DISSERTATION

EFFECTS OF INTERLEUKIN-1 RECEPTOR ANTAGONIST IN AGED RATS ON SPATIAL LEARNING IN THE MORRIS WATER MAZE

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY BRANDI SCRUGGS ENTITLED EFFECTS OF INTERLEUKIN-1 RECEPTOR ANTAGONIST IN AGED RATS ON SPATIAL LEARNING IN THE MORRIS WATER MAZE BE ACCETPED AS FULFILLING IN PART THE REQUIREMENT FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

EFFECTS OF INTERLEUKIN-1 RECEPTOR ANTAGONIST IN AGED RATS ON SPATIAL LEARNING IN THE MORRIS WATER MAZE

Aging is a prevalent problem that has stimulated a great deal of research recently. Various changes in the physiological and psychological functioning of the aged individual have led to growing research interest in improving the quality of life for the elderly. The current study focused on age-related deficits in cognition, particularly learning and memory. One factor linked to age-related changes in learning and memory is Interleukin-1β (IL-1β), which has been found in increased concentrations in aged animals, and has been shown to inhibit long-term potentiation and learning. The current study attempted to improve learning and memory in aged animals in the Morris water maze by injecting the Interleukin-1 receptor antagonist (IL-1ra) intracerebroventricularly to block the effects of IL-1β. Concentrations of IL-1β in the brains of old versus young animals were also measured. It was expected that old animals would have the highest levels of IL-1β compared to young animals. It was also expected that IL-1β concentration would be negatively correlated with performance in the Morris water maze. Results showed that injections of IL-1ra did not effectively improve the learning of aged animals in the water maze. Results also showed that, contrary to previous research, young animals had the highest levels of IL-1β in their brains, while aged animals had the lowest

concentrations. IL-1β concentrations were not correlated with performance in the water maze. Various interpretations of these results are discussed, as well as recommendations for future research.

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Chapter 1: Introduction

The problem of aging has become a pressing one in recent decades. Physiological and psychological changes associated with aging, as well as disease states that become more prevalent when growing older have raised concern over the quality of life for older individuals. It is believed that this upsurge in concern about aging and age-related issues is due to the fact that the number of aged people in our country has increased exponentially in recent times. It is estimated that the number of Americans over the age of 65 years has grown from 3.1 million in the year 1900 to almost 35 million in the year 2000. The number of people over age 65 years is projected to double to 69.4 million by the year 2030, when those born of the "baby boom" generation will reach age 65 (Whitbourne, 2002). Interestingly, not only is there an incredible growth of the aged population in our country, but the older population itself is getting older. For example, the oldest-old (people over age 85 years) are the fastest growing segment of our population (Hayflick, 1994). In fact, in 1985 the 65-74 year-old age group (17 million) was nearly eight times larger than in 1900, while the 75-84 year-old age group (8.8 million) was 11 times larger, and the 85+ year-old age group (2.7 million) was more than 22 times larger than in 1900 (Woodruff-Pak, 1988). This huge increase in the percentage of our population entering their elderly years is thought to be due to the alleviation of high infant and teenage mortality rates, better health care, and healthier lifestyles.

Because so many of our population are now facing old age, a great deal of recent research has focused on determining what aging is, and how it affects both body and

mind. Speculation about aging in general is rooted in ancient Greece. From that time and up to the scientific era, many myths about aging were generated, perhaps to relieve anxieties about growing old and contemplating death (Gruman, 1966). Since the dawn of the scientific era, these myths about aging have been somewhat eliminated by the vast quantities of research that have been conducted on the physiological processes of aging, and their psychological effects. However, it has proven difficult to operationally define aging, because there are no set physiological processes or psychological effects that have been determined for all people and that occur on a set timeline. Different researchers have different definitions of aging, depending on their research perspective, although most aging researchers agree that aging can be conceptualized on three primary levels: biological, psychological, and social. Biological aging is viewed in terms of the decline of the organism. Aging begins when some apex in physiological functioning is reached, and the physiological systems begin to decrease in functioning. Psychological aging refers to the adaptive capacity of an individual's behavior over time, and social aging deals with the social habits and observances of the individual in reaction to society and social norms (Woodruff-Pak, 1988). Because this paper will be concerned primarily with the biological and psychological aspects of aging, we will focus on a psychobiologically-based definition.

In keeping with a psychobiologically-based definition of aging, Strehler (1982) suggested that aging must include four conditions. First, aging must be deleterious, and reduce functioning. Second, aging must be progressive, and take place gradually. Third, aging must be intrinsic, and not be due to a modifiable environmental agent. And last, aging must be universal, so that all members of a species show the same deficits with

advancing age. This operational definition is useful because it defines what is meant by biological aging with precision, and it excludes other biological phenomena with which aging is often confused, such as diseases (Hayflick, 1994). It is important to note that not all time-dependent changes should automatically be considered fundamental changes associated with aging. Also, aging changes usually become manifest after maturity, although their genesis may have taken place earlier (Arking, 1991).

Theories of Aging

As much as a specific definition of aging has been hard to universalize, so has a specific theory of what biologically happens to organisms as they age. It is implicit that in order to change the course of any biological phenomenon, such as the onset of age changes, for our benefit, the phenomenon itself must first be understood. Unfortunately, we are a long way from a definitive understanding of what specific biological phenomenon causes us to age. There are many different theories, which are divided into two basic groups: those based on a preexisting biological plan, and those based on random biological events. All of these theories suffer from a major flaw: any of the proposed causes of aging may result from a more fundamental, although unidentified, cause. In addition, many of the theories proposed in the preexisting plan category overlap into the random events category, and vice versa. In other words, random events can influence a preexisting plan of aging, or a preexisting plan of aging can influence a set of random events. Most aging researchers overcome these problems by acknowledging that there is probably not a single cause of aging, and that several biological mechanisms for aging are probably operating simultaneously (Hayflick, 1994). Perhaps the more

fundamental problem with the following theories is that none has enough evidence to prove it unequivocally correct or incorrect.

Aging by Design

This general category of theories on the biological cause of aging assumes that organisms have some sort of biological clock that induces a series of chemical or physical changes at a particular time during the development of the organism that produce aging, and eventually death. These theories include those in which age changes are determined by turning on/off certain genes or by certain hormones secreted at a particular time by the hypothalamus or pituitary. One such example of a theory of programmed aging is the gene theory. This theory proposes that there is one (or more) gene in the organism that "turns on" the mechanisms for aging after some specified period of development. Once this gene is activated, the organism begins to decline through the aging process, toward death. Researchers who subscribe to this theory agree that there is probably more than one gene that would be responsible for this process; however, no progress has been made on identifying such genes (Woodruff-Pak, 1988).

Potential support for this theory includes the occurrence of programmed cell death, which is a phenomenon universally accepted by biologists. It occurs during development of the fetus (for example), when millions of cells that were just formed die in order to provide for appropriate formation of the embryo. For example, billions of neurons are formed in the developing nervous system of the fetus; however most of them are destined to die as the brain is completed. The concept of a genetic blue-print for activation of the aging process correlates well with this phenomenon of programmed cell

death. A potential problem with this theory is that there were never enough elderly members of a species around in order for evolution to select for the aging process. Also, definitive experimental support has not yet been provided (Hayflick, 1994).

Another example of a theory of programmed aging could be found in neuroendocrine aging. Many changes in the neuroendocrine system are associated with the aging process, such as the decreases in gonadotropin-releasing hormone and estradiol associated with menopause. It is unclear whether these neuroendocrine changes are the cause or the effect of the aging process. However, some researchers believe that the hormone dehydroepiandrosterone (DHEA) secreted by the adrenal gland may be a potential hormonal cause of aging. This hormone is found in greater amounts in younger adults, which then declines with age. Also, when administered to aged mice, DHEA seemed to reduce the incidence of breast cancer, delay immune system dysfunction, and increase longevity (Hayflick, 1994). Perhaps there is a genetic code that programs the adrenal gland to decrease production of DHEA at a certain time, which then causes the physiological deficits seen with aging. Whether DHEA truly is a "youth-producing" hormone or not, research in this area could potentially support the theory of a program for biological aging.

Aging Based on Random Events

In contrast to the theories which propose that there is a biological program for aging, these theories propose that random events influence our physiology such that our body ages. In other words, they attribute age-related changes to a random accumulation of insults that disrupts normal functioning of our DNA, protein synthesis, or immune

system. For example, the wear-and-tear theory states that aging occurs due to the wearing down of our normal cellular mechanisms. Support for this theory includes the example of the fruit fly which, when young, can beat its wings approximately 2 million times and sustain flight for about 110 minutes. As the fruit fly ages, however, it can only beat its wings approximately 170,000 times and sustain flight for 19 minutes. Proponents of the wear-and-tear theory postulate that the wing muscle cells of the fruit fly undergo wear and tear from use and toxic environmental insults, so that they deteriorate and lose efficiency over time. However, this theory does not explain why the wing muscle cells deteriorate, so it has lost popularity in recent years (Hayflick, 1994).

Another example of a random events theory of aging is the cross-linkage theory, proposed by Bjorksten in 1974, which argues that, with age, certain proteins become cross-linked. This occurs when the molecules of important tissues, like collagen, become irreparably bound together. The cross-linkage of molecules could then interfere with the normal processes of these cells, such as waste removal and nutrient provision (Hayflick, 1994). Cross-linkages have been identified in such important molecules as nucleic acids associated with DNA, and collagen in the lungs, heart, muscle, and lining of the walls of the blood vessels (Woodruff-Pak, 1988). Although this is an intriguing theory, there has not been specific experimental support for cross-linkages actually producing specific age-related changes.

A more popular example of a random events theory of aging is the somatic-mutation theory, which states that aging is due to chromosomal mistakes that accumulate with time and disrupt functioning in important physiological systems. This theory was proposed by Cutis and Miller in 1971 when they observed that older mice and mice of a

short-lived strain incurred more spontaneous chromosome abnormalities. How these chromosomal abnormalities occurred over time, and how they might specifically contribute to the aging process was not specified (Woodruff-Pak, 1988).

The error-catastrophe theory, proposed by Orgel in 1963, is related to the somatic-mutation theory, in that it proposes that some error in the production of proteins accumulates over time and produces aging of the organism. However, instead of chromosomal abnormalities occurring over time, this theory proposes that errors in the translation of the DNA sequence into a protein occurs over time. This mutation would then lead to the production of a faulty enzyme that is pivotal in the production of other important proteins, such as membrane-bound receptors and channels. Therefore, when this faulty enzyme is produced, it leads to the creation of other faulty proteins. This creates a cascade of errors that produces aging of the organism, and, ultimately a failure of the system (Arking, 1991). This theory has also lost supporters due to the lack of definitive experimental support, and because the theory does not hypothesize what causes the errors in the translational system.

One theory which could potentially answer this question of cause associated with the somatic mutation and error-catastrophe theories is the very popular free-radical theory, proposed by Gordon in 1974. The free-radical theory enjoys a great deal of popularity because it is more specific and testable, and because it can be applied to explain a number of other theories, such as the somatic mutation or error-catastrophe theories. According to this theory, damage to DNA and other protein-production systems are caused by the conversion of normal cellular components by oxygen into free radicals. These compounds are highly reactive, and can self-propagate, where a recently-turned

free radical can then produce more free radicals. Important cellular components that are highly susceptible to free radical damage include cell membranes, DNA, protein, and mitochondria. These structures are essential to cell life; therefore if they are damaged by a high concentration of free radicals, the cell will not function properly and could die. The free radical theory of aging posits that the accumulation of such damage to cell membranes, DNA, and mitochondria leads to the decrease in physiological functioning associated with aging.

Support for this theory includes the finding that free radicals produce cross-links in some molecules, and can damage DNA. Other support comes from research on naturally-occurring free radical blockers: the antioxidants. Antioxidants are produced in the body, and are absorbed through our diet. Examples include vitamins E and C. These chemicals inhibit the production of free radicals, and the cellular damage associated with free radical production, by preventing oxygen from combining with susceptible molecules. Experiments that have attempted to test the free-radical theory of aging by utilizing antioxidants primarily focus on feeding animals a diet high in antioxidants and observing if they have a longer life span. Results in this area have been mixed, but they tend to show that animals fed antioxidants do live longer than control animals (Hayflick, 1994).

One final theory that proposes that aging is due to a random accumulation of errors is the autoimmune theory, proposed by Walford in 1969. This theory states that the immune system, which normally produces antibodies to fight off foreign invaders (such as bacteria or viruses), begins to produce antibodies to itself. In other words, the immune system fails in its function because it can no longer distinguish "self" from "non-self".

The attacks of our own immune system upon our bodies then produce the deficits seen with aging. Support for this theory include two major findings: that our immune system does decrease in proper functioning as we age, and that many elderly people do develop autoimmune disorders, in which they produce antibodies that incorrectly attack "self" proteins. However, the theory is fundamentally flawed because some animals do not have malfunctioning immune systems, and yet they still age (Hayflick, 1994). The theory is also flawed because, like so many other theories of aging, it does not explain why the immune system begins to fail in the first place.

Physiological and Psychological Changes Associated With Aging

More important than the definition of aging or the specific biological cause of aging, to the general public, are the physiological and psychological changes that are a normal part of the aging process. Some of the more commonly recognized age changes include graying hair, loss of strength and stamina, farsightedness, a decrease in height, and menopause. These seemingly inevitable changes in our bodies (and minds!) are accompanied by thousands of tiny changes that occur in all our organs and tissues, most of them too small for us to notice, until they take a toll on our quality of life. The next section will focus on these changes that are associated with growing older, including cardiovascular and respiratory changes, digestive, excretory, and endocrine changes, reproductive and immune system changes, musculoskeletal and mobility changes, and sensation and perception changes. The section will then conclude with a discussion of nervous system and cognitive changes, as they are directly relevant to the current study.

Because age-related changes in learning and memory are so pivotal to this study, they will be discussed further in a separate section.

Cardiovascular and Respiratory System Changes

Cardiovascular and respiratory changes due to aging can be some of the most detrimental to the aged person, because they are often the cause of death for the elderly. Cardiovascular changes associated with aging include sarcopenia of the heart muscle, in which there is a progressive loss of mass in the muscle cells of the heart. As sarcopenia of the heart continues, there is an increase in the connective tissue and fat, which causes the left ventricle to become stiff and less compliant. The pericardium also becomes stiffer, which can again contribute to loss of compliance in the ventricles (Kitzman and Edwards, 1990). This leads to a reduced and delayed filling of the left ventricle (Schulman, 1999). The cardiac muscle also becomes less responsive to neural stimulation from the heart's own pacemaker cells, which normally initiate each contraction (Fleg, et al., 1994). Changes in the blood vessels also occur, such as an increase in the thickness of the tissue which comprises the arterial pipeline. This results in a narrowing of the diameter of the artery and increased rigidity, which can intensify the risk of atherosclerosis (Hayflick, 1994). Aging also increases the aged individual's general risk and vulnerability to cardiovascular diseases, such as atherosclerosis, hypertension, myocardial infarction, and congestive heart failure (Whitbourne, 2002).

Respiratory system changes associated with aging can also significantly alter the quality of life for the elderly person. Such changes often seen with aging include "failing lung syndrome" in which there is a decrease in the lung's elastic recoil (Babb and

Rodarte, 2000). This decrease in elasticity means that less than the maximal amount of air can be brought in and out of the lungs. This can be particularly dangerous under conditions of physical exertion (DeLorey and Babb, 1999), although the changes are not otherwise noticed by the individual.

Digestive, Excretory, and Endocrine System Changes

Changes in the digestive system due to age are not normally very serious or life-threatening. Major changes include presbyesophagus, which literally means "old esophagus". Presbyesophagus refers to age-related changes in the esophagus in which the muscles to do not function effectively, which reduces the movement of food down the esophagus. This change does not generally interfere with normal digestive functioning in the aged person. A more significant change in the digestive system of the elderly is referred to as "anorexia of aging". This occurs when older adults fail to eat a sufficient amount to meet their body's nutritional needs. This condition is often not due to the normal aging process; instead it is associated with disease states that cause the individual to lose their appetite (Whitbourne, 2002).

In terms of the urinary system, the bladder is usually the organ most affected by age. The aged bladder's normal function of expanding to hold stored urine without discomfort, and emptying completely when voiding are compromised over time. Thus, adults over the age of 65 experience a reduction in the amount of urine they can store, and more urine is retained in the bladder after voiding, both of which can lead to unwanted accidents. The kidneys do not show age-related changes on a day-to-day basis, however, during times of physical stress, they can become compromised. When the

elderly individual is physically stressed due to high temperatures, extreme exertion, or illness the kidneys often do not function properly. The outcome of this kidney failure can be fatigue, changes in body chemistry, and deleterious changes in bodily fluid levels (Whitbourne, 2002).

Endocrine system changes are among some of the most important age-related changes that an individual will experience. These changes can occur at many levels. There may be alterations in the brain structures that control secretion by the glands, there may be changes in the glands themselves, or there may be changes in the way the target organs respond to hormones. The secretion of many hormones, including testosterone, insulin, and thyroid and growth hormones decreases with aging. In addition, there is a decline in the number and/or efficiency of the target receptors for these hormones with age (Hayflick, 1994). The effect of aging on the hypothalamic-pituitary-thyroid (HPT) axis can be a significant one, because changes in the secretion of hormones (such as thyroid releasing hormone, thyroid stimulating hormone, and thyroxine) by these structures can affect the body's basal metabolic rate. The HPT axis normally regulates the body's metabolic rate, which slows down as we age. Therefore, changes in the secretion of hormones from these structures could be responsible for this significant age-related change (Whitbourne, 2002).

Another endocrine system which can be significantly affected by aging is the hypothalamic-pituitary-adrenal (HPA) axis. These structures are self-regulating so that a negative feedback mechanism that controls the levels of cortisol being released by the adrenal glands causes the hypothalamus to cease production of adrenocorticotropin hormone (ACTH). This negative feedback mechanism insures that the levels of cortisol

do not rise too high. Many aging researchers believe, however, that the negative feedback can become insensitive with age, so that cortisol levels can become out of control. If the cortisol levels are allowed to rise unchecked, they can produce a number of detrimental physiological effects, such as damage to the thymus gland, depression of the immune system, and damage to the nervous system (Seaton, 1995). This theory is called the glucocorticoid hypothesis, and it proposes that many of the negative changes related to age, such as decreases in cognition, increases in fat deposits, and decreases in the immune response are due to an insensitivity of the negative feedback mechanism for cortisol (Wilkinson, Peskind, and Raskind, 1997).

Reproductive and Immune System Changes

Intimately related to the endocrine system changes discussed above are the changes in the reproductive system due to aging, since many of the components of the reproductive system are also part of the endocrine system. For the female, the changes in her reproductive system with age can be some of the most significant of her adult life. These alterations are hallmarked by menopause, which is the point in a woman's life when menstruation stops permanently, and she is unable to reproduce. The age of onset for menopause varies among individuals; however, the average age is 50 years. It is unknown what the initial trigger for menopause is; whether it is a reduction in the ovarian follicles, or a change in the central nervous system that halts the production of gonadotropin-releasing hormone from the hypothalamus (Wise, Krajnak, and Kason, 1996). Menopause is associated with fluctuating (and ultimately decreasing) levels of estrogen, which can cause fatigue, hot flashes, insomnia, memory loss, depression, and

loss of bone strength. Menopause is also associated with an increased risk for atherosclerosis, high blood pressure, and other cardiovascular diseases. Following menopause there are decreases in the release of estradiol (a form of estrogen) and testosterone (Whitbourne, 2002).

