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The effects of chronic simvastatin treatment on the expression of behavioral symptoms in a transgenic mouse model of Huntington's disease

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List of Abbreviations

HD ..... Huntington's Disease
CAG ..... Cytosine, Adenosine, Guanine
Htt ..... Huntingtin
mHtt ..... Mutant Huntingtin
Rhes ..... Ras Homologue Enriched in the Striatum
NES ..... Nuclear Export Signal
HSP ..... Heat Shock Protein
UPS ..... Ubiquitin-Proteasome System
mTor ..... Mammalian Target of Rapamycin
FPP ..... Farnesylpyrophosphate
GGPP ..... Geranylgeranylpyrophosphate
AD ..... Alzheimer's Disease
FTI ..... Farnesyl Transferase Inhibitor
GGTI ..... Geranylgeranyl Transferase Inhibitor
DPTI ..... Dual Prenyl Transferase Inhibtor
NC ..... Non-carrier
ANOVA ..... Analysis of Variance

Abstract

Huntington's disease (HD) is a heritable, neurodegenerative disorder characterized by motor, cognitive, and psychiatric disturbances. An unstable CAG expansion within the gene normally encoding for the Huntingtin protein is responsible. The expanded mutant form of Huntingtin and the putative protein co-factor Rhes interact and cause cell death within the striatum. We hypothesized chronic treatment with simvastatin, a cholesterol lowering drug, would disrupt the biosynthetical pathway which gives both Rhes and its target cells binding sites and render Rhes inactive. Healthy and HD mice were treated with simvastatin or a vehicle. Animals' motor behavior was assessed with three separate tests over the first four months of life. No significant differences were found between the HD groups; however, the HD treated animals' performance on the rotarod test, at month 4, was intermediate between healthy mice and HD vehicle treated mice. The results hint at simvastatin's therapeutic potential, but are interpreted cautiously.

Huntington's disease; Rhes; statin; mevalonate; Huntingtin protein; rotarod

Introduction

Huntington's Disease (HD) is a debilitating neurodegenerative condition with no cure or treatment that can delay or prevent the onset of symptoms. It is a progressive, dominantly inherited disorder that interferes with the motor, cognitive, and psychiatric functioning of afflicted individuals. The disease stems from a single mutation on the short arm of human chromosome 4. The C (Cytosine) A (Adenosine) G (Guanine) trinucleotide repeat in this region encodes for a poly-glutamine stretch of the protein huntingtin (Htt) and, under normal conditions, may have up to 34 repeats. An extension beyond 37 glutamines is unstable and results in a mutated form of huntingtin (mHtt), ensuring HD. The mHtt protein undergoes changes in makeup and shape, causing the formation of intracellular protein aggregates. Presently, researchers are fervently attempting to uncover the either destructive or protective nature of such aggregates. While mHtt has emergent toxic capabilities, it simultaneously prevents the function of Htt, which aids activity, and survival of the neurons that are susceptible in HD (Cattaneo, Zuccato, and Tartari, 2005). Although huntingtin is expressed ubiquitously throughout the body, the hallmark of HD neuropathology is severe loss of neuronal cells only in the striatum and, to a lesser extent, the cortex (Reiner et al., 1988), suggesting that there is a permissive cofactor within cells of these regions. The subcortical striatum is a portion of the basal ganglia, a set of structures responsible for behavioral activation (Kandel, Schwartz, & Jessell, 2000.) In humans, the striatum is comprised of the caudate nucleus and putamen. These structures receive input from the cortex, the thalamus, and the substantia nigra pars compacta. Striatal output eventually reaches the cortex via connections in the thalamus, substantia nigra pars reticulata, and the globus pallidus. Within the striatum, GABAergic medium spiny neurons are particularly vulnerable, experiencing up to a 95% loss in advanced stages of HD (Graveland,

Williams, & DiFiglia, 1985; Albin, Reiner, Anderson, Dure, Handelin, Balfour et al., 1992). The striatum is also home to the preferentially expressed protein Rhes (Ras homologue enriched in the striatum). The exact mechanisms of HD neuronal degeneration is unknown, but many have implicated the intracellular inclusion bodies exhibited in affected neurons and some have named the intriguing Rhes as a possible cofactor. These are just two possible targets for developing therapeutic strategies.

George Huntington's original description of the disease offers a glimpse into what life is like for those carrying the HD mutation (1872). He detailed a familial condition entailing involuntary, choreic movements, behavioral and psychiatric disturbances, and an ultimate dementia state. Although dance-like motor perturbations were noted and remain the hallmark feature, giving HD it's original namesake of Huntington's chorea, the complex and pervasive nature of the disease is now fully appreciated. With a lifetime prevalence rate of 1 in 10,000 for those of European descent, symptoms begin to emerge in middle adulthood, between ages 30 and 50, for the majority and gradually progress for the next 10-15 years until death (Craufurd, Thompson, & Snowden, 2001). The process is extremely distressing and even painful. As Huntington initially accounted, suicide is often an accompanying feature of the illness. Indeed, there is evidence of increased suicide rates among HD patients, particularly for those in the early stages of the disease (Schoenfeld, Myers, Cupples, Berkman, Sax & Clark, 1984). Studies on the sequence of symptom onset have yielded inconsistent findings, although many agree that some compilation of psychiatric, behavioral, and cognitive changes frequently present before motor disturbances (de Boo, Tibben, Hermans, Jennekens-Schinkel, Maat-Kievit, Roos, 1999; Lawrence, Hodges, Rosser, Kershaw, ffrench-Constant, Rubinsztein et al., 1998; Roos, 2010). A survey of the literature has found largely varied estimates of lifetime prevalence rate among HD

patients for psychiatric symptoms, between 33% and 76%, based on varying assessment methodologies (van Duijn, Kingma & van der Mast, 2007). Such symptoms are often considered to be the most troubling facet of HD, over motor symptoms, and are an important point of consideration when caregivers and patients are deciding on hospitalization options (Hamilton, Salmon, Corey-Bloom, et al., 2003). Three clusters of psychiatric features, each with its own longitudinal trajectory, have emerged in the research: apathy, irritability, and depression. Apathy tends to continually progress over time and through all stages of the disease. Irritability increases throughout the disease, but more so in early stages. Lastly, depression, the most common psychiatric symptom, is a point of contention. Many hold that depression is most common in the middle stages of the disease, while others point out increased rates even in pre-clinical samples (Julien, Thompson, Yardumian, Snowden, Turner & Craufurd, 2007; Paulsen, Nehl, Hoth, Kanz, Benjamin, Conybeare et al., 2005). Demonstrated declines in depression have been attributed to anti-depressant medication relief (Thompson, Harris, Sollom, Stopford, Howard, Snowden & Craufurd, 2012). Psychiatric conditions may deteriorate so greatly in later stages of the disease that psychosis emerges. In 5%-16% of HD patients, a psychosis similar to that of schizophrenia occurs, complete with paranoid delusions and auditory hallucinations (Tsuang, Almqvist, Lipe, Strgar, DiGiacomo, Hoff et al., 2000)

Early cognitive changes are regularly cited by clinicians and family members (Kirkwood, Siemers, Hodes, Conneally, Christian & Foroud, 2000). Neuropsychological testing has shown early deficits in visuo-spatial skills, affective processing, concentration and executive functioning. It has been argued that the pattern of cognitive weaknesses is distinct from other basal ganglia disorders and cortical dementias (Lawrence, Sahakian, Hodges, Rosser, Lange & Robbins, 1996). Specific affected abilities include, but are not limited to, pattern recognition,

spatial working memory, information processing speed and capacity, planning, problem solving, concept formation and decision making. As time goes on, memory deficits come to the forefront of clinical presentation. Short-term and long-term memory show decline, in memory acquisition, delayed recall, and with contention, recognition (Zakzanis, 1998). Brain imaging analyses have revealed that progression of subtle cognitive deficits to more severe impairments results from the progression of deterioration from striatal to extra-striatal tissue, namely the insular lobe, to the frontal and temporal cortex. Specifically, the connectivity in the fronto-striatal circuit is thought to be the driving force behind HD cognitive deficits (Montoya, Price, Menear & Lepage, 2006; Peinemann, Schuller, Puhl, Jahn, Weindl & Kassubek, 2005).

