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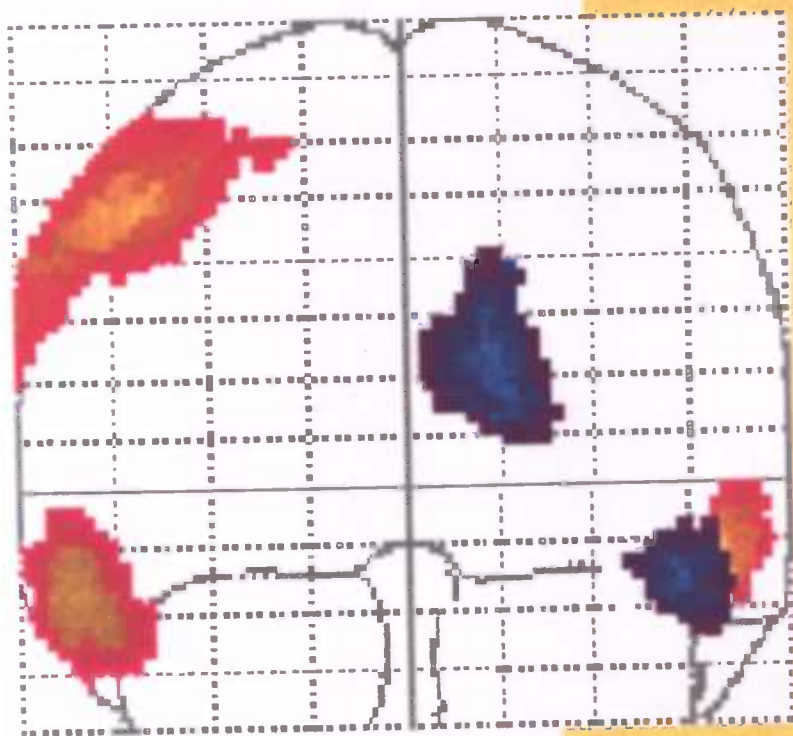
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GRADUATION ESSAY

A.A.T.S. Reinders

*Identity state-dependent processing of neutral  
and traumatic scripts in Dissociative Identity  
Disorder as assessed by PET*  
(Positron Emission Tomography)



Cognitive science and engineering  
University of Groningen

RUG

February 1999

Trefwoorden

SPM - dataanalyse?

DID identiteit problemen

? MPD

PET - hersen scan / order 2016

Positron Emission Tomography



TCW  
9/6/52

*To*

*My brother Pieter*



## Table of Contents

	Page
<b>Preface</b>	4
<b>Chapter I: PET</b>	5
§1.1: Applications	5
§1.2: Preparation of positron-labeled compounds	5
§1.3: $H_2^{15}O$	5
§1.4: Gamma rays	6
§1.5: The PET scanner	7
§1.6: 2-D/3-D scanners	8
§1.7: PET-scanners in Groningen	8
§1.8: PET data correction and image reconstruction	9
§1.8.1: Data correction	9
§1.8.2: Image reconstruction	9
<b>Chapter II: DID</b>	11
§2.1: DID-patients	11
§2.2: Why PET-investigation?	11
§2.3: PTSD	11
§2.4: Hypotheses	12
§2.5: Aim	12
§2.6: DID brain studies in literature	13
<b>Chapter III: Patients and methods</b>	14
§3.1: Patients	14
§3.2: Scripts	14
§3.3: Emotional rating scales	14
§3.4: Scanning procedure	14
§3.5: PET Camera	15
§3.6: Radiation	15
§3.7: Patient and scan selection	15
<b>Chapter IV: Data processing</b>	19
§4.1: Background correction	19
§4.2: Center images	19
§4.3: Picture checks on translation and rotation	19
§4.4: Transformation	20
§4.5: Realignment	20
§4.6: Spatial normalisation	20
§4.7: Smoothing	20
§4.8: Statistical analysis for DID-PET data	21
§4.9: Coordinates and Talairach atlas	32
§4.9.1: Use of the atlas	32
§4.9.2: Crisis in Normalisation?	32
§4.10: SPM in a nutshell	37
§4.10.1: Introduction	37
§4.10.2: General statistics	37
§4.10.2.a: t test	37
§4.10.2.b: Least squares fit	37
§4.10.3: SPM, the general linear model (GLM)	37
§4.10.4: t statistics and contrasts	39
§4.10.5: Z scores	40
<b>Chapter V: SPM96 problems and solutions</b>	44
§5.1: SPM96 problems with $HR^+$ data	44
§5.2: Statistical comparison of SPM95 and SPM96	46
§5.3: Optimisation of the spatial normalisation	47

§5.3.1: Options within SPM96	47
§5.3.2 Results	48
§5.3.3: Effects on statistical analysis using 3x4x3 deformation anatomical normalisation	50
§5.3.4: SPM-mailing list	51
§5.4: Rotations	56
<b>Chapter VI: Results</b>	61
§6.1: Results	61
§6.1.1: Text effects	61
§6.1.2: Identity state effects	61
§6.1.3: Grouped comparisons	62
§6.2: Discussion	62
<b>Acknowledgements</b>	68
<b>Appendix A: Manual</b>	69
<b>Appendix B: Premature article</b>	90
<b>References</b>	93

## Preface

This essay was written to complete my education in Cognitive science and Engineering at the University of Groningen. After my propaedeutics in Applied Physics, I switched to Cognitive science and engineering. My interests appeared to be more in the direction of human processing. To stay on the technical side I preferred Cognitive science and Engineering above Psychology. To fulfill my interests in psychological disorders I followed some courses in Psychology. The question always appeared: how does the brain work? Because of this interests I wanted to graduate in the neuro-imaging field (fMRI, PET or ERP). After having had several conversations with different professors I finally found a subject that was appealing to me.

I was put in contact with professor J.Korf and J.Quak. The last mentioned was working on a Positron Emmision Tomography (PET) investigation project called identity state-dependent processing of neutral and traumatic scripts in Dissociative Identity Disorder. The first six month we worked together on the project and three patients were investigated. Because of time restrictions I worked alone on contract basis, but with guidance, by the time of September.

In total nine patients were investigated. Two had to be excluded. Results as shown in Chapter VI are with seven patients. More patients will be investigated to strengthen statistical power and to obtain better (other) results. I wish to stress that results shown are preliminary and subject to changes. Parameters other than regional cerebral blood flow (rCBF) were examined during the investigation. These parameters are named in chapter III to give a complete picture of the PET investigation protocol. However, results are not included because they are beyond reach of my graduation investigation, which only concerns PET investigation and PET data analysis.

Chapter I describes how PET works. Chapter II gives information about Dissociative Identity Disorder and literature. Chapter III describes patients and methods of the PET investigation. Chapter IV describes data processing and SPM96. In Chapter V problems I encountered during data analysis (SPM96) and answers that I obtained are described. In Chapter VI preliminary results are presented. The Appendices contain a manual that I wrote for the PET center in Groningen and a scheme for an article.

I hope the readers enjoys reading this graduation essay, just as I enjoyed working on the graduation project.

# Chapter I: PET

## §1.1: Applications

To investigate brain (re)activity in patients with dissociative identity disorder, Positron Emission Tomography (PET) is applied. Measurements are done on patients in different identity states, listening to autobiographical texts. The goal is to increase objectivity of identity dependent information processing in this psychiatric disorder.

PET has been widely applied, in both research and clinical environments to provide a window on the living brain. To name a few examples: to explore the functional organization of the intact brain during its development and during periods of neuronal plasticity, to investigate compensatory responses on lesions or surgical resection, to examine brain responses to sensory, motor, cognitive and drug stimulations, to identify specific disease states (Parkinson, dementia) and also in different clinical applications, as oncology, cardiology and neurology.

PET is a powerful biological imaging tool to assay biochemical and physiological processes in living tissue in a quantitative and non invasive way. The physiological process is the blood flow in the cerebral cortex, named regional cerebral blood flow (rCBF). To detect this regional brain function a radio tracer is used. The radio nuclei decay resulting in emittance of radiation. This radiation can be detected outside the body. The concentrations involved are in the nanomolar or picomolar range. So there is no significant mass disturbance on the biological system under investigation. In recent years different biological probes have been synthesized with positron-emitting isotopes to broaden the possibilities for observing and trying to understand the living functioning human brain.

The brain is a complex network in which all structural subunits can communicate with each other directly or indirectly, so it is necessary to monitor local and distributed functions. Combining functional PET data with a MRI or CT scan allows structure/function relationships (interrelated functions of the brain) to be identified. The ECAT Exact HR+ PET scanner at Groningen makes an attenuation scan (transmission scan) measured by three internal rotating  $^{68}\text{Ge}/^{68}\text{Ga}$  block sources.

## §1.2: Preparation of positron-labeled compounds

Isotopes of carbon ( $^{11}\text{C}$ ), nitrogen ( $^{13}\text{N}$ ) and oxygen ( $^{15}\text{O}$ ) serve as positron emitters for PET. These are isotopes of the major constituents of the body. For the experiment described later, the isotope  $^{15}\text{O}$  is used. This has a half-life of 2.07 minutes and a maximum possible positron energy (endpoint energy) of 1720 keV. The range (path length of travelling positron before it is annihilated) is on the average 1.44 mm. Water containing  $^{15}\text{O}$  is specifically used in rCBF investigations.

For creation of the positron emitting isotope a cyclotron is used which bombards appropriate non radioactive (stable) target atoms with protons or deuterons accelerated to high energies. These high energies are necessary to overcome repulsive electrostatic and nuclear forces of the target nuclei so that a nuclear reaction can take place. Because of the relatively short half-lives, isotopes are produced close to the PET-scanner. In Groningen the cyclotron is positioned in the cellar, the laboratory and the PET-scanner are situated above the cyclotron so the isotopes need only transportation upwards into the laboratory to prepare the substance for injection.

## §1.3: $\text{H}_2^{15}\text{O}$

For the production of  $^{15}\text{O}$ , a deuteron (a nucleus consisting of a proton and a neutron) and a  $^{14}\text{N}$  atom are needed. The deuterons are accelerated in the cyclotron to obtain a particle beam for bombardment of the target material. The target material is stable nitrogen-14 gas outside the cyclotron. The following reaction takes place (there is a brief introduction about atoms and nuclei a little further):



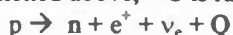
After the production of  $^{15}\text{O}$ , it has to be separated from the target material. The radiofarmacon,  $\text{H}_2^{15}\text{O}$ , must be formed by a chemical reaction. This is achieved in the laboratory in a leadcell with the use of robot arms. Robots need to be used because of high levels of radiation. The robot arm can be operated from a distance by a computer. Time for syntheses amounts to three half-lives of the material. After preparation of the radiofarmacon it has to be purified to obtain the needed product without other materials (for example radioactive metabolites). After this step the radiofarmacon is ready for injection.



$\text{H}_2^{15}\text{O}$  is introduced into the patients body by intravenous injection.  $\text{H}_2^{15}\text{O}$  (radioactive water) has been chosen for its similarity to naturally occurring biochemical matters in the human body. This radioactive labeled compound is used to trace biological processes in the brain. The tracer diffuses into the whole body according to delivery, uptake and metabolism. Its spatial distribution and concentration will thus in general vary with time. Only very small doses of this radiofarmakon are used.

$\text{H}_2^{15}\text{O}$  is a positron emitting matter. This is due to  $^{15}\text{O}$ . First a brief intermezzo about what an atom looks like so that the reader may get some idea about the processes involved in radiation. Basically an atom consists of three types of particles: protons, neutrons and electrons. By convention protons are positively charged. Electrons have an equally large but negative charge. Neutrons however do not carry charge; they are neutral particles. Now atoms can be considered as being a positively charged core (the nucleus) enveloped with a negatively charged cloud of electrons. The nucleus consists of protons and neutrons whereby the last mentioned serve as some kind of glue to keep the protons together (particles with equal charge repel each other). The three particles also have so called anti-particles which play a role in nuclear reactions. One anti-particle is important in the nuclear reaction that will be discussed later. This is the anti-particle to the electron: the positron. Particles have the interesting property that they can annihilate with their own anti-particle. Roughly speaking, both particle and anti-particle disappear and two photons take their place. A short elaboration on that subject will follow in the next section.

As mentioned above,  $^{15}\text{O}$  is radioactive since it emits positrons. The following reaction takes place:



This may look rather abstract, but the explanation is as follows: a proton (denoted by p) is converted into a neutron (n), a positron ( $e^+$ ), an electron-neutrino ( $\nu_e$ ) and kinetic energy. Remember that a positron is the anti-particle to the electron and therefore, since the electron has negative charge, the positron has positive charge. This explains the (+) sign. Kinetic energy is related to velocity. When a particle has velocity it means that it also has kinetic energy. If the velocity increases, so does kinetic energy. The electron-neutrino is a particle that is virtually undetectable (this also means that it has no interaction with the patients body i.e. it is harmless). Indeed the only reason that the existence of this particle was, in earlier times, even considered by physicists (Pauli 1930) is that it is the only explanation for the fact that the velocity of the positron is not always the same at each measurement. This phenomenon occurs because the available kinetic energy has to be divided up between the positron and the neutrino instead of only the positron.

The  $^{15}\text{O}$  nucleus 'loses' a proton and 'gains' a neutron, effectively converting it into a nitrogen nucleus ( $^{15}\text{N}$ ). This process takes place with a half-life of 127 seconds, which means that after this amount of time half of the  $^{15}\text{O}$  has been converted into  $^{15}\text{N}$ . The positron 'bounces about' in the body until it has lost most of its velocity. The distance it travels before that happens is typically less than 2 mm. It then annihilates with an electron into two gamma photons, which is the subject of the next section.

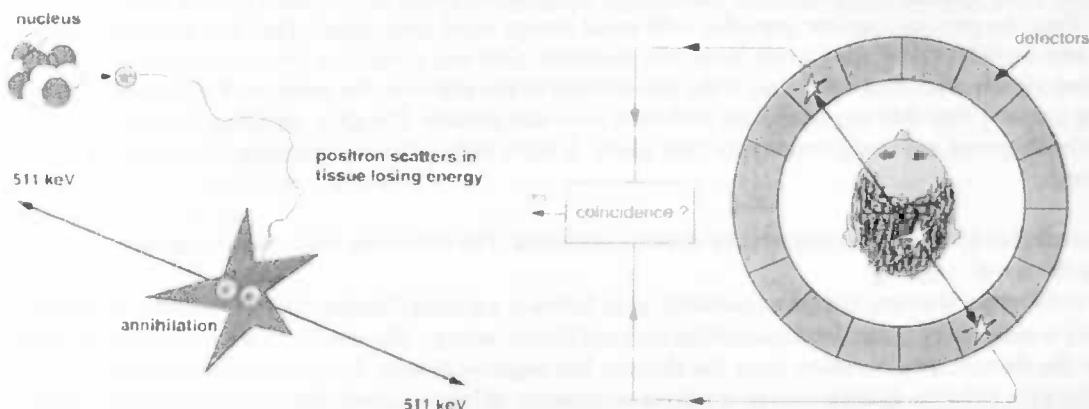
#### §1.4: Gamma rays

In all physical processes there are physical quantities that must be conserved. The most important ones for this discussion are: energy, momentum and angular momentum. The laws of conservation of these quantities dictate how the dynamics of a process behaves. In the case of electron-positron annihilation this means that following Einstein's law of equivalence of mass and energy ( $E = mc^2$ ) the mass of both particles is converted into energy. Because of conservation laws this must result into two or three photons. Since the probability of two photons is extremely large compared to the probability of three, it is safe to say that the result will be two photons of equal energy (511 keV) moving in opposite direction. Here the unit keV (kilo electronvolt) is introduced which is a ubiquitous unit of energy in the world of nuclear and subatomic physics. Photons can be looked upon (physically not entirely accurate) as being light-particles. Gamma photons have very high energy which can be illustrated by noting that photons in sunlight (visible light) have energies of approximately 2 eV (250.000 times smaller). Note that  $1\text{keV} = 1000\text{eV}$ .

Rays of these gamma photons have little interaction with matter so there is a high probability of escaping the body without attenuation and thus for possible detection by external detector systems. The detection takes place by the positron camera. The positron camera consists of thousands of scintillation detectors positioned in multiple rings. Every detector is linked with detectors on the other side. If two opposite positioned detectors at the same time (within 5-15ns) detect a photon of energy 511 keV an annihilation is counted. By measuring the signal of the two gamma quanta (location of both gamma photons), the line on which the annihilation (positron emission) took place can be determined (strictly speaking one isolated photon cannot be measured, instead there is an intensity of photons that exceeds some threshold value). This line is called 'the line of response' or LOR.

By placing the detectors in a circle around the body, most of the arisen LOR's can be measured. Because of placement of more than one ring next to each other a PET camera is able to make a lot of recordings at the same time and at different angles. By combining lines of response from many different angles, the data can be reconstructed into cross-sectional images using conventional mathematical algorithms. The count density in the resulting images, reflect the concentration of positron-emitting probe in the tissue.