Reproductive changes in men due to aging tend to be less significant. Unlike women, healthy men are capable of reproduction until very old age. The aging of the male reproductive system involves gradual changes over time during the later years of adulthood. Sperm are relatively unaffected by age, in that the production of sperm is well maintained, and there are no major changes in sperm morphology or motility. There can be extensive changes in the prostate gland with age, because, in the human male's elderly years the prostate loses the ability to secrete fluid and shows signs of deterioration. Hard masses may appear, and the connective tissue loses elasticity. The consequence of these changes is that semen is not propelled with as much force during ejaculation (Whitbourne, 2002). Many men also develop benign prostatic hypertrophy, characterized by an increase in the prostate's volume (Arenas, et al., 2001). Lastly, there is an age-related decrease in the secretion of testosterone, a condition referred to by some aging researchers as "andropause" (Morley, 2001).

The immune system of both sexes undergoes significant losses in function with age. This is in concert with conventional wisdom that indicates that elderly people are more susceptible to influenza and cancer. Immune system changes with age include a decline in T cell functioning, which renders the system less able to deal with newly encountered antigens (Miller, 1996). The ability to produce B cells is also reduced with age (Whitbourne, 2002). These changes in B cell and T cell functioning may be due to a

degeneration of the thymus gland after sexual maturation. Elderly people are also more susceptible to autoimmune disorders, in which the immune system fails to recognize "self" proteins, and begins to attack the individual's body. In fact some researchers believe that this malfunction in the antibody system could contribute to the elderly person's increased risk of cancer, because the antibodies do not destroy cancer cells like they did during the individual's youth (Hayflick, 1994).

While many of the components of the immune system decline with age, some have been shown to increase in functioning with age, often to the detriment of the individual. For example, cytokines have been shown to increase in concentration with age. Cytokines are chemicals that are released in the body; their sources include several different cell types (e.g. leukocytes, macrophages, and neutrophils). Cytokines also act on several different cell types, such as T-lymphocytes, B-lymphocytes, and natural killer cells (Dinarello, 1991). The interleukins, including interleukin-1 and interleukin-6, are examples of such cytokines that increase with age, and may potentially have damaging effects on various systems of the aging individual (Whitbourne, 2002). Interleukin-1 will be discussed in more detail in reference to the current study.

Musculoskeletal and Mobility Changes

Another physiological system that shows significant changes with age, which can severely impair the quality of life for the aged individual, is the musculoskeletal system. Changes in the musculoskeletal system can lead to mobility problems, even with only normal age-related changes. However, changes in this system can also lead to more significant problems in the form of musculoskeletal disorders.

Common changes in the musculoskeletal system due to normal aging include a progressive loss of muscle mass, known as sarcopenia. Sarcopenia results in the progressive reduction in the number and size of muscle fibers, particularly the fast-twitch fibers, which are responsible for speed and strength (Morley, Baumgartner, Roubenoff, Mayer, and Nair, 2001). Losses in bone tissue are also seen with aging. Bone loss begins in the fifties, and can lead to diminished bone strength, making the elderly individual more susceptible to fracture (Hayflick, 1994). The cartilage of older people is also affected by age, as it tends to become cracked, frayed, and calcified, which can interfere with the diffusion of nutrients and waste products to and from the cells, causing cartilage cell death (Arking, 1991).

All of these changes in the musculoskeletal system can lead to decreases in overall mobility for the aged person. In fact, decreased mobility is one of the most significant concerns for the elderly, with a significant percentage of the elderly population suffering from problems walking (Simoneau and Leibowitz, 1996). Common problems with mobility include increased sway, decreased balance, which contributes to a greater risk of falling, and changes in gait pattern, such as decreased gait velocity and decreased step length (Wall, Hogan, Turnbull, and Fox, 1991). In most cases these decreases in mobility are associated with normal (and pathological) age-related changes in the musculoskeletal system and nervous system.

Elderly people are also at higher risk for incidences of musculoskeletal disorders. The most common disorder seen in aged adults is arthritis. Arthritis is a common disorder that can range in severity from a minor limitation to an extreme disability. It refers to a set of conditions that affects the joints and surrounding tissue, resulting in pain, stiffness,

and swelling. The most common form of arthritis in the elderly is osteoarthritis, which is a painful degenerative disease that typically develops in joints that are repeatedly injured over time during physical activity. Eventually the injury wears away the cartilage that cushions the joint, which allows the ends of the bones to rub together. Another common form of arthritis is rheumatoid arthritis, which is an inflammatory disease of the joints. This disorder is an autoimmune disease in which the immune system attacks the cells of the joint, causing inflammation and a weakening of the muscles and tendons surrounding the joint (Whitbourne, 2002).

Aging is also associated with pathological changes in the musculoskeletal system and loss of mobility due to another common disorder: osteoporosis. Osteoporosis is caused by a loss of bone mineral content. The rate of osteoporosis rises in increasingly older age groups, and is most commonly found in older women. The loss of bone strength due to osteoporosis increases the aged female's risk of bone fracture (Whitbourne, 2002).

Sensation and Perception Changes

Changes in the sensory system of the older adult range from only slightly distracting, to severe. The most significant changes, and those that seem to most significantly affect the functioning of the individual, are seen in the visual and auditory sensory systems. Changes in the olfactory, gustatory, and vestibular systems tend to have less of an impact on everyday life.

For the visual system, many of the effects of aging begin in the forties, about a decade before they become noticeable to the individual (Nomura et al., 2000). Aging of the cornea causes a thickening (Weale, 1982) and yellowing (Lerman, 1984) of this

transparent, light refracting structure. The iris also becomes weaker with age, due to atrophy of the muscle cells. This causes the pupil to become less circular (Wyatt, 1995) and smaller (Morgan, 1986). This change is most noticeable under conditions of low illumination (Winn, Whitaker, Elliot, and Phillips, 1994). The lens yellows and becomes thicker and less flexible with age (Kashima, Trus, Unser, Edwards, and Datiles, 1993). Adding to this problem, the ciliary muscles that control adjustment of the shape of the lens weaken over time (Marmor, 1977), which decreases the ability of the eye to accommodate to changes in visual focus (from far to near or vice versa). The retina does not undergo significant age-related changes. There is a minimal age-related loss of foveal cones, with a higher loss seen in the periphery. There is, however, a more marked decrease in rod density (approximately 30%) near the central retina with age (Curcio, Millican, Allen, and Kalina, 1993).

Perceptual changes that accompany these visual system structural changes include presbyopia and decreased visual acuity. Presbyopia is a visual condition associated with the age-related changes in the lens. When the lens becomes less flexible, and the ciliary muscles cannot effectively change the shape of the lens, the lens is no longer able to effectively perform its function of changing shape in order to bring near objects into focus. This condition affects approximately 92 percent of persons aged 75 and over (Hayflick, 1994). These changes in the visual system structures also cause a decrease in visual acuity with age. It has been well established that there is a consistent decline in visual acuity after the age of 40 (Pitts, 1982).

In addition to changes in accommodation and visual acuity, elderly adults also have decreased contrast sensitivity and dark adaptation. The loss of contrast sensitivity

makes them less able to discern contrast patterns involving medium to thin areas of dark and light (Panek, Barrett, Sterns, and Alexander, 1977). With aging, color vision also changes modestly, mostly due to the yellowing of the lens, which reduces the individual's ability to discriminate colors at the short wavelength end of the spectrum (Knoblauch et al., 1987). Lastly, motion perception is also sensitive to the effects of aging. For example, Trick and Sliverman (1991) showed that aged adults have a higher threshold for the discrimination of motion in a random dot sequence.

For the auditory system, age-related changes can have just as much impact as changes in the visual system. And, like the visual system, the auditory system is a complex one, meaning that there are many structures that might be influenced by the effect of age. Interestingly, the middle ear structures are not greatly affected by age, and they do not contribute greatly to age-related hearing loss (Whitbourne, 2002). The tympanic ring or the ossicles of the middle ear can become calcified with age, which can cause a reduction in the amplitude of the auditory signal (Belal, 1975).

More significant changes occur in the inner ear, where the basilar and tectorial membranes become less flexible with age (Hansen and Reske-Nielsen, 1965). Hair cell loss is also very prevalent (Bhattacharyya and Dayal, 1989), especially near the base of the cochlea, which is the region believed to code for higher frequencies of sound (Johnsson and Hawkins, 1972). These changes can result in a significant loss of hearing in later life, particularly a condition called presbycusis, which is characterized by a loss of auditory sensitivity, especially to higher frequencies (Arlinger, 1991). This deficit can be very debilitating because many speech sounds are within the higher frequency range.

In other words, many aged adults who are experiencing presbycusis will have significant trouble understanding what other people are saying to them (Whitbourne, 2002).

Changes in the gustatory sensory system with age are not as extensive as was once thought. There does not seem to be a loss in the number of taste buds in older people, although they do experience a slight decrease in their ability to discriminate the five primary tastes (sweet, salty, bitter, sour, and umami). In contrast to the gustatory system, the olfactory and vestibular systems do undergo significant changes with age. The olfactory abilities of elderly people decrease, because the olfactory epithelium shrinks, and the number of olfactory receptors is reduced. This usually leads to a decline in the individual's sensitivity to odors and odor discrimination (Whitbourne, 2002). The vestibular system also changes markedly with age, when the number of sensory cells is reduced, and the fluid surrounding these cells in the utricle and saccule begins to deteriorate. These malfunctions in the vestibular system usually manifest to the older adult as dizziness and vertigo, which can contribute to the individual's increased risk of falling (Whitbourne, 2002).

Nervous System Changes

Age-related changes in the nervous system are considered by some to be the most significant changes that occur during the later years of a person's life. They can involve many different central nervous system structures, and can have widespread effects on other physiological systems and various overt behaviors. New studies using brain imaging are clarifying that there is considerable variability within individuals in terms of specific changes in the brain during the aging process. There is also considerable

variability in the changes seen in each structure within one individual (Whitbourne, 2002). This makes unraveling the specific nervous system changes that are a universal part of the aging process difficult to determine.

In terms of gross structural changes, it has long been known that the brain loses approximately 10 percent of its total weight during the aging process. This loss of tissue is particularly noticeable in the cerebral cortex, where the sulci widen as the gyri lose tissue mass (Hayflick, 1994). Gyral atrophy is most likely to be noted in the frontal and temporal regions of the cortex, although this varies from individual to individual (Scheibel, 1996). It is possible that this decrease in brain tissue with age may be due to a loss of neurons. Research results examining this issue indicate that decreases in neuronal density do occur in certain regions of the cerebral cortex and limbic system structures (Arking, 1991). It has also been shown that the majority of tissue loss in the brain occurs in the grey matter up to approximately age 50, after which the white matter begins to deteriorate (Scheibel, 1996).

Loss of tissue in specific areas of the central nervous system can vary depending on which structure is being examined, which individual is being tested, or even which research technique is being used. For example, there have been widely varied reports of age-related damage to the cerebral cortex. Some recent MRI studies have reported that there is a 1 percent loss of tissue in the frontal cortex with each decade of aging (De Santi et al., 1995) while others report a much higher 10 percent loss in the frontal cortex per decade (Eisen, Entezari-Taher, and Stewart, 1996). PET studies have also been mixed; some report a decrease in frontal lobe metabolic activity with age (Salmon, et al., 1991), while others have reported that there is no regional metabolic change with age (de Leon,

Ferris, George et al., 1983). In spite of these differential findings most researchers agree that there is at least some structural loss in the cerebral cortex with age, particularly in the frontal and temporal lobes.

The limbic system shows varying degrees of age-related change. Golomb et al. (1993) demonstrated some hippocampal atrophy (approximately 10 to 40 percent) in almost one-third of the subjects (aged 55-84 years) examined. These losses were shown to increase to up to 52 percent in certain areas of the limbic system, including the subiculum and dentate gyrus (West, 1993). The changes seen in the amygdala may be more complicated. Researchers have shown that there is some degree of degradation of amygdaloid neuronal processes, but the number of afferent peptidergic and cholinergic afferent fibers does not seem to be appreciably altered (Scheibel, 1996).

Most structures in the brain stem do not show significant losses with age. However, there are exceptions to this. In the hypothalamus, most age-related changes have been localized to the suprachiasmatic nucleus (SCN) and the sexually dimorphic nucleus (SDN). Several researchers have reported a decrease in the number of neurons in the SCN, which becomes particularly noticeable after age 80 (Swaab et al., 1993). In such individuals the cell damage is associated with changes in circadian rhythms (Mirmiran et al., 1992). The SDN of the hypothalamus appears to be unaltered until after the age of 50 in men, when the number of neurons begins to decrease rapidly. This dramatic decrease in cell numbers occurs in females after age 70, and probably accompanies the hormonal changes associated with old age (Hofman and Swaab, 1989). The substantia nigra (SN) also shows a slight response to normal aging, as does the locus coeruleus of the pons (LC). Fearnely and Lees (1990) noted a loss of approximately 4.7 percent of neurons in

the caudal SN with each decade of advancing age. The LC was shown to lose an average of 20 percent of neurons in aged individuals (Marcyniuk, Mann, and Yates, 1989). The cerebellum is also thought to incur age-related changes, because it loses both Purkinje and granule cells over time (Scheibel, 1996).

These age-related changes in specific regions of the brain are often accompanied by modifications in neurotransmitters and overall electrical activity. There is a striking reduction in acetylcholine in the hippocampus with age, perhaps reflecting the decrease in tissue seen in that region. There is also a reduction in dopamine secretion in the substantia nigra and striatal pathway. Norepinephrine and serotonin release are also reduced in portions of the brainstem, and gamma-aminobutyric acid (GABA) is reduced in the thalamus (Whitbourne, 2002). These changes in neurotransmitter secretion are often accompanied by a reduction in the expression of the receptors and their binding affinity for the neurotransmitters (Petkov, Petkov, and Stancheva, 1988). General electrical activity (as measured by the electroencephalogram [EEG]) also decreases with age. The dominant brain wave rhythm of young adults is slightly faster than 10 cycles per second. This dominant brain wave rhythm slows with age because, for example, by the age of 65 aged adults' brain wave rhythms have slowed to nine cycles per second (Woodruff, 1985).

These regional changes in brain structure and function may not necessarily reflect neuronal death. Recent research indicates that the significant loss of tissue seen in certain brain regions, and their associated loss of function, may be due to the degeneration of dendrites and dendritic spines, rather than the death of entire neurons. Dendrite systems are one of the most significant anatomical indicators of neural function because they are

the primary recipient of the synaptic input impinging on each cell (Scheibel, 1996). In humans, the number of dendrites and dendritic spines decreases with age, affecting some areas more than others. Some brain areas show decreases of up to 25 percent (Cotman and Holets, 1985). These changes undoubtedly lead to an attenuation of the postsynaptic cell's response to synaptic input from the presynaptic cell, especially in its capacity to integrate large amounts of afferent information (Scheibel, 1996).

Other cellular changes that may contribute to regional structure/function deficits include neurofibrillary tangles and senile plaques. These structural anomalies are one of the pathological signs of Alzheimer's disease, however they are also found (in a much lower concentration) in the brains of healthy aged individuals. Neurofibrillary tangles represent a very damaging cell body inclusion, which means they are most likely contributing to the age-related damage of brain structures in which they are located. Senile plaques consist of a core of β-amyloid protein surrounded by partially destroyed neurites and microglia, all of which contribute to a progressive and slowly widening focus of tissue death (Scheibel, 1996). These abnormal structures are found primarily in the cortical pyramidal cells, the hippocampus, and in frontal and temporal cortical association areas (Greenfield et al., 1967). Another cellular anomaly that is found in the majority of neurons in the aging nervous system is lipofuscin (Borst, 1992). It is unknown what role these structures play, if any, in the loss of tissue and neuronal function seen with age.

Cognitive Changes

The focus of many research studies (including this one) has been on cognitive changes that accompany the aging process. For many individuals, these deficits are the most debilitating, and cause the most distress as they get older. For this reason, researchers spend a great deal of time attempting to describe age-related changes in cognition, and to explain their causes, in the hopes of discovering a way to alleviate the distress of the elderly person who is experiencing them. Because this experiment focused on deficits in learning and memory due to aging, the majority of this section will be devoted to that topic. The first part of this section will concentrate on describing other losses in cognitive ability seen with aging, including deficits in reaction time, attention, problem solving, and intelligence. The next part of this section will focus on age-related deficits seen in learning and memory and their specific biological causes.

Other Cognitive Changes.

Increased slowness is one of the major changes in cognition seen with aging. For example, between the ages of 20 and 60, human reaction time falls by an average of 20 percent in tasks of psychomotor functioning (Coni, Davison, and Webster, 1992). Reaction time is also impaired in aged rats (Burwell and Gallagher, 1993). Tests of attention also indicate that this important cognitive task is impaired with age. Older subjects tend to experience fatigue during an attention task more quickly than younger subjects. They are also more likely to be distracted during the task. Older adults also have much more trouble solving complex problems. Elderly people are more likely to be thrown off the correct solution to a problem by irrelevant and redundant information (Coni, Davison, and Webster, 1992). This may be due to the aforementioned problems:

perhaps they have trouble solving difficult problems because they take more time to do so, and because they find their attention wandering more often.

Another classic pattern of cognitive aging involves the changes seen in intelligence. Elderly individuals experience a decrease in fluid intelligence over time, which reflects their decreased ability to take in information quickly and deal with it efficiently. Tests of intelligence involving skills which are unlearned or dealing with new information in a novel manner are considered tests of fluid intelligence. As a person ages, their abilities on these types of tests decreases. On the other hand, crystallized intelligence actually improves with age. Crystallized intelligence reflects the individual's accumulated vocabulary, mathematical ability, and other stored episodic information (Woodruff-Pak, 1988).

Learning and Memory Changes.

The majority of research on cognitive changes seen with aging has focused on learning and memory deficits. Pioneering studies by Ruch (1934) and Gilbert (1941) showed that, relative to young adults, older adults exhibit a decline in performance on tests of learning and memory capabilities (Kubanis and Zornetzer, 1981). This decline in learning and memory is a common phenomenon in many species (Craik, 1977). Specifically, older individuals experience difficulty with acquiring new information, and also with the retrieval of information already stored in memory. This deficit is more pronounced in certain types of learning and memory, and shows up in varying degrees depending on the individual (Woodruff-Pak, 1988).

Examples of the varying degree of age-related deficits in learning and memory are in perceptual learning and stimulus-response learning. Perceptual learning is the ability to

improve the processing of sensory stimuli. Older adults are capable of perceptual learning, but they tend not to do as well on perceptual learning tasks as younger subjects. For example, practice does improve the visual detection of target stimuli in older subjects; however they usually do not ever perform at the same level as young subjects (Willott, 1999).

