Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

Brain Research

Research Report

The persisting effects of electroconvulsive stimulation on the hippocampal proteome

Sinead M. O'Donovan[a], Shane O'Mara[a], Michael J. Dunn[b], Declan M. McLoughlin[a,c,*]

[a]Trinity College Institute of Neuroscience, Trinity College Dublin, Dublin 2, Ireland
[b]Conway Institute, University College Dublin, Belfield, Dublin 4, Ireland
[c]Department of Psychiatry, Trinity College Dublin, St. Patrick's University Hospital, James's Street, Dublin 8, Ireland

ARTICLE INFO

Article history:
Accepted 13 October 2014
Available online 22 October 2014

Keywords:
Electroconvulsive therapy
Electroconvulsive stimulation
2D-DiGE
LC-MS/MS
Actin

ABSTRACT

Electroconvulsive therapy (ECT) is the most acutely effective treatment available for severe depression. However, its mechanism of action is not fully understood. Elucidating the protein changes induced in the brain by ECT will enhance our understanding of this antidepressant therapy. Electroconvulsive stimulation (ECS), the animal analogue of ECT, was administered to rats to determine the proteomic changes induced in the hippocampus, a region of the brain implicated in the biology of depression and its treatment. Two-dimensional difference in gel electrophoresis (2D-DiGE) and liquid chromatography tandem mass spectrometry (LC-MS/MS) methods were applied to identify differentially expressed proteins following acute ($\times 1$ treatment), chronic ($\times 10$ treatments) or chronic$^{+4\ weeks}$ ($\times 10$ treatments plus 4 weeks later) ECS. Administration of acute, chronic and chronic$^{+4\ weeks}$ ECS induced significant changes in multiple DiGE gel protein spots. Interestingly, the largest number of differentially expressed protein spots was identified following chronic$^{+4\ weeks}$ ECS. Following protein identification by LC-MS/MS, gene ontology analysis primarily implicated proteins with cytoskeletal and metabolism-related roles in the action of ECS. Immunoblotting confirmed the changes in abundance of the cytoskeletal protein actin following chronic$^{+4\ weeks}$ ECS. Overall, chronic$^{+4\ weeks}$ ECS was particularly effective at inducing longer-lasting changes in the abundance of hippocampal proteins with cytoskeletal and metabolism roles. These results suggest a role for persisting cytoskeletal-related neuroplastic changes in the action of ECS and may be informative as to the antidepressant mechanisms of ECT in patients with depression.

*Corresponding author at: Department of Psychiatry, Trinity College Dublin, St. Patrick's University Hospital, James's Street, Dublin 8, Ireland.
E-mail address: d.mcloughlin@tcd.ie (D.M. McLoughlin).

http://dx.doi.org/10.1016/j.brainres.2014.10.020
0006-8993/

1. Introduction

Mental disorders accounted for 7.4% of total disease burden in 2010 with depression expected to be the world's second leading contributor to the global burden of disease by 2020 (Whiteford et al., 2013; World Health Organisation, 2011). Electroconvulsive therapy (ECT) has been available to patients for over 75 years and is recognised as the most acutely effective treatment available for severe depression. ECT is often administered to antidepressant-drug resistant patients and has been reported to be more effective than pharmacotherapy at reducing depressive symptoms (UK ECT Review Group, 2003).

The traditional proteomic methods of two dimensional poly-acrylamide gel electrophoresis (PAGE) (2D-PAGE) and 2D-DiGE are powerful techniques that have successfully identified large numbers of brain proteins in previous studies exploring the effects of antidepressant action. Studies of molecular changes induced by different types of antidepressant treatments, including pharmacotherapy (Marais et al., 2009), herbal antidepressants (Pennington et al., 2009) and exercise (Kirchner et al., 2008), have highlighted a range of brain protein abundance changes that may be of relevance in elucidating antidepressant mechanisms of action. Interestingly, cytoskeletal proteins and proteins involved in metabolic processes are among those most commonly identified. They appear to be of particular relevance when investigating antidepressant action of pharmacotherapies, including fluoxetine and nortriptyline (Guest et al., 2004; Piubelli et al., 2011a, 2011b).