By the use of mathematical techniques, an image can be constructed. If the difference in detection time is known then the 'time-of-flight' is known. By the use of fast  $\text{BaF}_2$  detectors a time resolution of 0.5 seconds is possible. The spatial resolution of the PET scanner is about 5 mm.

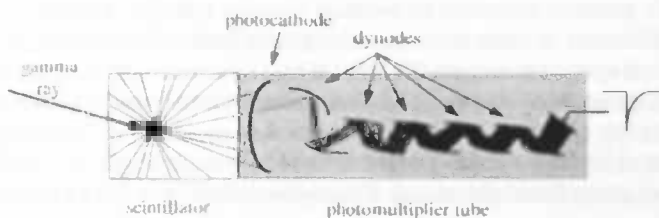


### §1.5: The PET scanner

The PET-scanner is a imaging instrument which provides tomographic measurements of the concentration of positron-labeled compounds in tissue. The PET-scanner provides spatial information but also has a temporal resolution in the order of minutes which allows for following biological processes.

The two key parameters for a PET-scanner are spatial resolution (the ability to accurately locate events) and sensitivity (the number of events registered per unit dose injected). Sensitivity is important because the signal to noise ratio, and therefore image quality in PET, depends on the number of counts detected. This number depends in turn on the injected dose, the scan length (depending on the kind of tracer injected), the efficiency of the detectors and the angle at which the detectors subtend to the subject. The spatial resolution of the scanner depends on the resolution of the detectors used. For the the single element detectors of width  $d$ , the spatial resolution is about  $d/2$ . Therefore it is important to use small detector elements to achieve high spatial resolution. Independent of the kind of receptor the aim is to detect as many of pairs of 511-keV gamma rays emitted from the body as possible and to determine their interaction position as accurately as possible.

Detectors used in PET-scanners are based on crystalline materials, called scintillators, which are coupled to photomultiplier tubes. These detectors have a relatively good detection efficiency for high energy gamma rays. When a gamma photon interacts in the scintillating crystal, some of the energy is converted into a flash of visible light (a process involving excitation of electrons from the valence band of the crystal which in turn excite electrons from luminescence centers in the band gap which decay trough photon emission). The amount of light produced for a 511 keV interaction consists of no more than a few thousand photons and is therefore extremely weak. To produce a measurable signal, a photomultiplier tube is used to detect this scintillation light. Light that falls onto the photocathode of the photomultiplier tube frees electrons from the material, which are accelerated by a high electric field through several multiplying stages (one primary electron produces  $10^6$  secondary electrons) to the output. In this way a detectable current pulse is produced.



The scintillating material used for the PET-scanner in Groningen is Bismuth Germanate (BGO), with an effective atomic number of 74. This high effective atomic number provides a good stopping power at 511 keV. However to detect 95% of the 511 keV gamma rays, a 3 cm thickness is required. BGO has a relative high pulse decay time of 0.30  $\mu$ s compared to 0.23  $\mu$ s of NaI. But because of its higher stopping power it has replaced NaI. The emission wave length is 480 nm. These factors impose restrictions on the spatial resolution.

There are two physical factors that limit the spatial resolution. The 180° emission of 511 keV gamma rays is not exact because the positron and electron are not completely at rest when they annihilate. The deviation from 180° can be represented by a Gaussian function with a FWHM of 5.7 mrad. The limit this imposes on the spatial resolution of a PET scanner depends on the diameter of the system and is 2 mm for a 80-90-cm-diameter PET system. The second effect is caused by the fact that the positron travels some distance before combining with an electron and annihilating into the 511 keV gamma rays. This positron range is isotope dependent and is related to the kinetic energy with which the positrons are emitted. The higher their energy the further the positrons will travel before annihilating. Positron range effects degrade the spatial resolution by 0.4 to 1.5 mm. Fortunately, the angular errors in 511 keV gamma ray emission and positron range effects are not additive but rather convolved together.

It is an unfortunate fact that most PET studies can not be reconstructed at the resolution that the scanner is capable of. Some smoothing has to be applied to the data during the reconstruction process to improve the signal to noise ratio at the cost of spatial resolution. The limitation on improving the spatial resolution is not the resolution of the detectors but the number of coincidence events recorded to form the image. It is impractical to increase the injected dose because of dose considerations and dead time. Dead time is the finite time the scanner needs to process each event and is 'dead' for this time.

### §1.6: 2-D/3-D scanners

PET scanners collect data by recording the coincidence combinations between detectors on opposite sides of a ring of detectors. PET scanners have multiple rings of detectors to allow simultaneous collection of multiple planes of images. However, each ring of detectors acts independently, only recording data from the image plane they represent. This is called 2D (two-dimensional) scanning and it is very wasteful because most of the gamma ray pairs are not emitted normal to the imaging planes and are therefore rejected.

3D data acquisition has been developed to collect and reconstruct all gamma ray pairs which intersect the PET scanner. This increases the number of gamma rays collected and therefore increases the sensitivity of the PET scanner (by a factor five). This allows a reconstruction of the data set close to the resolution limit of the PET scanner. Because of the sensitivity increase the injected dose can be reduced or the scanning time can be reduced. Comparing 2D scanning and 3D scanning shows a dramatic increase in signal to noise.

A disadvantage is the large size of the data sets and the more computationally intensive reconstruction algorithm that is required. Another disadvantage is an increase of detections of scattered events (gamma rays which scatter in the body before reaching the detectors, leading to mispointed events).

### §1.7: PET-scanners in Groningen

The PET-center in Groningen has two PET-scanners, a cyclotron, a laboratory and a data analysis room all situated in each others vicinity. Both the scanners are from Siemens, the ECAT 951/31 whole body positron camera (camera I) and the Siemens EXACT HR<sup>+</sup> (camera II). The HR<sup>+</sup> has a greater axial length at the PET-ring system than the ECAT 951. In the 3D-mode of the HR<sup>+</sup> the sensitivity is 7 times better for a statistical accurate measurement. But there is more acceptance of scattered radiation. More information of the camera's is given below:

	<u>ECAT 951/31</u>	<u>EXACT HR<sup>+</sup></u>
number of rings/material:	2 blocks, 16 ring BGO	4 blocks, 32 ring BGO
number of planes:	31	63
number of detectors:	8192	18432
axial length:	10.8 cm	15.5 cm
diameter head opening:	102 cm	83 cm (detectors closer to axis)
spatial resolution:	6-7 mm FWHM	4-5 mm FWHM
2D/3D:	2D	2D/3D

kcps/ $\mu$ Ci/ml:

115.000

2D: 200.000

3D: 900.000

## §1.8: PET data correction and image reconstruction

### §1.8.1: Data correction

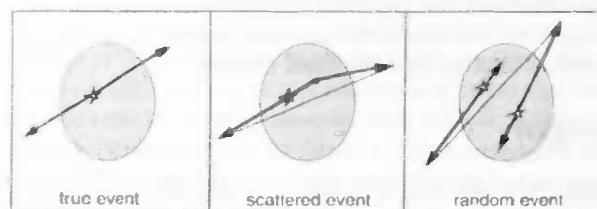
To obtain the full quantitative PET images (correct relationship between the concentration of the biological probe and the count density measured in the PET images) a number of correction procedures must be applied to the raw PET data as in the flow chart below.

#### DATA ACQUISITION

- 1) on-line randoms correction
- 2) on-line deadtime correction
- RAW PET DATA (SINOGRAMS) →
- 3) detector normalization
- 4) scatter correction
- 5) attenuation correction
- IMAGE RECONSTRUCTION →
- 6) image calibration

The largest of the correction factors is to correct for gamma ray attenuation by the tissue. Gamma rays emitted from the edge of the brain (outwards) are more likely to reach the detectors than those emitted from the center of the brain because they have less tissue to travel through. Only one in five gamma ray pairs emitted from the center of the brain will make it out of the head without being lost to attenuation. The attenuation correction can be calculated based on the contours of the head and the known attenuation coefficients of the brain tissue and skull at 511 keV (using an external positron-emitting source).

Events detected by the PET camera can be contaminated by two classes of events. The first is accidental (random) coincidences, this happens when two unrelated gamma rays happen to strike detectors within the coincidence timing window (finite temporal width) of the system. The second class of events are the scattered events in which one or both of the 511-keV gamma rays scatter on their way out of the head, but are still detected. Such events will be incorrectly positioned and result in a reduction of contrast in the images. Several techniques for scatter correction are available and are particularly important in 3D PET studies. Effects of scattered and random coincidence events are known as the "noise effective count rate".



Another correction is needed at higher count rates because the limit, due to electronics, on the ability of the PET scanner to distinguish each individual event then becomes apparent. This is called 'dead' time and results in a count rate dependent loss of events. It can be corrected by measuring the count rate on the detector and having knowledge of the 'dead' time.

### §1.8.2: Image reconstruction

After the application correction factors the images are reconstructed from raw PET data to allow the density in PET images to be expressed in  $\mu$ Ci/ml. For structures in the order of magnitude of the resolution of the PET scanner, errors in the activity concentration can occur because of resolution effects. Activity from small structures is then 'smeared' over an area that is larger than the structure itself, reducing the apparent count density. Since the ECAT Exact HR<sup>+</sup> scanner in Groningen has a spatial resolution of 4-5 mm, any structure less than 1cm across will suffer from resolution effects. Unfortunately this includes many structures in the brain.

The process of image reconstruction is to convert the events detected by the PET scanner (projection data) into cross sectional images of the brain representing the distribution of the positron-emitting tracer (activity). Only the LOR is known. The raw data for a ring of PET detectors is represented as sinograms. A sinogram is a 2D matrix of projection data. The columns represent the angle of the LOR and the rows represent the displacement from the center of the field of view (position from the center of the head). Coincidences between a given detector pair are represented by a single element in the sinogram matrix.

During this reconstruction a Hanning filter is applied, modified by an exact choice of window function to reduce the amplitude at high frequencies. Since noise is a high frequency phenomenon this helps to reduce the noise level in the reconstructed images. However, sharp detail in the images is represented by high frequency as well so increase of the signal to noise ratio comes at the expense of degrading the spatial resolution. The exact choice of window function can be adjusted as desired.

## Chapter II: DID

### §2.1: *DID-patients*

Dissociation involves failure to associate information and experience in ways that would be normally expected. In dissociative identity disorder this failure is extreme. Apart from amnesia and depersonalisation DID is characterised by identity fragmentation, related to the existence of dissociative identities.

Patients with DID usually have two or more distinct identities also called personalities, alters, parts or aspects. Each of the identities has its own pattern of perception, reaction and thinking about itself. Switches between states are manifest by nonlinear changes in a number of variables, including access to memory, attention and cognition, regulatory physiology, and a sense of self. These fluctuating states of consciousness result in changing identity notion and changing access to autobiographic memory.

DID can be a consequence of severe and chronic traumatising starting at an early age (childhood trauma). This traumatising can evoke dissociative identity formation resulting in "traumatic" identities with their own particular reaction to, recollection of, or emotional responses to the traumatic memories. Furthermore "neutral" identities exist with no, or in any case less, recollection of these memories. These defensive (neutral) states will be activated by traumatic labeled stimuli.

Clinical observations are that reactions of patients with DID on identical stimulations are dependent on the identity they experience at that moment. This is specific for stimulations that are associated with traumatic memory as reported by the patients. Clinical observations lead also to the notion that if the patient is in an identity which is familiar with traumatic memory and the patient is exposed to the traumatic memory, a higher level of arousal (fear, tension) is present than when the patient is in a identity wherein he or she is not aware of the traumatising.

In "apparently normal" identity states patients can perform functions in daily life. In these states access to traumatic memories is restricted, or totally inhibited, as is emotional responsiveness. Thus, these neutral identities are depersonalised, numbed and amnesic with respect to trauma.

### §2.2: *Why PET-investigation?*

Dissociative disorders are unsatisfactorily understood phenomena, resulting in theoretical debates and discussions, hindering and even harming diagnostic and treatment of DID-patients. Some critics think that the disorder is a matter of self suggested effects. However according to DID experts the disorder can be seen as a complex post-traumatic stress disorder.

For DID patients and clinicians it is very important to obtain clearness about the authenticity of dissociative phenomena, validity of diagnosing DID and validity of traumatic memory reported by DID patients. Objective research is needed. This can be done by investigating state dependent brain activations according to neutral and traumatic stimulation. Nowadays it is not known if state dependent reactivity exists and if it does, which brain areas are involved. DID is described in many case studies, but almost no quantitative psychophysiological multi-subject studies have been reported.

PET was chosen above fMRI since fMRI is less suitable. The tube of the fMRI machine is long and narrow which could have been very frightening for DID patients. Because of the long tube, less contact between therapist and patient would have been possible. However, this contact between therapist and patient is essential during the investigation. The fMRI machine produces more noise than a PET machine, interfering with auditory stimuli.

### §2.3: *PTSD*

There are indications that dissociative identity disorder (DID) can be viewed as a complex post-traumatic stress disorder (PTSD) (e.g. Spiegel, 1984). A symptom provocation study of post-traumatic stress disorder using the script-driven imagery technique was performed by Rauch et al <sup>A17</sup>. Subjects were scanned after hearing audio taped autobiographical narratives of the traumatic events that precipitated their PTSD (traumatic condition) as

well as narratives of neutral life events (control conditions). Our DID-PET investigation is comparable to this investigation.

The PET investigation on PTSD patients<sup>A17</sup> indicates that texts concerning autobiographic traumatic memory give cause to activations in different brain areas compared to texts referring to neutral autobiographical life events. Increases in normalized blood flow were found for the traumatic text condition compared with control conditions (neutral text and teeth-clenching) in right-sided limbic and paralimbic territories, including anterior cingulate cortex, right/posterior orbitofrontal, right anterior temporal and insular cortex, as well as the right amygdala. Amygdala activation reflected its role in threat assessment and emotional memory. Paralimbic activation was consistent with its important role in mediating anxiety or arousal non-specifically. The visual cortex was also activated. Decreases were found in left inferior frontal cortex, including Broca's area, and middle temporal cortex. Broca's area is a higher cognitive function. Finding decreased bloodflow in this area is consistent with the observation that patients with PTSD have difficulty structuring recollections of their traumatic histories cognitively.

These results suggest that emotions associated with the PTSD symptomatic state are mediated by the limbic and paralimbic systems within the right hemisphere. Activation of visual cortex may correspond to the visual component of PTSD re-experiencing phenomena.

In the previous mentioned article all subjects were traumatised and had PTSD. This can be compared to our investigation with the traumatic identities. Neutral identities (which function as the control group) can perhaps be compared to traumatised subjects without PTSD. Shin et al<sup>A20</sup> used 15 adult victims of childhood sexual abuse, 8 with PTSD and 7 without PTSD (to serve as a traumatic exposed control group). PET data of the traumatic vs control condition, showed significantly larger rCBF increases in orbitofrontal and anterior temporal cortex, as well as larger decreases in Broca's area for the subjects with PTSD in comparison with the non-PTSD group. The non-PTSD group exhibited a larger increase in rCBF within anterior cingulate cortex than did the PTSD group. This raises the possibility that failure to adequately recruit this structure plays a role in the disease (PTSD) process.

Because of indications that DID can be viewed as a complex PTSD, the possibility exists that results of our DID-PET investigation show overlap with the above mentioned findings.

Anxiety is connected with DID, findings in anxiety studies might also be found in our DID study. A PET study<sup>A18</sup> to determine the shared mediating neuroanatomy of anxiety symptoms across three different anxiety disorders was performed. Results indicated activation in right inferior frontal cortex, right posterior medial orbitofrontal cortex, bilateral insular cortex, bilateral lenticulate nuclei and bilateral brain stem foci during the symptomatic versus control conditions. The results suggests that elements of the paralimbic belt, right inferior cortex and subcortical nuclei mediate symptoms across different anxiety disorders. An anxiety-induction PET study<sup>A20</sup> of normal subjects demonstrated increased rCBF in anterior cingulate cortex and a so called claustrum-insular-amygdala territory.

## **§2.4: Hypotheses**

According to clinical and empirical findings one may hypothesize that if DID patients, which are in a personality state aware of traumatic memories, will show brain activations corresponding to PTSD patients exposed to their traumatic memories. These brain activations will not be shown being exposed to neutral texts or in the neutral identity, which is not familiar with, or affected by, the traumatic memories. Probably the neutral text in traumatic identity and text effects in neutral identity show different state dependent activated brain areas.

If these effects are found a striking phenomenon is established namely differential dissociation, thus state dependent brain(re)activity. These findings could contribute to increase objectivity on the subjective experience of patients with DID.

## **§2.5: Aim**

Study brain activity (rCBF) in DID patients, listening to autobiographic texts in different identity states (traumatised or neutral), to show objectivity identity state dependent information processing in this psychiatric disorder.