Stimulus-response learning refers to classical conditioning, in which a subject learns an association between two stimuli, and operant conditioning, in which a subject learns to produce a voluntary response based on the consequences of that response. Adults over the age of 40 have been found to have a lower rate of classical conditioning than adults in their 20's. This difference becomes magnified as age increases into the 60's (Woodruff-Pak, 1988). For example, consistent findings in eyeblink conditioning experiments have shown that the rate of conditioning (number of tone-air puff pairings to reach a criterion level of learning) typically slows with age. Age-related changes in the cerebellum probably contribute to this decline in classical conditioning, because the cerebellum has been shown to be instrumental in learning this association (Willott, 1999). As noted earlier, some age-related changes in the cerebellum have been found, particularly in the Purkinje cells of the cerebellar cortex (Duara, London, and Rapoport, 1985). Changes in the amygdala are probably also involved in age-related deficits in certain types of classical conditioning, such as fear conditioning (Davis, 1992). The amygdala is a pivotal structure in learning the association between a harmless stimulus and a harmful one, therefore changes in this brain structure may also contribute to differences seen in the classical conditioning of older adults.

Older subjects also tend to make more errors and acquire operant conditioning responses more slowly, particularly when the task is more difficult. For example, old rats demonstrate poorer avoidance conditioning than young rats. In other words, old rats make more mistakes when required to perform a behavior in order to remove a negative stimulus (negative reinforcer). Experiments using positive reinforcers (such as food or money) with human subjects have shown that acquisition of these learned associations is slower with age (Willott, 1999). Changes in brain structures that may contribute to this deficit include the basal ganglia, which are structures shown to be important in the operant conditioning of motor responses. Many studies have demonstrated age effects in the basal ganglia, including receptor loss and other alterations in the dopaminergic circuits (Powell, Buchanan, and Hernandez, 1991).

The effect of aging on short term memory (or working memory) has been studied extensively. The principal finding is that deterioration of short term memory with age is task dependent. For example, some investigators have shown no significant age differences in performance on simple tasks such as serial recall of a list of digits (Drachman and Leavitt, 1972), while others have shown reliable deficits on tasks requiring repetition of a list of items in reverse order (Mueller, Rankin, and Carlomusto, 1979). Any age-related performance deficits were originally attributed to a faster rate of decay of short term memory in older subjects (Fraser, 1958), but it is now more accepted that interference by intervening material and an attention deficit is responsible for forgetting (Kubanis and Zornetzer, 1981).

Like short-term memory, the effects of aging on long-term memory vary. Most investigators agree that there is an impairment of long-term memory with aging, however

this impairment also seems to be task dependent (Kubanis and Zoretzer, 1981). For example, Glibert (1941) showed a deficit in elderly people in paired-associate learning which utilized tests of recall of lists of words. Craik (1968) also presented word lists of varying lengths and found that older subjects recalled fewer words from the lists. However, in other studies which simply required elderly subjects to recognize words from a list of memorized words among distracter items, the older subjects showed no deficit (Schonfield and Robertson, 1966). Many studies have shown this lack of an age difference on tests of long-term memory that use recognition tasks (Woodruff-Pak, 1988).

Different forms of long-term declarative memory also show varying degrees of decline with aging. For example, episodic memory typically becomes poorer with age, whereas declines in semantic memory tend to be minimal. Numerous studies on rodents have shown that spatial learning and memory are impaired with age, similar to the deficits seen when the hippocampus is lesioned in young subjects. For example, old rodents learn Morris and radial arm mazes more slowly than young ones, although again the degree of deficit can vary depending on the individual animal (Willott, 1999). Spatial learning deficits accompany old age in humans as well. For example, older people tend to acquire less knowledge about novel environments, such as learning to navigate a new travel route in everyday life (Wilkniss, Jones, Korol, Gold, and Manning, 1997).

This deficit in long-term declarative memory that accompanies aging may be due to age-related changes in the hippocampal formation and surrounding structures. The hippocampal formation and neighboring regions of the medial temporal lobe are essential for consolidating information into long-term declarative memory, especially for learning

tasks involving spatial memory or memory after a delay. Lesions of the hippocampus (and surrounding structures) in young humans and animals cause deficits in spatial learning that are similar to those deficits seen due to aging. Therefore, it makes sense that some sort of age-related damage to the hippocampus plays a causal role in declines in spatial memory seen with aging (Willott, 1999), especially because the hippocampus is particularly susceptible to damage due to aging (Lynch, 1998). As mentioned previously, the hippocampus undergoes serious hippocampal damage in old age, as shown by MRI and CT scan. Approximately 80 percent of men ages 77 to 88 exhibit hippocampal atrophy. Forty percent of women ages 77 to 88 show the same type of damage (Golomb et al., 1993). Landfield, Rose, Sanles, Wohlstadter, and Lynch (1977) reported a depletion of hippocampal pyramidal cells in aged rats. Cholinergic neurotransmission in the hippocampus declines with age (Morgan and May, 1990), and degenerating axons have been observed in the hippocampus and associated fiber tracts of old rats (Greene and Naranjo, 1987). Bondareff and Geinisman (1976) reported an age-related loss of synapses in the dentate gyrus of the hippocampus, and Nunzi, Milan, Guidolin, and Toffano (1987) showed that dendritic spines of the hippocampus are lost as rats age. In addition, the firing patterns of hippocampal place cells, which fire in a particular pattern when an animal is in a particular area of space and are important in rodent spatial learning, are altered with age. The average firing rates of hippocampal place cells during active behavior has been shown to be the same in young and old rats; however, the specificity (total area of the maze over which a given cell fired) and reliability (consistency with which the cell fired to the same location) are both reduced in old rats (Barnes, 1998).

Age-related changes in the cerebral cortex, particularly the frontal and temporal lobes, may also contribute to deficits in long-term declarative learning and memory. For example, some PET studies have found the temporal and frontal cortex to be vulnerable to age-related metabolic deficits (De Santi et al., 1995). In fact, patients with frontal lobe damage and healthy older people tend to have difficulty on many of the same neurological tests (Moscovitch and Winocur, 1992). MRI studies have shown that the frontal cortex in particular (especially the prefrontal cortex) is susceptible to atrophy with age (Coffey et al., 1992). Also, many cortical neurons exhibit substantial abnormalities and/or loss of dendrites, dendritic spines, and presynaptic terminals with age (Cotman and Holets, 1985).

Long-term potentiation (LTP) is a cellular mechanism many believe to be the basis for learning and memory. LTP occurs when increased stimulation of circuits within the hippocampus leads to long-term physiological changes in neurons, such as increased synaptic strengths or plasticity. LTP also declines with age. For example, many different groups of researchers have shown that aged rats with spatial learning deficits also have decreased maintenance of LTP (Landfield et al., 1978; Barnes, 1990; Lynch and Voss 1994; Murray and Lynch, 1998). Whether this deficit in LTP is solely restricted to the maintenance of LTP, or if it also affects induction of LTP as well has not yet been established (Lynch, 1998). The NMDA receptor has been shown to play an essential role in the establishment of LTP (Carlson, 2002), therefore changes in the NMDA receptor with age may contribute to the age-related deficit in LTP. Several researchers have shown that NMDA receptor function changes with age. For example, NMDA receptor binding

decreases with increasing age (Magnusson and Cotman, 1993) and NMDA receptor density decreases with increasing age (Bonhaus et al., 1990).

The appropriate lipid environment of the plasma membrane of the neuron is crucial for optimum functioning of membrane proteins, like the NMDA receptor. If the environment of the neuronal membrane is compromised, then LTP is likely to be compromised as well. Membrane lipids can be compromised by the introduction of free radicals, which produce lipid peroxidation of the membrane, and decrease membrane arachidonic acid. Therefore, age-related increases in free radicals could induce lipid peroxidation and decreases of arachidonic acid in the neuronal membrane. Arachidonic acid was shown to be significantly lower in aged rats, especially those who failed to sustain LTP (Lynch and Voss, 1994). A potential cause of such increases in free radicals, lipid peroxidation, and decreases in membrane arachidonic acid in the aged brain is discussed in the next section.

Current Study

The current study attempted to investigate one possible cause of learning and memory deficits in aged rats. Interleukin-1β has been shown by many researchers to play a role in declines in learning and memory, especially with age. The next section will review background research on this potentially very important neurochemical, and present the specific research hypotheses for the current experiment.

Interleukin-1

One promising line of research in the investigation of cognitive deficits due to aging has been the effect of cytokines on learning. Cytokines are chemicals that are released in the body; their sources include several different cell types (e.g. leukocytes, macrophages, and neutrophils). Cytokines act on several different cell types, such as T-lymphocytes, B-lymphocytes, and natural killer cells (Dinarello, 1991). Cytokines are also important in immune function and have been shown to be important in the bi-directional communication between the brain and the immune system. Interleukin-1 (IL-1) is a cytokine believed to act directly on the central nervous system and have an effect on learning and memory. Glial cells and neurons synthesize IL-1, and IL-1 receptors are found throughout the brain (Dinarello, 1991). An important location for IL-1 receptors is the hippocampus, which is, as previously mentioned, one of the structures in the brain known to be important in learning and memory (McNamara & Skelton, 1993). Cunningham, Wada, Carter, Tracey, Battey and DeSouza (1991) reported a large number of IL-1 receptors located in the dentate gyrus and CA3 Schaffer collateral pathway of the hippocampus, and Dinarello (1991) found high concentrations of these receptors on hippocampal neurons.

Interleukin-1 has two isoforms: IL-1 beta (IL-1β) and IL-1 alpha (IL-1α). Both isoforms of the IL-1 family are distinct gene products, yet recognize the same surface receptors and share some of the same biological activities (Dinarello, 1991); however, it is the IL-1β isoform that has received the most attention in studies of learning and memory deficits.

Interleukin-1β

Murray & Lynch (1998) showed an increase in the concentration of IL-1β throughout the hippocampus, particularly in the dentate gyrus, with increasing age. Low concentrations of IL-1β in these brain regions can be neuroprotective, but evidence suggests that in higher concentrations, such as those found in aged rats, IL-1β may induce neurodegenerative changes (Hopkins & Rothwell, 1995). It is these neurodegenerative changes that could decrease an aged animal's ability to learn and remember.

An important neurodegenerative change produced by IL-1β in the hippocampus is through its effect on long-term potentiation (LTP). As mentioned above, age produces a decrease in synaptic plasticity and membrane permeability in the hippocampus (Pitler & Landfield, 1990), as well as a decrease in neuronal ability to sustain LTP (Murray & Lynch, 1997 & 1998). IL-1β could possibly be involved in this age-related decrease in LTP, because IL-1β is known to inhibit LTP in the mossy fiber CA3 pathway of the mouse hippocampus (Katsuki, Nakai, Hirai, Akaji, Kiso, & Satoh, 1990) and enhance synaptic inhibitory mechanisms (Bellinger, Madamba, & Siggins, 1993). Intracerebroventricular injection (ICV) of IL-1β was shown to have little effect on the induction of LTP, but IL-1β significantly inhibited maintenance of LTP (Murray & Lynch, 1998).

Another important neurodegenerative change that IL-1β could induce at the level of the membrane of the neuron is the release of reactive oxygen species, the process which produces free radicals (as mentioned in the above section on the free radical theory of aging). The formation of free radicals is commonly thought to be involved in age-related changes in the brain. Nathan and Tsunawaki (1986) showed that the biological

activities of IL-1β include the release of reactive oxygen species. Furthermore, Murray and Lynch (1997) showed that lipid peroxidation was increased by IL-1β, and that this effect was inhibited by the antioxidant vitamin E, suggesting that the effect of IL-1β was mediated by reactive oxygen species. This finding is especially important in the context that IL-1β also inhibits long-term potentiation (LTP).

The precise mechanism whereby free radicals inhibit LTP is not known. It may be that they oxidize the NMDA receptor complex and reduce its contribution to the increased synaptic response associated with LTP. It is also possible that the peroxide formed by the free radicals interferes with one of several second messenger systems thought to be involved in LTP (Pellmar, Hollinden, & Sarvey, 1991). There is also evidence that free radicals induce oxidation of arachidonic acid, thereby decreasing membrane concentration, and inhibiting LTP (Lynch & Voss, 1994). Additionally, results from Choi & Yu (1995) demonstrated free radicals play a role in neuronal cell death and age-related neuronal deficits resulting from oxidative damage. This compilation of evidence indicates that increases in free radicals in the brain have an overwhelming inhibitory effect on LTP, and possibly a deleterious effect on learning.

Murray and Lynch (1998) demonstrated another degenerative change IL-1β has on neuronal membranes that may also lead to an age-related deficit in learning. These researchers found ICV injection of IL-1β induced a significant increase in lipid peroxidation. They suggest this effect is related to the IL-1β-induced release of reactive oxygen species and significantly decreased concentrations of membrane arachidonic acid in neurons of the hippocampus. They also found that changes in aged rats (as compared to young rats) indicate a significant increase in reactive oxygen species, lipid

peroxidation, and decreases in membrane arachidonic acid, as well as impairments in LTP. This information is important because increases in lipid peroxidation and decreases in arachidonic acid are known to have a degenerative effect on hippocampal neurons that could lead to a decrease in cognitive functioning

Behavioral Effects of Interleukin-1β

The deleterious effects of IL-1β on learning have also been demonstrated behaviorally. For example, Oitzl, van Oers, Schobitz, & de Kloet (1993) showed that central injection of IL-1β disrupted learning of spatial navigational information in the Morris water maze task. Similarly, Gibertini, Newton, Friedman, & Klein (1995) showed that peripheral injections of exogenous IL-1β inhibited learning in the Morris water maze task. In this study, Gibertini and colleagues demonstrated that the effects of exogenous IL-1β, as well as endogenous IL-1β produced in response to an injected infection of *Legionella pneumophilia*, on task acquisition in mice were neutralized by the peripheral injection of an antibody raised against IL-1β, which binds to IL-1β and interferes with its function.

Based on previous research, it seems that the increased levels of IL-1β present in aged animals may have a degenerative effect on the neurons of the hippocampus, and thereby impact learning and memory. A next logical step in investigating IL-1β's role in aging was to examine the effects of blocking the function of IL-1β on the learning of aged animals in a behavioral task. The task chosen for this study was the Morris water maze since the hippocampus has been implicated as mediating learning this task (e.g. MacNamara & Skelton, 1993).

Interleukin-1 Receptor Antagonist

The antagonist that was used in the current research was the IL-1 receptor antagonist (IL-1ra). All cytokines, including IL-1, are self-regulating through the action of opposing cytokines and antagonists. IL-1ra was the first described of such naturally occurring antagonists. Other cytokines, viral products, and acute phase proteins induce IL-1ra production. This indicates that IL-1ra may be produced *in vivo* in many inflammatory and infectious diseases (Arend, Malyak, Guthridge, and Gabay, 1998). IL-1ra has been identified as a polypeptide member of the IL-1 family which specifically inhibits IL-1 by occupying the IL-1 receptor(s) with nearly the same affinity as IL-1 but without the accompanying agonist activity, and without affecting other cytokines or binding to IL-1 itself (Dinarello, 1991; Arend et al, 1998). It is unknown if the function of IL-1ra is solely to regulate the effects of IL-1α and β in normal or pathological conditions (Arend et al, 1998).

However, the availability of recombinant IL-1ra has provided a useful experimental tool for investigating the endogenous action of IL-1 (Bianchi, Sacerdote, and Panerai, 1998). Therapeutically, administration of IL-1ra has been shown to be beneficial in many experimental animal models of disease. For example, intravenous (IV) administration of IL-1ra has been shown to be beneficial in treatment of various forms of arthritis in rodents, septic shock, bacterial meningitis, and colitis (Arend et al, 1998). IL-1ra has also proven to be important in attenuating the suppression of LTP believed to be caused by IL-1 (Cunningham, Murray, O'Neill, Lynch, and O'Connor, 1996). Behavioral responses to endotoxin are attenuated in response to administration of IL-1ra (Bluthe, Dantzer, and Kelley, 1992), as is escape learning failure following inescapable tail shock

and the decrease in contextual fear conditioning caused by social isolation (Maier, and Watkins, 1995; Pugh, Nguyen, Gonyea, Fleshner, Watkins, Maier, and Rudy, 1999). IL-1ra has been identified in the brain, but how regulation of synthesis occurs and its functional importance is unknown. mRNA for IL-1ra has been localized in rat brain by in situ hybridization in the paraventricular nucleus of the hypothalamus, the hippocampus, and the cerebellum (Licinio, Wong, and Gold, 1991). It has also been shown that neuronal cell death in the brain induced by focal cerebral ischemia or exitotoxic damage is reduced by central injection of IL-1ra (Relton and Rothwell, 1992). Therefore, endogenous production of IL-1ra may be an important anti-inflammatory response to ischemia, as well as playing a role in regulation of the neuroendocrine system (Arend et al, 1998). Because of IL-1ra's demonstrated role in blocking the effects of IL-1 (and therefore IL-1β) both peripherally and centrally, it was the perfect candidate to test as an antagonist to IL-1β in aged animals in order to evaluate the effects of blocking IL-1β on learning.

Hypotheses

After reviewing the background research, it was predicted that when the IL-1 receptor antagonist was injected ICV into aged rats, it would block the damaging effects of IL-1β on hippocampal neurons, and thereby enhance learning. It was expected that when the animals were tested in the Morris water maze learning task, the animals receiving IL-1ra would perform better than the animals that did not receive IL-1ra. Superior learning was defined as shorter escape latencies, and decreased cumulative and average proximity in the water maze during acquisition and decreased cumulative and

average proximity from the former escape platform location during the probe trial. It was also predicted that the aged animals receiving the IL-1ra would not be significantly different from the aged control animals.

A second objective of this experiment was to examine if there was an age difference in the performance of rats in the Morris water maze, comparing young rats to old. It was hypothesized that, because the young control rats would potentially have lower levels of IL-1β than the old control rats, the young control rats would perform significantly better than the old control rats in the Morris water maze during the acquisition and probe phases. Superior performance was again defined as shorter escape latencies, and decreased cumulative and average proximity in the water maze during acquisition and decreased cumulative and average proximity from the former escape platform location during the probe trial. It was also hypothesized that the performance of the young control rats would not be significantly different from the performance of the aged control rats during the cued phase

The last objective of this experiment was to determine if there was an age difference in the protein concentration of IL-1β in the brains of old versus young rats, and to correlate these concentrations with performance of the rats in the water maze (IL-1β quantitation). First, it was hypothesized that there would be a significant difference in the protein concentrations of IL-1β between groups, with the aged groups showing higher concentrations compared to the young groups. It was expected that the young control group would have the lowest concentration of IL-1β, while the aged groups would not be different from one another in their concentration of IL-1β. It was also predicted that the concentrations of IL-1β found the in the brains of the aged animals would be correlated

with their performance, such that increased concentration would be related to decreased performance.

Chapter 2: Method

Subjects

Forty-five male Fischer 344 rats acquired from the National Institute of Aging (Bethesda, MD) served as subjects for this study. Upon arrival, the animals were housed in pairs in plastic hanging cages containing wood shavings as bedding material. Animals were allowed *ad libitum* access to lab chow, and free access to water throughout the experiment. A continuous 12: 12h light/dark cycle was maintained in the room where the subjects were housed. The animals were divided into three groups of 15 rats each: young control, aged control, and aged experimental. Thirty-one rats survived the surgical procedure and were available for behavioral testing. Ten rats composed the young control group, and were approximately three months of age at the start of the experiment. This group received control intracerebroventricular (ICV) injections of saline (2 μl) and was run in the same behavioral protocol as the aged animals. The second group, the aged control group, consisted of 11 rats (approximately 25 months of age at the start of the experiment) and received saline control ICV injections (2 μl). The third group, the aged experimental group, consisted of ten rats (approximately 25 months of age at the start of the experiment). This group received ICV injections of the IL-1ra antagonist (0.50 μg in 2 μl).

