Title

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Journal

Annals of Neurology, 73(5)

ISSN

0364-5134

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Publication Date

2013-05-01

DOI

10.1002/ana.23860

Peer reviewed

Published in final edited form as:
Ann Neurol. 2013 May ; 73(5): 637–645. doi:10.1002/ana.23860.

Hippocampal demyelination and memory dysfunction are associated with increased levels of the neuronal microRNA miR-124 and reduced AMPA receptors

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Abstract

Background—Hippocampal demyelination, a common feature of postmortem multiple sclerosis (MS) brains, reduces neuronal gene expression and is a likely contributor to the memory impairment that is found in greater than 40% of individuals with (MS). How demyelination alters neuronal gene expression is unknown.

Methods—To explore if loss of hippocampal myelin alters expression of neuronal microRNAs (miRNA), we compared miRNA profiles from myelinated and demyelinated hippocampi from postmortem MS brains and performed validation studies.

Findings—A network-based interaction analysis depicts a correlation between increased neuronal miRNAs and decreased neuronal genes identified in our previous study. The neuronal miRNA miR-124, was increased in demyelinated MS hippocampi and targets mRNAs encoding 26 neuronal proteins that were decreased in demyelinated hippocampus, including the ionotrophic glutamate receptors, AMPA 2 and AMPA3. Hippocampal demyelination in mice also increased miR-124, reduced expression of AMPA receptors and decreased memory performance in water maze tests. Remyelination of the mouse hippocampus reversed these changes.

Conclusion—We establish here that myelin alters neuronal gene expression and function by modulating the levels of the neuronal miRNA miR-124. Inhibition of miR-124 in hippocampal neurons may provide a therapeutic approach to improve memory performance in MS patients.

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Contributions:
RD designed, conducted the experiments and analyzed the data. AC, MVR, SAD helped with the in situ hybridization and immunohistochemistry experiments. AMC, KD, DDE, BB and WBM helped with generation of the mouse model, mouse injections and behavioral testing. SMS and RJF helped with the procurement of control and MS tissues. ML and SEB performed the bioinformatic analysis. BDT and RD wrote the manuscript.

Completing Interests:
None

Keywords

Multiple sclerosis; myelin; microRNA

Introduction

Multiple Sclerosis (MS) is an inflammatory demyelinating and neurodegenerative disease of the central nervous system (CNS). MS affects more than two million people worldwide and over 400,000 individuals in the United States [1,2], where it is the leading cause of non-traumatic neurological disability in young adults. Greater than 65% of MS patients become cognitively impaired, with more than 40% having memory dysfunction [3,4]. Recently, there has been increased interest in the role of hippocampal pathology and memory dysfunction in MS patients [4-7]. While levels of neuronal genes are decreased in demyelinated hippocampus, the underlying mechanisms responsible for these gene changes remain to be identified. Neuronal genes are both increased and decreased in demyelinated hippocampi and neuronal loss is modest [5]. This implies that demyelination modulates the transcription and/or translation of neuronal genes.

MicroRNAs (miRNAs) are a class of short, non-protein coding RNAs, capable of decreasing mRNA translation by binding to 3′ UTR of mRNAs [8]. miRNA's are critical regulators of the development and maturation of neurons and oligodendrocytes [9-12]}. Changes in levels of miRNAs and their target genes have been reported in a variety of neurological diseases including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), Tourette's syndrome, and schizophrenia [13-16]. In addition, argonaut, a protein that regulates the processing of miRNA's is mutated in Fragile-X-syndrome [17]. miRNA profiling of white matter lesions from postmortem MS brains revealed distinct miRNA profiles in active and inactive demyelinated lesions possibly reflecting increased numbers of infiltrating immune cells in acute lesions compared to increased reactive gliosis in the chronic lesions [18]. miRNA profiles are also altered in peripheral blood monocytes from MS patients, but most of the altered miRNA's differed from those altered in acute or chronic MS brain lesions (reviewed by [19]). Increased levels of miRNAs in rodent brain can constrain synaptic plasticity and memory function [20-22] and may have similar effects in AD brains [23,24]. miRNA changes in myelinated or demyelinated MS hippocampus have not been reported. Compared to myelinated hippocampus from postmortem MS brains, we previously reported that demyelinated hippocampi contained a reduction in both mRNA and protein levels for genes essential for glutamate neurotransmission, glutamate homeostasis, axonal transport and memory [5]. These molecular changes were accompanied by a significant loss of synaptic density in the demyelinated hippocampus. To investigate possible mechanisms by which myelin regulates neuronal gene expression, we performed a comprehensive comparison of miRNAs in myelinated and demyelinated hippocampi from postmortem MS brains. Our results show that hippocampal demyelination leads to an up regulation of several neuronal miRNAs including miR-124. Using an animal model of hippocampal demyelination/ remyelination, we show increased miR-124 and reduced mRNA and protein levels of AMPA receptors in demyelinated hippocampus. In addition, mice with demyelinated hippocampi had reduced memory performance in water maze tests. Remyelination returned memory performance and miRNA and AMPA receptor levels to those observed in control hippocampus. These data highlights how myelin can influence neuronal gene expression by regulating levels of neuronal miRNA's.

