Running Head: Effects of Neurogenesis on Pain Behavior

The Relationship Between Neurogenesis and Pain Behavior

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Abstract

One of the most prominent discoveries of recent neuroscience is the finding that neurogenesis, the formation of new neurons, can occur in the adult brain. While it was previously thought that neurogenesis occurs only in developmental stages, recent breakthrough research has revealed novel analyses about the brain, specifically its inherent plastic nature. Plasticity is not just an attribute of the brain; many bodily mechanisms exhibit plastic properties, as well, including the pain pathway. Sensitization, or the lowered sensitivity threshold of pain-responsive receptors with increased stimulation, is one such example of plasticity in the pain response. Interestingly, mechanisms of pain and neurogenesis have been shown to be connected. Research indicates an inverse relationship between pain and neurogenesis: fewer neurons will form as pain is enhanced. Patients with Alzheimer's disease, a disease in which adult neurogenesis occurs at a slower rate than normal, report a higher pain tolerance. The present study was designed to investigate if the administration of galantamine and nicotine, both of which are acetylcholine agonists but have opposing effects on neurogenesis, have effects on pain behavior associated with varying degrees of neurogenesis. We hypothesize that if neurogenesis increases with galantamine administration and decreases with administration of nicotine, the mice should display significantly more pain behavior in galantamine conditions when compared to both nicotine subjects and controls because of the increased number cells involved in the pain pathway that form in conjunction with new neurons. Such findings would establish a correlation between either increased pain threshold or analgesia and drug-induced neurogenesis. The general trend observed indicated that with increased neurogenesis, the subject exhibited more pain during the formalin test. Limitations and future research are discussed.

The Relationship Between Neurogenesis and Pain Behavior

One aspect of neuroscience that is well studied is the pain pathway; how one perceives, responds, and physiologically reacts to pain, as well as various factors that influence an individual's response to pain. This paper reviews such factors, including stress, depression, and neurogenesis, the formation of new neurons in the brain. It is important to study these bodily mechanisms both independently and in relation to one another so as to best comprehend the underlying processes of these sensations. Pain has been shown to have hindering effects on adult neurogenesis, indicating a correlational relationship between new neuron formation and nociception. Furthermore, antidepressants have been demonstrated as analgesics as well as effective treatments of the side effects of Alzheimer's disease, a disease in which neurogenesis occurs at a slower rate than normal. Patients with Alzheimer's disease also often report a higher pain tolerance. Such findings encourage further research to determine if increased pain threshold is a direct effect of the disease itself or a bodily response to the medications that Alzheimer's patients receive. Our experiment was designed to further investigate this connection between pain and neurogenesis by observing the effects of induced neurogenesis on subsequent pain behaviors. By utilizing Alzheimer's medication as a means of inducing varying degrees of neurogenesis, we found a direct correlational relationship between neurogenesis and pain behavior. Such findings imply that effects on pain threshold are likely due to the properties of the disease itself rather than the medications taken to counter the disease.

Pain

Sternbach (1968) defines pain as an abstract notion referring to 1) a personal feeling of hurt, 2) a damaging stimulus that leads to tissue damage, and 3) a series of reactions that occur in

order to protect the individual from further injury. There are many aspects of pain to account for when investigating this phenomenon. Pain has both a sensory component as well as an emotional component. Always an unpleasant sensation, pain involves an individual's affective response. It is important, then, to differentiate between pain "sensation" and pain "perception." Sensation, the physical component, also known as nociception, is described as impending or resulting tissue damage resulting from an alteration in the activity of neurons, which, in turn, is sent as sensory information to the brain and spinal cord. Conversely, perception is the individual's subjective experience that depends on the brain collecting information from incoming afferent neurons (Sternberg, 2007). Thus, in observing pain behaviors in an experimental setting, researchers are studying the subjective translation of incoming peripheral sensations to specific locations of pain perceived on (or in) the body. Sensation, on the other hand, can be observed using brain imaging techniques which show neural activity of the brain. Both perception and sensation are important in the study of pain, but their occurrences are not dependent on one another. An individual can experience perception with seemingly harmless sensations, as in the disorder known as allodynia (Basbaum & Jessell, 1991), and the body also can experience sensory stimulation without translating this as "pain" to the brain, as occurs during the "fight or flight" response or an adrenaline rush that produces stress-induced analgesia.

In studying pain, the different types of pain that an individual can encounter are important to understand. In transient pain, there is no damage observed in the body tissue, but nociceptors are activated and pain is briefly perceived. Acute pain involves body tissue injury and activated nociceptors at the injury site. Often, the body will heal itself of acute pain with time. Chronic pain entails a possible exceeding of the body's abilities to heal itself, and frequently emerges because of previous injury or disease. It differs from acute pain in that the

body often is unable to replace the functions that are operating during normal homeostasis (Loeser & Melzack, 1999).

There are also different types of pain sensations, which are perceived according to the type of nociceptor that is activated. Afferent nociceptors have little to no myelin, and, as a result, conduct information very slowly. Thermal nociceptors are sensitive to extreme heat or cold, while mechanical nociceptors are active with extreme pressure forced on the skin. Polymodal nociceptors react with intensive thermal, mechanical, or chemical stimulation. (Loeser & Melzack, 1999)

When any one of these nociceptors' receptors is activated, the body responds with a conversion of the sensory energy to a depolarizing electrical potential. This electrical signal is then transmitted in the nerve through the dorsal root ganglia, which contains the sensory nerve fibers known as C-nociceptors that respond to noxious stimuli. The axons of the cell bodies of the dorsal root ganglia connect by way of synapses to other cell bodies located in the dorsal horn of the spinal cord. Secondary projections, whose fibers cross the midline of the spinal cord, turn upwards and send information from the spinal cord to the thalamus in the brainstem, where it then proceeds to specific cortices with higher-order functioning (Sternbach, 1968). Additional projections target including the limbic system, which manages the emotional response to noxious stimuli (Basbaum & Jessell, 1991). It is important to note that once sensory information is relayed to the brain, the brain's role is not only to translate this information, but it also must compare this information with stored memories containing comparable information in order to produce the appropriate, adaptive behavioral response (Melzack & Wall, 1973).

A recent meta-analysis conducted by Peyron, Laurent, & Garcia-Larrea in 2000 reviewed the literature on the areas of the brain that are activated during a painful experience using

positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) technology. Both PET and fMRI techniques measure the brain activity of awake, alert humans. Areas showing activation during acute pain include the thalamus and the anterior cingulate cortex, which also showed increased blood flow (and thus, activation), during analgesia. Allodynia revealed an activated thalamus, insular cortex, and the secondary somatosensory area. Brain imaging responses considered the emotive, cognitive, and sensory components of pain processing. The authors also suggest that a structure can simultaneously be involved in both pain response and pain inhibition.

The limbic system regulates the affective response to pain (Sternberg, 2007). The cingulate gyrus and the insular cortex are also central elements in the process of pain perception. The insular cortex is involved in the autonomic, reflexive portion of the pain response, while the cingulate gyrus receives projections from the thalamus and relays these projections to the prefrontal cortex, which stores the information as pain, thus completing the input pathway (Nagai, Kishi, & Kato, 2007). The limbic system has been shown to be involved in the pain pathway in many ways. Removal of amygdala tissue has revealed decreased responsiveness to noxious stimuli, indicating an involvement of the limbic system in the affective, unpleasant component of pain (Melzack & Wall, 1973).

Modulation of Pain

The body is capable of preventing or delaying the perception of pain based on the surrounding environmental factors. A stressor can activate the "fight-or-flight" response, in which case the stress signal from the hypothalamus transfers downward to the midbrain, then the medulla, and finally to the spinal cord where signal-negating synapses are constructed. Neurons

whose specific job it is to transmit pain are located in the spinal cord dorsal horn, but during this stress response, these neurons' messages are stifled (Sternberg, 2007).

Nociceptors are activated by injurious stimuli, and the damaged tissue becomes more sensitive to pain. Known as sensitization, the threshold level at which these receptors are stimulated is lowered. Phenomena such as sensitization reveal the plasticity of the pain pathways. When the pain perception threshold is effectively lowered, this is called hyperalgesia. Central sensitization, in addition, results from synapse use as well and leads to a strengthening of the input-to-spinal cord connection. Thus, these synapses are now more effective and a greater reaction responds to a much weaker stimulus than previously established (Sternberg, 2007).

In studying pain modulation, researchers must account for psychological influencing factors, including expectation, arousal, and alertness, presumably because such factors are the reason pain modulation systems have evolved. What can be painful to an individual in one situation can go virtually unnoticed when central nervous system processing is altered by such environmental and psychological factors. Pain modulation theory takes into account this ability to both sense and inhibit noxious stimuli. The Gate Control Theory, originated by Melzack and Wall in 1962, explained pain modulation as the balance among noxious stimuli-sensitive neurons and nonnoxious stimuli-sensitive neurons. According to Basbaum and Jessell (1991), while activated non-nociceptors "close" the "gate" that leads to the dorsal horn of the spinal cord, nociceptors "open" it. The gate, the entrance to the spinal cord, mediates the transmission of injurious input. In other words, the gate is closed (and injurious input is not transmitted) when neurons that do not transmit noxious stimuli inhibit the effects of those that do, and the gate is open (and input is transmitted) when nociceptors inhibit the effects of non-nociceptors. Therefore, even neurons not activated by noxious stimuli play a role in analgesia.

Pain Behavior

In its investigations, the coherent theory of pain must incorporate how an individual responds to pain and what factors influence varying behaviors. Researchers can assess pain behavior by investigating the pain behavior of mice and extending these findings to human behavior. Moreover, pain is an adaptive experience, and the mechanisms utilized in the pain response are highly conserved across species. Therefore, animal models help researchers understand the underlying mechanisms that control pain responses and reactions. Before the 1970s, tests consisted of direct noxious stimuli applied to the animal's tail or placing them on hot plates at various temperatures. Such brief experimental paradigms proved to be irrelevant for clinical research of human pain given that they were measures of transient, fleeting pain rather than chronic pain. Researchers, however, then changed their focus to chronic pain, and the onset of analgesia, or pain relief, was difficult to study with transient pain tests. In addition, physical restraint, as exhibited in some of these tests, could lead to stress and discomfort, possibly resulting in unwanted pain and anguish for the animal which could greatly skew results (Dubuisson & Dennis, 1977).

An increasingly common testing model for inducing pain in laboratory mice is the formalin test, first used by Dubuisson and Dennis in 1977. This test consists of administration of formalin, an inflammatory noxious stimulus, into one paw and the experimenters observe the pain behavior following the injection. Typically, the test is scored by surveying behaviors such as favoring, elevation, and licking or biting the injured paw (Abbott, Franklin, & Westbrook, 1995). Previous studies have shown a biphasic response to formalin injection, indicating separate neuronal processes, and this response is generally divided into the initial, phasic excitatory reaction and the prolonged, tonic period of painful sensation (Dickenson & Sullivan, 1976). The

first phase, characterized by the initial rapid escalation of behavior and then a gradual subsiding, lasts approximately five minutes. The second phase, a plateau of behavior, lasts from 20-35 minutes. Tjolsen, Berge, Hunskaar, Rosland, & Hole (1992) attributed the early phase to the activation of C-nociceptors in response to the noxious stimulus, and attribute the late phase to both the inflammatory response and synaptic plasticity changes in the dorsal horn of the spinal cord. This two-part time course has been observed, with minor variations, in the rat, monkey, cat, guinea pig, and mouse (Wheeler-Aceto & Cowan, 1991). Wheeler-Aceto and Cowan (1991) observed licking and flinching in both the first and second phases, while Coderre, Fundytus, McKenna, Dalal, & Melzack (1993) detected lifting in addition to licking and shaking/flinching in the second phase. In fact, it has also been shown by Rosland, Tjolsen, Maehle, & Hole (1990) that different concentrations of formalin have varying effects on the two phases, a consideration to be taken when studying either one of the phases in isolation from the other. The advantages of this test are discussed by Dubuisson and Dennis (1977). Subjective evaluation was a possible confound in tests run prior to the formalin test, so the formalin test was designed with a previously-agreed upon series of pain ratings. Additionally, restraint of the animal is diminished or eliminated completely, and the noxious stimulus induces continuous, nontransient pain. Thus, the results can better represent and apply to human models of chronic pain. Finally, formalin has been proven to be more effective than other noxious stimuli in observing behavioral responses (Wheeler-Aceto & Cowan, 1991).

