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SYNERGISTIC EFFECTS OF DIET AND EXERCISE ON HIPPOCAMPAL FUNCTION IN CHRONICALLY STRESSED MICE

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Abstract—Severe chronic stress can have a profoundly negative impact on the brain, affecting plasticity, neurogenesis, memory and mood. On the other hand, there are factors that upregulate neurogenesis, which include dietary antioxidants and physical activity. These factors are associated with biochemical processes that are also altered in age-related cognitive decline and dementia, such as neurotrophin expression, oxidative stress and inflammation. We exposed mice to an unpredictable series of stressors or left them undisturbed (controls). Subsets of stressed and control mice were concurrently given (1) no additional treatment, (2) a complex dietary supplement (CDS) designed to ameliorate inflammation, oxidative stress, mitochondrial dysfunction, insulin resistance and membrane integrity, (3) a running wheel in each of their home cages that permitted them to exercise, or (4) both the CDS and the running wheel for exercise. Four weeks of unpredictable stress reduced the animals' preference for saccharin, increased their adrenal weights and abolished the exercise-induced upregulation of neurogenesis that was observed in non-stressed animals. Unexpectedly, stress did not reduce hippocampal size, brain-derived neurotrophic factor (BDNF), or neurogenesis. The combination of dietary supplementation and exercise had multiple beneficial effects, as reflected in the number of doublecortin (DCX)-positive immature neurons in the dentate gyrus (DG), the sectional area of the DG and hippocampal CA1, as well as increased hippocampal BDNF messenger ribonucleic acid (mRNA) and serum vascular endothelial growth factor (VEGF) levels. In contrast, these benefits were not observed in chronically stressed animals exposed to either dietary supplementation or exercise alone. These findings could have important clinical implications for those suffering from chronic stress-related disorders such as major depression.

Key words: stress, neurogenesis, hippocampus, exercise, dietary supplements, psychological depression.

INTRODUCTION

Chronic stress can have a profoundly negative impact on the brain and contributes to a number of psychological disorders including major depression (McEwen, 2003; Miller and Hen, 2015). Evidence from rodents and primates links severe and prolonged elevation of glucocorticoids (corticosterone, cortisol) to hippocampal damage (Sapolsky, 1985; Sapolsky et al., 1995). This damage includes synaptic atrophy (Watanabe et al., 1992; Magariños et al., 1997) and reduced neurogenesis (Watanabe et al., 1992; Gould et al., 1992, 1997, 1998). Glucocorticoids bind extensively in the healthy hippocampus to both glucocorticoid and mineralocorticoid receptors (Reul and De Kloet, 1985; Aronsson et al., 1988), and chronic stress can reduce the number of hippocampal mineralocorticoid receptors (López et al., 1998). Normally, the hypothalamic–pituitary–adrenal (HPA) axis shows habituation to repeated exposure to a stressor, such that glucocorticoid elevation following the stressor diminishes, but the reduction in hippocampal mineralocorticoid receptors can lead to impairment of this habituation (Cole et al., 2000).

These detrimental effects on the hippocampus correlate with behavioral signs of major depressive disorder. Evidence indicates that the effects of serotonergic antidepressant medications rely upon intact adult hippocampal neurogenesis (Malberg et al., 2000; Santarelli et al., 2003; Sahay et al., 2011). For example, social isolation stress induced anhedonia and depression-like behavior in monkeys; this was alleviated by fluoxetine treatment, which also upregulated neurogenesis (Perera et al., 2011). However fluoxetine's behavioral antidepressant effect was abolished by focal hippocampal X-irradiation (Perera et al., 2011), which is highly toxic to immature neurons (Snyder et al., 2001; Winocur et al., 2006). Similarly, chronic unpredictable mild stress induced

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Abbreviations: BDNF, brain-derived neurotrophic factor; CDS, complex dietary supplement; CUS, chronic unpredictable stress; DCX, doublecortin; DG, dentate gyrus; HPA, hypothalamic–pituitary–adrenal; IGF-1, insulin-like growth factor-1; mRNA, messenger ribonucleic acid; VEGF, vascular endothelial growth factor.

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anhedonic behavior in mice, accompanied by a 30% reduction in neurogenesis and habituation of the hippocampal inhibitory influence on the HPA axis (Surget et al., 2011). This habituation was evident in a reduction in the number of newly born neurons in the dentate gyrus (DG) activated by dexamethasone, a synthetic glucocorticoid. Moreover, disruption of neurogenesis by irradiation impaired the ability of fluoxetine to restore hippocampal modulation of HPA activity during chronic stress, suggesting that this modulation may depend upon neurogenesis. Although it is challenging to study human neurogenesis, a number of studies have associated the number and duration of depressive episodes with loss of hippocampal volume and memory function (see e.g. Sheline et al., 1999; MacQueen et al., 2003).