Striatal degeneration is directly responsible for the prototypical symptom of HD, chorea, as well as many other motor features. Chorea is defined as unwanted, involuntary movements of extremities that can be described as dance-like. Beginning in distal extremities, like fingers and toes, as well as small facial muscles, these twitches develop from mild to moderate classification (Roos, 2010). Other early, even pre-clinical indications are abnormal saccadic eye movements, brisk muscle stretches, and decreased ability to quickly alternate movements (Penney et al., 1990). Slower reaction times have also been cited, but like other early indicators of imminent disease, the literature is inconsistent on this point (de Boo, Tibben, Hermans, Maat, & Roos, 1998)de Boo, Tibben, Lanser, Jennekens-Schinkel, Hermans, Maat-Kievit & Roos, 1997; Kirkwood, Siemers, Bond, Conneally, Christian & Foroud, 2000). Although accounts of early and pre-manifest motor symptoms are continually revised, all of the above are accepted as eventual symptoms. In addition, akinesia, or problems with initiating movement, bradykinesia, slowness in performing movement, and dystonia, abnormal and contorted posture are prominent features (Rosas, Salat, Lee, Zaleta, Pappu, Fischl et al., 2008).

Huntingtin

Htt is an elusive protein, of which the structure and function are not completely understood. Unlike other proteins with a high molecular weight (~348 kDa), it is completely soluble and is expressed throughout the human and rodent body, both in and out of the nervous system (Cattaneo, Zuccato, and Tartari, 2005). The highest concentrations are found in the central nervous system and the testes (Gil & Rego, 2008). It is made up of 3,144 amino acids, of which only a few motifs have been identified. Htt is known to consist of the poly-glutamine section which holds the HD mutation, an adjacent region rich in the glutamate derived, non-essential amino acid proline, and 4 clusters of HEAT repeats (named for the first 4 proteins they were found in: Htt, Elongation factor 3, the A subunit of protein phosphatase 2A, and TOR1), sequences of ~40 amino acids that are duplicated at various points along the protein structure (Andrade, Petosa, O'Donoghue, Muller, & Bork, 2001; Li & Li, 2004).

The function of Htt has only recently begun to be understood. Its wide distribution throughout the cell has complicated the search for function. It is localized to both nuclear and cytoplasmic structures, such as the endoplasmic reticulum, the Golgi apparatus, synaptic vesicles, and mitochondria (DiFiglia, Sapp, Chase, Schwarz, Meloni, Young et al., 1995; Hilditch-Maguire, Trettel, Passani, Auerbach, Persichetti, & MacDonald, 2000; Kegel, Meloni, Yi, Kim, Doyle, Cuiffo et al., 2002; Li, Plomann, & Brundin, 2003). Information on the structure and interacting proteins of Htt has helped elucidate its function. The poly-glutamine tract begins at the 18^{th} amino acid position and, when in normal or wild-type-type form, elongates to no more than 34 repeats (Huntington's Disease Collaborative Research Group, 1996). Earlier studies suggested that the multiple glutamines formed a "polar zipper" facilitating binding between Htt and transcription factors which also contained a poly-glutamine stretch (Perutz et al., 1994). In

fact, Htt interacts with multiple partners (Harjes & Wanker, 2003; Zuccato, Valenza, & Cattaneo, 2010). The poly-proline rich portion of the protein is thought to be necessary for the formation of inclusion bodies, perhaps by affecting solubility (Steffan et al., 2004). HEAT repeats are present in many other large eukaryotic proteins involved in transport processes in the nucleus and the cytoplasm as well as chromosome separation, indicating this may be also true of Htt (Neuwald & Hirano, 2000).

Further evidence for the role of Htt in transporting processes stems from its Carboxyl-terminal nuclear export signal (NES) and a less active, non-conventional nuclear localization signal. Their presence allows for the movement of Htt to and from the nucleus, likely as part of a larger nuclear-cytoplasmic shuttling complex. In mHtt, the NES is cleaved away, leaving the protein able to enter the nucleus, but not exit (Xia, Lee, Taylor, Vandelft, & Truant, 2003).

In addition to development and transport processes such as protein and vesicle trafficking, Htt is thought to be involved in cytoskeletar anchoring, postsynaptic signaling, transcriptional regulation, and serves an anti-apoptotic role intracellularly (Caviston & Holzbaur, 2009; Gil & Rego, 2008; Kegel, Meloni, Yi, Kim, Doyle, Cuiffo et al., 2002; (Rigamonti et al., 2000). HD neurodegeneration is now known to be a result of both a loss of the diverse wild-type-type Htt functioning and a toxic gain of functioning of mHtt (Landles & Bates, 2004). The many proposed mechanisms leading to the devastating degeneration are all rooted in the decreased expression of functional Htt and the increased expression of pathological mHtt.

mHtt Aggregation

Early investigations of HD neuropathology in mice and humans described the presence of intra- and extra-nuclear inclusion bodies within neurons of all cortical layers and medium sized striatal neurons (Davies, Turmaine, Cozens, DiFiglia, Sharp, Ross et al., 1997; DiFiglia, Sapp,

Chase, Davies, Bates, Vonsattel & Aronin, 1997). These aggregates differ in composition, with nuclear bodies consisting of NH-terminal mHtt fragments and extra-nuclear bodies consisting of both fragments and full length mHtt (Cooper, Schilling, Peters, Herring, Sharp, Kaminsky, et al., 1998; Martindale, Hackam, Wieczorak, Ellerby, Wellington, McCutcheon, et al., 1998). Their presence is due to the expanded polyglutamine portion of mHtt, which induces a conformational change that is thought of as the starting point for downstream pathogenesis. Normally, exon 1 of Htt, made up of 17 polyglutamine residues, has an N-terminal α helical structure. The shape is flexible and transforms into a well-defined α helix, a random coil, or a loosely-bound and extended loop conformation at various, randomly determined points along the glutamine chain. This flexibility creates a low threshold for interference from outside influences, such as length of polyglutamine tract and neighboring protein motifs (Kim, Chelliah, Kim, Otwinowski, Bezprozvanny, 2009). Not surprisingly, mHtt's first exon, which contains upward of 17 residues, has its conformational flexibility taken advantage of. In this case, the random coil portion of the polyglutamine expansion misfolds into a hairpin structure, described as two anti-parallel β strands and a sharp turn (de Mezer, Wojciechowska, Napierala, Sobczak, & Krzyzosiak, 2011).