Animal models help in understanding the principal neuronal mechanisms at work in mammalian species, including humans. In researching pain, animal models have been particularly useful in establishing not only factors which influence behavioral responses, but also structural changes in the brain that occur from such manipulations. Animal models as shown by

Iyengar, Bymaster, Wong, Simmons, & Ahmad, (2002) displayed the effects of antidepressants on pain behavior in the formalin test situation. Researchers administered the dual-action antidepressant duloxetine, which inhibits the reuptake of norepinephrine and serotonin, and observed significantly less paw licking in the second phase of the pain behavior response.

It is important to study the effects of stress on pain responses because the stress response and the pain pathways are closely linked. By minimizing pain of the subject, the experimenter should realize he is probably simultaneously minimizing stress, as pain is a stressful phenomenon. However, an interesting study conducted by Seo et al. (2006) investigated the effects of immobilization-induced stress on pain behaviors in mice. Mice in conditions of immobilization displayed a reduction in pain behaviors during the second phase of the formalin test. Stress-induced analgesia was said to be the mechanism at work when such pain behaviors were diminished, acting as evidence for the link between pain and stress, which appear to have both a direct and indirect relationship: while minimizing pain can minimize stress, stress-induced analgesia is evidence for a reduction in pain threshold with increased stress. It is important to note the flexibility of the pain pathways. Situational factors can instigate responses which are appropriate and in accordance with the surrounding circumstances, thereby further citing the plasticity inherent in the pain response system.

Brain Plasticity and Pain

Brain plasticity allows for neural enhancement and development, but this malleability can also have harmful consequences. Plotsky (1998) affirms that negative experiences early in life can lead to certain changes in the neurophysiology of the brain which, in turn, may be a significant factor involved in later life mental states like depression and post-traumatic stress disorder. He showed that both early maternal separation and early pain experiences had an effect

on pain thresholds before and after puberty, indicating an effect of the "permanent trace" of early traumatic memories on brain plasticity and development. Similarly, Sternberg, Scorr, Smith, Ridgway, & Stout (2005) noted pain behavior differences in adulthood with mice that underwent neonatal surgery such that behavioral responses decreased after surgery, implying an increase in pain threshold. These findings were suggested to be attributed to changes in the stress system, showing further indication of the malleability of the interaction between the brain and these response mechanisms. Much research has shown that experiencing pain in early life leads to alterations in neurodevelopmental functioning (ie, Page, Blakely, & Kim, 2005). Thus, although the connection between brain plasticity and pain in early life is well established, that between adulthood plasticity and pain needs further development.

Central nervous system plasticity has been observed in adult animals using enriched environment conditions. In such condition, the animals' housing arouses the senses, either by means of exercise, toys, or even stimulating patterns on the cage walls. Such conditions have been shown to affect dendritic growth (Leggio et al, 2005) and influence opioid sensitivity, and thus, analgesic activity (Smith et al, 2005). Shum et al. (2007) also demonstrated the effects of an enriched environment on brain plasticity, specifically the anterior cingulate cortex, a region well known for its involvement in the pain pathways. The authors hypothesized the cingulate cortex plasticity due to enriched environment would lead to changes in pain behavior. Results showed enhanced synaptic plasticity as well as an increased sensitivity to inflammation, both acute and long-term, with exposure to enriched environments. In addition, enriched environments led to an increase in long-term potentiation (a form of cellular learning), and a decrease in long-term depression (the weakening of neuronal connections). These findings imply a possible involvement of synaptic plasticity to the threshold-lowering phenomenon of sensitization. Thus,

there appears to be a positive correlation between enriched environment and pain sensitivity, and development in enriched conditions seemingly has an effect on the physical structure of the anterior cingulate cortex and the neuronal mechanisms involved in learning. This study establishes that plasticity in the brain, particularly of those mechanisms involved in learning, are correlated to enriched environment-induced pain sensitivity.

Similarly, Fessler and Beatty (1976) tested pain threshold using electrical shock in mice that experienced early life enrichment, and also tested the amount of exploring the mice exhibited in an open-field test situation. Mice were raised either in isolation or with one other mouse of the same sex. They found that those animals in the enriched condition showed less activity in the open-field test, based on degree of exploration observed. Moreover, the enriched mice were also shown to have a lower threshold to the electric shock stimulus. These findings suggest that both limiting sensory input and being socially constrained early in life have an impact on later life sensitivity to pain. Perhaps by stimulating the senses early in life, the enriched mice's "adaptation levels", a term coined by Sackett in 1965, were higher than the isolated mice's. Therefore, the open-field and shock tests were less of a sensory surprise to these mice, eliciting a lower, less intense response. These results are supported by previous findings, which imply that the sensation system consists of two overlapping receptive areas, one for mild stimuli and one for intense stimuli (Melzack & Wall, 1973). Such findings can be thought of in terms of stress: mice raised in enriched environments are perhaps utilizing the receptive area for more mild stimuli because their high adaptation level acts as a buffer to the usual stress of a sensory surprise. In observing plastic changes in the brain buffering the effects of an arduous experience, one can comprehend a possible analogous development in terms of neuron formation leading to changes in pain thresholds and subsequent behaviors.

In taking a different approach to the study of pain behavior, Lore (1969) looked at pain avoidance in mice in enriched and restricted environments when placed in a novel setting. Previous research showed that isolation-raised animals were seemingly insensitive to pain when in stressful situations. It was hypothesized that animals raised in isolation but were not placed in stressful situations would behave differently in reaction to noxious stimuli. Experimenters observed the number of times the mouse's nose touched a nearby open flame. Subjects who were raised in a restricted environment touched the flame the most while in the novel cage, indicating that the pain threshold increase might be explained by an amplification of emotional reactivity. Perhaps, then, enriched environment animals exhibit less fear of potentially painful stimuli because of their higher adaptations levels, which entails that a restricted environment can be considered a stressful one. Again, it is reasonable to view higher adaptation levels as responsible for changes in pain behaviors which occurring in conjunction with synaptic plasticity.

The unquestionable finding from research on enriched environment effects is that the brain is not a static entity. Structures in the brain are capable of changing size and density; cells are perpetually being born while others are simultaneously dieing. Such changes can lead to new insights about how the brain functions, or ceases to function. Devor (1991) discusses chronic pain in the aging population with respect to age-related cell loss, which he proposes has resemblance to tissue damage-related cell loss. Specifically, he looked at dorsal root ganglia, the cell bodies of the peripheral afferents, which are the primary nociceptors in the pain pathway (Sternberg, 2007), and the alterations that occur in them after an axon is severed, including abnormal discharge firing, enhanced cross-excitation with nearby neurons, and the induction of cell death. Such changes, Devor proposes, may play a role in atypical sensory processes associated with axotomy and nerve injury, including chronic pain. Because dorsal root ganglia

degeneration occurs in both damaged tissue and the aged, it is suggested that "spontaneous cell death" occurring in the aged might cause these similar changes as abnormal firing and cross-excitation, leading to sensory vulnerability, and thus, chronic pain. This study exhibits the comparable cell loss following tissue damage as well as in the aging brain, revealing evidence for brain plasticity. In addition, the sensory vulnerability of the aged population displays the similarly inherent plastic nature of the pain system.

It is clear that the pain pathways are involved in processes that are either the cause of or are susceptible to change. Additionally, the anterior cingulate cortex has been shown to have plastic qualities when impacted by an enriched environment. Early pain has been shown to impact the plastic nature of the brain by affecting later-life responses, and enriched environment conditions have been shown to influence pain behavior, threshold and relief. Because of the inherent plasticity of the brain as well as the pain pathways, one can deduce that a structure specifically involved in the pain pathway must also be plastic in nature.

The hippocampus, located in the temporal lobe, plays a role in long term memory as well as spatial learning and chronic pain perception. McEwen (2001) affirms that electrically stimulating the hippocampus leads to increase in pain threshold, implying a decrease in sensitivity to pain. Furthermore, the plasticity of the hippocampus is demonstrated by its involvement in coping with stress and allostatic load. In such processes, glucocorticoids, which are adrenal steroids, adversely react to the body undergoing a stressful, demanding situation. Adrenal steroids are also known to participate in the regulation of neurogenesis of the dentate gyrus, part of the hippocampal formation where new cell growth is originated. McEwen (2001) also notes that injecting an anesthetic into the dentate gyrus leads to pain relief. Such findings indicate the clear connection between pain threshold, neurogenesis, the dentate gyrus, and

hippocampal plasticity as demonstrated by the stress response. Analgesic effects caused by numbing the dentate gyrus act as additional evidence for the connection between neurogenesis and pain response. It is reasonable to hypothesize a direct relationship will result from the present study; with decreased neurogenesis, decreased pain sensations result.

Though it is contested, experiments indicate that the function of the newly formed neurons may deal with learning enhancement. However, it must be noted that neurogenesis in adulthood is a relatively new idea and thus a chronological narrative of how it came to be an accepted theory is important to understanding the foundation of neurogenesis itself.

Neurogenesis

Throughout the history of neuroscience, it was agreed that new cells could not be formed in the brain and brain damage was irreversible. However, with the progress in technology of the 1960s and 70s, experiments began illustrating that self-repair was possible in some areas of the brain. Axon regrowth in the brain and spinal cord was found to some extent after damage from brain trauma. Some scientists, however, were still skeptical. Eckenhoff and Rakic (1988) observed progenitor cells in the dentate gyrus. Using autoradiography to identify neurons, they found no new neurons produced after puberty in the rhesus monkey. However, Gould, Reeves, Graziano, & Gross (1999a) suggests that these findings were a result of autoradiography not accounting for all the newly formed cells. In addition, there is a possibility of cell death of those cells that were labeled between the time of injection and sacrifice since the animals were raised past maturity, which is over 3 years of age. It was not until the turn of the 21st century that researchers discovered new neurons in the hippocampus of the postmortem human brain. Since that time, much research has been done on neurogenesis, learning and memory, and the effects that living situations, stress, gender, and countless other variables have on this phenomenon. For

example, a 1997 study revealed a 60% increase in neurogenesis in mice raised in enriched environments (Eriksson et al, 1998).

Neurogenesis illustrates the malleable, plastic nature of the brain. It consists of three phases: proliferation, migration, and differentiation. To guarantee survival of the new cell, migration and differentiation are of the utmost importance (Abrous et al, 2002). Neurogenesis occurs on the barrier between the hilus and the granule layer of the dentate gyrus. Stem, or precursor, cells, born in the subventricular zone of the cortex, divide into both new stem cells as well as progenitor cells. The progenitor cells then migrate to the olfactory bulb via the rostral migratory stream (Altman, 1969), and continue to the granule layer where they differentiate either into neurons or glial cells (van der Borght, Eggen, Van der Zee, unpublished). The dentate gyrus of the hippocampal formation, produces neural stem cells in its subgranular zone, a site of neurogenesis. The subventricular zone, located throughout the lateral walls of the lateral ventricles in the brain, also provides neural stem cells for neurogenesis to occur.

To test for newly formed cells, 5-bromo-2'-deoxyuridine, or bromodeoxyuridine (BrdU) is administered, followed by an immunohistochemistry procedure, which reveals the daughter cells of mother progenitors. BrdU operates by integrating onto the DNA of the new cells that is then passed on to their daughters as a substitute for the thymidine nucleoside. Researchers are later able to locate proliferating cells as well as the lineage of cells that resulted from mitosis by administering BrdU antibodies to detect the BrdU on the DNA (Gould, McEwen, Tanapat, Galea, & Fuchs, 1997). However, BrdU labeling indicates that a cell has proliferated, or that the DNA of an injured cell is being repaired (Jin, Xie, Ou Mao, Greenberg, 2006), not necessarily that it has become a functional neuron or glial cell.

Gould et al. (1999a) investigated neurogenesis specifically in the primate brain using NeuN (neuronal nuclei) markers, a standard antibody used to detect neurons after mitosis has occurred. Many cells that contained BrdU were also comprised of round or oval nuclei, a feature typically found in mature neurons. In finding that new cells formed and proliferated, since these cells had neuronal-like properties, the theory that neurogenesis could occur during adulthood in the primate was supported. However, although these cells had features similar to neurons, the function of these cells is still unknown. Thus, Gould cautions that it might be considered premature to label these cells as neurons, indicating that more research is needed.

