International Journal of Neuropsychopharmacology (2012), **15**, 1541–1546. © CINP 2012
doi:10.1017/S1461145712000272

BRIEF REPORT

CINP

Early increase in marker of neuronal integrity with antidepressant treatment of major depression: ^{1}H-magnetic resonance spectroscopy of *N*-acetyl-aspartate

Matthew J. Taylor[1,3], Beata R. Godlewska[1], Ray Norbury[1,2], Sudhakar Selvaraj[1,3], Jamie Near[1] and Philip J. Cowen[1]

[1] *University Department of Psychiatry, Warneford Hospital Oxford, UK*
[2] *Oxford Centre for Clinical Magnetic Resonance Research (OCMR), John Radcliffe Hospital, Oxford, UK*
[3] *Department of Psychosis Studies, Institute of Psychiatry, King's College, London, UK*

Abstract

Increasing interest surrounds potential neuroprotective or neurotrophic actions of antidepressants. While growing evidence points to important early clinical and neuropsychological effects of antidepressants, the time-course of any effect on neuronal integrity is unclear. This study used magnetic resonance spectroscopy to assess effects of short-term treatment with escitalopram on *N*-acetyl-aspartate (NAA), a marker of neuronal integrity. Thirty-nine participants with major depression were randomly assigned to receive either 10 mg escitalopram or placebo daily in a double-blind, parallel group design. On the seventh day of treatment, PRESS data were obtained from a $30 \times 30 \times 20$ mm voxel placed in medial frontal cortex. Age and gender-matched healthy controls who received no treatment were also scanned. Levels of NAA were significantly higher in patients treated with escitalopram than in either placebo-treated patients ($p < 0.01$) or healthy controls ($p < 0.01$). Our findings are consistent with the proposition that antidepressant treatment in depressed patients can produce early changes in neuronal integrity.

Received 30 November 2011; Reviewed 4 January 2012; Revised 23 February 2012; Accepted 27 February 2012; First published online 26 March 2012

Key words: Antidepressant, depression, magnetic resonance spectroscopy, *N*-acetyl-aspartate.

Introduction

There is increasing interest in the potential neuroprotective or neurotrophic action of antidepressant treatments (Schmidt & Duman, 2007). *N*-acetyl-aspartate (NAA) is a marker of neuronal integrity, synthesized within neuronal mitochondria (Moffett *et al.* 2007), that can readily be measured using the safe, non-invasive technique of magnetic resonance spectroscopy (MRS). If antidepressant treatment acts via a neurotrophic mechanism, increased levels of NAA might be observed. Indeed, in animal studies, it has been found that antidepressant treatment can prevent stress-induced decreases in NAA (van der Hart *et al.* 2002). In addition, some clinical reports have noted increases in NAA in frontal cortex with antidepressant treatment over periods of several weeks (Gonul *et al.* 2006; Huang *et al*. 2010; Jang *et al.* 2006) although there are also some negative findings (Henigsberg *et al*. 2011; Kaymak *et al*. 2009).

Clinical trial evidence shows that antidepressants such as selective serotonin reuptake inhibitors (SSRIs) have an early onset of clinical effects detectable with standard rating scales within 1 wk (Taylor *et al.* 2006). Consistent with this, early effects on behavioural and neural aspects of emotional processing are observed after even a few days administration of antidepressants (Harmer *et al.* 2004). If neurotrophic effects are mechanistically important in therapeutic response, they should also be evident after short-term treatment of depression with an SSRI. Escitalopram is a clinically effective antidepressant and the most selective of the SSRIs for the serotonin transporter. We hypothesized that 7 d treatment with escitalopram

Address for correspondence: Dr M. J. Taylor, Institute of Psychiatry, Department of Psychosis Studies PO63, London SE5 8AF, UK.
Tel.: +44 20 7848 0363 *Fax*: +44 20 7848 0976
Email: matthew.j.taylor@kcl.ac.uk

would lead to an increase in cortical NAA levels. Accordingly, the aim of the present study was to compare the neurochemistry of the medial prefrontal cortex in two groups of depressed patients, one unmedicated and one who had received short-term treatment with the SSRI, escitalopram.