Surgery

Two weeks prior to the behavioral testing, animals were anesthetized [100 mg/kg ketamine mixed with 10 mg/kg xylazine injected intraperitoneally (IP)] and a 23-gauge stainless steel guide cannulae was inserted stereotaxically into the lateral ventricle for ICV injections, using Bregma as a reference. Coordinates for cannulae placement were 2 mm lateral to midline, 0.6 mm posterior to Bregma, and 3.2 mm under the surface of the skull with toothbars 5 mm above the interaural line (according to procedures outlined in Bluthe, et al., 1992). Half of the cannulae were implanted to the left of Bregma, and the other half were placed to the right of Bregma, selected randomly. The cannulae were secured in place via Superglue, a method previously found to be successful in our lab. Unfortunately, the Superglue did not adequately secure the cannulae.

Because the method used to secure the cannulae was unsuccessful, verification of the precise placement of the cannulae was impossible. To resolve this problem, the animals were re-anesthetized, using ketamine/xylazine (1.0 ml/kg for old animals IP and 1.52 ml/kg for young animals IP) and atropine (.093 ml/kg sub-cutaneous) to counteract breathing problems associated with the anesthetic. We then replaced the cannula on the opposite side of Bregma (from previous placement) and secured it to the animal's skull using dental acrylic and screws. Animals were given four days of recovery time before behavioral testing. During recovery, the anti-inflammatory drug banamine (1.1 mg/kg sub-cutaneous) was injected twice a day, and the analgesic Tylenol + codeine was provided in the animals' water bottles.

Apparatus

All animals were tested in the same room (approximately 12 x 20 ft) and in the same apparatus. A circular, aluminum Morris water maze, measuring 183 cm in diameter and 61 cm in height, was used as the test apparatus. The tank was filled with water (~18°C) to a height of 30 cm, and non-toxic, black tempera paint (Dry Temp, Palmer Paint Products) was used to dye the water black. The escape platform was a black, circular platform (10 cm diameter) that was placed 1 cm beneath the surface of the water in one quadrant of the tank. The distance from the center of the platform to the side of the tank was maintained at 41 cm. The escape platform was kept in the same location in the tank throughout the acquisition phase. The tank was located in a room with a variety of distal, spatial navigational cues, including posters on the walls. A large white curtain was used to obscure experimenters from the animal's view during the experiment.

A video tracking system (PolyTrack Video Tracking System, San Diego Instruments, San Diego CA) was used to record escape latency (time to find the escape platform) and to record the movement of the animals within the maze during each trial. A computer outside of the maze area tracked the animals within the maze via a CCD camera mounted above the water maze. This system also monitored the animal's movements from quadrant to quadrant for data analysis. The tracking system also corrected for each animal's start position (start quadrant) in the maze by estimating the most efficient swim path to the escape platform based on that animal's swim speed, and subtracting that amount of time from the track in the beginning of the animal's swim.

Behavioral Testing

Upon arrival, animals were housed in pairs, and were weighed and given free access to food and water in their home cages. The animals were handled for five days to habituate them to experimental procedures. The subjects were then assigned to their experimental conditions, in which the young animals were assigned to the young control group, and the old animals were randomly assigned to old control, or old experimental groups. Following habituation, the animals underwent the stereotaxic surgery to implant cannulae into the lateral ventricle for ICV injections. Ventricle placement was verified at the time of injection by the presence of free gravity flow of saline through tubing attached to the cannula. All injections were given over a two-minute period using a 33-guage microinjection needle inserted through the guide cannula. The injector was left in place for two minutes after the injection was completed. Injections were given each day, approximately 15 minutes before behavioral testing.

All animals were tested for spatial learning using a conventional Morris water maze task. During the experiment, the animals were given ICV injections of either saline (control groups) or the IL-1ra (experimental group) at least 15, and no longer than 30 minutes prior to being placed in the maze. The experiment consisted of 11 sessions in which Days 1-10 were acquisition, and Day 11 was a cued phase. Every odd day (e.g. Days 1, 3, 5, etc.) during acquisition (Days 1-10) consisted of only three trials. Every even day (e.g. Days 2, 4, 6, etc.) contained a probe trial (see below) between the second and third trial of acquisition, therefore these sessions consisted of four trials. There were a total number of 41 trials per animal during the experiment.

Phase 1: Acquisition. The first phase of the experiment (Days 1-10) was acquisition during which the individual animals were randomly placed in one of the three quadrants of the maze that did not contain the platform. The animals were carefully placed in the water facing the wall of the maze to prevent experimenter bias (e.g. inadvertently pushing the animal toward the escape platform). The rats were allowed to swim for 60 seconds, or until they found the platform. If the animal located the platform before 60 seconds, they were allowed to remain on the platform for 30 seconds in order to become aware of the distal cues in the room and the relative location of the platform. After its time on the platform, the animal was removed from the maze and put under a heating lamp for a 60-second intertrial interval. If the rat did not find the platform in 60 seconds, the animal was manually placed on the platform and allowed to remain there for 30 seconds, after which it was placed under the heat lamp for 60 seconds. During the intertrial interval, any feces were removed from the maze to minimize any olfactory cues left by the animal. Following the intertrial interval, the animal was again randomly placed in one of the non-platform quadrants for the next trial. Latency to locate the platform, measured in seconds, as well as a record of each animal's swim path within the maze (including cumulative and average proximity) was recorded by the computer. If the session was a non-probe session (odd-numbered days), then after each animal completed all three acquisition trials of the session, it was towel dried and returned to its home cage.

Phase 2: Probe Trials. If the session was a probe trial session (even-numbered days), the third trial (between the second and third trial of acquisition) was reserved for the probe trial. In the probe trial, the platform was removed from the maze and the rats were required to swim in the maze for 60 seconds. They were then placed under the heat

lamp for 60 seconds, after which they were removed from the heat lamp and placed into the maze for the fourth trial of the session. The purpose of the probe trial was to record the animals' behavior when the platform was not in the tank, thereby preventing chance encounters with it. Cumulative and average proximity of swim path to the previous location of the platform was recorded, by measuring the distance between the animal and the former platform location every frame (18 times per second) during the 60-second trial period. The fourth trial of these sessions was considered a retraining trial, during which the platform was returned to its previous location and the trial proceeded as other acquisition trials. Because these trials were considered retraining, the data from the fourth trial on even numbered days was analyzed separately.

Phase 3: Cued Trials. Day 11 was a cued phase, in which the motor and sensory abilities of the animals were assessed by placing a distinct proximal cue within the maze. During these six cued trials, a flag was placed on the escape platform (approximately 6 inches from the top of the platform) to serve as a proximal cue. This flag was approximately 3 inches wide by 2 inches high and colored red and white (to provide contrast against the black tank and water). The platform was initially raised 2 cm above the water, however old animals had trouble climbing onto the platform, so the platform was lowered to the same level as the water for subsequent cued trials. The platform was randomly placed into different quadrants within the maze to prevent information learned during acquisition from causing a bias during cued trials. To maintain procedural consistency throughout the experiment the rats were permitted 60 seconds to locate the platform, and 30 seconds to remain on the platform, followed by a 60 second intertrial

interval under the heat lamp. Escape latencies, and cumulative and average proximities were measured as previously described.

IL-1β Quantitation

Following completion of behavioral testing, all animals were sacrificed and the brains were removed. The hippocampus was dissected out and chopped into 350 μm slices using a tissue chopper. The slices were then divided into four tubes for protein analysis, washed three times in a Krebs solution (Krebs buffer with 2 mM $CaCl_2$) on ice, and stored in 1ml Krebs solution containing 10% DMSO at –80 degrees C.

For protein analysis, the tissue was thawed quickly and washed three times in Krebs solution. After washing, the supernatant was removed and 400 μl of Krebs solution was added. The tissue was then homogenized using a sonicator. For the protein assay, 360 μl of phosphate buffered saline (PBS), 35 μl of Krebs solution, and 5 μl of the homogenate were mixed in an aliquot for the sample dilution. Six standards were also prepared for comparison of protein concentration using 95% PBS and 5% Krebs solution (buffer) mixed with bovine serum albumin (BSA). The standards consisted of 0, 2, 5, 10, 20, or 30% BSA. The protein analysis was performed using a Bio-Rad kit (Bio-Rad, Hercules, CA). One hundred μl of sample or standard solution was added to each well in triplicate in a 96-well plate. Five μl of 0.2 M NaOH was then added to each well, and the plate was shaken for 15 minutes. After shaking, 25 μl of reagent A and 200 μl of reagent B (Bio-Rad, Hercules, CA) were added to each well. The plate was then shaken for five minutes, and absorbance was read at 750 nm within 30 minutes. Standards were used to convert sample optical density to mg/ml protein. Based on the concentrations of total

protein present in each aliquot, Krebs solution was added to achieve the desired protein concentration of 1.5 mg/ml protein (based on protocol from personal communication with Lynch, 2002). Samples were then refrozen and stored at –80 degrees C.

An Enzyme-Linked Immunosorbant Assay (ELISA) was then used for quantitative analysis of the IL-1β concentration present in the hippocampi of both the young and aged animals. The Quantikine M rat IL-1β immunoassay kit for the ELISA was purchased from R & D Systems (Minneapolis, MN). The assay employed the quantitative sandwich enzyme immunoassay technique. An affinity purified polyclonal antibody specific for rat IL-1β was pre-coated onto ninety-six-well plates. Standards, controls, an enzyme-linked antibody specific to IL-1β, substrate solution, and stop solutions were also provided in the kit.

The procedure for IL-1β quantitation was performed according to the procedure from Murray and Lynch (1998). Diluted samples were thawed to room temperature. 100μl of samples, controls (from the kit), or IL-1β standards (also from the kit) were added in triplicate to each well of the 96-well plates, on which an affinity purified polyclonal antibody specific for rat IL-1β was pre-coated (from the kit), and incubated for 2 hours at room temperature. Plates were washed four times using a plate washer, and a biotinylated second primary antibody, which was conjugated to the detection agent (horseradish peroxidase), was added to each well, after which the incubation continued for another 2 hours at room temperature. The plates were again washed four times, and 100μl of substrate (tetramethylbenzidine and stabilized hydrogen peroxide liquid substrate) were added to each well, with incubation following for 30 minutes at room temperature. Finally, the reaction was ended by adding H_2SO_4 (stop solution), and

absorbance was read at 450 and 540 nm within 30 minutes. Standards were used to convert sample optical density to μg IL-1β/μg protein. While the ELISA was run, another protein concentration analysis was run (according to the above protocol) to determine final, post-dilution protein concentration, so IL-1β concentration could be corrected for total protein concentration.

Because there was so much variability in IL-1β concentration within groups, as well as within individual animals (within triplicates), it was decided that the ELISA should be re-run to provide more accuracy in the results. Using hippocampi dissected out of spare animals from another project (n=2), we attempted to modify the ELISA protocol to obtain more stable results within individual animals' data (triplicates). The whole hippocampus was placed in 320μl of 0.5x PBS, which created a hypotonic environment, and caused membranes to lyse, allowing access to intracellular stores of IL-1β as well as extracellular IL-1β. No washes were used (as in the previous protocol). The tissue was sonicated, and a protein analysis was performed (see above protocol). Samples were then diluted to reach a total protein concentration of 7.33 mg/ml (based on the protocol of Vezzani, Conti, De Luigi, Ravizza, Moneta, Marchisi, and De Simoni, 1999). The ELISA protocol was then run as before (see above).

Based on more stable results from this practice ELISA, the new protocol was used to determine IL-1β concentration in the undissected hippocampus of the original animals. The opposite hippocampus (from the one used previously) was dissected on ice and placed in 320 μl of 0.5x PBS. The sample was then sonicated and protein analysis was performed on the samples (see above), in order to determine total protein concentration. Samples were then diluted based on this data to achieve a total protein concentration of

7.5 mg/ml. The ELISA was then performed on the samples according to the previous protocol (see above), as well as a second protein analysis to determine true protein concentration post-dilution.

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) was used for statistical analyses of the data. Means were compared for 1) latency to find the platform, 2) cumulative proximity from the platform, and 3) average proximity from the platform, for young control versus aged control versus aged experimental groups for the acquisition phase of the experiment. A 3x10 mixed design analysis of variance (ANOVA) for group by trials was then performed on the obtained means for each of these dependent measures. For the probe trials, means were compared for the cumulative proximity from the former platform location and average proximity from the former platform location among groups. A 3x5 mixed-design ANOVA for group by trials was then conducted on the obtained means for individual animals for each of these measures. For the cued phase, means were compared for the latency to find the platform, cumulative proximity from the platform, and average proximity from the platform among groups. A 3x6 mixed-design ANOVA for group by trials was then conducted on the obtained means for individual animals for each of these measures.

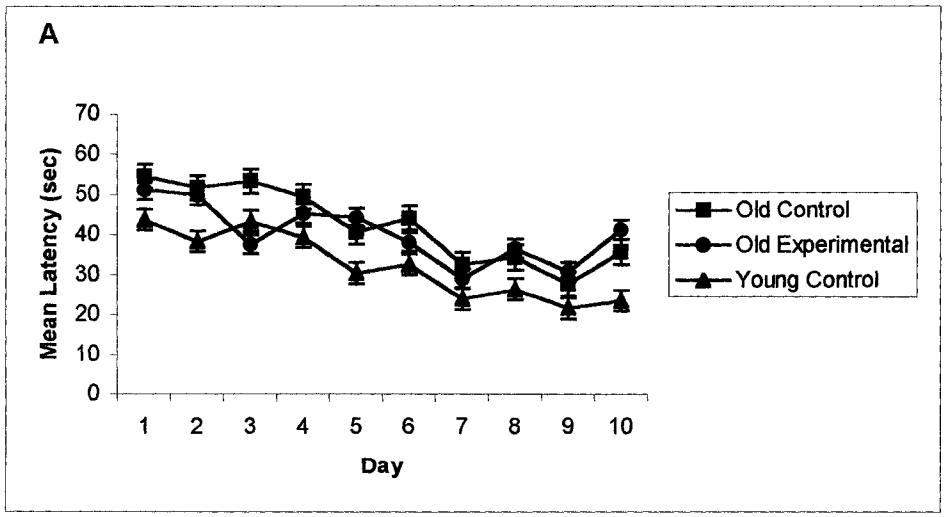
For the IL-1β quantitation (ELISA), mean IL-1β protein concentrations (pg/mg of tissue) were calculated, and a one-way ANOVA comparing groups was performed. A correlational analysis was also performed to examine the relationship between IL-1β protein concentrations and performance in the water maze. Pearson's correlation

coefficient was used to determine if there was a significant relationship between IL-1β concentration and the performance of each animal in the water maze during each phase (acquisition, probe, and cued).

Chapter 3: Results

Acquisition Phase

After the acquisition phase (Days 1-10) was completed, the mean latency for each day across 3 trials for each group was calculated. The mean latencies and standard errors of the mean (SEM) for the aged experimental group, the aged control group, and the young control group across all days of acquisition are shown in Figure 1A. As shown in Figure 1B, the mean latency (collapsed across days) for the old control group was 42.41s (SEM = 3.04s), while the mean latency for the old experimental group was 40.39s (SEM = 2.35s), and the mean latency for the young control group was 32.34s (SEM = 2.66s). Therefore, the young control group performed better than either of the aged groups, because the young group had a much lower latency to find the escape platform.



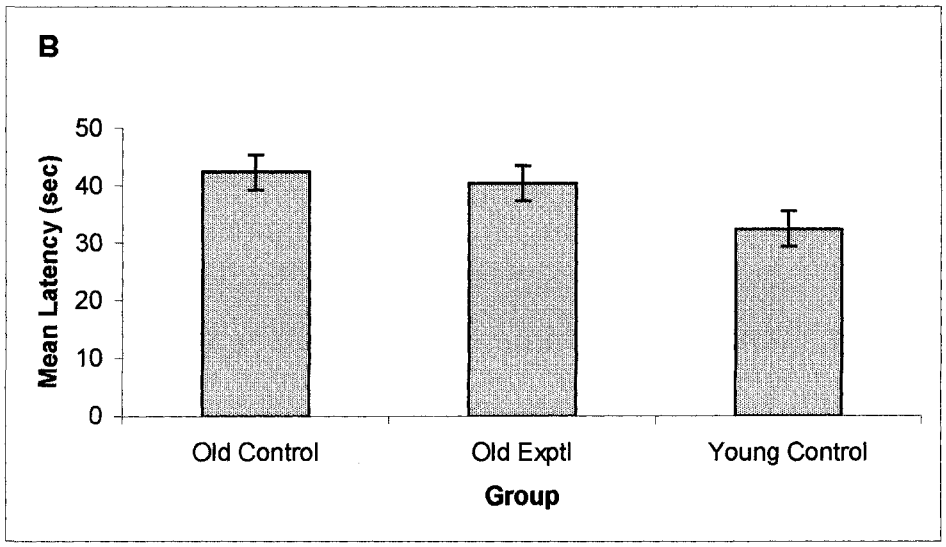


Figure 1: Acquisition training in the Morris water maze. *Panel A*: Shown is the old control group (squares) injected with saline ICV, versus the old experimental group (diamonds) injected with interleukin-1 receptor antagonist (IL-1ra) ICV, versus the young control group (triangles) injected with saline ICV. Symbols denote mean latency to find the hidden escape platform across animals, 3 trials per day. Error bars represent standard error of the mean. *Panel B*: Shown are the old control group, old experimental group, and young control group. Bars represent mean latency averaged across all days of acquisition. Error bars represent standard error of the mean.

A mixed design ANOVA was performed on these means and yielded a significant difference among groups due to treatment, $F(2, 27) = 9.23$, $p < .001$. Post-hoc analysis of the treatment effects (using Tukey's HSD) showed a significant difference between the old control group and the young group, and between the old experimental group and the young group. There was not a significant difference between the old control group and the old experimental group (as shown in Table 1).

Post-hoc Comparison	Mean Difference	Significance
Old Cont to Old Exptl	2.0198	.675
Old Cont to Young	10.072*	.001
Old Exptl to Young	8.052*	.009

Table 1: Shown is the mean difference among the old control, old experimental, and young control groups for latency during the acquisition phase. * indicates significance at the $p < .05$ level.

Also, as can be seen in Figure 1A, performance of the animals in all groups significantly improved across days of acquisition, $F(9, 243) = 13.26$, $p < .0001$, with the young group

showing the most improvement. There was not a significant interaction between treatment and performance across days, $F(18, 243) = .95$, $p < .51$.

