Multiple system atrophy (MSA) is a progressive neurodegenerative disease that clinically presents with parkinsonian, cerebellar, and autonomic features. α-Synuclein (αsyn), encoded by the gene SNCA, is the main constituent of glial cytoplasmic inclusion (GCI) found in oligodendrocytes in MSA, but the methods of its accumulation have not been established. The aim of this study is to investigate alterations in regional and cellular SNCA mRNA expression in MSA as a possible substrate for GCI formation. Quantitative reverse transcription polymerase chain reaction (qPCR) was performed on postmortem brain samples from 15 MSA, 5 IPD, and 5 control cases to investigate regional expression in the frontal and occipital regions, dorsal putamen, pontine base, and cerebellum. For cellular expression analysis, neurons and oligodendrocytes were isolated by laser-capture microdissection from five MSA and five control cases. SNCA mRNA expression was not significantly different between the MSA, IPD and control cases in all regions (multilevel model, \( P < 0.14 \)). After adjusting for group effect, the highest expression was found in the occipital cortex while the lowest was in the putamen (multilevel model, \( P < 0.0001 \)). At the cellular level, MSA oligodendrocytes expressed more SNCA than control oligodendrocytes and expression in MSA neurons was slightly lower than that in controls, however, these results did not reach statistical significance. We have demonstrated regional variations in SNCA expression, which is higher in cortical than subcortical regions. This study is the first to demonstrate SNCA mRNA expression by oligodendrocytes in human postmortem tissue using qPCR and, although not statistically significant, could suggest that this may be increased in MSA compared to controls.

Key words: α-synuclein, multiple system atrophy, oligodendrocytes, glial cytoplasmic inclusions, laser-capture microdissection
The expression profile of the aim of this study was to investigate the regional and cellular expression of proteins relating to MSA. Overexpression in oligodendrocytes (Jin et al. 2008; Kingsbury et al. 2004; Miller et al. 2005; Solano et al. 2000). Unravelling the cellular expression profile of proteins in MSA will help to shed light on the possible contribution of overexpression in oligodendrocytes as a mechanism for GCI formation. Therefore, the aim of this study was to investigate the regional and cellular expression profile of SNCA in MSA subtypes and controls using qPCR.

### Materials and Methods

#### Tissue Collection

Samples were obtained from brains donated to the Queen Square Brain Bank (QSSBB) for Neurological Disorders, Department of Molecular Neuroscience, UCL Institute of Neurology, London. The donations were made according to ethically approved protocols, and tissue is stored under a license issued by the Human Tissue Authority (No. 12198). One half of the brain is sliced in the coronal plane, flash frozen, and stored at −80°C on arrival to the QSSBB, while the other half is fixed in 10% buffered formalin. Tissue was sampled from the frozen half brain.

#### Case Selection

MSA cases were pathologically typed based on published criteria into mixed, SND and OPCA subtypes (Ozawa et al. 2004). Five MSA-SND, five MSA-OPCA, five MSA-mixed subtypes were selected and sex and age matched to five IPD and four normal control cases to study regional zsyn mRNA expression (Table 1). For cellular mRNA expression, five MSA-mixed subtype and six neurologically normal controls were used to isolate neurons and oligodendrocytes (Table 2).

#### Quantitative PCR

qPCR was performed on Stratagene MX3000p (Agilent technologies, CA) using 50-ng cDNA and Power SYBR Green Master Mix (Applied Biosystems) according to the manufacturer's instructions. All primers were run at the following thermal profile: one cycle at 95°C for 10 min followed by 40 cycles at 95°C for 15 min and

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**Regional Sampling, RNA Extraction, and Reverse Transcription**

Frozen tissue (~100 mg) from the posterior frontal region (cortex and subcortical white matter), occipital region (cortex and subcortical white matter), dorsal putamen, pontine base, and cerebellum (white matter) was collected and homogenised using TissueRuptor (Qiagen, Germany). RNA extraction was carried out using RNasy Mini Kit (Qiagen, Germany) and cDNA synthesized using SuperScript® VILO™ cDNA Synthesis Kit (Invitrogen, UK) according to the manufacturer's instructions.

