LSHM-CT-2004-512039

NeuroNE

Molecular mechanisms of neuronal degeneration: from cell biology to the clinic

Final Activity Report

January 2005 to June 2009

Executive summary for the whole project

The NeuroNE network is a large Network of Excellence with 20 academic partners, 5 SMEs and one Management Team. Its scientific **scope covers the mechanisms of neurodegeneration and neural repair. In neurodegenerative** disease the mechanisms of degeneration in Alzheimer's, Parkinson's, Huntington's disease and in ALS are not fully understood. Members of the network are in the forefront of research into the cause of neurodegeneration and the development of treatments to prevent it. Neurodegenerative disease and acute brain or spinal cord injury can produce considerable disability in patients. At present it is not possible to repair damage to the CNS. Members of the network are developing methods to promote axon regeneration and the replacement of neurons and glia in the damaged nervous system.

This is the final report from the NeuroNE Network of Excellence. In this section we summarize lessons learned from running this large network over four years. The detailed reporting on network activities is in later sections.

- Overall NeuroNE was a success, proving that with appropriate management large networks can be very effective.
- The key to running a large network is use of a variety of networking tools to ensure frequent communication between partners. A substantial budget needs therefore needs to be set aside for networking activities
- Because budgets for projects and programmes are limited the natural tendency of applicants is to put all the money into scientific work. It is essential that large projects have a substantial networking budget. This might be put as requirement in the applications process, and grants which lack a sufficient budget for effective networking should not be funded.
- There is no point having networks in which the partners do not have post-docs or PhD students. Without junior scientists working full time on network projects there is insufficient reason for the partners to collaborate, and the result will just be a few meetings.
- The partners are very busy with multiple calls on their time, so their involvement in a network will be sporadic. The post-docs are working full time in the network and their time is more flexible. Networking activities are therefore best focussed on the post-docs, who then communicate their enthusiasm and involvement to the PIs.
- A problem with focussing on post-docs is that only a few stay with the network for its full timespan. This problem can be overcome as long as the post-docs meet fairly often and the new arrivals can be briefed by the existing post-docs.
- **The most successful networking tools in NeuroNE have been**:-
  1) Regular web seminars, with the post-docs as speakers
  2) An internal collaborative grants programme, with the grant applications coming from two or more post-docs from different labs. The grants (30'000 and 50'000 euros) were to pay for travel, consumables, assistance.
  3) Ready availability of travel money for collaborative meetings and projects
  4) Several meetings a year- one large plenary and around three focussed workshops

5) Prizes for best post-doc posters and projects (to pay for visits to international conferences such as SFN)
6) Availability of technical platforms for collaborative projects
7) Social events at network meetings

- NeuroNE meetings have been very successful because they are sufficiently large to have a critical mass as scientific meetings. Smaller projects cannot achieve this. Clustering projects in the same field for annual meetings will add value.
- A successful outreach programme is very good for network morale and identification, and is also a good networking tool, because the junior scientists become involved.
- Restarting a new outreach programme for each network is time-consuming and difficult. It would be useful to have some carry-over into future networks, and also some existing structures with which new networks can work
- Experienced management is essential for running large projects and networks. Projects that are not associated with an experienced management company are liable to fail. Applicants for grants need advice on how to link up with good management companies
- The EU science programme by establishing networks of scientists has truly reshaped the European scientific community and greatly strengthened science within the community. It has been one of the great successes of the EU. The budget for collaboration should not be decreased. The European Research Council's grants to individual labs is not a substitute.

