Voluntary exercise alters adaptive immunity prior to injury

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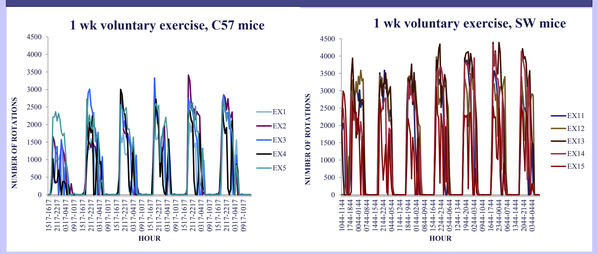
Introduction

- Despite the progress that has been made with regard to the prevention, treatment, and management of stroke, it remains a major cause of death and disability. Severity of stroke is important to consider as severe deficits can leave patients facing life-long mental and physical disability [1].
- Previous clinical research has demonstrated the importance of exercise prior to stroke in the reduction of stroke severity and achievement of better long term outcomes [2]. Studies examining stroke in rats show that exercise prior to stroke is associated with reduced ischemic volumes and reduced neurologic deficits [3].
- The Stowe lab is interested in the effect of exercise preconditioning on the inflammatory response pre and post-stroke in mice. Earlier studies have shown that in absence of injury, exercise modulates T cell and B cell phenotypes, induces leukocytosis, and increases acute phase reactants [4]. How these changes are related to a protective phenotype in the setting of stroke has yet to be determined.
- This study established an exercise preconditioning protocol to test the hypothesis that exercise mediates protection from stroke by altering the immune profile prior to injury to reduce inflammation post-infarct.

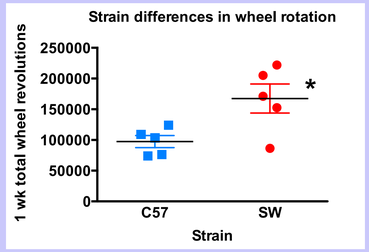
Aims

- Establish a voluntary exercise protocol in mice within the Stowe lab.
- Characterize changes in the B cell phenotype following 3 weeks of voluntary exercise in mice.

PATTERNS SEEN IN VOLUNTARY EXERCISE



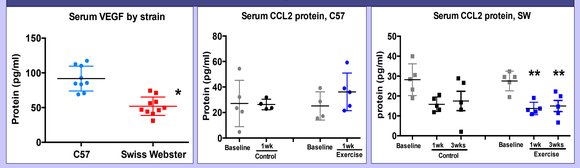
These figures demonstrate the cyclical nature of the animals' exercise pattern. The highest number of rotations are achieved between 6PM and 6AM, during the animals' dark cycle. Individual variation is also present in total number of rotations.



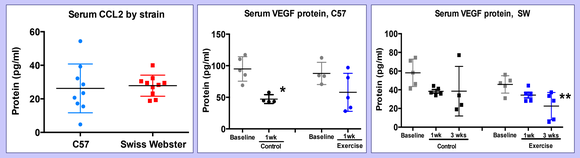
SW mice achieve significantly higher rotations than C57 mice at 1 week of total rotations.

Results

ELISA, Peripheral Blood

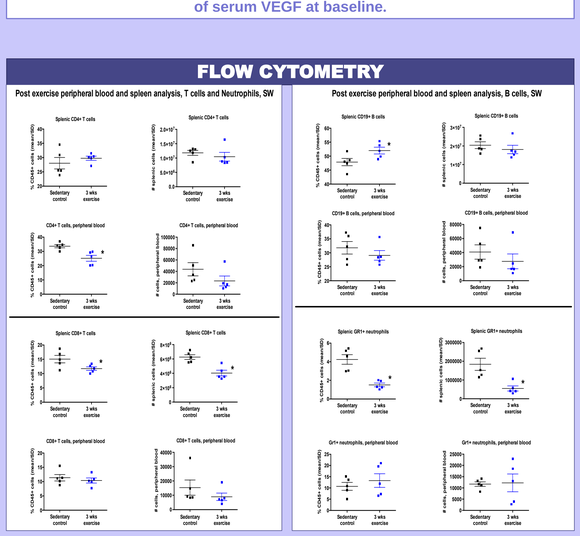


Serum CCL2, a chemokine for monocytes, memory T cells, and dendritic cells is significantly decreased in SW mice following voluntary exercise and is not significantly changed in C57 mice. There is no significant strain difference in CCL2 levels at baseline.



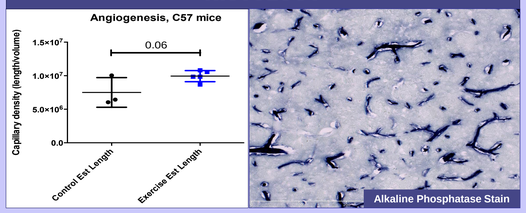
Serum VEGF, a protein that stimulates angiogenesis and vasculogenesis, is significantly down regulated in SW mice following voluntary exercise and in C57 mice following sedentary activity. SW mice have significantly lower levels of serum VEGF at baseline.

