

When motor neurones damaged by MND are investigated a number of different pathologies are seen, suggesting that all cases of MND do not result from only one common disease process but from any of several possible disease processes. Some researchers suspect some of these disease processes are actually links in different chains of processes which, if any single process is interrupted, eventually causes the whole chain to fail and results in the death of motor neurones.

The diagram, below, shows four very simplified "chains," any one of which could affect the survival of a motor neurone.

In the upper left part of the diagram too much glutamate could be released by the astrocytes, so flooding the transporter mechanisms and allowing glutamate to arrive at the neurone faster than is safe. This could either cause damage to the transporter systems or cause the neurone to overreact and become damaged.

In the upper right part of the diagram; stopping the release of "used" glutamate by the neurone could cause it to keep firing until it exhausts itself and dies in the process. Failure of the transport or re-uptake mechanisms could leave too much glutamate in the environment of the motor neurones so poisoning them and causing death.

On the lower left of the diagram a simplified description of how a cell obtains energy and generates free radicals is shown. Damage to the food transport system or the cytochrome chain

would starve the cell of energy while failure of the SOD1 scavenging system to control free radicals could result in damage to cell structures such as the membranes, or even the cell's DNA.

In the lower centre half damage to the mechanisms that control either sodium or calcium could affect the neurone directly and again cause catastrophic damage.

The detail of these chains of events is much more complex than is shown in the diagrams and, indeed, these are not the only chains of events known to exist in motor neurones.

The important point to note is that in this simplified diagram alone there are at least fourteen distinct places where an interruption to one process in any of the four chains could result in the death of the motor neurone. It is for reasons like these that many scientists are questioning whether MND is one disease or many different diseases which just happen to affect motor neurones.

Some of the current areas of research and investigation are mentioned here to illustrate the wide ranging complexity of the investigations into this disease.

Free Radicals and Oxidative Stress

All cells generate toxic metabolites (think of these as the "exhaust" from your car and the consequences to yourself and your passengers should your car not be properly ventilated). In the cell, these "exhaust products" are carbon dioxide, water and sometimes "free radicals."

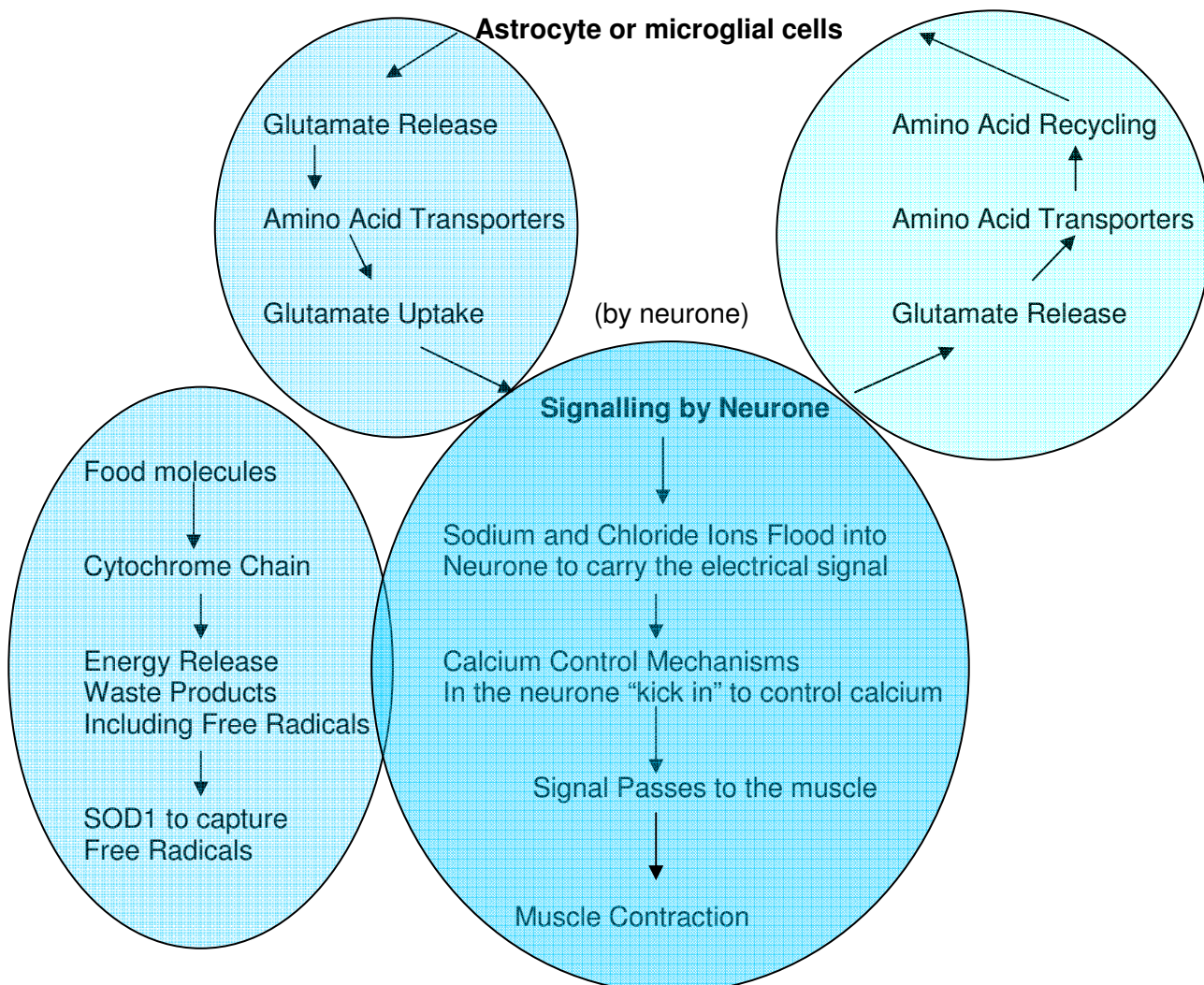
MND Scotland is the working name of the Scottish Motor Neurone Disease Association, the only charity funding research and providing care and information for those affected by MND in Scotland.