Material and Methods

All postmortem brains were collected as part of the tissue procurement program approved by the Cleveland Clinic Institutional Review Board. All patient demographics, tissue processing, RNA isolation, RT-PCR and western blot analysis have been previously described [5]. MicroRNA arrays were performed by LC Sciences, Houston, TX and the bioinformatic analysis was performed using iCTNet [25], a plug-in for cytoscape software (www.cytoscape.org). In-situ hybridization was performed using a modified in situ protocol and LNA-modified oligonucleotide probes (miRCURY, Exiqon, Denmark). All animal experiments were performed in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the Cleveland Clinic Foundation using six week old C57BL/6 male mice purchased from Jackson laboratories (Bar Harbor, ME). Full experimental details are available in the *S1 Supplemental Materials and Methods*.

Results

Demyelination affects neuronal miRNAs in MS hippocampus

We earlier reported changes in mRNA and protein levels of neuronal genes following demyelination in MS hippocampus [5]. To determine underlying regulatory mechanisms that could control neuronal gene expression, we compared levels of mature miRNAs in myelinated and demyelinated hippocampi from postmortem MS brains. Compared to myelinated hippocampi, 4 miRNAs were significantly decreased and 7 miRNAs were significantly increased in demyelinated hippocampus (**Fig. 1A**). Using a bioinformatic approach we integrated these 11 miRNA's with neuronal specific mRNA transcripts that were significantly decreased in previous gene profiling comparisons of myelinated and demyelinated MS hippocampi [5]. For inclusion, these miRNA-mRNA interactions had to be reported in at least 2 independent miRNA target databases. This network analysis show interactions between the altered miRNA's detected in the present study with the altered neuronal mRNAs identified in our previous study [5] (**Supplemental Fig. 1**). To strengthen this interaction, the miRNA-mRNA network (miRNAs in circles, mRNAs in triangles) was further merged with protein-protein interactions (retrieved from Human Protein Reference Database, blue lines) as described previously [25]. In this bioinformatic screening, 9 of the 11 altered miRNAs (miR-24, miR-143, miR-124, miR-30d, miR-379, miR-138, miR-181a, miR-181c and miR-204) in demyelinated MS hippocampus show significant association with protein-protein interactions (**Supplemental Fig. 2**). Among these 9 miRNAs, 5 were increased (miR-24, miR-143, miR-124, miR-30d, miR-379) and found to be enriched in hippocampal neurons using fluorescent in situ hybridization.

Representative data shows localization of miR-24 (**Fig. 1B**-myelinated, **1C**-demyelinated**)** and miR-30d (**Fig. 1D**-myelinated, **1E**-demyelinated**)** in MS hippocampus. miRNAs and their predicted targets that were significantly changed in MS demyelinated hippocampus are shown in Supplemental Table 1. We also verified the cellular localization of the target mRNAs in the Allen Brain Atlas, an online resource for mRNA cellular distribution based upon in situ hybridization (http://www.brain-map.org/). The query showed that 33 out of the 37 target mRNAs were expressed by neurons in rodent brain (Supplemental Table 1). Interestingly, 26 of these mRNAs contained miR124 binding sites (Supplemental Table 1) in their 3′UTR region, including the glutamate receptor subunits AMPA1, AMPA2 and AMPA3. Among the 26 increased mRNAs, 24 were expressed by neurons and 14 were predicted to have a miR-181a binding site (Supplemental Table 1). These data support the possibility that miR124 and miR181a regulates neuronal gene expression in demyelinated hippocampal neurons.