Factors That Promote and Inhibit Neurogenesis

Many mechanisms and systems within the brain influence and can even be contingent on one another. Although this can complicate studies, it also reveals much unknown information about interdependent relationships occurring in the brain. The same is true for neurogenesis. Occurring in the hippocampus, which is involved in numerous and important functions, neurogenesis inevitably affects and is affected by many factors.

In studying pain, Delgado (2004) reviews the effects of antidepressants on pain relief. Both serotonergic and noradrenergic neurotransmitters not only treat depression but also operate in analgesia by lowering sensitivity to pain. Because the effects of depression have been shown to alter the structure and volume of the brain, the importance of studying pain and depression in a joint manner is highlighted. Reports showing that antidepressants stimulate neurogenesis, and that neurogenesis might be an essential mechanism involved in the performance of antidepressants (Delgado, 2004) illuminates the link between pain and neurogenesis. In addition, Kempermann and Kronenberg (2003) suggest antidepressants to be therapeutic for Alzheimer's

patients, who display lower levels of neurogenesis since such treatments have been shown to increase proliferation of hippocampal progenitor cells.

As briefly mentioned above, a connection has been shown between neurogenesis and learning involving the hippocampus. In another study (Gould, Beylin, Tanapat, Reeves, & Shors, 1999b), hippocampus involvement in learning was investigated with regards to neurogenesis. By using BrdU staining, the outcome of the new cells produced could be determined. In order to manipulate learning, rats performed hippocampus-dependent behavioral tasks (i.e. navigation in the Morris water maze) and hippocampus-independent behavioral tasks (i.e. Morris water maze with cues given). Results showed an increase in the number of neurons in the dentate gyrus in hippocampus-dependent learning rats, but when comparing rats in the non-hippocampus-dependent learning condition to controls, no significant difference was found. Though the hippocampus was activated during all learning tasks, a significantly greater number of neurons were only found in the hippocampus-dependent condition when compared to both the control and the comparison condition. These results revealed a direct relationship between learning using newly formed cells in the hippocampus. Though the function of new neurons is unknown, the authors propose that these cells of neuronal maturity have a function in spatial learning.

Previous research shows stress hormones to have an effect of granule neuron synthesis in the hippocampal dentate gyrus. Gould et al. (1997) studied the effects of stress on neurogenesis in the tree shrew, an animal that phylogenically falls between primates and insectivores. The experimenters decided the tree shrew would be a good candidate for studying psychosocial stress because a dominant/inferior relationship is immediately created among same-sex dyads and causes much stress in the subordinate individual. Thus, BrdU labeling was executed in the dentate gyrus cells of the stressed animals, and cells were found to have been greatly decreased

in number when compared to animals that underwent NMDA receptor blockage. NMDA is an amino acid that mimics glutamate, which has been shown to be released in stressful situations. Activation of NMDA receptors, Gould et al. hypothesize, leads to a decline in neurogenesis because substances normally released from nearby cells that stimulate mitosis are being inhibited, thus exhibiting neurogenesis under the influence of stress as well as NMDA receptor activity.

In further citing a relationship between stress, and hippocampal plasticity, Duric and McCarson (2006) noted the effects of the hypothalamic-pituitary-adrenal (HPA) axis, which is involved in the stress response pathway, on hippocampal structure, and compared these effects to those found after chronic pain induction. Using BrdU-labeling techniques, they observed structural changes in the hippocampal dentate gyrus. BrdU-positive cells contained characteristics typical of immature cells, which are known to likely differentiate into neurons. Pain induction did lead to a decrease in the number of such cells thereby indicating a decrease in neurogenesis. This study reveals the similarities seen in the pain and stress response systems in hindering the amount of new neurons formed in adulthood. It has been shown that normal effects of stress can be evaded by the changes in brain plasticity, as demonstrated by enriched environment studies, the parallel relationship seen between the pain and stress systems acts as further evidence that the pain pathway may also be altered by changes in plasticity.

While stress can affect neurogenesis, other potent agents can influence the degree of this phenomenon, as well. Nicotine is an alkaloid that acts on the receptors of nicotinic acetylcholine (nAChRs), which are also activated by the neurotransmitter acetylcholine (ACh). This neurotransmitter has also been shown to have effects on neurogenesis. In a 2005 study, Mohapel, Leanza, Kokaia, & Lindvall demonstrated decreased neurogenesis in the rat dentate gyrus when

the cholinergic input to the forebrain was lesioned and an enhancement in neurogenesis when a cholinergic agonist was administered. Interestingly, the authors attribute changes in dentate gyrus neurogenesis to the cholinergic system directly affecting cell proliferation and the short-term, rather than long-term, survival of the neuron. Evidence of this supposition is seen in the recognition of certain ACh receptors located on the newly born cell, along with the observation that the early phases of neurogenesis are affected by changes in the cholinergic system. The authors also noted the deleterious effects of ACh lesions on spatial memory. This finding coincides with past research done by Gould et al. (1999b) that suggested an involvement of mature neurons to spatial learning, and is further illuminated by recognizing the involvement of the hippocampus in both spatial learning and neurogenesis. Additionally, Bartus (1981) observed decline in cognitive functioning with cholinergic antagonist administration, further citing the influence of acetylcholine on brain functioning.

It has been proposed that nicotine is responsible for the reduction of neural marker expression, affecting migration of the cell and, thus, the cell's survival. Moreover, nicotine has also been shown to be a lethal agent to immature cells during development by stimulating apoptosis of these progenitor cells in the hippocampus (Abrous et al., 2002). The beneficial effects of nicotine in small doses have also been described, indicating the protective role it can play on brain tissue. In fact, to treat age-related brain damage and to improve cognition, drugs with nicotinic properties have been recommended. However, past research implies a negative effect of nicotine in high doses on learning and memory.

Abrous et al. (2002) examined the dentate gyrus and the subventricular zone for neuronal markers, neurogenesis, and cell death. Results indicated nicotine self-administration led to a great decline in neurogenesis and neural markers in the dentate gyrus as well as an increase in

cell death. Such effects were not found in the subventricular zone, however. Researchers believe these findings are the result of these two areas under independent controls. In support of this hypothesis, previous studies indicate neurogenesis in the dentate gyrus, not in the subventricular zone, to be influenced by learning, aging, and stress hormones while proliferation in the subventricular zone, and not in the dentate gyrus, has been shown to be modified by growth factors. These findings reveal the contribution of nicotine dose-dependently on the cognitive impairments observed during withdrawal in chronic smokers and, thus, nicotine's effect on hippocampal plasticity.

Another study revealing similar results was done by Shingo and Kito in 2005. Rather than self-administration, the rats were given intraperitoneal injections of nicotine, after which the hippocampi were inspected for neuronal (NeuN) and glial (GFAP) markers. The authors discuss the inducing effects of nicotine on the expression of the mRNA of insulin-like growth factor (IGF-1) in the rat hippocampus, reiterating the beneficial effects of nicotine in small doses. To analyze the manifestation of the markers, antibodies against the compounds utilized were given in order to bind to the antigens, thus revealing the compounds' presence or absence. Findings showed a negative correlation between neuronal-marked cells in the dentate gyrus and nicotine dosage such that higher doses were associated with lower numbers of labeled cells. This trend was not seen with the glial markers, indicating a non-involvement of nicotine in astrocyte formation. This study confirmed prior results that illustrate a negative effect of nicotine on maturing neurons in large doses. In designing our study, we chose to utilize nicotine because the effects on neurogenesis are clearly heavily dependent on the dosage administered. In addition, the studies mentioned here demonstrated such effects of nicotine in rats, whereas the present study observed mice. Therefore, in an effort to extend the current research of neurogenesis

enhancement with this alkaloid, we hope to clarify the margin of effectiveness of nicotine on enhancing neurogenesis in mice.

Mohapel et al. (2005) discuss the possible implications of nicotine effecting neurogenesis on Alzheimer's disease patients because of the loss of hippocampal functioning that occurs in the disease as well as the involvement of ACh in such functioning as learning and memory. Alzheimer's disease has become the most common cause of dementia, or gradual cognitive functioning decline (Hill, 2008). Although there is currently no cure for Alzheimer's disease, research on possible treatments have shown to be promising in delaying the symptoms. With the development of an experimental model, it has been postulated that the impairment of learning and memory functions in Alzheimer's patients may result from ACh depletion that leads to reduced neurogenesis. These findings clarify the involvement of cholinergic functioning on memory and learning, and, therefore, neurogenesis. However, ACh is not the only link between Alzheimer's disease and neurogenesis.

Alzheimer's Disease. The neurodegenerative disease known as Alzheimer's disease involves the slow but gradual deterioration of the brain, beginning with the hippocampus. This deterioration is caused by the build up of senile plaques, which are deposits of amyloid in the gray structure of the brain. These characteristic plaques have been suggested to be lethal to neuron survival, leading to progressive death of neurons, and thus accounting for the neurodegenerative nature of the disease. Research pertaining to the effects of Alzheimer's disease on neurogenesis has been considered in both human and animal models, though results have varied depending on the species. Jin et al. (2004a) examined 25 postmortem human brains, 14 of which were diagnosed with Alzheimer's disease. In comparison to the brains without neurological disorders, the hippocampi of the Alzheimer's patients showed a greater expression

of markers that indicate immature neuronal proteins as well as an augmentation in the number of cells containing these markers. This finding greatly contrasts to results of mouse models, in which an Alzheimer's disease model is associated with a decrease in hippocampal neurogenesis. However, the authors relate their findings to similar results observed in patients that have undergone an ischemic stroke. These patients have demonstrated increased neurogenesis as well, implying a compensatory role to counterbalance the lost neurons from the trauma. Previous research has confirmed neuronal migration to the ischemic areas of the cortex, entailing repair of the damaged tissue. The authors believe a similar mechanism is involved in the brains of the Alzheimer's patients; neurons lost due to degeneration are being replaced by new cells. Of course cognitive impairments are not improved with Alzheimer's disease, as normal neurogenesis enhancements have demonstrated (in enriched environments, for example), because the number of neurons lost outweighs the number of compensatory neurons. The authors suggest many explanations for this, including the possibility that too many cells are lost that can be compensated for, the possible ineffectiveness of the not fully developed or incorrectly developed neurons formed, or the potentially toxic microenvironment that a brain with Alzheimer's may provide these neurons, leading to their inevitable deaths.

In a study comparable to Jin et al. (2004a), Donovan et al. (2006) studied the relationship between Alzheimer's and neurogenesis using the PDAPP mouse model, in which the mouse's genes overexpress the mutant human amyloid precursor protein (APP). In this model, the brain has "an age-related accumulation of Aβ plaques, hippocampal pathology, and cognitive decline" (Donovan, 2006), thus mirroring the deficits involved in Alzheimer's. However, results were contrary to those found by Jin et al. (2004a and 2004b). A reduction of 50% in neurogenesis was found in the old mice but not the young mice, and new neurons in the subgranular zone displayed

irregular maturation. Interestingly, there was an increase in neuron count in the outer granule cell layer, but these cells did not survive to maturity. Because of the involvement of neurons in hippocampal functioning, the abnormalities observed in this experiment are postulated to be a factor in hippocampus malfunctioning seen in the mouse model.

Jin et al. (2004a) justify the varying results found among models of humans and mice by explaining that certain mouse models do not emulate familial or sporadic Alzheimer's, which signify the genetically-inherited, rare form and the late-onset, common form, respectively. To further investigate the species- and model-dependent differences in results, Jin et al (2004b) studied a transgenic mouse model that strongly represents human mutations found in early-onset Alzheimer's. Results corresponded to their earlier findings of increased production of new neurons in both the dentate gyrus and the subventricular zone. While other transgenic mouse models mimicking Alzheimer's have exhibited a reduction in neurogenesis, the outcomes seem to be dependent on the mutation type present on the amyloid precursor protein, an indicator of Alzheimer's disease. Because the expression of the disease in these transgenic mice is similar to that seen in human patients with Alzheimer's, the authors believe that enhanced neurogenesis is an intrinsic characteristic of the disease, and that the increased neuron count is a mechanism of restitution to account for the lost neurons that accompany the disease as well. As mentioned above, the brain is capable of full restoration of lost neurons when repairing damaged tissue from ischemia, and the authors are confident that this could, too, be possible with Alzheimer's treatments. The inhibition of the neurotransmitter glutamate, mentioned above with respect to NMDA imitation, acts as a neurogenesis stimulant, suggesting an involvement of faulty transmission of glutamate in eliciting neurogenesis in Alzheimer's patients. Finally, in discussing drug treatments, the authors mention acetylcholinesterase (AChE) inhibitors, which are

essentially agonists of acetylcholine because they inhibit the breakdown of ACh. AChE inhibitors are recommended because cholinergic receptors have been found in neuron progenitor cells and have also been linked to cell proliferation.