MND Factsheet 26 Current Research

Free radicals in this context are a destructive form of oxygen that can also be used by the cell under normal circumstances to fight disease. However, an overproduction of free oxygen radicals can result in cell damage and death. As might be expected, several defences against such a process exist. A major one is the superoxide dismutase enzyme (SOD-1) discussed elsewhere. Mutations in the SOD-1 gene could allow excessive free radical accumulation in the cell, damaging the neuron. Researchers have documented the existence of excessive levels of proteins damaged by free oxygen radicals within neurons in ALS,

suggesting that either the neuron synthesises excessive levels of free radicals or it is incapable of disposing of those that are normally produced.

Therapies geared to reducing oxidative stress are in development, including gene therapy and new drugs. The properties of certain foods and vitamins are also studied for their ability to reduce free radicals. Vitamin E's antioxidant properties made it the prescribed therapy for people with MND one hundred years ago and it is still in use today, although with little apparent effect in hindering the course of the disease.



Some simplified chains of reactions needed for normal motor neurone functioning

The information in this leaflet is believed to be accurate at the time of production. MND Scotland cannot give detailed medical advice, this leaflet should be regarded only as general background information.

Immunological Factors

In recent years, researchers have considered how injured motor neurons might spur an immune response that could contribute to, and even perpetuate, a cascade of cell death in the nervous system. The nervous system's immune cells, called microglial cells and astrocytes, can respond to an injured neuron in a way that can be either beneficial or harmful. This response by microglial cells has been implicated as a trigger of programmed cell death (PCD), a mechanism that is useful on the small scale to clear away damaged cells, but devastating on a large scale as it ripples through the nervous system killing motor neurons.

Neurotrophic Factors

These important chemicals help in the growth and maintenance of motor neurons and have been shown to enhance motor neuron survival in mice with a variety of motor neuron disorders. Although it is not clear how deficiencies of neurotrophic factors may affect human motor neurons, several attempts have been made to determine whether neurotrophic factors can slow the rate of progression of MND by first testing these agents in animal models of the disease.

Researchers are also investigating how the neurotrophic factors including brain derived neurotrophic factor (BDNF) and "cytokine ciliary neurotrophic factor" (CNTF) interact with metal ions in cells, and how metal ions can thereby have enhanced toxic effects in a cell depending on the neurotrophic factors present. By developing drugs to manipulate the interactions of neurotrophic factors, researchers hope to learn about and gain

some control over this potentially useful aspect of the disease process.

Gene therapies are also in development to promote the levels of beneficial neurotrophic factors. Recently, the gene for insulin-like growth factor 1 (IGF-1) was successfully delivered in ALS mice using a viral vector, with the successful result of prolonging the life of the mice.

Viral Vectors

Viruses are parasites which reproduce themselves by hijacking the reproductive mechanisms of a host cell to manufacture virus particles. They do this by injecting their own DNA into the cells of the host organism where the viral DNA inserts itself into the DNA of the host cell. Once the viral DNA is in place it releases signals which cause the host cell's reproductive apparatus to copy the viral instructions over and over again making more and more virus particles. In the case of the virus responsible for cold-sores (Herpes simplex) the host cell makes so many copies of the virus that the host cell swells up until it bursts releasing cell fluid loaded with newly made virus particles.

The ability of viruses to insert their DNA into that of a host cell makes them ideal carriers (vectors) for genes we might want to insert into a healthy cell. In theory a healthy copy of a human gene is inserted into the viral DNA so that when the virus DNA inserts itself into the host DNA the healthy copy of the human gene is used.