Loss of myelin leads to increased neuronal miR-124 in multiple sclerosis brain

Among miRNA's that were increased in demyelinated hippocampi from postmortem MS brains, miR-124 was the most intriguing as it had 1) the largest number (26) of predicted neuronal mRNA targets that were significantly decreased in demyelinated hippocampi from MS brains and 2) increased levels of miR124 have been correlated with decreased synaptic plasticity and reduce memory performance in rodents [20,22,23]. To confirm the increase in miR-124 levels in demyelinated hippocampi, we used RT-PCR and detected a 4.5 fold ($p=0.02$) increase in miR-124 levels in demyelinated MS hippocampi compared to myelinated hippocampi (**Fig. 2A**). Using a combined immuno-insitu protocol we determined that miR-124 is expressed in myelinated (**Fig. 2B**) and demyelinated (**Fig. 2C**) hippocampus and enriched in hippocampal neurons (**Fig. 2D-E**). Interestingly, miR-124 expression was confined to neurons in MS hippocampus (**Fig 2D-E**). Recently, increased miR-124 levels have been associated with microglial quiescence and suppression of experimental autoimmune encephalomyelitis in mice [26]. We did not detect miR-124 expression in glial cells in tissue sections from either myelinated or demyelinated MS hippocampus. We next inquired if loss of myelin in cortical and sub-cortical white matter regions also leads to increased levels of miR-124. In control brain tissue, levels of miR-124 were significantly increased in cortical grey matter compared to sub-cortical white matter (**Fig. 2F**) supporting its neuronal enrichment. The expression of miR-124 in white matter in MS brains was primarily due to its presence in white matter neurons as shown by fluorescent in situ hybridization (**Fig. 2G**). Levels of miR-124 as measured by RT PCR were also significantly increased in cortical lesions, compared to control or myelinated MS cortex **(Fig. 2F)**. Similar to demyelinated hippocampal neurons, miR-124 was enriched in neurons in both myelinated (**Fig. 2H**) and demyelinated MS cortex (**Fig. 2I**). These studies establish that the neuronal enriched miRNA, miR-124, is significantly increased in demyelinated hippocampi and cortex from postmortem MS brains. Despite significant activation of microglia and reactive astrogliosis in postmortem MS tissue sections, our in situ hybridization studies failed to detect miR-124 in glial cells. Our data support earlier studies, which detected low levels of miR-124 in acute or chronic MS white matter lesions [18].

Rodent miR-124 levels increase following hippocampal demyelination and inversely correlate with memory performance and levels of AMPA receptors

The miRNA changes described above were generated from demyelinated hippocampi obtained from individuals with a chronic MS disease course. To help delineate primary vs. secondary neuronal miRNA changes in postmortem MS brains, we analyzed a mouse model of hippocampal demyelination using dietary cuprizone combined with intraperitoneal injection of rapamycin. Rapamycin reduces endogenous remyelination and establishes a more consistent baseline of demyelination. Compared to rapamycin treatment only, (**Fig. 3A**), 12 weeks (**Fig. 3B**) of cuprizone/rapamycin treatment significantly reduced hippocampal myelin by 95%. Upon returning cuprizone/rapamycin-treated mice to a normal diet for 6 weeks, remyelination was prominent (**Fig. 3C**) and myelin levels returned to 61% of control levels (**Fig. 3D**). We next investigated if 12 weeks of cuprizone/rapamycin treatment leads to decreased memory performance. Using the Morris water maze test, we examined spatial memory in cuprizone/rapamycin mice. Rapamycin-treated control mice and cuprizone/rapamycin-treated mice showed similar latencies in finding a visible platform, supporting normal visual and motor functions in cuprizone/rapamycin-treated mice. Mice with 12 weeks of cuprizone/rapamycin treatment took significantly longer times to reach a submerged platform compared to control mice (**Fig. 3E**). This spatial memory impairment was reversed in mice with remyelinated hippocampi (**Fig. 3E**). These data support the possibility that hippocampal demyelination is responsible for the memory dysfunction observed in these mice. We next examined whether miR-124 was increased in demyelinated mouse hippocampi. Levels of miR-124 was increased 2.6 fold ($p=0.038$) in demyelinated

mouse hippocampus and returned to control levels following remyelination (**Fig. 3F**). miR-124 expression was confined to neurons in control (**Fig. 3G**), demyelinated (**Fig. 3H**) and remyelinated (**Fig. 3I**) rodent hippocampus. The results establish that demyelination increases expression of the neuronal miRNA, miR-124 and remyelination reverses this change.