A 2006 study by Jin et al. further studied drugs being used to treat Alzheimer's in order to better understand the repairing of the brain that takes place with their administration. The authors reason that drugs found to enhance neurogenesis will be therapeutic to Alzheimer's patients because they will further perpetuate the cell replacement naturally occurring in the brain. AChE inhibitors and an NMDA receptor agonist were administered in vitro to mouse cerebral cortical cell cultures and in vivo to mouse dentate gyrus and subventricular zone. BrdU-labeling and protein markers were the means of establishing neurogenesis. All compounds were shown to increase BrdU-labeled cell count, which also exhibited indicators of immature neuronal progenitors, both in vivo and in vitro, including galantamine, an allosteric enhancer of nicotinic cholinergic activity. Because in vivo and in vitro findings were similar, the authors suggest that these drugs elicit responses in the cell that are independent of non-neuronal cells that would exist in this environment in an organism. Of course, varying percentages of enhancement were found. Both dentate gyrus and subventricular zone cell count was increased the most by galantamine, due to its ability to enzymatically arouse nicotine receptors. Cognitive impairment found in Alzheimer's patients is thought to be improved by augmenting cholinergic transmission, supported by findings that lesions to the cholinergic input to the brain lead to a decrease in neurogenesis, as previously noted. In utilizing drugs used to treat Alzheimer's disease in the present study, we hope to implement further the compensatory processes that such cholinergic agonists help to promote.

The majority of Alzheimer's patients are older than 55 years of age, implying that it is a disease that, for the most part, occurs in the aging brain. Of course, much research has also been done with respect to the aging brain without a neurological disorder. Harkins and Price (1992) discuss the experience of pain in older adults as being less severe than in younger adults, most likely due to the changes in the nociceptive system that occur naturally, similar to changes in vision and hearing. In addition, when studying pain in the elderly other factors must be taken into account including comorbidity, mental health and history, and daily life.

Pain and Alzheimer's disease. Alzheimer's disease affects both mental and physical functioning. A major side effect of Alzheimer's is the chronic pain that often accompanies the degenerative aspect of the disease. Therefore, studying how Alzheimer's patients' respond to pain automatically and affectively can lead to effective advances in treatment. Benedetti et al. (1999) demonstrated pain tolerance differences among Alzheimer's patients, and found that tolerance to noxious stimuli increased as severity of the disease worsened. They monitored EEG testing and found that increased changes in EEG and a higher rating on the Mini Mental State Examination, an assessment of cognitive function and a standard tool used to screen for dementia, gave rise to higher pain tolerance, which is considered an emotional aspect of pain. However, similar data did not emerge when observing pain thresholds, a physiological component. These results imply that the cognition/emotion aspect of pain experience is impaired in Alzheimer's patients. This study acts as further evidence of the direct relationship between pain sensitivity and neurogenesis. By observing that the more severe the disease, the higher the patient's pain tolerance, one can deduce that a decrease in neurogenesis thus is correlated to a decrease in pain sensitivity.

To extend these findings, Rainero, Vighetti, Bergamasco, Pinessi, & Benedetti (2000) observed the autonomic and perceptual responses to pain in twenty Alzheimer's patients, either just above the threshold or at twice the pain threshold. With intensities just above the pain threshold, patients exhibited diminished autonomic responses when compared to controls, but pain perception remained normal. When the pain stimulus was increased to twice the pain threshold, patients' physiological responses were increased so as to be comparable to controls, though with a somewhat weaker increase in heart rate, and pain perception appeared to be blunted. From this, the authors suggest that autonomic responses vary depending on the intensity of the noxious stimulus, and, in Alzheimer's disease, the thresholds for activating the autonomic system and pain are increased. The authors rationalize that, in general, elderly subjects report less pain to experimentally applied stimuli than young subjects, perhaps due to the age-related dementia, which effects overall sensory perception, that occurs naturally in normal subjects. These results stand as further evidence confirming a detachment of one's affective and physical elements during the experience of pain, as well as how changes occurring in the brain can affect one's physiological and affective responses to pain.

Along with changes in pain perception and the neuronal loss that occurs with age, there also exists cognitive decline and memory loss in the naturally aging brain. According to Sternberg, Martinez, Gold, & McGaugh (1985), the brains of humans, monkeys, and rodents all progressively lose the ability to make new memories with age. This decline in cognitive abilities has been attributed to the deterioration of the cholinergic system in the forebrain. Memory impairment has also been demonstrated as a result of administration of cholinergic antagonists (Bartus, 1981), suggesting that an increase of activity at the cholinergic receptors may lead to

repairs in such memory deficits. Such findings imply that the cholinergic system is vulnerable in not only in individuals with Alzheimer's but with normally-aging individuals, as well.

Pain and Neurogenesis

The cholinergic system is involved in both neurogenesis and pain, but pain can also be an influential factor on levels of neurogenesis itself. Current research analyzing the effects of pain on neurogenesis is revealing much new information regarding the pain pathways and what mechanisms are involved with hippocampal plasticity. As previously discussed, Duric and McCarson (2006) demonstrated that pain can induce changes in the hippocampus and neurogenesis similar to those induced by stress. Results indicated that in both nociception and immobilization conditions, the amounts of the compounds examined found in the hippocampus were significantly decreased. This study reveals the debilitating effects of pain not only on neurogenesis, but also the therapeutic effects of antidepressant medication, given the comorbidity of chronic pain and depression. This extends previous findings that depression can decrease hippocampal volume and antidepressants can act as analgesics. Although there does not seem to be enough evidence of direction of causality, a correlation between pain, neurogenesis, and depression medications seems to be evident.

The hippocampus has been shown to be involved in both neurogenesis and pain responses (Yang, Xiao, &Xu, 2007). The experimenters investigated the CA1 region of the hippocampus and the effects of ACh, muscarinic ACh receptor (mAChRs) agonists and mAChR antagonists on pain responses. Previous research has shown mAChR agonists to be effective treatments for pain and can possibly cause increased pain thresholds and desensitization, further implicating ACh involvement with plasticity of the pain system (Dussor, Helesic, Hargreaves, & Flores, 2004). Yang et al. (2007) expanded on this finding, in accordance with previous research

indicating a hippocampal role in pain perception, response behaviors, and, more significantly, neurons of the hippocampal CA1 region's involvement in nociception. Researchers know that a family of mAChR genes can be found in the hippocampus, but the relation between the cholinergic pathway and nociception is still unclear. This study was designed to test the effects of ACh, an mAChR agonist, and an mAChR antagonist on neuronal firing in the CA1 of the hippocampus. To examine neuronal firing, experimenters observed pain-excited neuron (PEN) and pain-inhibited neuron (PIN) activity in response to a noxious stimulus. Results indicated that with administration of both ACh and the mAChR agonist, PIN firing increased and PEN firing decreased, while the mAChR antagonist produced opposite effects. From these observations, the authors concluded a cholinergic involvement the pain pathway, namely the transmission of information into the CA1 region of the hippocampus, and mAChR agonists that are present in the CA1 act as analgesic agents. This study acts as further evidence that cholinergic agonists lead to a decrease in the pain pathway response.

Dussor et al. (2004) also demonstrated the effects of nicotinic acetylcholine receptors on nociception. This study revealed mediation of nAChRs in the enhancement of Calcitonin gene related peptide (CGRP) release, which has been shown to be involved in the processes of neurogenic inflammation. Therefore, while analgesic responses have been shown to be influenced by ACh and nicotinic acetylcholine, the amount of new neuron growth has been shown to be vulnerable to nicotine levels as well. Previous research also reveals the involvement of ACh in cognition and learning and such deficits in Alzheimer's disease. Maelicke (2000) placed emphasis on the reduction in nAChRs in an Alzheimer's-afflicted brain, and the possible role of nicotine in treatment of Alzheimer's disease. Because many treatments are transient due to a development of tolerance to the drug, specifically acetylcholinesterase (AChE) inhibitors or

other ACh agonists, Maelicke proposes allosteric modulation of nAChRs, which would entail a different binding site than ACh. Maelicke also utilizes galantamine, previously noted as an allosteric activator of nAChRs, and demonstrates its possible role in enhancing nAChR synthesis due to avoidance of densensitization and, thus, its therapeutic value as an Alzheimer's treatment. These findings also further implicate the plasticity of the brain as exhibited both in desensitization of nAChRs and in the counter-prevention of such desensitization. Maelicke (2000) demonstrates that cholinergic agonists can be used to enhance brain plasticity, as well as related cognitive functioning.

To reiterate the findings mentioned above, ACh agonists have been shown to be effective in treating Alzheimer's disease by acting to increase neurogenesis (Jin et al, 2004b), and both lesioning ACh input (Mohapel et al, 2005) and high doses of nicotine (Shingo & Kito, 2005) have been shown to be detrimental to new neuron growth. Such findings reveal the connection between neuron formation and cholinergic pathways. In addition, increased numbers of cholinergic receptors has been shown to increase threshold of pain (Yang et al., 2007). Furthermore, research reveals manipulations of the pain pathway and its plasticity by stress (Page et al., 2005) and depression (Keefe et al., 1986), as well as enriched environment living conditions (Shum et al, 2007), all of which have established connections to neurogenesis. Because pain- and stress-induction have been shown to have similar outcomes, (Duric & McCarson, 2006), and stress has been shown to decrease neurogenesis (Gould et al., 1997), it is reasonable to believe there is a relationship between neurogenesis and pain as well.

Analgesic effects caused by numbing the dentate gyrus act as additional evidence for the connection between neurogenesis and pain response (McEwen, 2001). Thus, a direct relationship indicating decreased neurogenesis may result in decreased pain sensations can be established.

Duric and McCarson also provided strong evidence for an effect of pain on hippocampus plasticity and neurogenesis. Therefore, speculation about neurogenesis influencing the behavioral response to noxious stimuli has previously been put forward, and past research findings indicate a possible direct connection between these two phenomena.

The present study was designed to investigate if the administration of galantamine and nicotine, both of which are ACh agonists but can have opposite effects on neurogenesis, provided that the dosage of nicotine is high enough to cause damage. Research on Alzheimer's treatment refers to AChE inhibitors, which are essentially agonists of acetylcholine because they inhibit the breakdown of ACh. AChE inhibitors are advocated because cholinergic receptors have been found in neuron progenitor cells and have also been linked to cell proliferation. Galantamine, a known AchE inhibitor, has been shown to have positive effects on new neuron growth (Maelicke, 2000). Galantamine has also been shown to play a possible role in preventing receptor densensitization by enhancing nAChR synthesis (Maelicke, 2000), further linking its effects to brain plasticity. Conversely, nicotine, in large doses, has been shown to have debilitating effects on neurogenesis in rats (Shingo & Kito, 2005), as it functions to block the nicotinic acetylcholine receptor at large quantities. In fact, this blockage is the reason for nicotine's toxicity (Wikipedia.org). However, nicotine has also been shown to have positive effects on neurogenesis in small doses (Maelicke, 2000), so testing this effect in the present study will reveal directionality of nicotine effects on neurogenesis in mice.

We chose to study the effects of nicotine and galantamine on pain behavior in the formalin test because, unlike many other pain test designs, formalin testing induces consistent, long-term pain, which better resembles clinical pain than the transient pain induction observed with hot plate and tail withdrawal tests. We believe administration of these cholinergic agonists

will reveal different degrees of neurogenesis in addition to corresponding differences in pain behavior compared to the control subjects, the saline condition. We hypothesize that if neurogenesis increases with galantamine administration, the mice should display significantly more pain behavior in galantamine conditions when compared to both nicotine subjects and controls. This is based on the supposition that increased neurogenesis will lead to an increase in pain. Support for this conjecture can be seen in Shum et al. (2007), in which an increase in plasticity of the cigulate cortex and pain sensitization were observed with exposure to enriched environment. Similarly, Rainero et al (2000) revealed findings indicating a lowering of pain perception in Alzheimer's patients, thus strengthening the correlation of decreased sensation of pain with decreased neurogenesis. We hypothesize that this trend will be seen with our data because as new neurons form, the number of receptors that are sensitive to pain also increases, and so the animal with the most neuron formation will also be most sensitive to pain. Results indicating that the animals in the galantamine condition display more pain behavior as well as more neuron formation, our study will validate a direct relationship between degree of neurogenesis occurrence and pain threshold.

