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# Parallel changes in cortical neuron biochemistry and motor function in protein-energy malnourished adult rats

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ABSTRACT

While protein-energy malnutrition in the adult has been reported to induce motor abnormalities and exaggerate motor deficits caused by stroke, it is not known if alterations in mature cortical neurons contribute to the functional deficits. Therefore, we explored if PEM in adult rats provoked changes in the biochemical profile of neurons in the forelimb and hindlimb regions of the motor cortex. Fourier transform infrared spectroscopic imaging using a synchrotron generated light source revealed for the first time altered lipid composition in neurons and subcellular domains (cytosol and nuclei) in a cortical layer and region-specific manner. This change measured by the area under the curve of the $\delta(CH_2)$ band may indicate modifications in membrane fluidity. These PEM-induced biochemical changes were associated with the development of abnormalities in forelimb use and posture. The findings of this study provide a mechanism by which PEM, if not treated, could exacerbate the course of various neurological disorders and diminish treatment efficacy.

## Introduction

Protein-energy malnutrition (PEM), **a combined deficiency state with depletion of energy stores and tissue proteins (**Ramirez-Zea and Caballero, 2014**),** can have harmful effects on the developing and adult brain. PEM in the developing child (Georgieff, 2007), primate (Enwonwu and Worthing, 1973), rodent (Ahmad and Rahman, 1975; Lee et al., 1999) and swine (Badger and Tumbleson, 1974) results in enduring aberrant changes in neuron and glial proliferation, dendritic arbors and spine density, and brain chemistry in cortical and subcortical regions, and cerebellum (Levitsky and Strupp, 1995). Consequently, PEM causes long lasting emotional, motor and cognitive impairments (Kar et al., 2008; Strupp and Levitsky, 1995).

The adult brain also appears to be vulnerable to PEM, although the data are more scarce. Abnormalities in sensory-motor and cognitive functions have been reported in both preclinical and clinical studies of adulthood PEM (Andrade and Paula-Barbosa, 1996; Lukoyanov and Andrade, 2000; Merli et al., 2013). In older adults, some clinical studies have associated PEM with sensory-motor (Challa et al., 2007; Chen et al., 2016) and cognitive deficits (Challa et al., 2007), whereas others observed preserved cognitive capabilities (Chen et al., 2016). Since it is challenging to isolate the true effects of PEM on neurological function in clinical studies because of co-existing co-morbidities or micronutrient deficiencies (Bemeur, 2013; Merli et al., 2013), many investigators employ animal models of PEM. PEM-induced morphological and biochemical changes in adult hippocampus (Andrade et al., 1995; Ji et al., 2008; Lukoyanov and Andrade, 2000), subiculum (Andrade et al., 1998) and cerebellum (Paula-Barbosa et al., 1989) in rodent studies are consistent with data from young rats. The hippocampus has been most investigated, and PEM-induced structural alterations in hippocampal neurons are associated with abnormalities in hippocampal-dependent behavior (Lukoyanov and Andrade, 2000). However, it has been suggested that the vulnerability of the adult brain to PEM varies by brain region and that cortical regions contributing to sensory-motor functions are less affected (Andrade et al., 1998). The data of Viana et al. (1997**)** on muscarinic acetylcholine receptors in adult rat brain also support this idea, although they initiated the PEM during prenatal life. Unfortunately, the interpretation of some of the key preclinical findings in the adult is limited by the dietary interven-

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tions employed. That is, the findings cannot be exclusively attributed to PEM because the control and experimental diets differed substantially in the content of other nutrients in addition to protein and energy (Andrade and Paula-Barbosa, 1996; Andrade et al., 1995, 1998; Lukoyanov and Andrade, 2000; Paula-Barbosa et al., 1989, Viana et al., 1997). In addition, clear evidence of compromised protein-energy status was missing from a number of these studies.

Despite this uncertainty on the biochemical and morphological composition of the adult protein-energy malnourished cortex, abnormalities in sensory-motor function do appear to be characteristic of adulthood PEM. Poor scores for activities of daily living have been reported in the malnourished adult human (Challa et al., 2007; Chen et al., 2016). We detected a variety of sensory-motor abnormalities that developed at different stages of PEM in adult rats (Alaverdashvili et al., 2015) and found that PEM exacerbated motor abnormalities caused by stroke in the motor cortex (MtCtx) of adult rats (Alaverdashvili and Paterson, 2014; Matwee et al., 2014). While muscle dysfunction can contribute to motor abnormalities (Alaverdashvili et al., 2015; Norman et al., 2011), this only partially explains the PEM-induced sensory-motor deficits (Alaverdashvili et al., 2015). Therefore, we hypothesized that PEM also exerts direct effects on cortical neurons.