Given the relationship between miRNA and mRNA in MS hippocampus shown in Supplemental Fig. 2, we explored the possibility that increased levels of miR-124 could have a direct regulatory role on mRNA's that encode proteins involved in synaptic plasticity. Major targets of miRNA-124 include the AMPA glutamate receptors, GRIA1, GRIA2 and GRIA3. Levels of these three AMPA receptors were decreased in MS demyelinated hippocampus in postmortem MS brains [5] suggested that increased expression of miR-124 could lead to reduced levels of AMPA receptors. We next asked whether AMPA receptors were altered in our rodent hippocampal demyelination/remyelination model. Levels of the AMPA receptor subunits GRIA1 and GRIA2 were significantly decreased in demyelinated rodent hippocampus (**Fig. 3J**). Levels of GRIA3 were also decreased in mouse hippocampus, but did not reach statistical significance. Importantly, the levels of these receptors increased upon remyelination and correlated with decreased levels of miR-124. miRNAs decrease gene expression by binding to 3′untranslated region (UTR) sequences of target genes. Sequencing of the 3′ UTR of the three AMPA receptors in control and MS patients identified miR-124 complementary binding sites (**Supplemental Fig. 3**). We tested whether miR-124 could repress AMPA receptor expression by placing their 3′UTR segments downstream of a cytomegalovirus (CMV)-driven luciferase reporter and performed reporter assays in HEK293 cells transfected with a miR-124 mimic. The presence of miR-124 significantly decreased the luciferase activity of reporters containing the AMPA1, AMPA2 and AMPA3 3′UTR segments that were predicted to bind miR-124 (**Fig. 3K**). Mutations of these miR-124 binding sequences abolished the repressive activities of all three AMPA receptor luciferase reporters (**Fig. 3K**). These results support direct binding of miR-124 to the 3′UTR of mRNA encoding the three AMPA receptors. Next we asked if binding of miR-124 to the 3′UTRs of AMPA receptors cause down-regulation of AMPA receptor mRNA levels. Transfection of primary neurons with a miR-124 mimic led to a significant (5.4 fold, p=0.004) increase in levels of miR-124 (**Supplemental Fig. 4**) and a significant decrease in AMPA1, 2 and 3 mRNA levels when compared to neurons that were not transfected with the miR-124 mimic (**Fig. 3L**). Addition of a miR-124 inhibitor that blocks endogenous miR-124, however abolished this decrease and led to a significant increase in AMPA receptor mRNA levels. Introduction of a scrambled miRNA did not alter AMPA receptor mRNA levels (**Fig. 3L**). Collectively our results indicate that miR-124 binds to and reduces neuronal AMPA receptor mRNA in primary neuronal cultures. The increase of miR-124 following hippocampal demyelination may therefore play a role in affecting memory by decreasing levels of AMPA receptors.

Discussion

The present study supports the concept that loss of myelin reduces hippocampal function by altering expression of neuronal miRNAs. Increases in the neuron specific miRNA, miR124, can decrease expression of neuronal genes including AMPA receptors. Hippocampal demyelination negatively impacts spatial memory performance in mice. This decrease in spatial memory also correlates with increased expression of miR-124 and decreased expression of AMPA receptors in hippocampal neurons. Remyelination enhanced memory performance, increased levels of AMPA receptors and decreased levels of miR-124. We therefore propose that increased miR-124 negatively impacts memory performance by downregulation of AMPA-mediated glutamate signaling.

In addition to increasing the speed of nerve conduction, myelin plays a significant role in maintaining the integrity and long-term survival of axons [27]. The myelin proteins, myelin-associated glycoprotein (MAG), proteolipid protein (PLP) and 2′3′-cyclic nucleotide 3′-phosphodiesterase (CNP), play a role in providing this trophic support and this function appears independent of any role in myelin sheath formation [27,28]. Studies have focused on how the loss of myelin causes axonal or neuronal degeneration, which is considered the major cause of permanent neurological disability in primary diseases of myelin (for reviews, see [27,29,30]). From a mechanistic point of view, recent studies support the transfer of lactate from oligodendrocytes to axons. This lactate may be a substrate for axonal ATP production and essential for axonal viability [7,31,32]. We propose an additional mechanism whereby myelin regulates neuronal gene expression by regulating the expression of neuronal miRNA's. Our studies have leveraged miRNA and mRNA data bases in human and rodent hippocampi with and without myelin. We identify miR124 as a major negative regulator neuronal gene expression (26 out of 33) in demyelinated MS hippocampus. miR-124 targets the 3′UTR of AMPA receptors and can decrease AMPA receptor reporter mRNA expression in *in vitro* assays. In addition, neuronal miRNA's were decreased in demyelinated hippocampus and target 3”UTR's of neuronal genes that are increased in demyelinated hippocampus. miR-181a was significantly decreased in demyelinated hippocampi and this miRNA targets a majority (14 out of 24) of the hippocampal neuronal genes (Supplemental Table 1) that were reported to be significantly increased by demyelination [5]. While miRNA's are regulated by demyelination and remyelination, other neuronal genes decreased in demyelinated hippocampi [5], such as KIF1A, do not appear to contain 3” UTR sequences targeted by miRNA's identified in this study. Demyelination is likely to influence neuronal gene expression by additional mechanisms that regulate gene transcription. In addition to the primary effect of demyelination, loss of synapses following demyelination could also negatively impact expression of synaptic and neuronal genes.

When investigating diseased brain tissue where the proportion of individual cell types can change, it is imperative to establish which cell type is expressing individual miRNA's that decrease or increase. For example, if a miRNA is enriched in oligodendrocytes it would be decreased in MS lesions where oligodendrocytes are destroyed, but it would have no affect on mRNA translation in that lesion. Similar concerns could be raised regarding the increase in miR-124 in MS lesions as previous studies have reported miR-124 controlling activation of microglia [26]. Microglia are known to be activated in some MS lesions. Therefore, we developed a combined immunocytochemistry-insitu hybridization protocol for identifying cell types expressing individual miRNAs in rodent and human brain sections. Using this protocol, miR-124 expression was highly enriched in neuronal cells in both rodent and human brain and increased in neurons in demyelinated hippocampi and cortex (Figure 2). Our studies provided a list of mRNA's that 1) were increased or decreased in demyelinated hippocampus and 2) contained 3′ UTR sequence targeted by miRNA's that were increased or decreased in demyelinated hippocampus. Based upon analysis of the Allen in situ hybridization Brain Atlas (http://www.brain-map.org/), mRNA's that are enriched in neurons are highlighted in Supplemental Table 1. Therefore, we are confident that the altered miRNA's and mRNA's reported in Supplemental Table 1 are enriched in neurons.