Methods

Subjects

Sixty adult CD-1 mice were supplied by Harlan Sprague-Dawley (Indianapolis, Indiana). However, during drug injection procedures, 12 mice died due to various complications, making the final N=48. All experimentation conducted with these animals was in compliance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and was approved by the Institutional Animal Care and Use Committee of Haverford College. The mice were between 12 and 14 weeks old, and were given daily supplies of food from the Harlan

Teklad 8604 diet and tap water. They were housed in cages with 3-5 other mice in a room of normal temperature (approximately 70 degrees Fahrenheit) with a 12 hour light/dark cycle beginning at 8:00 in the morning. All injections were done during the light cycle. The male mice generally weighed 40 ounces, and the female mice weighed between 30 and 35 ounces. The weights of the mice did not vary significantly over the two-week period.

Procedure

Drug administration. On the first day of the two weeks of their drug administration, the mice were given an intraperitoneal BrdU (300 mg/kg) injection. Injections were administered intraperitoneally, following Gould et al (1997) protocol. BrdU (minimum 99% HPLC) labeling will allow two weeks for the labeled cells to divide or not, which indicated the degree of neurogenesis in the various conditions. Males were administered an injection volume of 10 mg/mL of BrdU and females were administered 9 mg/mL. Twenty mice (10 males and 10 females) were randomly assigned to each of the drug conditions, and were administered an injection volume of 10 mL/kg daily of either saline (10 mg/kg), galantamine (10 mg/kg), or nicotine (5 mg/kg). All drugs were purchased from Sigma in St. Louis, MO.

Formalin test. For formalin testing, the saline condition consisted of 15 subjects, nicotine consisted of 16 subjects, and galantamine contained 17 subjects (N=48). Because some mice died from aggressive cagemate attacks, we attempted to separate the instigator from the rest so as to not have injuries and wounds interfere with pain testing. To test for pain, the formalin test was administered to all 48 of the mice. In order to avoid analgesic effects of the drugs, the subjects were only tested after a 24-hour period of their last drug injection. Following the two weeks of daily injections, the plantar surface of the hindpaw was injected with formalin (5% in 20uL), after which half of the subjects were euthanized and perfused. These 30 brains were extracted for

quantification of neurogenesis. The formalin test was conducted using a black and white video camera to ensure accuracy. No experimenters were present in the room at the time of behavior observation since human presence has been shown to be fearful for animals and may alter pain behaviors in the mice (Vierin & Bouissou, 2003). The camera was secured under a clear glass table on which the mice were placed. This angle maximized the area of examination and facilitated behavioral observations. Video footage of pain behavior was later coded for behaviors such as paw licking, flicking and lifting, the three most common actions detected during the formalin test (Tjolsen et al, 1992). Coders watched the first five seconds of every twenty second bin in the early phase, from 0-10 minutes, and then again in the late phase, from 20-40 minutes. The 10 minute interphase not observed because of lack of behavior typically found during this time, as affirmed in Wheeler-Aceto and Cowan (1991). Coders marked each five-second bin with a dichotomous variable indicating presence of absence of behavior; the number of behaviors within the five-second interval was not recorded.

Histological procedures. As soon as the subject completed the forty-minute formalin test, it was sacrificed. Anesthetic procedures began with an injection of pentobarbital (100 mg/kg), after which half of the animals underwent a perfusion surgery and the other half were sacrificed using CO2 asphyxiation. In total, thirty mice were perfused, 10 per condition which each contained five males and five females. Before perfusion surgery was initiated, experimenters verified the depth of the anesthesia to assure the animal could no longer feel pain. If the subject flinched, the experimenters waited to proceed until the animal passed the tail-pinch test before starting the surgery. The transcardiac perfusion began with a cross-body incision was made across the lower torso, followed by a perpendicular incision towards the head. The diaphragm and liver were repositioned so as to allow access to the heart. By cutting the atrium, the blood in

the heart is allowed to escape. Following this incision, a needle was placed in the heart and the blood in the circulatory system was first replaced with .1M phosphate-buffered saline (PBS), and then with paraformaldehyde in order to fix the neural tissue. Following the perfusion, the brains were extracted and frozen for a 4 week period, after which the hippocampus of one hemisphere was sliced into 40 μm sections using a neural tissue slicer, the Lancer Vibratome (series 1000) in a 100mL bath of PBS (.1M). Every twelfth slice was mounted onto glass slides **(**Fisherbrand) to undergo BrdU immunohistochemistry procedures.

Immunohistochemistry protocol. The brains were processed immunohistochemically for BrdU labeling. Slides were first rinsed in PBS (.1M), and were then placed in a microwave while bathing in .1M citric acid for antigen unmasking and background reductioning. After another .1M PBS rinse, the tissue was first digested in trypsin, a mixture of .1M TB, trypsin, and 10% $CaCl_2$, followed by DNA denaturing in 2N HCl. The slides were incubated overnight in mouse anti-BrdU, a product of mouse anti-BrdU, PBS, and 10% Tween-20, at 4 degrees Celsius. The following day, the slides were again rinsed in .1M PBS and incubated in biotinylated horse anti-mouse antibody for one hour. After this step was completed, the slides were again incubated for one hour in avidin-biotin-horseradish peroxidase complex, which was furnished by a Vector kit. Finally, the tissue was rinsed in DAB, a mixture of distilled water, a diaminobenzadine tablet, and a urea hydrogen peroxide tablet. To stain the granule cell layer of the hippocampus, a cresyl violet counterstaining procedure was utilized, in which the slides were bathed in ethyl alcohol, cresyl violet and citrisolv for varying amounts of time.

All 30 slides were then coverslipped with Esco cover glass slides. Using a Nikon Eclipse E200 microscope, experimenters counted BrdU labeled cells based on past analyses (Gould et al, 1997). The experimenters recognized dark, brownish purple, circular cells within the granule cell

layer as being labeled with BrdU. Cells more than two cell-lengths outside or inside the granule layer were not valid for analysis. The experimenters were blind to the condition of the mice, and were not made aware of the conditions until the cell counting was complete.

Results

Pain behavior data analysis. To test the effects of neurogenesis-enhancing or –reducing drugs on pain behavior, a mixed factorial ANOVA test was used. Between-group variables examined were drug condition (3 levels), sex (2 levels), and experimenter (2 levels), and the within-group variable examined was phase (2 levels). The behavior data revealed significant results and a variety of interactions. A significant main effect was found for phase, $F_{1,36}=103.61$, $p<.01$. During phase one, animals were reported to display behaviors on an average of 20.29 (SD=.79), while during phase two, behaviors averaged 34.61 (SD=7.60). Interestingly, over the two phases, a near significant trend occurred implicating a general increase in pain behavior in mice of the two drug conditions compared to the control subjects (See Figure 1). Results revealed a significant interaction between phase and condition, $F_{2,36}=5.615$, $p<.05$. In phase one, the subjects displayed pain behaviors averaging 21.60 (SD=1.37), 19.04 (SD=1.35), and 20.22 (SD=1.24) in the saline, nicotine, and galantamine conditions, respectively. In phase two, subjects displayed an average of 29.15 (SD=2.87), 36.58 (SD=2.83), and 38.09 (2.60) behaviors in the saline, nicotine, and galantamine conditions, respectively (See Figure 2).

Table 1 displays the significant mean difference observed in late phase data between galantamine and saline, as well as the near significant mean difference between nicotine and saline. An interaction was seen between phase and sex, $F_{1,36}=4.37$, $p<.05$. Generally, males displayed fewer behaviors in both phase one and two, and females showed a greater difference in observed behaviors between phases than males (See Figure 3 for means). Table 2 displays post-

hoc mean differences found for sex for near significant early phase and significant late phase, $p<.05$. A significant interaction was also seen between phase and experimenter, $F_{1,36}=5.71$, $p<.05$, such that Experimenter A reported more behaviors in both phases than Experimenter B (See Table 3 for means).

A significant interaction was also observed between phase, sex, and condition, $F_{2,36}=4.86$, $p<.05$. For females, in phase one, the differing pain behaviors displayed was much more prominent than those reporting during phase two. Overall, males displayed less behavior, but behaviors reported in pzhase two are more prominent than in phase one (See Table 4 for means). Finally, an interaction was also seen between phase, condition, and experimenter, $F_{2,36}=3.31$, $p<.05$. Overall, experimenter A reported more than experimenter B (See Figure 4). However, both experimenters' analyses showed trends progressing in the same direction (See Table 5 for reported means). In our data analysis, we discounted the late phase behavioral data of one mouse, a male in the saline condition, because the data collected of his reported behaviors averaged 2.2 standard deviations from the mean. Therefore, this subject was considered an outlier and the late phase data was not included in our analysis.

BrdU cell counting data analysis. Data was examined using a three-way factorial ANOVA. The between-group variables were drug condition (3 levels), sex (2 levels), and experimenter (2 levels). Original calculations of area (μm^2), obtained using ImageJ software, computed total number of detected cells in the total area. However, in order to calculate density values across conditions, the volume of the subgranular zone was estimated. The area reported in ImageJ was multiplied by 40 μm, the thickness of each slice, yielding values of density in cells per volume (μm^3). Density values were recorded in the range of 10^{-6}, so in an effort to facilitate interpretations of the data, reported means have been multiplied by 10^7.

A significant main effect of conditions was found, $F_{2,18}=4.25$, $p<.05$. Cell count observed from animals in the saline, nicotine, and galantamine conditions estimated 8.40 (SD=1.98), 11.01 (SD=1.98), and 16.42 (SD=1.98), respectively. In addition, there was a main effect of experimenter, $F_{1,18}=5.51$, $p<.05$, where Experimenter A reported an average of 14.63 (SD=1.62) labeled cells and Experimenter B reported an average of 9.26 (SD=1.62) cells. There was no main effect found for sex, nor was there a significant interaction between sex and condition or experimenter and condition.

A strong trend, however, can be seen among the differing conditions. Saline appeared to have the lowest BrdU-labeled cell count (M=8.40, SD=1.98), followed by nicotine (M=11.01, SD=1.98), and galantamine averaged the highest count of cells (M=16.42, SD=1.98) (See Figure 4). In addition, significant mean differences were found between saline and galantamine, M=8.02 (SD=2.81), $p<.05$ (See Figure 5), and a near significant difference between galantamine and nicotine, M=5.40 (SD=2.81), $p=.07$.

Discussion

The results of our data were consistent with our hypotheses regarding both behavioral data and neurogenesis data. General trends indicate that with enhanced induced neurogenesis, pain sensitivity also increases. The animals in the control saline condition showed the least amount of pain behaviors during the late phase, as well as the fewest number of BrdU-labeled cells. In contrast, the galantamine animals displayed the most pain behaviors during the late phase, and the highest number of new neurons formed. In both behavioral data and neuron formation increase, nicotine condition animals fell between the other two conditions. Finding that galantamine was significantly different from the control supports our hypothesis that with increased neuron formation, nociception also increases because of resultant newly formed cells

that participate in the pain pathway. In addition, although nicotine was not found to have significantly different effects than galantamine or the control, the directionalities of both analyses were the same; as neurogenesis increased, so did pain sensitivity. These results suggest a possible link between drug-induced neurogenesis and pain threshold.

A significant main effect was found for phase, but this was not surprising because the first phase was half the length of time as the second. Therefore, there are twice as many observed times found in phase two than phase one, so a significant difference found between the two is self-evident. Interestingly, we found all of the significant results for the behavioral data in the second phase of the formalin test. This might be due to the differing structures responding in the two phases. Coderre et al (1993) observed additional behaviors in phase two that did not occur in phase one. Perhaps, then, the increase in behaviors observed accounts for the significant results seen only in phase two. From the neurobiological perspective, Tjolsen et al (1992) attribute phase two responses to changes in the dorsal horn and peripheral inflammation. Phase one behaviors, on the other hand, are under the control of chemically stimulated nociceptors. It is possible, then, that the phase differences found are due to the spinal cord plasticity changes that only appear during phase two. Additionally, research attributes the lack of effect during the early phase to the actual substances involved in each phase. For example, substance p, a neuropeptide, has been shown to participate in the early phase, while the neurotransmitter, serotonin, participates in the late phase. Serotonin has been shown to regulate behaviors such as anger and aggression, but substance p is involved more directly with pain and nociception (Wikipedia.org), thereby leading to more efficient operation of antinociception.