## §2.6: DID brain studies in literature

Only a few brain studies have been carried out on patients with DID. However no PET-study is found in the literature. Most of these studies concern electric evoked responses (EEG <sup>A3,4,7,9</sup>, AEP <sup>A14</sup>). Brain regions probably involved with MPD according to electric evoked responses studies are: left hemisphere (temporal, hippocampal) <sup>A4</sup> and left temporal and right posterior regions <sup>A7</sup>.

Two articles <sup>A9,24</sup> and a paper <sup>A23</sup> have been written on SPECT (Single Photon Emission Computerized Tomography) neuro-imaging with DID patients. The paper on SPECT imaging in DID <sup>A23</sup>, described a case study testing a host personality and two alter personalities. In this study the SPECT imaging was performed on five separate occasions in three distinct personality states. Regional cerebral blood flow changes were observed in both parietal lobes and bilateral frontal lobes. One personality state showed normal perfusion in all regions. The patterns were producible for both alter personality states. This leads to the conclusion that there is a correlation between cerebral blood flow pattern and different personality states in DID.

Previous studies have also reported fluctuations in cerebral blood flow in patients with DID concerning the temporal lobe. In the SPECT study <sup>A24</sup> significant blood flow increases were shown in four different alter personalities. This study describes a SPECT study in one patient performed on four separate occasions on four distinct personality states. Significant regional cerebral blood flow changes were observed in the left temporal lobe. The mean increase in activation in the left temporal lobe in the four personality states was 10.7%. The authors of this article stated that these findings suggests that the temporal lobe may be a mediating factor in the psycho-physiology of MPD. This study suggests that the temporal lobes play a role in the pathogenesis of MPD.

The other article <sup>A9</sup> has reported fluctuations in cerebral blood flow between personality states in patients with MPD. In the study cerebral blood flow was measured in a patient during two different personalities, the same patient after recovery and in three control subjects. Personality change was associated with no significant alterations in cerebral blood flow except right temporal and occipital hyper-perfusion. The right-sided temporal and occipital brain areas showed increased blood flow. Both sided parietal regions also showed increased blood flow. The right hemisphere is more related to experience of emotions and is associated with memory processes. This increase in temporal lobe blood flow is indicative of functional over-activity.

Various EEG studies were performed over the years. Putnam <sup>A15</sup> found in MPD subjects significant differences in brain electrical activity but not in subjects without MPD. Various activated brain areas have been found in the different EEG studies. For example left hemisphere (temporal, hippocampal), left temporal and right posterior regions, but also low central activity and frontal and occipital high. Frequency analyses show significant differences among personalities, due to changes in emotional states. As Putnam stated in 1986 "the most reported abnormality is bilateral temporal lobe slowing (EEG)". These findings agree with two out of three SPECT studies.



## **Chapter III: Patients and methods**

### **§3.1: *Patients***

Nine female volunteers aged between 26 and 60 took part in the study which was approved by the academic hospital ethics committee of Groningen, the Netherlands. Subjects were free of neurologic illness and gave written informed consent to participate in the study after the experimental procedure and radiation risks were explained. Other alter personalities were informed and inquired during a session with the therapist and gave oral agreement to participate.

Entry criteria included Dissociative Identity Disorder criteria according to DSM-IV (American Psychiatric Association, 1994) and those determined in the Structured Clinical Interview for Dissociative Disorders (SCID-D, Steinberg; Steinberg, Cichetti, Buchanan, Hall, Rounsaville, 1993). Patients were only asked to participate if they had successfully went through treatment on stabilisation and symptom reduction and if treatment had progressed to the phase which involves exposure to traumatic memories.

Inclusion criteria required presence of at least two dissociative identities, one trauma related and one non trauma related, capable of being activated in cooperation with their therapist who was present at the subject's bedside during the study. Subjects were able to switch between personality states during the investigation. Two personalities participated in the investigation. Both identities subjectively aged 10 years or more. The so called traumatic identity (re-)experienced the traumatic event, which occurred at an age of 10 or later, and was emotionally affected by the traumatic text. The second identity was emotionally unresponsive to the traumatic text because of amnesia or depersonalisation. Interference of other identities was absent if one of the participating identities was activated, according to information supplied by the therapist.

Exclusion criteria were pregnancy, traumatic experiences considering a hospital, systemic or neurological illness and not mastering the Dutch language.

### **§3.2: *Scripts***

Scripts of the patients' past personal events were prepared by patient and therapist. One traumatic and one neutral text were developed. The traumatic text described a traumatic event which occurred at age 10 or later in the patients life. The neutral text described an autobiographic neutral event. Each script was audio-taped in a neutral voice by the therapist for playback in the laboratory setting. Both scripts were 120 seconds in duration.

### **§3.3: *Emotional rating scales***

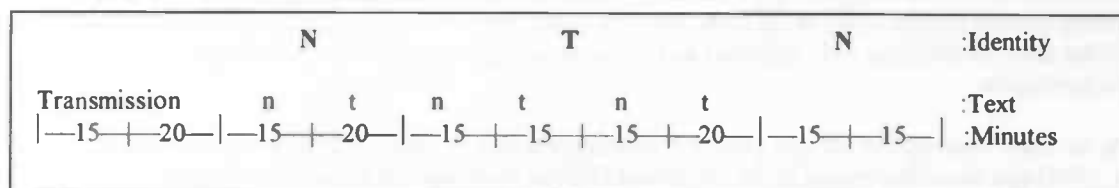
Each patient rated the severity of various emotional dimensions. These emotional dimensions consisted of fear, sorrow, somberness, anger, shame and disgust. The questionnaire was taken for each condition on separate subjective 0- to 10-point analog scales, by the therapist. Anchor points were provided by the therapist, where 0 indicated complete absence and 10 indicated maximum possible for the specified emotion. With regard to re-experiencing phenomena during the study each patient was asked which sensory modalities were involved, also in a 0- to 10- point analog scale. The questioned sensory modalities consists of images, touch, sounds, smells, tastes, pain, physical deafening, physical stiffening, physical paralysis and physical restlessness. Results will not be shown.

### **§3.4: *Scanning procedure***

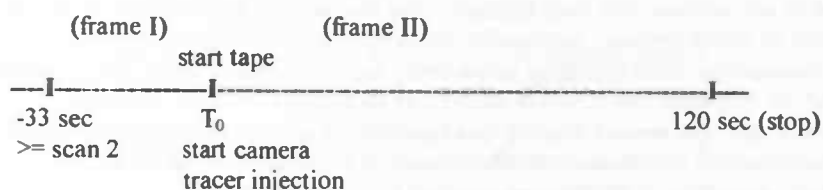
After a first visit, a few days or hours before the PET investigation to habituate to the PET environment, patients with DID were scanned 8 times. Before PET investigation an intravenous infusion was brought in. This was done in both arms. One infusion for injection of radioactive water (right arm) and one to draw blood from (left arm). During transmission scan no radioactive water was injected. A transmission scan is meant to chart brain structures. It is an anatomical scan, not a functional scan. The functional PET-scans were mapped onto this anatomical scan. To do this correctly, patients were instructed not to move their heads. During the transmission scan the complete procedure was practiced once with the neutral tape.

After a transmission scan four different conditions were obtained twice, resulting in eight scans. The complete scanning sequence was Nn, Nt, Tn, Tt, Tn, Tt, Nn and Nt. The first character (capital) indicates the personality

state (Neutral or Traumatic) and the second character (minor) indicates the content of the text (neutral or traumatic). Four scans corresponded with the traumatic personality state and four scans corresponded with the neutral personality state. During the four scans each personality state listened to a neutral autobiographic text (two times) and to a traumatic autobiographic text (two times). Time between each text amounted to 15 or 20 minutes, as shown in the time scanning sequence below. Time between scans amounted to 15 minutes. If a personality switch needed to be acquired, 5 more minutes were needed for the switching procedure, time between scans amounted to 20 minutes.

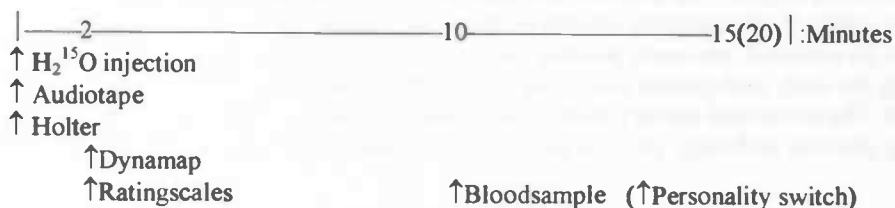


#### PET scan protocol



Two frames were obtained. The first frame contained information about radiation remained from the previous scan, the second scan contained count data of patients listening to autobiographic texts. The first frame was used for background radiation correction for the second frame. The first frame started 33 seconds before injection of the radio tracer. As the tracer was injected the second frame started. At the same time the therapist received a sign to start the audiotape and mark the Holter (ECG registration) by pressing a button. The audiotape and the second frame both had a duration of 120 seconds. Immediately following the end of the script the researcher received a sign of the therapist to serve the dynamap. Heart rate, systolic and diastolic blood pressure were measured by means of the Dynamap. At the same time the therapist debriefed the patient regarding her subjective experiences by means of the ratingscales. Blood was drawn by a venous catheter, ten minutes after the start of the scan (audiotape). A personality switch was obtained if the next scan was the third or seventh in order. Total investigation time amounted to almost three hours.

#### Complete scan procedure:



#### Dynamap:

\*Bloodpressure: Systolic, Diastolic

\*Heart rate measurement

Holter: Continuous heart rate variability

Bloodsample: Cortisol, ACTH, Noradrenaline, Adrenaline, Dopamine

Ratingscales: emotional response and sensory perception

### §3.5: PET Camera

Subjects underwent PET scanning in a Siemens ECAT Exact HR<sup>+</sup> PET scanner, which acquires 63 slices over an axial length of 15.5 cm. The 32 ring BGO detectors after reconstruction supplied 32 straight planes and 31 crossplanes over an axial length of 15.5 cm, resulting in 63 slices. Before emission scanning an attenuation scan (transmission scan) measured by three internal rotating <sup>68</sup>Ge/<sup>68</sup>Ga block sources was performed. Each PET data acquisition run consisted of 2 frames, the first of 33 seconds and the second of 120 seconds duration. The second scan contained information to form images of relative blood flow after background radiation correction using frame one. Data was acquired in the three-dimensional mode.

At the moment of intravenous injection of H<sub>2</sub><sup>15</sup>O (a dose of 500 MBq), the 120-seconds-scan acquisition started. After 23 seconds maximum count rate in the brain was achieved. Images were reconstructed with a 3D reconstruction algorithm using a Hanning filter and attenuation corrected by means of the transmission scan. Measured coincidences were globally corrected for count loss caused by electronic dead time, not for scattered radiation or random coincidence. The resolution of the raw images was 5 mm.

### §3.6: Radiation

The total amount of radioactive matter injected had a radiation load of 1.5 milliSievert (mSv), this is 8 times 500MBq. This radiation load was within limits of the World Health Organization (WHO). The radiation level in Holland from the cosmic radiation is about 2-2.5 mSv a year. The WHO has limited the radiation to 5 mSv for experiments on volunteers.

### §3.7: Patient and scan selection

During the scan procedure therapists watched for undesirable interference by other identity states. If interference was noticed it was noted down on the rating scale list and mentioned to the researcher. Scans with undesirable interference by other identity states were not included in statistical analysis. Urine was investigated on benzodizepines, ethanol and drugs. If urine results were not as desired the subject was excluded from the statistical analysis data set. Because of these restrictions two patients were excluded. One patient showed urine drugs levels above threshold and one patient had enlarged ventricles (a picture is shown in §5.4).

Overview of patients and scans after selection on identity interference, drugs abuse and neurological illness:

**Tabel I**

Patient: I	II	III	IV	VII	VIII	IX
scan						
1)	Nn	Nn	Nn	Nn	#	#
2)	Nt	Nt	Nt	Nt	Nt	#
3)	Tn	Tn	Tn	Tn	Tn	Tn
4)	Tt	Tt	#	Tt	Tt	#
5)	Tn	Tn	Tn	Tn	Tn	#
6)	Tt	Tt	Tt	Tt	Tt	Tt
7)	Nn	Nn	Nn	#	Nn	#
8)	Nt	Nt	Nt	#	Nt	Nt

An overview of specifications is given in the table below.

# **Patients overview**

Scan date	I 20-3-98	II 21-4-98	III 14-5-98	IV 22-10-98	V 23-10-98
Medication	prozac	<26-3:fevarin	mixtard	#	ranitidine
Physical			Diabetes 2.5 year TIA, 1 day paralysed		several concussions
Urine	#	#	Ethanol<0.1g/l	Ethanol <0.1g/l Benzo:<40ug Kreat.:317 Ja	Ethanol <0.1g/l Benzo:47ug Drugs b) ?
Both alters right-handed?	?	Yes	?		
Scanprocedure:					
Blood	Yes	Yes	Yes	No blood after scan 2	No
Transmissionscan	#	#	Intf. 2 alters	Intf. 1 alter a)**	Intf.div.alter
1) Nn	#	#	#	#	Intf.div.alter =>ignore scan
2) Nt	#	#	#	#	#
3) Tn	#	#	#	#	#
4) Tt	#	#	Tape got stuck 30 sec => ignore scan	#	#
5) Tn	#	#	#	#	#
6) Tt	#	#	#	#	#
7) Nn	#	#	#	Intf. a) => ignore scan	#
8) Nt	#	#	#	Intf. a) + Tt => ignore scan	#
Remarks:				a) = defender ** causes amnesia during investigation	b) Drugs: Cocaine:42ug Opiates:60ug Amfetamines:664ug  Drugs levels too high => ignore patient

PETcenter-staff  
1)  
upper 20  
planes empty  
2)  
scan 1=120sec  
scan 2-8=100sec

Urine determinaton:  
Lowest border and Threshold

Benzo: O=40; T=200  
Cocain: O=30; T=300  
Opiates: O=50; T=300  
Amfetamine: O=150; T=500  
Cannabis: O=13; T=50  
Kreatinine: O=250  
<250 urine thinned

Values between O and T  
=> benefit of the doubt

# Patients overview

Scan date	VI 28-10-98		VII 28-10-98	VIII 30-10-98	IX 11-11-98
Medication	Ventricels	#		d)	#
Physical	enlarged				
Urine	Ethanol <0.1g/l	Ethanol <0.1g/l Benzo:87ug Drugs c)		Ethanol <0.1g/l	
Both alters right-handed?	?	?		Yes	?
Scanprocedure:	no head band => rotated	no head band => rotated			
Blood	Yes	Yes		Yes	No
Transmissionscan	#	#		#	2 times
1) Nn	#	#		Intf. =>ignore scan	knows trauma =>ignore scan
2) Nt	#	#		#	knows trauma =>ignore scan
3) Tn	#	#		#	#
4) Tt	#	#		pee pressure	moved head =>ignore scan
5) Tn	#	#		#	Intf. =>ignore scan
6) Tt	#	#		#	#
7) Nn	#	#		Intf. Tt =>ignore scan	#
8) Nt	#	#		Intf. Tt + e) =>ignore scan	#
Remarks:		c) Drugs: Cocain:63ug  Cannabis:26ug Amfetamines:100ug Methylamfetamines:100ug  Enlarged ventricels => ignore patient		d) Medication promethazin 75mg dalmadorm 66mg nozinan 25mg apirine e) defect tape condition again 2x water	after scan3 + scan6 raised head
PETcenter-staff	t=12.01 hose from pump	scan6 water delayed			scan8 water delayed

Urine determinaton:  
Lowest border and Threshold

Benzo: O=40; T=200  
Cocain: O=30; T=300  
Opiates: O=50; T=300  
Amfetamine: O=150; T=500  
Cannabis: O=13; T=50  
Kreatinine: O=250  
<250 urine thinned

Values between O and T  
=> benefit of the doubt

## Chapter IV: Data processing

Recent data could be copied from the general data directory, which contains all data from camera II. Older data was stored at optical disk (OD) or CD. All scan data is stored there permanently. Before data could be entered into SPM96 different preparation steps needed to be performed. These steps are described below. A more extended explanation of these steps is given in the manual (written for the PET center in Groningen) in Appendix A, at the end of this graduation essay. The SPM interface is described at the end of this chapter, Addition IV.d.

### §4.1: Background correction

The datafile from camera II contained two frames. The first frame contained measured background radiation (radiation left from the previous injection) and the second frame contained the information needed. The program used to correct for this background radiation was *BackgroundCorr*. This program needs an input file (`-i "nameold.v"`) which contains the two frames and returns an output file (`-o "namenew_b.v"`) which contains only one frame. Two frames become one. The second frame was corrected for remained radiation. It was also necessary to integrate the two files to one file for SPM, because the statistics in SPM one needs one file per scan. The first dynamic scan did not need background correction, it contained just one frame.

### §4.2: Center images

Images need to be centered to the same point of departure. All images, also from other experiments were centered to the same location in the defined plane. In one experiment the head could be at a position for example more left in the plane than in another experiment. For camera II data the program *CenterImage* was used (which uses an input (`-i "name_b.v"`) and an output file (`-o "name_c.v"`)). All the images were now centered in the same way and the same location in the defined plane. It was important that the images were centered to the same basis for normalisation and statistical analysis. For comparison of scans and subjects in SPM96 the images need to be at the same point of departure.