Aggregates are thought to be formed by separate, but likely simultaneous, pathways. The first involves proteolysis, the process by which proteins are degraded either partially into peptides, or completely into their amino acids. Specifically, NH-terminal mHtt is cleaved by two classes of enzymes, caspases (notably caspase-3 and caspase-6) and calpains, which leave behind mHtt fragments that have a propensity for diffusing into the nucleus and aggregating (Gafni & Ellerby, 2002; Goldberg, Nicholson, Raper, Kalchman, Koide, Graham, et al., 1996; Graham, Deng, Slow, Haigh, Bissada, Lu, et al., 2006; Sun, Fan, Balciunas, Cooper, Bitan, Steavenson et al., 2002; Wellington, Ellerby, Hackam, Margolis, Trifiro, Singaraja, et al., 1998). These

fragments recruit further proteases, thereby creating a positive feedback loop which results in amplified aggregation until ultimately, some propose, the cell dies (Gil & Rego, 2008). Secondly, aggregation is dependent upon protein misfolding which Htt normally protects against (Fink, 1999). In the absence of mHtt, chaperones, Heat-shock protein 70 (Hsp70) and heat-shock protein 40 (Hsp40), coordinate to correct misfoldings and maintain the solubility of Htt (Hartl & Hayer-Hartl, 2002). If misfoldings remain after these chaperones are active, the ubiquitin-proteasome system (UPS) degrades the target proteins (Voges, Zwickl, & Baumeister, 1999). However, if the misfolded protein, in this case mHtt, is being generated faster than the chaperone-UPS buffer can act, aggregation will begin, only to be further exacerbated by the sequestering of precious chaperones and proteasomes within the aggregates themselves (Hay, Sathasivam, Tobaben, Stahl, Marber, Mestril et al., 2004; Martin-Aparicio, Yamamoto, Hernandez, Hen, Avila, Lucas, 2001). While mHtt's misfolding contributes to aggregation, Htt inhibits the activity of certain fragment inducing caspases and interacts with corrective chaperones, demonstrating that such aggregation is the result of both a gain and loss of function.

An ongoing controversy exists over the role of mHtt aggregates in HD neurodegeneration. Earlier research labeled mHtt aggregates as cytotoxic. This argument centers around the presence of intracellular aggregates in several neurodegenerative diseases and the correlation between aggregation and cell death (Yang, Dunlap, Andrews, & Wetzel, 2002). Indeed, aggregates do seize important classes of proteins that also contain a poly-glutamine tract and facilitate or regulate transcription, as well as parts of the essential proteasome mentioned above (Cha, 2007). Overexpression of such proteasome components reduces aggregation and prolongs cell life (Carmichael, Chatellier, Woolfson, Milstein, Fersht & Rubinsztein, 2000, Vacher, Garcia-Oroz, Rubinsztein, 2005). Others contend that aggregation is a noncausal,

incidental phenomenon of cellular dysfunction (Kuemmerle, Gutekunst, Klein, Li, Li, Beal et al., 1999). Evidence for a more "side-effect" role is found in studies of human post-mortem HD brains that demonstrate the highest number of aggregates in the cortex, rather than the most severely affected area, the striatum (Gutekunst, Li, Yi, Mulroy, Kuemmerle, Jones, et al., 1999). It has also been shown that neuropil aggregates of the dendrites and dendritic spines are much more common in HD brains than the seemingly more detrimental nuclear aggregates, and that peripheral nervous system tissue displays a number of mHtt inclusion bodies (Moffitt, McPhail, Woodman, Hobbs, & Bates, 2009). Furthermore, a mouse model containing a particular fragment of the full length HD gene indicates it is not the aggregates themselves that are driving neuronal loss. The model displays early and widespread neuronal nuclear inclusions, but no signs of neuronal dysfunction or death (Slow, Graham, Osmand, Devon, Lu et al., 2005).

Still a third argument is made for a neuroprotective capacity of aggregates. Aggregation sequesters mHtt and decreases the amount of diffuse or soluble form of mHtt in other cell locations. Alterations in the length, and thus aggregation, of mHtt have shown related decreases in the number of neuronal aggregates and death (Ratovitski et al, 2009). Moreover, survival analysis has shown that inclusions actually predict cell survival, indicating the presence of aggregation could be a coping mechanism of the cell (Arrasate et al., 2004). Interestingly, the same sequestration by mHtt aggregates affects mammalian target of rapamycin (mTOR) (Ravikumar et al., 2004). This kinase is a negative regulator of autophagy, the process by which cells clear mHtt and alleviates toxicity and behavioral symptoms in HD (Jia, Hart, & Levine, 2007).

Rhes

In the midst of still a largely unknown function of Htt and mHtt's aggregates, clues can be found in an emerging line of research devoted to a possible co-factor in HD pathology, the protein Rhes. As evident by its name, Rhes or the Ras homolog enriched in the striatum, is selectively located in areas most vulnerable to HD degeneration. It is an intermediate sized guanine-nucleotide binding protein (Falk, Vargiu, Foye, Usui, Perez, Danielson et al., 1999). Similar to prototypical Ras proteins, Rhes has GTP binding and effector domains and a CAAX box (composed of a Cysteine which attaches to membranes, two aliphatic amino Acids, and one of several amino acids, X). It also has an extended C terminal which adds to its molecular weight (Harrison, 2012). The function of Rhes remains unclear but it is involved in dopamine-mediated signaling and behavior (Harrison & LaHoste, 2006; Spano, Branchi, Rosica, Pirro, Riccio, Mithbaokar et al., 2004). Rhes mRNA in the striatum was decreased by depleting the striatal dopamine input.

Research from Johns Hopkins University's group has substantially added to the literature on Rhes and HD. In vitro studies demonstrated that Rhes binds to both Htt and mHtt, but holds preference for mHtt. Notably, Rhes does not bind to ataxin, another protein containing a poly-glutamine repeat that is charged with neurodegeneration in spinocerebellar ataxia, indicating a role specific to HD pathology. The most influential finding from their work, however, was that the overexpression of Rhes in cells containing mHtt decreased cell survival by 50%. This decrease was not found when either Rhes or mHtt was overexpressed singly, nor was it shown in cells expressing both Rhes and Htt. Depletion of Rhes using RNA interference increased rates of cell survival. Likewise, they found that, in mHtt knock-in striatal cells, overexpression of Rhes further decreased cell survival by 60% overall. There were no toxic effects found when

overexpression was conducted in Htt knock-in cells (Subramaniam, Sixt, Barrow & Snyder, 2009).

The researchers subsequently demonstrated that Rhes augments neuronal death by influencing sumoylation. Sumoylation is a post-translational modification process in which a small ubiquitin-like modifier is covalently attached to various proteins and a change in functioning results. When mHtt undergoes sumoylation, aggregation is reduced and neurotoxicity is increased (lending support to the notion that non aggregated, soluble mHtt is toxic) (Steffan, Agrawal, Pallos, Rockabrand, Trotman, Slepko et al., 2004). Rhes enhances sumoylation of mHtt, but not Htt, in a time and concentration dependent manner; thus, the potential cofactor is responsible for the disaggregation of mHtt bodies and consequential cytotoxicity (Subramaniam, Sixt, Barrow & Snyder, 2009). Work from our laboratory (Baiamonte, 2012) corroborates a cytotoxic Rhes scenario. Novel in vivo work demonstrates that HD mice without the Rhes gene are less susceptible to the motor deficits and neuropathological markers of HD.

Mevalonate Pathway

The mevalonate pathway is one of the most studied biological synthesis pathways in the human body. The pathway is named for the 3-hydroxy-3-methylglutaryl- CoA (HMG-CoA) derivative, mevalonate. It is the foundation for the production of essential compounds such as dolichols, ubiquinones and, most notably, cholesterol. These structures and their products serve several key functions like steroid and hormone production, synthesis of glycoproteins, cell respiration, and lastly, maintaining membrane integrity (Fritz, 2009). Furthermore, the pathway produces two isoprenoids, or prenyl groups: farnesylpyrophosphate and geranylgeranylpyrophosphate. Both are implicated in post-translational modifications of small

GTP-binding proteins, like those in the Ras super family (Takai, Sasaki & Matozaki, 2001). Such proteins are pivotal in intracellular signaling, cell growth and differentiation, and gene expression (Goldstein & Brown, 1990; Hinson, Chambliss, Toth, Tanaka & Gibson, 1997).

