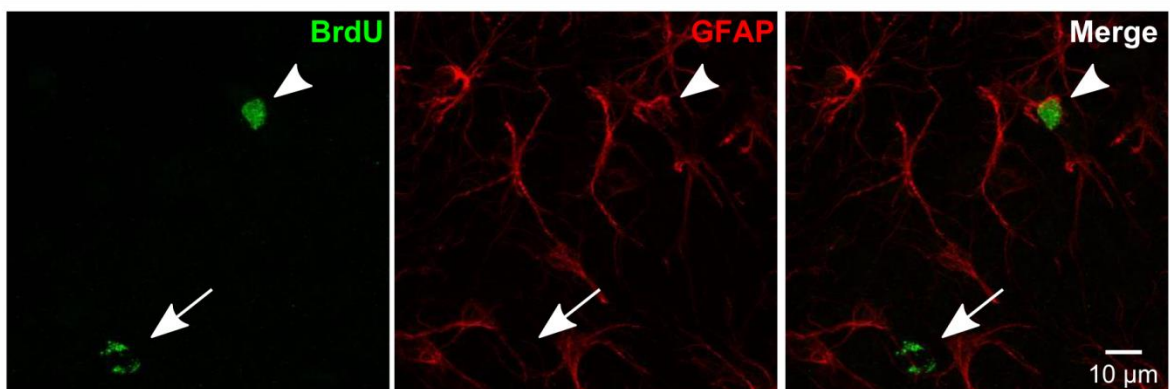
similarly. However, the SGZ-derived neurospheres may have responded differently. Some labs have claimed to culture SGZ-derived cells without contamination from the nearby SVZ but it is currently controversial (Walker and Kempermann, 2014; Devesa et al., 2014; Jhaveri et al., 2014). One study treated SGZ-derived neurospheres with cirazoline or prazosin and did not show a difference in the proportion of cells differentiating into neurons (βIII-tubulin) or astrocytes (GFAP) (Jhaveri et al., 2014). This report did not assess differentiation *in vivo*.

The lack of a change in the number or percent of BrdU$^+$ cells after 4 wks suggests that α_{1A}AR treatment does not increase, and may decrease, cell survival. Sixty percent of newly generated cells in the adult mouse do not survive past two wks (Dayer et al., 2003). An assay of activated caspase 3 (AC3) or TUNEL for apoptotic cell death would be another way to assess cell survival. It is possible that α_{1A}AR activation could be inducing division of neural stem cells rather than neural progenitor cells. In that case, it is possible the stem cell pool was exhausted leading to an apparent decrease in survival (Ables et al., 2010). To address this, Nestin GFP mice could be used to count Type 1 (NSC) and Type 2 (NPC) cells by morphology and determine whether Type 1 cells are decreased after chronic α_{1A}AR activation.

In addition, we previously showed that cirazoline treatment increases the density of BrdU$^+$ cells in the adult mouse dentate gyrus, which could have confounded our results in the current study (Gupta et al., 2009). Because cirazoline treatment began 4 wks prior to BrdU injection, a higher number of proliferating cells in the cirazoline-treated group were likely labeled. The percentage of cells labeled with both BrdU and DCX partially addresses this and boosts the finding that α_{1A}AR activation did not affect cell survival. To confirm,

future studies should include a paradigm in which control and cirazoline-treated groups are injected with BrdU at a 2 hr timepoint and a separate cohort of mice injected and allowed to survive for 4 wks. Comparison of the number of BrdU$^+$ cells at 2 hr and 4 wks would better address the question of whether α_{1A}AR activation influences cell survival.

DCX is often used as a proxy for BrdU as a measure of proliferation because it labels only immature neurons. Surprisingly, there was no difference in the number of DCX$^+$ cells in the cirazoline-treated group. There was, in fact, a trend toward fewer DCX$^+$ cells after α_{1A}AR treatment and the effect size of these results was very large. Additional control animals to reduce the variability in that group would clarify these findings.

Our previous work showed a higher density of BrdU$^+$ cells in the SGZ. For a better comparison, density of DCX$^+$ cells in the current samples could be analyzed but would require confocal-quality fluorescent imaging of the samples to accurately determine the dentate gyrus volume. Another possibility is that chronic cirazoline treatment accelerated the maturation of new cells which resulted in lower numbers of DCX$^+$ cells after 4 wks (David et al., 2009; Wang et al., 2008). If that was the case, we would expect a higher number of either mature neurons or astrocytes, which was not seen. It is also possible that differentiation of the immature neurons was stunted, keeping them in an immature state. Approximately 25 percent of adult-born cells are of an unknown phenotype so there is a remote possibility that cirazoline increased cell fate toward an unknown phenotype. As yet, it is not possible to answer this question.

Our hypothesis was that cirazoline would push differentiation toward astrocytes rather than neurons. There was no difference in the number or percent of cells that were

BrdU$^+$GFAP$^+$ vs BrdU$^+$NeuN$^+$. The percent of new neurons was similar to published research but the percent of new GFAP+ astrocytes was half of what previous studies have found. This could be due to the difficulty of quantifying double-labeling of BrdU and GFAP in brightfield and epifluorescence. Imaging with confocal-like quality would alleviate this issue. To confirm the finding of astrocytes, double-labeling for BrdU and S100β would be informative. Most GFAP$^+$ cells 4 wks after BrdU administration are also S100β$^+$ (Steiner et al., 2004). In addition, S100β does not label neural stem cells, which may provide a more accurate assessment of differentiated cells.

The results of the current study are interesting and important despite the lack of statistical significance. The large effect size of the DCX results coupled with the trend toward fewer BrdU$^+$DCX$^+$ cells implies a potential effect of chronic α_{1A}AR activation on the rate of differentiation. Coupled with our previous work showing an increase in the density of BrdU+ cells in the SGZ of CAM-α_{1A}AR mice, this could mean that the proliferating cells are kept in the immature neuron state rather than continuing through differentiation. In recent years, the field of adult neurogenesis and learning has shown an importance for adult-born cells not surviving past the first few weeks (Denny et al., 2012; Drew et al., 2013). These new cells have increased plasticity and are important for retaining memories, particularly during pattern separation. If chronic α_{1A}AR activation is indeed keeping cells in this immature state, it could potentially explain the enhancements in learning and memory we have previously reported (Doze et al., 2011).

Overall, our results suggest that α_{1A}AR activation does not affect survival or the final cell fate in the adult mouse dentate gyrus during adult neurogenesis. In the future,

additional animals and the proposed experiments could clarify the effect of cirazoline on cell

survival and differentiation.

CHAPTER 5

LONG-TERM ALPHA$_{1B}$-ADRENERGIC RECEPTOR ACTIVATION SHORTENS LIFESPAN WHILE ALPHA$_{1A}$-ADRENERGIC RECEPTOR STIMULATION PROLONGS LIFESPAN IN ASSOCIATION WITH DECREASED CANCER INCIDENCE

Abstract

The α_1-adrenergic receptor (α_1AR) subtypes, α_{1A}AR and α_{1B}AR, have differential effects in the heart and central nervous system. Long-term stimulation of the α_{1A}AR subtype prolongs lifespan and provides cardio- and neuro-protective effects. We examined the lifespan of CAM-α_{1B}AR mice and the incidence of cancer in mice expressing the constitutively active mutant (CAM) form of either the α_{1A}AR (CAM-α_{1A}AR mice) or α_{1B}AR. CAM-α_{1B}AR mice have a significantly shortened lifespan when compared with wild type (WT) animals; however, the effect was sex dependent. Female CAM-α_{1B}AR mice lived significantly shorter lives while the median lifespan of male CAM-α_{1B}AR mice was not different when compared with WT animals. There was no difference in the incidence of cancer in either sex of CAM-α_{1B}AR mice. The incidence of cancer was significantly decreased in CAM-α_{1A}AR mice when compared with WT and no sex dependent effects were observed. Further study is warranted on cancer incidence after activation of each α_1AR subtype and the effect of sex on lifespan following activation of the α_{1B}AR. The implications of a decrease in cancer incidence following long-term α_{1A}AR stimulation could lead to improved treatments for cancer.

Introduction

Epinephrine and norepinephrine are catecholamines that act as chemical messengers. They are synthesized in the adrenal medulla and in adrenergic neurons in the brain, respectively, as well as in post-ganglionic neurons in the sympathetic nervous system. In the periphery, both epinephrine and norepinephrine help regulate heart rate and blood vessel constriction and modulate the force of heart contraction and physiological arousal. When stressed, the body releases both chemicals to mediate the fight-or-flight response. All of these effects are a result of epinephrine or norepinephrine binding to adrenergic receptors (ARs), of which there are three families: α_1, α_2, and β.

Our lab has found differential effects of activating either the α_{1A} or α_{1B}AR subtype. Long-term stimulation of the α_{1A}AR, either pharmacologically or through transgenic manipulation, increases adult neurogenesis, reduces depression and anxiety-like behavior, and enhances learning and memory in the mouse (Gupta et al., 2009; Doze et al., 2011, 2009). Mice with a constitutively active mutant form of the α_{1A}AR (CAM-α_{1A}AR) also live significantly longer lives when compared with wild type (WT) animals of the same background (Doze et al., 2011). In contrast, chronic activation of the α_{1B}AR increases depression-like behavior (Doze et al. 2009). Chronic α_{1B}AR activity also leads to age-related apoptotic neurodegeneration, a synucleinopathy with Parkinson-like movement deficits similar to human multiple system atrophy (Papay et al., 2002; Zuscik et al., 2000). The neurodegeneration begins in areas of the brain with a high density of α_{1B}ARs and leads to a substantial loss of dopaminergic neurons in the substantia nigra. The α_1AR-selective antagonist terazosin protects against α-synuclein aggregates, neurodegeneration, and

92

partially rescues the movement deficits. Terazosin treatment also ameliorated the α_{1B}AR

activation's detrimental effect on early mortality; however, animals were only followed up

to 70 wks of age. Taken together, these results suggest the α_1AR subtypes mediate

neurogenesis and neurodegeneration which is in line with their role in proliferation in the

peripheral nervous system.

The α_1ARs can mediate proliferation and cell growth in the periphery (Michelotti et

al., 2000; Hoffman and Hu, 2000). α_1AR activation stimulates DNA synthesis in human

vascular smooth muscle cells through a PI3 kinase and MAPK pathway (Hu et al., 1996;

Hoffman and Hu, 2000). It also induces the expression of the proto-oncogenes *c-fos* and *c-

jun* in arterial smooth muscle (Okazaki et al., 1994). In a Rat-1 fibroblast microarray, the

α_1AR subtypes preferentially induced transcription of genes that regulate the cell cycle

(Gonzalez-Cabrera et al., 2004). The microarray results suggested α_{1A} and α_{1D}ARs halt the

cell cycle at the G1-S checkpoint. α_{1B}AR expression led to cell cycle progression through the

G1-S checkpoint by inducing transcription of cdk-6- and cyclin E-associated kinases. This cell

cycle progression is evident in Rat-1 and NIH-3T3 fibroblast cells transfected with α_{1B}ARs

which enhances focus formation after agonist stimulation. The cells are also tumorigenic

when injected into nude mice, implicating the gene as a potential proto-oncogene (Allen et

al., 1991; Gonzalez-Cabrera et al., 2004). That is the only study to date that has examined *in

vivo* tumor formation mediated by the α_1AR subtypes. The role of the α_{1A}AR subtype in

cancer incidence has not been examined prior to the current study.

Our hypothesis was that long-term transgenic activation of the α_{1A} and α_{1B}ARs

would lead to a decrease and increase in the incidence of cancer, respectively. It was

expected that chronic activation of the α_{1B}AR would lead to a shorter lifespan due to cancer and neurodegeneration. In this study, we followed transgenic mice overexpressing the α_{1A} or α_{1B}AR throughout their lifespan. We also completed necropsy on subsets of each group to assess cancer incidence. Surprisingly, we found that chronic α_{1B}AR activation had no significant effect on cancer but did reduce longevity, presumably secondary to increased neurodegeneration. Importantly, we observed that long-term α_{1A}AR stimulation was associated with a significantly lower incidence of cancer and longer lifespan.

Methods

Animals

This study used transgenic mice overexpressing a constitutively active mutant (CAM) form of either the α_{1A} or α_{1B}AR that were created on a B6CBA background. The CAM receptor genes were expressed under the endogenous promoters and the animals have been previously characterized (Zuscik et al., 2000; Rorabaugh et al., 2005). The longevity part of the study included 235 mice and the pathology study included 157 mice with some overlap. Animals were bred and provided with identifying ear tags at the Cleveland Clinic Foundation and transferred to the University of North Dakota. Both facilities are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). 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.

Mice were housed in translucent, polycarbonate boxes, 17 × 28 × 13 cm, 1-5 mice per box with one rodent clubhouse. Animals were maintained on a 12 h light-dark cycle

with lights on at 0700. Harlan diet 8640 and water were provided *ad libitum* (Harlan,

Indianapolis, IN). The temperature was held constant at 22 °C and the humidity at 23-27%.

Longevity

Animals were observed daily by facility personnel but handled only for cage changes

and clinical assessments. All staff members who observed and handled the mice were

trained to detect and record signs of illness and notified the veterinarian and research team

immediately regarding sick animals. The veterinarian and researchers examined the mice

for severity of illness or impairment and animals were euthanized if they were not likely to

survive for another 48 h. The likelihood of survival was based on the occurrence of at least

two of the following clinical signs set forth by The Jackson Laboratory (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 that had ulcerated or were bleeding (Ray et al., 2010). The date of death for each

mouse was logged and the number of days lived was calculated and used for analysis. The

mean age at death for the euthanized mice was not significantly different from the mice

which died spontaneously, therefore euthanized mice were included in the analysis.

Pathology

Mice were removed from their cages as soon as possible after death and frozen at -

20 °C until pathological analysis. Animals sacrificed at a younger age were included in the

analysis to increase statistical power (See Appendix A for further information). At necropsy,

the mice were visually inspected and the exterior palpated to assess skin condition and any

outward signs of abscess, disease, or tumors. The abdomen and thoracic cavities were

opened and organs inspected and removed. Tumors were digitally photographed *in situ* and

after removal with a measurement scale clearly visible. Samples of the heart, lungs, spleen,

kidney, liver, and intestine were immersion-fixed in neutral buffered 10% formalin for at

least 24 h. Samples were dehydrated in graded ethanol, cleared in xylene, infiltrated, and

paraffin embedded. Serial sections were cut at 3-5 μm, stained with hematoxylin and eosin,

and evaluated for cancerous cells on a Carl Zeiss Axioskop 50 microscope (Zeiss, Germany).

Classification of tumors was based on the current World Health Organization Classification

of Tumors with modifications, as needed, for mouse tissue. The primary diagnosis for each

animal was used for analysis; tumor burden was not assessed.

Statistical Analysis

Survival was analyzed using Kaplan Meier survival curves and the log-rank (Mantel-

Cox) test with GraphPad Prism 5.04 (San Diego, CA). The median lifespan was the point at

which the fractional survival of each curve equaled 50 percent. Cancer incidence was

analyzed using a χ^2 test on raw data but is presented as percentages for clarity. Mice still

alive at the end of the study were interval-censored. An unpaired t-test was used for

comparison of maximal lifespan. Significance levels were set at $p < 0.05$.

