Exercise protects against inflammation by modulating rat hippocampal NF-kB and nucleus accumbens FosB/ΔFosB

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Abstract

It is well-established that exercise exerts beneficial effects on the central nervous system, such as protecting against neuronal aging and inducing stress-resilience. However, significant individual differences exist in exercise motivation, and the underlying mechanisms linking exercise to brain health remain unclear. Using rat voluntary wheel running, we modeled for acute exercise: rats were allowed to run during a 5-hour session after 4 days of training and subsequent 2 days of rest. They were then grouped based on running distances. With high throughput active kinome profiling on rat hippocampus, we discovered exercise significantly reduces the activity of several kinases in the NF-kB activation pathway responsible for cellular inflammation, including COT, IKK, PKD, and RIPK. Subsequent validation with transcriptional analysis showed that with an acute bout of running, NF-kB activity in the hippocampus is significantly repressed compared to sedentary controls, especially in high-running rats. Additionally, we found that acute exercise induces FosB/ΔFosB expression in the nucleus accumbens, which drives motivation in reinforced behaviors. This is intriguing because while FosB/ΔFosB canonically responds to repeated and chronic exposure to certain antidepressants or drugs of abuse, here we show that a single period of exercise is sufficient to induce its expression. In summary, we propose that acute exercise may exert anti-inflammatory effects via downregulation of NF-kB pathway in the hippocampus, and the motivation underlying exercise may be related to FosB/ΔFosB expression in the nucleus accumbens. Future directions include explicating individual differences in exercise motivation and examining other brain regions.

Background and Introduction

FosB/ΔFosB
ΔFosB is a transcription factor derived from alternative splicing of FosB. Like other Fos family proteins, FosB/ΔFosB are both responsive to acute neuronal perturbations. While FosB levels are prone to flucuations, the more stable ΔFosB is more likely to accumulate over time[4]. They have been implicated to mediate neural plasticity[4,6], stress[3], as well as reward, motivation, and addiction[1].

NF-kB
NF-kB is a well-studied transcriptional factor that mediates the cellular responses to external stimuli such as inflammation, immunity, growth, and apoptosis. Persistently active NF-kB is found in various inflammatory and disease states[5].

Previous Data
In our previous experiments, high throughput active kinome profiling on sedentary and running rat hippocampus showed significant reduction in the activity of several kinases in the NF-kB pathway (COT, IKK, PKD, and RIPK). This study aims to validate the downregulation of NF-kB in hippocampus after running, and to further explicate both the mechanisms linking exercise and brain health, as well as the molecular basis of motivation to exercise.

Materials and Methods

Animals
Adult male Sprague-Dawley rats from Charles River Laboratory were used for all experiments. Rats were housed by Des Moines University Animal Care Facility under 12-hour light-dark cycle with unlimited access to food and water.
Acute Exercise Model
Rats were given access to voluntary running wheels (Lafayette Instrument, 1.1m diameter in-cage running wheels) for 4 days (training). Wheels were subsequently locked for 2 days (rest). Prior to sacrifice, rats were allowed to run for 5 hours during the beginning of their wake cycle. (Figure 1)
Immunohistochemistry
Fixed frozen sections were incubated overnight in FosB (5G4) primary antibody (Cell Signaling #2251), then labeled with Alexa Fluor 568 secondary antibody.
NF-kB Assay
Nuclear fractions of hippocampus samples were extracted with the Nuclear Extraction Kit from Cayman Chemical. Then, NFkB activity were quantified by the NF-kB Transcription Factor Assay Kit from Cayman Chemical.
Imaging and Statistical Analysis
Sections were imaged with Zeiss Axioscope Fluorescent Microscope and quantified with ImageJ. Statistical analysis was carried out with Prism 8. Unpaired, two-tailed t-tests with equal standard deviations were used to assess statistical significance between independent experimental groups. All reported significance levels represent two-tailed values.

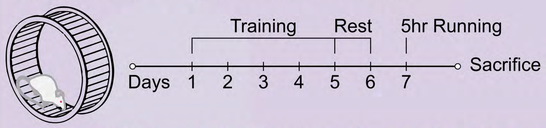


Figure 1. Acute exercise model consists of training for 4 days, rest for 2 days, and acute 5 hour running prior to sacrifice.