Following the pattern of Ras proteins, Rhes shares a chemical structure that involves prenylation (Hancock, Magee, Childs & Marshall, 1989; Vargiu, De Abajo, Garcia-Ranea, Valencia, Santisteban, Crespo et al., 2004). Prenylation, or isoprenylation, is the process by which a prenyl group, farnesylpyrophosphate (FPP) or geranylgeranylpyrophosphate (GGPP), is added to the C-terminal cysteine(s) of peptides and proteins by farnesyltransferase or geranylgeranyltransferase, respectively. The hydrophobic prenyl groups serve several purposes. They may act as anchors, tying together the target protein and a cell membrane or two separate proteins. In fact, the majority of prenylated proteins are localized to cell membranes. After prenylation, Ras proteins undergo proteolysis and then C-terminal methylation, which is stimulated by GTP binding in membranes (Backlund, Simonds & Spiegel, 1990; Zhang & Casey, 1996). Proteolysis and methylation facilitate proper membrane association, but the initial prenylation is essential.

Given that the mevalonate pathway and its numerous metabolites influence such a large and diverse group of proteins, much interest lies in the therapeutic potential of mevalonate interference. Two main classes of interfering pharmaceuticals exist: those that inhibit CAAX box post-translational modification and those that inhibit the pathway's key enzyme, HMG-CoA reductase. The first group interferes with the intermediate processes. Farnesyltransferase inhibitors (FTIs), geranylgeranyltransferase inhibitors (GGTIs), and dual prenyltransferase inhibitors (DPTIs) prevent the prenylation of proteins. By preventing the transfer of farnesyl, geranygeranyl groups, or both, to CAAX box motifs, these drugs also prevent the localization of

affected proteins to cell membranes and thereby interfere with signal transduction. Since small GTP proteins, like H-, K-, and N-Ras, are the most common culprits of oncogenic mutations, there has been an impressive exploration of oncogenic therapies utilizing FTIs, GGTIs, and DPTIs (Gao, Liao & Yang, 2009). In vitro and in vivo studies have exhibited antitumor effects of FTIs and GGTIs. FTIs have been successful in directly inhibiting tumor development in a majority of cancer cell lines (Sepp-Lorenzino, Ma, Rands, Kohl, Gibbs, et al., 1995). Similarly, animal models have shown that FTI driven inhibition of Ras prenylation is a valid means of therapy in colon, pancreatic, lung, prostate, bladder, and breast cancer (Ayral-Kaloustian & Salaski, 2002).

Acting at the beginning point of the pathway are HMG-CoA reductase inhibitors, or statins. HMG-CoA reductase serves as the pathway's rate limiting enzyme, transforming HMG-CoA into mevalonate (Kuzuyama & Seto, 2012). Lipid lowering statins are widely used to treat hypercholesterolemia, reducing the likelihood of heart attacks and stroke. Statins have also been explored in AD models, and recently, have been named as a possible treatment for HD symptoms. Alzheimer's research has generated several notable findings. Epidemiological studies have shown that chronic statin use was associated with decreased risk for AD (Jick, Zornberg, Jick, Seshadri & Drachman, 2000). In vitro experiments showed that statins reduce amyloid beta protein production, which composes the hallmark plaques of AD pathology (Simons, Keller, De Strooper, Beyreuther, Dotti & Simons, 1998). In corroboration, statins drive decreases in amyloid beta peptides in vivo (Fassbender, Simons, Bergmann, Stroick, Lutjohann, Keller, et al., 2001). Statins could also have therapeutic effects based on their regulation of isoprenoids. Mevalonate derived compounds, FPP and GGPP, but not cholesterol, are dramatically elevated in human AD brains. In vivo treatment with Simvastatin, a highly effective statin, significantly

reduced both isoprenoids in mice (Eckert, Hooff, Strandjord, Igbavboa, Volmer, Muller & Wood, 2009). Clinical trials using statins have shown mixed results, though the cholesterol pathway hypothesis of AD remains prominent (Hoglund & Blennow, 2007; Shepardson, Shankar & Dennis, 2011).

Because HD and AD are both neurodegenerative diseases involving intranuclear inclusion bodies and possible Ras contribution, HD research is following suit and beginning to explore what the mevalonate pathway contributes to disease. In an inducible mHtt model, increased expression of mHtt in striatal cells reduced the transcription of several genes involved in the mevalonate pathway, notably the gene encoding for HMG-CoA reductase (Sipione, Rigamonti, Valenza, Zuccato, Conti, Pritchard, et al., 2002). Similar expression effects have been found in the brains of HD humans and mice that have an overexpression of exon 1 fragment of the mHtt gene (Mangiarini, Sathasivam, Seller, Cozens, Harper, Hetherington et al., 1996). Valenza and colleagues also found that altered cholesterol biosynthesis results in lower overall cholesterol mass in cultured mHtt cells, cortical and striatal tissue of HD mice (2005). Importantly, by adding exogenous cholesterol into striatal mHtt neurons, they were able to rescue the cells in a dose dependent manner.

In contrast, Del Toro and colleagues (2010) argue that augmented cholesterol homeostasis in HD takes the form of cholesterol accumulation and altered cellular distribution which contributes to excitotoxicity. Because accumulation has been documented in striatal neurons and tissue of full length mHtt mice, researchers aimed to visualize and quantify lipid distribution within striatal mHtt cells (Trushina, Singh, Dyer, Cao, Shah, Parton, et al., 2006). Compared to cultured Htt cells, mHtt cells showed a 24% increase in cholesterol, distributed in the plasma membrane and intracellular deposits. Similarly, in vivo striatal neurons of full length

mHtt mice exhibited a 25.7% increase in total cholesterol. Not surprisingly cultured striatal cells and neurons that contained mHtt also demonstrated an increase in cholesterol-rich, highly ordered portions of the plasma membrane and those in the cytosol. Healthy Htt cells have only sporadic places of highly ordered domains, with more fluid membranes in the cytosol. Because NMDA receptors are located within such high cholesterol areas, known as lipid rafts, the extent of membrane order influences how susceptible neurons are to NMDA mediated excitotoxicity. Administration of simvastatin caused decreases in plasma membrane order, reduced lipid rafts, and protected against NMDA excitotoxicity in both Htt and mHtt cells. Interestingly, simvastatin treatment did not reduce overall cholesterol levels, supporting earlier work which suggested simvastatin redistributes plasma cholesterol content in other brain areas without increasing total levels (Burns, Igbavboa, Wang, Wood & Duff, 2006; Paolisso, Sgambato, De, Gambardella, Verza, Varricchio, & D'Onofrio, 1991; Thelen, Rentsch, Gutteck, Heverin, Olin, Andersson, et al., 2006).

In addition to altering membrane configuration, the statins' early point of interception in the mevalonate pathway allows the drugs to block downstream protein prenylation. Statins prevent the synthesis of isoprenoids farnesylpyrophosphate and geranylgeranylpyrophosphate, which as described above, are necessary for the anchoring prenylated proteins to membranes or other proteins (Jasinska, Owczarek & Orsulatk-Michalak, 2007). Whereas FTIs, GGTIs, and DPTIs inactivate the enzymes that achieve prenylation, statins eliminate the prenyl groups themselves. When normally prenylated proteins do not have the machinery to attach to membranes, their following signaling processes are prohibited. Rhes, like all Ras superfamily members, are among such prenylated proteins. In fact, non-pharmacological disruption of Rhes farnesylation prevented its sumoylation and subsequent disaggregation of mHtt, as well as Rhes-

mediated cytotoxicity (Subramaniam, Sixt, Barrow & Snyder, 2009). Statins offer a clinically relevant means to inhibition of Ras prenylation, an important step toward unlocking a treatment for not just AD, but HD as well.