FLOW CYTOMETRY



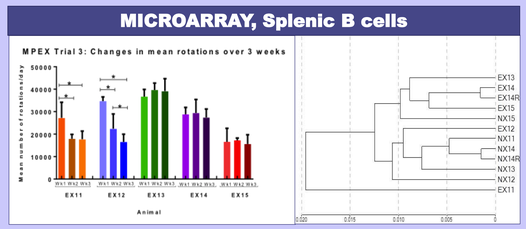
Voluntary exercise in SW mice showed decreases in percent and raw number of splenic neutrophils and CD8+ T cells (both $p<0.01$), with a concomitant increase in B cell representation ($p<0.05$). Peripheral blood samples demonstrated a decrease in percent CD4+ T cells ($p<0.01$). There were no significant changes in peripheral blood levels of CD8+ T cells, CD 19+ B cells, or Gr1+ neutrophils.

HISTOLOGY

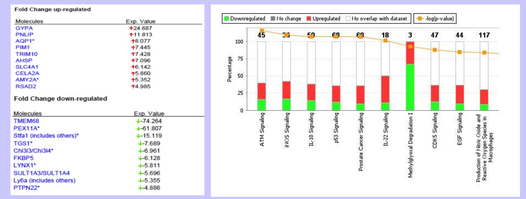


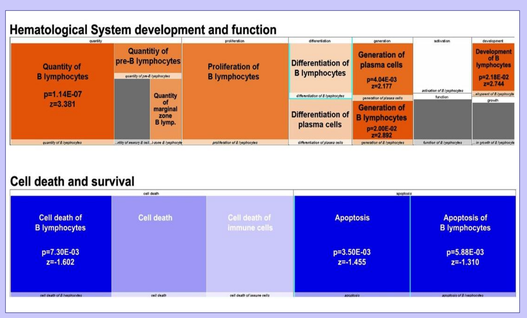
Voluntary exercise is correlated with a trend increase in cortical angiogenesis ($p<0.06$).

MICROARRAY, Splenic B cells



SW mice showing the highest level of consistency in their exercise over a 3 week period also demonstrate closer genetic linkage post-exercise.





There is significant up regulation of 1844 genes and significant down regulation of 1333 genes in the B cells of SW exercise mice over the control, with activation of several B cell pathways.

Methods

Voluntary exercise training: Mice were placed overnight in individual cages with computer monitored running wheels unlocked for 3 weeks. Voluntary running activity is associated with an enhanced effect on neurogenesis compared to forced exercise training [5]. Sedentary control mice were housed in identical cages with locked running wheels.

Immunohistochemistry to quantify angiogenesis and neurogenesis: Animals were overdosed with isoflurane, transcardiac perfused (20ml PBS, 40ml 4% paraformaldehyde), and brains removed, cryoprotected, and sectioned at a 20-µm thickness. Sections were rinsed (PBS) and blocked (10% NGS, 0.2% Triton, 10mM PBS). Capillaries were stained with Alkaline phosphatase and hippocampal neuron counts were estimated utilizing the Cresyl Violet stain. Images were captured using a Nanozoomer (Hamamatsu) and quantified using Stereo Investigator [6].

Quantification of leukocyte diapedesis using flow cytometry: Submandibular peripheral blood collections were pooled (3 mice/sample), resuspended (50µl PBS) and transferred to FACS tubes. Animals were perfused (20ml PBS), and brains dissected (3 mice/sample/hemisphere). Lymphocytes were isolated from brain tissue with 70:30 discontinuous Percoll gradient and lysate collected for protein analysis. Nonspecific binding was blocked with FcRyII/III. Antibodies identified T cells, B cells, macrophages, dendritic cells (DC) and microglia. The B and T cell staining panels reflected markers that are useful in tracking both B cell depletion, and CNS B cell lymphocyte dynamics in mice [7]. Samples were run on a BD-FACS Aria flow cytometer (UTSW core), and analyzed (FlowJo, Tree Star Inc).

Quantification of serum or brain lysate protein by ELISA: After peripheral blood collection, each sample was centrifuged (14000 rpm, 2–3 min). Quantitative immunoassays of these proteins were performed with the Enzyme-Linked Immunosorbent Assay (ELISA) kit (R&D Systems) according to the manufacturer's instructions. Prepared serum or brain lysate (the latter collected during flow cytometry) were aliquoted on the coated immunoassay plates, a biotinylated detection antibody applied, and development with an avidin-horseradish peroxidase conjugate was performed. A colorimetric substrate was applied and the intensity was read by a microplate reader, thus converting absorbance to concentration.