**Cellular Sampling, RNA Extraction, and Reverse Transcription**

Neurons from the pontine base (~1,000 cells) and oligodendrocytes from the cerebellar white matter (~1,000 cells) were isolated in five MSA and six control cases using the PixCell II laser-capture microdissection (LCM) system (Arcurtus Engineering, Mountain View, CA). Neurons were identified using toluidine blue stain and oligodendrocytes using a rapid immunohistochemistry (IHC) protocol as previously described (Waller et al. 2012). Briefly, 7-μm frozen section collection was carried out on uncharged, sterile glass slides and warmed to RT for 30 sec. The sections were then fixed in ice-cold acetone for 3 min and immunostained using oligodendrocyte-specific protein (OSP) primary antibody (Abcam, UK, ab7474) and VECTASTAIN Elite ABC kit (Vector labs, Burlingame, CA, PK-6101). Sections were blocked with normal goat serum (2%) for 3 min then incubated in OSP (1:10) for 3 min at RT. After a brief rinse with tris-buffered saline (TBS), sections were incubated in 5% biotinylated secondary antibody for 3 min at RT, then rinsed again with TBS. The avidin–biotin complex solution was then added to the section for 3 min at RT then rinsed off. DAB peroxidase substrate kit (Vector labs, Burlingame, CA, SK-4100) was used to visualize the reaction by incubating section for 3 min at RT. The sections were rinsed with TBS, dehydrated in graded alcohol (70, 95, 100, 100% for 1 min each), cleared in two changes of xylene and allowed to air dry in an air-flow hood for an hour prior to LCM. This rapid IHC protocol was carried out under sterile conditions and using diethylpyrocarbonate (DEPC)-treated water. RNA extraction of LCM samples was carried out using the Arcurtus PicoPure RNA isolation kit (Applied Biosystems, UK, KIT0204) and cDNA synthesized using SuperScript® VILO™ cDNA Synthesis Kit (Invitrogen, UK) according to manufacturer's instructions. RNA quality and concentration was then assessed using the Nanodrop and Agilent Bioanalyzer 2100. Maintenance of RNA quality was adequate as assessed by the RIN of samples before and after LCM was performed. Prior to LCM, samples had a mean RIN of 4.7 (range 4.2–5.8) declining to 2.6 (range 2.0–3.1) after LCM and this is in keeping with previous findings (Waller et al. 2012).
6°C for 1 min. Three reference genes, UCB, TBP, and GAPDH, were determined as appropriate to normalize regional expression data using qBase plus software (Biogazelle, Belgium). Due to limited sample volume, one reference gene (UBC) was used to normalize LCM expression data. Primers for the gene of interest (GOI), SNCA, were designed in house while the reference genes were obtained from RTprimerDB (Table 3).

Expression Analysis and Statistics
Standard curves were used to extrapolate expression value of each gene in each region or cell. The GOI was then normalized to the geometric mean of the reference genes as recommended by Vandesompele et al. (2002). A multilevel statistical model was used for regional expression analysis while the Mann–Whitney U test was used to analyze cellular expression results with the significance levels set at $P < 0.05$.

Results
Regional SNCA mRNA expression was examined in the posterior frontal region, occipital region, dorsal putamen, pontine base, and cerebellar white matter of three MSA subtypes (mixed, SND, and OPCA), IPD, and control cases (Fig. 1). SNCA expression level was highest in IPD and lowest in MSA-SND, however, data analysis using a multilevel statistical model showed that there was no statistically significant difference between the different groups (multilevel statistical model, $P = 0.14$). After adjusting for group effect, the highest expression was found in the occipital cortex while the lowest was in the putamen. The differences between regions were found to be statistically significant (multilevel statistical model, $P < 0.0001$).

| Case | Age | Sex | Diagnosis | MSA pathological type | PMI (hr) |
|------|-----|-----|-----------|-----------------------|----------|
| 1    | 56  | F   | MSA       | Mixed                | 28       |
| 2    | 62  | M   | MSA       | Mixed                | 20       |
| 3    | 64  | F   | MSA       | Mixed                | 52       |
| 4    | 66  | M   | MSA       | Mixed                | 105      |
| 5    | 70  | F   | MSA       | Mixed                | 65       |
| 6    | 50  | F   | MSA       | SND                  | 4        |
| 7    | 54  | F   | MSA       | SND                  | 47       |
| 8    | 58  | F   | MSA       | SND                  | 11       |
| 9    | 63  | M   | MSA       | SND                  | 27       |
| 10   | 72  | M   | MSA       | SND                  | 50       |
| 11   | 56  | M   | MSA       | OPCA                 | 37       |
| 12   | 60  | F   | MSA       | OPCA                 | 62       |
| 13   | 61  | M   | MSA       | OPCA                 | ND       |
| 14   | 64  | M   | MSA       | OPCA                 | 35       |
| 15   | 66  | F   | MSA       | OPCA                 | 74       |
| 16   | 61  | F   | IPD       | NA                   | 26       |
| 17   | 63  | M   | IPD       | NA                   | 37       |
| 18   | 65  | M   | IPD       | NA                   | 43       |
| 19   | 67  | F   | IPD       | NA                   | 108      |
| 20   | 70  | M   | IPD       | NA                   | 75       |
| 21   | 57  | M   | Control   | NA                   | 79       |
| 22   | 69  | M   | Control   | NA                   | ND       |
| 23   | 71  | M   | Control   | NA                   | 39       |
| 24   | 73  | F   | Control   | NA                   | 24       |