The mean cumulative proximity from the escape platform for each day across three trials for each group was also calculated. The mean cumulative proximity and standard errors of the mean for the aged experimental group, the aged control group, and the young group across all days of acquisition are shown in Figure 2A.As shown in Figure 2B, the mean cumulative proximity (collapsed across days) for the old control group was 28104.86 tracker units (SEM = 1909.89 tracker units), while the mean cumulative proximity for the old experimental group was 28599.13 tracker units (SEM = 1470.83 tracker units), and the mean cumulative proximity for the young control group was 28249.56 tracker units (SEM = 3261.87 tracker units). Therefore, none of the groups performed significantly better than any other group, because all groups had a similar cumulative proximity from the escape platform. A mixed design ANOVA was performed on these means and yielded a non-significant difference among groups due to treatment, $F(2, 27) = .01$, $p < .99$. However, as can be seen in Figure 2A, performance of the animals in all groups significantly improved across

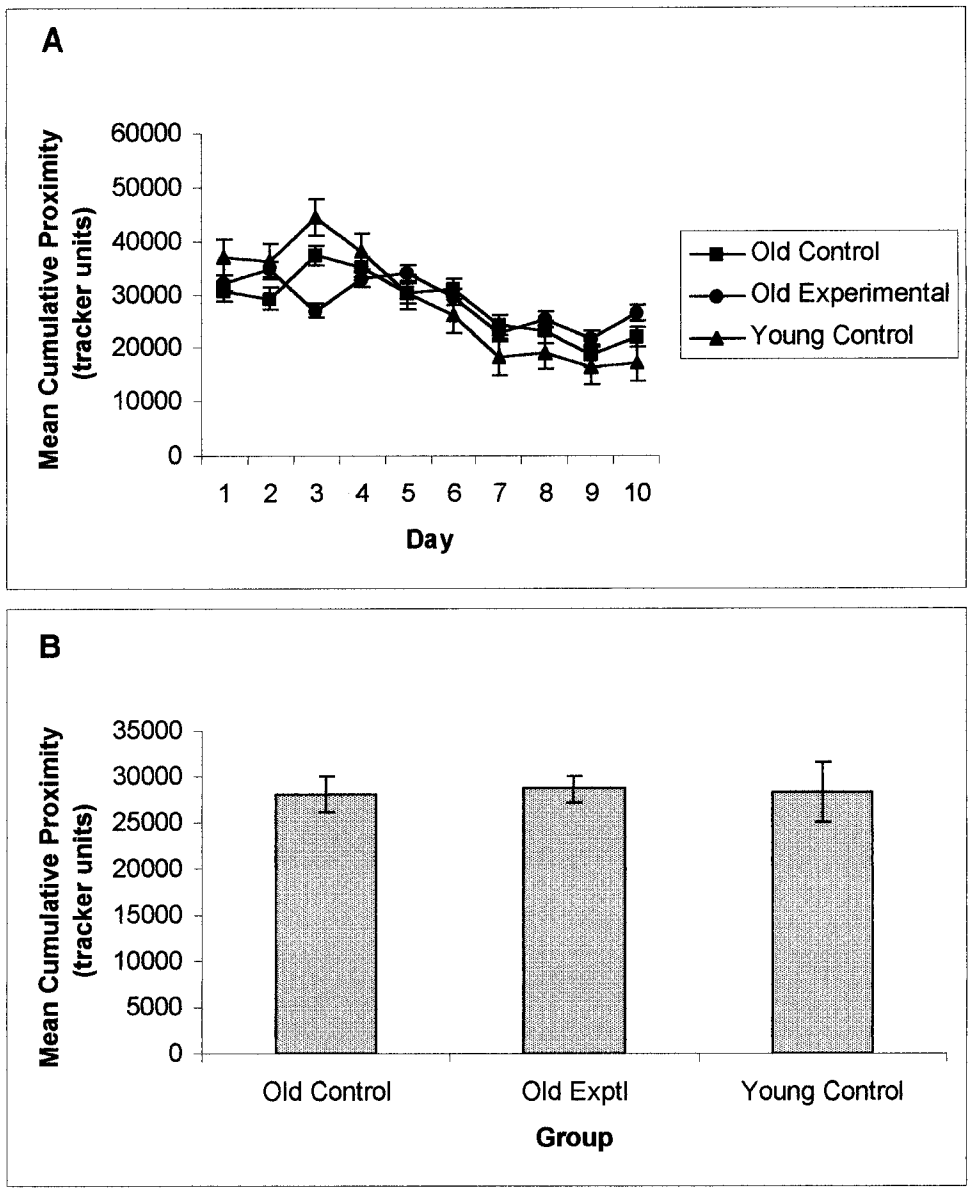


Figure 2: Acquisition training in the Morris water maze. *Panel A*: Shown is the old control group (squares) injected with saline ICV, versus the old experimental group (diamonds) injected with interleukin-1 receptor antagonist (IL-1ra) ICV, versus the young control group (triangles) injected with saline ICV. Symbols denote mean cumulative proximity from the hidden escape platform across animals, 3 trials per day. Error bars represent standard error of the mean. *Panel B*: Shown are the old control group, old experimental group, and young control group. Bars represent mean cumulative proximity averaged across all days of acquisition. Error bars represent standard error of the mean

days of acquisition, $F(9,243) = 9.15$, $p < .0001$. There was not a significant interaction between treatment and performance across days, $F(18, 243) = 1.28$, $p < .20$.

Lastly, the mean average proximity from the escape platform for each day across three trials for each group was calculated. The mean average proximity and standard

errors of the mean for all groups across all days of acquisition are shown in Figure 3A.As shown in Figure 3B, the mean average proximity (collapsed across days) for the old control group was 69.30 tracker units (SEM = 3.15 tracker units), while the mean average proximity for the old experimental group was 69.03 tracker units (SEM = 2.62 tracker units), and the mean average proximity for the young control group was 61.00 tracker units (SEM = 3.72 tracker units).

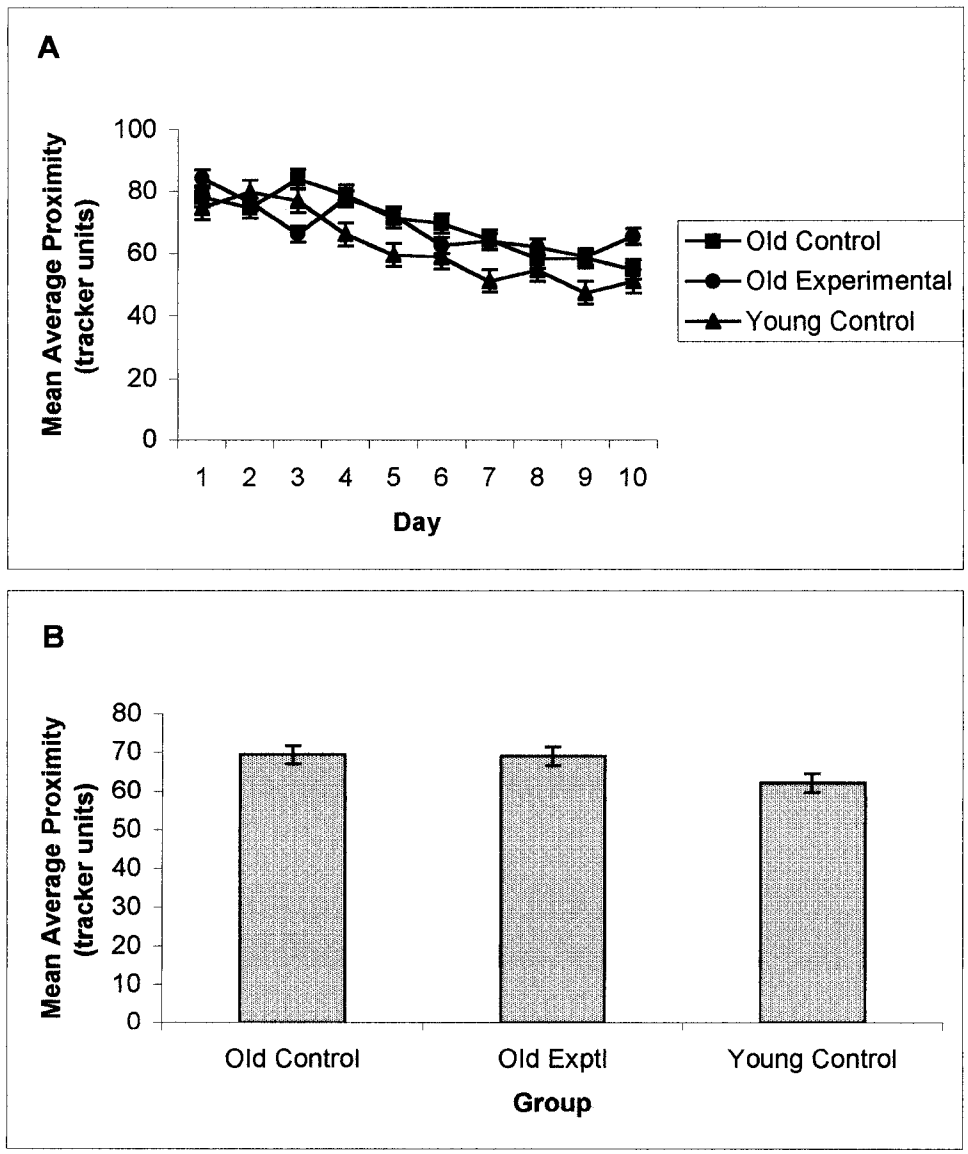


Figure 3: Acquisition training in the Morris water maze. *Panel A*: Shown is the old control group (squares) injected with saline ICV, versus the old experimental group (diamonds) injected with interleukin-1 receptor antagonist (IL-1ra) ICV, versus the young control group (triangles) injected with saline ICV.

Symbols denote mean average proximity from the hidden escape platform across animals, 3 trials per day. Error bars represent standard error of the mean. *Panel B*: Shown are the old control group, old experimental group, and young control group. Bars represent mean average proximity averaged across all days of acquisition. Error bars represent standard error of the mean.

Post-hoc Comparison	Mean Difference	Significance
Old Cont to Old Exptl	.272	.994
Old Cont to Young	7.303*	.033
Old Exptl to Young	7.031*	.047

Table 2: Shown is the mean difference among the old control, old experimental, and young control groups for average proximity during the acquisition phase. * indicates significance at the $p < .05$ level.

Therefore, the young control group performed slightly better than either of the aged groups, because the young group had a lower average proximity from the escape platform. Even though this difference is small, when a mixed design ANOVA was performed on these means it yielded a significant difference among groups due to treatment, $F(2, 27) = 4.37$, $p < .02$. Post-hoc analysis of the treatment effects showed a significant difference between the old control group and the young group, and between the old experimental group and the young group. There was not a significant difference between the old control group and the old experimental group (as shown in Table 2). Also, performance of the animals in all groups significantly improved across days of acquisition, $F(9, 243) = 12.61$, $p < .0001$ (see Figure 3A). There was not a significant interaction between treatment and performance across days, $F(18, 243) = 1.35$, $p<.16$.

Probe Phase

To evaluate the effect of the IL-1β receptor antagonist on performance during the probe trials (trial 3 on even-numbered days during acquisition), each group's cumulative proximity from the former platform location was averaged across each probe trial. The mean cumulative proximity and standard errors of the mean for each group across all of

the probe trials are shown in Figure 4A. As shown in Figure 4B, the mean cumulative proximity (collapsed across trials) for the old control group was 36997.54 tracker units (SEM = 2164.81 tracker units), while the mean cumulative proximity for the old experimental group was 38662.85 tracker units (SEM = 2104.39 tracker units), and the mean cumulative proximity for the young control group was 47045.04 tracker units (SEM = 2665.42 tracker units). Therefore, the old control group performed better than the young control and the old experimental groups, because it had a lower cumulative

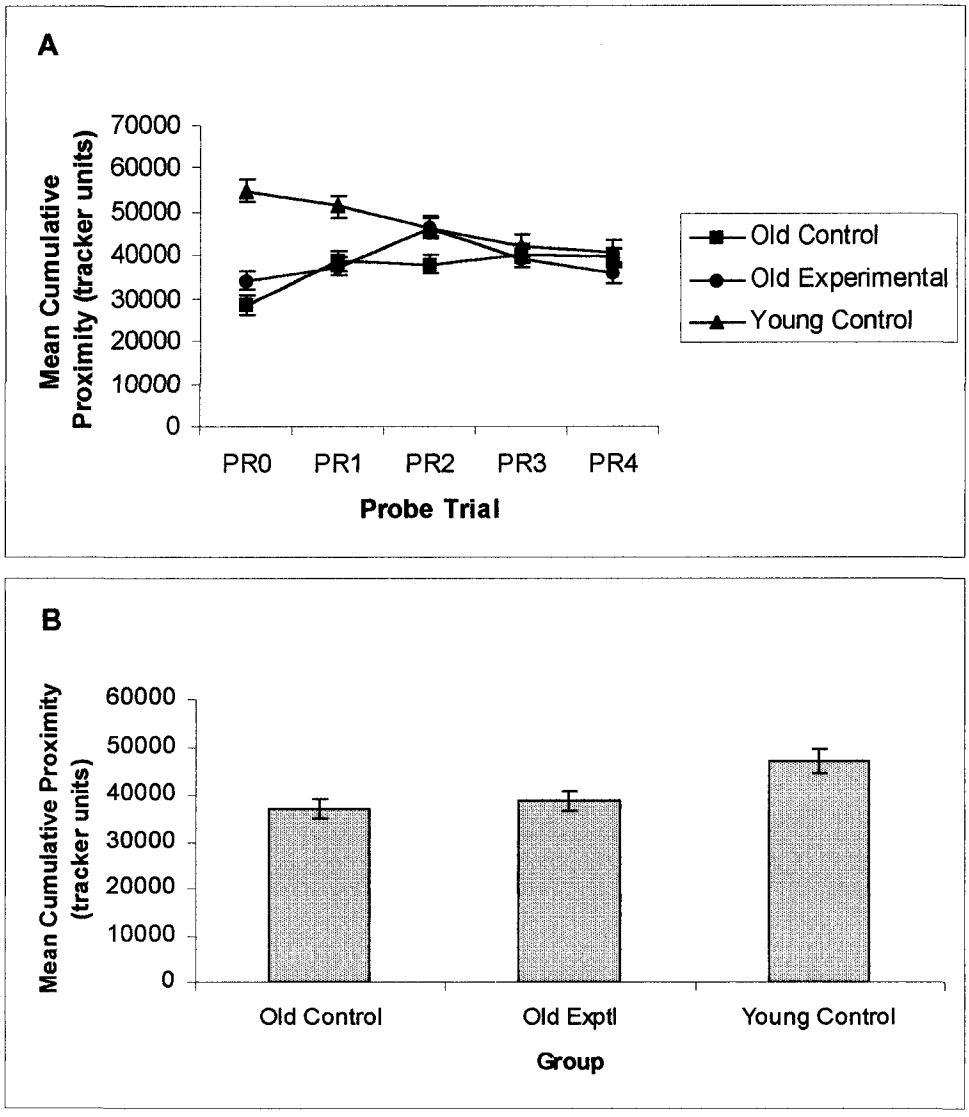


Figure 4: Probe phase in the Morris water maze. *Panel A*: Shown is the old control group (squares) injected with saline ICV, versus the old experimental group (diamonds) injected with interleukin-1 receptor

antagonist (IL-1ra) ICV, versus the young control group (triangles) injected with saline ICV. Symbols denote mean cumulative proximity from the former platform location across animals, for each probe trial. Error bars represent standard error of the mean. *Panel B*: Shown are the old control group, old experimental group, and young control group. Bars represent mean cumulative proximity averaged across all probe trials. Error bars represent standard error of the mean.

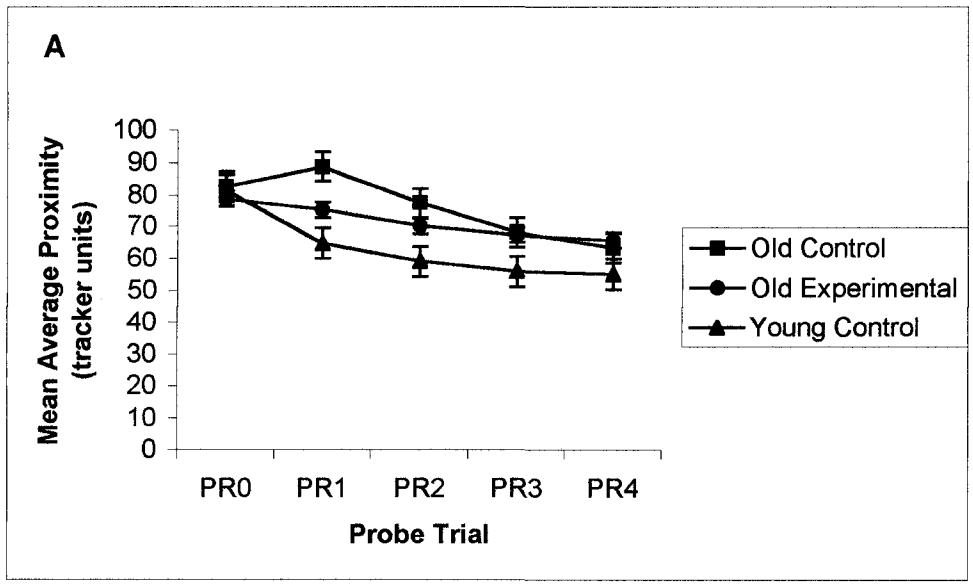
proximity from the former escape platform. The old experimental group also performed better than the young control group, because it had a lower cumulative proximity from the former platform location. A mixed-design ANOVA was performed on these means and revealed a significant difference among groups, $F(2, 27) = 3.91$, $p = .03$. Post-hoc analysis showed there was a significant difference between the old control group and the young group. There was not a significant difference between the old experimental group and the young group, or between the old experimental group and the old control group (see Table 3).

Post-hoc Comparison	Mean Difference	Significance
Old Cont to Old Exptl	-1665.314	.890
Old Cont to Young	-9867.497*	.034
Old Exptl to Young	-8202.183	.096

Table 3: Shown is the mean difference among the old control, old experimental, and young control groups for cumulative proximity during the probe phase. * indicates significance at the $p < .05$ level.

There was no significant difference across probe trials, $F(4, 108) = 1.03$, $p = .40$, indicating that the performance of all animals (across groups) did not change significantly from probe trial to probe trial. There was a significant interaction between treatment and trial, $F(8, 108) = 2.69$, $p = .01$ (see Figure 4A). Interaction analysis showed that there was a significant treatment effect on the first probe trial (PR0). The old control group was significantly different from the young group [Mean Difference = 7.37, $p < .05$] and the old experimental group was significantly different from the young group [Mean Difference = 5.75, $p < .05$].

In addition, for the probe trials each group's average proximity from the former platform location was averaged across each probe trial. The mean average proximity and standard errors of the mean for each group across all probe trials are shown in Figure 5A. As shown in Figure 5B, the mean average proximity (collapsed across trials) for the old control group was 76.07 tracker units (SEM = 4.58 tracker units), while the mean average proximity for the old experimental group was 71.48 tracker units (SEM = 2.40 tracker units), and the mean average proximity for the young control group was 63.33 tracker units (SEM = 4.80 tracker units).