Method

Design

Drug-free depressed patients were randomly assigned to receive 10 mg escitalopram or placebo daily for 7 d in a double-blind, parallel group design. Both groups were then scanned on the seventh day of treatment. The escitalopram dose was chosen as this is the manufacturer's recommended treatment dose for major depressive episodes. The elimination half-life after multiple dosing is about 30 h. Ratings of mental state were obtained using the 17-item Hamilton Depression Rating Scale (HAMD-17; Hamilton, 1967), Beck Depression Inventory (BDI; Beck *et al.* 1961) and the State-Trait Anxiety Inventory (Spielberger *et al.* 1970). An age and gender-matched group of healthy controls were scanned on a single occasion and received no treatment.

Participants

Thirty-nine participants with major depression and 27 healthy controls completed the MRS protocol. Controls were recruited through university volunteer lists and advertising in local newspapers. Depressed patients were recruited from a number of sources, including newspaper advertisements as well as referral from other clinicians. All participants were assessed for the presence of current and past psychiatric disorder with the Structured Clinical Interview for DSM-IV (First *et al.* 2002). The depressed patients met criteria for a primary diagnosis of major depressive disorder. Exclusion criteria for the study were as follows. For depressed patients: suffering from psychosis or substance dependence as defined by DSM-IV; being at clinically significant risk of suicidal behaviour; having contraindications to escitalopram treatment; or being treated with psychotropic medication <3 wk before the study (5 wk in the case of fluoxetine). For healthy volunteers: current or past history of Axis I disorder as defined by DSM-IV. For both groups: major somatic or neurological disorders; pregnancy or breast-feeding; contraindications to magnetic resonance imaging or concurrent medication, which could alter emotional processing. No minimum HAMD-17 score was required. The study was approved by the Oxford Research Ethics Committee and all participants gave written informed consent. Participants were reimbursed for their time and any other expenses.

MRS methodology

Scanning was performed on a 3T Siemens Trio (Erlangen, Germany) system with a head gradient coil (Magnex Scientific, Oxford) and a 12 channel head-only receive array RF coil. Data were acquired from a $30 \times 30 \times 20$ mm voxel placed in the medial prefrontal cortex anterior to the genu of the corpus callosum (Supplementary Fig. S1). The voxel was positioned manually by reference to an axial T_1-weighted gradient-echo image.

PRESS data (Bottomley, 1987) with water suppression were acquired (TE 30 ms, TR 3 s, 64 averages). PRESS data were analysed with LCModel (Provencher, 1993) using a set of simulated metabolite basis spectra. Concentrations relative to creatine (Cr) were obtained for NAA, the total of NAA and *N*-acetylaspartylglutamic acid (NAA + NAAG), choline, inositol and total combined glutamate and glutamine (Glx). Concentration estimates with Cramer–Rao lower bounds (CRLB) $>20\%$ were rejected as unreliable. In a subset of participants (29 patients, 15 controls) PRESS-J data (Hurd *et al.* 2004) were also acquired (TE 35–185 ms, 10 ms increments, total 128 acquisitions, TR 3 s). Estimates of glutamate relative to Cr were obtained using AMARES (Vanhamme *et al*. 1997). Estimates with damping factor >55 were excluded. T_1-weighted structural images of whole brain were acquired with 1 mm³ voxel resolution. FSL FAST was employed to segment the structural brain images into grey matter, white matter and cerebrospinal fluid, to allow estimation of voxel composition. Group differences were tested using the general linear model in PASW Statistics v18 (SPSS Inc., USA) employing analysis of variance or analysis of covariance as appropriate with *post-hoc t* test. Results were tested for sensitivity to the inclusion or exclusion or age, gender and voxel grey matter content as covariates. A conventional two-tailed statistical significance threshold of $p = 0.05$ was applied throughout.