NA: not applicable; ND: not documented; PMI: post-mortem interval.
Next SNCA expression in neurons and oligodendrocytes isolated by laser capture was explored in the pontine base and cerebellar white matter, respectively, as these regions are affected in MSA. SNCA expression was detected in neurons and oligodendrocytes of both MSA and control cases, with the highest level of expression being found in MSA oligodendrocytes (Fig. 2A). The fold changes in mRNA expression between the different cell types and groups were also calculated (Fig. 2B). Although none of the differences identified reached statistical significance, there was a slight increase in expression in MSA oligodendrocytes as compared to control oligodendrocytes (fold change = 0.7, Mann–Whitney U test, \( P = 0.18 \)). Expression in MSA neurons was slightly decreased as compared to control neurons (fold change = -0.4, Mann–Whitney U test, \( P = 0.46 \)). Comparing the different cell types within the same groups revealed that control oligodendrocytes express 0.3 more SNCA than control neurons (Mann–Whitney U test, \( P = 0.46 \)). The greatest difference was seen between MSA oligodendrocytes and neurons where expression in MSA oligodendrocytes was 3.1 times higher than MSA neurons (Mann–Whitney U test, \( P = 0.16 \)).

**Discussion**

\( \alpha \)-Syn has been localized in the brain to neuronal presynaptic terminals and may play a role in neuronal plasticity, vesicular transport, and membrane interaction (Iwai et al. 1995; Kahle et al. 2000; Reynolds et al. 2011). There has been great interest in the possible mechanisms by which \( \alpha \)-Syn forms primarily oligodendrogial inclusions in MSA since its identification as the main constituent of the GCIs. Understanding the expression pattern of SNCA in MSA at both regional and cellular levels is essential to determine if changes in expression play a role in MSA pathogenesis and whether this influences regional vulnerability to disease. The regional expression analysis in this study now demonstrates that SNCA expression level varies across different brain regions in control, MSA, and IPD groups. The greatest expression is found in the occipital grey matter and the lowest level in the putamen. However, SNCA mRNA expression is not significantly different between the MSA subgroups, IPD and control cases in all regions. The expression trend found in MSA and control cases used in this study is similar to that documented in expression databases such as UKBEC and Allen Brain Atlas, which analyze gene expression in control human brains (Hawrylycz et al. 2012; Trabzuni et al. 2011). The similarity in trend, where SNCA is generally expressed more in cortical regions than subcortical and cerebellar regions, has at least two main implications. The first is that the vulnerability of StrN and OPC regions is not associated with a higher baseline expression of SNCA. It could be hypothesized that the concentration of GCIs in specific regions is due to higher expression levels of SNCA in normal conditions in those regions that is exacerbated by disease. Our data do not support this hypothesis as SNCA mRNA levels are higher in cortical than subcortical regions in both normal and disease cases, while GCI pathology is greater in subcortical and hindbrain structures such as the StrN and OPC regions in MSA. This also indicates that the disease does not cause an alteration in expression levels in areas of greater vulnerability. The absence of a difference in SNCA expression levels between the different MSA groups, IPD, and controls may imply that factors

| Case | Age (yr) | Sex | Diagnosis | MSA pathological type | PMI (hr) | RIN before LCM | RIN after LCM |
|------|----------|-----|-----------|-----------------------|----------|----------------|--------------|
| 1    | 50       | M   | MSA       | Mixed                 | 30       | 4.8            | 2.6          |
| 2    | 70       | F   | MSA       | Mixed                 | 65       | 5.8            | ND           |
| 3    | 56       | M   | MSA       | Mixed                 | 75       | 4.7            | ND           |
| 4    | 64       | F   | MSA       | Mixed                 | 99       | 4.4            | 2.8          |
| 5    | 64       | M   | MSA       | Mixed                 | 100      | 5.1            | 3.1          |
| 6    | 69       | M   | Control   | NA                    | ND       | 4.4            | ND           |
| 7    | 82       | F   | Control   | NA                    | ND       | 4.8            | 3.6          |
| 8    | 73       | F   | Control   | NA                    | 24       | 5.3            | 2.0          |
| 9    | 85       | M   | Control   | NA                    | 78       | 4.2            | 2.2          |
| 10   | 80       | F   | Control   | NA                    | 49       | 3.9            | 2.5          |
| 11   | 83       | M   | Control   | NA                    | 63       | 4.2            | 2.3          |

NA: not applicable; ND: not documented; PMI: post-mortem interval.
contributing to pathogenic mechanisms of MSA may be further downstream and not at the transcriptional level and could suggest that impaired αsyn degradation may contribute to the disease. Another possibility is that any differences at the transcriptional level are masked by the cellular heterogeneity of the samples. To overcome this problem, we used LCM to obtain samples highly enriched in neurons and oligodendrocytes.