The role of miR-124 in regulating hippocampal function in MS brains is supported by studies that correlate increased miR-124 and reduced synaptic plasticity [21]. In addition, a previous study correlated decreased hippocampal levels of miR-124 with enhanced memory performance [33] in mice. These data together with decreased miR-124 expression and increased memory/learning by remyelination in our rodent model raise the possibility that selective inhibition of miR-124 in hippocampal neurons could enhance cognitive performance in MS patients. Minimal neuronal loss in demyelinated MS hippocampus [5] identifies the demyelinated hippocampal neuron as a viable and abundant therapeutic target.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors like to thank Dr. Richard M Ransohoff, MD, Dr. Bruce T Lamb, Ph.D and Richard M Rudick, MD for helpful comments on the manuscript. The authors would like to thank Cynthia Swanger, Life Banc for the MS tissue collection program, Olga N. Kokiko-Cochran for assisting with the rodent behavioral study, Giuxiang Xu for the primary neuronal cultures and Dr. Christopher Nelson for manuscript editing.

Funding: The work was supported by NMSS RG-4280 (RD), NIH NS35058 and NIH NS38667 (BDT).

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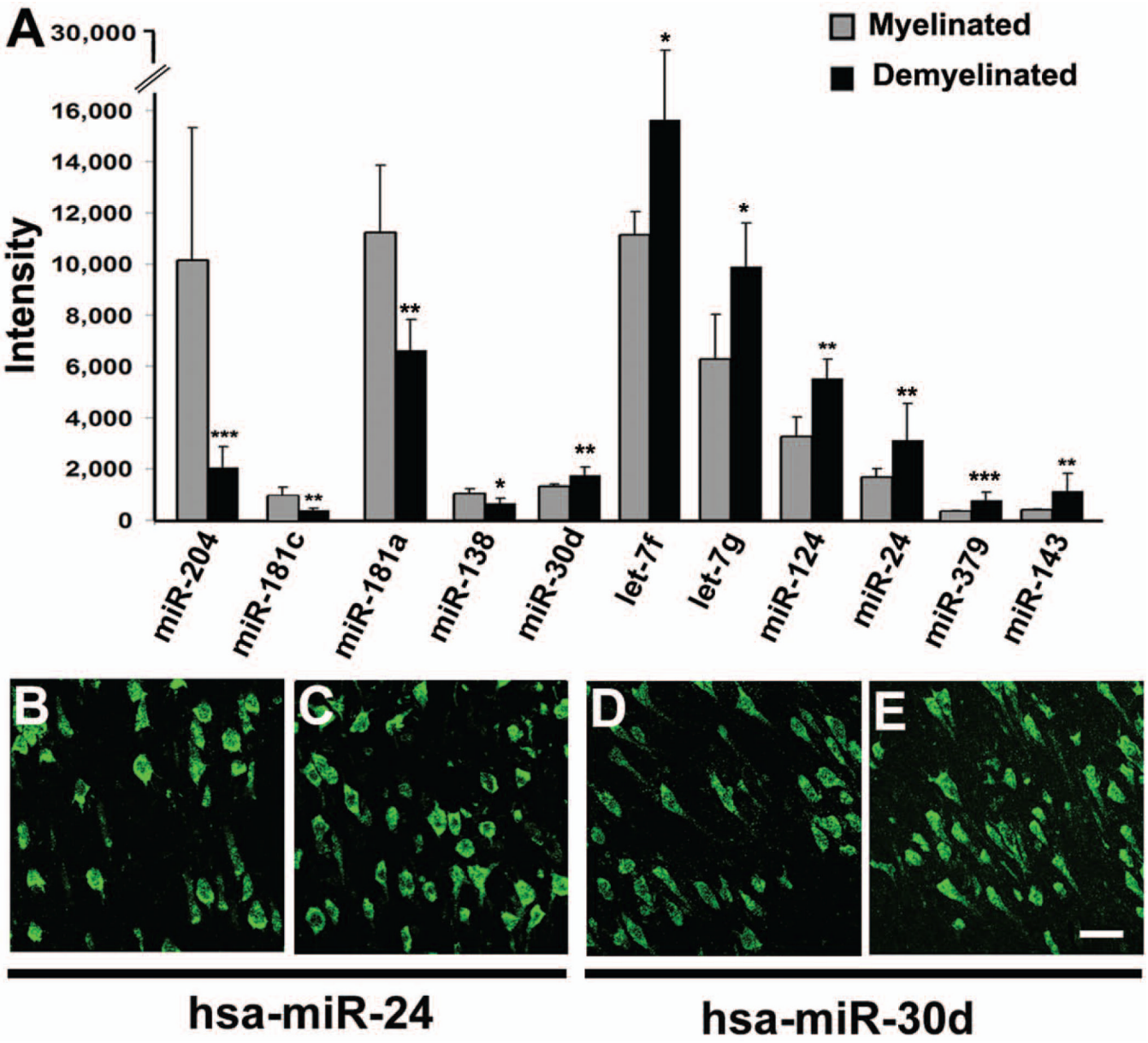