### §4.3: Picture checks on translation and rotation

At the PET-center two programs, *Imagetool* and *CAPP5*, were available to check the images on translations and rotations with the eye. The format of the data from camera II can not be read by *Imagetool*. The data has to be transformed from Ecat7 to Ecat6 format with the program *Ecat7to6* (`-i "oldname_c.v" -o "newname_c6.img"`), before the images are checked in *Imagetool*. If the pictures were checked in *CAPP5* with data format Ecat7, the transformation from Ecat7 to Ecat6 format was made afterwards. Data always has to be transformed into Ecat6 format. The program *cti2spm* cannot read Ecat7 format data.

With the program 'Imagetool' images could be checked for translations and rotations. According to old opinions the maximum rotation that could be corrected by SPM95 itself with the realignment and normalisation was about 6-7 degrees. If the pictures were rotated more than 7 degrees SPM95 was not able to handle this anymore and a rotation program was needed, *Imagetool*.

*CAPP5* is the other program to check the images. This program uses Ecat7 format files to check the pictures. All steps before SPM could be checked in *CAPP5*. When files were saved in *CAPP5* they were always in ECAT7 format. With the 'Slice Viewer' (comparable with *imagetool*), different files can be checked on position changes and rotations. With 'Reslicer', rotations could easy be performed. The rotations could be done in the three planes:

- 1) Coronal : rotations over the y-axis.
- 2) Trans-axial : rotations over the z-axis.
- 3) Sagittal : rotations over the x-axis.

Rotated image files that were saved in *CAPP5* could not be used SPM96 because of resolution loss (63 slices to 45 slices and again to 63 slices) and rotations outside the defined areas of *CAPP5*. Statistical calculations are very inaccurate (see chapter about rotations).

However according to new opinions (A.Paans, P.Maguire and myself: see also §5.4) SPM96 could find the ACPC line very well. This means no rotations have to be performed. The normalisation in SPM96 can handle rotations up to 45 degrees.

#### §4.4: Transformation

Transformation from *cti* to SPM96 format(*cti2spm* "name\_c6.img" "name\_c\_t.img") ,using Ecat6 data format, was needed to obtain a data format which could be used in SPM96. Together with the image (.img) files now also the header files (.hdr) were created which were needed in SPM96. Finally data could be entered into SPM96.

#### §4.5: Realignment

The program SPM96 was started for the realignment. Three boxes appeared at the left upper window: Spatial, Statistics and Results. In the Spatial Box are three items: "Realign", "Normalize" and "Smooth". These three steps were handled before statistical analyses.

Realignment is a transfer from the scans to (for example) the middle scan. The middle scan is the first scan to enter the realignment. The middle scan functions as reference point, to which the other scans are realigned. The middle scan usually lies in the middle of movement. During the scanning procedure the head may shift a little bit. The order of scans imported (in SPM) must be the same for each subject.



For example in the realignment procedure scan 2 is shifted down a little and scans 4 and 5 are shifted up. The movements can take place in the x,y,z-direction. Rotation also occurs in three directions: movement of the nose towards the ears (roll), towards the chin/forehead (pitch) or a rotation towards the shoulders (yaw).

In the realignment procedure the first scan chosen was the scan to which the other scans were realigned. It is recommended to select the middle scan as the first scan, using *cti2spm* transformed image files. Only the mean image needs to be created. The graphics (below) present the translation and rotation of the head after the procedure. The brain was re-sliced to 43 slices. A second realignment was needed for a better result. The first scan chosen was the *mean\*.img* created in the first realignment. The next scans were the same scans as used in the first realignment. One scan more was selected than in the first realignment, this was the mean image. Images were now nicely re-placed to the position of the first image selected.

#### §4.6: Spatial normalisation

Normalisation concerns a mapping of scanned individual brains to a template brain. Parameters needed to do this were obtained from the mean image created during the realignment. The template concerns a standard brain. The brains of the subjects from the experiments were standardized to this standard brain.

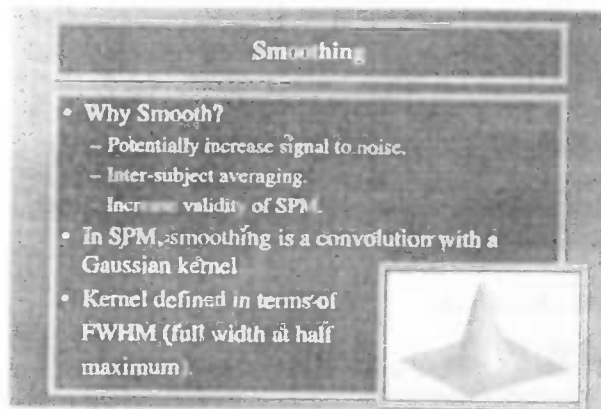
At first the stereotaxic normalisation was not satisfactory. The problems with the normalisation that occurred was stretching of the data. The plastic anatomical transformation (warping or local deformation) of the stereotaxic normalisation was adjusted to optimize the normalisation. This considered the deformation of structures in the head. This consisted of stretching and shrinking of the structures in the head, in three directions *x\*y\*z*, to map the structures in the head as precisely as possible to the *PET.img* template. The nonlinear basis functions in the default spatial optimisation menu, were set from 4x5x4 to 3x4x3 non-linear basis functions. Investigations done about this subject are handled in the §5.3.

During normalisation parameters were determined from the *mean\*.image* scan formed in the realignment. These parameters were used to perform the mathematical normalisation. The image to write normalised (perform normalisation on) were the scans after the realignment. The template to map the images on, was the *PET.img*. The result were normalised and realigned image files named *nr\*.img*.

#### §4.7: Smoothing

With smoothing local contrasts will be reduced, this means noise will be reduced. However, the signal to noise ratio will increase. Details on smaller scale (i.e. noise) will disappear as will variations. Within this smaller scale some information will also be lost. However, on a larger scale there is no information loss, details will stay visible. With smoothing the number of pixels remains the same. In SPM smoothing is a convolution with a Gaussian Kernel.

A smoothing filter of width FWHM (Full Width Half Maximum) of 20mm (this is a large smoothing factor) was chosen (smaller smoothing factors will be tried). The Exact HR<sup>+</sup> has a resolution of 5mm so, a smaller filter (for example 8mm) could also be used. When choosing a larger number to smooth with, the resolution (sharpness) becomes smaller. The localisation (accuracy) becomes worse because a larger area is taken into account to obtain significance. So the sensitivity becomes better when you take little areas together. With a smoothing factor of 12, the smearing is less. So if an area is significant it is sharper. A smoothing factor of 12 is recommended to look at small regions with a lot of data.



#### §4.8: Statistical analysis for DID-PET data

After Realignment and stereotaxic normalisation of data, statistical analysis for seven subjects was performed with the remaining scans (Tabel I in §3.7). Seven subjects were left, so the Multi-subject option was chosen with replications, explanation of the SPM96 interface and buttons in Addition IV.d. Each scan was repeated. Factors chosen were: images scaled to an overall grand mean of 50, a gray matter threshold of 80% of the whole brain mean and an AnCova global normalisation. Two contrasts were used: 1 - 1 (first condition - second condition) and -1 1 (second condition - first condition). During the scanning procedure eight scans were obtained. Each of the four conditions obtained twice. This led to the following series:

Scan nr.:	1	2	3	4	5	6	7	8
Condition:	Nn	Nt	Tn	Tt	Tn	Tt	Nn	Nt

For statistical analysis the following set of comparisons were made:

- 1) Identity effects (text independent):
  - 1,2,7,8 vs 3,4,5,6
  - Nn, Nt vs Tn, Tt
- 2) Text effects (identity independent)
  - 1,3,5,7 vs 2,4,5,6
  - Nn, Tn vs Nt, Tt
- 3) Situation matched effects:
  - 1,7 vs 4,6
  - Nn vs Tt
- 4) Effects of texts within identities
  - Same identity, different text:
    - a) 1,7 vs 2,8  
Nn vs Nt
    - b) 3,5 vs 4,6  
Tn vs Tt
- 5) Effects of identities over the texts
  - Same text, different identity (reaction of the different identities on the same text):
    - a) 1,7 vs 3,5  
Nn vs Tn
    - b) 2,8 vs 3,5  
Nt vs Tt
- 6) 2,8 vs 3,5  
Nt vs Tn



The most interesting comparisons made were 4) (text effects within identities) and 5) (effects of identities over the text). For each comparison two statistical calculations were made, one for each contrast.

In SPM96 the effects of the four conditions were estimated on a pixel-by-pixel basis using parametric maps. Global differences were first covaried out and comparisons of the conditions (multi subject different conditions with replications) were then made using t-statistics. Computed paired t values were converted into z scores, using Z-statistics, producing statistical parametric maps. These statistical parametric maps show all Z scores above threshold value  $Z = 2.33$ . Calculated p values and Z scores are printed into the SPM postscript file (spm.ps). The spm.ps file shows information involved in the statistical calculations. Three images of the head are shown, which can be build to form an imaginary glass brain. The spm.ps output file containing realignment, normalisation and statistical analysis is shown below, along with explanations in boxes.

## Image realignment

```

1 /home/simone/DIS/ 4_c_timg
2 /home/simone/DIS/ 1_c_timg
3 /home/simone/DIS/ 2_c_timg
4 /home/simone/DIS/ 3_c_timg
5 /home/simone/DIS/ 5_c_timg
6 /home/simone/DIS/ 6_c_timg
7 /home/simone/DIS/ 7_c_timg
8 /home/simone/DIS/ 8_c_timg

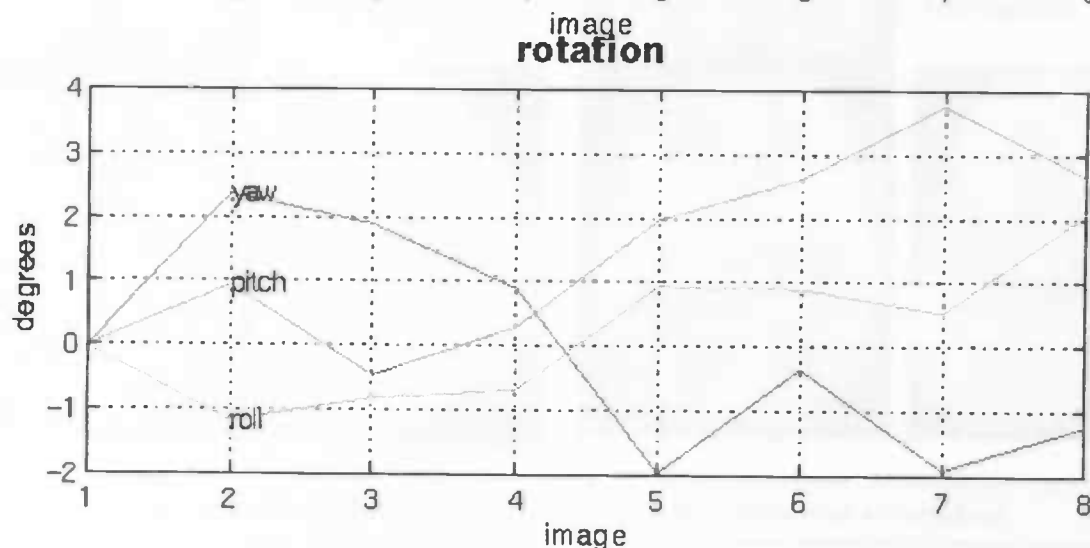
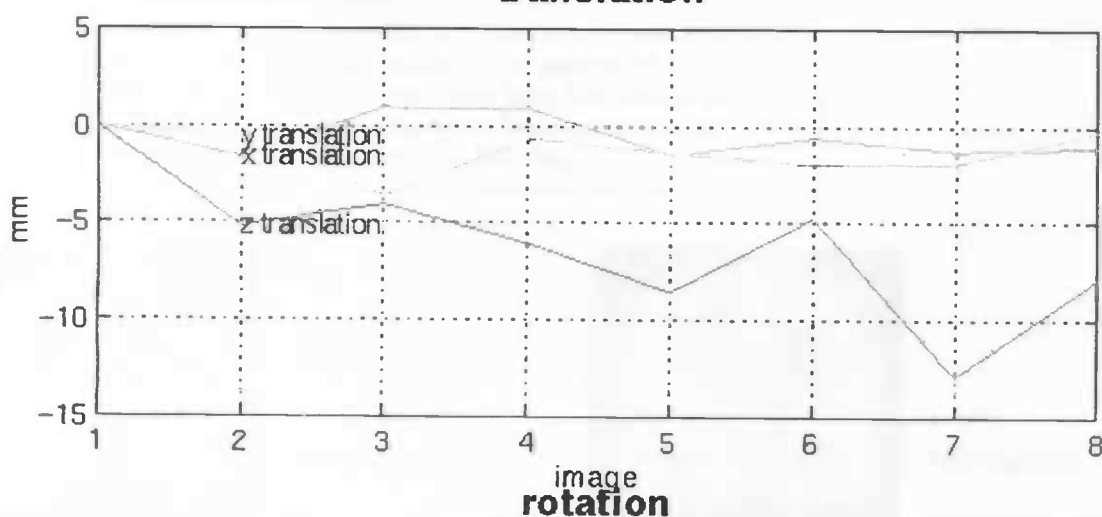
```

The first scan selected is the middle scan in time.  
\_c means centered image, \_t means cti2spm transformed

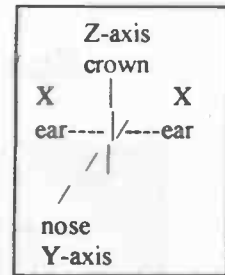
Translation of images to the middle scan:  
Red = x-axis, Green = y-axis, Blue = z-axis  
Horizontal: imagenumber  
Vertical: translation in mm, usually between +10 and -10

One can read for every scan the translation over the three axis copared to the previous scan.

### translation



# Image realignment



```

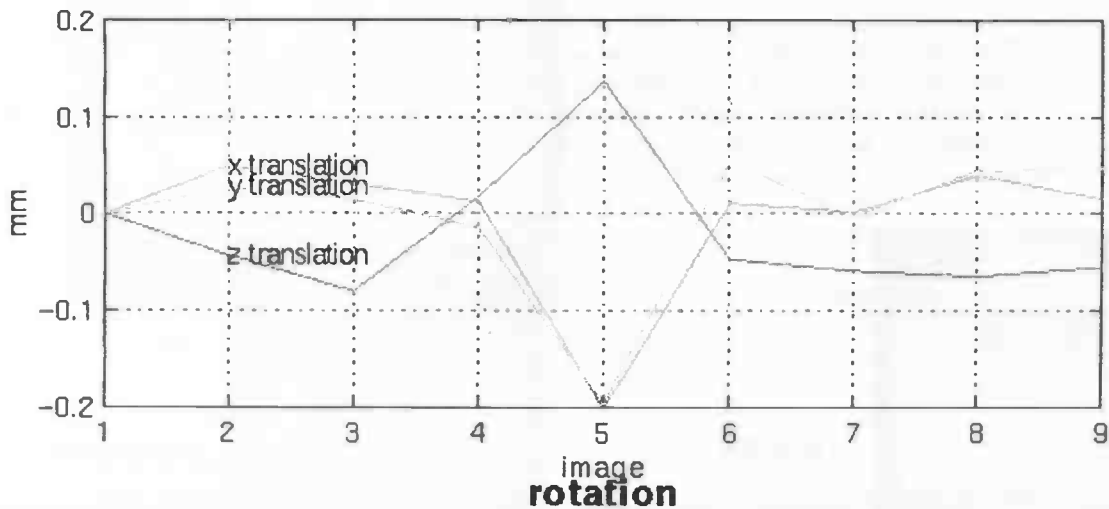
1 /home/simone/DIS/mean/ 4_c_timg
2 /home/simone/DIS/ 1_c_timg
3 /home/simone/DIS/ 2_c_timg
4 /home/simone/DIS/ 3_c_timg
5 /home/simone/DIS/ 4_c_timg
6 /home/simone/DIS/ 5_c_timg
7 /home/simone/DIS/ 6_c_timg
8 /home/simone/DIS/ 7_c_timg
9 /home/simone/DIS/ 8_c_timg
    
```

Rotation occurs in three directions:

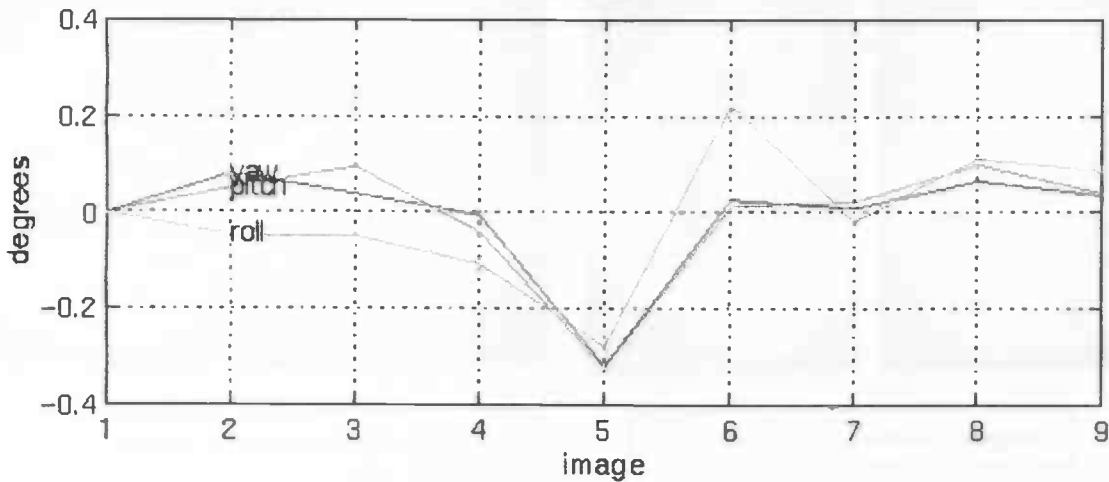
- 1) movement of the nose towards the ears (roll): rotation about the z-axis.
- 2) movement of the nose towards the chin/forehead (pitch): rotation about the x-axis.
- 3) movement of the head towards the shoulders (yaw): rotation about the y-axis.