It has long been suggested that mature oligodendrocytes lack αsyn (Jin et al. 2008; Miller et al. 2005; Solano et al. 2000). However, cell-culture evidence indicates that rat and mouse oligodendrocytes express αsyn protein and mRNA, and in some cases this expression is transient, appearing in precursor cells and declining as they mature (Culvenor et al. 2002; Nielsen et al. 2006; Richter-Landsberg et al. 2000). To date, there is one published study addressing the question of SNCA mRNA expression in MSA oligodendrocytes (Miller et al. 2005). This study used double-labeling in situ hybridization of SNCA and proteolipid protein (PLP), an oligodendrocyte marker, and showed that SNCA is not expressed in oligodendrocytes in MSA or control brains. As ISH has limited sensitivity, the findings of this study do not exclude the possibility of SNCA mRNA expression by oligodendrocytes (Deglincerti and Jaffrey 2012; Miller et al. 2005).

Analyzing SNCA expression in LCM-isolated neurons and oligodendrocytes, our data indicate that this highly conserved protein is expressed in human oligodendrocytes and that expression in MSA oligodendrocytes is greater than control oligodendrocytes. In our hands, LCM isolation of OSP-positive oligodendrocytes provides an oligodendrocyte-enriched sample (Waller et al. 2012). This suggests that SNCA overexpression in MSA oligodendrocytes may be a possible mechanism contributing to GCI formation. The cellular expression of αsyn in MSA in this study may also explain the pathological profile of the disease. GCIs are found in greater abundance than NCIs in MSA, and this greater susceptibility of oligodendrocytes to inclusion formation may be a reflection of overexpression of αsyn by oligodendrocytes in MSA.

The novel findings of this LCM study demonstrating expression of SNCA mRNA in oligodendrocytes open the door to further exploration of the molecular pathogenesis of MSA. Expanding the current study to examine cellular SNCA mRNA expression in other affected and unaffected brain regions in MSA may help to elucidate the selective vulnerability of StrN and OPC regions. In addition to affected and unaffected brain regions, examination of SNCA expression in MSA cases with short disease duration and long disease duration may provide further insight into the role of SNCA mRNA expression on clinical outcome. A further step would be to characterize SNCA mRNA expression.
in oligodendrocytes with and without GCIs to determine the contribution of SNCA expression to inclusion formation in oligodendrocytes. In addition to GCI+/- oligodendrocytes, SNCA mRNA expression may also be explored at different stages of oligodendrocyte maturation. The oligodendrocyte lineage comprises precursor, immature, mature nonmyelinating, and mature myelinating cells and zsyn protein in GCIs has been found in mature but not pre-

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FIGURE 1: SNCA mRNA regional expression (A) Expression between the different cases (adjusted for regions) shows no statistically significant differences between the groups although the lowest level of expression was found in MSA-SND and the highest in IPD cases (P = 0.14). (B) After adjusting for case type, a statistically significant difference in SNCA expression between regions was found (P < 0.0001), with the lowest expression in the dorsal putamen and the highest in the occipital cortex. Multi-level model test with significance level set at P = 0.05. The boxplot show median values as the line within the box, the box reflects the interquartile range and the whiskers the range of the values. PFR: posterior frontal region; OR: occipital region; Put-D: dorsal putamen; CBM: cerebellum; Ctx: cortex; WM: white matter; MSA: multiple system atrophy; SND: striatonigral degeneration; OPC: olivopontocerebellar; IPD: idiopathic Parkinson’s disease; SNCA: α-synuclein.

FIGURE 2: SNCA mRNA cellular expression (A) SNCA is expressed in neurons and oligodendrocytes of both MSA and control cases. Expression in neurons is greater in controls as compared to MSA (P = 0.47), in contrast to oligodendrocytes where expression is greater in MSA (P = 0.18) but these results did not reach statistical significance. (B) Bar graph representing fold change of expression values between the different cases and cell types. No statistically significant differences were found although there was a trend toward increased expression in MSA oligodendrocytes when compared to control oligodendrocytes (P = 0.18), MSA neurons (P = 0.16) and control neurons (P = 0.18). In contrast, there was a slight decrease in zsyn expression in MSA neurons as compared to control neurons (P = 0.46). The greatest difference seems to be the increase in expression in MSA oligodendrocytes compared with MSA neurons. Mann- Whitney U test with significance level set at P = 0.05. The boxplot shows median values as the line within the box, the box reflects the interquartile range and the whiskers the range of the values. Oligo: oligodendrocytes; MSA: multiple system atrophy; SNCA: α-synuclein.
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