<div align="center">

Results

</div>

Chronic Activation of the α_{1B}AR Leads to Decreased Body Weight Starting During Middle Age While Long-Term α_{1A}AR Stimulation Does Not Affect Adult Weight

We weighed cohorts of mice at various ages to rule out increased lifespan due to

caloric restriction because acute activation of the α_{1A}AR can suppress appetite (Davies and

Wellman, 1992; Morien et al., 1993). Just after weaning, CAM-α_{1A}AR ($p < 0.05$) and CAM-

α_{1B}AR mice ($p < 0.001$) weighed significantly less than WT mice (Figure 28). Between the 2nd
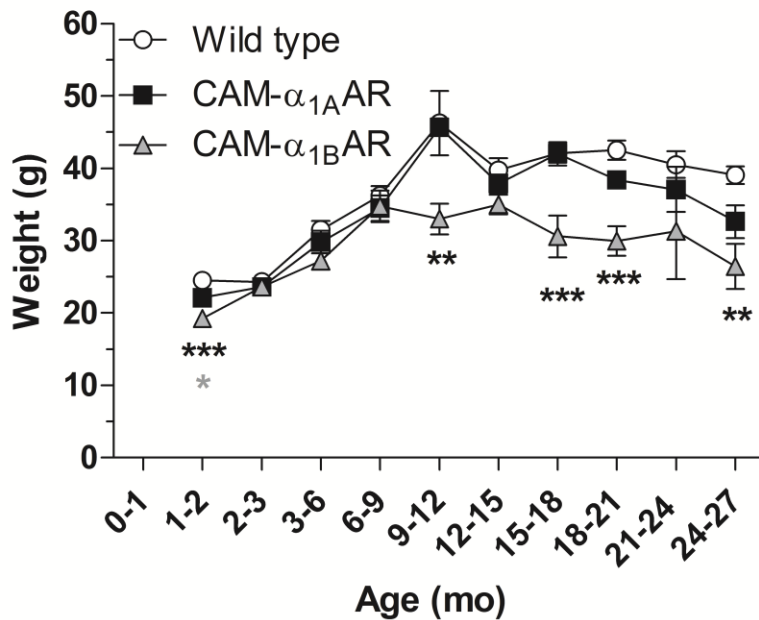
Figure 28. Weight across the lifespan. CAM-α_{1B}AR mice weighed significantly less than WT mice at most time points after 9 mo of age * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

mo and the end of the 8th mo of age there were no significant differences in weight

between groups. However, CAM-α_{1B}AR animals weighed significantly less when compared

with WT mice starting at approximately 9 mo of age. The 21-24 mo age range had a low

number of CAM-α_{1B}AR animals and no significant differences in weight were observed.

CAM-α_{1A}AR Mice Have an Increased Lifespan While CAM-α_{1B}AR Mice Have Shorter Lives

We previously reported that CAM-α_{1A}AR mice have a significant increase in median

and maximal lifespan when compared with WT animals (Doze et al., 2011). The CAM-α_{1A}AR

longevity data included here is the previously published data and is included only for

comparison. There was no significant difference between lifespans of previously published

WT animals and WT mice that have died since; therefore, both groups are included here to

provide statistical power (Figure 29, Table 3, Appendix A). Comparison of WT mice excluding

previously published data and CAM-α_{1B}AR mice can be found in Appendix A.

Within the WT group, there was no significant difference between female (711 d)

and male mice (721 d, χ^2 = 2.300, df = 1, p = 0.129). The median lifespan of CAM-α_{1B}AR mice

(637 d) was significantly shorter than WT animals (719 d, χ^2 = 7.194, df = 1, p = 0.007), a

decrease of approximately 11% (Figure 29, Table 3). There was no significant difference in

lifespan between the male CAM-α_{1B}AR mice (652 d) and male WT animals (721 d, χ^2 =0.251,

df = 1, p = 0.616). Female CAM-α_{1B}AR mice (619 d) lived significantly shorter lives than

female WT animals (711 d, χ^2 = 9.415, df = 1, p = 0.002).

Maximal lifespan is an index of slowed aging and is calculated by comparing the ages

of the 10% longest living mice in each group. The maximal lifespan of CAM-α_{1B}AR mice was

significantly shorter than WT mice (p = 0.025). Female CAM-α_{1B}AR mice had a significantly

shorter maximal lifespan than female WT animals (p = 0.009). However, the maximal

lifespan of male CAM-α_{1B}AR mice was significantly increased when compared with male WT

mice (p = 0.004).

The sigmoidal shape of all three survival curves suggests deaths were not due to a

terminal infectious agent or a disease causing rapid death (Van Zwieten et al., 1981). The

CAM-α_{1B}AR curve begins to deviate from the WT curve at approximately 9-10 mo (270-300

d) of age suggesting normal development to that point. The longest living CAM-α_{1B}AR

animals lived to similar ages as WT animals, likely due to incomplete penetrance of the
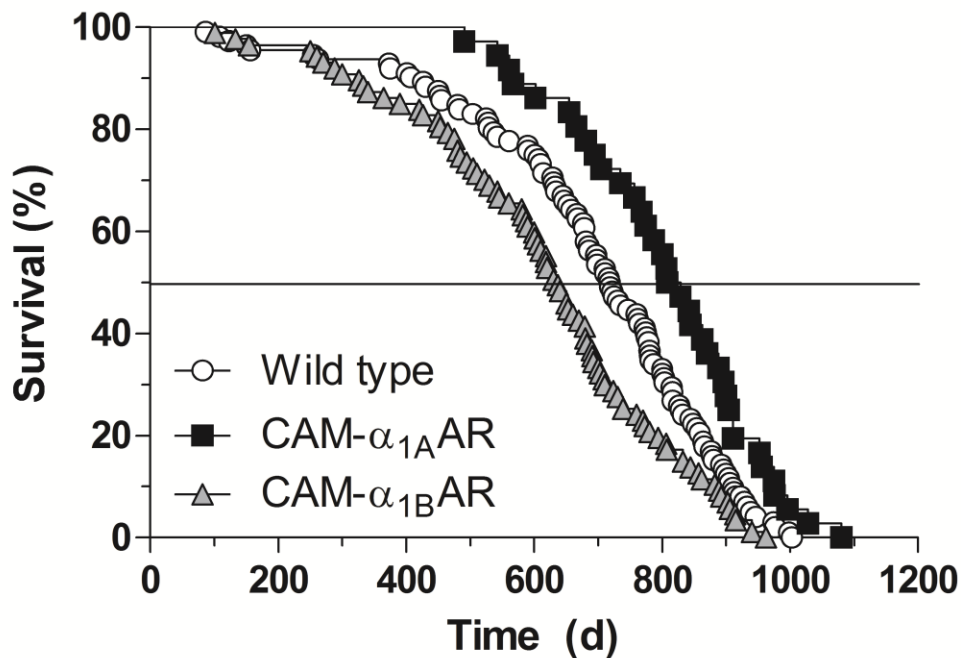
Figure 29. Kaplan-Meier survival plots. CAM-α_{1B}AR mice (n = 87) have a shorter lifespan than WT mice (n = 112, p = 0.007). As previously published, CAM-α_{1A}AR animals (n = 36) live significantly longer than WT mice (p = 0.004) (Doze et al., 2011).

transgene in those animals. Similarly, the CAM-α_{1A}AR curve shifts closer to WT at the end of life stage.

Chronic α_{1B}AR Stimulation Has No Significant Effect on the Incidence of Cancer While Activating α_{1A}ARs Decreases Cancer Incidence

The overall cancer incidence between CAM-α_{1B}AR (n = 34) and WT mice (n = 70) did not differ (χ^2 = 0.1242, *df* 1, p = 0.7245, Figure 30). In contrast, CAM-α_{1A}AR mice (n = 53) had a significantly lower overall incidence of cancer than WT animals (n = 70) (χ^2 = 17.83, *df* 1, p < 0.0001). There was no significant difference in cancer incidence between male and female animals for any of the groups.

The most common diagnoses among WT mice were epithelial and hematological cancers (Table 4). CAM-α_{1A}AR and CAM-α_{1B}AR animals had a similar incidence for the type

99

Table 3. Lifespan data of CAM-α_{1B}AR, WT, and CAM-α_{1A}AR mice

Genotype	Median (d)	Mean (d)	S.D. (d)	S.E.M. (d)	95% CI (d)	90th Percentile (d)	Deaths (n)
CAM-α_{1B}AR	637	617	200	21	575 - 660	890	87
Male	652	635	204	29	576 - 694	907	48
Female	619	595	196	31	532 - 659	882	39
Wild type	719	689	203	19	651 - 727	912	112
Male	721	674	195	28	618 - 729	875	50
Female	711	701	210	27	648 - 754	932	62
CAM-α_{1A}AR	819	806	146	24	757 - 856	982	36
Male	822	821	150	35	746 - 895	999	18
Female	819	792	145	34	720 - 864	928	18

of cancers as WT mice. Epithelial cancers included adenocarcinoma of the lung, renal cell carcinoma, hepatocellular carcinoma, and neuroendocrine carcinoma. Hematological cancers included lymphoma and leukemia involving various organs. The only mesenchymal cancer was spindle cell sarcoma. Non-cancerous epithelial lesions included hepatocellular and small intestine adenomas and one case of hepatocellular hyperplasia. The only non-cancerous hematological finding was lymphoid hyperplasia. Mesenchymal tumors included hemangioma and a non-cancerous fibroma. Non-tumor lesions included pathologies such as pulmonary edema, chronic inflammation, glomerular disease, cardiomyopathy, and heart thrombi. Representative images of common diagnoses are shown in Figure 31.
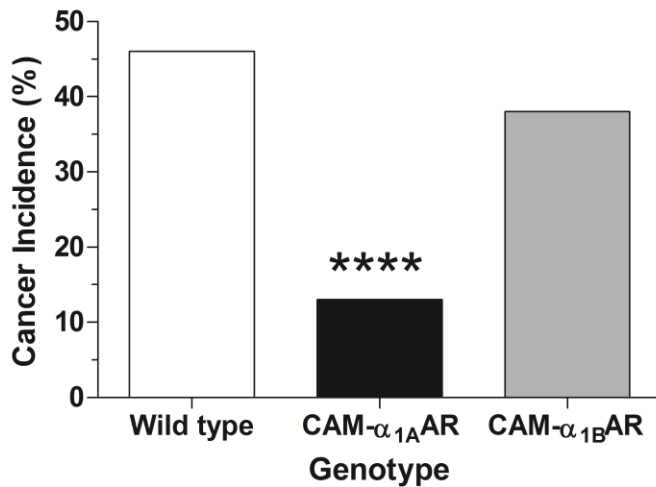
Figure 30. Cancer incidence. CAM-α_{1A}AR (n = 53) mice had a significantly lower incidence of cancer when compared with WT animals (n = 70, p < 0.0001). The incidence of cancer in CAM-α_{1B}AR mice did not differ from that of WT mice (n = 34, p = 0.7245).

Discussion

It is well documented that caloric restriction increases lifespan in many species including rodents (Reviewed in Masoro, 2005). Injection of α_1AR agonists systemically or into the paraventricular nucleus of the hypothalamus can cause appetite suppression in acute studies (Davies and Wellman, 1992; Morien et al., 1993). In our lab, long-term treatment (8-9 wks) with cirazoline, an α_{1A}AR-selective agonist, has not reduced food or water intake nor reduced body weight (Doze, Goldenstein, and Collette, unpublished data). To our knowledge, food intake has not been studied in CAM-α_{1A}AR or CAM-α_{1B}AR mice. However, because even modest caloric restriction can reduce body weight we weighed cohorts of mice to determine whether transgenic activation of either α_1AR subtype decreased body weight with a subsequent increase in lifespan (Colman et al., 2009). There

Table 4. Pathology of WT, CAM-α_{1A}AR, and CAM-α_{1B}AR mice at death

	B6CBA WT (n = 70)	CAM-α_{1A}AR (n = 53)	CAM-α_{1B}AR (n = 34)
Cancerous	45.7% (32)	13.2% (7)	38.2% (13)
Epithelial	25.7% (18)	5.7% (3)	23.5% (8)
Hematological	18.6% (13)	7.5% (4)	8.8% (3)
Mesenchymal	1.4% (1)	0.0% (0)	5.9% (2)
Noncancerous	54.3% (38)	86.8% (46)	61.8% (21)
Benign tumor			
Epithelial	7.1% (5)	3.8% (2)	0.0% (0)
Hematological	2.9% (2)	3.8% (2)	11.8% (4)
Mesenchymal	0.0% (0)	3.8% (2)	2.9% (1)
Non-tumor lesions	35.7% (25)	49.1% (26)	35.3% (12)
No abnormal findings	8.6% (6)	26.4% (14)	11.8% (4)

was no difference in body weight between CAM-α_{1A}AR and WT mice except one timepoint after weaning. Body weight of the CAM-α_{1B}AR animals was significantly lower when compared with WT mice after weaning and again after approximately 9 mo of age. However, the lifespan of CAM-α_{1B}AR mice was decreased rather than increased so the effect was not positive on lifespan. Food intake was not assessed during the longevity study so we cannot definitively conclude that the α_{1A}AR-stimulated lifespan increase was not through a caloric reduction mechanism but it seems unlikely because the CAM-α_{1A}AR animals were of normal weight.
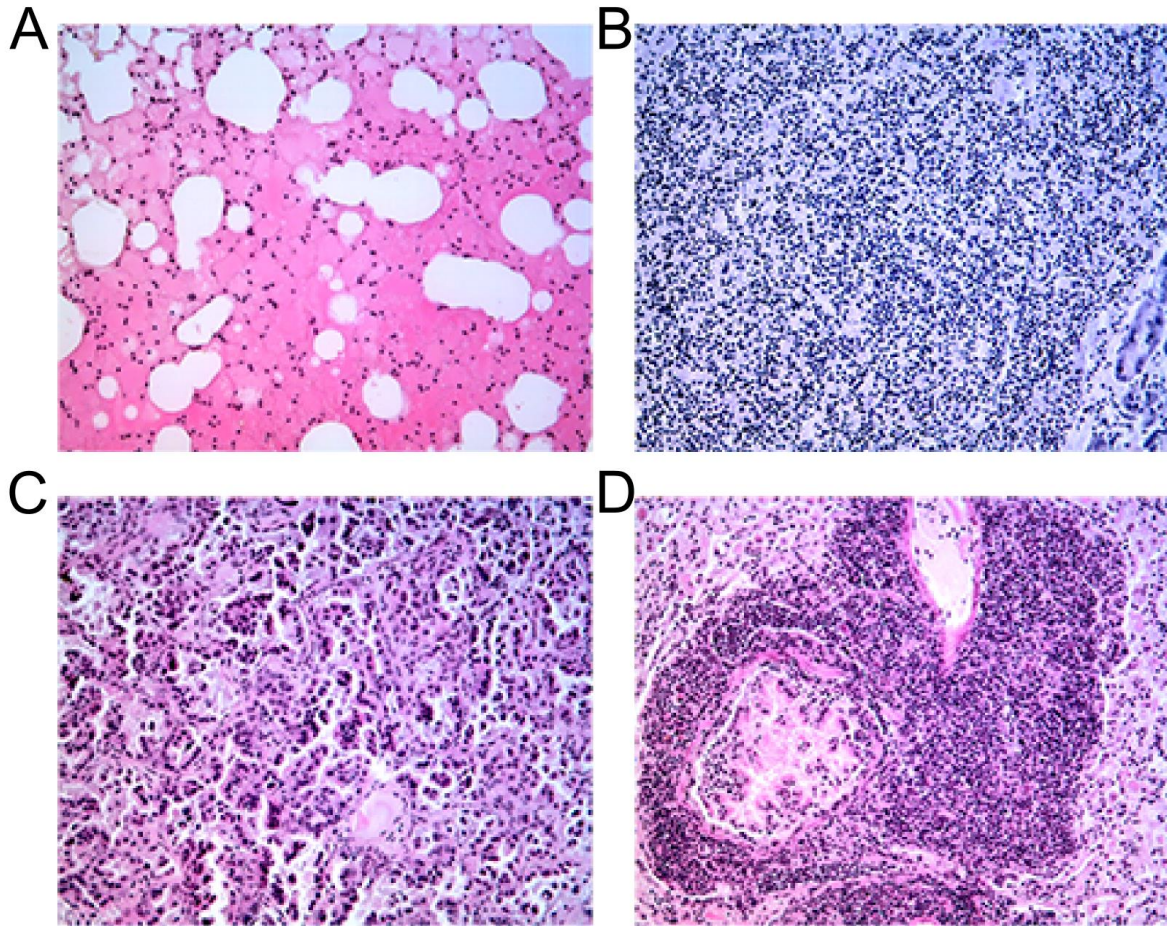
Figure 31. Representative photomicrographs of the most common pathological findings at death. A) Pulmonary edema in a CAM-α_{1A}AR mouse, B) lymphoma in a WT mouse, C) adenocarcinoma in a WT mouse, and D) lymphoma of the lung in a CAM-α_{1B}AR mouse.

Previous studies of increased lifespan in mouse found a 15-80 percent extension, depending on the intervention (Blüher et al., 2003; Brown-Borg et al., 1996). Shortened lifespan is not reported as often but in certain transgenic models death *in utero* or within the early postnatal period is not uncommon if the mutated gene is involved in critical developmental processes (Thyagarajan et al., 2003). In our study, CAM-α_{1B}AR mice had an 11 percent decrease in lifespan when compared with WT animals but the cause of the decrease is still unknown. Some genetic models with shortened lifespan showed

accelerated aging or increased cancer incidence with subsequent lifespan decreases of 25-50 percent (Rudolph et al., 1999; Keyes et al., 2005). We observed signs of accelerated aging including reduced body weight, alopecia, and spine curvature in our model but did not examine other factors such as bone density or cellular senescence (de Boer et al., 2002; Sun et al., 2004). Cancer incidence was similar to WT mice. Another factor which must be considered in mouse models of aging is the background strain because the baseline lifespan is variable.