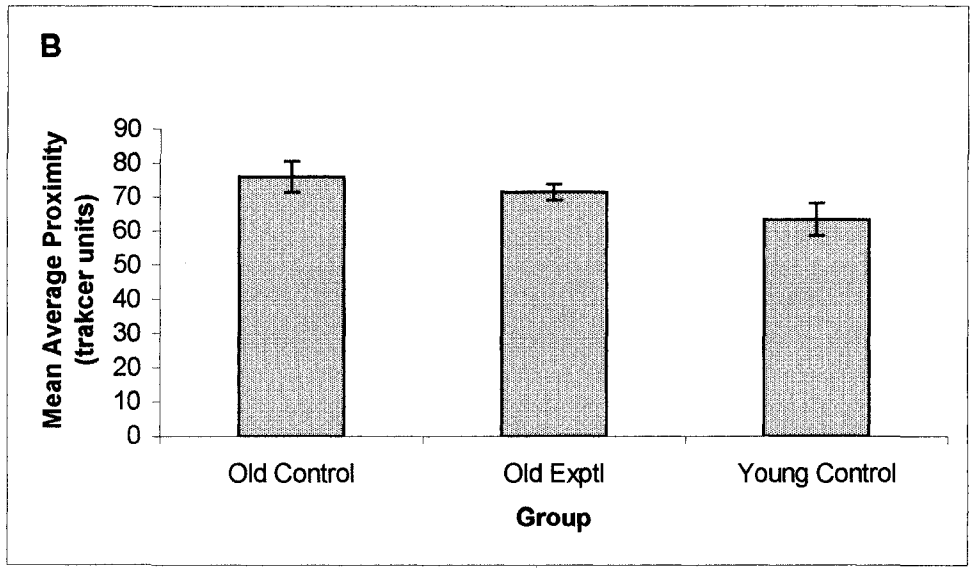


Figure 5: Probe phase in the Morris water maze. *Panel A*: Shown is the old control group (squares) injected with saline ICV, versus the old experimental group (diamonds) injected with interleukin-1 receptor antagonist (IL-1ra) ICV, versus the young control group (triangles) injected with saline ICV. Symbols denote mean average proximity from the former platform locations across animals, for each probe trial. Error bars represent standard error of the mean. *Panel B*: Shown are the old control group, old experimental group, and young control group. Bars represent mean average proximity averaged across all probe trials. Error bars represent standard error of the mean.

Therefore, the young control group performed better than either of the aged groups because it had a lower average proximity from the former platform location. The old experimental group also performed better than the old control group because it had a lower average proximity from the former platform location. We speculated that the inconsistent results between cumulative proximity and average proximity reflected the use of the correction for start position. Because the tracking system corrects for each animal's start position by estimating their most efficient path to the platform and subtracting that amount of time from the beginning of the animal's actual track, a slower swimmer (such as the aged animals) would have more time subtracted from their tracks than faster swimmers (such as the young animals). For cumulative proximity, the measurements of distance from the platform are summed over the animal's total time swimming. Therefore, a slower swimmer would have a lower cumulative proximity

simply because they had more information subtracted from their summed track than the faster swimmers. This might explain why the old animals had a lower cumulative proximity than the young animals in the probe phase. A mixed-design ANOVA was performed on these means and revealed a significant difference among groups, $F(2, 27) = 5.32$, $p = .01$. Post-hoc analysis showed there was a significant difference between the old control group and the young group. There was not a significant difference between the old experimental group and the young group, or between the old experimental group and the old control group (see Table 4).

Post-hoc Comparison	Mean Difference	Significance
Old Cont to Old Exptl	4.588	.462
Old Cont to Young	12.735*	.009
Old Exptl to Young	8.147	.124

Table 4: Shown is the mean difference among the old control, old experimental, and young control groups for average proximity during the probe phase. * indicates significance at the $p < .05$ level.

Also, as can be seen in Figure 5A, performance of the animals in all groups significantly improved across probe trials, $F(4, 108) = 9.69$, $p = .0001$, and there was not a significant interaction between treatment and trial, $F(8, 108) = 1.22$, $p = .30$.Retraining trials (trial 4 on even-numbered days during acquisition) were not statistically analyzed because these trials were designed to prevent extinction of platform searching behaviors. As expected, the data from the retraining trials were consistent with data collected during the acquisition phase.

Cued Phase

For the cued trials (six trials on Day 11), mean latency to locate, cumulative proximity from, and average proximity from the raised and flagged platform were calculated. These means and the standard error of the means are represented in Figures 6-

8. It was expected that there would not be a significant difference among groups on these trials since any differences in these comparisons would be attributed to differences in the underlying motivation, sensory, or motor abilities among the three groups.

First, the mean latency to locate the cued platform was calculated for each group. The mean latency and the standard errors of the mean across all cued trials are shown in Figure 6A.As shown in Figure 6B, the mean latency (collapsed across trials) for the old control group was 33.94s (SEM = 3.29s), while the mean latency for the old experimental group was 36.81s (SEM = 3.99s), and the mean latency for the young control group was 15.35s (SEM = 4.22s). Therefore, the young control group performed significantly better than either of the aged groups, because the young group had a much lower latency to find the visible platform. These means were evaluated using a mixed design ANOVA, which yielded a significant difference among groups, $F(2, 27) = 18.07$, $p = .0001$. Post-hoc analysis revealed a significant difference between the old control group and the young group, and between the old experimental group and the young group. There was not a significant difference between the old control group and the old experimental group, as shown in Table 5. Also, as can be seen in Figure 6A, performance of the animals in all groups was significantly different across cued trials, $F(5, 135) = 4.19$, $p = .001$. However, in contrast to other phases, this difference reflected a decrease in performance for the aged groups, because they showed an increase in latency to find the visible platform across cued trials. The young group showed no change in performance across trials. There was a significant interaction between treatment and cued trial, $F(10, 135) = 2.22$, $p = .02$. Interaction analysis showed there was a significant effect of treatment on cued trials five and six. On cued trial five, the old control group was significantly different

from the young group [Mean Difference = 5.68, $p < .05$] and the old experimental group was significantly different from the young group [Mean Difference = 5.51, $p < .05$]. On cued trial 6, the old control group was significantly different from the young group

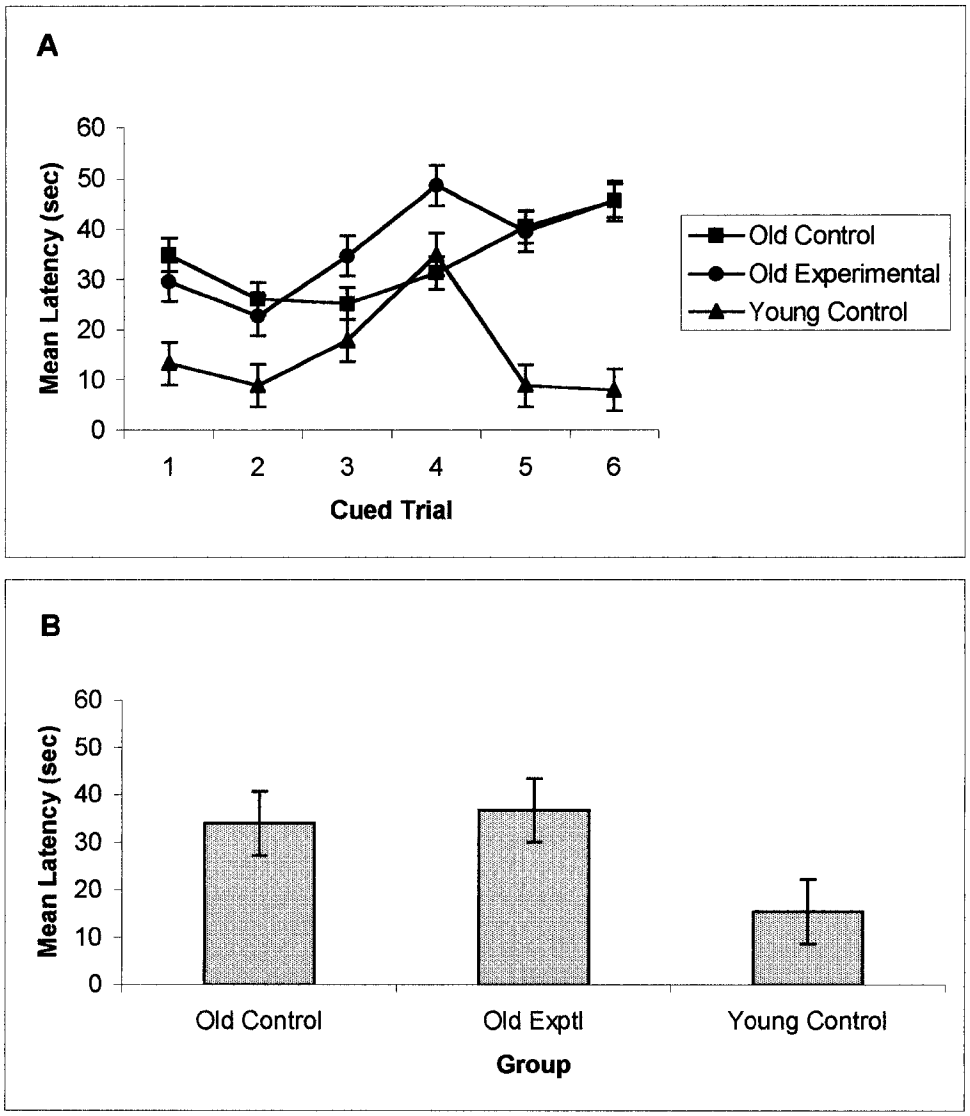


Figure 6: Cued phase in the Morris water maze. *Panel A*: Shown is the old control group (squares) injected with saline ICV, versus the old experimental group (diamonds) injected with interleukin-1 receptor antagonist (IL-1ra) ICV, versus the young control group (triangles) injected with saline ICV. Symbols denote mean latency to find the visible escape platform across animals, for each cued trial. Error bars represent standard error of the mean. *Panel B*: Shown are the old control group, old experimental group, and young control group. Bars represent mean latency averaged across all cued trials. Error bars represent standard error of the mean.

Post-hoc Comparison	Mean Difference	Significance
Old Cont to Old Exptl	-2.878	.716
Old Cont to Young	18.588*	.0001
Old Exptl to Young	21.466*	.0001

Table 5: Shown is the mean difference among the old control, old experimental, and young control groups for latency during the cued phase. * indicates significance at the $p < .05$ level.

[Mean Difference = 6.76, $p < .05$] and the old experimental group was significantly different from the young group [Mean Difference = 6.77, $p < .05$].

Next, the mean cumulative proximity from the cued platform was calculated for each group. The mean cumulative proximity and the standard errors of the mean across all cued trials are shown in Figure 7A.As shown in Figure 7B, the mean cumulative proximity (collapsed across trials) for the old control group was 22673.53 tracker units (SEM = 1960.82 tracker units), while the mean cumulative proximity for the old experimental group was 22290.55 tracker units (SEM = 2182.36 tracker units), and the mean cumulative proximity for the young control group was 8057.99 tracker units (SEM = 2.66 tracker units). Therefore, the young control group performed significantly better than either of the aged groups, because the young group had a much lower cumulative proximity from the visible platform. A mixed-design ANOVA was performed on these means and revealed a significant difference among groups, $F(2, 27) = 24.91$, $p = .0001$. Post-hoc analysis showed there was a significant difference between the old control group and the young group and between the old experimental group and the young group. There was not a significant difference between the old experimental group and the old control group, (see Table 6). There was not a significant difference across cued trials, $F(5, 135) = 1.83$, $p = .11$, meaning that the performance of all groups did not change

across cued trials. There was not a significant interaction between treatment and trial, $F(10, 135) = 1.29$, $p = .24$ (see Figure 7A).

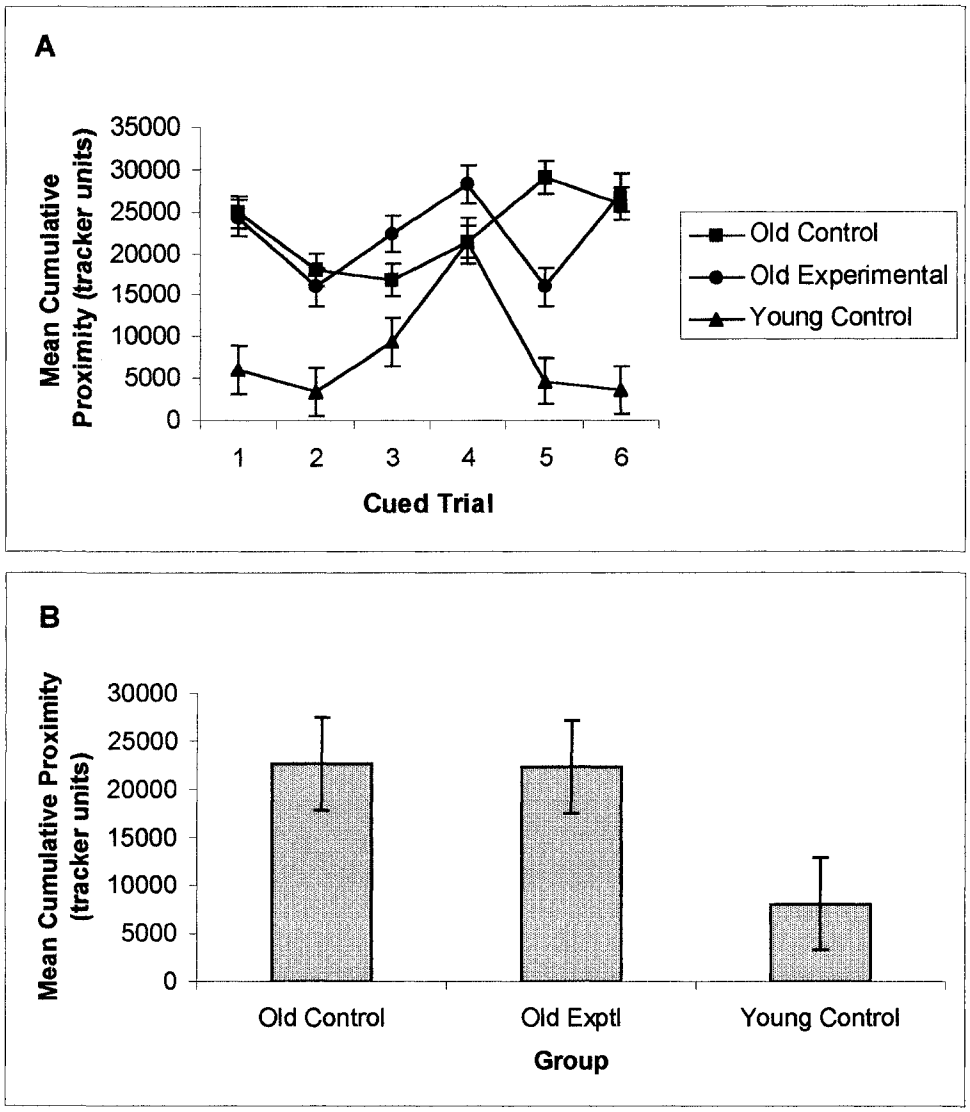


Figure 7: Cued phase in the Morris water maze. *Panel A*: Shown is the old control group (squares) injected with saline ICV, versus the old experimental group (diamonds) injected with interleukin-1 receptor antagonist (IL-1ra) ICV, versus the young control group (triangles) injected with saline ICV. Symbols denote mean cumulative proximity from the visible escape platform across animals, for each cued trial. Error bars represent standard error of the mean. *Panel B*: Shown are the old control group, old experimental group, and young control group. Bars represent mean cumulative proximity averaged across all cued trials. Error bars represent standard error of the mean.

Post-hoc Comparison	Mean Difference	Significance
Old Cont to Old Exptl	382.9755	.984
Old Cont to Young	14615.839*	.0001
Old Exptl to Young	14232.563*	.0001

Table 6: Shown is the mean difference among the old control, old experimental, and young control groups for cumulative proximity during the cued phase. * indicates significance at the $p < .05$ level.

Lastly, the mean average proximity from the cued platform was calculated for each group. The mean average proximity and the standard errors of the mean across all cued trials are shown in Figure 8A.As shown in Figure 8B, the mean average proximity

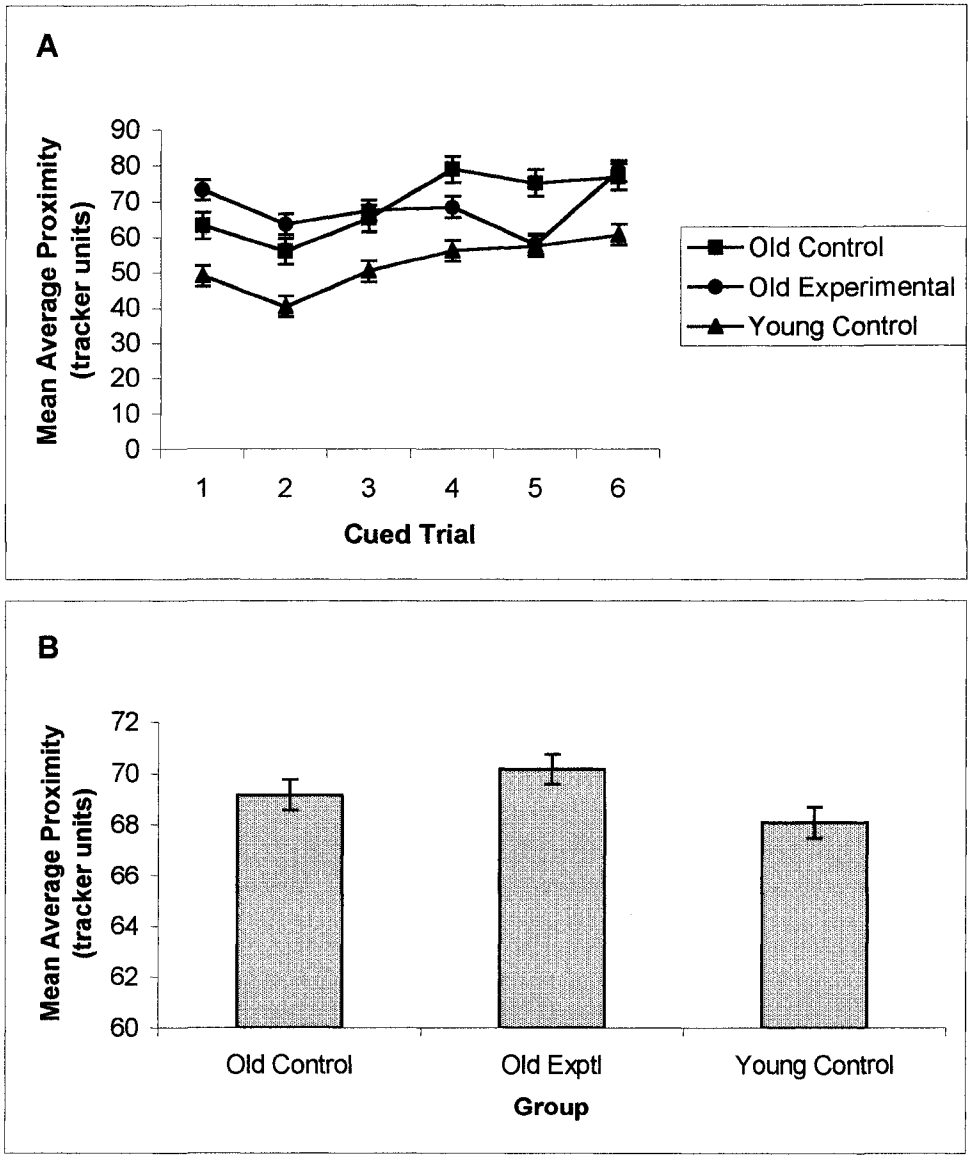


Figure 8: Cued phase in the Morris water maze. *Panel A*: Shown is the old control group (squares) injected with saline ICV, versus the old experimental group (diamonds) injected with interleukin-1 receptor antagonist (IL-1ra) ICV, versus the young control group (triangles) injected with saline ICV. Symbols denote mean average proximity from the visible escape platform across animals, for each cued trial. Error bars represent standard error of the mean. *Panel B*: Shown are the old control group, old experimental

group, and young control group. Bars represent mean average proximity averaged across all cued trials. Error bars represent standard error of the mean.