As mentioned earlier, the research on which the design of this study was based observed the effects of nicotine on neurogenesis in rats. Our dosage, then, was not one that had been used

previously, and so the direction that neurogenesis and corresponding pain behaviors would take with administration of nicotine was unclear. Previous literature has revealed that administration of this drug can have debilitating effects on neurogenesis in large doses (Abrous et al., 2002), but enhancing effects in small doses (Dussor et al, 2004) in rats. Because our results indicated an increase in BrdU-labeled cells, we can conclude that our dosage of nicotine (5 mg/kg) is to be considered a small dose for mice. Again, it is important to realize that different rodent animal models have displayed various outcomes with regards to pain and neurogenesis. In addition, it should be reiterated that nicotine, only in large doses, is toxic, so the subjects of this condition were not receiving a harmful dosage of this alkaloid.

The interaction between phase, condition, and experimenter was an interesting finding because although experimenter biases are often a potential confound in experimental analyses, the bias seen in our results was consistent throughout the analyses. In other words, although the experimenters had made judgments using different criteria, these biases remained constant throughout both behavior analysis and cell counting analysis. Experimenter A reported generally more data than experimenter B, but there was no significant interaction with experimenter, so if any biases were present they were insignificant. In addition, subjects were evenly distributed to each experimenter, so both experimenters analyzed the same number of subjects in each condition and of each sex. This explains the experimenter differences seen, and also confirms the validity of our findings because both experimenters reported similar trends in the data.

Research on Alzheimer's disease medication indicates that cholinergic receptor agonists can be used as treatment in minimizing symptoms (Jin et al, 2004a), and because of the prevalence of chronic pain in Alzheimer's patients (Benedetti et al, 1999), as well as the analgesic effects seen in antidepressants, which can also be shown to treat neuropsychiatric

symptoms of dementia **(**Sink, Holden, & Yaffe, 2005**),** it is reasonable to expect that drugs effective in treating the degenerative aspect of Alzheimer's may also have analgesic effects. Carstens, Anderson, Simmons, Carstens, and Jinks (2001) demonstrate an analgesic property of nicotine with chronic application of the drug. However, in the present study, the formalin test was administered 24 hours after the last drug injection, so as to completely avoid the potential analgesic effects. Over the two-week period, the drugs were expected to only manipulate neurogenesis, but even if an analgesic effect had occurred, the 24 hour period between drug injection and the pain testing situation ensured that the drug would no longer be present in the system. Therefore, any significant effects found from data analysis are likely due to the drugs interacting directly with the processes of new neuron formation.

Past research has illustrated that females exhibit enhanced neurogenesis compared to males (Tanapat, Hastings, Reeves, & Gould, 1999), and evidence of sex hormones regulating neurogenesis in the hippocampus (McEwen, 2004) acts as further support of the existence of such sex differences. However, no such interaction between sex and neurogenesis was seen in our results. This might be due to our small sample sizes; for each condition, there were only five female and five male subjects within the neurogenesis segment of analysis. However, the more plausible reason for the lack of significance here is that this interaction appears to occur only when utilizing certain animal models. Tanapat et al, (1999), for example, reveal an effect of ovarian hormone levels on new neuron formation in the adult rat. In fact, ovariectomies led to decreased BrdU-labeled cell counts. However, Lagace, Fischer, and Eisch (2007) assert that such findings do not occur in mice. In observing the C57BL/6 strain in mice, no such sex differences were found, and ovariectomies did not influence production of neurons. Therefore, it can be

concluded that our findings are consistent with past research indicating the lack of effects of sex on neurogenesis in mice.

Our findings did, however, indicate a possible sex difference within the behavior analyses. During phase one, males and females demonstrated similar amounts of pain behaviors. However, the mean differences between sexes of phase two was much larger. This finding may indicate a gender difference for the late phase of the formalin test. General trends imply that females exhibited more pain behaviors in the late phase than males. This is consistent with previous research on gender differences seen in pain perception and inhibition, indicating females are more sensitive to pain (Holdcroft & Berkley, 2005) and therefore display more behaviors in response to noxious stimuli. Sternberg (1995) reveals sex-related differences in types of pain testing. Findings indicate female mice display fewer pain behaviors than male subjects in the formalin test, but males show similar behaviors when administered other tests, like the hot plate test. However, our data analysis suggested females exhibited greater differences in pain behaviors between phase one and two than males did. This disparity in findings exposes the dependence on the type of test (and the type of pain) the subjects are experiencing. Analyzing the existence of sex differences might reveal more information regarding the pain pathway in response to different types of pain, along with more knowledge about the physiological differences between males and females.

Another possible explanation for discrepancies in results is formalin concentration. Such sex differences are only seen with high concentrations of formalin (10%), and, in fact, with low concentrations (.1%) the opposite trend occurs: males show more responses to pain (Aloisi, Sacerdote, Albonetti, and Carli, 1995). It is possible that our concentration of formalin was significantly higher than that of Sternberg (1995) and so an opposite trend occurred.

Interestingly, Mogil, Chesler, Wilson, Juraska, and Sternberg (2000) noted that sex differences in hot plate tests are not regulated by the estrous cycle in females, which has served as a potential explanation for sex differences seen in neurogenesis. We can conclude, then, that in analyzing pain testing, drug concentration and effects of pain type reveal new insight to possible explanations of observed interactions, including sex differences.

Limitations and Possible Confounds. While our research methods were carefully designed and based on previous research of a similar form, there were some aspects of the design that were unavoidable and may or may not have affected our results. For instance, sample size if often a limitation of experimental analyses. Because of time constraints, we could only run pain behavior analysis on a maximum of 60 mice and perfuse 30. Therefore, our BrdU cell counting conditions only contained 5 males and 5 females each, making it difficult to form extensive generalizations about our results.

The use of mice as an animal model for neurogenesis should be thoroughly examined, as there are many things to consider that could prove to be problematic in later analysis. As mentioned earlier, sex differences in neurogenesis do not emerge at all in the mouse model, but in most other rodent models clear differences are seen. Additionally, sex differences of pain behaviors only arise when the research design allows for it (i.e. the type of pain being induced); if not then the results are often difficult to interpret. Therefore, researchers must thoroughly examine all options before deciding on an animal with which to base their experiment. Perhaps we would have seen different results if we used a rat or shrew animal model. Such species-dependent differences make it difficult to make generalizations about the findings because other trends found might also be species-dependent. Therefore, research involving the brain and response behaviors should be studied in a wide variety of animal models. In studying the mouse

brain in this experiment, we have thereby extended the literature to include an additional species. It should also be reiterated; results from animal models should not automatically be extended to human research. Much of human behavior and brain functioning cannot relate to animal models, but animal models are a useful starting point to understanding the mechanisms underlying human functioning.

One interesting observation made during the drug administration period was the display of torso contractions seen only in females of the nicotine condition. We considered the possibility of the dosage of nicotine as being an irritant, and thus this small portion of our subject pool was experiencing brief, transient pain daily. However, this was rare and an unavoidable confound. No research reviewed stated that nicotine would cause acute pain upon intraperitoneal injection. In fact, previous research protocol indicated intraperitoneal injection of nicotine to be a useful practice, and such findings of irritation were not reported. From this, we can presume that either this went unreported because it was believed to be of no consequence, or else supplementary research should be done on the matter. However, because females in the nicotine condition were not shown to display a significantly different amount of behaviors than those in the other two conditions, it is unlikely that this brief exposure to acute pain had any major effects at all, especially because our findings were consistent with past research which did not utilize nicotine.

In coding the video footage of pain behavior, both experimenters were assigned the same number of subjects from each condition and sex. However, the coding was not done blindly, and so experimenter biases, although not seen to be significant in our data, may have slightly skewed results. However, at the point in our research during which the video coding took place, our belief was that nicotine might have debilitating effects on neurogenesis. Because the trends of

our results indicate that nicotine, in fact, had augmenting effects on neurogenesis, the likelihood of experimenter bias affecting coding results is very slim. In addition, we coded five-second bins of time every 20 seconds for the first ten minutes and the last twenty minutes. While this is a commonly used methodology, recording data for every five-second interval might strengthen our findings. In addition, rather than record number of behaviors, we noted only whether or not the subject displayed a pain behavior during the five minute bin. The behavior itself (licking, lifting, or flinching) was not recorded. Past studies reveal that recording a time course of behaviors and ascribing weighted scores to each behavior have been useful techniques (Wheeler-Aceto & Cowan, 1991). Perhaps with a coding protocol utilizing all of these techniques, our data might have shown stronger correlations.

Although we followed the standard protocol for DAB staining, our background staining was much darker than we expected it to be. Even after doing an additional run and altering our methodology accordingly, the dark background staining added to the difficulties of locating BrdU-labeled cells. Because the background staining was almost the same shade of brown that the BrdU-marked cells were meant to be, it is likely that our cell counts were greatly underestimated. Although the trends seen in our results revealed significance and directions consistent with our hypotheses, perhaps with better staining techniques, our results would have yielded even greater significance and stronger trends.

Another likely confound of our study can be seen in the delicate nature of brain tissue. The slices cut were very delicate and easily and often split into pieces as it was being sliced, or sometimes the slices would fracture during the transportation from well to slide. In addition, after staining the slices, we found many of them to be cracked, which made it difficult to count the

cells because much overlap resulted from the fissures. However, it is not likely that the data was greatly affected by this limitation because, again, our results showed significant trends.

While BrdU labeling techniques have been utilized in many past experiments, and support for such procedures in signifying neurogenesis exists, Gould and Gross (2002) discuss possible problems with BrdU labeling. When BrdU labeling was first introduced, it was thought to be toxic and inaccurate at high levels. However, it was found that lower levels of BrdU may not be as effective in adult brains as in less developed brains due to differences in blood flow, blood-brain barrier development, and changes in metabolism. Therefore, it is possible that BrdU labeling at certain dosages is not accounting for every new neuron, nor is it accounting for neurogenesis in other areas of the brain. In addition, certain experimental manipulations, including the use of anesthetics, may also affect the degree of BrdU uptake. To solve this, higher dosage of BrdU is recommended, but this solution may not override the effects that some manipulations have on blood flow. To account for this, we administered a higher dosage of BrdU (300 mg/kg), which Gould & Gross (2002) have shown to be the most effective dose. High doses, however, were once considered to be toxic, and though it was shown to not apply to rats (Cameron & McKay, as cited in Gould & Gross, 2002), it is still possibly true for mice. Therefore, we administered a moderate dose so as to compensate for both of these possible confounding effects; doses of 600 mg/kg have been shown to yield fewer numbers of labeled cells than administering 300 mg/kg. In addition, Gould and Gross (2002) propose that, while new cells generated in adulthood are always being created, they are also always dying. Thus, they discourage lengthy survival after BrdU injection because it is possible that cells that were labeled have already died. Although the two week interlude we waited before brain extraction is doubtfully considered a “lengthy” period, the possibility of cell death prior to perfusion is a

likely, though unavoidable, confound, and thus it is possible that we underestimated the number of new neurons.

Future research. Our findings have extended the literature regarding a link between the pain pathways and neuron formation in the hippocampal dentate gyrus in mice. Our results yielded interesting trends regarding neurogenesis-enhancing drugs and subsequent increased pain behaviors. However, there is much still to be studied on this matter. Although the formalin testing situation is an established and widely accepted pain behavior assessment, it has been made clear that different types of pain yield various results. Therefore, administering a variety of pain tests that stimulate thermal, chemical, or mechanical nociceptors in conjunction with BrdU labeling procedures could present new information regarding pain and neurogenesis. Future research could determine whether the increased pain behaviors observed with neurogenesis increase are a result of the type of pain experienced. For example, an experiment could be designed in which similar neurogenesis-enhancing procedures were applied prior to tests of visceral pain, rather than inflammatory, tonic or phasic pain as demonstrated by the formalin test situation. If analogous trends were to transpire, one might conclude that changes occurring in the dentate gyrus have an overarching effect on the pain pathway, generalizing beyond affecting responses to inflammatory and tonic nociception as well as further supporting the findings of this study. In addition, to expand this study, drugs which inhibit neurogenesis, for example, higher, but not toxic, doses of nicotine, could serve as further evidence for the correlation between pain and neurogenesis.