The aim of this study was to explore if PEM in the adult provokes changes in the MtCtx neuronal biochemical profile that parallel changes in sensory-motor function. The biochemical signature of cortical neurons was imaged and quantified at cellular and subcellular levels using Fourier transform infrared spectroscopy coupled with a focal plane array detector using a synchrotron generated light source (S-FTIR-FPA). S-FTIR-FPA imaging has been previously validated to study subcellular lipid and protein biochemistry of individual healthy and diseased brain cells (Hackett et al., 2013, 2015; Kastyak-Ibrahim et al., 2012; Liao et al., 2013). PEM was induced by feeding rats a low protein diet, and protein-energy status was evaluated by food intake, body weight, serum acute-phase reactants and corticosterone concentrations, and the degree of liver steatosis. Finally, we investigated glial cell activation and the relationship among PEM-induced neuronal, glial and systemic alterations and behavioral changes in the sensory-motor domain as well as habituation.

## Materials and methods

### *Subjects*

Eleven week old, male Sprague-Dawley rats (n=10) were obtained from Charles River (QC, Canada) and maintained in a colony room with a 12 h light/12 h dark cycle (07:00–19:00) with controlled temperature and humidity. **The rats served as sham surgical controls for another study investigating the influence of PEM developing before stroke on motor recovery (**Supplementary material**). The rats underwent sham surgery on Day 28 after diet assignment (see Experimental design below).** Rats were housed in pairs in standard Plexiglas cages (39.5 cm×34.6 cm×22.7 cm) with absorbent bedding. To eliminate excessive fighting between cage mates, rats from one cage for each experimental group were separated. This work was approved by the University of Saskatchewan's Animal Research Ethics Board and adhered to the Canadian Council on Animal Care guidelines for humane animal use.

### *Nutritional intervention*

The rats (12–13 week old) were provided with regular rat chow (Prolab® RMH 3000) for 10 days. They were then acclimatized to a modified American Institute of Nutrition-93M diet containing 12.5% protein (NP; normal protein, control) supplied by Dyets Inc. (#1008790) (Alaverdashvili et al., 2015). The rats were trained on a horizontal ladder walking task for 14 days to prepare for later testing of skilled walking ability. They were food restricted (20 g $day^{-1}$ $rat^{-1}$) for the first 8 days to motivate them to walk back and forth across the ladder in exchange for sugar pellet rewards. This was followed by 6 days of ad libitum feeding to allow recovery from metabolic changes associated with food restriction. The rats, at 14–15 weeks of age, were then assigned ad libitum access to either the NP diet (n=5) or a matched low protein diet containing 0.5% protein (LP) (n=5) for 32 days. The NP and LP (Dyets Inc. #103271) diets contained 3.605 and 3.607 kcal/g, respectively. Adult, Sprague-Dawley rats fed the LP diet voluntarily reduce food intake, resulting in mixed protein-energy malnutrition (Alaverdashvili et al., 2015).

### *Experimental design*

The experimental design is summarised in Fig. S1. The rats were tested in the horizontal ladder walking task and cylinder task on Day 27 and Day 32 after diet assignment. On Day 32, the rats were anaesthetized with 4% isoflurane (1 L/min $O_2$), and humanely killed through decapitation, with the head immediately flash frozen in liquid nitrogen and stored at −80 °C. The brains were subsequently chiseled out from the frozen heads on dry ice and stored at −80 °C until sectioning (Alaverdashvili et al., 2014). Blood was collected by decapitation, allowed to clot for 30 min at room temperature, and centrifuged at 1,500×*g* at 4 °C for 10 min. Serum was removed and stored at −80 °C. The remaining body was flushed with intracardially administered saline (0.9%). Liver samples were dissected, transferred to liquid nitrogen, and stored at −80 °C.

### *Nutritional status*

Protein-energy status was measured by body weight, food intake, serum acute phase proteins, corticosterone, and liver lipid content (Supplementary material).

### *Functional tests*

#### *Cylinder task*

Spontaneous activity, balancing ability and habituation capability were assessed in a transparent Plexiglas cylinder (Fig. S3A) task for 5 min (Schallert and Woodlee, 2005; Alaverdashvili et al., 2015). The sum of the independent placements for the right and left forelimb and the simultaneous forelimb placements on the cylinder wall during rearing activity was used to quantify spontaneous forelimb activity (Schallert and Woodlee, 2005). Spontaneous exploratory activity was quantified by the total number of rears with and without forelimb support. Balancing ability was measured by the number of rears without forelimb support. Habituation was measured in two ways: 1) the decrease in spontaneous exploratory activity across the session (ΔRearing as %) calculated as: (Number of rears in the second half of the session/Number of rears in the first half of the session)×100; 2) the change in spontaneous exploratory activity across two sessions.

#### *Horizontal ladder walking task*

Skilled and coordinated movements of forelimbs and hindlimbs were evaluated in the horizontal ladder walking task (Metz and Whishaw, 2002). On each assessment day, five ladder crossings were video recorded and analyzed. Extra crossings were included if there were multiple stops or hesitations.

*Walking ability*. A foot fault scoring system (Metz and Whishaw, 2002) was used to assess (i) forelimb and hindlimb use (how the rat placed the foot on the rungs), (ii) forelimb and hindlimb placement accuracy (number of errors), and (ii) forepaw digit use (the way digits were placed on the rung). A 7-movement category was used to rate limb use: *(0) Total miss; (1) Deep slip; (2) Slight slip; (3) Replacement; (4) Correction; (5) Partial placement; (6) Correct placement*. Total errors