Figure 1. Neuronal miRNA expression in demyelinated hippocampi from multiple sclerosis (MS) brains

(**A**) Global miRNA profiling identified miRNAs altered in demyelinated hippocampus from postmortem MS brains. Compared to myelinated MS hippocampus (n=4), 4 miRNAs were decreased while 7 miRNAs were increased in demyelinated (n=4) MS hippocampus. (**B-E**) Altered miRNAs are expressed by hippocampus neurons. Fluorescent in situ hybridization (shown in green) of myelinated (**B, D**) and demyelinated (**C, E**) MS hippocampal sections with probes specific for the miRNAs, hsa-miR-24 (**B-C**) and hsa-miR-30d (**D-E**) show that both miRNA's are highly enriched in neurons. Scale Bars: B-E: 30μm; Error bars indicate + S.E.M. * $p<0.05$, ** $p<0.005$, *** $p<0.0005$.

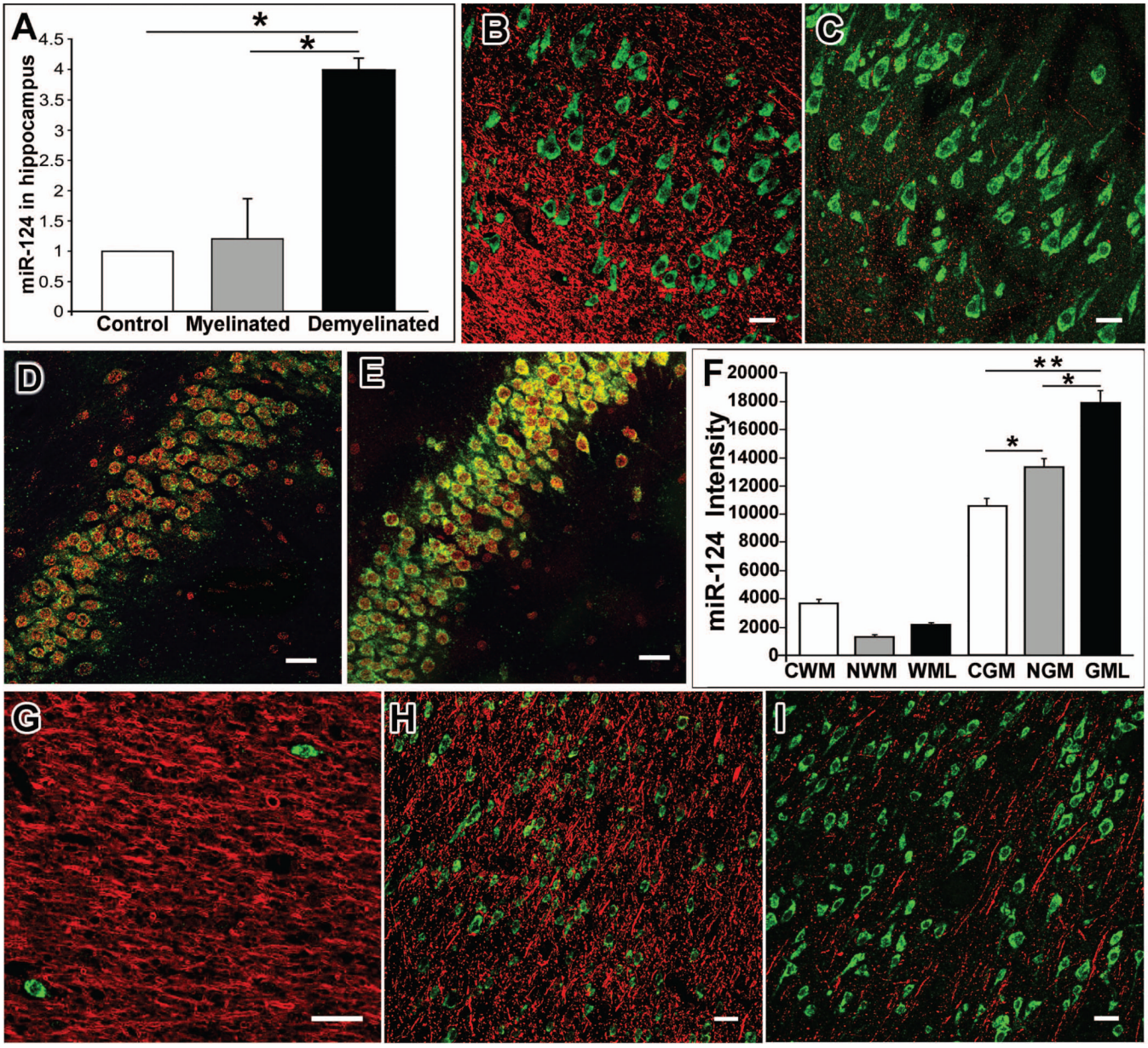

Figure 2. Demyelination in MS brains leads to increased neuronal expression of miR-124

(**A)** miR-124 levels are significantly increased in MS demyelinated hippocampus. Quantitative RT-PCR detected significant increase in level of miR-124 in demyelinated MS hippocampus (n=5) compared to control (n=5) and myelinated MS hippocampus (n=5). Fold changes represent differences in miR-124 levels compared to control brains. (**B-C**) Confocal images of MS hippocampus immunostained with an antibody specific to myelin basic protein (MBP; red) and fluorescent in situ hybridization for miR-124 (green) show abundant CA1 neurons expressing miR-124 in myelinated (B) and demyelinated hippocampus (**C**). (**D-E**) Confocal images stained for neuronal marker HuR (red) and miR-124 (green) show neuronal localization of miR-124 in the DG region of myelinated (**D**) or demyelinated (**E**) MS hippocampus. (**F**) miR-124 levels in white and grey matter tissues from human brain. Levels of miR-124 were measured in control white matter (CWM, n=5), MS normal white matter (NWM, n=5), white matter lesion (WML, n=5), control grey matter (CGM, n=5), MS

normal grey matter (NGM, n=5) and grey matter lesion (GML, n=5) using a quantitative RT-PCR. Intensity of miR-124 was significantly increased in MS demyelinated cortex compared to both control and myelinated MS cortex. (**G**) In MS white matter, levels of miR-124 was lower compared to cortical grey matter and expressed predominantly by neurons (MBP-red, miR-124-green) (**H-I**) Confocal images of MS sections immunostained with MBP (red) and hybridized for miR-124 (green) detect miR-124 in cortical neurons in MS myelinated (**H**) and demyelinated cortices (**I**). Scale Bars: B-E, G-I: 30μm; Error bars indicate + S.E.M. * $p<0.05$, ** $p<0.005$.

