DIFFERENTIAL EFFECTS OF SEX, STRAIN AND BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) ON RAT HIPPOCAMPUS FOLLOWING INDUCTION OF AUTOIMMUNE SYMPATHETIC DYSFUNCTION

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Differential Effects of Sex, Strain and Brain-Derived Neurotrophic Factor (BDNF) on Rat Hippocampus Following Induction of Autoimmune Sympathetic Dysfunction

Abstract

Autoimmune diseases occur from dysregulation of immune responses against self-antigens mediated by B-cells, T-cells or autoantibodies, and resulting in the body attacking its own cells and tissues. These diseases are observed in approximately 8% of the general population, affecting women and minorities disproportionally. It has been reported that 78% of those affected are women (Fairweather and Rose, 2004). Patients suffering from diseases such as rheumatoid arthritis, scleroderma, lupus, and multiple sclerosis often experience inflammation as a result of tissue deposits of autoantibodies (Tsokos, 2001). It has been reported that activated immune effectors are responsible for the vascular damage associated with inflammation via direct endothelial damage or the induction of proinflammatory cytokine release (Boin and Rosen, 2007). Proinflammatory cytokines enhance the activity of B-Lymphocytes which are responsible for the production of the autoantibodies that result in tissue activation when they bind to form immune complexes and then activate complement T cells that participate in the immune complexes responsible for the induction of immune responses. Some T-cell populations express the neurotrophin brain-derived neurotrophic factor (BDNF) (Muhallab et al., 2001). Previous experiments from our laboratory have demonstrated that hippocampal infusion of BDNF after induction of sympathetic autoimmunity via injection of complete Freund's adjuvant (CFA)-emulsified superior cervical ganglion (SCG) homogenates into hind paw results in inflammation (Kasselman et al., 2006). Supportive of the role of BDNF in inflammation are the findings that serum BDNF levels are decreased in patients suffering from rheumatoid arthritis after treatment with anti-inflammatory cytokines, as well as increased levels of BDNF during the acute and relapsing phase of multiple sclerosis (Caggiula et al., 2005). Lastly, consistent with the observed sex disproportionality in autoimmune

diseases, it has been reported that men show more variation in BDNF serum levels throughout a day, and that at points women express significantly higher levels of BDNF than men (Piccinni et al., 2008). In line with those findings, estrogen deprivation in female rodents decreases BDNF levels from baseline (Berchtold et al., 2001) and estrogen replacement reverses those effects (Allen and McCarson, 2004). Here we report the finding that hippocampal BDNF infusion after induced sympathetic dysfunction results in significantly greater inflammation in female than in male rats. These findings were accompanied by a tendency for female rats to show more cuffing of blood vessels with immune cells than males, as well as a tendency for the autoimmune-prone rat strain, Lewis rats, to be more affected than Sprague Dawleys. *Post hoc* observations revealed an unexpected neurodegenerative pattern following inflammation which only occurred in female rats, as well as a tendency for these effects to be exacerbated in the Lewis strain.

Introduction

Immunity is the natural process by which an organism's body defends itself against the presence of foreign bodies, such as bacteria and viruses, which may instigate detrimental effects for the organism. The process by which the organism's body protects itself is hence called an immune response. Immune responses to a wide variety of different foreign bodies typically involve the same elements, including an antigen, interacting populations of immune cells including T lymphocytes and B lymphocytes, messenger molecules such as cytokines, and complex formations at cell surfaces which initiate the signaling process, resulting in the production of antibodies (Mackay, 2000).

The immune system can become pathogenic when the body turns on itself and the immune system begins to recognize its own tissues as foreign, a process termed autoimmunity. In these special cases, an organism's immune system recognizes its own tissue as an antigen (autoantigen). This recognition process triggers the activation of B lymphocytes by T lymphocytes, just as occurs in immune responses to foreign antigens. Activated cytokines facilitate the production autoantibodies via complex formation on the cell's surface. Secreted autoantibodies can then collect in local tissues, thereby initiating an inflammatory response (Tsokos, 2001). These inflammatory processes result in local tissue damage, and can eventually lead to organ failure. The fact that inflammatory processes are often instigated by autoimmunity is not surprising as inflammation is one of the most common characteristics among the seventy clinically recognized autoimmune diseases such as rheumatoid arthritis (RA), multiple sclerosis (MS) and systemic lupus erythematosus (SLE). Collectively, autoimmune diseases such as the ones mentioned above affect about 5-8% of the general population in America alone, disproportionally affecting women and minorities more (National Institute of Health Report, 2000). Indeed, about 78% of those affected are women (Fairweather and Rose, 2004). Although much is known about the effects of autoimmune diseases in an organism's body, the precise intracellular processes

mediating these effects are yet to be elucidated. It's clear, however, that autoimmunity is driven, at least in part, by processes which vary between ethnicities, and which are differentially regulated by gender.

