Nitric Oxide Signalling: Nitric oxide (NO) is the signalling molecule originally identified as endothelium-derived relaxing factor (EDRF) mediating relaxation of blood vessels. It is a small, highly diffusible and reactive molecule with a short life-time, generated from arginine by the cytoplasmic enzyme nitric oxide synthase (NOS). Three NOS genes with distinct tissue localisation and properties are known, namely: endothelial, inducible and neuronal NOS (eNOS, iNOS & nNOS, respectively). Activation of eNOS and nNOS are classically Ca2+-dependent, with nNOS being closely coupled to Ca+2-permeable NMDA receptor (NMDAR), both of which are linked to postsynaptic densities (PSD-95) of the CNS through their mutual PDZ binding motifs. eNOS and nNOS generate low nanomolar concentrations of NO, whereas iNOS can produce micromolar levels. Such high concentrations affect down-stream signalling mechanisms, with low concentrations being neuroprotective and mediating physiological signalling (e.g. neurotransmission or vasodilatation) whereas higher concentrations are neurotoxic. Excessive activation of iNOS has been linked to several neurodegenerative disorders (see below).

The major physiologically relevant receptor for NO is soluble guanylyl cyclase (sGC) which mediates the production of cGMP from GTP. Down-stream transduction can be via cyclic nucleotide-gated ion channels, activation of protein kinase G and protein phosphorylation, or direct actions on proteins via S-nitrosylation and nitrotyrosination (Figure 1). Metabolism of cGMP by phosphodiesterases (PDE) suppresses NO/sGC signalling. There are 11 PDE genes with specific differential expression in nervous tissue. Signalling activity will then reflect the equilibrium between cGMP synthesis and degradation; for instance sildenafil/Viagra is an antagonist of PDE5, reducing degradation so that lower activity of sGC can achieve sufficient signalling to relax corpora cavernosa muscle and achieve erection.

There are several well characterised competitive antagonists for nNOS and sGC, and some allosteric modulators allowing pharmacological intervention. But physiological actions of NO are achieved at very low concentrations, so proof of endogenous

Figure 1: The NO signalling pathways.
Nitric oxide (NO) generation by physiological stimuli is difficult nNOS is widely distributed across the brain, but it is normally expressed in a subpopulation of neurons within a given region. Its mobility, unconstrained by cell membranes, allows action across a broad volume (hence the term 'volume transmitter') limited by inactivation (e.g. scavenging or degradation). It has long been postulated that NO could also act as a retrograde messenger, mediating transmission from target neurons back onto the synapse and regulating synaptic plasticity (for example in the hippocampus and cerebellum).

Nitric oxide signalling in the brain can modulate a range of processes such as various forms of plasticity (long term potentiation and depression, LTP and LTD) regulating rhythmic activity including gut motility, respiratory rhythm, circadian rhythms, locomotor and thalamic activation. There is strong evidence for involvement in learning and memory mechanisms through mediation of specific forms of LTP in the cerebellum, hippocampus and neocortex and LTD in the cerebellum. The cellular and molecular targets of nitric signalling pathways are also diverse and as yet incompletely resolved; there is evidence for modulation of postsynaptic transmitter release at excitatory glutamatergic and inhibitory GABAergic synapses, postsynaptic AMPAR phosphorylation and trafficking, calcium channels, potassium channels and interactions with other signalling pathways (such as mGlur, endocannabinoid and catecholamine). Our recent work in the auditory brainstem has highlighted the role of NO in regulating postsynaptic excitability via Kv3 voltage-gated potassium channels in activity-dependent auditory processing Enhanced synaptic transmission at the calyx of Held synapse onto principal cells of the medial nucleus of the trapezoid body (MNTB) causes NMDAR-mediated and calcium-dependent activation of postsynaptic nNOS. The NO acts in the target neuron and surrounding neurons to suppress voltage-gated potassium channels (particularly Kv3) through a slow time-course (15-30 minutes) phosphorylation mechanism which has a homeostatic-like function in matching postsynaptic excitability to the synaptic traffic. The broad expression of function in matching postsynaptic excitability via Kv3 voltage-gated potassium channels in fast-spiking interneurons to suppress voltage-gated potassium channels (particularly Kv3) through a slow time-course (15-30 minutes) phosphorylation mechanism which has a homeostatic-like function in matching postsynaptic excitability to the synaptic traffic.

So what are the processes whereby NO signalling might contribute to disease?

**Production of Reactive Nitrogen Species (RNS):** The term nitrosative stress describes this ability of NO and its derivatives (RNS) to damage proteins and DNA. A primary reaction is reaction of NO and O2- to form peroxynitrite (ONOO-). Generation of reactive oxygen species (ROS) occurs in every eukaryotic cell; electron 'leakage' from the mitochondrial electron transfer chain results in the production of superoxide (O2-) which is further degraded by antioxidants enzymes, catalase or glutathione peroxidase. Thus mitochondria are also a potential source of RNS. NO and ONOO- both inhibit the mitochondrial respiratory chain, reducing ATP production so that susceptibility to neurodegeneration shows complex dependence on local metabolic rates, oxygen availability, antioxidant activity (reduced glutathione) and cell stress signalling. Other effects of NO/ONOO- include release of Zn2+ from internal stores (such as metallothionein) with concomitant formation of S-nitrosothiol and neurotoxicity. Free Zn2+ induces respiratory block, opening of the mitochondrial permeability transition pore (mPTP), cytochrome c release, generation of ROS, and p38 MAP kinase activation leading to caspase-independent K+ efflux with cell volume loss and apoptotic-like death.

Further metabolic compromise may result from mitochondrial fragmentation. This is fast occurring within minutes after NMDAR activation and is considered a prelude to neurodegeneration and cell death. Increased mitochondrial fission in response to NO has been reported in AD, PD, amyotrophic lateral sclerosis (ALS) and...
Huntington’s disease (HD).

Fragmentation of postsynaptic excitability at a glutamatergic synapse.

Mediator of neuronal damage caused by Aβ protein modifications in Parkinson’s disease.

Nitrosative stress linked to sporadic Parkinson’s disease with special reference to Parkinson’s disease and amyotrophic lateral sclerosis.

Electrically active neurons degenerate when exposed to nitric oxide.