Results

The three groups were demographically similar (Table 1). After 1 wk of treatment, there was no significant difference in rating scale scores between those patients receiving placebo and those receiving

Table 1. Participant characteristics

	Placebo ($n=19$)	Escitalopram ($n=20$)	Healthy ($n=27$)
Age, yr	32.4 (12.0) range 18–56	30.6 (8.5) range 19–51	31.4 (11.1) range 19–58
Gender	9 male/10 female	8 male/12 female	9 male/18 female
HAMD-17: baseline	23.2 (4.6)	24.2 (5.7)	0.5 (0.7)
7 d	20.4 (4.2)	19.3 (7.9)	–
BDI: baseline	31.5 (9.7)	30.8 (9.6)	1.1 (1.3)
7 d	25.1 (9.0)	24.8 (12.0)	–
STAI state: baseline	49.2 (17.7)	47.5 (23.6)	27 (5.3)
7 d	47.3 (10.5)	49.9 (11.8)	–
Voxel grey-matter percentage	43 (5)	43 (4)	42 (5)
Voxel CSF percentage	21 (5)	22 (3)	21 (4)

HAMD-17, Hamilton Depression Rating Scale; BDI, Beck Depression Inventory; STAI, State-Trait Anxiety Inventory; CSF, cerebrospinal fluid.
Values are mean (S.D.).
No significant differences between patient groups.

escitalopram (HAMD-17 $p=0.59$; BDI $p=0.93$; state anxiety $p=0.49$; Table 1).

A significant effect of group was seen on NAA+NAAG/Cr (placebo 1.07 ± 0.16, escitalopram 1.18 ± 0.18, healthy 1.06 ± 0.16, $p=0.03$) and NAA/Cr (placebo 0.99 ± 0.16, escitalopram 1.13 ± 0.19, healthy 0.96 ± 0.18, $p<0.01$; Fig. 1) but not on other metabolites measured ($p>0.35$ in all cases). Levels of NAA/Cr were higher in patients randomized to 7 d treatment with escitalopram than in those receiving placebo ($p<0.01$) or in healthy controls ($p<0.01$). The same effect was seen with the more conservative NAA+NAAG/Cr measure ($p<0.05$ both cases). There was no difference between NAA/Cr or NAA+NAAG/Cr levels between the placebo group and healthy controls ($p>0.7$ both cases).

A small but statistically significant inverse effect of age on NAA/Cr was observed ($r=-0.26$, $p=0.03$) across the entire group. No age × group interaction on NAA/Cr was observed and the group effect remained with age as a covariate ($p<0.01$). No effect was seen of age on NAA+NAAG/Cr ($p=0.19$). No group effect on Glx/Cr was observed, but there was an inverse correlation between baseline depression rating scale score and Glx/Cr in the depressed group ($r=-0.39$, $p=0.015$).

Metabolite estimates were obtained with a high degree of confidence. Mean CRLB for NAA+NAAG/Cr estimates was 3.65% and for NAA/Cr estimates was 4.94% (see Supplementary Table S1).

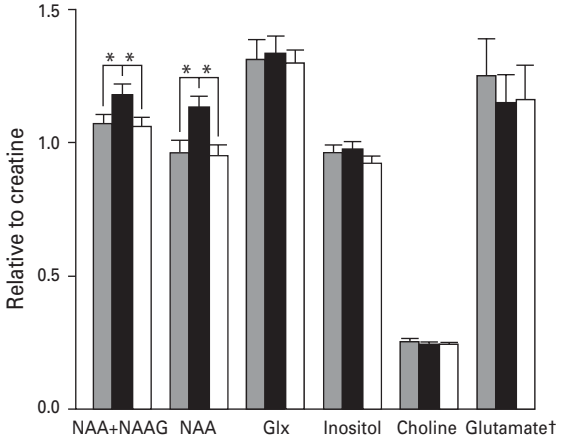

Fig. 1. Concentrations of magnetic resonance spectroscopy measures. Concentrations of *N*-acetyl-aspartate (NAA)+*N*-acetylaspartylglutamic acid (NAAG), NAA alone, total combined glutamate and glutamine (Glx), inositol, choline and glutamate are shown for depressed patients receiving placebo ($n=19$, ▒), depressed patients receiving escitalopram ($n=20$, ■) and healthy controls ($n=27$, □). Mean with S.E.M. * $p<0.05$ *post-hoc t* test; † glutamate data from subset (placebo $n=16$, escitalopram $n=13$, control $n=15$).