It is currently speculated that one of the possible cellular mechanisms involved in triggering autoimmunity occurs via the production of proinflammatory proteins such as cytokines. More specifically, abnormal T-lymphocyte activation by autoantigens is recognized to induce the production of cytokines such as interleukin-2 (IL-2) which in turn, via clonal expansion, induce the production of other proinflammatory cytokines including interferons (INFs) and tumor-necrosis factors (TNF) (O'shea et al., 2002). Evidence for the involvement of cytokines in inflammatory processes is provided by the observations that INF and TNF protein levels are expressed at significantly higher levels in MS patients during the relapse phase (when inflammation is a major characteristic) than for other stages of the disorder and for control groups (Caggiula et al., 2005). Further, it has been demonstrated that treatment with TNF blockers significantly improves disease activity and inflammation in patients suffering from RA (Del Porto el al., 2006). The findings that neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) modulate immune cell function (Schulte-Herbrüggen et al., 2007) have been of much interest to our laboratory. Specifically, reports that BDNF is produced by activated T cells, B cell and monocytes suggest the potential for immunomodulatory effects of these factors (Hohlfeld, 2004). Additionally, it has been reported that neurotrophic factors activate microglial cells, the nervous system's intrinsic immune cells (Mizoguchi et al., 2005) as well as eosinophils (Noga et al., 2002). Further, there is evidence that some immune cells express the receptors for neurotrophins, which provides evidence for the interaction between immune cells and the neurotrophin family of neurotrophic factors (Muhallab et al., 2001). In line with the findings that the neurotrophin BDNF stimulates the activation of immune cells, previous findings from our laboratory report that hippocampal inflammation results from direct infusion of BDNF after induced sympathetic dysfunction

(Kasselman et al., 2006). Taken together, the available literature seems to provide a link between neurotrophins, immune cells and inflammatory processes.

Kasselman et al. (2006) provided a link between BDNF and inflammatory processes similar to those observed in autoimmune diseases. In support of the involvement of BDNF in these processes are observations that BDNF is differentially expressed between females and males. Piccinni et al. (2008) reported that during the course of diurnal variation of plasma BDNF, there are periods of time at which females expressed significantly greater BDNF concentrations than men. This differential in BDNF expression appears to be related to hormonal differences between males and females. That is, estrogen deprivation via ovariectomy significantly decreased baseline levels of BDNF in the rat hippocampus, an effect that was reverted after estrogen replacement (Berchtold et al., 2001; Allen and McCarson, 2005). Likewise, it has been reported that both male and female patients suffering from autoimmune diseases have lower testosterone levels than controls, suggesting that testosterone may play a protective role in autoimmunity thereby favoring protection of males over females (Dane and Timur, 2005). In sum, it appears that the differential expression of BDNF between males and females is largely driven by sex hormones which protect males and predispose females for autoimmune reactivity.

Evidence from human clinical populations offers support for the involvement of neurotrophins in autoimmunity and related inflammation. However; this evidence is only correlational which prevents a causal inference. To achieve causal relationships, experimental animal models of autoimmunity are necessary and some advances have been made over the last decades. For example, one of the most widely studied models of autoimmunity is the experimental autoimmune encephalomyelitis (EAE) model of MS. In the EAE model, autoimmunity is induced by the intradermal transfer of myelin-basic-protein-reactive CD4+ T-cells mixed with complete Freund’s adjuvant (CFA) into a naïve animal. The result is a monophasic paralytic disease (Muhallab et al., 2002). In an attempt to develop a model for sympathetic

autoimmunity, our laboratory has developed a protocol whereby compensatory sympathetic hyperactivity, similar to what is observed in autoimmune diseases such as lupus and scleroderma, is induced in rodents. This sympathetic autoimmune model consists of surgically removing the superior cervical ganglia (SCG) from donor animals, homogenizing the SCG's in phosphate buffer saline (PBS) solution, emulsifying it with complete Freund's adjuvant (CFA) and lastly injecting it subcutaneously into the hind paws of naïve animals. In this experiment, we used the SCG-CFA model of sympathetic hyperactivity autoimmunity induction to assess the differential effects of BDNF-induced inflammation on rats as a function of sex and strain. Further, we sought to determine whether or not the BDNF protein needs to be biologically active or if the mere presence of the inactive recombinant human protein would trigger inflammation. First, we hypothesized that female rats would be more vulnerable to the brain inflammation caused by the sympathetic autoimmunity induction. Second, we hypothesized that inflammation would be more pronounced in Lewis rats, which have been shown to be more prone to autoimmune induction. Third, we predicted that only animals receiving the biologically active BDNF protein would develop an autoimmune response resulting in brain inflammation.

Methods

Animals. A total of ninety-six animals were used for this experiment. Forty-eight served as superior cervical ganglion donors and the rest were experimental animals. Of the forty-eight experimental rats, half were of the Lewis strain and the other half were Sprague Dawleys. Within each strain, there were an equal number of male and female rats. Ten animals did not survive until the end of the experiment (two male and five female Sprague Dawleys and three female Lewis), resulting in reduced animal numbers in some groups. Deaths occurred at different points in the experiment, but none before BDNF treatment was implemented. Animals were housed in groups of four on a 12:12 hour light:dark cycle (lights on 07:00) and provided food and water *ad libitum*. All procedures were

conducted with the approval of, and in compliance with, the animal welfare policies of the Institutional Animal Care and Use Committee of Queens College of the City University of New York.

Superior Cervical Ganglion Removal. Donor animals were overdosed with chloral hydrate-pentobarbital (2:1 pentobarbital to weight ratio). The incision area was cleaned with alcohol and hydrogen peroxide. We then made an incision across the neck exposing the muscles and thyroid gland. We separated the thyroid gland from underlying tissues and lifted it to allow access to the target ganglion. We next dissected down to the left superior cervical ganglion, isolated it from surrounding tissue and blood vessels, and used micro-scissors to remove it completely. This same procedure was repeated for the right superior cervical ganglion. Hence, superior cervical ganglia were collected bilaterally from each donor animal. All the collected ganglia were placed into holding vials and stored in a -80°C freezer awaiting emulsion preparation.