Second realignment:  
Realignment to the meanimage, created in the first realignment.

## translation



## rotation



## Spatial Normalisation

Image : /home/simone/DIS/mean 4\_c\_t.img

Parameters : /home/simone/DIS/mean 4\_c\_t\_sn3d.mat

### Linear {affine} component

$$X1 = -1.09 \cdot X + 0.31 \cdot Y + -0.11 \cdot Z + 9.93$$

$$Y1 = 0.31 \cdot X + 0.92 \cdot Y + -0.41 \cdot Z + -8.71$$

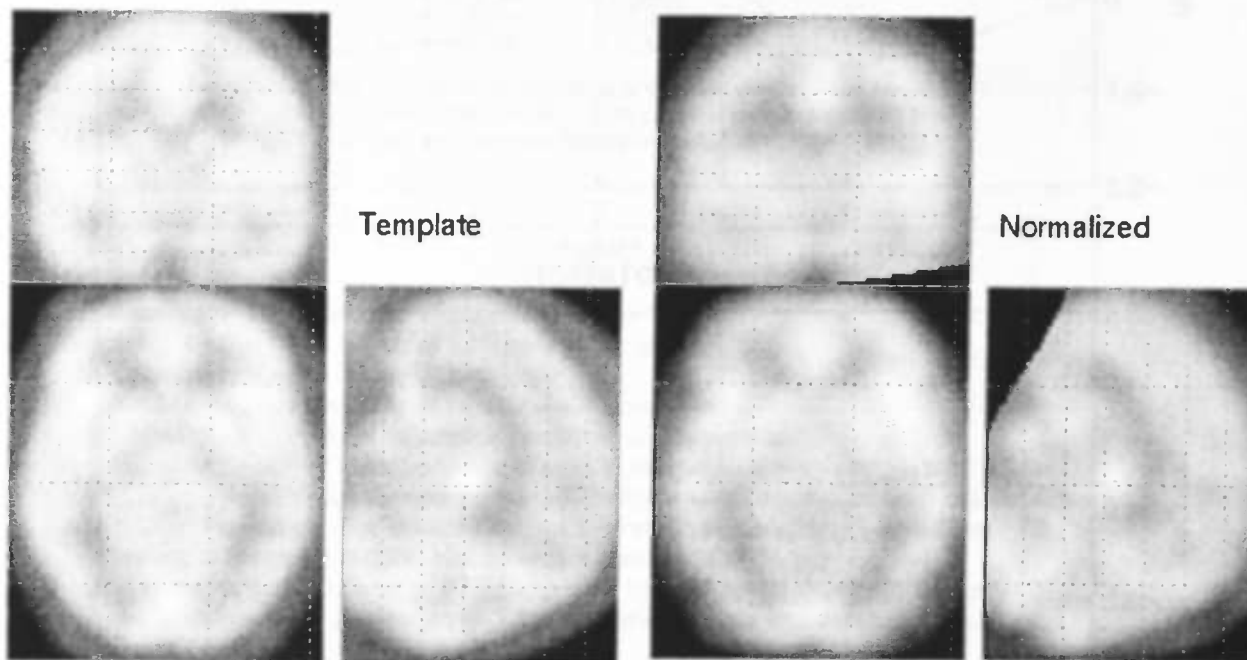
$$Z1 = 0.03 \cdot X + 0.47 \cdot Y + 1.09 \cdot Z + 8.70$$

This is the normalisation matrix.  
The given parameters are specific  
for this subject.

8 nonlinear iterations

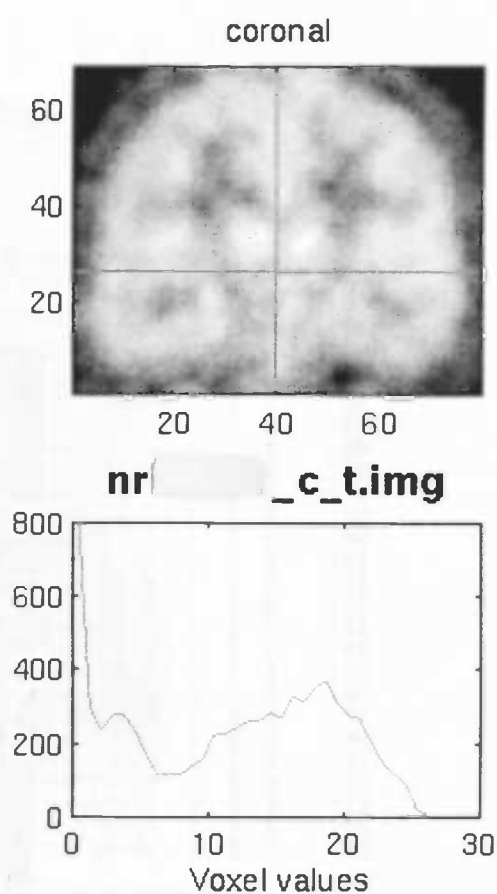
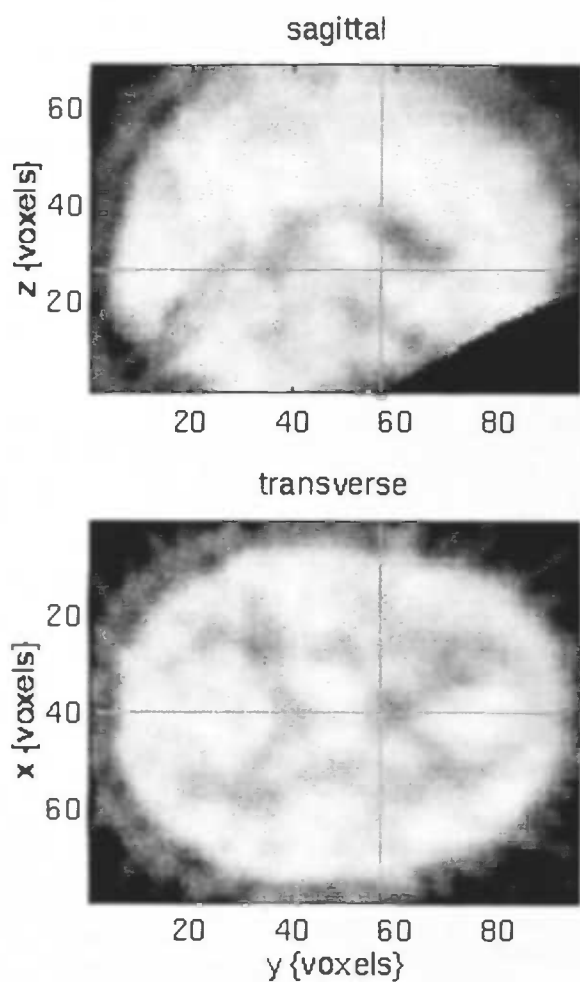
3 x 4 x 3 basis functions

These are the non-default values for the anatomical deformation, using 3x4x3 non-linear basis functions as adjusted before the normalisation. The 4x5x4 basisfunctions cause overstretched normalisation using HR<sup>+</sup> data.



Template: standard normalised brain

Normalised brain of the subject



These normalised pictures are obtained by displaying the normalised images per scan. This gives a clearer picture of the normalised brain per scan.

Histogram presentation of voxels with respective voxel values.

## Statistical analysis

Scan Index	Subject	Condition	Replication	Global	Base directory: /home/simone/DIS/Patienten/S20 Filename Tails
01 :	1	1	1	39.7471 /sn	NT1_c_timg
02 :	1	1	2	38.7996 /sn	NT2_c_timg
03 :	1	2	1	35.7027 /sn	TT1_c_timg
04 :	1	2	2	38.5978 /sn	TT2_c_timg
05 :	2	1	1	48.1865 /sn	1_c_timg
06 :	2	1	2	50.5257 /sn	TT2_c_timg
07 :	2	2	1	51.2212 /sn	TT1_c_timg
08 :	2	2	2	52.6348 /sn	TT2_c_timg
09 :	3	1	1	41.5764 /sn	1_c_timg
10 :	3	1	2	38.8933 /sn	2_c_timg
11 :	3	2	1	48.8658 /sn	2_c_timg
12 :	4	1	1	50.1338 /sn	TT1_c_timg
13 :	4	2	1	59.2633 /sn	TT1_c_timg
14 :	4	2	2	54.3018 /sn	TT2_c_timg
15 :	5	1	1	62.3433 /sn	1_c_timg
16 :	5	1	2	68.0882 /sn	2_c_timg
17 :	5	2	1	55.3659 /sn	1_c_timg
18 :	5	2	2	53.7178 /sn	2_c_timg
19 :	6	1	1	61.6306 /sn	1_c_timg
20 :	6	2	1	45.1677 /sn	1_c_timg
21 :	6	2	2	52.9196 /sn	1_c_timg
22 :	7	1	1	48.128 /sn	NT2_c_timg
23 :	7	2	1	54.1892 /sn	TT2_c_timg

Images scaled to an overall grand mean of 50

Gray matter threshold is 80% of the whole brain mean

Covariates are centered before inclusion in design matrix

### Explanation:

- Scan index = Number of scans for analyses in SPM
- Subject = Number of subjects used for analyses
- Conditions = Number of conditions
- Replication = Number of replications of conditions
- Global = Relative cerebral bloodflow during the scan
- Filename Tails = Filenames imported in SPM for statistical analyses.

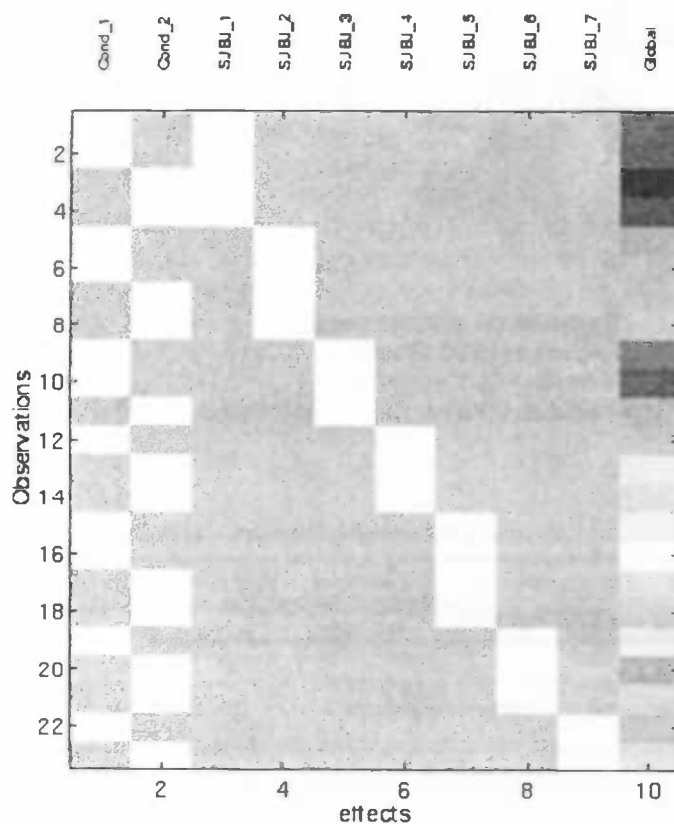
The above list shows a study with 7 subjects with 2 conditions, with and without replications. For example: subject 7 => two conditions, only 1 replication

Scaling of the global cerebral bloodflow to a grand mean of 50.

Gray matter threshold is set to 80%, this means 80%, all voxels above this 80% threshold are considered to be graymatter, so 20% of the brain is taken into statistical calculation.

Covariates: are not defined in this analysis.

## Design Matrix



Design: Multi-subject: with replications

Global normalisation: AnCova

### Parameters:

2 Condition + 0 Covariate + 7 Block + 1 Confound

= 10 parameters, having 9 degrees of freedom, giving 14 residual df (23 scans).

### Explanation:

The Design Matrix: In the subj\_number column, one white block is present, from here, going to the left, one can perceive the corresponding observation with the conditions.

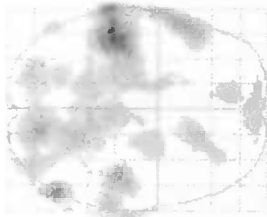
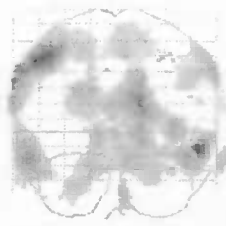
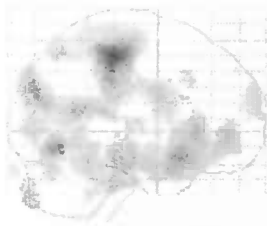
The "Global" column gives information about the cerebral bloodflow. A lighter square stands for more global cerebral bloodflow.

In this case the design is Multi-subject (here 7) with replications (some conditions were repeated)

Parameters: In this case analyses were made with 2 conditions, 0 covariates, 7 blocks (=7 subjects) and 1 confounded covariate (= global cerebral bloodflow)

df means degrees of freedom: in this case are 10 parameters involved ( $2+0+7+1=10$ ) this results in  $10 - 1$  (1 confound variable = gCBF) = 9 degrees of freedom, giving the number of scans(23) - 9 = 14 remaining degrees of freedom. So,  $df=14$ .

SPM{F}  $p < 0.05$ , df. 1.0, 14.0



Search volume: 233425 voxels  
 Image size: 79 95 68 voxels  
 Voxel size 2.0 2.0 2.0 mm  
 Resolution {FWHM} 16.4 17.4 17.4 mm

Results directory: /home/simone/DIS/Patienten

Contrasts	1	2
Cand_1	1	-1
Cand_2	-1	1

#### Explanation:

SPM{F} means a statistical parametric map computed with F statistical tests, areas represented have chance values from  $p < 0.05$  or smaller.

Resolution {FWHM} = Full Width Half Maximum

Smoothing is a convolution with a Gaussian kernel.

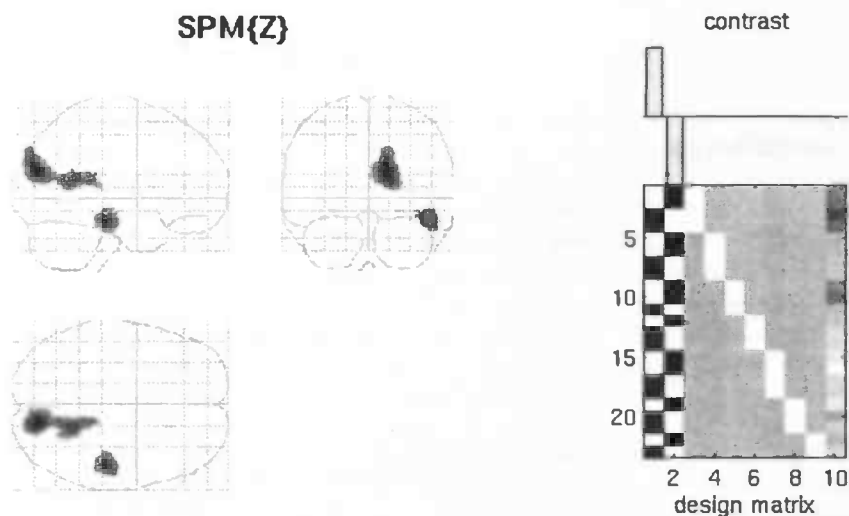
#### Two contrasts:

- 1)  $1 * \text{condition\_1} + (-1 * \text{condition\_2})$
- 2)  $-1 * \text{condition\_1} + (1 * \text{condition\_2})$

In this case this means:

- 1) NT - TT (See file list: condition1 = NT)
- 2) TT - NT (condition2 = TT)





**P values & statistics: /home/simone/DIS/Patienten**

set-level {c}	cluster-level {k,Z}	voxel-level {Z}	uncorrected k & Z			x,y,z {mm}		
0.950 (2)	0.457 (719, 3.11)	0.967 (3.11)	0.030	0.001	14	-82	24	
		0.993 (2.94)						
		0.996 (2.90)						
	0.962 (229, 2.96)	0.992 (2.96)	0.196	0.002	48	-24	-16	

**Explanation P values & statistics:**

The table exists of 5 columns:

**1) set-level {c}:**

The probability of getting (2) or more clusters by chance 0.950 (small values wished). {c} is the number of clusters bigger than the given Extent threshold {k} for the given Height threshold {u} (Height and Extent threshold at the bottom of the page).