Discussion

The main finding of this study was that after 7 d treatment of depression with escitalopram, elevated levels of NAA are observed in the medial frontal cortex. As NAA is one of the major peaks on proton MRS spectrum (Supplementary Fig. S1), quantitation can be

achieved with high accuracy, lending confidence to this result.

An increase in NAA in similar brain regions has been reported after longer-term response to a variety of antidepressants for major depression (Gonul *et al.* 2006) and after 12 wk treatment of obsessive–compulsive disorder with citalopram (Jang *et al.* 2006). However, not all have reported similar findings (Henigsberg *et al.* 2011; Kaymak *et al.* 2009), suggesting that patient heterogeneity might be a factor. For example, a significant proportion of our patients were drug naive and none was treatment-resistant. It should also be noted that we did not observe any effect on NAA in previous studies of citalopram in healthy volunteers (Taylor *et al.* 2008, 2010), which suggests there may be an interaction of treatment and underlying disease factors.

No difference in NAA levels was seen here between placebo-treated depressed patients and healthy controls. A lack of effect of depression itself on observed NAA levels is consistent with many prior studies that have found no significant effect of depression on NAA in this region (Yildiz-Yesiloglu & Ankerst, 2006). More recently, some studies have found decreased NAA in medial prefrontal cortex in particular populations. One study found decreased NAA + NAAG in chronic and relapsing–remitting subgroups, but not in the early phases of the illness (Portella *et al.* 2011). Decreased NAA was also found in a group of patients prior to electroconvulsive therapy treatment (Merkl *et al.* 2010). Thus, as with the effects of treatment on NAA, it is possible that a difference could have been observed if the patients studied had different clinical characteristics.

NAAG is a neuropeptide synthesized from NAA and glutamate and also found localized within neurons (Moffett *et al.* 2007). There is substantial overlap of MRS signal from NAA and NAAG. While the total combined signal is readily measured using MRS, errors in correctly attributing components of that signal to NAA or NAAG are a possible cause of erroneous findings. In these data, effects were observed whether NAA or NAA + NAAG was considered, which lends support to the effect seen.

The effect here was demonstrated in medial prefrontal cortex. Future studies could investigate a wider range of brain structures because effects on hippocampal and amygdala function are likely to be important in antidepressant actions (Schmidt & Duman, 2007). While one recent study of paroxetine for generalized anxiety found no effect on hippocampal NAA levels (Mathew *et al.* 2010), increases in hippocampal NAA with extended SSRI treatment have now been described in other patient populations (Duan *et al.* 2011; Huang *et al.* 2010).

This study reported concentrations relative to Cr, which is conventional. It is possible that apparent changes in NAA:Cr ratio could be explained by alterations in Cr levels. This seems unlikely to explain the effect seen here, since no change was observed in other metabolites, also referenced to Cr. Nevertheless, future studies could also reference concentrations to tissue water, to avoid this potential confound. The major methodological limitation of our study is the cross-sectional design. Thus, although we have a reasonable sample size, it is possible that the difference in NAA, which we detected at 7 d, is actually due to baseline differences between the subject groups. Clearly a further study with longitudinal design is necessary to control for this possibility and to allow for investigation of any link between the early neuronal effects observed here and eventual clinical response. Within these limitations, our findings are consistent with a neurotrophic hypothesis of antidepressant action.