The NeuroNE consortium

Further information: http://www.euneurone.net and http://theneuroneproject.org

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List of Partners

Partner number	Institution	Partner name	Post doc name
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2	EPFL	Patrick Aebischer	Chris Towne
3	UHEI	Hilmar Bading	Oliver Dick
4	UMG-GOE	Mathias Bähr	Katrin Eckerman
5	ULUND	Patrick Brundin Deniz Kirik	Denis Soulet (P Brundin) Lachlan Thompson (D Kirik)
7	FMI	Pico Caroni	Smita Saxena
8	UNMIL	Elena Cattaneo	Elena Lugani
9	TECHNION	Aaron Ciechanover	Yifat Herman
10	CEA	Nicole Déglon	Latifa Rbah
12	UMUENCHEN	Christian Haass	Richard Page
13	KI	Carlos Ibanez	Tatiana Sandoval
15	MPIN	Rüdiger Klein	Edgar Kramer
17	KCL	Nigel Leigh / Chris Miller	Arjuna Ratnayaka
18	VIB	Bart de Strooper	Lucia Chavez
19	CSIC	Jose Naranjo	Malgosia Palzcewska
20	UZH	Martin Schwab	Marija Petrinovic
21	UNITO	Piergiorgio Strata	Paolo Cesare
23	TROPHOS	Trophos	Serena Bielli
26	GENETRIX	Genetrix	-
27	PHARMAXON	Pharmaxon	-
28	MILTENYI	Miltenyi	Melanie Jungblut
29	CNRS	Harold Cremer	Antoine de Chevigny
30	VIMM	Ernesto Carafoli	Dmitry Lim
31	D-PHARM	D-Pharm	Shirley Abramovitch Michal Schwarz
32	BABRAHAM	Michael Coleman	Giacomo Morreale

Scientific achievements throughout the whole project

Achievements at the end of Year 1:

During its first year, a major aim of the network has been the integration of scientific activities. NeuroNE has recruited 20 young scientists who will work together, and act as the glue to cement collaborations between the laboratories. In order to provide them with the best research facilities, the network has set up six technical platforms which are available to all the members. These provide the most advanced facilities for genomics, proteomics, production of transgenic animals, high throughput screening, brain imaging and electron microscopy. There is a programme of collaborative visits, seminars and internet meetings. NeuroNE has a programme of technical workshops to spread the most advanced technology around its members. In the first period workshops on brain imaging and on nerve fibre damage in degenerative diseases.

Some highlights of the work of the network during the first year have been:

The causes and treatment of motorneuron disease. Motorneuron disease or ALS is a condition that leads to progressive paralysis and eventually death. NeuroNE members have identified a new genetic defect that can be responsible for the condition. This is a molecule called ALS2, which normally helps to control nerve fibre growth. It has also been shown that as the motorneurons become sick, nerve fibres gradually withdraw from their connections with muscles. Protection of the motorneurons in susceptible mice has been achieved in two ways. The first has been to crossbreed the mice with animals which have a mutation which preserves damaged nerve fibres. The second has been to develop gene therapy approaches using viral vectors. These vectors have been engineered to partly remove one of the toxic molecules that can cause the condition, SOD1.

Protein aggregation in Alzheimer's disease. A feature of Alzheimer's disease is the formation of aggregates of a toxic peptide, Abeta. This peptide is produce by chopping the larger APP molecule in two or more places. NeuroNE laboratories have been investigating the function of the enzymes that perform this cutting, known as secretases. Knockout animals have been made to find out the normal function of these secretases, and potential pharmaceuticals have been discovered that can alter their activity. A key technology is the ability to monitor the formation of the aggregates in live cells. Using new imaging methods NeuroNE scientists have developed a method to visualise the formation of aggregates.

Protection of brain cells. The death of neurons during degenerative diseases leads to the disabilities suffered by patients. NeuroNE scientists have investigated various new ways to keep cells alive. In Huntington's disease, it has been shown that the loss of a growth factor, BDNF, may be a major cause of the condition. Growth factors have also been shown to protect against motorneuron disease and to be involved in the early development of the cerebral cortex. Cells possess various molecules which can inhibit the cell death programme. The method by which one of these, XIAP, is controlled has been worked out. Calcium overload is extremely toxic to nerve cells, and often occurs during degenerative conditions. New mechanisms have been discovered for controlling the calcium level inside neurons.