In summary, statins hold promising potential for HD pathology on two fronts. First, statins' cholesterol lowering effects could attenuate cholesterol accumulations found in striatal neurons of HD patients and mice. A decrease in such accumulations is protective against NMDA-mediated excitotoxicity, one proposed mechanism of HD degeneration. More relevant to the presently proposed research, statins may pharmacologically interfere with the putative cofactor, Rhes. By compromising plasma membrane integrity and blocking prenyl group synthesis, statins can prevent the prenylation of Rhes, thus, rendering the striatally expressed protein inactive.

Purpose and Hypothesis

Current evidence implicates the soluble form of striatal mHtt and the protein Rhes, which adds to soluble levels, in HD degeneration. The majority of this work, with the exception of recent findings from our laboratory (Baiamonte, 2012), has been conducted in vitro. Therefore, in vivo exploration of Rhes' role in HD remains essential. The current study aimed to inactivate Rhes, and thus mHtt, via de-prenylation and/or removal of membrane localization using a clinically relevant mode, a commercially available and extensively researched statin, simvastatin. By administering chronic statin treatment, established Rhes prenylation and membrane localization was proposed to be eliminated and further prenylation to be prevented. Such an effect would hypothetically be manifested as decreased HD motor deficits. It was hypothesized that HD mice receiving the drug treatment would show significantly better performance on

measures of motor ability and motor related markers of neuropathology than HD mice receiving no treatment.

Methods

Animals

Breeding. All animals used in the study were derived from cross-breeding the wild-type genotype of a Rhes knockout line obtained from Dr. Daniela Spano (Spano, Branchi, Rosica, Pirro, Riccio, Mithbaokar et al., 2004) with R6/1 transgenic mice bearing the human mutated Huntington's allele (115 CAG repeats). The current breeding colony is maintained from an original HD R6/1 cohort purchased from The Jackson Laboratory (Bar Harbor, ME). All animals used were homozygous for the native Rhes allele, and either carried (hemizygous HD) or did not carry (NC) the mHtt gene. Of the 13 HD animals, 5 were placed in the drug treatment group, while 8 were used as controls. Of the 11 NC animals, 4 were treated with the drug and 7 were used as controls.

Material and Apparatus

Rotarod Apparatus. The rotarod device (Med Associates, Inc., Georgia, VT) is designed to assess animal motor coordination and balance. It is made of a suspended, rotating horizontal bar attached on both sides to the center of a 38 cm-wide metal wall. The walls, and thus, the bar, rotate at a rate of 16 revolutions per minute (rpm). The bar is 30 mm in diameter and positioned 27 cm above a floor of soft bedding material. The height of the bar is intended to encourage the animal to continue walking as to avoid a fall, but in the event of falling the bedding acts as a protective padding.

Suspended Bar. The suspended bar task is also used to measure motor coordination and balance. The set up consists of a wooden pole 1 cm in diameter positioned 30 cm. above a protective floor of animal bedding.

Triple Beam Balance. Animals' body weights were measured using an OHAUS® triple beam balance. Animals were placed in an attached, covered metal container which allows for proper measurement.

Analytical Balance. A Mettler Toledo AG64 scale was used to calculate all food and drug administration. The scale has the capacity to measure masses within 1 mg. It is further equipped with a glass shield which protects against drafts, minimizing environmentally caused fluctuations in reading.

Transgenic mouse dough. A soft dough diet, designed for transgenic mice (Bioserv, Frenchtown, NJ) was used as animal diet. The dough allows for even incorporation of drugs and, thereby, accurate dosing. The dough has been successfully used to deliver mevalonate interfering drugs (Capell, Olive, Erdos, Cao, Faddah, Tavarez et al., 2008).

Simvastatin treatment. Simvastatin (Zocor® Merck & Co., Inc. Whitehouse Station, NJ) was generously provided by Dr. Lee Roy Morgan.

Procedure

Genotyping.

Tissue collection and digestion. Tissue used for genotyping was collected from animal tail snips. Mice were anesthetized prior to tail biopsies using an intraperitoneal injection of ketamine/xylazine solution (100/8 mg/kg). Pinches to the tail and foot which elicit no reflex ensured proper depth of anesthetization. After deemed anesthetized, a sterile straight razor blade removed the end 2-3 mm of tail tissue. Wounds were cauterized and antibiotic ointment was

applied. The harvested tissue was immediately placed in a solution of 300µl of DirectPCR™ (Viagen Biotech Inc., Los Angeles, CA) lysis reagent and 11.5µl of proteinase K. Samples were incubated at 55˚C overnight while cell lysis occurs and then at 85° for one hour to deactivate the proteinase K. After tissue digestion is completed, purified DNA samples are stored at 4˚C indefinitely.

Polymerase Chain Reaction (PCR). To determine Rhes genotype, primers (Invitrogen, Grand Island, NY) for Rhes wild-type and Rhes EGFP, which recognizes only the null mutation of Rhes, were utilized. Primers for the mHtt allele were used to detect HD carrier animals. For PCR of the Rhes WT allele, 0.5 µl of DNA and 3 µl of both the sense and antisense primers were added to a solution containing 12.5µl GoTaq Green MasterMix ™ (400 µM dNTPs, 3mM $MgCl_2$, Taq Polymerase, and reaction buffer), and 12.5 µl of nuclease free H_2O. To amplify the HD gene, the same measurements of PCR ingredients were used, except for an increased amount of DNA, 2 µl, and a reduction of nuclease free H_2O from 12.5 µl to 9.5 µl.

Gel Electrophoresis. Ten µl of each PCR product were loaded into a 3% agarose gel. A 100 bp ladder was used in each gel to aid in the estimation of allele length. Gels were input with 35v of power for 5 minutes to ensure DNA migration out of each well and then with 95v until visible PCR products reached the edge of the gel. Afterwards, gels were stained with 0.5 µg/ml solution of ethidium bromide. Stained gels were placed on a Biorad™ UV lightbox in a darkened room which allows for visualization of DNA markers.

Drug Administration.

Due to the longitudinal design of the current study, statin treatment was delivered in the animals' food. Simvastatin was chosen over several other available statins based on its higher potency, greater capacity to cross the blood brain barrier, and more widespread use clinically

(Saheki, Terasaki, Tamai & Tsuji, 1994; Shepardson, Shankar, & Selkoe, 2011). Pills were crushed using a pestle and mortar, and then sifted to filter out the pill coating. Drug doses were measured and dyed using food coloring. Dyed drug was then mixed into mouse dough by hand. The dye enabled the preparer to thoroughly and evenly blend the drug and food mixture in proportions that take into account body weight and food consumption. Animals were treated with a clinically relevant dose of 80 mg/kg of body weight daily for 3 months, beginning on post-natal day 30. The literature shows simvastatin effects in daily doses ranging from .125 mg/kg to 100mg/kg (Aprahamian, Bonegio, Rizzo, Perlman, Lefer, Rifkin & Walsh, 2006, Zhang, Mao, Luo, Wei, Berggren-Soderlund, Nilsson-Ehle & Xu, 2011). HD and NC mice were fed transgenic mouse dough either unmodified or containing simvastatin according to random assignment.

Motor Assessment.

Rotarod Performance. Using the rotarod apparatus at a fixed speed of 16 rpm, motor coordination and balance was measured once every 30 days over a 4 month period. Latency to fall was recorded over three 60 second trials for each animal, with a 60 second rest period between each trial. If an animal remained on the rod after 60 seconds, it was removed and the time was recorded as 60 seconds. Based on established protocol and considering habituation effects shown in unpublished data of our laboratory, each animal underwent one day of habituation prior to the first day of behavioral testing and then a 60 second habituation trial prior to first testing trial. The best score over three trials was used for each animal in analyses.