Some investigations have reported lower levels of Glx in anterior brain regions in depressed patients but not all studies are in agreement (Yüksel & Öngür, 2010). No group effect on Glx was observed here, but the inverse correlation between depression severity and Glx suggests that an effect might have been seen if a more severely depressed group of patients had been studied. However, the correlation observed would not have survived correction for multiple comparisons, so interpretation of the finding needs to be cautious. We used PRESS-J to derive a measure of glutamate separate from Glx in a subgroup of patients but again found no difference between the depressed patients and controls. There is evidence from the study of Portella *et al.* (2011) that lowered Glx levels may be more apparent in patients with chronic depression, which might make such a change less likely to be detected in our particular patient group (see above). Better measures of glutamate in larger patient groups will be needed to address this issue more definitively.

The lack of effect of short-term SSRI treatment on glutamate and Glx in anterior brain regions is consistent with our previous studies in healthy volunteers (Taylor *et al.* 2010). Our findings are consistent with the proposition that antidepressant treatment in depressed patients can produce early changes in neuronal integrity. Future studies should clarify whether an early change in NAA could be a possible early biomarker of treatment effect in depressed patients.

Supplementary material

For supplementary material accompanying this paper, visit http://dx.doi.org/10.1017/S1461145712000272.

Acknowledgements

This study was funded by the Medical Research Council. Dr Taylor was a National Institute for Health Research Clinical Lecturer.

Statement of Interest

Professor Cowen has been a paid member of advisory boards of Eli Lilly, Servier, Wyeth and Xytis and has been a paid lecturer for Eli Lilly, Servier and GlaxoSmithKline. He has received remuneration for scientific advice given to legal representatives of GlaxoSmithKline. Dr Taylor's spouse is an employee of GlaxoSmithKline.

References

Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J (1961). An inventory for measuring depression. *Archives of General Psychiatry* **4**, 561–571.

Bottomley PA (1987). Spatial localization in NMR spectroscopy *in vivo*. *Annals of the New York Academy of Sciences* **508**, 333–348.

Duan D-M, Tu Y, Jiao S, Qin W (2011). The relevance between symptoms and magnetic resonance imaging analysis of the hippocampus of depressed patients given electro-acupuncture combined with fluoxetine intervention – a randomized, controlled trial. *Chinese Journal of Integrative Medicine* **17**, 190–199.

First MB, Spitzer RL, Gibbon M, Williams JBW (2002). *Structured Clinical Interview for DSM-IV-TR Axis I Disorders*. New York: Biometrics Research, New York State Psychiatric Institute.

Gonul AS, Kitis O, Ozan E, Akdeniz F, *et al*. (2006). The effect of antidepressant treatment on *N*-acetyl aspartate levels of medial frontal cortex in drug-free depressed patients. *Progress in Neuropsychopharmacology and Biological Psychiatry* **30**, 120–125.

Hamilton M (1967). Development of a rating scale for primary depressive illness. *British Journal of Social and Clinical Psychology* **6**, 278–296.

Harmer CJ, Shelley NC, Cowen PJ, Goodwin GM (2004). Increased positive *vs.* negative affective perception and memory in healthy volunteers following selective serotonin and norepinephrine reuptake inhibition. *American Journal of Psychiatry* **161**, 1256–1263.

Henigsberg N, Bajs M, Hrabac P, Kalember P, *et al*. (2011). Changes in brain metabolites measured with magnetic resonance spectroscopy in antidepressant responders with comorbid major depression and posttraumatic stress disorder. *Collegium Antropologicum* **35**, 145–148.

Huang Y, Chen W, Li Y, Wu X, *et al*. (2010). Effects of antidepressant treatment on *N*-acetyl aspartate and choline levels in the hippocampus and thalami of post-stroke depression patients: a study using (1)H magnetic resonance spectroscopy. *Psychiatry Research* **182**, 48–52.

Hurd R, Sailasuta N, Srinivasan R, Vigneron DB, *et al*. (2004). Measurement of brain glutamate using TE-averaged PRESS at 3T. *Magnetic Resonance in Medicine* **51**, 435–440.