Plasticity in the brain and spinal cord. After the brain or spinal cord are damaged, the nervous system can partly rewire itself to compensate for the lost cells. This process is known as plasticity. The nervous system of children is highly plastic, but in adults much of this plasticity is lost. Children are therefore better able to recover from damage to their brain and spinal cord. NeuroNE scientists have been working to understand the factors that control plasticity and develop ways of reactivating it in adults. It has been discovered that molecules that occupy the extracellular space between nerve cells, known as proteoglycans, are involved in turning off plasticity in adults and that digesting them with an enzyme, chondroitinase, can bring back plasticity. This enhances recovery after brain and spinal cord injury. New genes have been discovered that promote plasticity inside neurons.

Achievements at the end of Year 2:

During its second year, the priorities of the network have been the optimisation of the collaboration of the partners, the integration of the young scientists who have been recruited and the advancement of the scientific activities.

Integration activities

In the previous year NeuroNE recruited 22 young scientists to work on collaborative projects within the Network. During 2006 a priority was to achieve a close integration of these researchers, who act in many ways as the glue that holds the network together. This was achieved in several ways.

1. A monthly web-seminar series was established. Each month two of the young researchers give a seminar over the Marratech web-conference software. This was very successful, with good attendance and excellent seminars. It kept the researchers in regular contact.

2. Internal collaborative grants programme. At the 2006 plenary meeting the young researchers were informed that NeuroNE would provide funding of up to 150K€ total for collaborative projects within the network. The researchers discussed their applications at the plenary meeting and afterwards. These were scientifically refereed, and six were funded.

3. Plenary meeting. The first plenary meeting was held in London in February, attended by 101 researchers. This was a successful meeting which achieved good interaction between the partners, the integrative researchers, patients' organizations, and the NeuroNE administrative team.

4. Workshops. Three workshops were held in 2006. Dysfunction of axons and synapses in neurodegeneration ' organised by Ann Kato and Pico Caroni in Cambridge, Extracellular mediators and intracellular pathways determining axon growth and cell survival/death organized by Mathias Baehr and Ruediger Klein in Varenna, and Neural Stem Cells in Brain Repair, organized by Harold Cremer and Andreas Bosio in Cassis.

5. Seminar visits. The network funded a programme which allowed members to visit partner laboratories to deliver seminars

6. Collaborative visits. The Network provided funds for partners and integrative researchers to visit other laboratories within the network.

7. Platforms. The network provided seven technical platforms, based in the institutions of partners, for the use of the network.

Scientific activities

Some highlights of the work of the network during the first year have been:-

- Selective vulnerability of motoneurons and their axons in a mouse model of ALS
- Disruption of fast and slow axonal transport of APP and other molecules and mitochondria in the SOD1 mutant model of ALS
- Development of an adeno-associated virus vector for the knockdown of SOD-1 in ALS models, and demonstration that it is transported from muscles to the motor neurons.
- Reduction in cortical BDNF in post-mortem human brains
- Disturbance of calcium homeostasis and related molecules in Huntington's disease models
- Real time imaging of alpha-synuclein oligomerization and interactions with tau
- Mechanisms of recognition of the APP cleavage site the nicastrin component of the gamma secretase complex.
- Reduction of memory loss in an Alzheimer disease model mouse by a lipophilic transitional metal chelator.

- Identification, function and regulation of alpha and beta secretases, and role of beta secretase in myelination.
- Mechanisms of ubiquitination of XIAP and control of its role in prevention of cell death.
- Involvement of lipid rafts in axon growth cone regeneration
- Internalization of NogoA and MAG by neurons.
- Remapping of the sensory cortex after spinal cord injury
- Recovery of skilled limb function after peripheral nerve repair and treatment of the spinal cord with chondroitinase.
- Quantification of dopamine receptors in Parkinson's disease models with ^{11}C raclopride positron emission tomography
- Role of glial derived neurotrophic factor in the maintenance of substantia nigra dopaminergic neurons.

Achievements at the end of Year 3:

During its third year, the network has become a mature organisation with good communication between laboratories. Its priorities have been to deliver the planned scientific advances, to continue to provide excellent training to its participants and to expand its engagement with the public.

