



Brain Bank Bulletin

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The PDS Tissue Bank is sponsored by the Parkinson's Disease Society

The PDS Tissue Bank at Imperial aims to help understand what causes Parkinson's and assist in the development of better drug treatments by providing high quality brain tissue to researchers working in the field of Parkinson's and related neurological disorders. The Tissue Bank also aims to enhance the public's awareness of Parkinson's, promote the work of the Tissue Bank and increase the numbers of volunteers who are willing to sign up to the donor scheme. The Tissue Bank also aims to collect the tissue so that it is suitable for all research needs and that it is collected in the most ethical manner.

So many questions?

The Tissue Bank have recently sent out two questionnaires to our prospective donors. The first concerns the medical history, including all drug treatments, acute and chronic conditions and further details relating to dietary, occupational and family background. The second questionnaire, which is to be sent out at regular intervals to those enrolled in our donorship scheme, contains 100 questions which address symptoms that can arise while living with a diagnosis of Parkinson's. You might wish to know why we are putting more post through your door!

The Biography of the Brain

When a pathologist looks at any tissue sample, be it from the liver, kidney or the brain itself, it is essential a clear and accurate record of the medical history is provided and also, in many contexts, of the wider social background and lifestyle of the individual. For example, for a liver biopsy, it would be of utmost importance to know about alcohol intake and drug exposure as well as the travel history which may involve exposure to infections such as viral hepatitis. In the case of the kidney, a biopsy report will not prove fully helpful without details concerning drug exposure, blood pressure and the presence of conditions such as rheumatological disorders which are known to involve the kidney. Without this information, the pathologist may be able to give only a vague and incomplete diagnosis and will not know which specialised tests are indicated upon the tissue in question.

The nervous system and brain have so much more complexity than organs such as the liver and kidney and chronic disorders such as Parkinson's develop over decades with both genetic and environmental influences. Hence, it is easy to understand why detailed and accurate medical and other biographical facts must accompany each brain at the time of pathological examination and then into the realm of scientific research.

The Devil is in the Detail!

What sort of facts are needed to make a brain donation more important? It would be desirable to have an accurate family history since some genetic influences can increase the risk of having Parkinson's. If we can obtain information from individuals as far back as possible on family members who have had problems with tremor or with movement and memory, then researchers may be able to trace back the specific genetic influences. When we do not have the family history then a brain might be analysed erroneously as a case of 'sporadic' Parkinson's and thus the genetic story will be lost!

Leaping from genes to the environment, we know that certain occupations with chemical exposure and rural living carry a slightly higher risk for Parkinson's later in life. Smoking seems to be protective (but is not recommended for many other health reasons!) and there may be further risks associated with high blood pressure, diabetes, elevated cholesterol, head injuries a history of depression or anxiety and other factors. Simple facts such as caffeine intake, fruit and vegetable consumption can all prove helpful information for the research scientist.

Since Parkinson's affects many parts of the nervous system seemingly unrelated health facts can be of great value. For example sleep disorders, smell and taste function and bowel habit (with lifelong constipation implying a higher risk) are all pieces of information that point to involvement of specific areas of the peripheral and central nervous system in Parkinson's. These 'non-motor' features of the illness that exist alongside tremor, slowness and stiffness are finally receiving the attention that they deserve for arriving at better treatments and promoting more wide-ranging research that will address what are often very serious and troubling symptoms.

The pathologist and scientist also both need to know about complications of treatment because when these occur they may indicate changes in the brain secondary to the treatment or making the individual more vulnerable to develop complications.

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For example, confusion and hallucinations, dyskinesias or involuntary movements, freezing and on/off episodes may all have significance for specific brain regions and chemical activities and without detailed information the researcher cannot address these problems while studying the brain. Thus, if we lose the information about these problems during treatment we also lose the opportunity to understand them better when looking at brain tissue itself.

The Changing Story

We now know that Parkinson's is often far more than a problem with tremor and poor movement and we need to understand better how and why the condition can cause symptoms from memory loss to poor sleep, from skin rash to poor bladder control. While symptoms may begin with movement problems and tremor, the picture most often changes over time. To be sure that the researcher can find the exact area where specific problems are coming from we need to have an accurate story from each brain donor. Every patient has a different experience with Parkinson's and some symptoms are rare while others are more common. Without the individual story of the person living with Parkinson's there can be no accurate assessment of the brain leading to a better understanding of how the illness involves individual parts of the nervous system. This is the very knowledge that we need in order to discover better treatments and a cure.