The antidepressant impact of ECT on the brain can be studied using an animal analogue of treatment, electroconvulsive stimulation (ECS). The utility of ECS as a model for antidepressant treatment has been well established using behavioural, cellular and molecular measures (Li et al., 2007; O'Donovan et al., 2012, 2014). Proteomic analysis of the effects of ECS on the brain has only been studied to date in whole brain tissue (Lee et al., 2009), limiting the interpretation of the relevance of the identified proteins in ECS action. The hippocampus is one of the core brain regions implicated in depression and is among the best studied of the brain regions that may be relevant to antidepressant action (Campbell and Macqueen, 2004; Maletic et al., 2007). The global protein changes in brain regions that are implicated in depression, and may play a fundamental role in ECS antidepressant action, are not yet known.

Chronic ECT is an efficacious antidepressant treatment (UK ECT Review Group, 2003); however the acute and persisting effects of ECT are not as well studied. The first signs of patient improvement may be seen after a single ECT treatment (Moksnes and Ilner, 2010) while the long-term effects of chronic ECT result in effective treatment with a relapse rate of approximately 37% at 6 months (Jelovac et al., 2013).

The purpose of the present study, therefore, was to identify protein changes in rodent hippocampus that contribute to the acute, chronic and persisting effects of ECS.

2. Results

The number of protein spots found to have significantly altered levels following acute and chronic ECS is shown in Fig. 1. Applying univariate and multivariate analyses resulted in the comprehensive detection of spots that underwent changes following treatment. Univariate analysis detected 65 protein spots and multivariate analysis detected 27 protein spots where the levels were significantly differentially altered following acute ECS. Eighteen of these proteins were common to both analyses. Following chronic ECS administration, univariate analysis calculated 44 statistically significantly altered protein spots; 32 protein spots were highlighted following multivariate analysis and 16 spots were found after both analyses. In the chronic$^{+4\ weeks}$ group, four weeks after completion of the chronic ECS treatment, 128 spots were determined to be statistically significant after univariate analysis, 163 after multivariate analysis and 105 were common to both analyses.

2.1. *Protein spot selection for identification by mass spectrometry*

Protein spots identified as significant by both univariate and multivariate analyses were prioritised for identification by mass spectrometry where possible. A protein spot value of $p<0.05$ following one-way ANOVA or spot identification as a significant contributor to data variability by PLS-DA was necessary for selection for identification. The accuracy with which the spots could be picked from preparative 2D SDS-PAGE gels was a limiting factor when selecting spots for

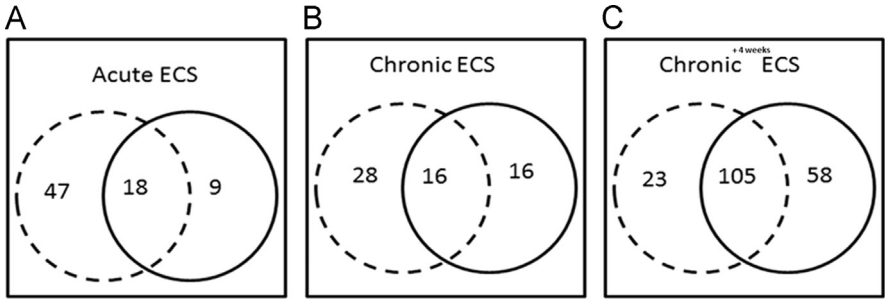

Fig. 1 – Protein spots identified by proteomic analysis. The numbers of altered protein spots identified by univariate analysis (broken-line circle), multivariate analysis (unbroken circle) or by both methods (overlapping sections) in animals treated with acute (A) and chronic ECS (B, C).