Cannulae Surgeries. We anesthetized adult Lewis and Sprague–Dawley rats (275–375 g) using sodium pentobarbital (75mg/kg). Animals were then shaved and treated with povidone–iodine solution. Incisions were made in the scalp and, after placing three anchor screws into the skull, a 4 mm indwelling cannula (Plastics One, Roanoke, Virginia) was placed - 2.6 mm ML and - 3.7 mm AP from bregma (Paxinos and Watson, 1986) into the left hippocampus. The cannulae were attached to heat-sealed polyvinyl catheters filled with sterile phosphate-buffered saline (PBS). We then attached the cannula to the skull using dental acrylic to secure the cannula to the anchor screws, sutured the incision with 3-0 nylon suture (Henry Schein, Melville, NY), and applied topical antibiotic cream. We recovered the rats under heat lamps until fully awake.

Pump Surgeries. 7 days after placing cannulae into the hippocampus, animals were re-anesthetized with 2.5% isoflurane. We placed an incision across the nape of the neck and withdrew the tubing attached to the cannulae. We next attached a 14 day, 0.5 µL/h osmotic mini-pump (Alza

Corporation, Mountain View, California) to the catheter end. Rats received one of the following infusions: Active BDNF (12μg/12μl day), Inactive BDNF (12μg/12μl day) or Phosphate Buffered Saline solution (12μl/day). We then placed the pump subcutaneously along the back, closed the incision with 3-0 nylon suture, and applied topical antimicrobial cream to the wound. Animals were then placed into resting cages and recovered under a heat lamp until fully awake.

Superior Cervical Ganglion-Complete Freund's adjuvant Emulsion. A total of forty-eight ganglia pairs were used for this emulsion. The ganglia were placed into a glass vial and mixed with 1mL of sterile phosphate-buffered saline (PBS). This mixture was then homogenized for a period of two minutes using a laboratory homogenizer. Using a glass Popper needle, the mixture from above was added to 1ml of complete Freund's adjuvant solution. A continuous back and forth motion with the Popper needles was performed until the emulsion solution was obtained. This emulsion solution was made on the same day when the hind paw injections took place.

Hind Paw Injections. All fifty-four experimental animals experienced a hind paw injection. For this procedure, animals were anesthetized via inhalation using isoflurane. The animal's left paw was cleaned with alcohol and hydrogen peroxide and injected using a 1cc syringe and a 25 gauge needle. Animals received one of the following injections: superior cervical ganglia-complete Freund's adjuvant mixture or complete Freund's adjuvant alone. Animals were then placed into the resting cages and allowed to recover under a heat lamp until awake.

Animal Sacrifices. Animals were deeply anesthetized and overdosed with Euthasol (Henry Schein, Inc., Melville, NY), transcardially exsanguinated with heparinized isotonic (0.9%) saline, and perfusion fixed with 4% paraformaldehyde, first in low pH buffer (acetate, 200 ml) and then in high pH buffer (borate, 200 ml). After fixation, brains were removed and placed in 30% sucrose in borate buffer for three to seven days at 4 °C.

Tissue Processing. Following fixation and immersion in 30% sucrose in borate buffer, rat brains were frozen and sectioned at 40 μm on the coronal plane using a sliding microtome. Frozen sections were stored at –20 °C in cryoprotectant solution (Watson et al., 1986) until mounted on gelatin-coated slides and stained with cresyl violet for evaluation of the hippocampus and surrounding structures.

Tissue Analysis. Brain sections were analyzed using a Nikon microscope (Morell Instruments, Melville, NY) by an experimenter blind to experimental conditions. The presence or absence of blood vessel cuffing was determined based on the whether or not immune cells surrounded the vascular walls (See figure 1), where immune cells were identified based on morphological characteristics. Similarly, hippocampal inflammation was assessed by the presence of immune cells within the hippocampal parenchyma, again differentiating immune cells based on morphological features. Lastly, assessment of hippocampal neurodegeneration was based on the density of pyramidal cells in Ammon's horn. The severity coding for each measure was determined on a 4 point scale (0=none, 1=mild, 2=moderate and 3=extreme) based on the descriptive language of the original blind tissue assessment.

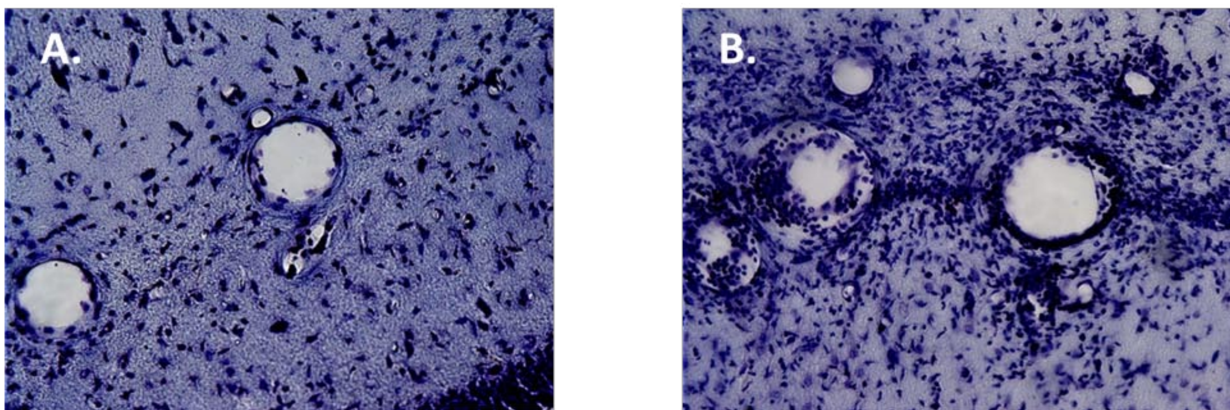

Figure 1. Uncuffed blood vessel in the cresyl violet-stained hippocampal fissure (A) and presence of immune cells surrounding vasculature from the same region (cuffed) (B).