Therefore, the 100 questions will be sent out to our donors on a regular basis so that we can learn about facts as diverse as bowel and bladder function, drooling and poor speech, memory and concentration, dizzy spells related to low blood pressure, sleep and dreams and many other things including drug treatments for Parkinson's and other illnesses. With these facts describing all symptoms and side effects the Tissue Bank will be able to provide researchers with sufficient knowledge to ensure that each brain donation is made as valuable as possible.

Dr Ronald Pearce – Clinical Neurologist



Research Project Report: Studies on the pathological basis of dementia and visual hallucinations in PD

Parkinson's disease (PD) has been characterised as a movement disorder with the main clinical features of resting tremor, bradykinesia, rigidity and abnormalities of gait, balance and posture. This underestimates the complexity of what is a multi-system disorder with many important non-motor features. Among the most prominent non-motor complications of the disease are dementia and visual hallucinations. The prevalence of dementia in PD has been reported to be between 12 and 41% with PD patients having a six-fold risk for dementia over age-matched controls. Prevalence figures for visual hallucinations are in the range of 6-60%.

Pathologically, PD is characterised by loss of cells in the substantia nigra pars compacta. However, it is now apparent that there is a widespread pathology in PD that greatly exceeds the classical brainstem 'locus classicus' of the substantia nigra pars compacta. Abnormal

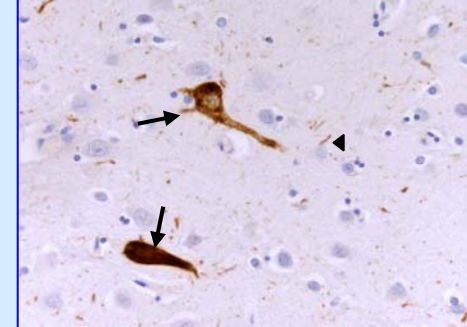


Fig 2 The entorhinal cortex of a Parkinson's disease patient containing tau positive neurofibrillary tangles (shown with the arrows) and neuropil threads (arrowhead).

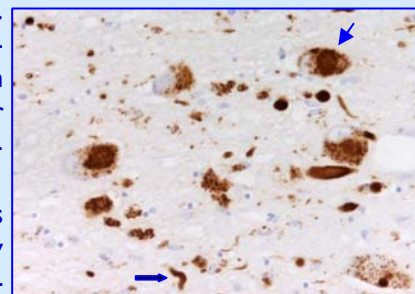


Fig 1. The nucleus basalis of Meynert from a patient with Parkinson's disease contains alpha-synuclein positive Lewy bodies (shown with the arrow) and Lewy neurites (arrowhead).

accumulation of a protein called alpha-synuclein is a histopathological marker of the disease with Lewy Bodies and Lewy Neurites containing alpha-synuclein (Fig 1). Nonetheless, other abnormally deposited proteins can also be found such as hyperphosphorylated tau and beta-amyloid that are characteristic neuropathological hallmarks of Alzheimer's disease (Fig 2 and 3). These abnormal proteins can be found in areas other than the substantia nigra and we wanted to investigate how the location of these abnormal proteins correlate with the prevalence of dementia and hallucinations. This will help us understand how we may stave off the progress of PDD and prevent hallucinations.

We designed a study selecting PD cases to isolate clinical features of interest and study the underlying anatomical and pathological basis. The medical histories of 81 cases from the UK Parkinson's Disease Society Tissue Bank were retrospectively examined and

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a severity score for dementia and visual hallucinations attributed without prior knowledge of the neuropathological diagnosis. Subsequently, cases with neuropathologically and clinically confirmed PD were grouped on the basis of clinical phenotype representing the extremes of a spectrum for dementia and visual hallucinations. Hence, we investigated the type of pathology and areas in which it was present in the following groups:

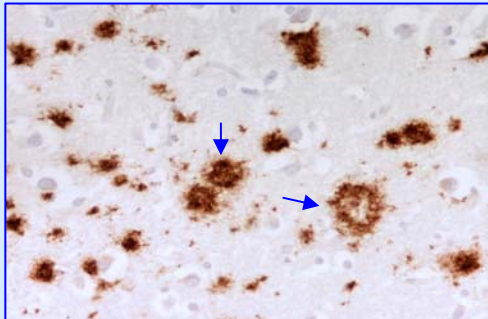


Fig 3. The cingulate gyrus of a Parkinson's disease patient containing beta-amyloid

- 1) without dementia and visual hallucinations (9 cases),
- 2) with severe visual hallucinations and no dementia (5 cases),
- 3) with severe dementia and no visual hallucinations (4 cases) and
- 4) with severe dementia and visual hallucinations (12 cases).