There have not been any studies yet on the lifespan of the B6CBA mouse, which is a cross between the C57BL/6 and CBA strains. The median age of C57BL/6 mice varies depending upon environment but ranges from 682-930 d (Ikeno et al. 2005; Selman and Withers 2011, and Reviewed in Nadon et al. 2008). In a study of 31 inbred strains at Jackson Labs, C57BL/6J mice had median ages of 866 d for males and 901 d for females (Yuan et al., 2009). In the same study, CBA/J mice had median ages of 679 d for males and 644 d for females. Our WT B6CBA animals had a median age of 719 d, which is between the two strains, but slightly closer to the CBA/J median age.

Prior to this work, survival had been assessed in CAM-α_{1B}AR mice, then called T1 mice, for up to 70 wks (Papay et al., 2002). However, no previous studies followed all animals until the natural date of death. In the current study, the survival of CAM-α_{1B}AR animals began to deviate from WT mice at approximately 9 mo of age, the timing of which coincides with the pathological appearance of neurodegeneration and the age at which body weight begins to differ (Papay et al., 2002). The α_1AR antagonist terazosin protects these mice against the weight loss and neurodegeneration. Terazosin also increased survival

rates in the T1 mice but the animals were not followed until natural death so the effect of terazosin on full lifespan is unknown. The combination of that study and the current results suggest the effects of α_{1B}AR over-activation can cause a shortened lifespan. The α-synucleinopathy induced by chronic activation of α_{1B}ARs is similar to multiple system atrophy (Zuscik et al., 2000; Papp et al., 1989). Multiple system atrophy can lead to early death in humans by sudden cardiopulmonary arrest or pneumonia (Papapetropoulos et al., 2007). In our study, there was no increased incidence of either event in CAM-α_{1B}AR mice. The mechanism by which α_{1B}AR activation can decrease lifespan is still unknown.

The sex differences in median and maximal lifespan in CAM-α_{1B}AR mice were an unexpected result, particularly because cancer incidence was not different for either sex. However, we did not examine cancer progression over time, which could have clarified whether cancer in the female mice was more aggressive with a quicker latency to death. We have not found sex differences in other studies using the CAM-α_{1B}AR mice (Doze et al., 2009). There is a dearth of data on sex differences in general in the scientific literature and only one published study on sex differences in relation to the adrenergic receptors (Novakova et al., 2010). In this report, female mice had higher basal α_1AR receptor densities in the lung and immobilization stress decreased the level of all three α_1AR subtypes. In male mice, only the α_{1A}AR level was decreased following stress. In the current study, shortened lifespan in the female CAM-α_{1B}AR animals may have involved hormonal changes mediated by the α_{1B}AR. There are noradrenergic innervations to the breast and ovarian follicles, where norepinephrine release can increase the levels of estradiol and progesterone and a decrease of sympathetic input can slow tumor growth (Romeo et al., 1991; Piccinato et al.,

2012). Furthermore, the norepinephrine reuptake inhibitor desipramine promotes breast cancer progression with α_2AR activation however, phenylephrine, a non-selective α_1AR agonist, did not have an effect (Szpunar et al. 2013). It's possible the α_{1B}AR is involved in hormone-related cancer progression but further work should be done to clarify if there is a role for the receptor.

The decrease in lifespan in the CAM-α_{1B}AR mice is unlikely due to heart failure, which is not present in this mouse model. While animal studies suggest that chronic activation of the α_{1B}AR subtype may be "bad" or maladaptive for the heart, the amount of dysfunction does not lead to heart failure if overexpression is more physiologically relevant or the heart is not stress-induced. CAM-α_{1B}AR mice also have a mild cardiac hypertrophy, decreased cardiac output, some diastolic dysfunction, and inflammation. These conditions did not, however, progress to heart failure on their own (Zuscik et al., 2001; Yun et al., 2003). Other groups have demonstrated that myocyte-targeted CAM-α_{1B}AR also induces a mild hypertrophy when the receptor is only overexpressed 3-fold or less, as it is in our mouse model, but only progressed to heart failure after blood pressure overload (Milano et al., 1994). Therefore, chronic stimulation of the α_{1B}AR leads to hypertrophy and some cardiac dysfunction but only induces heart failure when the receptor is artificially overloaded. As this aging study only used non-stressed mice, it is unlikely that CAM-α_{1B}AR mice die younger because of heart failure. Furthermore, in the present study one case of heart-related dysfunction was noted in CAM-α_{1B}AR mice, a heart thrombus.

On the other hand, previous studies have suggested the CAM-α_{1A}AR mice are cardio-protected, which may contribute to its increased longevity (Reviewed in Perez and Doze

2011). While CAM-α_{1A}AR mice also display cardiac hypertrophy as in CAM-α_{1B}AR mice,

α_{1A}AR activation results in positive adaptation of the heart to protect against ischemic

damage through preconditioning via cardiac protective IL-6 and JAK/STAT pathways or by

preventing apoptosis due to increased glucose uptake (Rorabaugh et al. 2005; Papay et al.

2013, Perez, unpublished data). In the current study, one case of heart dysfunction was

found in CAM-α_{1A}AR mice: endocarditis/ myocarditis.

The decrease in lifespan in CAM-α_{1B}AR mice does not appear to be due to an

increase in cancer incidence. *In vitro*, α_{1B}AR overexpression and activation results in focus

formations (Allen et al., 1991). In addition, the cells form tumors when injected into

immune-compromised mice. Due to the tumorigenic quality of transplanted α_{1B}AR-

expressing cells, we hypothesized CAM-α_{1B}AR mice would have an increased incidence of

cancer. Surprisingly, there was no significant difference in cancer incidence between CAM-

α_{1B}AR mice and WT animals. The α_{1B}AR can regulate the cell cycle through cdk-6 and cyclin

E-associated kinases but the α_{1B}AR effect on proliferation may be cell type specific

(Gonzalez-Cabrera et al., 2004). For example, in CHO cells transfected with the α_{1B}AR the

cell cycle was stopped when activated by phenylephrine (Shibata et al., 2003). In TRAMP

cells which expressed mostly the α_{1B}AR subtype, doubling time was faster with decreasing

α_{1B}AR and increasing α_{1A}AR and α_{1D}AR expression (Shi et al., 2007). However, the cancerous

state is highly complex and does not solely depend on a dysregulation of the cell cycle. Ours

is the first study to explore the effect of α_{1B}AR activation in cancer in an immunocompetent

mouse strain.

In most cell types studied, the α_{1A}AR subtype increases the levels of the cdk inhibitor p27Kip1 and halts the cell cycle at the G1-S checkpoint (Gonzalez-Cabrera et al., 2004; Saeed et al., 2004; Shibata et al., 2003). In our study, there was a significant reduction in cancer incidence in CAM-α_{1A}AR mice, which could be due to this cell cycle stoppage. The cell type specificity of α_1AR subtype localization, downstream pathways, and subsequently physiological effects are imperative to the mechanism behind the reduction. For example, in human prostate cancer cells, the α_1AR selective antagonist naftopidil stops growth through the same mechanism by which agonists halt the cycle, via p27Kip1 (Kanda et al., 2008). These contradictions are perplexing much as contradictory studies were prior to the delineation of the three α_1AR subtypes. The answers may lie in the regulators of G-protein signaling (RGS), reactive oxygen species, and cell type specific downstream effects (Abramow-Newerly et al., 2006; Hu et al., 1999; Shi et al., 2006).

Previous studies have implicated other ARs in longevity function, but these studies indicated shortened lifespan due to disease onset rather than increased lifespan. β_2ARs promote aging and reduced lifespan mostly due to adverse effects on cardiac and pulmonary functions and its age-related impairment to regulate insulin secretion (Gao et al., 2003; Santulli and Iaccarino, 2013; Santulli et al., 2012). However, it could also be due to polymorphisms in the receptor. For example, one study compared β_2AR polymorphisms and found two variants that were associated with increased longevity in the Han Chinese population (Zhao et al., 2012). These polymorphisms reduced translational efficiency and receptor expression in transfected cells suggesting that decreased β_2AR function promoted

longevity. In contrast, our results are the first to report that increasing AR activity would promote a longer lifespan, but specifically through the α_{1A}AR subtype.

Human longevity has continued to increase as sanitation, healthcare, and modern medicine have evolved. Ideally, increased longevity will also translate into improved health in later years. Cancer is one of the leading causes of death worldwide with approximately 50% of cases occurring in persons older than 65 years of age. Current therapeutics are not ideal because they kill all dividing cells indiscriminately which can lead to uncomfortable and dangerous side effects. Preferential activation of the α_{1B}AR subtype has negative effects in the heart and brain and may increase proliferation in some cell types, while stimulating the α_{1A}AR shows positive effects in these areas. The current work is the first to show that chronic activation of the α_{1B}AR can shorten lifespan. Whether this is due to accelerated neurodegeneration and/or other disease process(es) is still unknown. Importantly, we found that long-term α_{1A}AR stimulation significantly decreases cancer incidence which may account in part for our previous finding that it increases lifespan. While more work is needed, it is clear that activating the α_1AR subtypes leads to differential effects. α_{1A}AR subtype-specific therapeutics may lead to improved cancer therapeutics with fewer adverse effects.

CHAPTER 6

DISCUSSION

There are several key findings herein on the role of α_1AR subtypes in brain, and

potentially peripheral, function. α_{1A}AR activation does not influence survival and cell fate

during adult neurogenesis. However, it does affect survival at the systemic level by

increasing median and maximal longevity. Behaviorally, the lack of α_{1A}AR function does not

reliably change levels of anxiety or depression in relation to WT mice. In slightly older α_1AR

mice, anxiety levels are higher in the zero maze if the mice have not had seizures. Lack of

α_{1B}AR function reduces depression-like behavior but does not change anxiety-like behavior

when compared with WT. Both KO groups had deficits in novel object recognition based on

their memory of the original object failing to be higher than chance level. Lack of α_{1A}- or

α_{1B}AR function does not change spatial memory or cognitive flexibility in the Morris water

maze. Despite this, α_{1A}AR-KO mice did not significantly increase path efficiency over the

course of testing. Overall, the combined results are an important step in determining the

role of α_1AR function.

Contribution to the field

The field of α_1ARs and brain/ behavioral function was previously limited by the use

of non-selective agonists and antagonists that cross the blood brain barrier. Though

activation and KO mouse models have been available for over a decade, they are

predominantly used to study cardiac and cardiovascular function in the periphery. Our work

110

with transgenic animals is currently the leading research in the field in the area of brain and behavioral function of α_1ARs. In the context of adult neurogenesis, norepinephrine is the only main neurotransmitter in which the function has not been studied in depth. No other lab has published research regarding the effect of the α_1AR subtypes' role in differentiation *in vivo*. Additionally, previous research suggested the α_{1B}AR as a proto-oncogene based on *in vitro* work and subcutaneous injection of cancer cells (Allen et al., 1991). Our results show this does not appear to translate to the whole animal. Therefore, the research presented in this dissertation added a significant amount of new knowledge and proposes new avenues for study based on this knowledge.

Learning and memory

The role of the α_1AR and α_{1A}AR subtypes in learning and memory has not been clear and has been somewhat controversial due to conflicting results. Research examining the role of the receptors in prefrontal cortex-based learning and memory suggests that α_1ARs and even the α_{1A}AR subtype may be detrimental to learning and memory (Gibbs and Summers, 2001; Gibbs and Bowser, 2010; Mao et al., 1999; Arnsten et al., 1999; Arnsten and Jentsch, 1997; Torkaman-Boutorabi et al., 2014). The role of the receptor subtypes could be region-based: activation in the prefrontal cortex could be disruptive while activation in the hippocampus is enhancing. However, many of the studies that found deficits used only acute administration of agonists and antagonists, and they were mostly not selective for the receptor subtypes. In an early study with α_{1B}AR-KO mice, KO enhanced object recognition memory but impaired spatial memory when compared with WT mice (Spreng et al., 2001).

Our prior research showed that chronic α_{1A}AR activation, either using cirazoline or CAM activation of each subtype, enhanced both learning and memory in several measures. Our hypothesis for the experiments herein was that KO of the α_{1A}AR subtype would worsen learning and memory measures; a hypothesis that was not upheld by the results except in the novel object recognition test. We also hypothesized that α_{1B}AR-KO would mirror the learning and memory results of the Spreng study: enhanced novel object recognition but impaired spatial memory (Spreng et al., 2001). Instead, we found impaired novel object recognition (though not in relation to WT) and no difference in spatial memory compared with either the WT or α_{1A}AR-KO mice. A limitation of using KO mice is the potential for other pathways to compensate for the missing receptor. To confirm the findings presented herein, further research could use α_1AR subtype-selective antagonists via a ventricular cannula or a conditional KO mouse model. However, in combination with the conflicting mood results, the possibility that the α_1AR subtypes interact to mediate behavioral effects is intriguing.

Depression and anxiety

Depression and anxiety are highly intertwined and often comorbid in humans. While the α_1AR subtypes have not been previously studied in the context of depression and anxiety by other labs, there is evidence for α_1AR involvement in the mechanism of antidepressant action. Antidepressants with α_1AR antagonist properties can block anxiety and depressive-like behavior in animal models (Danysz et al., 1986; Kakui et al., 2009; Kitada et al., 1983). The α_1AR agonist, phenylephrine, potentiates the effect of the atypical antidepressant lamotrigine (Puumala et al., 1996). Furthermore, α_1AR subtype activation

increases the density of α_{1A}- and α_{1B}ARs in specific brain regions (Hanft and Gross, 1990; Perry et al., 1990).

Our earlier research showed that chronic α_{1A}AR activation decreases the basal level of depression and anxiety when compared with WT mice. Based on these results and the work of others, we hypothesized that α_{1A}AR-KO mice would have increased levels of depression and anxiety-like behaviors when compared with WT and α_{1B}AR-KO mice would have decreased levels of depression. We did not expect a difference in anxiety behavior in α_{1B}AR-KO mice in comparison to WT mice because we had seen no differences in anxiety after α_{1B}AR activation. The results were interesting. α_{1A}AR-KO did not change anxiety levels except in one test after removing mice that had experienced seizures. α_{1B}AR-KO mice had increased anxiety-like behavior in one parameter of the elevated zero maze compared with WT but a decreased depression level. Because depression and anxiety are inter-related, these conflicting results are difficult to interpret. One main difference between the CAM transgenic mice and the KO mice is that in the CAM mice both receptors are still present but at differing ratios of expression and activation. One explanation for the behavioral results of the current studies is that of *in vivo* receptor heterodimerization. This idea that the receptors dimerize to affect function and surface expression is particularly intriguing when exploring the mood results. This will be an important avenue in future research and is already being pursued in at least one other lab (Collette, personal communication).

In order to make strong conclusions about using α_{1A}AR activation as a treatment for depression additional research must be undertaken. Our previous and the current research show α_{1A}AR activation does, while α_{1A}AR-KO does not, change basal levels of depression.

Future research needs to include more ethologically valid experiments. One such possibility is to use a chronic mild stress paradigm to induce depression in rodents to model stress-induced depression in humans. Treatment could include a selective α_{1A}AR agonist or antagonist into the lateral ventricles via a cannula. Behavioral testing would show whether activation can rescue a depression phenotype or if blocking α_{1A}AR function would further increase depression behaviors.

Adult neurogenesis

Chronic activation of the α_{1A}AR *in vitro* and *in vivo* increases proliferation and directs cell fate in neurosphere culture (Gupta et al., 2009). Neurospheres cultured from normal adult mouse SVZ stimulated with phenylephrine, an α_1AR agonist, induces differentiation into glial cells. Neurospheres isolated from neonatal CAM-α_{1A}AR transgenic mice increases the differentiation markers Dlx2, Mash1, and NeuroD. Neurospheres from α_{1A}AR-KO mice increase expression of Nestin, a stem cell marker, suggesting a reversion to a less differentiated state. In contrast, a recently published paper used SGZ-derived neurospheres to study the role of adrenergic receptors on proliferation and cell fate (Jhaveri et al., 2014). Successfully deriving neural stem cells from the hippocampus without SVZ contamination is highly controversial. However, using cirazoline on the neurospheres, there was no difference in the percentage of cells differentiating into astrocytes (S100β). They also did not see an increase in the number of new neurons (using βIIItubulin as a marker).