Statistical Analysis. Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS) software version 11.5 (SPSS Inc., Chicago, Illinois). Chi-Square analyses were

computed to investigate whether distributions of categorical variables differed among groups (scores for inflammation, vascular cuffing, and neurodegeneration). A $p < .05$ was considered statistically significant for all analyses.

Figures. Figures 1 and 14 depict representative images of vascular cuffing and group hippocampal tissue respectively. Figures 2-13 are presented as percent frequency distributions of observed effects.

Results

A priori comparisons of subjective evaluations of hippocampal tissue for the intensity of blood vessel cuffing, inflammation and post hoc analysis of neurodegeneration were computed using a 3X3 Chi Square analysis. The three independent measures included in these analyses were the animal's strain, sex and treatment.

Vascular Cuffing. A Chi Square analysis of observed frequency distributions revealed a significant difference between sexes in the frequency of observed blood vessel cuffing, (x^2 (1, N = 38) = 3.87, p < .049). Female rats had significantly more instances of blood vessel cuffing than male rats. Figure 2 shows the observed percent frequencies of blood vessel cuffing for female and male rats for each strain. Statistical analysis for vascular cuffing severity revealed no significant difference between sexes in the intensity of observed blood vessel cuffing, (x^2 (3, N = 38) = 4.18, $p < .243$), suggesting that sex was more likely to drive the occurrence of cuffing than its severity. Figure 3 shows the frequencies of blood vessel cuffing intensity for female and male rats.

No significant difference in blood vessel cuffing distribution (x^2 (1, N = 38) = .106, $p < .744$) or intensity (x^2 (3, N = 38) = 2.42, $p < .491$) was observed between the two animal strains. Figure 4 shows the percent frequency distribution of blood vessel cuffing severity for each strain.

No significant differences in tissue damage occurrence (x^2 (1, N = 38) = 5.99, $p < .113$) or intensity (x^2 (9, N = 38) = 15.78, $p < .072$) was observed between the different treatment groups. However, there was a tendency for both active and inactive BDNF infused groups to express greater and more intense blood vessel cuffing. Figure 5 shows blood vessel cuffing severity for each treatment group.

Figure 2. Percent frequencies of blood vessel cuffing for Sprague Dawley male and female rats (A) and Lewis male and female rats (B) for each treatment group.

Figure 3. Percent frequency of blood vessel cuffing severity for male and female rats.

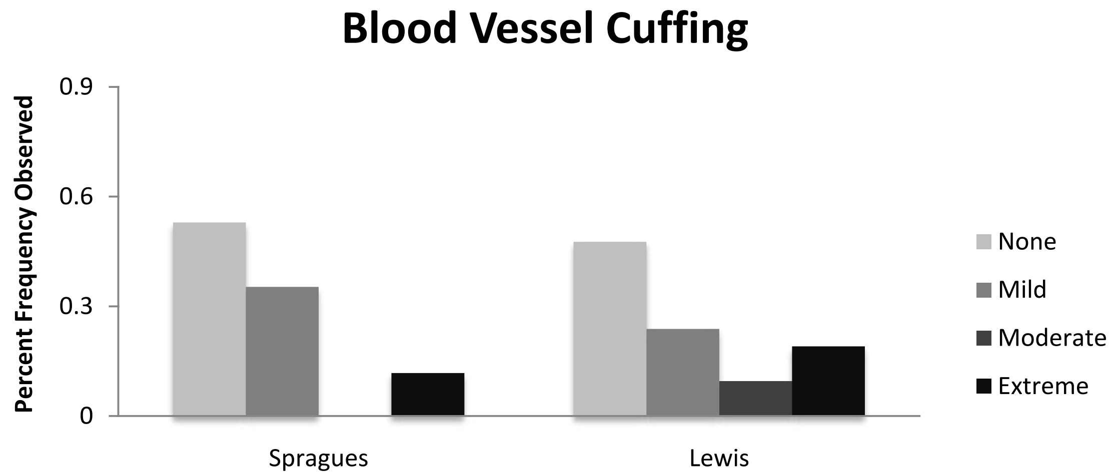

Figure 4. Percent frequency of blood vessel cuffing severity for each animal strain.

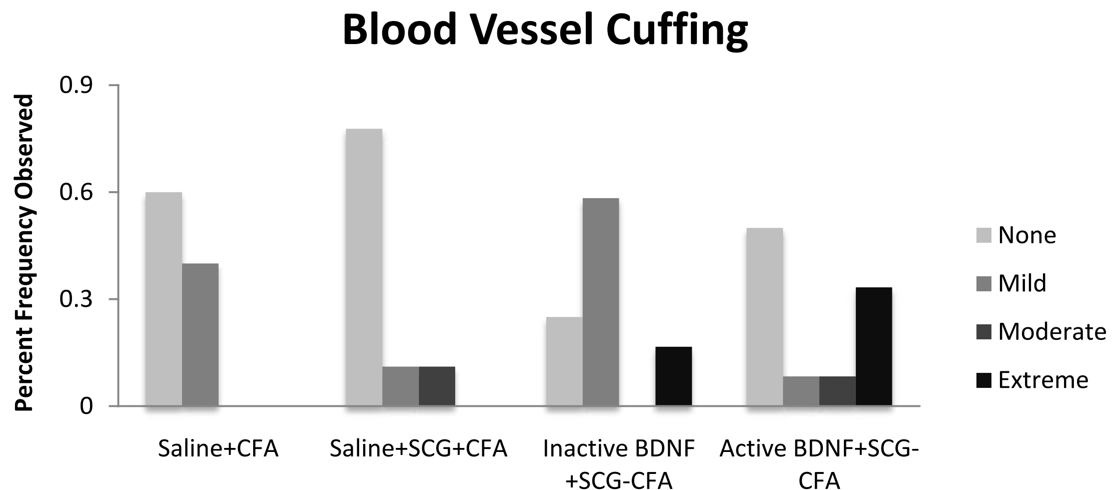

Figure 5. Observed percent frequency of blood vessel cuffing severity for each treatment group.