(collapsed across trials) for the old control group was 69.16 tracker units (SEM = 3.70 tracker units), while the mean average proximity for the old experimental group was 68.19 tracker units (SEM = 2.91 tracker units), and the mean latency for the young control group was 52.36 tracker units (SEM = 2.98 tracker units). Therefore, the young control group performed better than either of the aged groups, because the young group had a much lower average proximity from the visible platform. A mixed-design ANOVA performed on these means showed a significant difference among groups, $F(2, 27) = 11.18$, $p = .0001$. Post hoc analysis yielded a significant difference between the old control group and the young group, and between the old experimental group and the young group. There was not a significant difference between the old experimental group and the old control group (see Table 7).

Post-hoc Comparison	Mean Difference	Significance
Old Cont to Old Exptl	.967	.965
Old Cont to Young	16.802*	.001
Old Exptl to Young	15.836*	.001

Table 7: Shown is the mean difference among the old control, old experimental, and young control groups for average proximity during the cued phase. * indicates significance at the $p < .05$ level.

The ANOVA also showed that there was a significant difference across cued trials, $F(5, 135) = 5.57$, $p = .0001$, where the young control and old control groups performed worse across trials, reflected by their increased average proximity from the visible platform, but the old experimental group's performance did not change across trials. There was not a significant interaction between cued trial and treatment (see Figure 8A).

IL-1β Quantitation

The first ELISA, performed according to the procedure from Murray and Lynch (1998), yielded initial IL-1β protein concentrations (pg/100μl) based on the mean optical density reading (nm) averaged over three wells for each animal. These values were then corrected for total protein concentration, obtained in a separate protein analysis, to acquire the final IL-1β protein concentrations (pg/mg of protein) for each animal. As plotted in Figure 9, the mean IL-1β concentration for the old control group was 419.70 pg/mg (SEM = 84.53 pg/mg). The mean IL-1β concentration for the old experimental group was 516.96 pg/mg (SEM = 274.96 pg/mg). The mean IL-1β concentration for the young group was 779.10 pg/mg (SEM = 440.78 pg/mg). A one-way ANOVA performed on these means showed no significant difference in the IL-1β protein concentrations among groups, $F(2, 27) = .42$, $p = .66$.

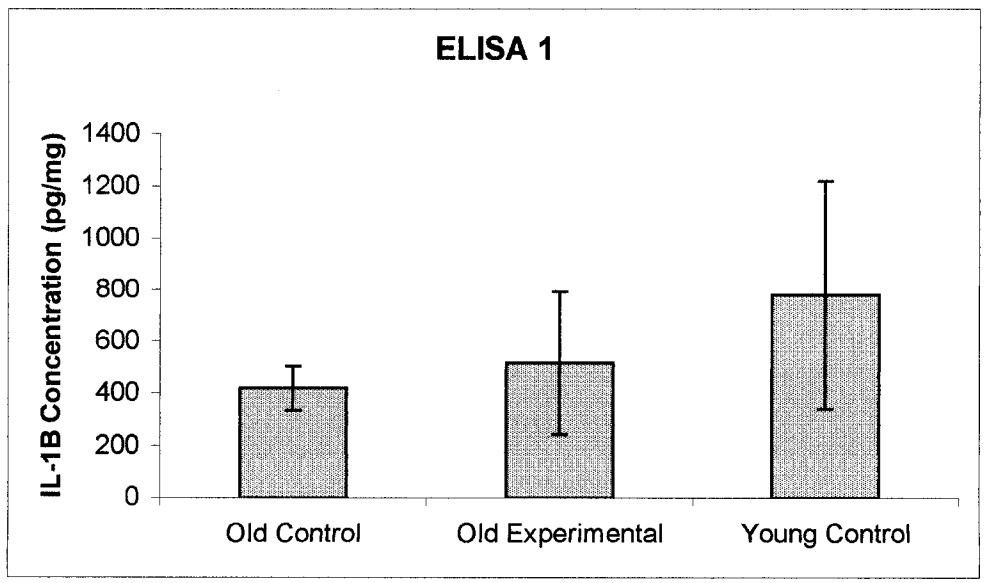


Figure 9: Shown is the mean IL-1β concentration (pg/mg) for the old control, old experimental, and young control groups for the first ELISA. Error bars represent standard error of the mean.

Because there was so much variability in IL-1β concentration results within groups, as well as within individual animals (within triplicates), for the first ELISA, a second ELISA was conducted using hippocampi dissected out of spare animals from another project (n=2) to produce more accurate results. Based on more stable results from this practice ELISA, the new protocol was used to determine IL-1β concentration in the undissected hippocampus of the original animals (third ELISA). The data from this second ELISA was not analyzed.

The third ELISA, performed using the undissected hippocampus from each animal, yielded IL-1β protein concentrations (pg/100μl) based on the mean optical density reading (nm) for each animal. These values were then corrected for total protein concentration, done in a separate protein analysis, to obtain the final IL-1β protein concentrations (pg/mg) for each animal. The mean IL-1β for the old control group was 32.74 pg/mg (SEM = 45.86 pg/mg). The mean IL-1β concentration for the old experimental group was 264.82 pg/mg (SEM = 94.09 pg/mg). The mean IL-1β concentration for the young group was 317.35 pg/mg (SEM = 113.08 pg/mg) (see Figure 10). A one-way ANOVA performed on these means showed a near significant difference in the IL-1β protein concentrations among groups, $F(2, 27) = 3.28$, $p = .053$. Post-hoc analysis showed that there was a near significant difference between the old control group and the young control group (see Table 8). There was no significant difference between the old control group and the old experimental group, or between the old experimental group and the young control group.

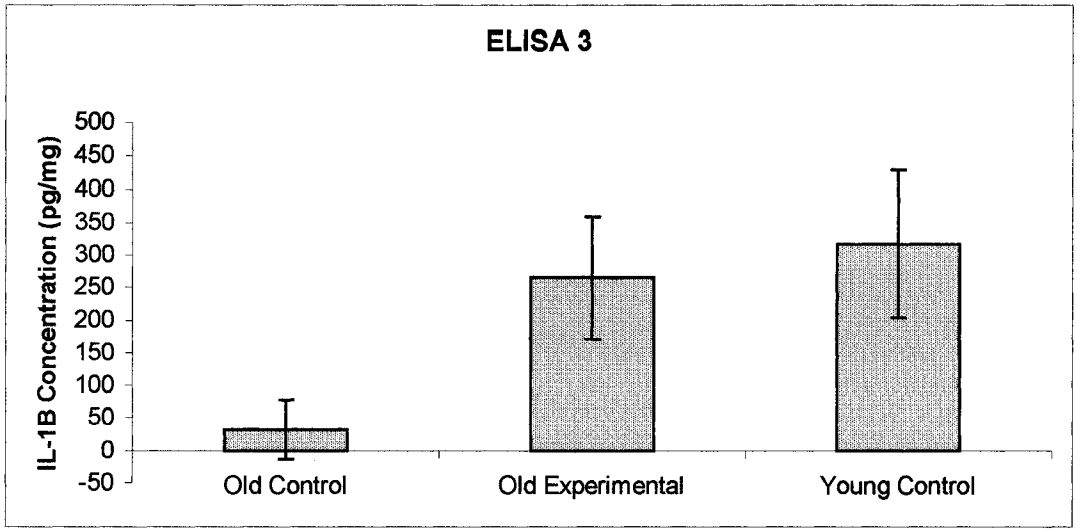


Figure 10: Shown is the mean IL-1β concentration (pg/mg) for the old control, old experimental, and young control groups for the third ELISA. Error bars represent standard error of the mean.

Post-hoc Comparison	Mean Difference	Significance
Old Cont to Old Exptl	-232.082	.137
Old Cont to Young	-284.6088	.065**
Old Exptl to Young	-52.527	.905

Table 8: Shown is the mean difference among the old control, old experimental, and young control groups for IL-1β concentration during the third ELISA. * indicates significance at the $p < .05$ level. ** indicates significance at the $p < .10$ level.

Due to the near significant differences found among groups for IL-1β protein concentrations in the third ELISA, a correlational analysis was performed to examine the relationship between these IL-1β protein concentrations (pg/mg) and performance in the water maze. Pearson's correlation coefficient was used to determine if there was a significant relationship between IL-1β concentration and the performance of each animal in the water maze during each phase (acquisition, probe, and cued). For the acquisition phase, no significant relationship was found between IL-1β concentration and any of the dependent measures (latency, cumulative proximity, or average proximity) (see Table 9). For the probe phase, no significant relationship was found between IL-1β concentration and any of the dependent measures (cumulative proximity or average proximity) (see

Table 10). Lastly, for the cued phase, no significant relationship was found between IL-1β concentration and any of the dependent measures (latency, cumulative proximity, or average proximity) (see Table 11).

	Latency	Cumulative Proximity	Average Proximity	IL-1β Concentration
Latency	1	.604*	.879*	-0.082
Cumulative Proximity	.604*	1	.681*	0.201
Average Distance	.879*	.681*	1	-0.009
IL-1B Concentration	-0.082	0.201	-0.009	1

Table 9: Shown is the correlation matrix analyzing the relationship between IL-1β concentration and performance in the water maze, as indicated by latency, cumulative proximity, and average proximity from the hidden platform location, for the acquisition phase. * indicates significance at the $p < .05$ level.

	Cumulative Proximity	Average Proximity	IL-1β Concentration
Cumulative Proximity	1	0.13	0.22
Average Distance	0.13	1	-0.132
IL-1B Concentration	0.22	-0.132	1

Table 10: Shown is the correlation matrix analyzing the relationship between IL-1β concentration and performance in the water maze, as indicated by cumulative proximity and average proximity from the former platform location, for the probe phase. * indicates significance at the $p < .05$ level.

	Latency	Cumulative Proximity	Average Proximity	IL-1β Concentration
Latency	1	.909*	.597*	-0.203
Cumulative Proximity	.909*	1	.715*	-0.092
Average Distance	.597*	.715*	1	0.033
IL-1B Concentration	-0.203	-0.092	0.033	1

Table 11: Shown is the correlation matrix analyzing the relationship between IL-1β concentration and performance in the water maze, as indicated by latency, cumulative proximity, and average proximity from the visible escape platform, for the cued phase. * indicates significance at the $p < .05$ level.

Chapter 4: Discussion

The objective of this experiment was three-fold. First, the objective was to examine if there was an age difference in the performance of rats in the Morris water maze, comparing young rats to old (effect of age). The second objective was to examine if intracerebroventricular (ICV) injections of an Interleukin-1 receptor antagonist (IL-1ra) would improve the learning of aged rats in the Morris water maze (effect of treatment). The last objective in this experiment was to determine if there was an age difference in the protein concentration of IL-1β in the brains of old versus young rats, and to correlate these concentrations with performance of the rats in the water maze (protein quantitation). Each of these objectives will be discussed separately.

Behavioral Effects

Effect of Age

The first objective of this experiment was to examine if there was an age difference in the performance of rats in the Morris water maze, comparing young rats to old. Previous research has shown that aged rats have impaired performance in spatial learning tasks, such as the Morris water maze (Willott, 1999; Gallagher, Burwell, and Burchinal, 1993). Previous research has also shown that aged rats have higher concentrations of IL-1β in their brains as compared to young rats (Murray and Lynch, 1998), and that higher concentrations of IL-1β impair LTP (Katsuki et al., 1990) and learning (Oitzl et al., 1993; Gibertini et al., 1995; Maier, and Watkins, 1995; Pugh,

Nguyen, Gonyea, Fleshner, Watkins, Maier, and Rudy, 1999). Therefore, it was hypothesized that, because the young control rats would potentially have lower levels of IL-1β than the old control rats, the young control rats would perform significantly better than the old control rats in the Morris water maze during the acquisition and probe phases. However, it was also hypothesized that the performance of the young control rats would not be significantly different from the performance of the aged control rats during the cued phase. Each of these phases will be discussed separately.

Acquisition Phase.

For the acquisition phase, it was hypothesized that young control animals would show superior learning to the aged control animals, demonstrated by their significantly lower latency to locate the escape platform, significantly lower cumulative proximity from the escape platform, and significantly lower average proximity from the escape platform. Results from the experiment did support this hypothesis. For example, the young control group did have a significantly lower mean latency than the old control group. Interestingly, results also showed that the young control group was not significantly different from the old control group for cumulative proximity to the escape platform. This might indicate that the old control animals performed better than the young control animals during acquisition, because the cumulative proximity measure takes into account latency of the swim, as well as distance from the platform. The young animals had a lower latency, but a slightly higher cumulative proximity. The old animals had just the opposite: a higher latency, but a slightly lower cumulative proximity. Therefore, in order for the cumulative proximity of the two groups to be approximately equal, the old animals must have had a much lower distance from the escape platform

added over their longer time in the maze, while the young animals must have had a much higher distance from the platform added over their shorter time in the maze. This could indicate better learning for the aged animals. On the other hand, results also showed that the young control group had a significantly lower mean average proximity than the old control group. Average proximity averages out the effects of latency, so the longer latencies for the old animals versus the shorter latencies for the young animals made no difference for this measure. Based on this measure, the young control animals performed better during the acquisition phase than the old control animals. Therefore, it seems that age did have a significant effect on the performance of aged animals during the acquisition phase, because they performed worse than the young animals on two of the three dependent measures.

Probe Phase.

For the probe phase, it was hypothesized that young control animals would show superior learning to the aged control animals, as demonstrated by their significantly lower cumulative proximity from the former escape platform location, and their significantly lower average proximity from the former escape platform location during probe trials. Results for this phase were mixed; some results did support the hypothesis, but some did not. For example, there was a significant difference between the old control group and the young control group for cumulative proximity from the former escape platform. The young control group actually had a significantly higher mean cumulative proximity than the old control group. These results did not support the hypothesis. However, results also showed that there was a significant difference between the old control group and the young control group for average distance from the former platform location. This time,

the young control rats did have a significantly lower mean average proximity than the old control rats. These results did support the hypothesis. Therefore, age may have had a significant effect on the performance of aged animals during the probe phase, as compared to young animals, although the direction of this effect is different for the different measures.

Cued Phase.

For the cued phase it was hypothesized that the old control animals would not be significantly different from the young control animals. Therefore, we did not expect any differences between the groups in their latency to find the visible platform, cumulative proximity from the visible platform, or average proximity from the platform. Differences in performance during cued trials would reflect a possible difference in the motivation, or visual/motor abilities between the two groups, rather than a difference in learning ability during the acquisition trials. Results did not support this hypothesis. For example, there was a significant difference between the old control and the young control groups on their latency to find the visible escape platform. The young control group had a significantly lower latency than the old control group. For cumulative proximity from the visible platform, the young control group had a significantly lower mean cumulative proximity than the old control group. Lastly, results for the cued phase showed that the young control group had a significantly lower mean average proximity than the old control group. Therefore, age did have a significant effect on the performance of aged animals during the cued phase, as compared to young animals. This may indicate a difference in the motivation or visual/motor capabilities between the two groups, rather than a difference in their learning ability.

Interpretations.

Based on the above results, the hypothesis that old control rats would perform significantly worse than young control rats in the Morris water maze was proven correct for the acquisition and probe phases. However, because the old rats also performed significantly worse than the young rats during the cued phase, we cannot attribute the differences in performance during the acquisition and probe phases to a difference in learning ability. The cued phase was designed as a test of motivation and visual/motor abilities. By using a visible platform, we can test if the animals can see a cue and swim towards it effectively, and if they are motivated to do so. Because the aged animals performed much worse during the cued phase, we must conclude that they have different motivation or visual/motor capabilities than the young animals. The same kind of age-related cued trial deficit has been shown in other studies. For example, research has shown that albino strains of rats (like the Fischer 344 rats used in this study) have age-related impairments in cue learning, and that these deficits seem to be pronounced in those animals with the most spatial learning deficits (Clark, Magnusson, and Cotman, 1992). Therefore, old Fischer rats may not have a learning deficit compared to young rats, instead their performance may only reflect different age-related visuomotor or motivation capabilities.

Another possible explanation may be due to the fact that the cannulae were falling out after the first surgery. It is conceivable that these cannulae caused severe damage to the brains of the animals, which could have also impaired their performance in the water maze, in terms of their learning or visuomotor abilities. This might conceivably have impaired the old animals more than the young because young animals tend to have a

higher capacity for brain plasticity after lesion (Hoff, Scheff, Bernardo, and Cotman, 1982) that would help them to recover from such injury. Therefore, the young animals might not have been as affected by the surgical complications. This is only speculation, however, because we did not examine the brains of either the young or old animals for any type of damage.

Effect of Treatment

The second objective for this experiment was to examine if intracerebroventricular (ICV) injections of an Interleukin-1 receptor antagonist (IL-1ra) would improve the learning of aged rats in the Morris water maze. Previous research has shown that aged animals have naturally higher concentrations of IL-1β than young animals (Murray and Lynch, 1998), and that higher concentrations of IL-1β can impair performance in the Morris water maze (Oitzl et al., 1993; Gibertini et al., 1995). It has also been shown that ICV injections of the IL-1 receptor antagonist blocks endotoxin-induced IL-1β function in the brain and improves learning in those animals with higher concentrations (Maier, and Watkins, 1995; Pugh, Nguyen, Gonyea, Fleshner, Watkins, Maier, and Rudy, 1999). Therefore, it was hypothesized that ICV injections of IL-1ra would sufficiently block the effects of IL-1β in old rats, thereby improving their spatial learning in the Morris water maze. The aged experimental rats were expected to perform significantly better in the water maze than the aged control rats. The behavioral component of this experiment was broken down into three phases, which will each be discussed separately.

Acquisition Phase.

For the acquisition phase, it was hypothesized that aged animals receiving the IL-1ra would show superior learning, demonstrated by their significantly lower latency to locate the escape platform, significantly lower cumulative proximity from the escape platform, and significantly lower average proximity from the escape platform compared to aged control animals. Results from the experiment did not support this hypothesis. For example, there was not a significant difference between the old control group and the old experimental group on their mean latency from the escape platform. Old experimental rats had only a slightly lower mean latency to find the escape platform than old control rats. Results also showed that the old experimental rats were not significantly different from the old control rats on their cumulative proximity from the platform. The old experimental rats had a slightly higher mean cumulative proximity than old control rats. Lastly, results also showed that there was not a significant difference between the groups on average proximity from the escape platform. The old experimental rats had only a slightly lower mean average proximity than the old control rats. Therefore, the injections of the IL-1ra did not effectively improve learning in the old experimental group during the acquisition phase.

Probe Phase.