Suspended Bar Performance. After all rotarod assessments were completed for a test day, animals underwent suspended bar testing. Animals were placed on the edge of a 30 cm high wooden dowel facing an open space. Latency to turn 180 degrees or to fall off was recorded. Inconsistent findings exist on how healthy mice behave in such a situation, but the original report

holds that animals will attempt to turn away from the dangerous edge. HD mice are known to lose their balance while managing the dowel (Mangiarini, Sathasivam, Seller, Cozens, Harper, Hetherington et al., 1996). Like the rotarod task, animals were habituated on the day prior to the start of testing. However, no 60 second practice trials were given on the first test day. The scoring of 3 trials were collapsed for a maximum daily score of 180 seconds.

Clasping Behavior. After each animal completed the suspended bar task, their limb movements during midair, ground-facing suspension were assessed. Healthy wild-type animals normally splay their limbs outward, while HD animals tend to clasp their front and back limbs inward (Mangiarini, Sathasivam, Seller, Cozens, Harper, Hetherington et al., 1996; Rubinsztein, 2002). Every animal was assessed during a 10 second trial, with 10 second rest periods in between. For each limb that was pulled in toward the body, the animal received a score of 1. Thus, every trial offered an opportunity to gain a maximum score of 4, while every testing day may have resulted in a score of 12. Animals were habituated on the day prior to their first day of testing.

Body Weight Assessment.

On each test day, animals' body weights were measured following the suspended bar task. Differences between HD and wild-type type animals in weight gain and overall weight have been demonstrated. HD mice have been shown to stop gaining weight when their symptoms begin and progressively lose weight as the disease advances (Mangiarini, Sathasivam, Seller, Cozens, Harper, Hetherington et al., 1996). Furthermore, the novel dough has a higher fat concentration than typical mouse diets contain, necessitating the assessment of weight gain and loss and comparison to previous research.

Results

The data were first examined for statistical outliers. Due to small sample sizes, extreme values were left unchanged, with one exception. A single NC control animal performed two standard deviations below the mean at month 4, a critical time point for detecting treatment effect, and was removed from all analyses. Analyses were performed using SPSS for Windows (version 19.0) with the probability of a Type I error set at 0.05. Data for each dependent variable was analyzed in two ways. First, a 2 x 2 x 4 mixed design Analysis of Variance (ANOVA) was conducted and then a two-factor, 4 x 4 mixed design ANOVA. The first analysis contained two between-subjects factors, treatment group assignment and HD carrier status. In the latter analysis, there was one between-subject factor, group assignment (4 levels consisting of HD vs NC animals in either a treatment or control group) and one within-groups factor, age at testing (1-4 months). Separate mixed ANOVAs were performed on rotarod, suspended bar, clasping, and body weight data. Significant F-ratios were followed by Tukey's post hoc tests to control for family wise error. Significant interactions of group and age at testing were subjected to tests of simple effects.

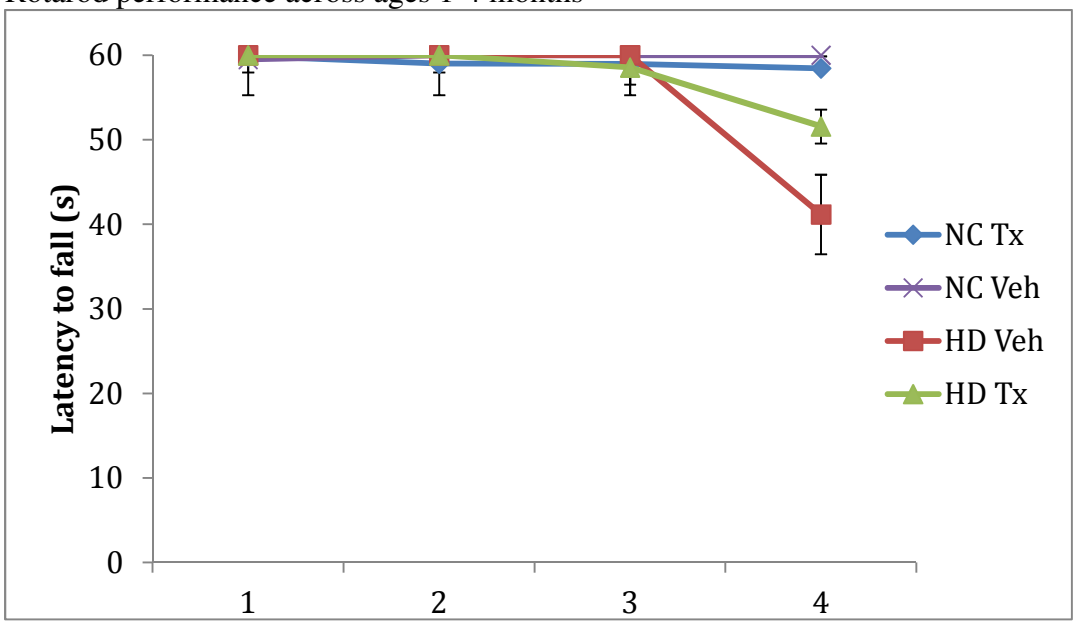
Analysis of Rotarod Performance

Rotarod performance data are summarized in Figure 1. Each animal's best performance, measured as the longest latency to fall from the rod, from three test trials was used. Analysis with a 2 x 2 x 4 ANOVA yielded a non-significant three-way interaction of treatment group, HD carrier status and testing age [Greenhouse-Geisser F (1.05, 21.05) = 1.29, p = .271]. Using a 4 x 4 design, a significant two-way interaction of group, including both treatment and HD carrier status groups, and testing age occurred [F (3.16, 21.05) = 3.40, p = .035], indicating that the groups differed in how their respective performance changed across the 4 months. Mauchly's test of sphericity indicated the data violated the assumption of equal variances among group

differences. The Greenhouse-Geisser F ratio was interpreted to correct for this violation. The conservative Tukey's HSD post-hoc tests subsequently revealed that although NC animals performed better than HD animals, and HD animals treated with the drug outperformed non-treated HD animals, the group mean differences were not significant when analyzed across all time points. However, a follow up one-way ANOVA of rotarod performance at only month 4, revealed significant group differences [$F(3, 23) = 3.19$, $p = .046$]. Post-hoc tests confirmed group differences in performance. The mean difference between the worst performing group consisting of HD untreated animals ($M = 41.18$, $SD = 18.32$) and the better performing groups made up of NC treated ($M = 58.46$, $SD = 4.08$, $p = .063$) and untreated animals approached significance ($M = 60$, $SD = 0$, $p = .095$). The HD animals treated with simvastatin ($M = 51.58$, $SD = 12.68$) performed at an intermediate level between NC groups and HD untreated animals (Figure 2). Lastly, comparison of group differences shows that HD treatment group was not significantly different from the HD control group.