Hippocampal Inflammation. A Chi square analysis of observed distribution of frequencies revealed that tissue inflammation did not differ significantly between female and male rats (x^2 (1, N = 38) = 2.54, p < .11). However, inflammation intensity was significantly different between female and male rats (x^2 (3, N = 38) =8.87, p < .031). Female as compared to male rats experienced more intense

instances of hippocampal inflammation. Figures 6 and 7 show the percent frequency distributions of hippocampal inflammation and severity of the inflammation respectively for each sex.

Instances of hippocampal inflammation (x^2 (1, *N* = 38) =.001, $p < .973$) and severity (x^2 (9, *N* = 38) =8.92, $p < .444$) did not differ across strains. Figure 8 shows the percent frequency of inflammation severity for each strain.

Percent frequency of hippocampal inflammation (x^2 (1, *N* = 38) = 0.88, $p < .831$) and severity (x^2 (9, *N* = 38) =8.92, $p < .444$) did not differ across treatment groups. Figure 9 shows the percent frequency of inflammation severity for each treatment group.

Figure 6. Percent frequency distributions of hippocampal inflammation for male and female Sprague Dawley rats (A) and Lewis rats (B).

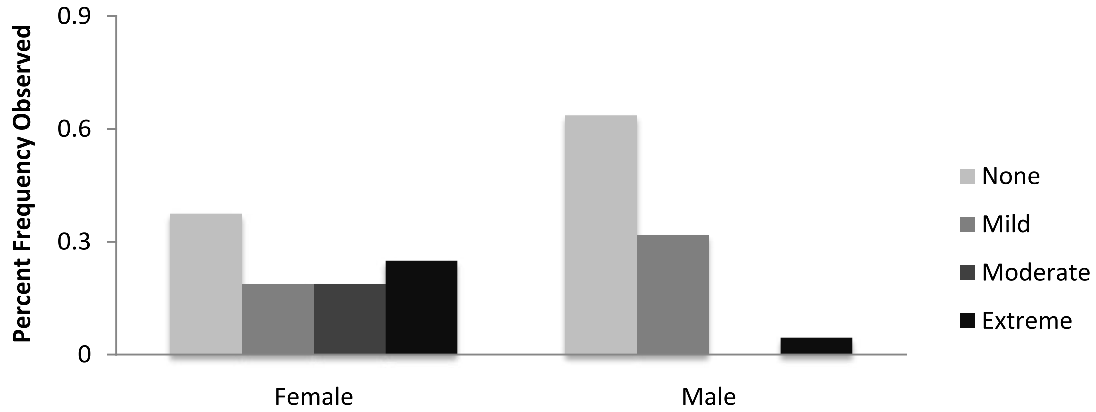

Figure 7. Percent frequency distribution of hippocampal inflammation severity for male and female rats.

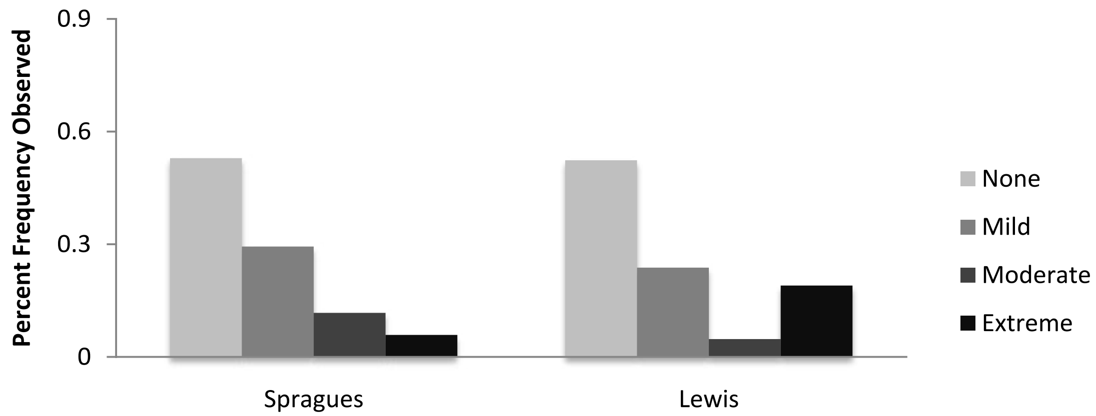

Figure 8. Percent frequency distribution of hippocampal inflammation severity for each rat strain.

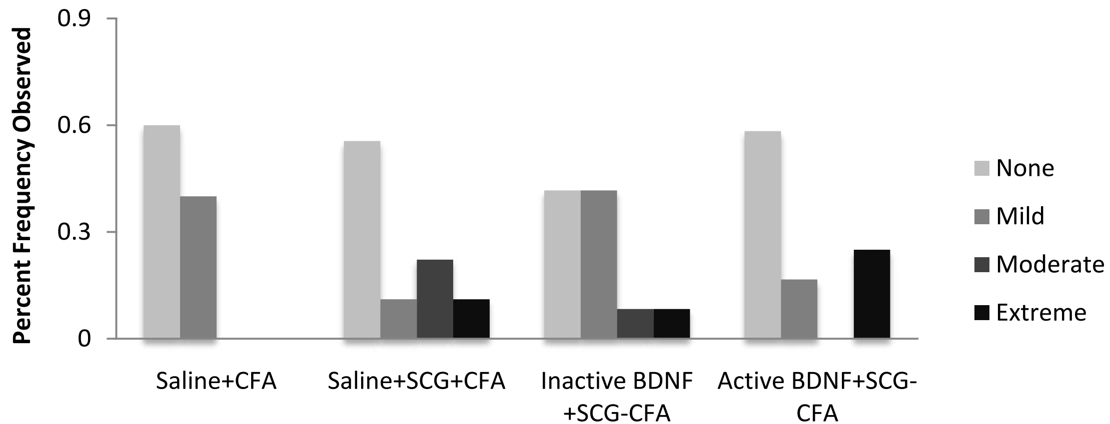

Figure 9. Percent frequency distribution of hippocampal inflammation severity for each treatment group.