Two factors that can enhance neurogenesis and offset stress and depression are dietary antioxidants (Lau et al., 2005; Valente et al., 2009) and long-term aerobic exercise (van Praag et al., 1999, 2005; Creer et al., 2010; Déry et al., 2013; Winocur et al., 2014). Voluntary aerobic exercise enhanced neurogenesis in rodents for up to 9 months (Merkley et al., 2014). In humans, exercise was found to increase serum brain-derived neurotrophic factor (BDNF; Erickson et al., 2011), DG blood volume (indicative of angiogenesis; Pereira et al., 2007) and memory scores on a behavioral test of pattern separation (Déry et al., 2013). Diet and exercise affect biochemical processes and signaling pathways that are also altered in age-related cognitive decline. These include neurotrophin expression (Fahnestock et al., 2012), cellular oxidative stress (Valente et al., 2009), inflammation (Goshen et al., 2008) and mTOR regulation (Ota et al., 2014). In rodents, a complex dietary supplement (CDS) greatly ameliorated age-related physiological and cognitive decline in transgenic growth hormone mice (a model of accelerated aging) and aged wild-type controls (Lemon et al., 2003). When aged mice received the same supplement from weaning onward, they performed as well as young mice on the hidden platform version of the Morris water maze (Aksenov et al., 2013) – a test on which younger mice typically outperform older ones. Aged mice who received the CDS also had larger brains than age-matched non-supplemented controls. The same CDS also protected mice from radiation-induced DNA damage and immunological apoptosis (Lemon et al., 2008a,b).

The CDS (Table 1) was designed to target five major mechanisms associated with aging: inflammation, oxidative stress, mitochondrial dysfunction, insulin resistance and membrane integrity. Although this approach may not identify contributions of any one ingredient, mounting evidence supports the potent neuroprotective effects of CDSs exhibiting some overlap in ingredients or physiological targets (Milgram et al., 2002; Parachikova et al., 2010). Broad-spectrum, antioxidant-rich micronutrient supplementation also shows promise in treatment of mood disorders, while single nutrient supplements generally produce weak results (Rucklidge and Kaplan, 2013; Popper, 2014). For example, pre-partum micronutrient supplementation lessens the risk and severity of postpartum depression (Leung et al., 2013).

Table 1. Ingredients included in the complex dietary supplement

Ingredient	Daily dose for a 35 g mouse
Acetyl-L-Carnitine	14.4 mg
Acetylsalicylic Acid	2.5 mg
Alpha-Lipoic Acid	0.72 mg
β-Carotene	50 IU
Bioflavonoids	4.32 mg
Chromium picolinate	1.44 μg
Cod Liver Oil	5.04 IU
Coenzyme Q10	0.44 mg
DHEA	0.15 mg
Flax Seed oil	21.6 mg
Folic Acid	0.01 mg
Garlic	26.6 μg
Ginger	7.2 mg
Gingko Biloba	1.44 mg
Ginseng	8.64 mg
Green Tea Extract	7.2 mg
L-Glutathione	0.36 mg
Magnesium	0.72 mg
Melatonin	0.01 mg
N-Acetyl Cysteine	7.2 mg
Potassium	0.36 mg
Rutin	0.72 mg
Selenium	1.08 μg
Vitamin B1	0.72 mg
Vitamin B3	0.72 mg
Vitamin B6	0.72 mg
Vitamin B12	0.72 μg
Vitamin C	3.6 mg
Vitamin D	2.5 IU
Vitamin E	1.44 IU
Zinc	0.14 mg

Although both exercise and nutraceuticals can enhance hippocampal volume (Erickson et al., 2011) and neurogenesis (Lau et al., 2005), the two together may produce greater effects. The combination of an antioxidant-fortified diet and environmental enrichment reduced age-related cognitive impairment and increased BDNF levels in dogs more than did either treatment alone (Fahnestock et al., 2012). Beneficial interactions between combinations of dietary supplementation and environmental enrichment or exercise have also been observed in investigations of Alzheimer's disease (Pop et al., 2010) and synaptic plasticity (Wu et al., 2008). Although environmental enrichment and exercise affect neurogenesis via distinct pathways (neuronal survival and proliferation respectively; Olson et al., 2006), they are difficult to dissociate experimentally because environmental enrichment protocols typically have an exercise component. Conversely, exercise protocols enrich the animal's environment by affording it access to novel complex objects with which it can interact meaningfully.

We exposed mice to a complex series of unpredictable stressors while concurrently giving some of them the CDS, a running wheel in the home cage, or both. We predicted that dietary supplementation and aerobic exercise would synergistically mitigate the impact of chronic stress on the hippocampus. Specifically, we hypothesized that the combination of CDS and exercise would prevent depression-like