Figure 1

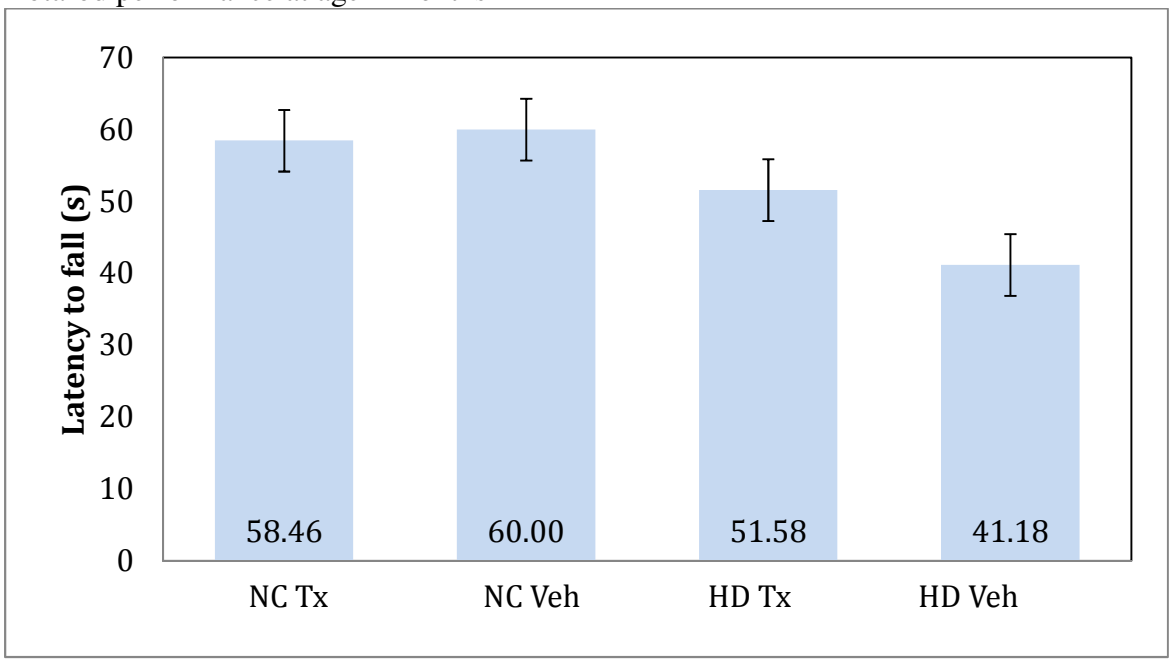
Rotarod performance across ages 1-4 months



Note: NC = Non-carrier, Tx = Treatment group, Veh = Vehicle/control group

Figure 2

Rotarod performance at age 4 months



Note: Means of groups are presented.

Analysis of Suspended Bar Performance

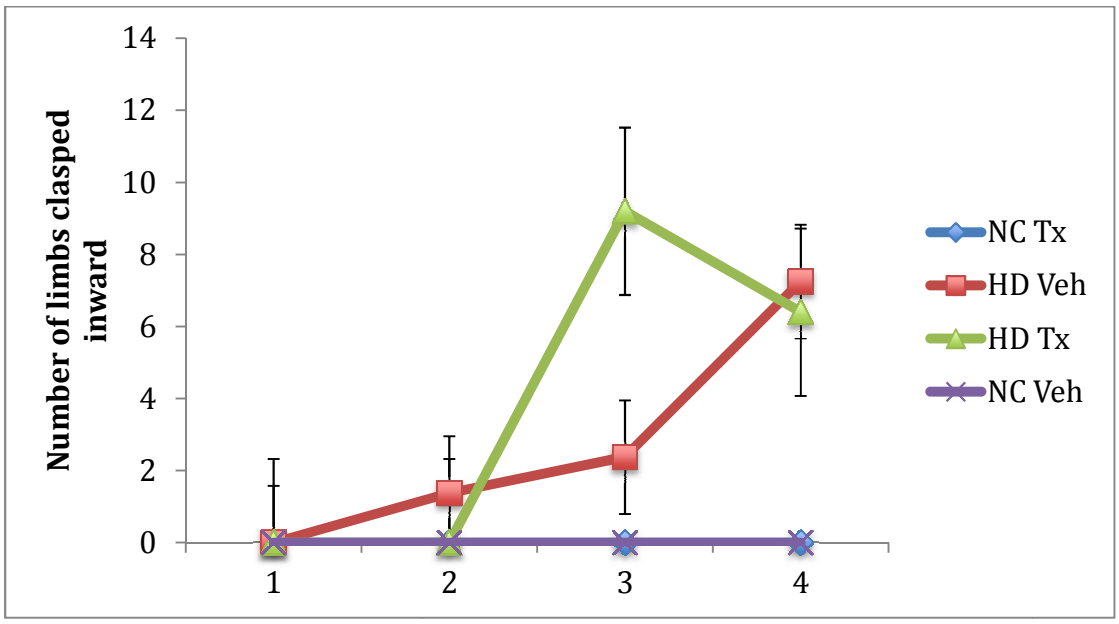
Neither a 2 x 2 x 4 ANOVA [Greenhouse-Geisser F (1.77, 36.88) = 2.51, p = .101]; nor the 4 x 4 design [Greenhouse-Geisser F (1.74, 34.87) = 2.44, p = .108] calculated for suspended bar performance showed a significant main effect of testing age. Analyses of interactions were also conducted and indicated no significant differences among groups in performance across all 4 months using a 2 x 2 x 4 design [F (1.77, 36.88) = .85, p = .42] or a 4 x 4 design [F (5.23, 34.87) = .46, p = .81]. Without significant interactions, post-hoc tests were not conducted.

Analysis of Clasping Behavior

Clasping data are summarized in Figure 3. The 2 x 2 x 4 ANOVA failed to show a significant three-way interaction between treatment group, HD status, and testing age [F (3, 63) = 2.54, p = .064]. Using a 4 x 4 design, a significant two-way interaction [F (9, 60) = 5.44, $p <$ .0001] was observed between group and testing age variables, indicating that some groups experienced an increase in clasping behavior over time, while others did not. Post hoc analysis of month 4 data, using a one-way ANOVA, indicated that, as expected, untreated HD animals (M = 7.25, SD = 6.04) showed significantly, or at a trend level, more clasping behavior than NC animals, both untreated (M = 0, SD = 0, p = .052) and treated (M = 0, SD = 0, p = .018). Similar to the rotarod data, treated HD mice (M = 6.4, SD = 5.18) displayed clasping levels that were between NC animals and the HD untreated group. However, the differences between HD treated mice and all other groups were not significant and the variability in all animals displaying clasping behavior was quite high.

Figure 3

Clasping behavior across ages 1-4 months



Analysis of Body Weight

The 2 x 2 x 4 ANOVA showed no interaction between treatment group, HD status, and testing age [Greenhouse-Geisser F (1.85, 37.03) = .98, p = .381]. The 4 x 4 ANOVA yielded a significant testing age x group interaction [F (5.56, 37.03) = 3.59, p = .008]. The Greenhouse-Geisser correction was again employed due to a violation of sphericity. Across all time points, no group's mean performance significantly differed from another, as assessed by Tukey's HSD. Examining only differences in weight at month 4, by means of a one-way ANOVA, no groups were found to differ significantly.

Discussion

Consistent with the current study's hypothesis, findings indicate that chronic simvastatin treatment was related to decreased motor deficits in animals carrying the HD gene, as measured by the rotarod task. The effects of the drug simvastatin, are visible at the animals' fourth month

of life. Whereas untreated HD mice showed significant motor impairment compared to treated NC mice, the motor performance of simvastatin-treated HD mice was not significantly different from controls. However, their performance fell between that of NC mice and untreated HD mice, being not significantly different from any other group, including untreated HD mice. While the HD control mice performed worse than both NC groups at the statistical trend level, the HD treated mice were considerably closer in performance to NC mice. Importantly, the data are consistent with recent findings from our laboratory, which show that depletion of the Rhes protein, by means of genetic manipulation, causes decreased HD-related motor deficits and, thus, better performance in the rotarod task, beginning at the 4 month age (Baiamonte, 2012). The effects of Rhes depletion remained at the fifth and sixth month tests. HD/Rhes depleted animals, which are analogous to the current study's HD treated animals, did ultimately show motor impairment compared to NC animals, though not until the age of five months.