Hippocampal Neurodegeneration. A *post hoc* Chi Square analysis of observed distribution of frequencies revealed a significant difference between sexes in percent frequency of observed neurodegeneration (x^2 (1, N = 38) = 13.93 $p < .000$) and severity (x^2 (3, N = 38) = 13.93 $p < .003$). Only female rats experienced neurodegeneration at all intensity levels, which was significantly greater than male rats for which neurodegeneration was never observed. Figures 10 and 11 show the percent frequency distributions for instances and severity of neurodegeneration respectively.

Neither cases of neurodegeneration (x^2 (1, N = 38) =.215, $p < .643$) nor severity of degeneration (x^2 (3, N = 38) = 3.08 $p < .379$) varied significantly across strains. Figure 12 shows the percent frequency distribution of neurodegeneration severity between the strains.

Neither the frequency of observed hippocampal neurodegeneration (x^2 (1, N = 38) = 2.57, $p < .463$) nor its severity (x^2 (9, N = 38) = 4.86 $p < .847$) varied significantly among the different treatment groups. Figure 13 shows the percent frequency of hippocampal neurodegeneration severity for each treatment group.

Figure 10. Percent frequency distributions of hippocampal neurodegeneration for male and female Sprague Dawley rats (A) and Lewis rats (B).

Figure 11. Percent frequency distribution of hippocampal neurodegeneration severity for male and female rats.

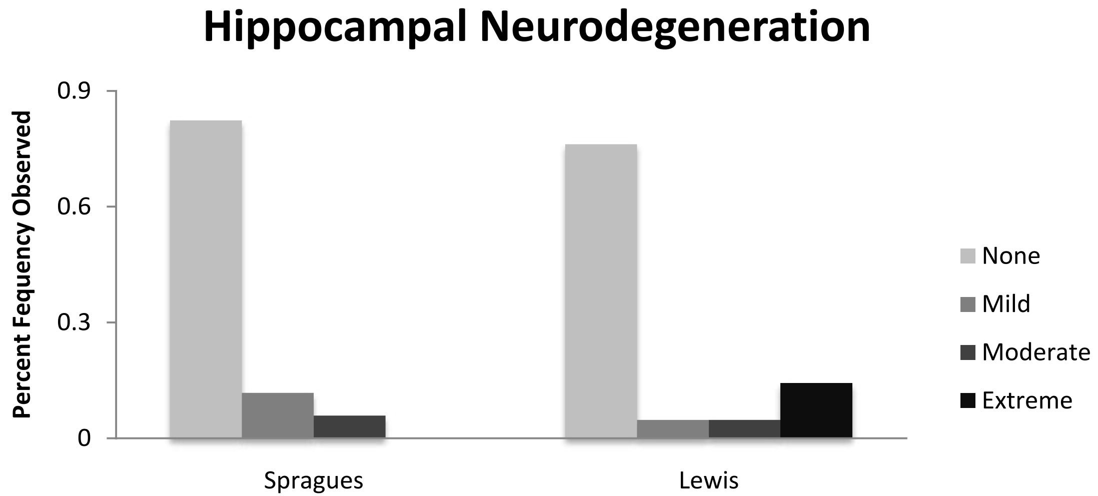

Figure 12. Percent frequency distribution of hippocampal neurodegeneration severity for Sprague Dawleys and Lewis rats.

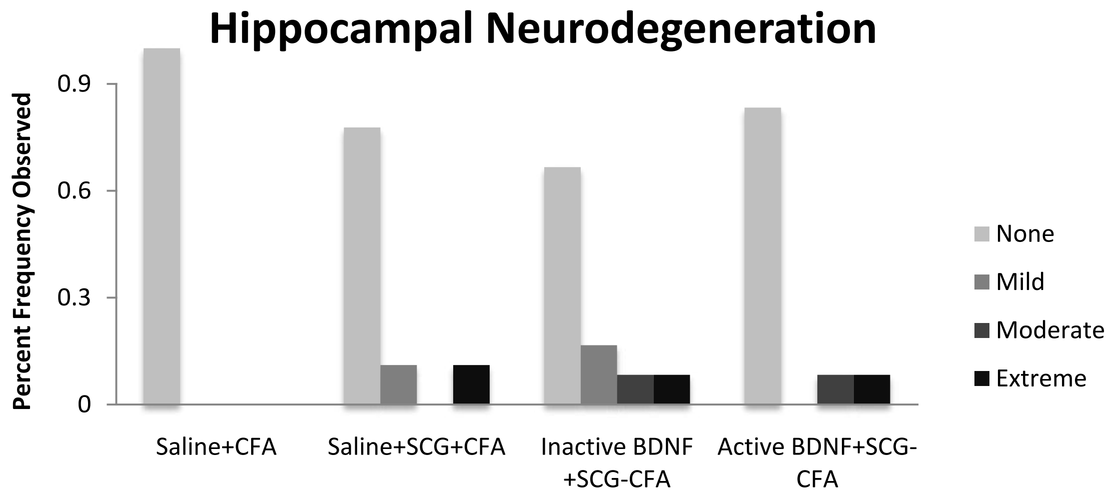

Figure 13. Percent frequency distribution of hippocampal neurodegeneration severity for each treatment group.