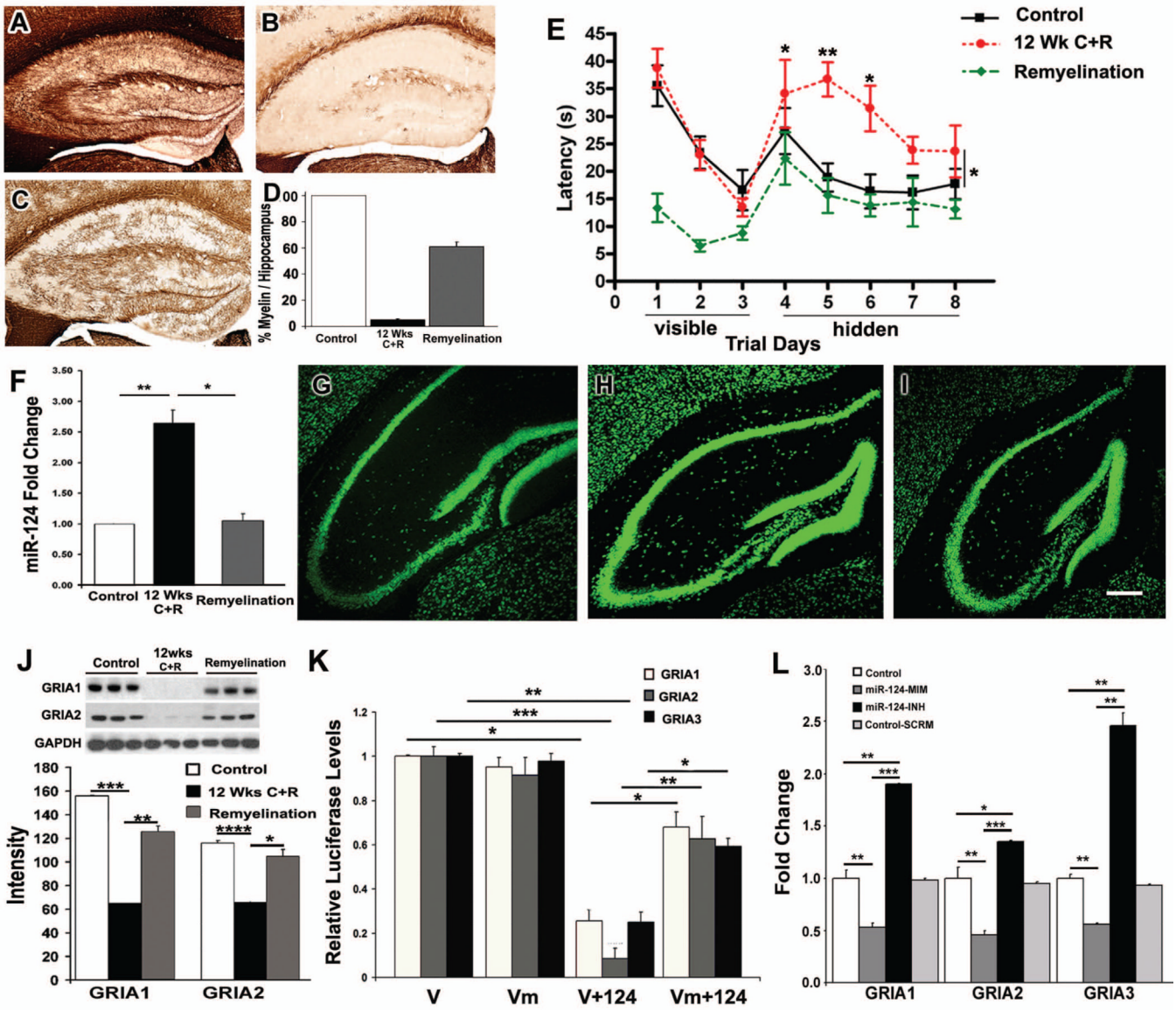

Figure 3. Dynamics of miR-124 changes in demyelinated and remyelinated rodent hippocampus

(**A-D**) Mice treated with cuprizone and rapamycin have hippocampal demyelination as shown by immunohistochemistry with proteolipid protein (PLP). Myelin was quantified in 10 individual hippocampi per group. Chronic treatment with cuprizone/rapamycin for 12 weeks shows 95% decrease in hippocampal myelin (**B**) compared to hippocampus from rapamycin only treated mice (**A**). In hippocampi that were spontaneously remyelinated for 6 weeks (**C**), myelin density was 61% of that found in control hippocampus (**D**). (**E**) Hippocampal demyelination was associated with significant loss of memory function. Mice (n=10/group) were tested in Morris water maze. Rapamycin injected control (black squares), chronically demyelinated (red circles) and remyelinated (green diamond) mice were exposed to the visible platform for 3 days followed by 5 days of hidden platform test. There was no difference in swim speeds between the animals during the visible and hidden platforms using a one-way ANOVA. Mice with demyelination of the hippocampus show decreased memory performance compared to control mice. Mice with remyelination did consistently better in finding the hidden platform than either demyelinated mice or controls. (**F**)

Demyelination of hippocampus led to significant increase in miR-124. Using quantitative RT-PCR, levels of miR-124 were significantly increased in demyelinated hippocampi and returned to control levels in remyelinated hippocampi (n=5/group). (**G-J**) Fluorescent in situ hybridization reveals abundant miR-124 positive