Integration activities

1. A monthly web-seminar series was established at the beginning of the network This has continued successfully. Each month one or two of the young researchers give a seminar over the Marratech web-conference software. There has been good attendance and excellent seminars. It has kept the researchers in regular contact.

2. Internal collaborative grants programme. One of the innovative instruments invented by the network has been an internal grants programme, available to applications by the post-doc scientists. After the first network plenary meeting grant applications were received and six projects totalling 150K€ for collaborative projects were funded following scientific refereeing. The progress of these projects was reviewed at the second plenary meeting, and a further grant round was approved. This has led to a further five projects being funded totalling 192.5K€. The exercise has been very successful in promoting collaboration between groups and for training the post-docs in grant writing.

3. Plenary meeting. The second plenary meeting was held in Barcelona in March 2007. Around 100 NeuroNE scientists attended, accompanied by local Spanish scientists who were invited, and representatives from charities involved in neurodegenerative conditions.

4. Workshops. Three workshops were held in 2007. Activity-dependent plasticity
June 21-23, 2007, Neuronal Calcium in Health and Diseas, October 12-14, 2007, Protein aggregation in neurodegenerative diseases, December 2-5th.

5. Seminar visits. The network funded a programme which allowed members to visit partner laboratories to deliver seminars

6. Collaborative visits. The Network provided funds for partners and integrative researchers to visit other laboratories within the network.

7. Platforms. The network provided six technical platforms, based in the institutions of partners, for the use of the network.

8. Schools education programme. NeuroNE has developed a programme suitable for school children aged six and above. Children are taught about the function of the brain and its various parts by building a brain out of air dough.

9. Science museum programme. NeuroNE has developed a set of activities suitable for demonstrations in science museums. Visitors can experience three methods that are used to assess brain function in patients with Alzheimer's, Parkinson's and Huntington's disease. They

perform computer-generated cognitive tests, tests of eye movement control and a test of the ability for rapid movement. This programme was taken to the Science museums in London and Madrid.

10. Dana Centre events. NeuroNE has participated in events in Dana Centres throughout Europe. Spinal cord superstars and Art and the Brain were two events in the London Dana Centre.

Scientific activities
Some highlights of the work of the network during the past year have been:-

1. **Biology and treatment of motoneuron disease.**
Several groups within the network research into motoneuron disease (ALS). Over the past year NeuroNE researchers have shown that much of the toxicity that kills motoneurons comes from the surrounding tissue rather than the cells themselves or the muscles that they connect to. Various inflammation-related changes have been found. Within the motoneurons damage to the nerve fibres is one of the first events, particularly through failure of the transport systems that take material to the ends of the nerve fibres. New mechanisms that lead to this damage have been discovered, together with a decrease in a calcium-related molecule that makes the motoneurons more vulnerable.

2. Huntington's disease is a genetic conditions that causes degeneration of neurons, usually starting in middle age. It is due to the attachment of excess DNA coding for polyglutamine repeats to the huntingtin protein. NeuroNE researchers have found two ways in which this mutation can lead to neuronal death. One discovery is that the mitochondria become very vulnerable to toxic insults, particularly those involving calcium. The second discovery is that production of a key growth factor, BDNF, and its receptor are affected in the Huntington brain.

3. For Parkinson's disease patients transplantation of immature dopaminergic neurons has provided a partial cure. Recent NeuroNE research has shown that in patients who had these transplants almost 20 years ago, the implanted cells are developing signs of Parkinson's disease. This is an important finding because it shows that the environment surrounding the affected neurons is a key factor in the cause of the condition. NeuroNE researchers are developing better forms of transplant by selecting the optimal cell type and by producing them from stem cells.

4. One of the phama companies associated with NeuroNE has developed compounds that localise to the membranes of neurons, and bind excess metal ions. This can prevent various toxic events in the cell, which depend on the activation of enzymes by these metal ions. Recent work has shown that these compounds are successful at slowing the development of Alzheimer's disease in animal models. Treated animals show improve cognitive skills and less pathology in the brain.