Statistical analysis: Power analysis based on previous results and published data determined the approximate number of animals with a 30% mortality rate. All assessments of histology and behavior were performed by technicians blinded to experimental condition. All between group differences were analyzed using student t-test, with Tukey post-hoc analysis, or one-way ANOVA (Prism). Significance was determined as $p < 0.05$.

Summary

- Previous studies have shown that exercise prior to stroke is associated with smaller infarct volumes and decreased deficits.
- Voluntary exercise in mice changes the cytokine and lymphocyte profiles in the spleen and peripheral blood.
- Specifically, the decrease in cytotoxic T cells, neutrophils, and CCL2 are consistent with an antinflammatory presentation and may play a role in the mechanism for exercise-mediated neuroprotection.

Conclusion & Future Direction

Three weeks of voluntary exercise in mice results in a change in the immune profile prior to a cerebral infarct occurring. Down regulation of neutrophils, cytotoxic T cells, and CCL2 suggest that this alteration in immunity is anti-inflammatory. Microarray analysis of isolated B cells showed an up regulation of genes associated with maturation and differentiation and down regulation of genes responsible for apoptosis and B cell death.

Current projects:

- Histologically stain SW brains with anti-BrdU for assessment of neurogenesis and CD31/BrdU staining for assessment of angiogenesis, both post-exercise.
- Run trials of SW animals with 3 weeks of exercise preconditioning followed by transient MCA occlusion in order to assess changes in the immune response following stroke.
- Compare the immune profile changes pre-stroke to the neurovascular protection occurring in exercise preconditioned animals following stroke, compared to sedentary controls
- Identify B cell subsets that are up regulated with exercise.

Future projects:

- In depth analysis into the genetic changes occurring at the level of B cells following exercise with microarray or RNAseq.
- Comparison of the immune changes following exercise to those following a repetitive hypoxia preconditioning protocol.
- Deplete only B cells in transgenic mice during exercise preconditioning, followed by stroke, to confirm the contribution of the altered B cells to stroke protection.

References

1. Guidelines for the Prevention of Stroke in Patients With Stroke or Transient Ischemic Attack: A Guideline for Healthcare Professionals From the American Heart Association/American Stroke Association *Stroke. 2011; 42: 227-276* Published online before print October 21, 2010, doi: 10.1161/STR.0b013e3181f7d043
2. Krarup, L.H., T. Truelsen, C. Gluud, G. Andersen, X. Zeng, J. Korv, A. Oskedra, and G. Boysen, *Prestroke physical activity is associated with severity and long-term outcome from first-ever stroke.* Neurology, 2008. 71(17): p. 1313-8. PMID:18936423.
3. Ding, Y., J. Li, X. Luan, Y.H. Ding, Q. Lai, J.A. Rafols, J.W. Phillis, J.C. Clark, and F.G. Diaz, *Exercise pre-conditioning reduces brain damage in ischemic rats that may be associated with regional angiogenesis and cellular overexpression of neurotrophin.* Neuroscience, 2004. 124(3): p. 583-91. PMID:14980729.
4. Zaldivar F, Wang-Rodriguez J, Nemet D, Schwindt C, Galassetti P, Mills PJ, Wilson LD, Cooper DM. *Constitutive pro- and anti-inflammatory cytokine and growth factor response to exercise in leukocytes.* J Appl Physiol 100: 1124–*1133*, 2006.
5. Luo, C.X., J. Jiang, Q.G. Zhou, X.J. Zhu, W. Wang, Z.J. Zhang, X. Han, and D.Y. Zhu, *Voluntary exercise-induced neurogenesis in the postischemic dentate gyrus is associated with spatial memory recovery from stroke.* J Neurosci Res, 2007. 85(8): p. 1637-46. PMID:17465031.
6. Stowe, A.M., E.J. Plautz, I. Eisner-Janowicz, S.B. Frost, S. Barbay, E.V. Zoubina, N. Dancause, M.D. Taylor, and R.J. Nudo, *VEGF protein associates to neurons in remote regions following cortical infarct.* J Cereb Blood Flow Metab, 2007. 27(1): p. 76-85. PMID:16639424.
7. Monson, N.L., P. Cravens, R. Hussain, C.T. Harp, M. Cummings, M. de Pilar Martin, L.H. Ben, J. Do, J.A. Lyons, A. Lovette-Racke, A.H. Cross, M.K. Racke, O. Stuve, M. Shlomchik, and T.N. Eagar, *Rituximab therapy reduces organ-specific T cell responses and ameliorates experimental autoimmune encephalomyelitis.* PLoS ONE, 2011. 6(2): p. e17103. PMID:21359213. PMCID:3040191.

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