The work presented herein examined the differentiation and survival stages of adult neurogenesis *in vivo*. Based on prior results, we hypothesized that newly created cells would differentiate preferentially into astrocytes rather than neurons in the adult mouse

SGZ. We found no difference in the number or percentage of new cells that became mature neurons, $BrdU^+NeuN^+$, or astrocytes, $BrdU^+GFAP^+$. There was a large effect size but no significant reduction in the number of immature neurons, $BrdU^+DCX^+$ cells 4 wks after BrdU injection. A reduction in immature neurons at this timepoint could mean that the rate of differentiation was faster after cirazoline treatment. If that was the case, the number of new mature neurons, $BrdU^+NeuN^+$, or astrocytes, $BrdU^+GFAP^+$, should have been increased and it was not. Taking the lack of increase in mature cell markers into account, it is also possible the rate of differentiation was decreased and newly generated cells did not progress past the immature neuron stage. This is also not likely because the percentage of mature neurons and astrocytes would have been decreased in the cirazoline group compared with controls and it was not. Analysis of DCX morphology of double-labeled cells could potentially answer this question. A better way would be to perform a time course of BrdU injections and cell counts. It is clear from the $BrdU^+NeuN^+$ and $BrdU^+GFAP^+$ results that the ratio of new cells is unchanged by chronic $\alpha_{1A}AR$ activation but the DCX results are very interesting and should be further studied.

Longevity and cancer

Ours is currently the only published research regarding the α_1AR subtypes and longevity (Collette et al., 2014). Survival to a 70 wk timepoint in another study was reduced to 75 percent in CAM-$\alpha_{1B}AR$ mice (Papay et al., 2002). In the current study, the number of early deaths in the CAM-$\alpha_{1B}AR$ was not quite that drastic but lifespan was significantly reduced compared with WT mice. One of the pioneers of AR research, Robert Lefkowitz, previously proposed that the $\alpha_{1B}AR$ was a proto-oncogene (Allen et al., 1991). This was

based on his lab's work showing that α_{1B}ARs formed foci when transfected into cells and tumors when injected subcutaneously into nude mice. In addition, the α_{1B}AR gene is associated with mitogenesis and cell cycle progression, which could lead to cancer (Gonzalez-Cabrera et al., 2004). The results herein, show that the *in vivo* picture is more complex. Chronic activation of the α_{1B}AR over the lifespan does not increase cancer incidence but it does decrease lifespan (Collette et al., 2014). Cancer is the most common cause of death in aged mice but our results show that activation of the α_{1B}AR is not responsible but could be contributing. CAM-α_{1B}AR mice develop a synucleinopathy similar to multiple system atrophy in humans which includes locomotor deficits, synuclein aggregates and neurodegeneration, autonomic dysfunction, and ultimately death (Papay et al., 2002). The decrease in lifespan after α_{1B}AR activation is likely due to this neurodegeneration but this was not tested in the current study.

Chronic α_{1A}AR activation in CAM-α_{1A}AR mice increases lifespan and significantly decreased cancer incidence in our population, which included mice at the end of life and a small number of younger mice. In this case, the correlation between increased lifespan and decreased cancer is an especially exciting finding and should be pursued. A potential mechanism could be regulation of the cell cycle by the α_{1A}AR subtype. In a gene microarray using Rat-1 fibroblasts, α_{1A}ARs were associated with cell cycle regulators that stop the cell cycle at the G1-S checkpoint (Gonzalez-Cabrera et al., 2004). Important factors to consider when expanding this line of study include using a prospective, rather than retrospective analysis of lifespan and cancer; removing confounding variables such as behavioral testing; and use transgenic animals of the same F generation (Appendix A). It would also be

interesting to use mice that already have cancer and treat with an α_{1A}AR agonist or antagonist. In studying peripheral cancers, such as in the present study, there is not a need for a drug to cross the blood brain barrier. The lab is now investigating the incidence of cancer in α_{1A}AR-KO and α_{1B}AR mice. The current study is a promising lead on a potential cancer prevention therapy in high-risk populations and expansion of this research would show whether it could also be used to stop cancer progression.

Conclusions

The progression of AR research has been akin to a funnel. Early on, it became clear there were different types of receptors with different downstream effects. It was thought that studying each of these types of receptors would clarify their roles in the body. However, it did not. Soon after, the subtypes were introduced but were difficult to study because there were no selective drugs. When drugs were developed they did not cross the blood brain barrier. Localization of the subtypes in the brain was even limited because there were not, and still are not, selective antibodies. The studies presented herein are an important step forward because they show the complexity of the α_1AR subtypes and suggest another level of specification. ARs are regulated by many factors including such basic input as how tightly an agonist binds which can activate a different G-protein. Future work will continue to whittle down the differences and explore how to best utilize the ARs as therapeutic targets. Our work suggests that α_{1A}AR-selective agonists could be promising treatments for diseases with cognitive disruptions, depression, anxiety, and even prevention of cancer. α_{1B}AR-selective antagonists may prevent synucleinopathic neurodegeneration and could be used in conjunction with α_{1A}AR agonists for depression.

117

APPENDIX

APPENDIX A

Lifespan, Cancer, and Loss of Phenotype

Data Collection

Data collection for the longevity study began prior to my arrival in the Doze lab; however, I did the majority of the data collection. I also verified dates of birth and death of all mice using mortality logs from the Center for Biomedical Research, shipment sheets from Cleveland Clinic, and cage cards. In some cases, there were mice with different birth dates in the same cage so if they did not have an ear tag at death they were not included because there was no way to verify the number of days lived.

Retrospective Analysis

The longevity study was a retrospective analysis of mice used in various behavioral studies over the span of several years. The mice were not included in any seizure studies nor were they given any treatments or drugs. A small retrospective analysis may be appropriate to provide data for a power analysis to determine how many animals would be needed for a full prospective study. However, standard procedure for a lifespan study is to predetermine the number of animals per group from the same F generation of breeding and begin tracking lifespan when they are the same age. Equal numbers of male and female mice would allow for reliable determinations of sex differences. Additionally, all confounding variables possible should be eliminated including behavioral testing. The mice should be allowed to age, undisturbed except for cage changes and food and water intake assessment, for their full lifespan.

Regarding Previously Published Lifespan Data

Previously published wild type (WT) mouse lifespan data was included in analysis for additional power. There was no significant difference between WT data that had been previously published and WT data gathered for additional WT mice (Doze et al., 2011). However, the data excluding the previously published WT data is presented here for completeness (Figure 32). When comparing only the non-published WT and CAM-α_{1B}AR lifespan there is not a significant difference in the number of days lived ($\chi^2(1) = 1.83$, p = 0.055).

Loss of Phenotype

During analysis of the longevity and pathology data, we had initially planned to include additional CAM-α_{1A}AR mice that had died since our 2011 publication, as we did with the WT data. However, I noticed a pattern that CAM-α_{1A}AR mice received from Cleveland Clinic after our initial longevity study were dying at much younger ages than previously observed.

Initially, we suspected a virus in the colony but further analysis revealed that only the CAM-α_{1A}AR mice were dying sooner. Analysis of the lifespan and F generation data showed a significant difference in lifespan in CAM-α_{1A}AR mice starting at the F16 generation (Figure 33A, $F(13, 69) = 12.49$, p < 0.0001), post-hoc comparisons in Table 5). By the F20 generation, some mice were not surviving past weaning or shortly after due to hydrocephaly and thus were not transferred to UND. For comparison, Jackson Laboratories refreshes their transgenic lines from cryo-storage after 10 generations to ensure a substrain does not
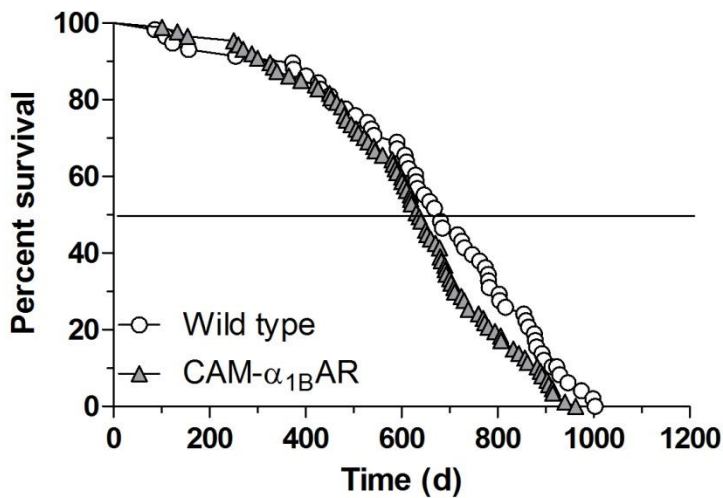
Figure 32: Kaplan-Meier survival plots. When the previously published WT mice were excluded from analysis there was no significant difference in lifespan between CAM-α_{1B}AR (n = 87) and WT mice (n = 58, p = 0.055).

develop due to random mutations, which can become fixed after 6-9 generations (The Jackson Laboratory, 2013).

CAM-α_{1A}AR mice included in the lifespan studies in the 2011 paper and for comparison in the 2014 paper were of the F5-F14 generation. CAM-α_{1B}AR animals were from the F2-F15 generation but most were in the F5-F8 range. We did not receive any CAM-α_{1B}AR mice bred past F11. There was a main effect of lifespan vs F generation in CAM-α_{1B}AR mice [$F(7, 37) = 3.29$, $p = 0.008$], and one post-hoc difference between F6 and F9 ($p < 0.05$). There were no differences between any other generations in CAM-α_{1B}AR mice (Figure 33B). For future reference, the lifespan vs F generation data, for which there were no significant differences, are also included for the α_{1A}AR-KO [$F(7, 42) = 0.59$, $p = 0.75$], α_{1B}AR-KO [$F(6, 39) = 0.52$, $p = 0.78$], and α_{1A}AR-EGFP [$F(3, 18) = 0.85$, $p = 0.48$, Figure 33C, D, and E].
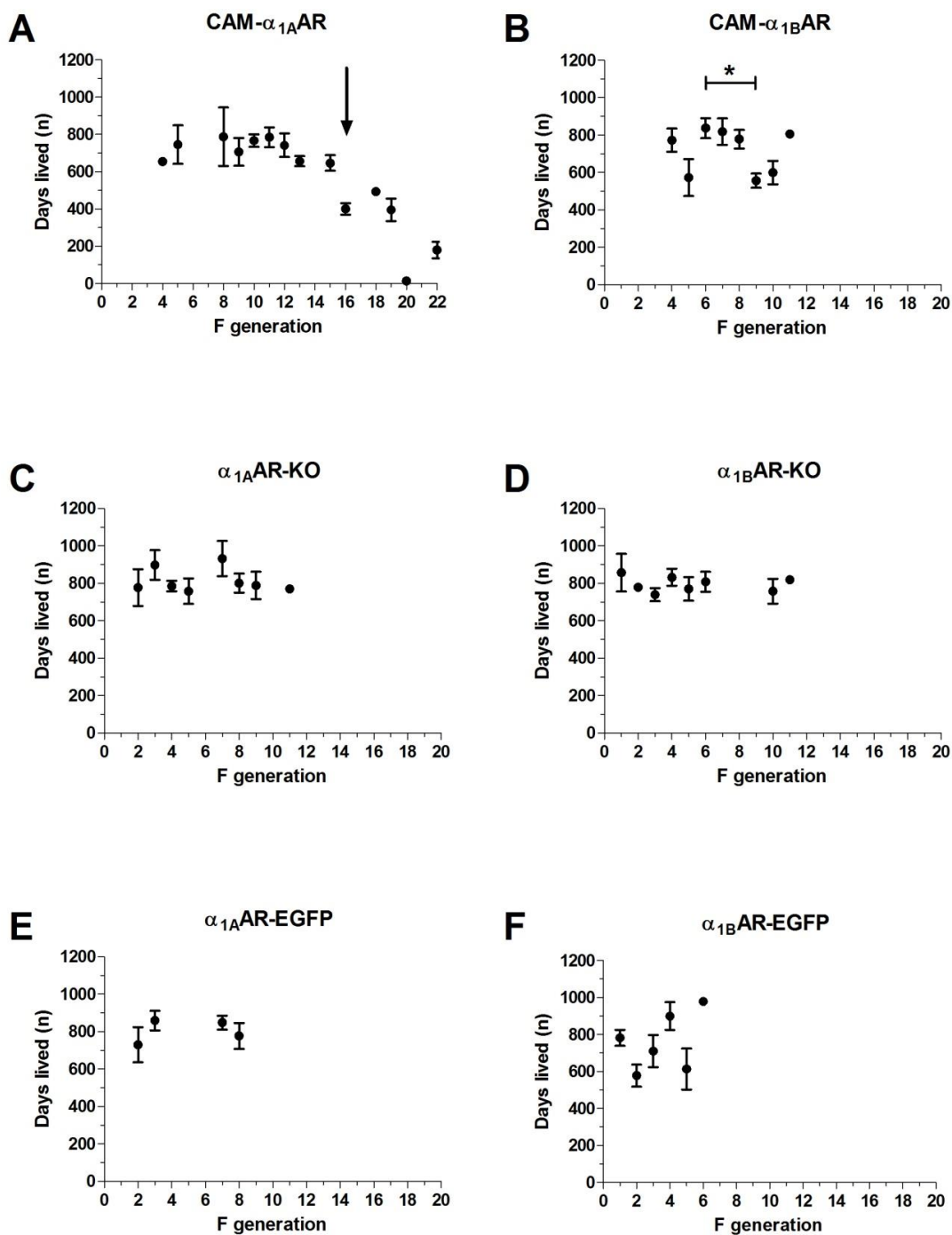
Figure 33: Age at death vs F generation. A) CAM-α_{1A}AR mice starting at the F16 generation lived significantly fewer days than mice of the <F15 generation. B) There was a significant difference in lifespan in CAM-α_{1B}AR mice of the F6 and F9 generation but no downward trend was observed. There were no significant differences in lifespan between the F generations of C) α_{1A}AR-KO, D) α_{1B}AR-KO, or E) α_{1A}AR-EGFP. There was a difference for F) α_{1B}AR-EGFP mice but no post-hoc differences.

Table 5: CAM-α_{1A}AR vs F generation post-hoc testing results.

Tukey's Multiple Comparison Test	Significant? P < 0.05?	Summary	95% CI of diff
4. vs 5.	No	ns	-543.2 to 361.2
4. vs 8.	No	ns	-610.4 to 343.0
4. vs 9.	No	ns	-457.2 to 351.8
4. vs 10.	No	ns	-550.3 to 323.5
4. vs 11.	No	ns	-583.2 to 321.2
4. vs 12.	No	ns	-514.2 to 338.5
4. vs 13.	No	ns	-479.0 to 474.4
4. vs 15.	No	ns	-385.8 to 400.4
4. vs 16.	No	ns	-141.4 to 648.1
4. vs 18.	No	ns	-361.7 to 682.7
4. vs 19.	No	ns	-214.7 to 622.7
4. vs 20.	Yes	***	203.1 to 1077
4. vs 22.	No	ns	-2.698 to 950.7
5. vs 8.	No	ns	-441.5 to 356.2
5. vs 9.	No	ns	-270.6 to 347.2
5. vs 10.	No	ns	-372.7 to 327.9
5. vs 11.	No	ns	-409.2 to 329.2
5. vs 12.	No	ns	-333.9 to 340.2
5. vs 13.	No	ns	-310.2 to 487.5
5. vs 15.	No	ns	-195.5 to 392.2
5. vs 16.	Yes	**	48.30 to 640.4
5. vs 18.	No	ns	-200.7 to 703.7
5. vs 19.	No	ns	-32.30 to 622.3
5. vs 20.	Yes	***	380.7 to 1081
5. vs 22.	Yes	***	166.2 to 963.8
8. vs 9.	No	ns	-262.8 to 424.7
8. vs 10.	No	ns	-361.1 to 401.6
8. vs 11.	No	ns	-396.2 to 401.5
8. vs 12.	No	ns	-323.4 to 415.1
8. vs 13.	No	ns	-295.0 to 557.7
8. vs 15.	No	ns	-189.3 to 471.3
8. vs 16.	Yes	**	54.80 to 719.2
8. vs 18.	No	ns	-182.5 to 770.9

Table 5 cont.