Similar to what was found in the Rhes depletion study, the current study failed to yield any significant effects of age or group in suspended bar performance. Although the suspended bar task was created to measure motor coordination, it is likely that the task did not capture such an ability, as even NC mice did not consistently display the measured behavior, turning in the opposite direction from a heightened ledge. Through observations, it was apparent that the mice, in fact, demonstrated more exploratory behaviors than evasive behaviors. It is possible that the mice became desensitized to the height of the ledge. Researchers have thought a heightened ledge produces an anxiety-like state in the mice, resulting in an attempt to flee the situation (Baiamonte, 2012). However, if no dangerous consequences are encountered initially without the mice successfully turning a full 180 degrees, continued anxiety when placed on the ledge and resulting turning behavior are unlikely. By even the first day of testing, all mice had received 3

trials of habituation, allowing reasonably sufficient time for animals to either learn a non-turning evasive technique, such as back pedaling a few centimeters from the ledge and then stabilizing oneself, or to become desensitized to the ledge in general.

Despite rotarod data that indicated decreased motor deficits in treated HD mice, clasping behavior, which is an indication of neurodegeneration, was not affected overall. At month 4, there was no difference between the treatment and control groups of HD animals. Both HD groups showed a tendency to pull their limbs inward toward their stomach, as opposed to splaying their limbs outward as the NC animals did, when suspended upside down in the air. Interestingly, the treated HD mice showed a decrease in clasping behavior from month 3 to month 4. These findings suggest that simvastatin may affect motor areas of the brain differentially. Although behaviors necessary to both the clasping and rotarod tasks are governed by the striatum and its neural projections, simvastatin could potentially influence Rhes neurons in some encapsulated striatal areas while not affecting others. HD is a progressive neurodegenerative disorder, and clinical findings hold that the progression's speed and specific course vary across individuals (Roos, 2010). HD patients show different patterns of symptom onset across their lifespan. Following this argument, even the HD mice studied here, which all have equal CAG repeat length known to influence progression, display varied deficits. For example, 3 animals of the HD control group failed to show any clasping behavior in the 4 months they were tested. Surely these animals will begin to show clasping behaviors at later time points as they lose more and more brain mass.

The current study found a lack of significant weight loss usually exhibited by HD animals when their symptoms begin. Neither treated nor untreated HD mice weighed significantly less than NC mice at any time point. The diet given to all animals in the study is likely responsible.

The soft dough has a higher fat content (14%) than regular hard pellet food commonly fed to experimental rodents (roughly 4%). Notably, the mice did not have unusually heavy weights and did not display any negative side effects of the food.

Taken together, the behavioral data point toward a drug effect limited in its capability to attend to HD motor deficits and neurodegeneration. Previous research has shown that Rhes acts as a potential cofactor, along with mHtt, to cause HD neurotoxicity. It is argued that Rhes binds to mHtt and enhances sumoylation, the post-translational modification which increases that amount of toxic soluble mHtt (Subramaniam, Sixt, Barrow & Snyder, 2009). Simvastatin, in this study, could possibly be attenuating HD motor deficits by preventing Rhes to attach to its normal membrane location prior to mHtt binding. The drug blocks the mevalonate pathway which generates the compounds used by Rhes to anchor to cell membranes. Simvastatin treatment could, in contrast, be acting via membrane alteration. By decreasing the lipid concentration of membranes, the drug treatment used here may be lowering the number of NMDA receptors and, in turn, decreasing NMDA-mediated excitotoxicity (Trushina, Singh, Dyer, Cao, Shah, Parton, et al., 2006). Given the contradictory results of drug treatment in the rotarod and clasping task, two measures shown to be influenced by Rhes protein levels, it is possible that simvastatin is not influencing Rhes activity at all.

Though the data are promising, they should be interpreted with caution, as the study has several shortcomings. First, the sample sizes were quite small, with unequal group numbers. In addition, no examination of simvastatin in brain tissue was conducted. While there is no reason to suspect that simvastatin, which holds a high potency, absorption rate, and ability to cross the blood-brain barrier, would not be reaching the target brain areas, such a confirmation is necessary.

There are many questions generated from this experiment. Future studies are needed to confirm the drug effect, gather details about its activity, and explore the potentially therapeutic use in clinical populations. To address this study's limitations, the drug's presence in the brain needs to be confirmed. By comparing neural sections following gross neuroanatomical markers, researchers can gain valuable information on simvastatin's penetration into separate areas with markedly different behavioral roles. In order to confidently conclude the drug effects these data suggest, a physiologically appropriate concentration of simvastatin needs to be demonstrated in the striatum. Examination is underway which will use homogenized brain tissue of various areas and employ thin layer chromatography, nuclear magnetic resonance spectroscopy, and high performance liquid chromatography to quantify drug concentrations.

Due to the patterns in behavioral measures which showed separation between HD and NC animals only beginning at month 4, the current animals will continue to be assessed at 5 and 6 months of life. As HD control mice continue to show declines, any protective drug effects will only become more apparent. There is considerable room to decline, as some HD control mice had not begun to show HD motor deficits or clasping behavior even at month 4. In light of the small sample sizes used, a continuation of drug effects requires HD treated animals to perform significantly better than HD control animals. The most supporting evidence for simvastatin's benefits would be generated if the HD treatment group maintained comparable behaviors to NC groups on the rotarod task. The exact mechanisms of action are also yet to be understood. Simvastatin could be influencing the putative co-factor Rhes, altering cell membrane integrity in general, or both. Further studies utilizing cell culture and Rhes protein visualization and localization are warranted.

Furthermore, exploration in a human clinical population may prove less straightforward. Although epidemiological studies propose links between statin use and decreased risk for neurodegenerative diseases (Jick, Zornberg, Jick, Seshadri & Drachman, 2000), little work has been done toward clinical trials of statins for relief in those that have such diseases. It should be noted that before such work can be conducted, more information is needed on statins' effects on the cognitive and psychiatric symptoms accompanying diseases like HD.

In conclusion, these findings hold promise for the development of pharmacologic therapies in HD. The novel treatment produced interesting differences in motor behavior within a small sample, despite variability in symptom severity. If subsequent testing at later ages produces similar results, simvastatin would be the first drug utilized to alleviate the motor symptoms of HD. This drug holds the capacity to cross the blood brain barrier and manipulate extra- and intra-cellular functioning. For a disease with no cure and no treatment targeting disease progression, simvastatin is a well-understood drug with great therapeutic potential.

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Institutional Animal Care and Use Committee

UNIVERSITY OF NEW ORLEANS

DATE: November 11, 2009

TO: Dr. Gerald LaHoste

FROM: Steven G. Johnson, Ph.D.
Chairman

RE: *IACUC Protocol* # **UNO-09-011**
Entitled: Breeding and Cross-breeding of Huntington's Disease Mice

Your application for the use of animals in research (referenced above) has been approved beginning November 11, 2009 and expiring November 10, 2012. Please note that an annual/final report must be provided to the UNO IACUC.

The University of New Orleans has an Animal Welfare Assurance on file with the Office of Laboratory Animal Welfare (OLAW), National Institutes of Health. The assurance number is A3299-01.

Institutional Animal Care and Use Committee

UNIVERSITY OF NEW ORLEANS

DATE: November 11, 2009

TO: Dr. Gerald LaHoste

FROM: Steven G. Johnson, Ph.D.
Chairman

RE: *IACUC Protocol* # **UNO-09-012**
Entitled: Rhes and Huntington's Disease

Your application for the use of animals in research (referenced above) has been approved beginning November 11, 2009 and expiring November 10, 2012. Please note that an annual/final report must be provided to the UNO IACUC.

The University of New Orleans has an Animal Welfare Assurance on file with the Office of Laboratory Animal Welfare (OLAW), National Institutes of Health. The assurance number is A3299-01.

Vita

The author was born in Wilmington, DE. She graduated cum laude from Elizabethtown College with her Bachelor's degree in psychology in 2008. She joined Dr. Gerald LaHoste's laboratory at the University of New Orleans, Applied Biopsychology program in 2010. She continues to pursue her Ph.D. while conducting Huntington's Disease research under Dr. LaHoste.