**2) cluster-level {k,Z}:**

Probability (0.457) of obtaining a cluster size of k (for example: k = 719 cluster in voxels). The voxel Z-value of 3.11, is the maximum Z-value within this cluster of connected voxels. In this column are {c} clusters.

**3) Voxel-level {Z}:**

It is the probability 0.967 of obtaining a Z-score of 3.11 (same Z score as in 2). This Z-score is the straight Z-score, only the neighbouring p-value (0.967) is corrected for multiple comparisons. The other two Z-scores are the two other highest maxima in the cluster that are at least 8 mm apart. Corrected (for the volume of the brain, i.e. for multiple comparisons): p < 0.05 for significance. A p < 2.00 means that no false positives are shown.

**4) Uncorrected k & Z:**

Uncorrected probabilities, looking at cluster/voxel in isolation, i.e. without a correction for the volume of brain analysed. The first p-value is the extent of the cluster (suprathreshold cluster size), the second p-value is for the height within the extent of the cluster (local maximum within the cluster). Both p-values are uncorrected.

So a significant cluster must have significant extent and height of voxels in isolation. Significant uncorrected p-values for the height: p < 0.001.

Height threshold {u} = 2.33, p = 0.010

Extent threshold {k} = 147 voxels, p = 0.298

Expected voxels per cluster, E(n) = 146.6

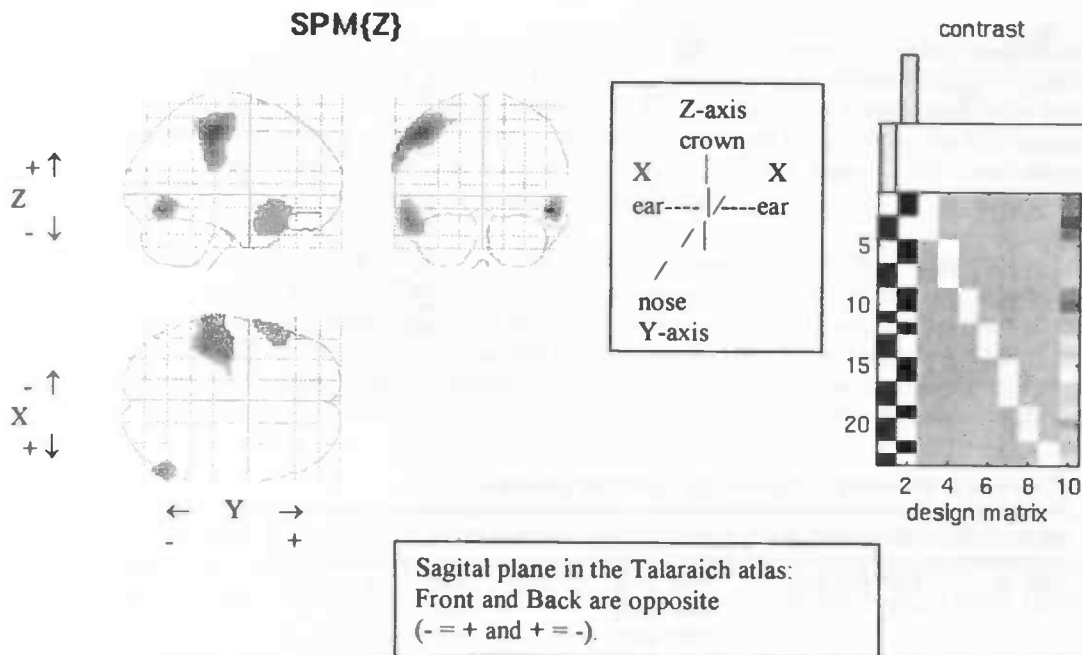
Expected number of clusters, E(m) = 4.7

Volume {S} = 233425 voxels or 376.6 Resels

Degrees of freedom due to error = 14.0

Smoothness FWHM {mm} = 16.4 17.4 17.4

{voxels} = 82 8.7 8.7



**P values & statistics: /home/simone/DIS/Patienten**

set-level {c}	cluster-level {k,Z}	voxel-level {Z}	uncorrected k & Z		x,y,z {mm}		
0.052 (3)	0.473 (177, 3.77)	0.401 (3.77)	0.254	0.000	54	-62	-10
		0.976 (3.07)		0.001	58	-72	-12
		0.997 (2.87)		0.002	58	-68	-4
	0.095 (1541, 3.42)	0.770 (3.42)	0.003	0.000	-46	-28	50
		0.792 (3.40)		0.000	-66	-34	40
		0.798 (3.39)		0.000	-52	-32	46
		0.981 (3.05)		0.001	-58	16	-18
	0.037 (376, 3.05)	0.998 (2.86)	0.104	0.002	-58	18	-10
		0.999 (2.75)		0.003	-50	24	-24

p-values in the columns 1 to 4 must be as small as possible.  
Then chances are smaller that clusters found, are based on coincidence.

Height threshold {u} = 2.33, p = 0.010  
Extent threshold {k} = 147 voxels, p = 0.298  
Expected voxels per cluster, E{n} = 146.6  
Expected number of clusters, E{m} = 4.7

Volume {S} = 233425 voxels or 376.6 Resels  
Degrees of freedom due to error = 14.0  
Smoothness FWHM {mm} = 16.4 17.4 17.4  
{voxels} = 82 8.7 8.7

SPM96 (simone) - 04/12/1998 (12:18)

E{m} = number of clusters expected on average  
Extend threshold {k} = spatial extent threshold, suprathreshold clusters are only shown if they exceed this size.

## §4.9: Coordinates and Talairach atlas

The fifth column of the statistical spm.ps file shows the coordinates (x,y,z{mm}) of the spatial location of the focus of activation in the brain. Coordinates can be looked up in the brain atlas: *Co-Planar Stereotaxic Atlas of the human brain; 3-dimensional proportional system: An approach to Cerebral Imaging* by Jean Talairach and Pierre Tournoux<sup>B9</sup>. This is the standard atlas for brain imaging studies. From this atlas the name of the anatomic brain area corresponding to the coordinate, can be obtained. Together with the brain areas the numbers of Brodmann's areas are given. Brodmann's map is a cyto-architectonic map which was constructed by examining the neurons of the neocortex to identify regions that seemed to have a unique organisation. Brodmann divided the brain at the central sulcus and then worked through each half in random order, numbering new conformations of cells as he found them. The atlas can only be used for localisation, the function of the different brain areas has to be obtained from literature (articles and books (e.g. Kolb and Whishaw<sup>B8</sup>)).

The Talairach and Tournoux atlas is based on the brain of only one French woman. The volumetric reconstruction took into account stereotaxic data from the original brain. The accurate reconstruction of the two cerebral hemispheres of anatomical zones and the location according to Brodmann's classification is given in figures I and II, Addition IV.b.

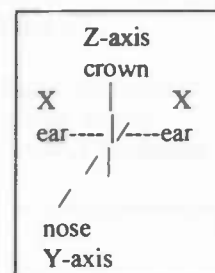
The human cerebral cortex is subdivided in four major lobes: the frontal lobe, parietal lobe, temporal lobe and occipital lobe. The frontal lobe contains the motor cortex and the prefrontal cortex. Brodmann's areas that belong to the frontal lobe are: area 4 (primary somatomotor area), area 6 (motor functions), area 8 (eye movement), areas 9 and 10 (prefrontal cortex), areas 44 and 45 (Broca's area) and area 47. The parietal lobe is specialised primarily for dealing with body information. Brodmann's areas that belong to the parietal lobe are: areas 1, 2 and 3 (primary somatosensory cortex), area 5, area 7 and areas 39 and 40 (associative functions). The temporal cortex is involved with auditory perception and some aspects of emotion (affect). Brodmann's areas that belong to the temporal lobe are: areas 41 (primary auditory receptive cortex), area 42 (auditory integration region), area 22 (auditory association area), area 21 and area 37 (auditory visual association area). The occipital lobe is involved with sense of vision. Brodmann's areas that belong to the occipital lobe are: area 17 (primary visual sensory area), area 18 (visual integration) and area 19. From the thalamus connections lead to the cortex, called corticothalamic connections. These connections are often reciprocal and maintain a topographic relationship between subdivisions of the thalamus and parts of the cortical area. If a cortical area is active, the adjacent white matter is occupied by fibers connected to a particular part of the thalamus.

Many structures in the brain are labeled according their location relative to one another. Conventional terms used to indicate anatomical direction are: inferior ((be)low), superior (top), lateral (side), medial (middle), ventral (bottom), anterior (front) and posterior (back). The brain is arranged symmetrically and consists of left and right sides. Two structures can lie ipsilateral (on the same side), contralateral (opposite sides) and bilateral (one on each side). Structures that are close to one another are said to be proximal; those far from each other are said to be distal. A projection that is approaching is said to be afferent; one that is leaving is said to be efferent.

### §4.9.1: Use of the atlas

A coordinate of three dimensions (x,y,z) can be looked up in three two-dimensional maps. One sliced along the X-axis (sagittal: ear to ear), one sliced across the Y-axis (coronal: nose to back of the head) and one along the Z-axis (transversal: crown to neck).

As an example: Obtaining the area accompanying the coordinate (3,-16,4), maps belonging to this coordinate are shown in Addition IV.c. One number of the coordinate equals the number of the two-dimensional map, the remaining two numbers indicate a location. The coordinate (3,-16,4) is located in the *dm* structure which is the dorsomedial nucleus. The three maps give an indication of the precise localisation of the coordinate within the dorsomedial nucleus. In this manner areas can be obtained from calculated coordinates in the SPM program.



### §4.9.2: Crisis in Normalisation?

During the normalisation brains of scanned subjects are matched onto a template. Shape, place and form of brain and brain structures from all subjects are now comparable to allow for statistical analyses. In SPM95 the template is based on the Talairach and Tournoux brain, the brain of one French female (also called single French

female brain on the SPM mailing list). Because normalisation is based on Talairach and Tournoux, the coordinates obtained from activated brain areas can be looked up in the Talairach and Tournoux atlas without any problems.

In SPM96 a so called MNI brain is used as a template. This template is the mean of 305 brains. According to an email discussion on the SPM-mailing list (June 1998: <http://www.fil.ion.ucl.ac.uk/spm/help>) the Talairach and Tournoux brain and the MNI brain appear not to be the same. The Talairach and Tournoux brain is smaller. Questions that appeared were "How can one compare coordinates obtained by matching the MNI template (ala SPM96) with coordinates published in the literature, supposedly in Talairach space?", "How can one compare results, listing Talairach coordinates, if the brain spaces are different?"

Answers from the creators of SPM in London: "...the templates available with SPM conform to the Talairach system" (x,y,z expressed system) "with varying degrees of 'closeness' to the atlas drawings. They are all variants of 'a' Talairach space. The most recent templates have been designed to make them compatible with the MNI 305 average and implicitly the ICBM atlases that will ensue in the future", "Given that the drawings in the T&T atlas do not conform to a template, the T&T space is operationally less useful than any other (because its relationship to other spaces is less easy to define)." They also state that it is an important component but no crisis in normalisation.

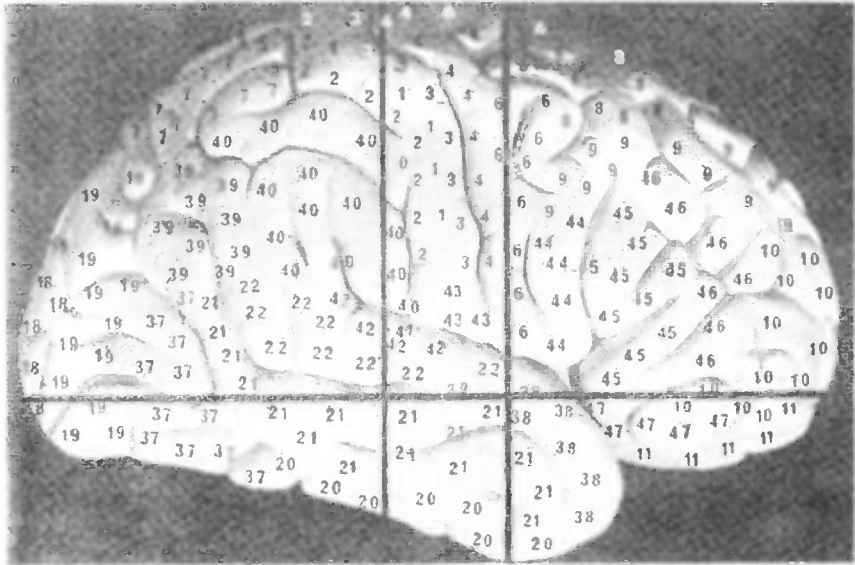
Some solutions were suggested. One research group takes the SPM95 template (Talairach compatible), using the spatial normalisation algorithm of SPM96 with an affine transform to map it onto the SPM96 template. Which resulted in formulae for translating between the two coordinate systems. After the application of the formulae, it appeared that the MNI brain is about 14% larger in the X and Z direction, also the Y origin shifts 3 mm.

Another research group created, from the MR scan made before the PET scan, an average MR for all the patients in that study and register the group PET data on the group MR data. This research group even states: "... Talairach coordinates could sometimes be at best misleading and at worst wrong." The activation place on the MR was not consistent with the Talairach indicating place. This research group also warns not to have blind faith in the Talairach coordinates.

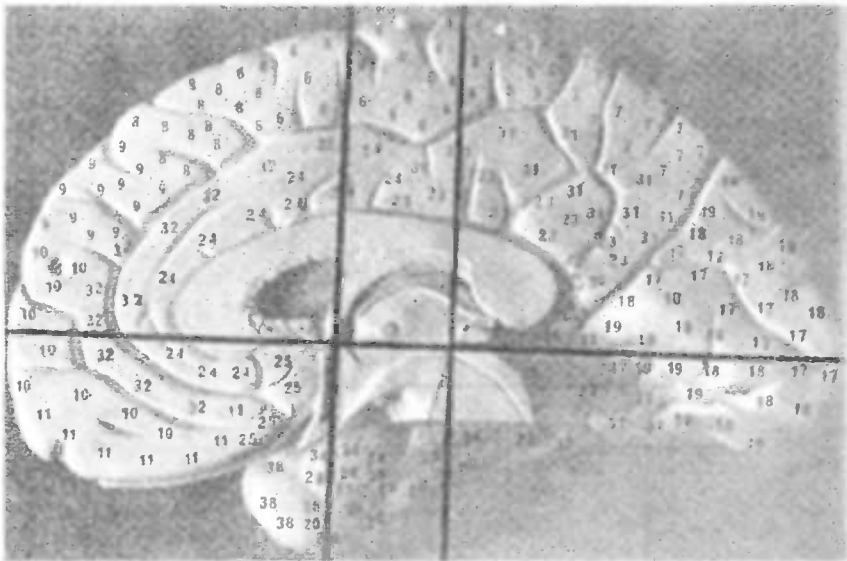
The 'mapping between MNI and Talairach space' problem also plays a role in this DID-PET investigation. SPM96 is used to perform the statistical analyses, but coordinates (obtained according to MNI template) are looked up in the Talairach atlas because no other brain atlas is yet available and no MRI's were obtained.