5. Plasticity is the process that alllows the damaged brain to compensate for damage by producing new circuits. In adults plasticity is much lower than in children, which means that adults have a limited ability to recover after brain or spinal cord damage. An enzyme called chondroitinase has been found to reactivate plasticity in the adult brain and spinal cord. This treatment alone produces some functional recovery after spinal cord injury. In the past year NeuroNE researchers have found that combining rehabilitation treatment with chondroitinase in order to drive the plastic changes produces a greatly increased degree of functional recovery.

Achievements at the end of Year 4:

During its last 18 months of activity, the network has show a high level of communication and interaction between the laboratories. Its priorities have been to deliver the planned scientific advances, to continue to provide excellent training to its participants and to expand and finish its engagement with the public.

Integration activities

1. The web-seminar series was continued. Every two months one or two of the young researchers give a seminar over the Marratech web-conference software. There has been good attendance and excellent seminars. It has kept the researchers in regular contact.

2. Internal collaborative grants programme. One of the innovative instruments invented by the network has been an internal grants programme, available to applications by the post-doc scientists. No grant was awarded in 2008 but the progress of these previous projects was reviewed at the third plenary meeting. The exercise has been very successful in promoting collaboration between groups and for training the post-docs in grant writing.

3. Plenary meeting. The third plenary meeting was held in Lisbon in April 2008. Around 110 NeuroNE scientists attended, accompanied by local Portuguese and Spanish scientists who were invited, and representatives from charities involved in neurodegenerative conditions.

4. Workshops. Three workshops were held in 2008.

5. Seminar visits. The network funded a programme which allowed members to visit partner laboratories to deliver seminars

6. Collaborative visits. The Network provided funds for partners and integrative researchers to visit other laboratories within the network.

7. Platforms. The network provided six technical platforms, based in the institutions of partners, for the use of the network.

8. Schools education programme. NeuroNE has developed a programme suitable for school children aged six and above. Children are taught about the function of the brain and its various parts by building a brain out of air dough.

9. Open Days in institutes. NeuroNE has developed a set of activities suitable for demonstrations in science museums and open days. Visitors can experience three methods that are used to assess brain function in patients with Alzheimer's, Parkinson's and Huntington's disease. They perform computer-generated cognitive tests, tests of eye movement control and a test of the ability for rapid movement. This programme was taken to the Science museums in London and Madrid.

10. NeuroNE DVD. NeuroNE has created a DVD to present the life of people affected by neurodegenerative diseases and to explain how networks of excellence, such as NeuroNE, can develop new therapeutic solutions for these patients.

Scientific activities

Some highlights of the work of the network during the past year have been:-

Early functional changes in models of neurodegenerative disease

1. Lack of sumoylation of alpha synuclein leads to increased toxicity on overexpression, and death of dopaminergic neurons in transgenic mice.
2. Endoplasmic reticulum stress precedes motoneuron death in three different transgenic models of motoneuron disease
3. In the SOD1 model of ALS anterograde transport of mitochondria is reduced but retrograde transport is unaffected, leading to depletion of axonal mitochondria.
4. Increased vulnerability of mitochondria to stress and to raised calcium in Huntington's disease models.

Common mechanisms in AD, PD, HD and ALS

1. Ubiquitin is degraded along with its substrate by the proteasome.

2. Mutant Huntingtin affects the surface trafficking of GluR2 AMPA receptors.
3. REST/NRSF in lymphocytes is a possible biomarker for early Huntington's disease
4. A new member of the sorting nexin family of molecules is an activator of alpha secretase, so diminishing production of Abeta.
5. Drugs that affect endosomal pH are novel modulators of Abeta production.

Neuronal vulnerability in PD, HD and ALS

1. An increase in ubiquitination precedes the onset of cell death in mouse models of ALS
2. Survival of dopaminergic neurons requires expression of the GDNF receptor ret and DJ-1, which modulates Akt signalling.
3. The mitochondrial toxin 3-proprionic acid is inactive in the absence of the calcium binding protein DREAM.