Immunostaining was used to assess the extent of alpha-synuclein, tau and beta-amyloid deposition in eight brain regions affected in PD and known to subserve cognitive function.

In our study we have found a clinical relevance of the abnormally deposited protein alpha-synuclein in the limbic system with respect to the occurrence of both dementia and hallucinations in PD. This system is a complex set of structures involved in emotions (e.g. fear, aggression), motivation, formation of memory and cognitive and attentional processing. In addition, it is highly interconnected with other brain structures that are also involved in cognitive functions (e.g. nucleus accumbens and prefrontal cortex). There is also evidence that the limbic system provides a custodial function for the maintenance of a healthy conscious state of mind.

An improved understanding of the anatomical and pathological basis of cognitive and psychiatric symptoms in PD may ultimately contribute towards the evolution of better treatment strategies for these aspects of the illness. By studying the relationship between centres in the brain that control behaviour in Parkinson's disease state, one will by inference add to a greater understanding of brain function in normal individuals.

Mr Michail Kalaitzakis PhD Student



Research Project Report: Parkinson's disease post-mortem brain tissue for RNA quality analysis

Introduction: Brain Net Europe II is a consortium of European tissue banks collecting human post-mortem brains for various research purposes. The aim of the consortium is to standardise experimental techniques by developing optimal protocols and spreading these across the network. This is important as it will allow researchers requiring tissue to obtain tissue from any of the Tissue Banks and be able to compare the research results. The UK Multiple Sclerosis and Parkinson's disease tissue banks at Imperial College London are members of this network providing human brain tissue for research.



Human post-mortem brain tissue is one of the most valuable resources available for research into human neurological disorders. The success of experiments and reliability of data obtained from a molecular analysis of the tissue will depend on the quality of genetic material (RNA) obtained from this post-mortem tissue. RNA will be analysed using different variables to see if any of the factors affect the quality of the RNA. This will give us information on how best to obtain and store tissue i.e. with time delays etc...

Experiment: Post-mortem brain tissue provided by the PD tissue bank was used in a collaborative experiment by the network members to determine the factors that affect the quality of RNA in the human post-mortem brain in order to determine its suitability for molecular biological analyses. The quality of RNA can be affected by biological and clinical factors, which include the;

- cause of death;
- post-mortem delay; and the
- method and duration of storage of brain tissue before it is used for RNA isolation.

This experiment was carried out to determine which of these ante and post-mortem variables affect the quality of RNA. A total of 149 snap frozen tissue samples were used in this analysis which included 70 samples from the UK MS and 32 samples from the UK PD tissue banks and 47 snap frozen tissue samples from Brain Net

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Europe participants. These 47 samples were provided by Barcelona (8 samples), Edinburgh (5 samples), Institutes of Psychiatry London (8 samples), Wuerzburg (8 samples), Paris (8 samples) and Munich (10 samples).

RNA was extracted from all the samples using a special kit, utilising methods previously optimised for the network. RNA quantity and quality was assessed using a piece of specialised equipment, the Agilent Bioanalyser. The data obtained was analysed against predetermined variables. As the information supplied by a majority of the participants was limited to:

- Age;
- Gender;
- Post Mortem (PM) delay;
- Disease type;
- Cerebral Spinal Fluid pH and;
- Freezer interval.

These were used as the main factors against which the quality of RNA was analysed. The statistical analysis of the data was carried out with advice and support from the Imperial College Statistical Advisory Service.

Results: This initial analysis suggests that gender, age, post-mortem delay, freezer interval and CSF pH do not have a significant effect on the quality of RNA. From these observations it can be concluded that it is possible to isolate adequate concentrations of good quality RNA from human post-mortem brain tissue even with longer PM delays and freezer intervals. Although variation in the quality of RNA between samples was observed, the factors analysed could not account for this variation. These results mean that the way tissue is currently being collected by the Tissue Banks, the method of storing, and PM delay times are appropriate to allow use of the tissue in molecular studies.

Future Research: We plan to carry out a prospective study by obtaining tissue samples from ten post-mortem brains from each of the brain banks that participated in this experiment. Also in the future we plan to carry out the first micro-array study to look at gene expression levels across diseases using the same test procedures. Gene expression analysis looks at which genes maybe 'switched on' or 'switched off' more in disease processes. In the past gene expression studies have been carried out on individual diseases like PD but this study will not only look at PD but also MS, Alzheimer's disease, Huntington's disease, Schizophrenia, Amyotrophic Lateral Sclerosis at the same time to see whether common gene changes occur in several diseases. This may help identify whether certain drug treatments may be effective in more than one disease.

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