In examining the formalin test specifically, future research could be done regarding whether or not the animal model being used yields differing results, especially because such variation is seen in neurogenesis research (Lagace et al, 2007). If brain plasticity varies among

species, it is likely that other factors, for example, bodily responses to stress, pain, or the drugs that influence such factors may also show variation across species. Research on neurogenesis, though it has seen much advancement in a relatively short period, is, in itself, in need of study expansion and extension. Because the role of the new neurons formed in adult neurogenesis is still unknown, it would be beneficial for future research to have the aims of determining their functions. It has been proposed that the function depends on the location of the new cells, so new neurons in the dentate gyrus would operate with other cells in the hippocampus as it regulates stress, learning, memory, or other functions. In fact, studies have shown that learning alters the amount of new hippocampal neurons by way of influencing cell proliferation and survival (Gould et al, 1999b). Gould and Gross (2002) cite studies that reveal an effect of exercise and enriched environments on both neurogenesis in the hippocampus and on the execution of a hippocampus-dependent task. This coincides with evidence reporting that in executing such tasks, neuron formation in adult rats increases compared to rats that do not perform hippocampus-dependent tasks (Gould et al, 1999). Conversely, reducing the number of new neurons in the dentate gyrus has been correlated to poor performance on a task dependent on the hippocampus. Together, these findings act as evidence of possible functional roles of the newly-developed hippocampal neurons in learning.

The dentate gyrus is not only involved in learning but also in the formation of new memories. Research has noted the deleterious effects of ACh lesions on spatial memory (Mohapel et al, 2005) as well as the involvement of mature neurons to spatial learning (Gould et al., 1999b). Future research could devote attention to the involvement of cholinergic receptors in pain perception and possibly antidepressant therapy. Acting as further evidence of a link between pain and neurogenesis, McEwen (2001) notes that injecting an anesthetic into the dentate gyrus

leads to pain relief. Upcoming research on pain and neurogenesis should focus on the dentate gyrus' function in the pain pathway as well as learning and memory. Perhaps the dentate gyrus is activated during the pain response. Future studies could establish whether or not granule cells have a functional role within the pain pathway. Such research would be devoted to discovering the exact function along with the underlying mechanisms within the dentate gyrus during the processes of nociception.

It is interesting to note the similarities between pain and stress, and learning that both of these factors having analogous effects on hippocampal plasticity and functioning (Duric & McCarson, 2006). While a possible explanation for the overlap of the two response mechanisms could be that pain is stressful, there seems to be a more intricate system at play. As mentioned previously, the function of these neurons can be dependent on their location. The dentate gyrus' role in stress responses indicates a possible function of these neurons in this system. Furthermore, glutamate binding to NMDA receptors is released during stressful situations and both stress and glutamate activation has been shown to decrease neurogenesis. Because a reduction in stress might also be involved in the brain-reparation system, it is reasonable, then, to propose that anti-stress drugs be considered in future research as possible treatments for Alzheimer's disease. Interestingly, Jin et al. (2004a) discuss the possibility of Alzheimer's treatment in NMDA antagonists because of the increased neurogenesis reported with NMDA receptor blockage, a point discussed earlier with regards to the article by Gould et al. (1997). Such findings can facilitate in expanding the literature on Alzheimer's treatments. Furthermore, Alzheimer's patients have been shown to report less pain (Rainero et al, 2000). Although elderly populations often report less pain than younger populations in clinical situations, (Harkins & Price, 1992), it would be beneficial to the study of pain to discover whether this change in pain is

due to the deteriorating effects on the nervous system seen in dementia and with increased age, or perhaps the medications used to treat disorders such as Alzheimer's are exhibiting analgesic effects. Should the former be established as an accurate underlying mechanism of pain relief in Alzheimer's patients, our hypothesis would be further supported in that a direct correlation would be demonstrated between neurogenesis decline and pain desensitization.

Stress (Gould et al, 1997) and pain (Duric & McCarson, 2006) have been shown to have negative effects on neurogenesis and neuron growth. In addition, research indicates that antidepressants relieve both pain (Iyengar et al, 2002) and stress that accompanies depression, in addition to enhance neurogenesis (Delgado, 2004). In addition, adrenal steroids, which are directly involved in the stress response and hippocampal plasticity, are also known to participate in the regulation of neurogenesis (McEwen, 2001). Further connections of these phenomena can be seen in enriched environment studies, where subjects raised in enriched conditions have exhibited a reduction in pain perception (Smith et al, 2005), memory and learning deficits caused by stress (Wright & Conrad, 2007), and enhanced dendritic growth (Leggio et al, 2005). Therefore, it is reasonable to assume that the effects of depression also influence pain, stress, and neurogenesis. The neurogenesis hypothesis of depression articulates this connection. Scientists are beginning to consider that the basis for major depressive disorder is rooted in a failure of adult neurogenesis. Two findings that support this theory are the change and loss in hippocampus structure and the neuronal cell death seen in depressed patients, along with the well-studied impact of antidepressants on neurogenesis and as Alzheimer's medication (Kempermann & Kronenberg, 2003). The compounds used in our study, nicotine and galantamine, are known AChE inhibitors, though their effects as analgesics or antidepressants have not been clearly established. While it is clear that these drugs encourage cell proliferation, future research could

address their possible role in preventing cell death, and related antidepressant, anti-stress, or analgesic effects.

Conclusion

In conclusion, our results serve as further evidence of a relationship between pain and neurogenesis, specifically the potential influence of drug-induced neurogenesis on subsequent tonic-pain behaviors. While there are minor limitations present in our study design, we believe that our results, significant and nonsignificant alike, are essential to the expansion of neurogenesis and pain research. Also of importance is the research regarding other possible correlating factors and, then, how the neural pathways interrelate. Research of this approach can facilitate the advancement of developing treatments for neurodegenerative diseases such as Alzheimer's by discovering how to stimulate neurogenesis or prevent the slowing of it, as well as how it is impacted by pain, stress, and depression. It has also been made clear that animal models employed for pain and neurogenesis research must be acutely examined prior to completion of experimentation because of varied effects found in different species and genetic strains. Ideally, research would reveal the most primate-like rodent brain with which to conduct experiments so the findings could be evaluated with respect to the human brain. The results found in this experiment act as evidence supporting past research which indicate a correlation between neurogenesis and pain sensation, as well as reveal new insight to directions of future research linking the function of the new neurons formed to pain and stress response mechanisms.

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Table 1

Condition A	Condition B	Mean Difference (A - B)	Std. Error	Significance
Galantamine	Saline	8.94*	3.87	.03
	Nicotine	1.51	3.84	.70
Nicotine	Saline	7.43	4.03	.07
	Galantamine	-1.51	3.84	.70
Saline	Nicotine	-7.43	4.03	.07
	Galantamine	-8.94*	3.87	.03

Table 1: This table displays the mean differences between the averaged behaviors found in each condition during the late phase of the formalin test.

Table 2

Sex A	Sex B	Mean Difference (A-B) in Phase 1	Std. Error	Sig.	Mean Difference (A-B) in Phase 2	Std. Error	Sig.
Female	Male	1.66	1.47	.26	7.46*	3.20	.03
Male	Female	-1.66	1.47	.26	-7.46*	3.20	.03

Table 2: This table displays the mean differences between both sexes found in each phase during the formalin test.

Table 3

Experimenter	Phase	Mean	Std. Error
A	1	23.23	1.09
	2	40.92	2.28
B	1	17.34	1.07
	2	28.30	2.25

Table 3: This table displays the averaged behaviors reported by each experimenter across phases during the formalin test.

Table 4

Condition	Sex	Phase	Mean	Std. Error
Saline	Female	1	22.25	1.77
		2	38.25	3.71
	Male	1	20.95	2.09
		2	20.50	4.39
Nicotine	Female	1	18.58	2.04
		2	38.67	4.28
	Male	1	19.50	1.77
		2	34.50	3.71
Galantamine	Female	1	22.40	1.58
		2	38.10	3.32
	Male	1	18.04	1.91
		2	38.08	4.00

Table 4: This table displays the averaged behaviors observed in each condition across sex and phase of the formalin test.

Table 5

Condition	Experimenter	Phase	Mean	Std. Error
Saline	A	1	25.88	2.16
		2	33.00	4.54
	B	1	17.33	1.68
		2	25.30	3.52
Nicotine	A	1	21.96	1.61
		2	41.92	3.38
	B	1	16.13	2.16
		2	31.25	4.54
Galantamine	A	1	21.87	1.83
		2	47.83	3.83
	B	1	18.58	1.68
		2	28.35	3.52

Table 5: This table displays the averaged behaviors reported by each experimenter across each phase of the formalin test.

Figure 1

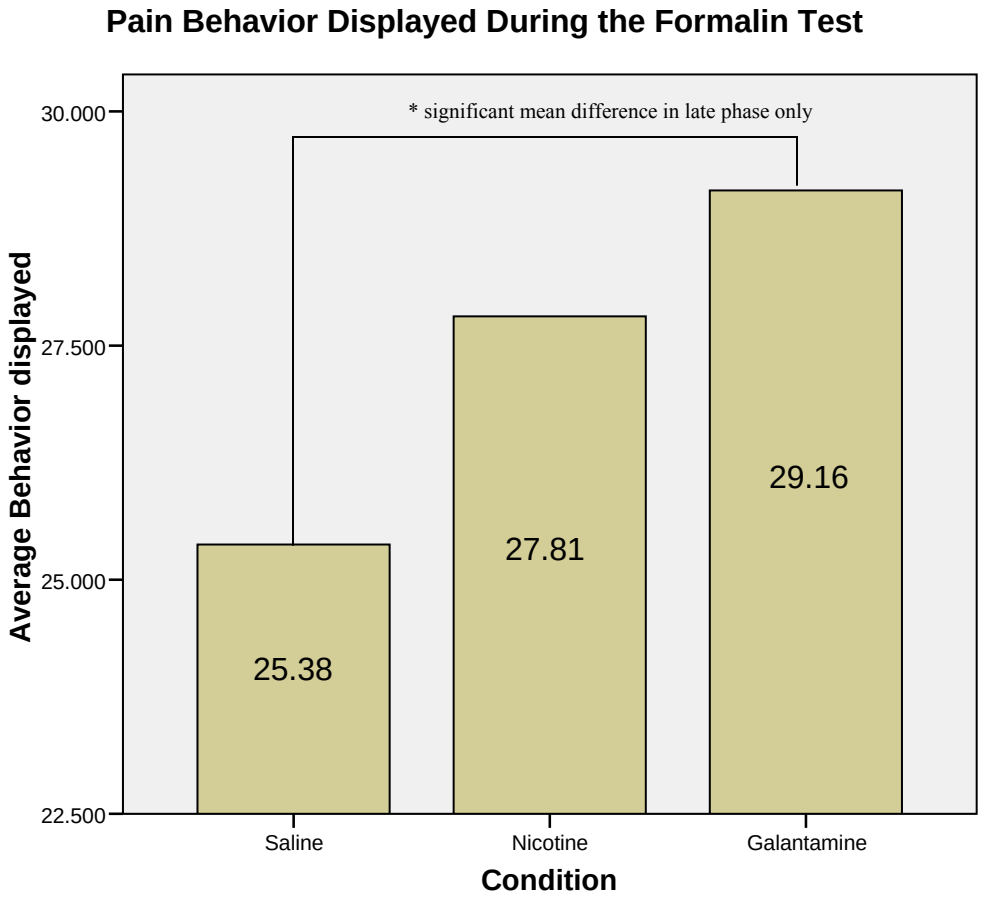

Figure 1: This graph shows the average amount of pain behaviors of the subjects during the formalin test. Although the overall differences between conditions were not significant, it is interesting to note the obvious trend being seen in this data. The trend suggests that the control subjects showed the least effect on pain behavior, nicotine subjects were slightly more sensitive to pain, and galantamine subjects became the most perceptive to pain sensations.