For the probe phase, it was hypothesized that aged animals receiving the IL-1ra would show superior learning, as demonstrated by their significantly lower cumulative proximity from the former escape platform location, and their significantly lower average proximity from the former escape platform location during probe trials compared to aged control animals. Results did not support this hypothesis. For example, there was not a

significant difference between the old control group and the old experimental group on cumulative proximity from the former escape platform. The old experimental group actually had a slightly higher mean cumulative proximity than the old control group. Results also showed that there was not a significant difference between the old control group and the old experimental group for their average proximity from the former platform location. In this case, the old experimental group had a slightly lower mean average proximity than the old control group. Therefore, the injections of the IL-1ra did not effectively improve learning in the old experimental group during the probe phase.

Cued Phase.

For the cued phase it was hypothesized that the aged animals receiving the IL-1ra would not be significantly different from the aged control animals. Therefore, we did not expect any differences between the groups in their latency to find, cumulative proximity or average proximity from the visible platform. Such differences would reflect a possible disparity in the motivation, or visual/motor abilities between the two groups, rather than in learning ability. Results did support this hypothesis. For example, there was not a significant difference between the old control and the old experimental groups in their latency from the visible platform. The old experimental group had only a slightly higher mean latency than the old control group. For the cumulative proximity from the visible platform, there was not a significant difference between the old control and the old experimental groups. The old experimental group had a slightly lower cumulative proximity than the old control group. Lastly, results for the cued phase showed there not a significant difference between the old control and the old experimental groups for their average proximity from the platform. The old experimental group again had only a

slightly lower mean average proximity than the old control group. Therefore, injections of IL-1ra did not change the motivation or visual/motor performance of the aged experimental group compared to the aged control group.

Interpretations.

Based on the above results, the hypothesis that the aged animals receiving injections of IL-1ra would perform significantly better than aged control animals was not supported. These unexpected results could have been due to a number of factors. First of all, as mentioned in the previous section, the aged animals performed significantly worse than the young animals during the cued phase. This indicates that there was a difference in the motivation or visual/motor capabilities between the old and young animals. This difference may reflect a potential age-related strain difference. It may be that, for Fischer 344 rats, they show a visuomotor or motivation difference with age, rather than a learning difference. This difference may also have been due to differential damage to the brains of the old animals caused by the cannulae falling out after the first surgery. If either of these scenarios were the case, then injections of IL-1ra would make no difference in the performance of the aged experimental animals, because their problem in the water maze was not with learning, it was with their motivation, vision, or motor abilities. IL-1ra has not been shown to effectively improve swimming/visual/motivation capabilities during cued learning in the Morris water maze (Yirmiya, Winocur, and Goshen, 2002). This is supported by the fact that the aged experimental performed at the same level as the aged control group during the cued phase. In other words, these two groups had basically the same visual/motor/motivation capabilities; IL-1ra did nothing to change that. But if the

aged animals were having visual/motor/motivation problems in the water maze task, IL-1ra might also do nothing to correct that.

It is also possible that IL-1ra produced agonistic activity in the hippocampus. Previous research had shown that IL-1ra produces selectively competitive antagonistic activity to IL-1β, with no reported agonist activity (Dinarello, 1991). However, recent research by Loscher, Mills, and Lynch (2003) raises the possibility that IL-1ra might produce some agonistic activity for IL-1β. They showed that stimulation of synaptosomes with IL-1ra *in vitro* mimicked the effects of IL-1β by decreasing glutamate release and increasing JNK phosphorylation. They also showed that IL-1ra mimicked the inhibitory effect of IL-1β on long-term potentiation in the hippocampus *in vitro*. If this were the case, it might explain why injecting IL-1ra ICV did not improve the learning of the old experimental group. Although this seems unlikely, because if IL-1ra in fact mimicked the effects of IL-1β on learning, the old experimental group would have been expected to perform significantly worse than the old control group, which they did not.

Another potential explanation of the insignificant difference between the aged experimental and aged control animals could be found in the length of time the animals were injected with the antagonist. As noted before, the changes that IL-1β could induce at the membrane of the neuron include the release of reactive oxygen species, a process commonly thought to be involved in age-related changes in the brain (Nathan and Tsunawaki, 1986). Murray and Lynch (1998) demonstrated some of the degenerative changes IL-1β has on neuronal membranes that would take time to show a behavioral effect on learning. These researchers found that intracerebroventricular (ICV) injection of IL-1β induced a significant increase in lipid peroxidation. They suggested this effect is

related to the IL-1β-induced release of reactive oxygen species and significantly decreased concentrations of membrane arachidonic acid in neurons of the hippocampus. This information is important because increases in lipid peroxidation and decreases in arachidonic acid are known to have a degenerative effect on hippocampal neurons that could lead to a decrease in cognitive functioning, and this type of damage can progress slowly over time. For example, Dinarello (1991) found that IL-1β binding in neutrophils resulted in increased arachidonic acid metabolism after more than 60 minutes of exposure *in vitro*. Oitzl et al (1993) found that IL-1β administered at least 60 minutes before training inhibited performance in the water maze the following day, but did not inhibit performance if given immediately before training. In other words, acquisition training had to occur after a delay from the injection time, and then results were only seen after a delay to training the next day. If the degenerative changes induced by IL-1β in aged animals take time to impair performance, it may also take time to correct these changes. Therefore, it may be necessary to employ a time-course injection of the antagonist to IL-1β that would occur before acquisition training so that the antagonist would be given sufficient time to reverse the damage already done to hippocampal neurons.

One study attempted to reverse the IL-1β-induced damage to neurons due to aging by giving dietary supplements of arachidonic acid to aged rats. The animals were then measured for their ability to sustain LTP. These dietary supplements were found to reverse the age-related changes in LTP (McGahon et al., 1997). These researchers utilized a time-course injection by giving the supplements over a period of eight weeks before they tested the animals' LTP. In other words, they reversed age-related deficits in LTP by using time-course dietary supplementation. Injecting an antagonist to IL-1β with

a considerable delay before water maze training has also been used with promising results. For example, Gibertini et al. (1995) injected an antibody to endogenous IL-1β at least 2 hours prior to training.

Another factor that may have negatively impacted the results is the variability in the performance of aged animals. Lindner (1997) showed that, while performance in the water maze generally declines with age, chronological age accounts for only a small amount of the variability among rats in water maze performance. He stresses that it is important to not only examine the average performance of a group, but also to note the variability seen among individual animals. Therefore, it is important to examine the factors which mediate individual differences in performance. In this experiment the actual concentration of IL-1β found in each animal's brain was measured; however, these results may have been inconsistent (see below). Therefore, differences that might be seen in IL-1β concentration in the individual animal's brains could be responsible for the variability seen in the performance of old rats in the water maze, and these differences may not have been accounted for.

IL-1β Quantitation

The last objective in this experiment was to determine if there was an age difference in the protein concentration of IL-1β in the brains of old versus young rats, and to correlate these concentrations with performance of the rats in the water maze (IL-1β quantitation). Previous research has shown that aged rats have higher concentrations of IL-1β in their brains compared with young rats (Murray and Lynch, 1998). Therefore, it was first hypothesized that there would be a significant difference in the protein

concentrations of IL-1β between groups, with the aged groups showing higher concentrations compared to the young group. It was expected that the young control group would have the lowest concentration of IL-1β, while the aged groups would not be different from one another in their concentration of IL-1β. This hypothesis was not supported, because, for the third ELISA, while a near significant difference was found between groups, this near significant finding did not reflect a difference in the expected direction. For example, the old experimental group was not significantly different from the old control group or the young control group. Also, unexpectedly, the old control group showed the lowest mean concentration, the old experimental group showed the next lowest mean concentration, and the young control group actually showed the highest mean concentration.

No one has ever tried to correlate IL-1β concentration with water maze performance before; however, it has been shown by previous research that higher concentrations of IL-1β impair performance in learning tasks, such as conditioned fear or the Morris water maze (Oitzl, et al., 1993; Gibertini et al., 1995; Maier, and Watkins, 1995; Pugh, Nguyen, Gonyea, Fleshner, Watkins, Maier, and Rudy, 1999). Therefore, for the protein quantitation a second hypothesis was proposed that IL-1β concentration would be negatively correlated with performance in the water maze. It was expected that as IL-1β protein concentration increased, water maze performance would decrease for each measure during each phase. This hypothesis was not supported because there was not a significant correlation between protein concentration and water maze performance on any measure during any of the phases.

Interpretations

Based on the above results, the hypotheses that the aged animals (both experimental and control groups) would have higher concentrations of IL-1β and that these higher concentrations would be negatively correlated with performance in the water maze were not supported. There are a number of reasons why this might have occurred. First of all, it is possible that the old control group did have a lower average concentration of IL-1β in their brains, with the young control group having the highest concentrations. However, this does not support what other researchers have found in the past. Murray and Lynch (1998) clearly demonstrated that aged animals have a significantly higher concentration of IL-1β than do young animals. Perhaps this discrepancy with previous research reflects a strain difference, as Murray and Lynch (1998) used Wistar rats, while this experiment utilized Fischer 344 rats. No other researchers have examined the levels of IL-1β present in the brains of aged animals in any other strains, so this may be a definite possibility. It is also possible that the old experimental animals had a higher concentration of IL-1β than the old control animals because injections of the IL-1ra caused an upregulation of IL-1β in those animals' brains. This seems unlikely, because previous research has shown that IL-1ra blocks the spontaneous production of IL-1 (Rambaldi, et al., 1990). However, it is still possible.

Another potential explanation for these results could be found in the raw data for this portion of the experiment. It can be observed from the raw data (see Appendix A) that the triplicates for each sample were often widely different for both the first and the third ELISAs. Triplicates are commonly performed in order to test for reliability of measurement across the 96-well plate. In other words, the ELISA for each sample is done

three times in order to test for measurement reliability that may be compromised due to experimenter error or malfunction in the provided testing apparatus (ELISA kit). Based on the triplicate information obtained for the protein analyses (see Tables 1 and 4 in Appendix A), it seems as if the experimenter methods were not compromised. The results for these analyses indicate that the triplicate results were very similar; therefore, at least for these measures, the experimenter (and the testing apparatus) was highly reliable. However, for the ELISA analyses, the results indicate that the triplicates were not very similar (see Tables 2 and 5 in Appendix A); therefore it is possible that either the experimenter was not reliable or the testing apparatus was not reliable. Because the protein analyses and the ELISA were performed at approximately the same time, it seems unlikely that the differences in the triplicates in the ELISA are due to experimenter error. It is more probable that the testing apparatus was compromised in some way. If the testing apparatus itself was malfunctioning, then it is not possible for there to be a usable correlation between ELISA measurements and behavioral performance in the water maze. A possible contradiction to this explanation might be found in comparing the first ELISA's results to the third. While both ELISAs showed variable results across triplicates, they both showed the same trend in terms of overall results. Both the first and third ELISAs showed that the old control animals had the lowest concentration of IL-1β, while the old experimental animals had the next lowest concentration, and the young control animals had the highest concentrations of IL-1β. Because these results were found twice using the same experimental apparatus, this may suggest that the ELISA kits were not compromised.

Conclusions

The above experiment was an excellent pilot experiment that will serve to guide future research in this area. Future research must concentrate on correcting potential procedural errors in order to eliminate potential experimental confounds. For example, it is a definite possibility that the behavioral component of this experiment would have gone much more smoothly if the surgeries had been performed only once. The stress of a second procedure could have produced a deficit in their water maze performance that did not reflect a learning deficit, but a visual or motor deficit. Proper surgical technique in the future could eliminate such a problem, as could allowing more recovery time from surgery.

Elimination of animals based on a criterion measure of performance could also address this potential source of experimental confound. If animals do not perform up to a certain (criterion) level on the cued trials, then they could be eliminated from statistical analyses. Unfortunately for this experiment, that might have eliminated all aged animals from analysis. If a replication of the behavioral component of this experiment (with more strict experimental control) still produces a difference between old and young animals on the cued trials, support would be added to the potential visual/motor/motivation difference between young and old Fischer 344 rats, rather than a learning impairment. Potential experimental confounds should also be eliminated in the future by testing the ELISA IL-1β protein analysis apparatus to make sure it is functioning properly before the experiment is begun. This would allow experimenters to be sure that results obtained from such measures were accurate.

Future research could also be directed toward investigation of the effectiveness of a time-course injection of IL-1ra. Based on previous research, it seems as if antagonizing IL-1β proves most effective when it is done over a length of time before behavioral (or LTP) analysis is performed. It is recommended that ICV injections of IL-1ra be given over a period of eight weeks (as in McGahon et al., 1997) before behavioral testing is started.

Whatever direction is taken, this field of research is extremely important to the area of normal memory loss associated with growing older. In addition, IL-1β has also been shown to be important in non-normal losses of cognitive ability associated with aging, especially that seen with Alzheimer's Disease (AD). The most likely explanation for the changes in memory due to AD pertains to damage to the hippocampus. For example, Hyman and Gomez-Isla (1998) showed that neuronal loss does not occur in the entorhinal cortex, which is associated with the hippocampal formation, in cognitively intact elderly, however, there is a vast neuronal loss in elderly patients with AD. It has also been demonstrated that IL-1β may play a role in AD and the associated neuronal loss. For example, the presence of IL-1β mRNA has been reported in the brains of Alzheimer's patients (Griffin, Stanley, Ling, White, MacLeod, Perrot, White, Araoz, 1989), but it is not yet known if the production of this cytokine is only a consequence of neuronal abnormalities, or if it has a causal role in the events related to AD. Contrary to these findings, Das and Potter (1995) found that the amyloid deposits that occur in AD contain, in addition to the β protein, lesser amounts of other proteins including α1-antichymotrypsin (ACT). These researchers found that IL-1 induced synthesis of ACT

and that the IL-1 induced expression of ACT may direct the production of mature amyloid filaments in the Alzheimer brain.

Although it is not clear if IL-1β has a direct effect on the brains of Alzheimer's Disease patients, it has been shown that IL-1β is important in the memory deficits associated with normal aging. Only through further study can the phenomenon of age-related memory loss be fully understood, and only then might changes be made in the quality of life expected for an ever-aging population.

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Appendix A

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0	0	.043	.041	.037	.053	.054	.050	.058	.056	.054
B	.097	.094	.098	.039	.032	.038	.045	.046	.027	.049	.048	.051
C	.147	.137	.155	.099	.096	.098	.035	.044	.040	.061	.079	.062
D	.312	.314	.315	.041	.038	.040	.049	.050	.019	.047	.045	.038
E	.616	.498	.640	.049	.032	.032	.024	.026	.032	.062	.067	.015
F	.722	.815	.065	.014	.036	.033	.008	.007	.031	.030	.043	.052
G				.036	.024	.030	.040	.068	.028	.035	.028	.044
H	.039	.034	.040	.014	.038	.022	.027	.031	.027	.025	.031	.020
I	0	0	0	.039	.036	.039	.091	.103	.100	.054	.052	.053
J	.167	.162	.146	.053	.053	.053	.308	.311	.305	.047	.038	.032
K	.617	.631	.651	.041	.040	.039	.826	.832	.797	.057	.039	.057

Table 1: Shown is the raw data (optical density) for the first protein assay. Light grey marked cells represent standard values. Blocked cells represent triplicate wells for each experimental sample.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Over	1.621	Over	.162	.401	.596	.124	.044	.139	.108	.041	.115
B	.779	.819	.325	.202	.049	.006	.018	.030	.030	.022	.032	.036
C	1.408	1.058	1.266	.038	.030	.116	.155	.034	.044	.190	.470	.638
D	.448	.214	.348	.079	.087	.073	.121	.114	.174	1.897	.730	.672
E	.308	.154	.120	.514	.247	.269	.043	.041	.053	.137	.077	.067
F	.178	.073	.072	1.032	.197	.033	.033	.033	.045	.058	.046	.043
G	.143	.058	.045	.175	.206	.193	.048	.044	.043	.113	.074	.075
H	0	-.001	.026	.076	.064	.041	.096	.093	.132	.054	.025	.111
I	Over	2.023	1.056	.285	.209	.025	-.05	-.06	-.08	-.065	.062	.048
J	1.611	1.489	1.086	.215	.064	.043	-.02	.006	1.374	.200	-.01	.348
K	.774	.819	.559	0	-.04	.046	.346	-.04	.054	.138	.042	-.03
L	.491	.468	.358	.462	.586	.202	.268	.072	.135	-.04	-.06	.04
M	0	.290	.246									

Table 2: Shown is the raw data (optical density) for the first ELISA. Light grey marked cells represent standard values. Dark grey cells represent control sample values. Blocked cells represent triplicate wells for each experimental sample.

	1	2	3	4	5	6	7	8	9	10	11	12
A	.786	.752	.568	.118	.077	.072	.596	.482	.518	.010	.082	.207
B	.402	.413	.299	.107	.034	.043	.123	.111	.116	.003	.069	.057
C	.231	.232	.187	0	.001	.006	.083	.080	.026	.371	.409	.560

Table 3: Shown is the raw data (optical density) for the second ELISA. Light grey marked cells represent standard values. Dark grey cells represent control sample values. Blocked cells represent triplicate wells for each experimental sample.

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	-.003	-.002	.233	.233	.237	.240	.240	.244	.218	.207	.220
B	.107	.105	.104	.279	.285	.279	.266	.275	.269	.267	.262	.267
C	.219	.215	.208	.257	.259	.256	.275	.274	.276	.300	.299	.303
D	.374	.272	.372	.315	.316	.304	.283	.283	.273	.292	.289	.282
E	.610	.623	.623	.331	.315	.303	.070	.068	.083	.275	.302	.282
F	.757	.652	.785	.294	.294	.288	.255	.263	.259	.274	.276	.276
G				.264	.259	.262	.280	.281	.271	.190	.193	.189
H	.308	.299	.301	.287	.275	.287	.291	.296	.280	.217	.206	.206
I	0	-.004	-.002	.368	.363	.367			.048			
J	.104	.101	.102	.615	.623	.606	.088		.058			
K	.216	.211	.210	.840	.826	.784	.100		.083			

Table 4: Shown is the raw data (optical density) for the last protein assay. Light grey marked cells represent standard values. Blocked cells represent triplicate wells for each experimental sample.

	1	2	3	4	5	6	7	8	9	10	11	12
A	.802	.656	.460	Over	Over	Over	.156	.159	.131	.109	.128	.133
B	.472	.266	.144	1.263	.332	.501	.283	.193	.201	.177	.195	.218
C	.381	.128	.055	1.999	1.977	1.966	.273	.224	.210	.150	.379	.253
D	.200	.069	-.04	.609	.383	.345	.794	.127	.046	1.94	1.951	1.948
E	.133	.022	.297	.366	.320	.281	.190	-.01	-.03	.548	.616	1.02
F	0	0	0	.129	1.85	.004	.184	.382	.145	.047	.093	2.01
G	.594	.543	.612	.317	.305	.424	.179	.108	.146	.623	1.07	2.09
H	.207	.164	.167	.073	.038	.230	.179	.173	.173	1.67	1.35	1.47
I	.95	.957	.763	.270	.146	.255	.674	.675	.659	-.08	-.09	-.07
J	.661	.532	.413	.130	.065	.229	.509	.384	.398	.114	.124	.332
K	.290	.254	.209	0	0	0	.442	.214	Un	Un	Un	Un

Table 5: Shown is the raw data (optical density) for the third ELISA. Light grey marked cells represent standard values. Dark grey cells represent control sample values. Blocked cells represent triplicate wells for each experimental sample.