Tukey's Multiple Comparison Test	Significant? P < 0.05?	Summary	95% CI of diff
8. vs 19.	No	ns	-22.68 to 698.0
8. vs 20.	Yes	***	392.3 to 1155
8. vs 22.	Yes	***	181.3 to 1034
9. vs 10.	No	ns	-346.7 to 225.3
9. vs 11.	No	ns	-387.2 to 230.6
9. vs 12.	No	ns	-304.8 to 234.5
9. vs 13.	No	ns	-293.4 to 394.1
9. vs 15.	No	ns	-153.2 to 273.2
9. vs 16.	Yes	***	89.85 to 522.3
9. vs 18.	No	ns	-191.3 to 617.7
9. vs 19.	No	ns	-0.6410 to 514.0
9. vs 20.	Yes	***	406.7 to 978.7
9. vs 22.	Yes	***	182.9 to 870.5
10. vs 11.	No	ns	-367.9 to 332.7
10. vs 12.	No	ns	-290.6 to 341.8
10. vs 13.	No	ns	-270.3 to 492.4
10. vs 15.	No	ns	-148.9 to 390.4
10. vs 16.	Yes	**	94.70 to 638.8
10. vs 18.	No	ns	-163.0 to 710.8
10. vs 19.	Yes	*	11.63 to 623.2
10. vs 20.	Yes	***	423.1 to 1084
10. vs 22.	Yes	***	206.0 to 968.8
11. vs 12.	No	ns	-293.9 to 380.2
11. vs 13.	No	ns	-270.2 to 527.5
11. vs 15.	No	ns	-155.5 to 432.2
11. vs 16.	Yes	**	88.30 to 680.4
11. vs 18.	No	ns	-160.7 to 743.7
11. vs 19.	Yes	*	7.696 to 662.3
11. vs 20.	Yes	***	420.7 to 1121
11. vs 22.	Yes	***	206.2 to 1004
12. vs 13.	No	ns	-283.7 to 454.7
12. vs 15.	No	ns	-157.1 to 347.4
12. vs 16.	Yes	**	86.38 to 596.0

Table 5 cont.

Tukey's Multiple Comparison Test	Significant? P < 0.05?	Summary	95% CI of diff
12. vs 18.	No	ns	-178.0 to 674.7
12. vs 19.	Yes	*	1.310 to 582.4
12. vs 20.	Yes	***	411.6 to 1044
12. vs 22.	Yes	***	192.6 to 931.1
13. vs 15.	No	ns	-320.6 to 339.9
13. vs 16.	No	ns	-76.54 to 587.9
13. vs 18.	No	ns	-313.9 to 639.5
13. vs 19.	No	ns	-154.0 to 566.7
13. vs 20.	Yes	***	261.0 to 1024
13. vs 22.	Yes	*	49.96 to 902.7
15. vs 16.	Yes	**	51.97 to 440.1
15. vs 18.	No	ns	-239.9 to 546.3
15. vs 19.	No	ns	-42.36 to 435.7
15. vs 20.	Yes	***	363.0 to 902.3
15. vs 22.	Yes	***	136.4 to 796.9
16. vs 18.	No	ns	-487.6 to 301.9
16. vs 19.	No	ns	-291.1 to 192.4
16. vs 20.	Yes	***	114.6 to 658.7
16. vs 22.	No	ns	-111.6 to 552.9
18. vs 19.	No	ns	-375.2 to 462.2
18. vs 20.	Yes	*	42.60 to 916.4
18. vs 22.	No	ns	-163.2 to 790.2
19. vs 20.	Yes	***	130.2 to 741.8
19. vs 22.	No	ns	-90.35 to 630.3
20. vs 22.	No	ns	-547.4 to 215.4

There was a main effect for α_{1B}AR-EGFP mice [$F(4, 15) = 3.56$, $p = 0.03$], however no post-hoc differences were found, Figure 33F).

Around the same time the shortened lifespan of CAM-α_{1A}AR mice was found, a 2 hr BrdU stereology pilot study was undertaken using CAM-α_{1A}AR mice of the F22-F23
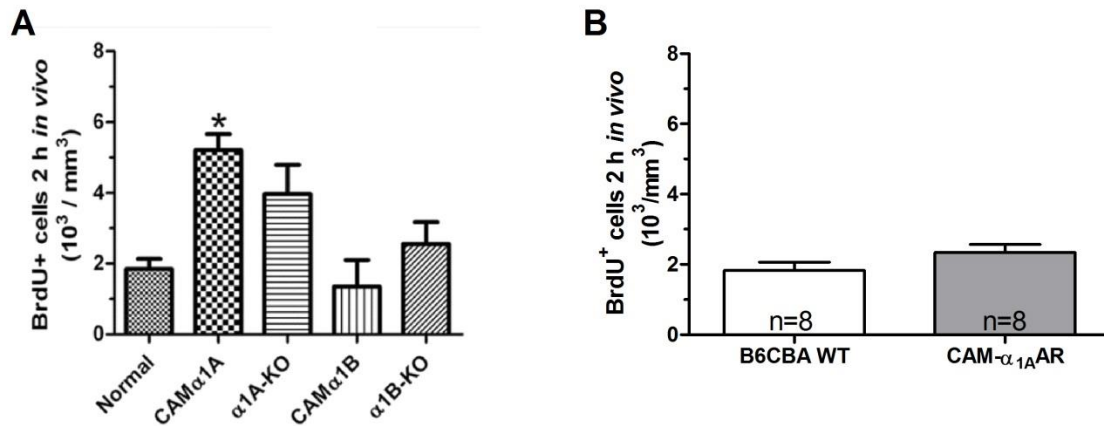
Figure 34: BrdU density in <F10 and F21-22 generation mice. A) CAM-α_{1A}AR mice of a generation <F10 had a significantly higher density of BrdU$^+$ cells after a 2 h chase period when compared with WT mice (Gupta et al., 2009). B) There was no significant difference in BrdU cell density after 2 h between CAM-α_{1A}AR of the F21-22 generation and WT mice.

generation. Surprisingly, in contrast to our previously published findings, no significant difference was found in BrdU density in the dentate gyrus after a 2 h chase period [$t(14)$ = 1.72, p = 0.10, Figure 34, Gupta et al., 2009). While sectioning brains for the stereology study hydrocephaly, in particular the lateral ventricles of CAM-α_{1A}AR mice were enlarged in comparison with WT mice (Figure 35). Discussions with Robert Papay in Dianne Perez's lab, who performed the sectioning for the 2009 neurogenesis paper, revealed that he had previously not noticed any gross differences between WT and CAM-α_{1A}AR brains.

To help confirm the possibility of a loss of phenotype we decided to use a behavioral test in which we had seen robust differences to determine if the behavioral phenotype had also changed. At the F12-14 generation, there was a main effect of genotype in time spent immobile when comparing WT, CAM-α_{1A}AR, and CAM-α_{1B}AR mice [$F(2, 36)$ = 29.26, p < 0.001]. Post-hoc testing showed that CAM-α_{1A}AR mice spent less time immobile (p < 0.001) and CAM-α_{1B}AR mice spent more time immobile (p <0.01) when compared with WT mice
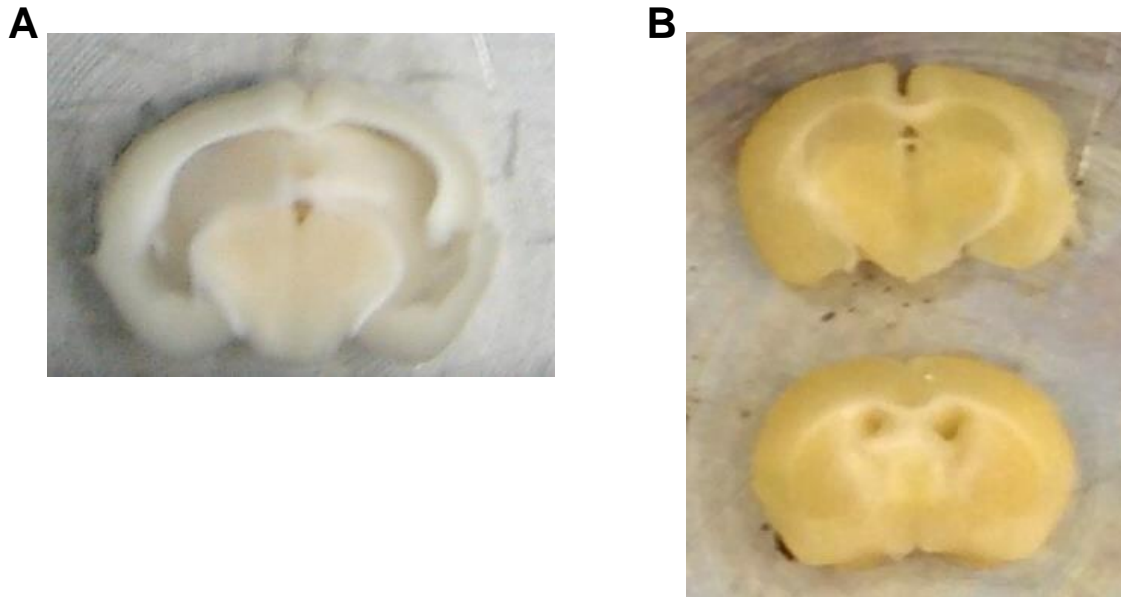
126

Figure 35: Lateral ventricle enlargement. The lateral ventricles of A) CAM-α_{1A}AR mice were enlarged in comparison to B) WT ventricles.

(Figure 36A, Doze et al., 2009). Additionally, CAM-α_{1A}AR mice spent less time immobile at the F15-16 generation when compared with WT [$t(50)$ =3.50, p < 0.001, Figure 36B, Doze et al., 2011). When F22-F23 generation CAM-α_{1A}AR mice were tested, there was no significant difference in the time spent immobile when compared with WT mice [$t(33)$ = 0.83, p = 0.41, Figure 36C]. In an attempt to refresh the CAM-α_{1A}AR line, the Perez lab bred CAM-α_{1A}AR mice of F20+ generation to WT mice. Some of the resulting animals were sent to UND and the tail suspension test was performed. No significant differences were found between WT and CAM-α_{1A}AR mice of this F3 generation [$t(25)$ = 0.78, p = 0.43, Figure 36D]. The next step to attempt to regain the phenotype seen previously in CAM-α_{1A}AR transgenic mice would be to either refresh the line from cryo-storage, if indeed there is such storage. Otherwise, the line must be restarted as it was originally.
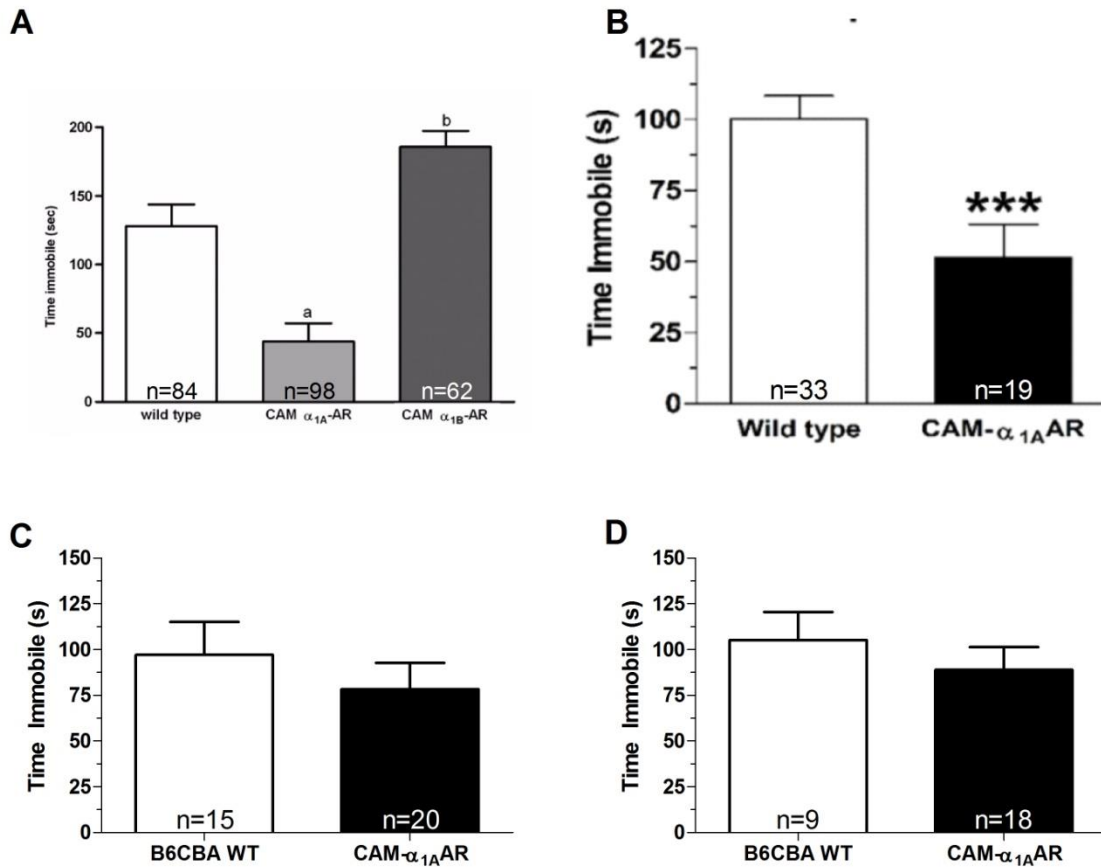
Figure 36: Tail suspension test in different F generations. CAM-α_{1A}AR mice spent A) less time immobile at the F12-F14 generation (Doze et al., 2009) and B) F15-F16 generation (Doze et al., 2011). There were no differences in time immobile between CAM-α_{1A}AR mice of the C) F22 generation or the D) refreshed line at F3.

Cancer Data

As per the Methods for Pathology, "Animals sacrificed at a younger age were included in the analysis to increase statistical power". During review prior to publication, one reviewer requested that additional animals, even if sacrificed at a younger age, be included to make a solid conclusion regarding cancer incidence in CAM-α_{1B}AR mice. Initially, the comparison between WT and CAM-α_{1B}AR mice for cancer incidence had a p value of 0.07, showing a trend toward less cancer in the CAM-α_{1B}AR group. Additional animals, including both mice

sacrificed at an earlier age and mice re-evaluated by Dr. Zhou via histology, were included in

the final published manuscript and in this dissertation.

REFERENCES

Ables, J. L., Decarolis, N. A., Johnson, M. A., Rivera, P. D., Gao, Z., Cooper, D. C., Radtke, F., Hsieh, J., and Eisch, A. J. (2010). Notch1 is required for maintenance of the reservoir of adult hippocampal stem cells. J. Neurosci. *30*, 10484–10492.

Abramow-Newerly, M., Roy, A. A., Nunn, C., and Chidiac, P. (2006). RGS proteins have a signalling complex: interactions between RGS proteins and GPCRs, effectors, and auxiliary proteins. Cell. Signal. *18*, 579–591.

Abrous, D. N., Koehl, M., Moal, M. L. E., and Moal, M. L. (2005). Adult neurogenesis: from precursors to network and physiology. Physiol. Rev. *85*, 523.

Acdx (2009). Norepinephrine. Wikipedia Commons Images. Available at: http://commons.wikimedia.org/wiki/File:Norepinephrine_structure_with_descriptor.svg.

Ahlquist, R. P. (1948). A study of the adrenotropic receptors. Am. J. Physiol. *153*, 586–600.

Aimone, J. B., Deng, W., and Gage, F. H. (2011). Resolving new memories: a critical look at the dentate gyrus, adult neurogenesis, and pattern separation. Neuron *70*, 589–596.

Allen Institute for Brain Science (2014). Allen Mouse Brain Atlas. Available at: http://mouse.brain-map.org.

Allen, L. F., Lefkowitz, R., Caron, M. G., and Cottecchia, S. (1991). G-protein-coupled receptor genes as protooncogenes: constitutively activating mutation of the alpha 1B-adrenergic receptor enhances mitogenesis and tumorigenicity. Proc. Natl. Acad. Sci. U. S. A. *88*, 11354–11358.

ALLHAT, L. F. (2000). Major cardiovascular events in hypertensive patients randomized to doxazosin vs chlorthalidone: the antihypertensive and lipid-lowering treatment to prevent heart attack trial (ALLHAT). ALLHAT Collaborative Research Group. JAMA *283*, 1967–1975.