Apoptotic factors, signalling pathways and cell cycling machinery in AD, PD, HD and ALS

1. Salubrinal relieves ER stress, and protects motoneurons in an animal model of ALS.

Growth factor receptor signalling in normal brain and in AD, PD, HD and ALS models and in human brain.

1. Perineuronal net formation is triggered by link protein expression, and animals lacking link protein lack perineuronal nets and show high levels of cortical plasticity into adulthood.
2. Alpa9 integrin is a tenascin-C receptor, absent in the adult CNS. Expression in adult neurons in vivo and in vitro enhances axon regeneration.
3. Nogo A is ▯ nternalized via pincher, and retrogradely transported to the neuronal cell body.
4. NogoA expressed by Purkinje cells inhibits climbing fibre synaptogenesis by enhances parallel fibre synaptogenesis. It may therefore mediate the competition between these two inputs.
5. In NogoA knockout mice multiple genes affecting actin dynamics in growth cones are altered.
6. Treatment with anti NogoA interacts with rehabilitation after spinal cord injury.
7. Anti NogoA clinical trial in human patients has successfully completed phase 1 with no treatment-related toxic effects. Phase 2 is now being planned.

Maintenance and production of animal models

1. A new model of Alzheimer's disease, in which neurons in the cortex and hippocampus are transduced to express mutant forms of tau with AAV vectors. Neurons show cell death with similar characteristics to AD.
2. A new drosophila model of neuronal death relevant to Parkinson's disease, in which eye neurons express knockouts or constitutively active forms of ret and DJ-1.

Production of cell models

1. Development of a method to generate and continuously propagate radial glial cells from ES cells or embryonic brain stem cells. The cells can be differentiated into neurons suitable for Huntington's disease studies.

Lentiviral vectors and delivery

1. In vivo transduction of Purkinje cells with GluRdelta2 lentivirus reveals that this channel-like molecule mediates climbing fibre synaptogenesis.
2. In vivo knockdown of GAP-43 in the olive reduces synapse formation by climbing fibres in the cerebellum.

Gene transcription in experimental systems

1. Changes in nuclear calcium levels due to synaptic activity changes 140 genes, 20 of which affect cell survival, 9 of which are strongly neuroprotective.
2. Comparison of gene expression in motoneurons that are affected early and relatively preserved in motoneuron disease in SOD1 mice.
3. Development of a magnetic cell sorting method for purifying neural stem cells from the immature CNS, followed by gene profiling.

In vivo imaging in Parkinson's and Huntington's diseases

1. Development of [18F] Fallypride imaging for estimating the survival and effects of dopamine or serotonin-rich embryonic ventral mesencephalic grafts.

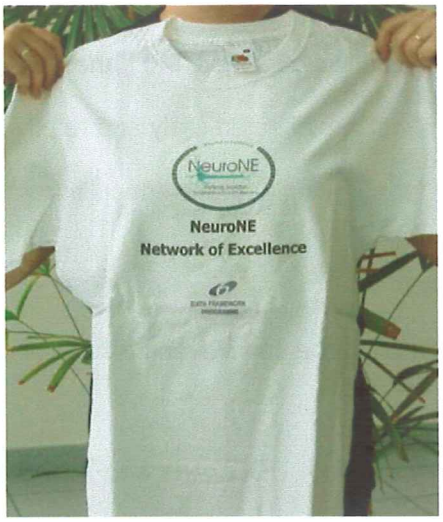
Neuroprotective and reparative approaches in spinal cord injury, SOD mice and animal models of neuropathic pain.