**Addition IV.b**

**Accurate reconstruction of the anatomical locations according to Brodmann of the brain of one french woman**

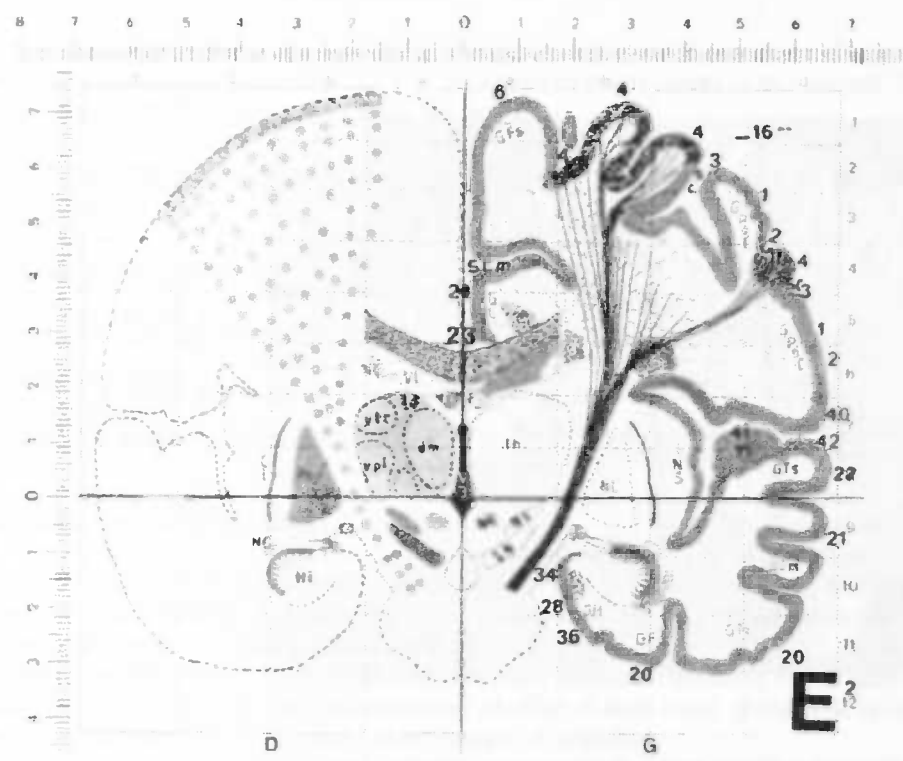
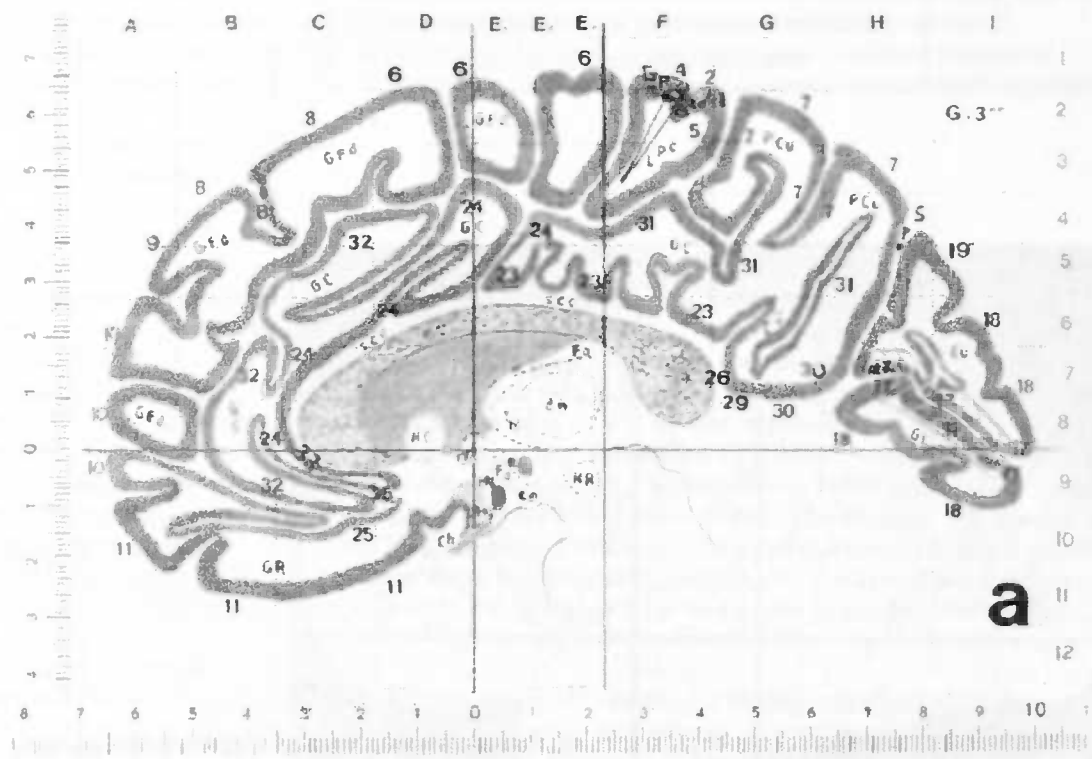


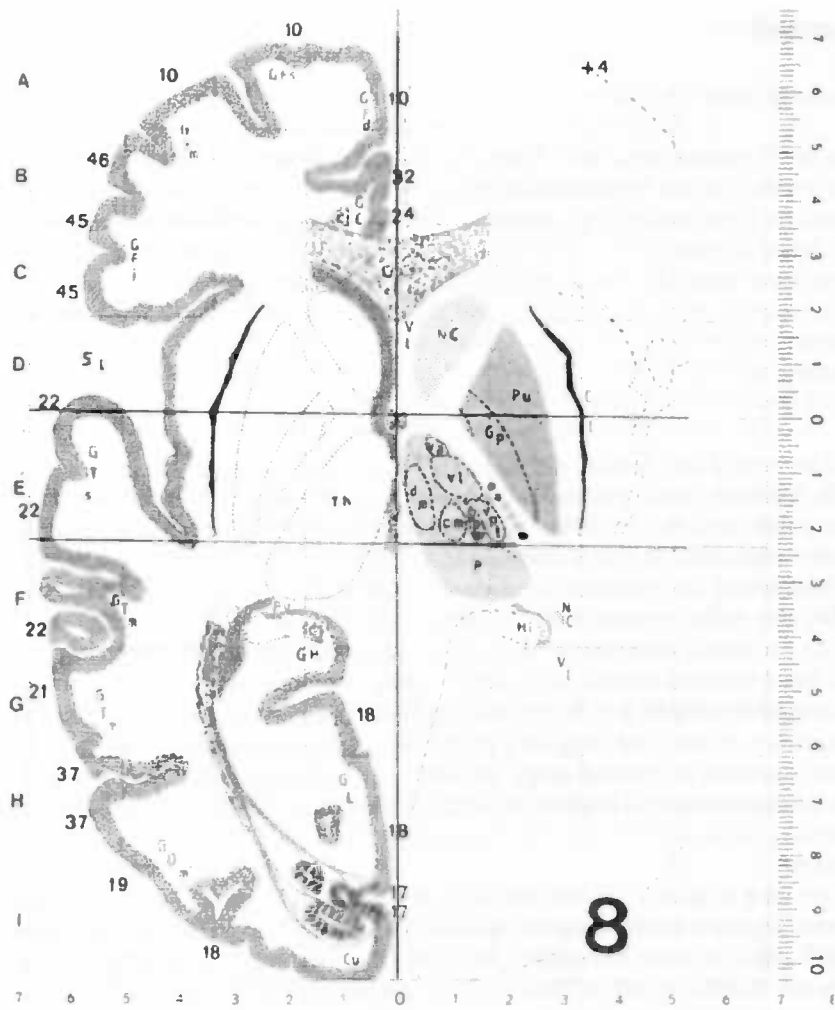
**Figure I:**  
Right Hemisphere.  
Lateral surface.  
Areas of Brodmann.



**Figure II:**  
Right Hemisphere.  
Medial surface.  
Areas of Brodmann

Addition IV.c





## §4.10: SPM in a nutshell

### §4.10.1: Introduction

SPM is the abbreviation for Statistical Parametric Mapping. Statistical parametric maps are spatially extended statistical processes that are used to test hypotheses about regionally specific effects in neuro-imaging data. Most parametric maps are based on linear models (for example ANOVA = analysis of variance, to obtain  $\sigma$ ), correlation coefficients (linear correlation between variables, e.g. voxels) and t-tests. Statistical parametric mapping uses the general linear model to create spatially extended statistical process (for an in-depth explanation of SPM see also <sup>B4</sup>).

### §4.10.2: General statistics

#### §4.10.2.a: t test

Testing with a t-test will be done if the standard deviation ( $\sigma^2$ ) (and so the standard variance ( $\sigma$ )) of the inquired variable is unknown. The standard variance must be estimated first (analysis of variance = ANOVA). The t distribution is comparable with the standard normal distribution except that the t distribution is broader (and therefore lower). To work with t tests a null hypotheses ( $H_0$ ) is needed and a critical area is defined if this hypotheses is rejected. With use of the estimated variance the t value of the variable,  $t^*$ , can be calculated. The critical area is bordered by the critical t value of the variable,  $t^c$ . If  $t^*$  (t value of the variable) is not included in the critical area,  $t^* < t^c$ , the variable is accepted on basis of  $H_0$ . This arbitrarily (almost normal) distributed t value needs to be transformed into a standard normal distribution, called z representation. This z representation is used for a standard normal distributed variable,  $z = N(\mu=0, \sigma=1)$  with  $\mu$  the mean and  $\sigma$  the variance. The Z score is coupled to this z representation. In the SPM program paired t values are computed and converted into Z scores, which are used to produce statistical parametric maps. In these statistical maps only Z scores above a default threshold are shown. This default threshold is set to a Z of 2.33, but can be changed to any desired threshold.

#### §4.10.2.b: Least squares fit

SPM also makes use of the least squares fit. If one independent variable X ('cause') influences another dependent variable Y ('result'), the way the variables influence each other is obtained by regression analysis. If the relationship between X and Y is linear, the method is called linear regression. The most important feature of a regression model is the non symmetric role of the variables. One variable is the cause of the other variable. The singular linear regression model is:  $Y = \alpha + \beta X + \epsilon$ .

The result, Y, is linear dependent of the variable X. The parameters  $\alpha$  and  $\beta$  need to be obtained with the least square method. A line through the results ( $X_i, Y_i$ ), the measured points, needs to be obtained. If a measured point  $X_i$  is assumed, then Y can be calculated by using the model:

$$Y = \alpha + \beta X + \epsilon$$

This gives  $Y^c$ , Y calculated.  $Y^c$  probably differs from the observed  $Y_i$ , belonging to  $X_i$ . The difference between  $Y_i$  and  $Y^c$  is given by:

$$\epsilon_i = Y_i - Y^c = Y_i - (\alpha + \beta X_i)$$

The sum of squares of  $\epsilon_i$  is given by:

$$\sum \epsilon_i^2 = \sum \{Y_i - (\alpha + \beta X_i)\}^2$$

This equation can be rewritten to obtain  $\alpha$  and  $\beta$ . The equations to obtain  $\alpha$  and  $\beta$  are:

$$\beta = (n \sum X_i Y_i - \sum X_i \sum Y_i) / (n \sum X_i^2 - (\sum X_i)^2)$$

$$\alpha = (\sum Y_i / n) - (\beta \sum X_i / n)$$

Visualizing these equations for  $\alpha$  and  $\beta$  can perhaps clear up calculations used in SPM routines (for more statistics see also <sup>B2,5</sup>).

### §4.10.3: SPM, the general linear model (GLM)

The statistical analysis must proceed the acquired data for evidence of an experimental induced effect at every intra-cerebral voxel individually. After reconstruction, realignment, stereotactic normalisation and smoothing, the data are ready for statistical analysis. This involves two steps:

- 1) A null hypothesis ( $H_0$ ), of no effect of covariates or other factors on the count rate in each voxel, is tested. Statistical evidence against this null hypothesis of no effect in each voxel, is obtained by complex mathematical computations. This results in an 'image' of statistics.
- 2) This image of statistics must be assessed, reliably locating voxels where an effect is exhibited whilst limiting the possibility of false positives.



The first step will be discussed below, explained in four steps.

Statistical parametric maps are given the acronym SPM{t}, this indicates that voxels have values distributed as a t-distribution under a null hypothesis. SPM analyses each voxel independently as:

- 1) Analysis of variance, separately at each voxel
- 2) Makes t statistics from the results of these analyses, for each voxel
- 3) Converts the t statistics to a Z score
- 4) Shows an image of the Z scores

SPM does an analysis of variance at each voxel entirely independently, in order to make its t statistics (and z scores). The *response variable* is some measured data for each observation (dependent variable). In SPM the *response variable* is counts data (counted gamma rays, representing rCBF) from a particular individual voxel for all the scans in our analysis. The *response variable* is data for all the scans for one voxel (i.e. all the observations). An observation is a voxel count value in the voxel analysed for one scan.

The *response variable* is given by  $Y_j$ , where  $j = 1, 2, 3, \dots, J$  indexes the observation (i.e. the scans) and proportional scaling is used to correct for global differences (i.e. global blood flow) between observations (scans).  $Y_j$  is a random variable, number of counts in scans. Suppose that for each observation there is a set of  $L$  ( $L < J$ ) *explanatory variables*, denoted by  $x_{jl}$  where  $l = 1, 2, 3, \dots, L$  indexes the *explanatory variables*. The *explanatory variables* may be continuous covariates, functions of covariates or they may be dummy variables indicating the levels of an experimental factor.

A general linear model (GLM) explains the variation in  $Y_j$ , in terms of a linear combination of the *explanatory variables* plus an error term:

$$Y_j = x_{j1}\beta_1 + \dots + x_{jl}\beta_l + \dots + x_{jL}\beta_L + \epsilon_j \quad 1)$$

Here the  $\beta_l$  are (yet) unknown parameters, corresponding to each of the  $L$  *explanatory variables*. The errors  $\epsilon_j$ , for each scan, are independent and normal distributed as:  $\epsilon_j = N(0, \sigma^2)$ . The use of the general linear model is also known as regression analysis.

The simplest example is linear regression. Here only one continuous *explanatory variable*  $x_j$  (dummy variable) is measured for each observation  $j = 1, 2, 3, \dots, J$ . The model, a straight line, is usually written as:

$$Y_j = x_j\beta + \mu + \epsilon_j \quad 2)$$

Equation 2) is the simplest form of equation 1). In the model  $\mu$  is the constant term, which is an unknown parameter. At  $x=0$ , the expected (intercept-) value of the line  $Y$  is determined by this parameter  $\mu$ .  $\beta$  is the regression slope of the line  $Y$ . So, the voxel's data for each scan  $j$  is approximated by some straight line relationship with the (*explanatory variable*) factor  $x_j$ , with a slope of  $\beta$  and an intercept on the y axis of  $\mu$ .

Equation 2) can be written as a general linear model of the form of equation 1) by use of a dummy *explanatory variable*. This is an indicator variable  $x_{2j}$  whose values are all one, because it is a dummy variable. Multiplying  $\mu$  by  $x_{2j}$  ( $\mu \cdot 1 = \mu$ ) in 2) gives:

$$Y_j = x_{1j}\beta + x_{2j}\mu + \epsilon_j \quad 3a)$$

Be aware of the fact that  $\mu$  is a constant. The  $\mu$  can also be represented as  $\beta_2$  which is a constant with the same value as  $\mu$ , but differently named. And  $x_{2j}$  equals one (in this case). This results in the equation, which is of the form of 1):

$$Y_j = x_{1j}\beta_1 + x_{2j}\beta_2 + \epsilon_j \quad 3b)$$

Equation 3b) can be rephrased in terms of matrices:

$$Y = X\beta + E \quad 4)$$

Where  $Y$  is the data matrix, a column vector (a vector is a  $1 \times n$  matrix) of observations.  $E$  is the column vector of error terms.  $\beta$  is the column vector of parameters.  $X$  is the design matrix. The size of  $X$  is  $J \times L$ , with the number of rows times the number of columns. The design matrix has one row per observation (i.e. scan) and one column per model parameter, also called every effect (factor or covariate) in the model (i.e.  $\beta$ 's).

An example: Two scans are obtained, scan 1 and scan 2. Two scans result in two equations:

$$\begin{aligned} Y_1 &= x_{11}\beta_1 + x_{21}\beta_2 + \epsilon_1 \\ Y_2 &= x_{12}\beta_1 + x_{22}\beta_2 + \epsilon_2 \end{aligned} \quad 5)$$

These two equations can be represented in matrices, as in equation 4):

$$\begin{bmatrix} Y_1 \\ Y_2 \end{bmatrix} = \begin{bmatrix} x_{11} & 1 \\ x_{12} & 1 \end{bmatrix} \begin{bmatrix} \beta_1 \\ \beta_2 \end{bmatrix} + \begin{bmatrix} \epsilon_1 \\ \epsilon_2 \end{bmatrix} \quad 6)$$

First a little about matrix calculation. Matrices are build up of rows (horizontal) and columns (vertical). Rows are aligned from top to bottom, columns from left to right. Sizes of matrices are defined by (number of rows) x (number of columns).

Say we have two matrices  $A = \begin{bmatrix} a & b \\ c & d \end{bmatrix}$  and  $B = \begin{bmatrix} e \\ f \end{bmatrix}$ . A is a 2x2 matrix and B is a 2x1 matrix. For matrix multiplication matrices must be of the form m x p and p x n. The number of columns of the first matrix (A) must equal the number of rows of the second matrix (B). The result is a m x n matrix. In our case a 2x1 matrix. The

multiplication result, of our two matrices, is:  $\begin{bmatrix} a*e + b*f \\ c*e + d*f \end{bmatrix}$ . The transpose of matrix A is  $A^T$ . This means that the

rows and columns are exchanged. A m x n matrix becomes n x m. For our matrices A and B:  $A^T = \begin{bmatrix} a & c \\ b & d \end{bmatrix}$  and  $B^T = \begin{bmatrix} e & f \end{bmatrix}$ . The inverse of a matrix  $A^{-1}$  is more complicated to calculate since for inverse-matrix calculation matrices must be of the form n x n. The inverse matrix of our matrix A:

$A^{-1} = \frac{1}{(ad-bc)} \begin{bmatrix} d & -b \\ -c & a \end{bmatrix}$ . Perhaps this very short introduction to matrix calculation can help later on (for more mathematics see also <sup>B1</sup>).