Arnsten, A. F. T., and Jentsch, J. D. (1997). The alpha-1 adrenergic agonist, cirazoline, impairs spatial working memory performance in aged monkeys. Pharmacol. Biochem. Behav. *58*, 55–59.

Arnsten, A. F. T., Mathew, R., Ubriani, R., Taylor, J. R., and Li, B. M. (1999). alpha-1 noradrenergic receptor stimulation impairs prefrontal cortical cognitive function. Biol. Psychiatry *45*, 26–31.

Ashton, R. S., Conway, A., Pangarkar, C., Bergen, J., Lim, K.-I., Shah, P., Bissell, M., and Schaffer, D. V (2012). Astrocytes regulate adult hippocampal neurogenesis through ephrin-B signaling. Nat. Neurosci. *15*, 1399–1406.

Blüher, M., Kahn, B. B., and Kahn, C. R. (2003). Extended longevity in mice lacking the insulin receptor in adipose tissue. Science *299*, 572–574.

De Boer, J., Andressoo, J. O., de Wit, J., Huijmans, J., Beems, R. B., van Steeg, H., Weeda, G., van der Horst, G. T. J., van Leeuwen, W., Themmen, A. P. N., et al. (2002). Premature aging in mice deficient in DNA repair and transcription. Science *296*, 1276–1279.

Borsini, F., Nowakowska, E., and Samanin, R. (1984). Effect of repeated treatment with desipramine in the behavioral "despair" test in rats: antagonism by "atypical" but not "classical" neuroleptics or antiadrenergic drugs. Life Sci. *34*, 1171–1176.

Brown-Borg, H. M., Borg, K. E., Meliska, C. J., and Bartke, A. (1996). Dwarf mice and the ageing process. Nature *384*, 33.

Bylund, D. B. (1988). Subtypes of alpha 2-adrenoceptors: pharmacological and molecular biological evidence converge. Trends Pharmacol. Sci. *9*, 356–361.

Bylund, D. B., Eikenberg, D. C., Hieble, J. P., Langer, S. Z., Lefkowitz, R., Minneman, K. P., Molinoff, P. B., Ruffolo, R. R., and Trendelenburg, U. (1994). International Union of Pharmacology nomenclature of adrenoceptors. Pharmacol. Rev. *46*, 121–136.

Can, A., Dao, D. T., Terrillion, C. E., Piantadosi, S. C., Bhat, S., and Gould, T. D. (2012). The tail suspension test. J. Vis. Exp., e3769.

Cavalli, A., Lattion, A. L., Hummler, E., Nenniger, M., Pedrazzini, T., Aubert, J. F., Michel, M. C., Yang, M., Lembo, G., Vecchione, C., et al. (1997). Decreased blood pressure response in mice deficient of the alpha1b-adrenergic receptor. Proc. Natl. Acad. Sci. U. S. A. *94*, 11589–11594.

Chalothorn, D., McCune, D. F., Edelmann, S. E., García-Cazarín, M. L., Tsujimoto, G., and Piascik, M. T. (2002). Differences in the cellular localization and agonist-mediated internalization properties of the alpha(1)-adrenoceptor subtypes. Mol. Pharmacol. *61*, 1008–1016.

Cherezov, V., Rosenbaum, D. M., Hanson, M. a, Søren, G. F., Thian, F. S., Kobilka, T. S., Choi, H., Kuhn, P., Weis, I., Kobilka, B. K., et al. (2007). High resolution crystal structure of an engineered human beta2-adrenergic G protein-coupled receptor. Science (80-.). *318*, 1258–1265.

Collette, K. (2010). Norepinephrine, through activation of α1A adrenergic receptors, stimulates the production of new neurons, leading to alleviation of depression and anxiety.

Collette, K., Zhou, X. D., Amoth, H. M., Lyons, M. J., Papay, R. S., Sens, D. a., Perez, D. M., Van Doze, A., and Doze, V. A. (2014). Long-term α1B-adrenergic receptor activation shortens lifespan, while α1A-adrenergic receptor stimulation prolongs lifespan in association with decreased cancer incidence. Age (Dordr). *36*, 1–10, 9675.

Colman, R. J., Anderson, R. M., Johnson, S. C., Kastman, E. K., Kosmatka, K. J., Beasley, T. M., Allison, D. B., Cruzen, C., Simmons, H. a, Kemnitz, J. W., et al. (2009). Caloric restriction delays disease onset and mortality in rhesus monkeys. Science *325*, 201–204.

Cottecchia, S., Schwinn, D. A., Randall, R. R., Lefkowitz, R., Caron, M. G., and Kobilka, B. K. (1988). Molecular cloning and expression of the cDNA for the hamster alpha 1-adrenergic receptor. Proc. Natl. Acad. Sci. U. S. A. *85*, 7159–7163.

Crawley, J. N., and Paylor, R. (1997). A proposed test battery and constellations of specific behavioral paradigms to investigate the behavioral phenotypes of transgenic and knockout mice. Horm. Behav. *31*, 197–211.

Cunha, M. P., Pazini, F. L., Oliveira, Á., Bettio, L. E. B., Rosa, J. M., Machado, D. G., and Rodrigues, A. L. S. (2013). The activation of α1-adrenoceptors is implicated in the antidepressant-like effect of creatine in the tail suspension test. Prog. Neuropsychopharmacol. Biol. Psychiatry *44*, 39–50.

Danysz, W., Kostowski, W., Kozak, W., and Hauptmann, M. (1986). On the role of noradrenergic neurotransmission in the action of desipramine and amitriptyline in animal models of depression. Pol. J. Pharmacol. Pharm. *38*, 285–298.

David, D. J., Samuels, B. A., Rainer, Q., Wang, J.-W. W., Marsteller, D., Mendez, I., Drew, M. R., Craig, D. a, Guiard, B. P., Guilloux, J.-P. P., et al. (2009). Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. Neuron *62*, 479–493.

Davies, B. T., and Wellman, P. J. (1992). Effects on ingestive behavior in rats of the alpha 1-adrenoceptor agonist cirazoline. Eur. J. Pharmacol. *210*, 11–16.

Day, H. E., Campeau, S., Watson, S. J., and Akil, H. (1997). Distribution of alpha 1a-, alpha 1b- and alpha 1d-adrenergic receptor mRNA in the rat brain and spinal cord. J. Chem. Neuroanat. *13*, 115–139.

Dayer, A. G., Ford, A. A., Cleaver, K. M., Yassaee, M., and Cameron, H. (2003). Short-term and long-term survival of new neurons in the rat dentate gyrus. J. Comp. Neurol. *460*, 563–572.

Deacon, R. M. J., and Rawlins, J. N. P. (2006). T-maze alternation in the rodent. Nat. Protoc. *1*, 7–12.

Denny, C. A., Burghardt, N. S., Schachter, D. M., Hen, R., and Drew, M. R. (2012). 4- to 6-week-old adult-born hippocampal neurons influence novelty-evoked exploration and contextual fear conditioning. Hippocampus *22*, 1188–1201.

Devesa, P., Agasse, F., Xapelli, S., Almengló, C., Devesa, J., Malva, J. O., and Arce, V. M. (2014). Growth hormone pathways signaling for cell proliferation and survival in hippocampal neural precursors from postnatal mice. BMC Neurosci. *15*, 100.

Diviani, D., Lattion, A. L., Larbi, N., Kunapuli, P., Pronin, A., Benovic, J. L., and Cottecchia, S. (1996). Effect of different G protein-coupled receptor kinases on phosphorylation and desensitization of the alpha1B-adrenergic receptor. J. Biol. Chem. *271*, 5049–5058.

Dixon, R. A., Kobilka, B. K., Strader, D. J., Benovic, J. L., Dohlman, H. G., Frielle, T., Bolanowski, M. A., Bennett, C. D., Rands, E., Diehl, R. E., et al. (1986). Cloning of the gene and cDNA for mammalian beta-adrenergic receptor and homology with rhodopsin. Nature *321*, 75–79.

Domyancic, A. V, and Morilak, D. A. (1997). Distribution of alpha1A adrenergic receptor mRNA in the rat brain visualized by in situ hybridization. J. Comp. Neurol. *386*, 358–378.

Doze, V. A., Handel, E. M., Jensen, K. A., Darsie, B., Luger, E. J., Haselton, J. R., Talbot, J. N., and Rorabaugh, B. R. (2009). alpha1A- and alpha1B-adrenergic receptors differentially modulate antidepressant-like behavior in the mouse. Brain Res. *1285*, 148–157.

Doze, V. A., Papay, R. S., Goldenstein, B. L., Gupta, M. K., Collette, K., Nelson, B. W., Lyons, M. J., Davis, B. A., Luger, E. J., Wood, S. G., et al. (2011). Long-term α1A-adrenergic receptor stimulation improves synaptic plasticity, cognitive function, mood, and longevity. Mol. Pharmacol. *80*, 747–758.

Drew, L. J., Fusi, S., and Hen, R. (2013). Adult neurogenesis in the mammalian hippocampus: why the dentate gyrus? Learn. Mem. *20*, 710–729.

Du, X.-J., Gao, X.-M., Kiriazis, H., Moore, X.-L., Ming, Z., Su, Y., Finch, A. M., Hannan, R. a, Dart, A. M., and Graham, R. M. (2006). Transgenic alpha1A-adrenergic activation limits post-infarct ventricular remodeling and dysfunction and improves survival. Cardiovasc. Res. *71*, 735–743.

Easson, L. H., and Stedman, E. (1933). Studies on the relationship between chemical constitution and physiological action: Molecular dissymmetry and physiological activity. Biochem. J. *27*, 1257–1266.

Von Euler, U. S. (1951). Hormones of the sympathetic nervous system and the adrenal medulla. Br. Med. J. *1*, 105–108.

Farrens, D. L., Altenbach, C., Yang, K., Hubbell, W. L., and Khorana, H. G. (1996). Requirement of rigid-body motion of transmembrane helices for light activation of rhodopsin. Science *274*, 768–770.

Finch, A., Sarramegna, V., and Graham, R. (2006). Ligand binding, activation, and agonist trafficking. In The adrenergic receptors In the 21st century, D. Perez, ed. (Totowa, NJ: Humana Press), pp. 25–85.

Furchgott, R. F. (1967). The pharmacological differentiation of adrenergic receptors. Ann. N. Y. Acad. Sci. *139*, 553–570.

Gao, X.-M., Agrotis, A., Autelitano, D. J., Percy, E., Woodcock, E. A., Jennings, G. L., Dart, A. M., and Du, X.-J. (2003). Sex hormones and cardiomyopathic phenotype induced by cardiac beta 2-adrenergic receptor overexpression. Endocrinology *144*, 4097–4105.

García-Sáinz, J. a, Vázquez-Prado, J., and Villalobos-Molina, R. (2000). Alpha 1-adrenoceptors: subtypes, signaling, and roles in health and disease. Arch. Med. Res. *30*, 449–458.

Garthe, A., Behr, J., and Kempermann, G. (2009). Adult-generated hippocampal neurons allow the flexible use of spatially precise learning strategies. PLoS One *4*, 13.

Gibbs, M. E., and Bowser, D. N. (2010). Astrocytic adrenoceptors and learning: alpha1-adrenoceptors. Neurochem. Int. *57*, 404–410.

Gibbs, M. E., and Summers, R. J. (2001). Stimulation of alpha1-adrenoceptors inhibits memory consolidation in the chick. Eur. J. Neurosci. *14*, 1369–1376.

Gilman, A. G. (1984). G proteins and dual control of adenylate cyclase. Cell *36*, 577–579.

Gonzalez-Cabrera, P. J., Shi, T., Yun, J., McCune, D. F., Rorabaugh, B. R., and Perez, D. M. (2004). Differential regulation of the cell cycle by alpha1-adrenergic receptor subtypes. Endocrinology *145*, 5157–5167.

Grzanna, R., and Fritschy, J. M. (1991). Efferent projections of different subpopulations of central noradrenaline neurons. Prog. Brain Res. *88*, 89–101.

Gupta, M. K., Papay, R. S., Jurgens, C. W. D., Gaivin, R. J., Shi, T., Doze, V. A., and Perez, D. M. (2009). α1-Adrenergic Receptors Regulate Neurogenesis and Gliogenesis. Mol. Pharmacol. *76*, 314.

Hanft, G., and Gross, G. (1990). The effect of reserpine, desipramine and thyroid hormone on alpha 1a- and alpha 1b-adrenoceptor binding sites: evidence for a subtype-specific regulation. Br. J. Clin. Pharmacol. *30 Suppl 1*, 125S – 127S.

Hedden, S. L., Bose, J., Downey, K., Gfroerer, J., Han, B., Tice, P., Hughes, A., Lipari, J., Rachel, K., Muhuri, P., et al. (2013). Results from the 2012 National Survey on Drug Use and Health: Mental Health Findings (Rockville, MD).

Hieble, J. P., Bylund, D. B., Clarke, D. E., Eikenburg, D. C., Langer, S. Z., Lefkowitz, R., Minneman, K. P., and Ruffolo, R. R. (1995). International Union of Pharmacology. X. Recommendation for nomenclature of alpha 1-adrenoceptors: consensus update. Pharmacol. Rev. *47*, 267–270.

Hillman, K. L., Doze, V. A., and Porter, J. E. (2007). Alpha1A-adrenergic receptors are functionally expressed by a subpopulation of cornu ammonis 1 interneurons in rat hippocampus. J. Pharmacol. Exp. Ther. *321*, 1062–1068.

Hillman, K. L., Knudson, C. a, Carr, P. a, Doze, V. A., and Porter, J. E. (2005). Adrenergic receptor characterization of CA1 hippocampal neurons using real time single cell RT-PCR. Brain Res. Mol. Brain Res. *139*, 267–276.

Hoffman, B. B., and Hu, Z. W. (2000). Alpha(1)-adrenoceptors (alpha(1)-AR) and vascular smooth muscle cell growth. Prostate. Suppl. *9*, 29–33.

Horie, K., Obika, K., Foglar, R., and Tsujimoto, G. (1995). Selectivity of the imidazoline alpha-adrenoceptor agonists (oxymetazoline and cirazoline) for human cloned alpha 1-adrenoceptor subtypes. Br. J. Pharmacol. *116*, 1611–1618.

Hosoda, C., Koshimizu, T.-A., Tanoue, A., Nasa, Y., Oikawa, R., Tomabechi, T., Fukuda, S., Shinoura, H., Oshikawa, S., Takeo, S., et al. (2005). Two alpha1-adrenergic receptor subtypes regulating the vasopressor response have differential roles in blood pressure regulation. Mol. Pharmacol. *67*, 912–922.

Hu, Z. W., Shi, X. Y., Lin, R. Z., and Hoffman, B. B. (1996). Alpha1 adrenergic receptors activate phosphatidylinositol 3-kinase in human vascular smooth muscle cells. Role in mitogenesis. J. Biol. Chem. *271*, 8977–8982.

Hu, Z. W., Shi, X.-Y. Y., Lin, R. Z., and Hoffman, B. B. (1999). Contrasting signaling pathways of alpha1A- and alpha1B-adrenergic receptor subtype activation of phosphatidylinositol 3-kinase and Ras in transfected NIH3T3 cells. Mol. Endocrinol. *13*, 3–14.

Huang, Y., Wright, C. D., Merkwan, C. L., Baye, N. L., Liang, Q., Simpson, P. C., and O'Connell, T. D. (2007). An alpha1A-adrenergic-extracellular signal-regulated kinase survival signaling pathway in cardiac myocytes. Circulation *115*, 763–772.

Ikeno, Y., Hubbard, G. B., Lee, S., Richardson, A., Strong, R., Diaz, V., and Nelson, J. F. (2005). Housing density does not influence the longevity effect of calorie restriction. J. Gerontol. A. Biol. Sci. Med. Sci. *60*, 1510–1517.

Izumi, J., Washizuka, M., Hayashi-Kuwabara, Y., Yoshinaga, K., Tanaka, Y., Ikeda, Y., Kiuchi, Y., and Oguchi, K. (1997). Protective effect of citalopram against the attenuation of the α1-potentiation of CAMP formation in Fischer 344 strain rats. Behav. Brain Res. *83*, 209–212.

Izumi, Y., and Zorumski, C. F. (1999). Norepinephrine promotes long-term potentiation in the adult rat hippocampus in vitro. Synapse *31*, 196–202.