neurons in control (**G**), and demyelinated (**H**) and remyelinated (**I**) hippocampi. (**J**) AMPA receptors are decreased in chronically demyelinated hippocampi. Protein levels of AMPA 1 and AMPA 2 receptors were decreased in demyelinated hippocampi and increased following remyelination. GAPDH was used as internal loading control. (**K**) miR-124 targets AMPA-specific glutamate receptors. HEK293 cells were transfected with luciferase reporter constructs carrying 3′UTR sequences of AMPA receptors with intact miR-124 binding site (V) or mutated miR-124 binding sites (Vm). Transfection of miR-124 mimic led to significant decrease in luciferase activity in all 3 AMPA receptor UTR constructs. The effect of miR-124 induction was not observed with the mutated 3′UTR constructs (Vm+124). (**L**) Introduction of miR-124 down-regulates AMPA receptor mRNA levels. Primary neurons (Control) were transfected with either miR-124 mimic (miR-124-MIM) or the synthetic endogeneous miR-124 inhibitor (miR-124-INH), or Cy5- labeled scramble miRNA (Control-SCRM). mRNA levels of the AMPA receptor 1, 2 and 3 were measured using RT-PCR. Addition of miR-124-mimic to primary neurons led to significant decrease in mRNA levels of all three AMPA receptors. Conversely, miR-124 inhibitor had opposing effect on the mRNA levels of the AMPA receptors leading to significant increase compared to controls. Changes in mRNA level of AMPA receptors were specific to introduction of miR-124 as introduction of the scrambled miRNA control had no effect on the mRNA level of the receptors. Scale bars: G-I 200um; Error bars indicate + S.E.M. *$p<0.05$, **$p<0.005$, ***$p<0.0005$, ****$p<0.00005$.