There are dangers in using viral DNA as a vector in this way. A number of genes are known to make biochemicals which prevent the growth and proliferation of tumours in the healthy body. The

dangers come from the possibility that the vector might insert itself into any one of these tumour suppressor genes and consequently switch it off, allowing the infected cell to become cancerous. Amongst other areas viral vector research is also looking at ways of directing where such vectors are inserted into human DNA to try to reduce these dangers.

Altered Protein and Neurofilament Metabolism

Advances in technology in the field of protein research have opened up new avenues into understanding the protein mechanisms involved in MND. Mass spectrometry has become very advanced enabling scientists to detect which proteins are present in an extremely small sample. Mass spectrometry is also providing researchers with the tools to develop a desperately needed simple, rapid test to diagnose MND by identifying proteins generated as a result of the disease. This test is undergoing clinical trials at the moment to test how reliable and effective it actually is. There are hopes that it can be used as part of the diagnostic process in the future when a small sample of cerebrospinal fluid could be taken and tested for the levels of certain proteins, so indicating whether or not MND is present.

Powerful electron microscopy can reveal the configuration and behaviour of proteins. Such technological advances provide insights into how proteins fold and unfold and interact with their environment.

A signature feature of ALS is the accumulation of neurofilaments in the motor neurons. These key neuronal proteins are believed to be responsible for maintaining the normal structure and

shape in healthy neurons. Studies making use of transgenic models to alter neurofilament expression reveal that abnormalities in the metabolism of neurofilaments, or the way in which neurofilaments interact with each other - or with other proteins, could play a role in the development of MND.

Many types of cellular proteins and enzymes may play a role in MND. Protein kinases are enzymes that regulate many cellular functions. Studies have revealed that abnormal levels of protein kinases exist in the nerve tissue of people who died of MND. By comparing this tissue with mouse models, researchers are investigating how abnormal levels of these important regulatory enzymes may trigger cell death in the motor neurons. They hope to find a way to counterbalance the protein kinase ratios and so prevent cell death from being triggered.

Proteosomes and protein chaperones are enzymes that shuttle proteins and chop them up into their basic components in order to clear away damaged proteins in the cell. Researchers are investigating these enzymes in mouse models and cell lines to understand their role in the pathogenesis of ALS and how they might be used therapeutically to hinder the disease.

By studying the proteins affected by genetic mutations in familial ALS, and the behaviour and interactions of the proteins involved in neurofilament aggregation in cells, researchers are gaining a better understanding of the role of abnormal protein mechanisms in MND.

Glutamate Excitotoxicity

Abnormalities in the handling of excitatory

amino acids, particularly glutamate, by the nervous system may be critical to the occurrence of MND. Damage to the normal "transporter" mechanisms, by which glutamate is removed from the nervous system, allows excessive glutamate to accumulate.

When motor neurons receive glutamate at their receptors, there is an influx of calcium ions into the cell. The motor neurons may not be able to deal with the excessive levels of calcium flooding in, resulting in damage.

Researchers are investigating ways to help the nervous system handle calcium and glutamate. Riluzole, the only drug currently available for the treatment of MND, shows very modest results. Its action is not well understood but is thought to perhaps affect glutamate uptake mechanisms in the motor neurone.

Genetic Factors

It is thought that five to ten percent of all MND cases are inherited and that between one fifth and one third of all inherited cases are due to mutations on the SOD1 gene. This means that between two-thirds and four-fifths of inherited cases are due to other genes

that can be passed from generation to generation in the same way as the SOD1 gene. Since people have two copies of the SOD1 gene and only one is normally mutated in people with inherited ALS the chances of a passing on the faulty gene are one in two, or 50/50.

Although about two dozen genes have been identified as being involved in different people with MND, the precise details of their involvement has not been fully discovered for many of them.

It is also possible that two or more different mutated genes might need to be inherited in some forms of sporadic MND. Situations like this are more difficult to identify in families since the chances of inheriting both faulty genes from a parent are $\frac{1}{2} \times \frac{1}{2}$ which is $\frac{1}{4}$ or a 1 in 4 chance of inheriting both copies. If three genes are involved the chances of inheriting all three faulty genes are 1 in 8. Four faulty genes would be 1 in 16 and so on.

Relatively low risks of inheriting all the faulty genes like this would make a familial form passed on this way very difficult to identify when the average family size is between two and three children.

Further Reading

Factsheet 10	Creatine
Factsheet 11	Vitamins and MND
Factsheet 16	Stem Cells
Factsheet 18	Clinical Trials
Factsheet 22	Riluzole
Factsheet 28	Complementary Therapies
Factsheet 39	Lithium

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