Jhaveri, D., Nanavaty, I., Prosper, B. W., Marathe, S., Husain, B. F. a, Kernie, S. G., Bartlett, P. F., and Vaidya, V. a (2014). Opposing effects of α2- and β-adrenergic receptor stimulation on quiescent neural precursor cell activity and adult hippocampal neurogenesis. PLoS One *9*, e98736.

Jones, B. E., Halaris, A. E., McIlhany, M., and Moore, R. Y. (1977). Ascending projections of the locus coeruleus in the rat. I. Axonal transport in central noradrenaline neurons. Brain Res. *127*, 1–21.

Jones, B. E., and Moore, R. Y. (1977). Ascending projections of the locus coeruleus in the rat. II. Autoradiographic study. Brain Res. *127*, 25–53.

Jones, L. S., Miller, G., Gauger, L. L., and Davis, J. N. (1985). Regional distribution of rat brain alpha 1-adrenergic receptors: correlation between (125I)-heat membrane binding and in vitro autoradiography. Life Sci. *36*, 45–51.

Kakui, N., Yokoyama, F., Yamauchi, M., Kitamura, K., Imanishi, T., Inoue, T., and Koyama, T. (2009). Anxiolytic-like profile of mirtazapine in rat conditioned fear stress model: Functional significance of 5-hydroxytryptamine 1A receptor and ??1-adrenergic receptor. Pharmacol. Biochem. Behav. *92*, 393–398.

Kanda, H., Ishii, K., Ogura, Y., Imamura, T., Kanai, M., Arima, K., and Sugimura, Y. (2008). Naftopidil, a selective alpha-1 adrenoceptor antagonist, inhibits growth of human prostate cancer cells by G1 cell cycle arrest. Int. J. Cancer *122*, 444–451.

Kaster, M. P., Raupp, I., Binfaré, R. W., Andreatini, R., and Rodrigues, A. L. S. (2007). Antidepressant-like effect of lamotrigine in the mouse forced swimming test: evidence for the involvement of the noradrenergic system. Eur. J. Pharmacol. *565*, 119–124.

Kempermann, G., and Gage, F. H. (2002). Genetic influence on phenotypic differentiation in adult hippocampal neurogenesis. Brain Res. Dev. Brain Res. *134*, 1–12.

Kempermann, G., Gast, D., Kronenberg, G., Yamaguchi, M., and Gage, F. H. (2003). Early determination and long-term persistence of adult-generated new neurons in the hippocampus of mice. Development *130*, 391–399.

Kessler, R. C., Berglund, P., Demler, O., Jin, R., Koretz, D., Merikangas, K. R. K., Rush, A. J., Walters, E. E. E., and Wang, P. S. (2003). National Comorbidity Survey Replication: the epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R). J. Am. Med. Assoc. *289*, 3095–3105.

Keyes, W. M., Wu, Y., Vogel, H., Guo, X., Lowe, S. W., and Mills, A. A. (2005). p63 deficiency activates a program of cellular senescence and leads to accelerated aging. Genes Dev. *19*, 1986–1999.

Kitada, Y., Miyauchi, T., Kanazawa, Y., Nakamichi, H., and Satoh, S. (1983). Involvement of alpha- and beta 1-adrenergic mechanisms in the immobility-reducing action of desipramine in the forced swimming test. Neuropharmacology *22*, 1055–1060.

Knauber, J., and Müller, W. E. (2000). Decreased exploratory activity and impaired passive avoidance behaviour in mice deficient for the α(1b)-adrenoceptor. Eur. Neuropsychopharmacol. *10*, 423–427.

Lalonde, R. (2002). The neurobiological basis of spontaneous alternation. Neurosci. Biobehav. Rev. *26*, 91–104.

Lands, A. M., Arnold, A., McAuliff, J. P., Luduena, F. P., and Brown, T. G. (1967). Differentiation of receptor systems activated by sympathomimetic amines. Nature *214*, 597–598.

Lefkowitz, R. (1998). G protein-coupled receptors. III. New roles for receptor kinases and beta-arrestins in receptor signaling and desensitization. J. Biol. Chem. *273*, 18677–18680.

Lie, D. C., Colamarino, S. A., Song, H., Désiré, L., Mira, H., Consiglio, A., Lein, E. S., Jessberger, S., Lansford, H., Dearie, A. R., et al. (2005). Wnt signalling regulates adult hippocampal neurogenesis. Nature *437*, 1370–1375.

Loughlin, S. E., Foote, S. L., and Grzanna, R. (1986). Efferent projections of nucleus locus coeruleus: morphologic subpopulations have different efferent targets. Neuroscience *18*, 307–319.

Maguire, M. E., Van Arsdale, P. M., and Gilman, a G. (1976). An agonist-specific effect of guanine nucleotides on binding to the beta adrenergic receptor. Mol. Pharmacol. *12*, 335–339.

Mao, Z. M., Arnsten, A. F., and Li, B. M. (1999). Local infusion of an alpha-1 adrenergic agonist into the prefrontal cortex impairs spatial working memory performance in monkeys. Biol. Psychiatry *46*, 1259–1265.

Masoro, E. J. (2005). Overview of caloric restriction and ageing. Mech. Ageing Dev. *126*, 913–922.

Mayorga, A. J., and Lucki, I. (2001). Limitations on the use of the C57BL/6 mouse in the tail suspension test. Psychopharmacology (Berl). *155*, 110–112.

McCune, S. K., Voigt, M. M., and Hill‡, J. M. (1993). Expression of multiple alpha adrenergic receptor subtype messenger RNAs in the adult rat brain. Neuroscience *57*, 143–151.

McKhann, G. M., Wenzel, H. J., Robbins, C. a., Sosunov, a. a., and Schwartzkroin, P. a. (2003). Mouse strain differences in kainic acid sensitivity, seizure behavior, mortality, and hippocampal pathology. Neuroscience *122*, 551–561.

Messenger, N. J., Rowe, S. J., and Warner, a E. (1999). The neurotransmitter noradrenaline drives noggin-expressing ectoderm cells to activate N-tubulin and become neurons. Dev. Biol. *205*, 224–232.

Michelotti, G. A., Price, D. T., and Schwinn, D. A. (2000). Alpha 1-adrenergic receptor regulation: basic science and clinical implications. Pharmacol. Ther. *88*, 281–309.

Milano, C. A., Dolber, P. C., Rockman, H. A., Bond, R. A., Venable, M. E., Allen, L. F., and Lefkowitz, R. (1994). Myocardial expression of a constitutively active alpha 1B-adrenergic receptor in transgenic mice induces cardiac hypertrophy. Proc. Natl. Acad. Sci. U. S. A. *91*, 10109–10113.

Minneman, K. P., Han, C., and Abel, P. W. (1988). Comparison of alpha 1-adrenergic receptor subtypes distinguished by chlorethylclonidine and WB 4101. Mol. Pharmacol. *33*, 509–514.

Mishima, K., Tanoue, A., Tsuda, M., Hasebe, N., Fukue, Y., Egashira, N., Takano, Y., Kamiya, H.-O., Tsujimoto, G., Iwasaki, K., et al. (2004). Characteristics of behavioral abnormalities in alpha1d-adrenoceptors deficient mice. Behav. Brain Res. *152*, 365–373.

Molchan, S. E., Lawlor, B. A., Hill, J. L., Martinez, R. A., Davis, C. L., Mellow, A. M., Rubinow, D. R., and Sunderland, T. (1991). CSF monoamine metabolites and somatostatin in Alzheimer's disease and major depression. Biol. Psychiatry *29*, 1110–1118.

Morien, A., McMahon, L., and Wellman, P. J. (1993). Effects on food and water intake of the alpha 1-adrenoceptor agonists amidephrine and SK&F-89748. Life Sci. *53*, 169–174.

Morrow, A. L., and Creese, I. (1986). Characterization of alpha 1-adrenergic receptor subtypes in rat brain: a reevaluation of [3H]WB4104 and [3H]prazosin binding. Mol. Pharmacol. *29*, 321–330.

Murchison, C. F., Zhang, X.-Y., Zhang, W.-P., Ouyang, M., Lee, A., and Thomas, S. A. (2004). A distinct role for norepinephrine in memory retrieval. Cell *117*, 131–143.

Nadon, N. L., Strong, R., Miller, R. A., Nelson, J., Javors, M., Sharp, Z. D., Peralba, J. M., and Harrison, D. E. (2008). Design of aging intervention studies: the NIA interventions testing program. Age (Dordr). *30*, 187–199.

Nagayasu, K., Kitaichi, M., Nishitani, N., Asaoka, N., Shirakawa, H., Nakagawa, T., and Kaneko, S. (2013). Chronic effects of antidepressants on serotonin release in rat raphe slice cultures: high potency of milnacipran in the augmentation of serotonin release. Int. J. Neuropsychopharmacol. *16*, 2295–2306.

Nalepa, I., Kreiner, G., Kowalska, M., Sanak, M., Zelek-Molik, A., and Vetulani, J. (2002). Repeated imipramine and electroconvulsive shock increase alpha 1A-adrenoceptor mRNA level in rat prefrontal cortex. Eur. J. Pharmacol. *444*, 151–159.

Northup, J. K., Sternweis, P. C., Smigel, M. D., Schleifer, L. S., Ross, E. M., and Gilman, A. G. (1980). Purification of the regulatory component of adenylate cyclase. Proc. Natl. Acad. Sci. U. S. A. *77*, 6516–6520.

Novakova, M., Kvetnansky, R., and Myslivecek, J. (2010). Sexual dimorphism in stress-induced changes in adrenergic and muscarinic receptor densities in the lung of wild type and corticotropin-releasing hormone-knockout mice. Stress *13*, 22–35.

O'Connell, T. D., Ishizaka, S., Nakamura, A., Swigart, P. M., Rodrigo, M. C., Simpson, G. L., Cotecchia, S., Rokosh, D. G., Grossman, W., Foster, E., et al. (2003). The alpha(1A/C)- and alpha(1B)-adrenergic receptors are required for physiological cardiac hypertrophy in the double-knockout mouse. J. Clin. Invest. *111*, 1783–1791.

O'Connell, T. D., Jensen, B. C., Baker, A. J., Simpson, P. C., Connell, T. D. O., Jensen, B. C., Baker, A. J., and Simpson, P. C. (2014). Cardiac alpha1-adrenergic receptors: novel aspects of expression, signaling mechanisms, physiologic function, and clinical importance. Pharmacol. Rev. *66*, 308–333.

Oh, J., McCloskey, M. A., Blong, C. C., Bendickson, L., Nilsen-Hamilton, M., and Sakaguchi, D. S. (2010). Astrocyte-derived interleukin-6 promotes specific neuronal differentiation of neural progenitor cells from adult hippocampus. J. Neurosci. Res. *88*, 2798–2809.

Ohashi, H., Nishikawa, K., Ayukawa, K., Hara, Y., Nishimoto, M., Kudo, Y., Abe, T., Arima, K., Wada, K., Aoki, S., et al. (2007). Alpha 1-adrenoceptor agonists protect against stress-induced death of neural progenitor cells. Eur. J. Pharmacol. *573*, 20–28.

Ohm, T. G., Busch, C., and Bohl, J. (1997). Unbiased estimation of neuronal numbers in the human nucleus coeruleus during aging. Neurobiol. Aging *18*, 393–399.

Okazaki, M., Hu, Z. W., Fujinaga, M., and Hoffman, B. B. (1994). Alpha 1 adrenergic receptor-induced c-fos gene expression in rat aorta and cultured vascular smooth muscle cells. J. Clin. Invest. *94*, 210–218.

Outschoorn, A. S. (1952). The hormones of the adrenal medulla and their release. Br. J. Pharmacol. Chemother. *7*, 605–615.

Palacios, J. M., Hoyer, D., and Cortes, R. (1987). [alpha] 1-adrenoceptors in the mammalian brain: similar pharmacology but different distribution in rodents and primates. Brain Res. *419*, 65–75.

Papapetropoulos, S., Tuchman, A., Laufer, D., Papatsoris, A., Papapetropoulos, N., and Mash, D. (2007). Causes of death in multiple system atrophy. J. Neurol. Neurosurg. Psychiatry *78*, 327–329.

Papay, R. S., Gaivin, R. J., Jha, A., McCune, D. F., Mcgrath, J. C., Rodrigo, M. C., Simpson, P. C., Doze, V. A., and Perez, D. M. (2006). Localization of the mouse α 1A-adrenergic receptor (AR) in the brain: α 1AAR is expressed in neurons, GABAergic interneurons, and NG2 oligodendrocyte progenitors. J. Comp. Neurol. *222*, 209–222.

Papay, R. S., Gaivin, R., McCune, D. F., Rorabaugh, B. R., Macklin, W. B., McGrath, J. C., and Perez, D. M. (2004). Mouse alpha1B-adrenergic receptor is expressed in neurons and NG2 oligodendrocytes. J. Comp. Neurol. *478*, 1–10.

Papay, R. S., Shi, T., Piascik, M. T., Naga Prasad, S. V, and Perez, D. M. (2013). α_1A-adrenergic receptors regulate cardiac hypertrophy in vivo through interleukin-6 secretion. Mol. Pharmacol. *83*, 939–948.

Papay, R. S., Zuscik, M. J., Ross, S. A., Yun, J., McCune, D. F., Gonzalez-Cabrera, P. J., Gaivin, R. J., Drazba, J., and Perez, D. M. (2002). Mice expressing the alpha(1B)-adrenergic receptor induces a synucleinopathy with excessive tyrosine nitration but decreased phosphorylation. J. Neurochem. *83*, 623–634.

Papp, M. I., Kahn, J. E., and Lantos, P. L. (1989). Glial cytoplasmic inclusions in the CNS of patients with multiple system atrophy (striatonigral degeneration, olivopontocerebellar atrophy and Shy-Drager syndrome). J. Neurol. Sci. *94*, 79–100.

Pei, L., Li, S., Wang, M., Diwan, M., Anisman, H., Fletcher, P. J., Nobrega, J. N., and Liu, F. (2010). Uncoupling the dopamine D1-D2 receptor complex exerts antidepressant-like effects. Nat. Med. *16*, 1393–1395.

Perez, D. M., and Doze, V. A. (2011). Cardiac and neuroprotection regulated by α(1)-adrenergic receptor subtypes. J. Recept. Signal Transduct. Res. *31*, 98–110.

Perez, D. M., Piascik, M. T., and Graham, R. M. (1991). Solution-phase library screening for the identification of rare clones: isolation of an alpha 1D-adrenergic receptor cDNA. Mol. Pharmacol. *40*, 876–883.

Perez, D. M., Piascik, M. T., Malik, N., Gaivin, R., and Graham, R. M. (1994). Cloning, expression, and tissue distribution of the rat homolog of the bovine alpha 1C-adrenergic receptor provide evidence for its classification as the alpha 1A subtype. Mol. Pharmacol. *46*, 823–831.

Perry, C., Kellar, J., Blendy, J. A., Grimm, L. J., Perry, D. C., West-Johnsrud, L., Kellar, K. J., Perry, C., and Kellar, J. (1990). Electroconvulsive shock differentially increases binding to alpha-1 adrenergic receptor subtypes in discrete regions of rat brain. J. Neurosci. *10*, 2580–2586.

Piatti, V. C., Ewell, L. A., and Leutgeb, J. K. (2013). Neurogenesis in the dentate gyrus: carrying the message or dictating the tone. Front. Neurosci. *7*, 50.

Piccinato, C. A., Montrezor, L. H., Collares, C. A. V, Vireque, A. A., and Rosa e Silva, A. A. M. (2012). Norepinephrine stimulates progesterone production in highly estrogenic bovine granulosa cells cultured under serum-free, chemically defined conditions. Reprod. Biol. Endocrinol. *10*, 95.

Pieribone, V. A., Nicholas, A. P., Dagerlind, A., and Hökfelt, T. (1994). Distribution of alpha 1 adrenoceptors in rat brain revealed by in situ hybridization experiments utilizing subtype-specific probes. J. Neurosci. *14*, 4252–4268.

Pizzanelli, C., Lazzeri, G., Fulceri, F., Giorgi, F. S., Pasquali, L., Cifelli, G., Murri, L., and Fornai, F. (2009). Lack of alpha 1b-adrenergic receptor protects against epileptic seizures. Epilepsia *50 Suppl 1*, 59–64.

Pohl, S. L., Birnbaumer, L., and Rodbell, M. (1971). The glucagon-sensitive adenyl cyclase system in plasma membranes of rat liver. I. Properties. J. Biol. Chem. *246*, 1849–1856.