Jang JH, Kwon JS, Jang DP, Moon W, *et al*. (2006). A proton MRSI study of brain *N*-acetylaspartate level after 12 weeks of citalopram treatment in drug-naive patients with obsessive-compulsive disorder. *American Journal of Psychiatry* **163**, 1202–1207.

Kaymak SU, Demir B, Oğuz KK, Sentürk S, Uluğ B (2009). Antidepressant effect detected on proton magnetic resonance spectroscopy in drug-naïve female patients with first-episode major depression. *Psychiatry and Clinical Neurosciences* **63**, 350–356.

Mathew SJ, Price RB, Shungu DC, Mao X, *et al*. (2010). A pilot study of the effects of chronic paroxetine administration on hippocampal *N*-acetylaspartate in generalized anxiety disorder. *Journal of Psychopharmacology* **24**, 1175–1181.

Merkl A, Schubert F, Quante A, Luborzewski A, *et al*. (2010). Abnormal cingulate and prefrontal cortical neurochemistry in major depression after electroconvulsive therapy. *Biological Psychiatry* **69**, 772–779.

Moffett JR, Ross B, Arun P, Madhavarao CN, Namboodiri AMA (2007). *N*-Acetylaspartate in the CNS: from neurodiagnostics to neurobiology. *Progress in Neurobiology* **81**, 89–131.

Portella MJ, de Diego-Adeliño J, Gómez-Ansón B, Morgan-Ferrando R, *et al*. (2011). Ventromedial prefrontal spectroscopic abnormalities over the course of depression: a comparison among first episode, remitted recurrent and chronic patients. *Journal of Psychiatry Research* **45**, 427–434.

Provencher SW (1993). Estimation of metabolite concentrations from localized *in vivo* proton NMR spectra. *Magnetic Resonance Medicine* **30**, 672–679.

Schmidt HD, Duman RS (2007). The role of neurotrophic factors in adult hippocampal neurogenesis, antidepressant treatments and animal models of depressive-like behavior. *Behavioural Pharmacology* **18**, 391–418.

Spielberger CD, Gorsuch RL, Lushene RE (1970). *The State-Trait Anxiety Inventory: Test Manual*. Palo Alto, CA: Consulting Psychologist Press.

Taylor M, Murphy SE, Selvaraj S, Wylezinska M, *et al*. (2008). Differential effects of citalopram and reboxetine on cortical Glx measured with proton MR spectroscopy. *Journal of Psychopharmacology* **22**, 473–476.

Taylor MJ, Freemantle N, Geddes JR, Bhagwagar Z (2006). Early onset of selective serotonin reuptake inhibitor antidepressant action: systematic review and meta-analysis. *Archives of General Psychiatry* **63**, 1217–1223.

Taylor MJ, Norbury R, Murphy S, Rudebeck S, *et al*. (2010). Lack of effect of citalopram on magnetic resonance spectroscopy measures of glutamate and glutamine in frontal cortex of healthy volunteers. *Journal of Psychopharmacology* **24**, 1217–1221.

van der Hart MGC, Czéh B, de Biurrun G, Michaelis T, *et al*. (2002). Substance P receptor antagonist and clomipramine prevent stress-induced alterations in cerebral metabolites, cytogenesis in the dentate gyrus and hippocampal volume. *Molecular Psychiatry* **7**, 933–941.

Vanhamme L, van den Boogaart A, Van Huffel S (1997). Improved method for accurate and efficient quantification of MRS data with use of prior knowledge. *Journal of Magnetic Resonance* **129**, 35–43.

Yildiz-Yesiloglu A, Ankerst DP (2006). Review of 1H magnetic resonance spectroscopy findings in major depressive disorder: a meta-analysis. *Psychiatry Research* **147**, 1–25.

Yüksel C, Öngür D (2010). Magnetic resonance spectroscopy studies of glutamate-related abnormalities in mood disorders. *Biological Psychiatry* **68**, 785–794.