Figure 2

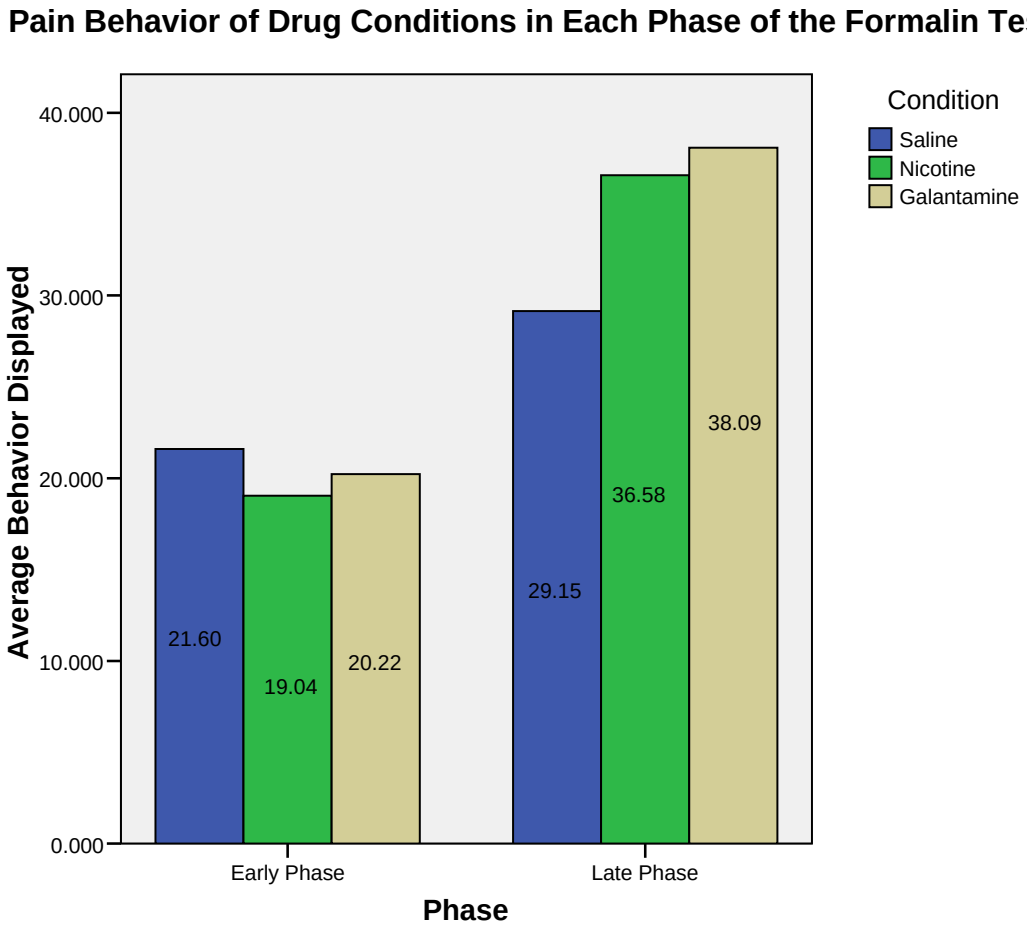

Figure 2: This graph shows the average number of pain behaviors of the mice in both phase one and phase two, in each of the three drug conditions. The late phase pain behavior resulted in a trend revealing the control condition as displaying the least amount of pain, significantly different from the galantamine condition, in which subjects displayed the most pain behaviors.

Figure 3

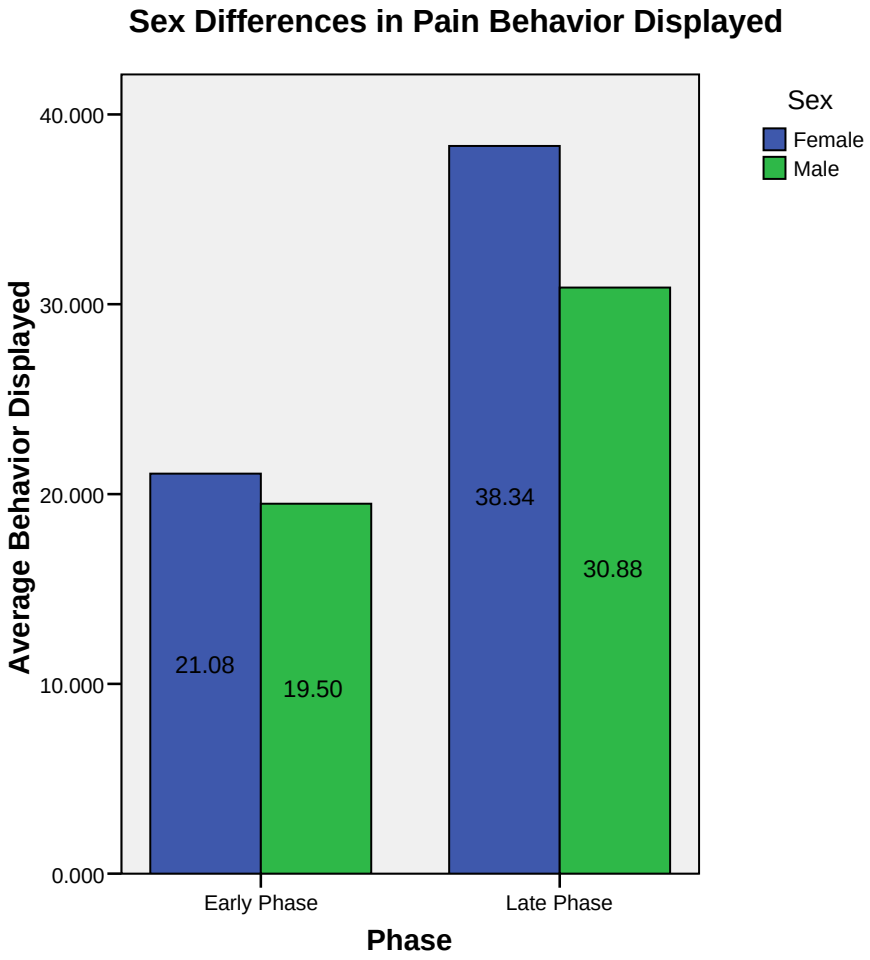

Figure 3: This graph reveals the sex-related differences in pain behavior displayed during the late phase of the formalin test. The female mice displayed more behaviors than the male mice, indicating a possible sex difference in pain perception during formalin testing.

Figure 4

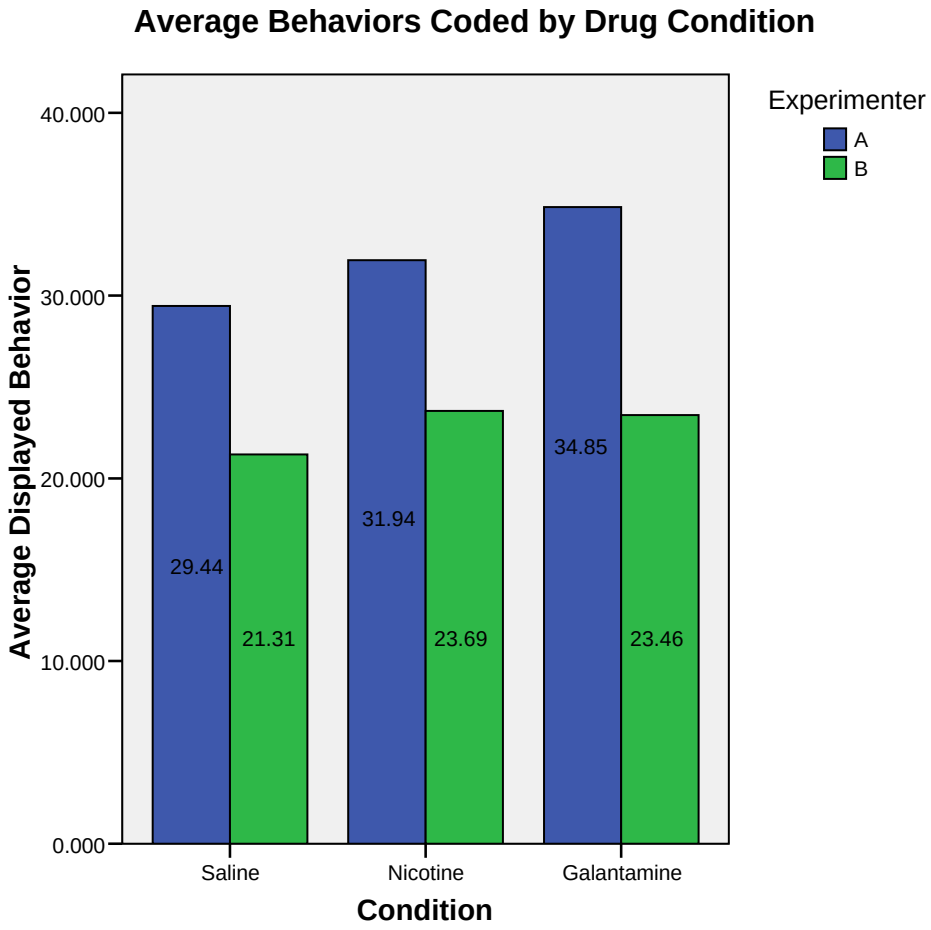

Figure 4: This graph reveals the general trend of the behavioral data video coding performed by each experimenter. Experimenter B coded using a more stringent protocol, but the resulting data was not affected by this difference in criteria because both experimenters coded across sex, phase and condition.

Figure 5

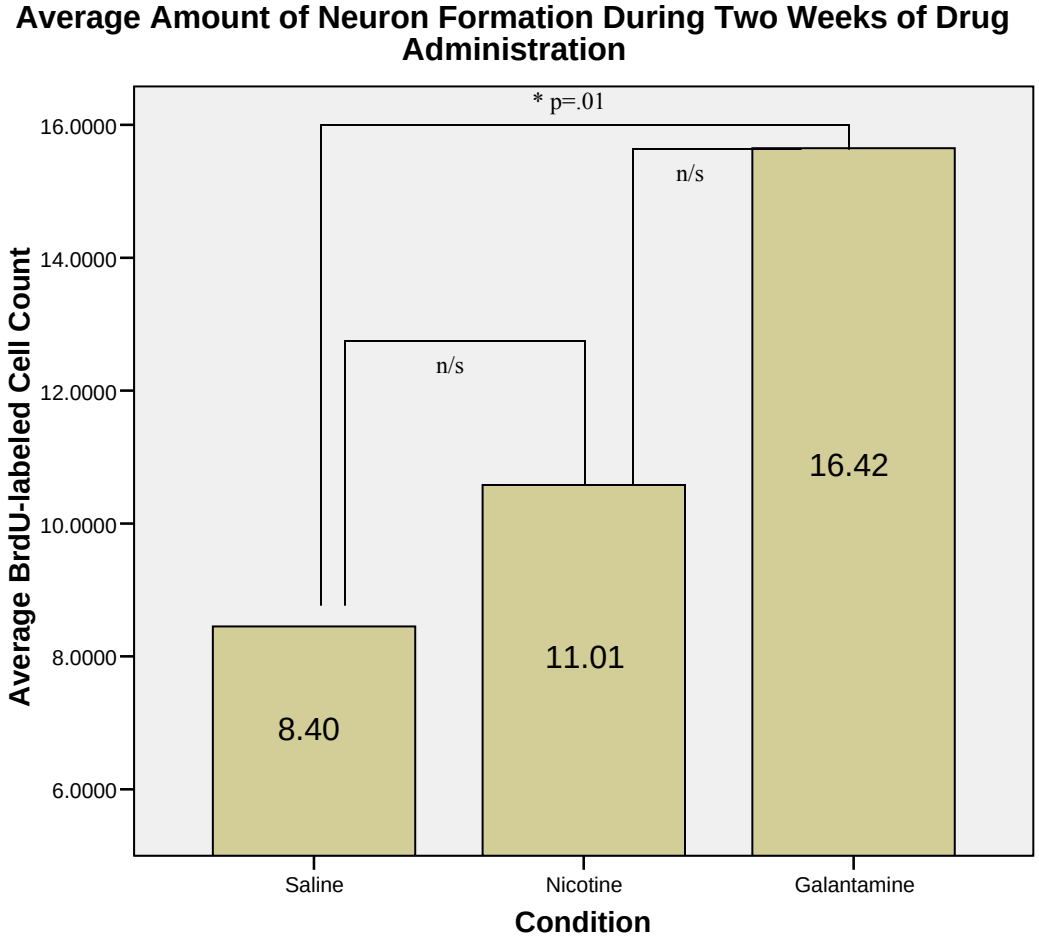

Figure 5: This graph reveals the results of the BrdU counting after two weeks of drug administration of the three conditions. Overall, a trend has appeared in which galantamine subjects exhibited significantly more BrdU-labeled cells than the control, and subjects in the nicotine condition exhibited an average of fewer cells than galantamine but more cells than the control.