NF-kB Downregulation in Hippocampus

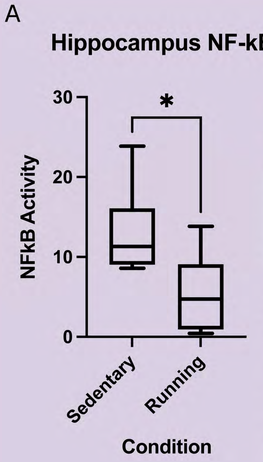
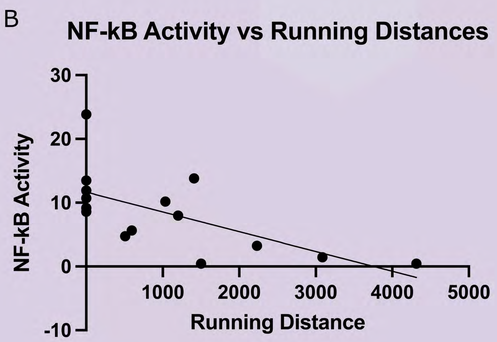


Figure 2. NF-kB activity is downregulated in hippocampus of running rats.

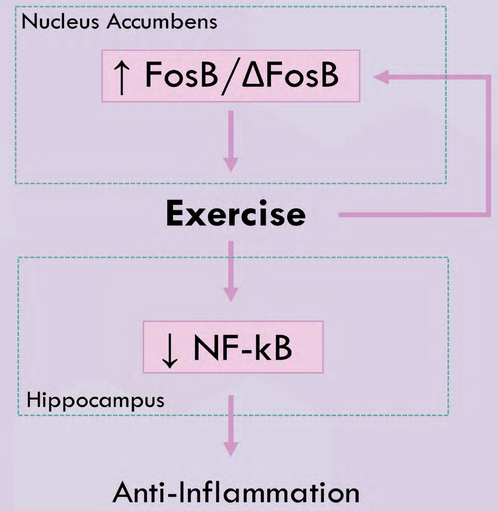
(a) Relative NF-kB activity standardized against positive control is reduced in the hippocampus of running rats. (n = 6 for sedentary, n = 9 for running, p = 0.013)

(b) Running distance during acute exercise period is inversely proportional to relative NF-KB activity. (n = 15, R-squared = 0.424)



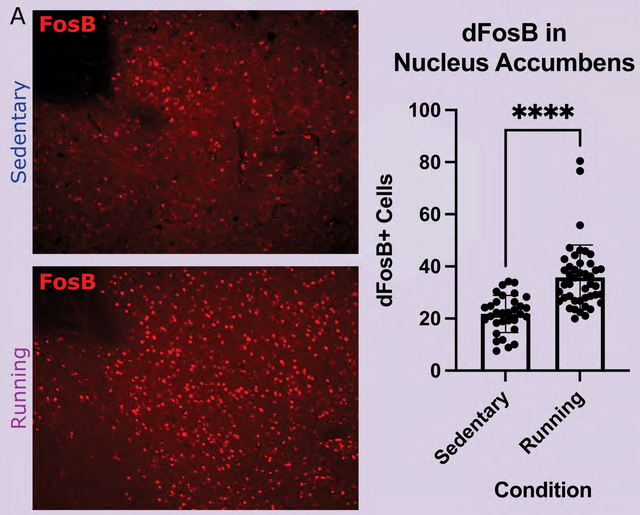
Discussion

FosB/ΔFosB. Here we report that merely one bout of acute running is sufficient to induce significant FosB/ΔFosB expression in the nucleus accumbens. As FosB/ΔFosB are known to drive motivation in reinforced behaviors[3,4], we propose that they may be involved in positive feedback driving motivation to exercise.



NFkB. We also validated previous high throughput active kinome profiling data with NF-kB assay and found significant downregulation of NF-kB in exercise. Thus, we propose acute exercise may exert anti-inflammatory effects via downregulation of hippocampal NF-kB pathway.

FosB/ΔFosB Upregulation in Nucleus Accumbens



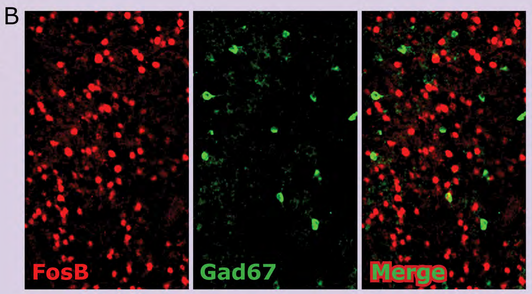


Figure 3. FosB/ΔFosB is upregulated in nucleus accumbens of running rats.

(a) Immunohistochemistry of FosB revealed significantly increased FosB expression in nucleus accumbens of running rats (n = 32 for sedentary, n = 44 for running, p <0.001)

(b) No obvious colocalization of FosB with interneuron marker Gad67. Further evaluation is needed to explicate the identities of FosB+ cells in context of exercise.

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