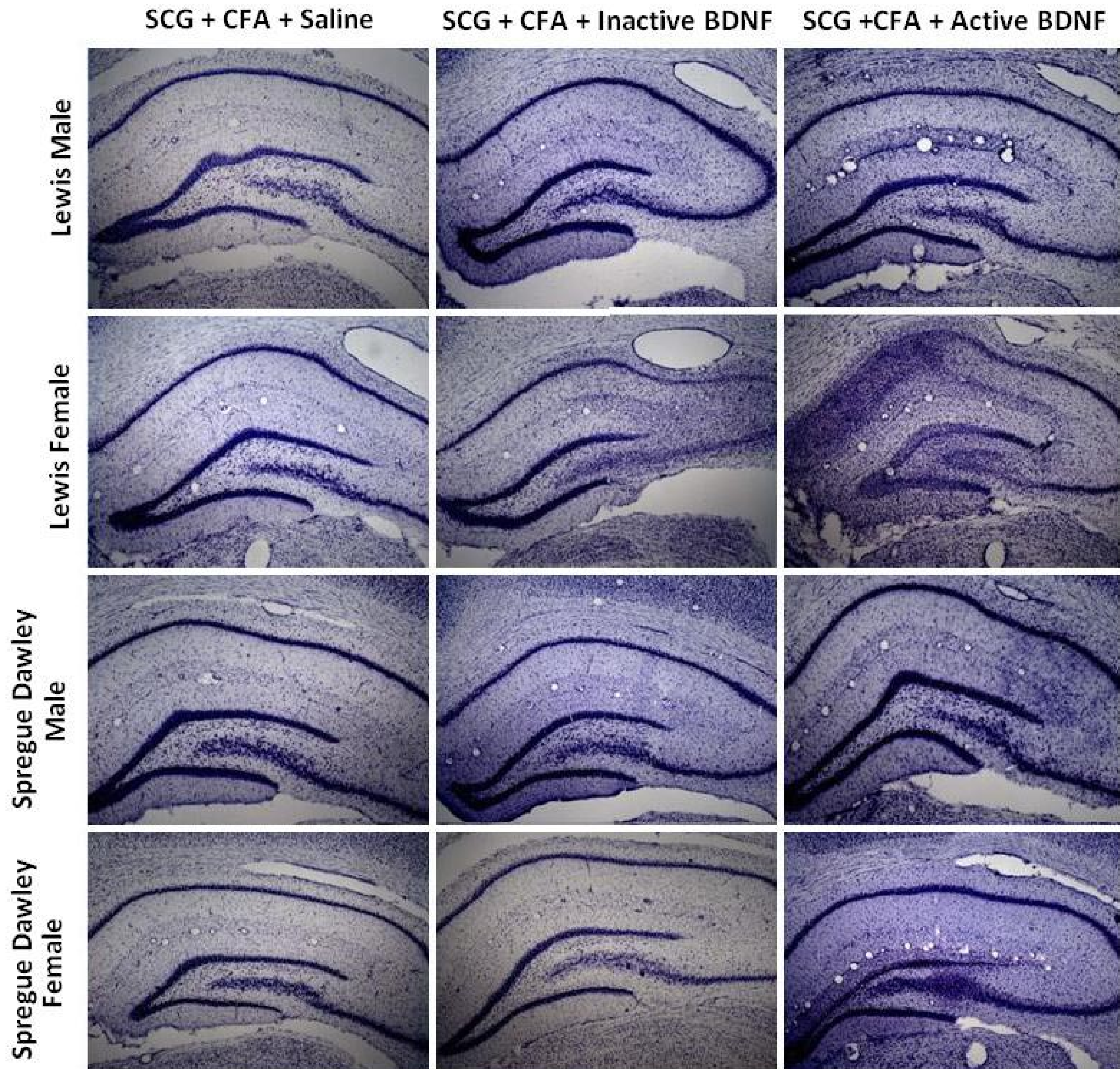

Figure 14. Photomicrographs of dorsal hippocampus representative of each experimental group. Note the presence of vascular cuffing and intense immune cell activity in the active BDNF groups as compared to the other groups. The PBS only control groups were similar in appearance to the CFA+saline group (data not shown).

Discussion

In the current study, we showed that BDNF-enhanced autoimmune inflammation was significantly modulated by sex, such that female rats consistently showed more inflammation than

males. Surprisingly, the autoimmune-prone strain of Lewis rats did not exhibit a significantly more aggressive immune response than the less autoimmune-prone Sprague-Dawley strain.

Immunity is the process by which the body protects itself from foreign molecules via the activation of lymphocytes and immune molecules such as cytokines. The problem arises when the body begins to recognize its own tissues as a foreign target and initiates a destructive process; this process is usually the result of abnormal lymphocyte activity and is termed autoimmunity. Autoimmune responses can be detrimental in that widespread inflammatory processes tend to be instigated, leading to the development of systemic autoimmune diseases. Autoimmune diseases such as multiple sclerosis, scleroderma and lupus are a major clinical issue for the general population that needs to be addressed using experimental models which allow causal inferences. The specific patterns of effects observed from these diseases could be used to provide clues into the etiology of autoimmunity. Some of the effects that may shed light on the origins of autoimmunity are the disproportionate prevalence patterns observed in ethnic minority groups and women. These observations point to a possible involvement of genetic risks as well as sex differences in the development of these disorders. This assumption is partly supported by the available literature. For example, activation of cytokines is in part mediated by neurotrophins such as NGF and BDNF and females express higher levels of BDNF than males which may place them at higher risk for the development of autoimmune diseases. Further, estrogen regulates concentration levels of BDNF in autoimmune inflammatory diseases whereas testosterone levels are lower in those same populations. However, much of the available data on autoimmunity has been collected from correlational studies that are largely based on clinical human populations which prevents causal relationships from being established.