1. In spinal cord injury combining specific rehabilitation with chondroitinase to induce plasticity produces excellent functional recovery, while either treatment alone is not effective.
2. Treatment with chondroitinase and rehabilitation is effective even when started one month after injury. This makes it possible to envision clinical trials that fit with the clinical schedule of patients.
3. Knockdown of SOD1 in motoneurons in the mutant SOD1 model of ALS does not protect the motoneurons, reinforcing the key role that glial expression of mutant SOD1 plays in the condition.
4. Identification of ER stress caused by misfolding to SOD1 and other proteins as the cause of cell loss in the SOD1 model of ALS.
5. Proof of efficacy of TRO19622 in two animal models of ALS.
6. Nmnat2 is an endogenous axonal survival factor which triggers Wallerian degeneration as it is depleted after axotomy, unless the action is blocked by the Wld mutation.
7. BAG-1 expression protects substantia nigra neurons in models of PD.
8. Optimisation of a protocol to generate dopaminergic neurons from human ES cells.
9. Demonstration that various lentiviral-mediated methods of knocking down mutant huntintin is protective in animal models of Huntington's disease.
10. Agrin is necessary for newly generated hippocampal neurons to form synapses and integrate into the adult dentate gyrus.

Selected pictures illustrating our outreach programme

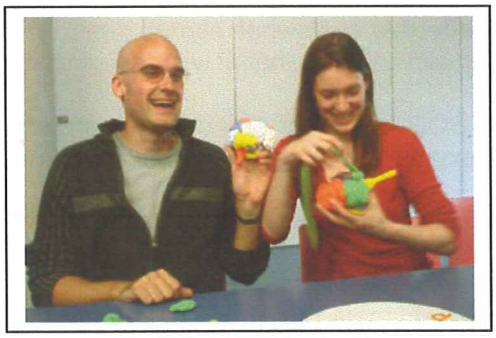
NeuroNE's T-Shirt wore by NeuroNE's scientists during the different public events organized by the network.

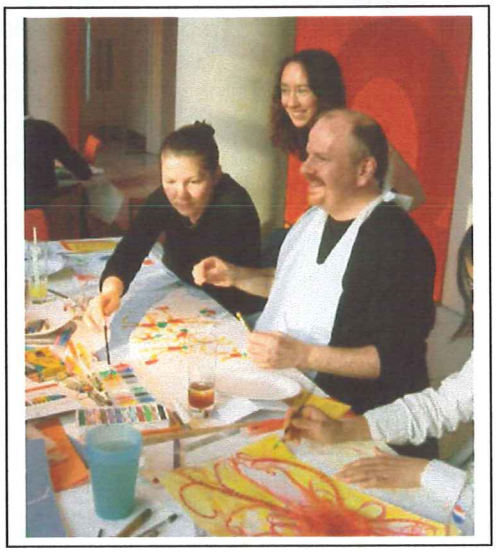
Front side



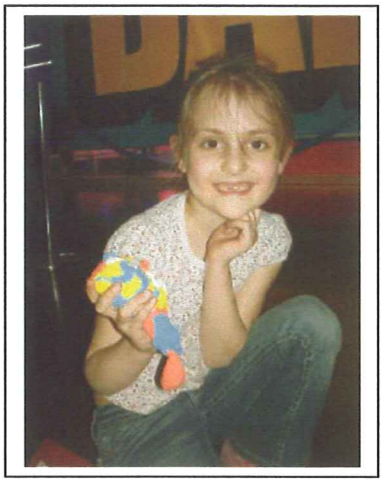
Back side

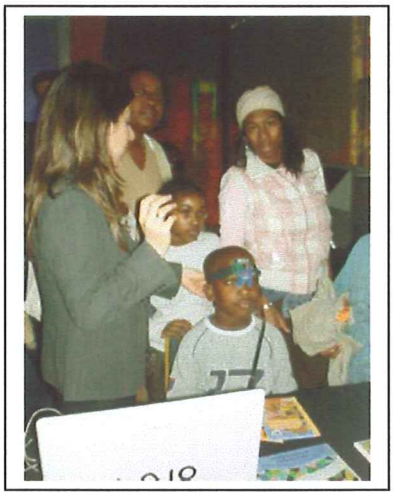
Dana Centre London





Science Museums (London & Madrid)





Art of the Brain workshops in primary schools (France, UK)