Price, R. R., Morris, D. P., Biswas, G., Smith, M. P., and Schwinn, D. A. (2002). Acute Agonist-mediated Desensitization of the Human 1a-Adrenergic Receptor Is Primarily Independent of Carboxyl Terminus Regulation: IMPLICATIONS FOR REGULATION OF 1aAR SPLICE VARIANTS. J. Biol. Chem. *277*, 9570–9579.

Pulvirenti, L., and Samanin, R. (1986). Antagonism by dopamine, but not noradrenaline receptor blockers of the anti-immobility activity of desipramine after different treatment schedules in the rat. Pharmacol. Res. Commun. *18*, 73–80.

Pussinen, R., and Sirviö, J. (1998). Minor role for alpha1-adrenoceptors in the facilitation of induction and early maintenance of long-term potentiation in the CA1 field of the hippocampus. J. Neurosci. Res. *51*, 309–315.

Puumala, T., Greijus, S., Narinen, K., Haapalinna, A., Riekkinen, P., and Sirviö, J. (1998). Stimulation of alpha-1 adrenergic receptors facilitates spatial learning in rats. Eur. Neuropsychopharmacol. *8*, 17–26.

Puumala, T., and Sirviö, J. (1997). Stimulation and blockade of alpha1 adrenoceptors affect behavioural activity, but not spatial working memory assessed by delayed non-matching to position task in rats. J. Psychopharmacol. *11*, 45–51.

Puumala, T., Sirviö, J., Ruotsalainen, S., and Riekkinen, P. (1996). Effects of St-587 and prazosin on water maze and passive avoidance performance of scopolamine-treated rats. Pharmacol. Biochem. Behav. *55*, 107–115.

Ramos, B. P., and Arnsten, A. F. T. (2007). Adrenergic pharmacology and cognition: Focus on the prefrontal cortex. Pharmacol. Ther. *113*, 523–536.

Rasmussen, S. G. F., DeVree, B. T., Zou, Y., Kruse, A. C., Chung, K. Y., Kobilka, T. S., Thian, F. S., Chae, P. S., Pardon, E., Calinski, D., et al. (2011). Crystal structure of the β2 adrenergic receptor-Gs protein complex. Nature *477*, 549–555.

Ray, M. A., Johnston, N. A., Verhulst, S., Trammell, R. A., and Toth, L. A. (2010). Identification of markers for imminent death in mice used in longevity and aging research. J. Am. Assoc. Lab. Anim. Sci. *49*, 282–288.

Riekkinen, M., Stefanski, R., Kuitunen, J., and Riekkinen, P. (1996). Effects of combined block of alpha 1-adrenoceptors and NMDA receptors on spatial and passive avoidance behavior in rats. Eur. J. Pharmacol. *300*, 9–16.

Roberts, D. J., and Waelbroeck, M. (2004). G protein activation by G protein coupled receptors: Ternary complex formation or catalyzed reaction? Biochem. Pharmacol. *68*, 799–806.

Rogers, D. C., Fisher, E. M., Brown, S. D., Peters, J., Hunter, a J., and Martin, J. E. (1997). Behavioral and functional analysis of mouse phenotype: SHIRPA, a proposed protocol for comprehensive phenotype assessment. Mamm. Genome *8*, 711–713.

Rokosh, D. G., and Simpson, P. C. (2002). Knockout of the alpha 1A/C-adrenergic receptor subtype: the alpha 1A/C is expressed in resistance arteries and is required to maintain arterial blood pressure. Proc. Natl. Acad. Sci. U. S. A. *99*, 9474–9479.

Romeo, H. E., Colombo, L. L., Esquifino, A. I., Rosenstein, R. E., Chuluyan, H. E., and Cardinali, D. P. (1991). Slower growth of tumours in sympathetically denervated murine skin. J. Auton. Nerv. Syst. *32*, 159–164.

Rorabaugh, B. R., Ross, S. A., Gaivin, R. J., Papay, R. S., McCune, D. F., Simpson, P. C., and Perez, D. M. (2005). alpha1A- but not alpha1B-adrenergic receptors precondition the ischemic heart by a staurosporine-sensitive, chelerythrine-insensitive mechanism. Cardiovasc. Res. *65*, 436–445.

Rowe, S. J., Messenger, N. J., and Warner, a E. (1993). The role of noradrenaline in the differentiation of amphibian embryonic neurons. Development *119*, 1343–1357.

Rudolph, K. L., Chang, S., Lee, H. W., Blasco, M., Gottlieb, G. J., Greider, C., and DePinho, R. A. (1999). Longevity, stress response, and cancer in aging telomerase-deficient mice. Cell *96*, 701–712.

Ruffolo, R. R., Waddell, J., and Ruffolo, R. (1982). Receptor Interactions Adrenergic Antagonist of Imidazolines. IX. Cirazoline is an Agonist and an Alpha-2 Adrenergic. J. Pharmacol. Exp. Ther. *222*, 29–36.

Saeed, A. E., Parmentier, J.-H., and Malik, K. U. (2004). Activation of alpha1A-adrenergic receptor promotes differentiation of rat-1 fibroblasts to a smooth muscle-like phenotype. BMC Cell Biol. *5*, 47.

Sahay, A., Scobie, K. N., Hill, A. S., O'Carroll, C. M., Kheirbek, M. A., Burghardt, N. S., Fenton, A. A., Dranovsky, A., and Hen, R. (2011). Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation. Nature *472*, 466–470.

Santulli, G., and Iaccarino, G. (2013). Pinpointing beta adrenergic receptor in ageing pathophysiology: victim or executioner? Evidence from crime scenes. Immun. ageing *10*, 1–13.

Santulli, G., Lombardi, A., Sorriento, D., Anastasio, A., Del Giudice, C., Formisano, P., Béguinot, F., Trimarco, B., Miele, C., and Iaccarino, G. (2012). Age-related impairment in insulin release: the essential role of β(2)-adrenergic receptor. Diabetes *61*, 692–701.

Sara, S. J., and Devauges, V. (1988). Priming stimulation of locus coeruleus facilitates memory retrieval in the rat. Brain Res. *438*, 299–303.

Schwinn, D. A., Lomasney, J. W., Lorenz, W., Szklut, P. J., Fremeau, R. T., Yang-Feng, T. L., Caron, M. G., Lefkowitz, R., Cottecchia, S., Leung, W. Y., et al. (1991). Molecular cloning and expression of the cDNA for the alpha 1A-adrenergic receptor. J. Biol. Chem. *265*, 8183–8189.

Segal, M., Markram, H., and Richter-Levin, G. (1991). Actions of norepinephrine in the rat hippocampus. Prog. Brain Res. *88*, 323–330.

Segal, M., Pickel, V., and Bloom, F. (1973). The projections of the nucleus locus coeruleus: an autoradiographic study. Life Sci. *13*, 817–821.

Selman, C., and Withers, D. J. (2011). Mammalian models of extended healthy lifespan. Philos. Trans. R. Soc. Lond. B. Biol. Sci. *366*, 99–107.

Shi, T., Duan, Z.-H., Papay, R. S., Pluskota, E., Gaivin, R. J., de la Motte, C. A., Plow, E. F., and Perez, D. M. (2006). Novel α1-Adrenergic Receptor Signaling Pathways: Secreted Factors and Interactions with the Extracellular Matrix. Mol. Pharmacol. *70*, 129–142.

Shi, T., Gaivin, R. J., McCune, D. F., Gupta, M. K., and Perez, D. M. (2007). Dominance of the alpha1B-adrenergic receptor and its subcellular localization in human and TRAMP prostate cancer cell lines. J. Recept. Signal Transduct. Res. *27*, 27–45.

Shibata, K., Katsuma, S., Koshimizu, T., Shinoura, H., Hirasawa, A., Tanoue, A., and Tsujimoto, G. (2003). Alpha 1-Adrenergic receptor subtypes differentially control the cell cycle of transfected CHO cells through a cAMP-dependent mechanism involving p27Kip1. J. Biol. Chem. *278*, 672–678.

Sirviö, J., and MacDonald, E. (1999). Central α1-adrenoceptors: Their role in the modulation of attention and memory formation. Pharmacol. Ther. *83*, 49–65.

Song, H., Stevens, C. F., and Gage, F. H. (2002). Astroglia induce neurogenesis from adult neural stem cells. Nature *417*, 39–44.

Spreng, M., Cottecchia, S., and Schenk, F. (2001). A behavioral study of alpha-1b adrenergic receptor knockout mice: increased reaction to novelty and selectively reduced learning capacities. Neurobiol. Learn. Mem. *75*, 214–229.

Stanasila, L., Abuin, L., Dey, J., and Cottecchia, S. (2008). Different internalization properties of the alpha1a- and alpha1b-adrenergic receptor subtypes: the potential role of receptor interaction with beta-arrestins and AP50. Mol. Pharmacol. *74*, 562–573.

Steiner, B., Kronenberg, G., Jessberger, S., Brandt, M. D., Reuter, K., and Kempermann, G. (2004). Differential regulation of gliogenesis in the context of adult hippocampal neurogenesis in mice. Glia *46*, 41–52.

Stone, E. A., Lin, Y., Sarfraz, Y., and Quartermain, D. (2011). Antidepressant-like action of intracerebral 6-fluoronorepinephrine, a selective full α-adrenoceptor agonist. Int. J. Neuropsychopharmacol. *14*, 319–331.

Strader, C. D., Sigal, I. S., Register, R. B., Candelore, M. R., Rands, E., and Dixon, R. A. (1987). Identification of residues required for ligand binding to the beta-adrenergic receptor. Proc. Natl. Acad. Sci. U. S. A. *84*, 4384–4388.

Sugimoto, Y., Yamamoto, M., Tagawa, N., Kobayashi, Y., Mitsui-Saitoh, K., Hotta, Y., and Yamada, J. (2011). Differences between mice strains in response to paroxetine in the forced swimming test: involvement of serotonergic or noradrenergic systems. Eur. J. Pharmacol. *672*, 121–125.

Suh, H., Deng, W., and Gage, F. H. (2009). Signaling in adult neurogenesis. Annu. Rev. Cell Dev. Biol. *25*, 253–275.

Sun, L.-Q., Lee, D. W., Zhang, Q., Xiao, W., Raabe, E. H., Meeker, A., Miao, D., Huso, D. L., and Arceci, R. J. (2004). Growth retardation and premature aging phenotypes in mice with disruption of the SNF2-like gene, PASG. Genes Dev. *18*, 1035–1046.

Swanson, L. W., and Hartman, B. K. (1975). The central adrenergic system. An immunofluorescence study of the location of cell bodies and their efferent connections in the rat utilizing dopamine-beta-hydroxylase as a marker. J. Comp. Neurol. *163*, 467–505.

Szot, P., White, S. S., Greenup, J. L., Leverenz, J. B., Peskind, E. R., and Raskind, M. a (2005). Alpha1-adrenoreceptor in human hippocampus: binding and receptor subtype mRNA expression. Brain Res. Mol. Brain Res. *139*, 367–371.

Tanoue, A., Nasa, Y., Koshimizu, T., Shinoura, H., Oshikawa, S., Kawai, T., Sunada, S., Takeoand, S., and Tsujimoto, G. (2002). The alpha1D-adrenergic receptor directly regulates arterial blood pressure via vasoconstriction. J. Clin. Invest. *109*, 765–775.

The Jackson Laboratory (2013). S.O.S. Colony Adrift: Protecting Mouse Colonies from Genetic Drift. In Webinar.

Thyagarajan, T., Totey, S., Danton, M. J. S., and Kulkarni, A. B. (2003). Genetically altered mouse models: the good, the bad, and the ugly. Crit. Rev. Oral Biol. Med. *14*, 154–174.

Toews, M. L., Prinster, S. C., and Schulte, N. A. (2003). Regulation of alpha-1B adrenergic receptor localization, trafficking, function, and stability. Life Sci. *74*, 379–389.

Torkaman-Boutorabi, A., Danyali, F., Oryan, S., Ebrahimi-Ghiri, M., and Zarrindast, M.-R. (2014). Hippocampal α-adrenoceptors involve in the effect of histamine on spatial learning. Physiol. Behav. *129*, 17–24.

Trivedi, M., Rush, A. J., Wisniewski, S. R., Nierenberg, A. A., Warden, D., Ritz, L., Norquist, G., Howland, R. H., Lebowitz, B., McGrath, P. J., et al. (2006). Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: implications for clinical practice. Am. J. Psychiatry *163*, 28–40.

Uberti, M., Hall, R. A., and Minneman, K. P. (2003). Subtype-specific dimerization of alpha 1-adrenoceptors: effects on receptor expression and pharmacological properties. Mol. Pharmacol. *64*, 1379–1390.

Vázquez-Prado, J., Medina, L. C., Romero-Avila, M. T., González-Espinosa, C., and García-Sáinz, J. A. (2000). Norepinephrine- and phorbol ester-induced phosphorylation of alpha(1a)-adrenergic receptors. Functional aspects. J. Biol. Chem. *275*, 6553–6559.

Vorhees, C. V., and Williams, M. M. T. (2006). Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nat. Protoc. *1*, 848–858.

Walker, T. L., and Kempermann, G. (2014). One mouse, two cultures: isolation and culture of adult neural stem cells from the two neurogenic zones of individual mice. J. Vis. Exp., e51225.

Wang, B. H. H., Du, X. J. J., Autelitano, D. J. J., Milano, C. A., and Woodcock, E. A. A. (2000). Adverse effects of constitutively active alpha-1B-adrenergic receptors after pressure overload in mouse hearts. Am. J. Physiol. Circ. Physiol. *279*, H1079–H1086.

Wang, J.-W., David, D. J., Monckton, J. E., Battaglia, F., and Hen, R. (2008). Chronic fluoxetine stimulates maturation and synaptic plasticity of adult-born hippocampal granule cells. J. Neurosci. *28*, 1374–1384.

Wang, P. S., Simon, G., and Kessler, R. C. (2003). The economic burden of depression and the cost-effectiveness of treatment. Int. J. Methods Psychiatr. Res. *12*, 22–33.

Wellman, P. J., and Davies, B. T. (1992). Reversal of cirazoline- and phenylpropanolamine-induced anorexia by the alpha 1-receptor antagonist prazosin. Pharmacol. Biochem. Behav. *42*, 97–100.

Wilson, K. M., and Minneman, K. P. (1989). Regional variations in alpha 1-adrenergic receptor subtypes in rat brain. J. Neurochem. *53*, 1782–1786.

Yuan, R., Tsaih, S.-W. W. S., Petkova, S. B. S., De Evsikova, C. M., Xing, S., Marion, M. A., Bogue, M. A., Mills, K. D., Peters, L. L., Bult, C. J., et al. (2009). Aging in inbred strains of mice: study design and interim report on median lifespans and circulating IGF1 levels. Aging Cell *8*, 277–287.

Yun, J., Gaivin, R. J., McCune, D. F., Boongird, A., Papay, R. S., Ying, Z., Gonzalez-Cabrera, P. J., Najm, I., and Perez, D. M. (2003). Gene expression profile of neurodegeneration induced by alpha1B-adrenergic receptor overactivity: NMDA/GABAA dysregulation and apoptosis. Brain *126*, 2667–2681.

Zhao, L., Yang, F., Xu, K., Cao, H., Zheng, G.-Y., Zhang, Y., Li, J., Cui, H., Chen, X., Zhu, Z., et al. (2012). Common genetic variants of the β2-adrenergic receptor affect its translational efficiency and are associated with human longevity. Aging Cell *11*, 1094–1101.

Zuscik, M. J., Chalothorn, D., Hellard, D., Deighan, C., McGee, a, Daly, C. J., Waugh, D. J., Ross, S. a, Gaivin, R. J., Morehead, a J., et al. (2001). Hypotension, autonomic failure, and cardiac hypertrophy in transgenic mice overexpressing the alpha 1B-adrenergic receptor. J. Biol. Chem. *276*, 13738–13743.

Zuscik, M. J., Sands, S., Ross, S. a, Waugh, D. J., Gaivin, R. J., Morilak, D., and Perez, D. M. (2000). Overexpression of the alpha1B-adrenergic receptor causes apoptotic neurodegeneration: multiple system atrophy. Nat. Med. *6*, 1388–1394.

Van Zwieten, M., Zurcher, C., Solleveld, H., and Hollander, C. (1981). Pathology. In Immunological Techniques Applied to Aging Research, W. Adler and A. Nordin, eds. (Boca Raton, FL: CRC Press, inc), pp. 2–33.