Since autoimmune diseases exert such a critically detrimental effect on the human general population, it is necessary to experimentally study the mechanisms underlying these diseases via the

development of animal models. Previous work from our laboratory has provided clues about some of the mechanisms underlying sympathetic autoimmune hyperactivity as is observed in diseases such as lupus and scleroderma. This evidence was obtained using a novel model consisting of subcutaneously injecting CFA-suspended superior cervical ganglia (SCG) homogenates from a donor animal into the hind paw of a naïve animal. This procedure induces sympathetic hyperactivity and concurrent infusion of BDNF into the hippocampus results in inflammation. In line with the correlational data from human clinical samples, this model suggests that the neurotrophin BDNF may provide a link between sympathetic dysfunction and inflammation, a common characteristic among autoimmune diseases. Based on that autoimmune model, we set out to test whether or not the model could be used to generalize observed differential sex and ethnic effects from human into animals. The model was developed using male Sprague Dawley rats, and in this report it is extended to Lewis rats as well as females rats from both strains.

Using the previously described sympathetic autoimmune hyperactivity model we assessed the differential effects of sex, strain and BDNF on rat hippocampus following induction of autoimmune sympathetic dysfunction. Here we present findings that offer support for the observed differential effect of autoimmunity on females. First, we show that female rats are significantly more affected than male rats by the sympathetic dysfunction as determined by greater susceptibility to vascular cuffing and more severe hippocampal inflammation patterns. In line with the observations of disproportional autoimmunity effects on ethnic groups genetically at risk, there were tendencies for vascular cuffing and hippocampal inflammation to be exacerbated in animals of the Lewis strain. This finding did not, however, achieve statistical significance. Lastly, as it was expected, there was greater inflammation for animals that were infused with the biologically active BDNF protein than the other groups. However, there were a significant number of cases where Lewis animals demonstrated sympathetic reactivity to the injection of the SCG-CFA mixture in the absence of BDNF. This control treatment does not induce

inflammation in Sprague Dawley rats, and may reflect an augmented non-specific response to exposure to recombinant human protein. That is, the response of the Lewis rats may have been an immune reaction to the presence of foreign (i.e. human) protein, rather than a specific response to the biological actions of BDNF.

Very interestingly, a striking and unexpected *post hoc* finding of hippocampal neurodegeneration was observed in female but was not in male rats of both strains. However, this finding of hippocampal neurodegeneration was in line with the observations by Kowal et al. (2004) who used an autoimmune model of lupus and found that immunization of mice while disrupting the blood brain barrier results in hippocampal neuron death. Kowal and colleagues determined that hippocampal neuron death was mediated by a cross reaction of antibodies and hippocampal neuron NMDA receptors. Further, Singh et al. (2008) reported a link between BDNF and sympathetic axon degeneration. More specifically, the authors found that during sympathetic axon competition, those axons that are better equipped for survival secrete BDNF which induces the degeneration of other, competing, axons. Hence, it would be interesting to identify the mechanisms by which the sympathetic autoimmune hyperactivity model is mediating the observed hippocampal neurodegeneration.

Interpretation of this experiment is limited by the small sample size per group. Each group consisted of only three animals, and deaths that occurred during the progress of the experiment resulted in some group numbers dropping to only one animal, while other groups had to be eliminated completely. For example, only one animal of three survived in the Sprague Dawley female group receiving the SCG-CFA emulsion and hippocampal infusion of saline and only two survived in the male group receiving the same treatment. Further, all animals in the female groups (Sprague Dawleys and Lewis) receiving only the CFA injection and hippocampal infusion of saline did not survive, therefore limiting our interpretations for females since that control group was excluded. Fortunately, the group

receiving saline and sympathetic autoimmunity turned out to serve as a good control, because inflammation was not observed in this group.

Future research using this autoimmune model may benefit from a replication of this experiment with a larger sample size and the inclusion of the CFA-only injection control group which was lacking in this experiment for female rats. The inclusion of that group will allow to determine if the observed Lewis' rats greater sympathetic reactivity could be induced simply by the injection of SCG, though this is unlikely given that most saline animals showed little or no inflammation. I

Another area for future work with this model is its use to study the mechanisms driving differential effects of autoimmunity between males and females. More specifically, future research may find it illuminating to assess the role of estrogen and testosterone in sex based differences in sympathetic reactivity. This is especially interesting when considering the findings that estrogen regulates hippocampal BDNF levels (Berchtold et al., 2001). By similar reasoning, it would be interesting to determine the role of exercise in autoimmunity since it has been reported that exercise can also regulate hippocampal BDNF levels (Mata et al., 2010).

Based on observations of BDNF involvement in activation of cytokines and inflammation following sympathetic stress, it would also be interesting to determine the cellular mechanisms by which BDNF mediates those inflammatory processes. For instance, BDNF binds to both TrkB and p75 neurotrophin receptors, and it is currently unclear which receptor mediates this effect (for review see Chao et al., 2005). In addition, BDNF modulates a large number of neuropeptides and neurotransmitters, including neuropeptide Y (Croll et al., 1992), which is co-released by the sympathetic neurons.

Lastly, in the current study we present the unexpected finding that the sympathetic autoimmune hyperactivity model manipulations result in hippocampal neurodegeneration that is

specific to female rats. Future research may seek to shed light on the mechanism that is causing the observed hippocampal neurodegeneration, especially in light of the findings that autoimmune patients often suffer from cognitive impairments (Kowal et al., 2004).

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