As an example the following values of the two scans are obtained: we have one person, two conditions, one scan per condition. The value of the voxel in scan 1 is 64 and during scan 2 the value is 74, 64 and 74 are the *response variables*. There are also two *explanatory variables*, namely a value of 3 for scan one and a value of 2 for scan two. The value of  $x_{2j}$  equals 1 and the value of  $\beta_2$  equals  $\mu$  (as mentioned above).

The data matrix Y is given by  $\begin{bmatrix} 64 \\ 74 \end{bmatrix}$ . The design matrix X is given by  $\begin{bmatrix} 3 & 1 \\ 2 & 1 \end{bmatrix}$ . The parameter matrix is given by  $\begin{bmatrix} \beta_1 \\ \mu \end{bmatrix}$ . The error matrix E, with one column and 2 rows is given by  $\begin{bmatrix} \text{error}_{\text{scan1}} \\ \text{error}_{\text{scan2}} \end{bmatrix}$ . If the matrix multiplication is applied, to following equations are obtained:

$$\begin{aligned} 64 &= 3\beta_1 + \mu + \text{error}_{\text{scan1}} \\ 74 &= 2\beta_1 + \mu + \text{error}_{\text{scan2}} \end{aligned} \quad 7)$$

Arriving at this point SPM does a least squares fit to obtain  $\beta$  and  $\mu$ .  $\beta$  and  $\mu$  make up the parameter matrix,

giving  $\beta: \begin{bmatrix} \beta_1 \\ \mu \end{bmatrix}$ , after minimizing the sum-of-squares and calculating  $\beta = (X^T X)^{-1} X^T Y$ . Now X, Y and  $\beta$  are known and  $\epsilon_i$ , the remaining errors, can be calculated by:  $E = Y - X*\beta$ . This is the same result as obtained from equation 7):

$$\begin{aligned} \text{error}_{\text{scan1}} &= 64 - 3\beta_1 + \mu \\ \text{error}_{\text{scan2}} &= 74 - 2\beta_1 + \mu \end{aligned} \quad 8)$$

In this equation both errors are the only unknown factors. These are the same as the distances of the voxel data values predicted by the least squares fitting line (as in § Least squares fit).

#### §4.10.4: t statistics and contrasts

As stated above: the simplest example is linear regression, as given in 2) ( $Y_j = x_j\beta + \mu + \epsilon_j$ ), where only one continous *explanatory variable*  $x_j$  (dummy variable) is measured for each observation  $j = 1, \dots, J$ . In the model  $\mu$  is the constant term (an unknown parameter). At  $x=0$ , the expected (intercept-) value of the line Y is determined by this parameter  $\mu$ .  $\beta$  is the regression slope of the line Y. So, the voxel counts for each scan j is approximated by some straight line relationship with the (*explanatory variable*) factor  $x_j$ , with a slope of  $\beta$  and an intercept on the y axis of  $\mu$ .

On the null hypothesis there is no relationship between the (*explanatory variable*) factor  $x_j$  and the voxel data ( $Y_j$ ). So,  $\beta$ , the slope of the line, will not be significantly different from zero. This can be tested by making a t statistics. The t-statistics in this case is to investigate the relation:

$$\beta_1 / (\text{standard error of the slope}) \quad 9)$$

The standard error is worked out with the original analysis of variance (ANOVA, to obtain  $\sigma$ ), using the matrix E (built up by  $\epsilon_j$ , see also 8)). If  $\beta_1$  is about zero the null-hypotheses is accepted. The result of t-statistics will be large and positive if the slope is significantly greater than 0, and large and negative if the slope is significantly less than 0.

To obtain  $\beta_1$  from equation 9) but also to calculate the standard error, a contrast is used. A contrast is a matrix, when multiplied by the parameter matrix  $\beta$  (containing  $\beta_1$  and  $\mu$ ), gives the numerator of the t-statistics. In this case the matrix is  $(1 \ 0)$ , because:  $(1 \ 0) * \begin{pmatrix} \beta_1 \\ \mu \end{pmatrix} = \beta_1$ .

The contrast or vector defines the linear compound (slope or  $\beta_1$ ) of parameters tested.

However, SPM will conceal constants, so it will conceal  $\mu$ , by terming it an 'effect of no interest', so effectively  $\beta$  becomes  $\beta_1$  and the contrast is  $(1)$ . This is just a contrast for a single covariate of interest in SPM. SPM will do the above ANOVA for every voxel in the brain. For every voxel, there will be a separate  $\beta_1$ . So in this example, with this contrast, for every voxel, t statistics will be obtained that are positive with positive slopes and negative with negative slopes. If we want to see voxels with slopes significantly less than 0, a contrast of  $(-1)$  has to be applied. Now negative  $\beta$ 's (in this case  $\beta_1$ ) become positive and therefore the negative t statistics become positive and get the same treatment obtaining Z scores and picture representation by SPM.

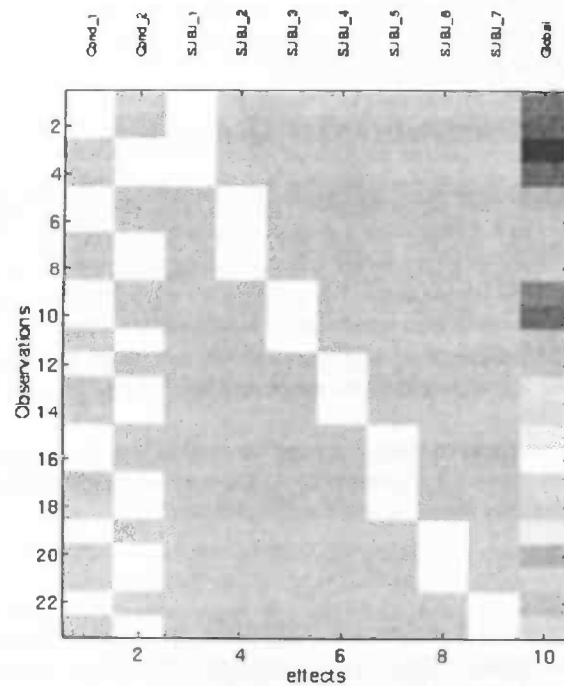
Or in other words: If the slopes of the lines are positive there is a positive proportionality between the (*explanatory variable*) factor  $x_j$  and the voxel data ( $Y_j$ ). If the slopes of the lines are negative there is a negative proportionality between the (*explanatory variable*) factor  $x_j$  and the voxel data ( $Y_j$ ).

#### §4.10.5: Z scores

Z scores are more amenable to later statistics than t-statistics. So, SPM will convert the t statistics to Z scores. Z scores are a way that SPM uses to display and analyse the p values from t-statistics. The p values from the t statistics need to be transformed into a standard normal distribution, called Z. This Z representation is used for a standard normal distributed variable,  $Z = N(\mu=0, \sigma=1)$  with  $\mu$  the mean and  $\sigma$  the variance. The Z score is coupled to this z representation. The Z scores are the numbers from the standard normal distribution ( $\mu=0, \sigma=1$ ) that would give the same p value as the t statistics.

In expanding the simplified example we could have 8 degrees of freedom (a df of 8) (this is not the case with the example given but that is why it functions as an simplified example, just to clear things up). We also could have a t value of a voxel of  $-2.9$ . This would give a one-tailed p value of  $p=0.01$ . The interpretation of this p value is that 1% of the cases by chance, with 8 df, we would find a t value less than or equal to  $-2.9$ . This is the same as saying that 1% of the area of the t distribution, with 8 df, lies below  $-2.9$ . The equivalent Z score is  $-2.33$ , because 1% of the normal distribution lies below  $-2.33$ . With a t value of  $+2.9$ , the p value will be 0.99. In 99% of the cases we would expect to find a t value less than or equal to 2.9 (in 1% of the cases we would find a higher p value). A p value of 0.99 gives us a Z score of  $+2.33$  (99% of the normal distribution lies below 2.33).

If we now ask SPM for a picture of the z statistics only z scores of 2.33 and higher are shown because the default z score, the threshold, is set to 2.33. This is exactly the same as showing t statistics more positive than 2.9. The design matrix is represented graphically by SPM. Every column is scaled so that the most negative number in the column will be nearest to black. The most positive will be nearest to white. An example of a design matrix:



Design: Multi-subject: with replications

Global normalisation: AnCova

**Parameters:**

2 Condition + 0 Covariate + 7 Block + 1 Confound

= 10 parameters, having 9 degrees of freedom, giving 14 residual df (23 scans).

**Design matrix:**

The first two columns represent the two conditions.  
Obtained by the contrast (1 -1) and (-1 1).

Seven subjects with 23 scans.

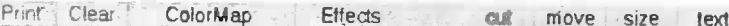
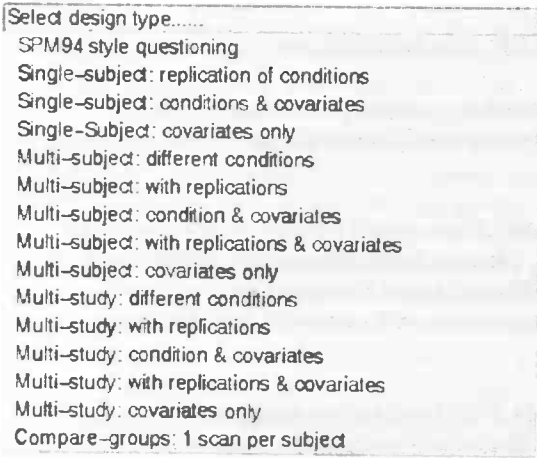
Two conditions, 0 covariates.

Residual degrees of freedom of 14:

$$23 - 9 = 14$$

$$(2 + 7 + 1 = 10 - 1 = 9)$$

#### Addition IV.d: The SPM96 interface



## SPM96

**This is SPM96**

This is SPM96, the public edition of SPM'96 - released 09/04/1997.  
Please refer to this version as "SPM96" in papers and communications,  
citing the "Wellcome Department of Cognitive Neurology"

SPW96 is backward compatible, in that each module will read files from previous versions. (E.g. SPW96 statistics will read normalised images from SPW95) However, it is recommended that you don't mix versions of the same module in a single analysis. (E.g. Don't mix SPW96 and SPW95 spatial normalisations in the same study group - the templates are different!) You should identify which versions of SPW were used for which stages of analysis in any publications.

A version choosing routine, `spm_choose`, is provided in the distribution. This presents a graphical menu of versions, which set the Matlab path and start the appropriate `spm`. Type '`spm_choose`' to use this facility. (System administrators need to edit the version and path definitions in `spm_choose.m` for this to work.)

Please take time to review the the help system, in particular the "About SPM" topic (spm.man).

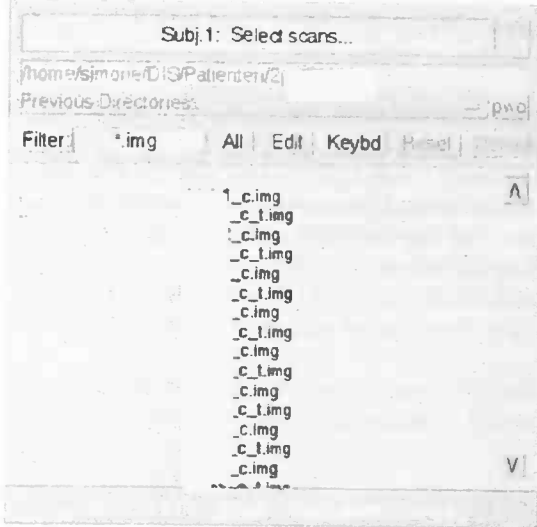
SPM96 is SPM96b with the public patches applied, together with some additional (cosmetic) improvements. The Contents.m file contains a list of the differences. Details of the SPM96b updates are on the SPMweb site

Further information may be found at the SPMweb site:  
<http://www.fil.ion.ucl.ac.uk/spm>  
 ...where details of the SPM email discussion list can be found:  
<http://www.fil.ion.ucl.ac.uk/spm/help>

Warmest regards,  
the authors

spm motd man

The **WIL** methods group : The Wellcome Department of Cognitive Neurology



This 'Pop-up screen' appears after selecting a statistical "design type".

**Blue Filter box:** letters can be typed included in the wanted file names (here all the \*.img files are represented)

**All:** selects all files

**Reset:** clears selected files

**Done:** confirm selected files, after clicking *done* there is no way back.

**All:** selects all files

**Reset:** clears selected files

**Done:** confirm selected files, after clicking *done* there is no way back.

## SPM96 interface explanation

The “Spatial” box contains options to adapt spatial properties (place and form) of images. The three most important items are:

- 1) “Realign”: Realignment is a transfer of coordinate system from the scans to those of one scan. This scan is the first scan to enter the realignment and functions as reference point to which the other scans are realigned. The movements can take place in the x,y,z-direction. Rotation also occurs in three directions: movement of the nose towards the ears (roll), towards the chin/forehead (pitch) or rotation towards the shoulders (yaw).
- 2) “Normalize”: Normalisation concerns a mapping of scanned individual brains to a template brain. Parameters needed to do this were obtained from the mean image, created during the realignment. The template contains a standard brain. The brains of the subjects from the experiments were standardized to this standard brain.
- 3) “Smoothing”: With smoothing local contrasts will be reduced, this means noise will be reduced. However, the signal to noise ratio will increase.

The “Analysis” box contains two options to perform analysis. If the “Statistics” option is chosen the blue window will pop up with the title “Select design type”. A design type can be selected. From this list three groups are the most important:

- 1) “Single-subject” (a statistical analysis which concerns only one subject),
- 2) “Multi-subject” (a statistical analysis which concerns more than one subjects) and
- 3) “Multi-study” (for group comparisons, e.g. a patient group and a control group). Different choices can be made per group. Selections can be made from
  - a) “different conditions” (different conditions e.g. listening and rest),
  - b) “replication” (if conditions are replicated),
  - c) “covariates” (a covariate is a factor that can be of influence on rCBF, e.g. task difficulty).

With the “Results” box, statistical results can be revised and manipulated, e.g. show results at a different threshold with the SPM{Z} option, to see more (lower Z score/p value threshold) or less (higher Z score/p value threshold) activation patterns.

In the box below “SPM for PET/SPECT” different options are available. For example a button “Ghostview” to view postscript files (e.g. the spm.ps file). With the button “Display”, files can be displayed e.g. original files, or normalised files. With the “Default” button, default values within SPM can be set to other values, e.g. the number of non linear basis functions (for the anatomical normalisation) can be set from 4x5x4 to 3x4x3. To leave the SPM program the button “Quit” is available.

In the window on the left side results are presented after execution of a SPM function, e.g. realignment, normalisation, statistical analysis, manipulation of thresholds, etc. At the top of the window some buttons are present, e.g. “print” (prints the content of the window to the spm.ps file), “clear” (removes the content of the window or clears a SPM function). The other buttons are used to manipulate the content of the window. The button on the right is sometimes useful to receive help.

## Chapter V: SPM96 problems and solutions

In this chapter problems with data and SPM96 will be discussed. All parts of investigation were done with three patients. In the first three sections investigations were done with the first three available patients. In the last section patients were included with maximal rotation.

### §5.1: SPM96 problems with HR<sup>+</sup> data

Data from the ECAT EXACT HR<sup>+</sup> camera is represented in kBq per pixel. The values are about 30.000 kBq per pixel. These large values caused warnings in Matlab: "matrix close to singular or badly scaled" after trying to realign or normalize images. If normalisation was carried out, strange images appeared. The normalized brain appeared to be like a mouse brain, it was too small. This mouse brain is represented on the next page, together with the SPM template (wished form of the brain) and the result after adjustments.

Graphics representing the voxel values have large numbers on the x- and y-axis. These values usually vary between 0 and 1000. In the problem case they sometimes represented values up to 15000. This means that in the brain voxel values were large (scaling factor) and the number of those high intensity voxels was large. In the mouse brain all the counts were compressed into the little brain. So, all counts were pressed into the voxels but all voxels were located in the little mouse brain. This resulted in a large number of voxels in a small brain. These graphics are on the next page.

Image pictures, before realignment and normalisation, were checked to establish possible problems. After transformation from *cti2spm* format the image (.img) files could be displayed in SPM96, using the "display" option within SPM96. The image files could be checked for correct scaling. If scaling was not correct (mouse brain) the scaling factor, which was between  $4.0e+05$  and  $6.0e+05$  ( $=6.0 \cdot 10^5 = 60.0000$ ) needed to be adjusted to values between 0 and 1. This means, in the example, division by a factor 100000. The "origin" was set (by SPM96) at 0,0,0. These values also needed to be adapted. This was done by hand, but a first good guess was 65, 70, 20. Changes were saved in the header file. This headerfile merges with the imagefile to represent pictures.

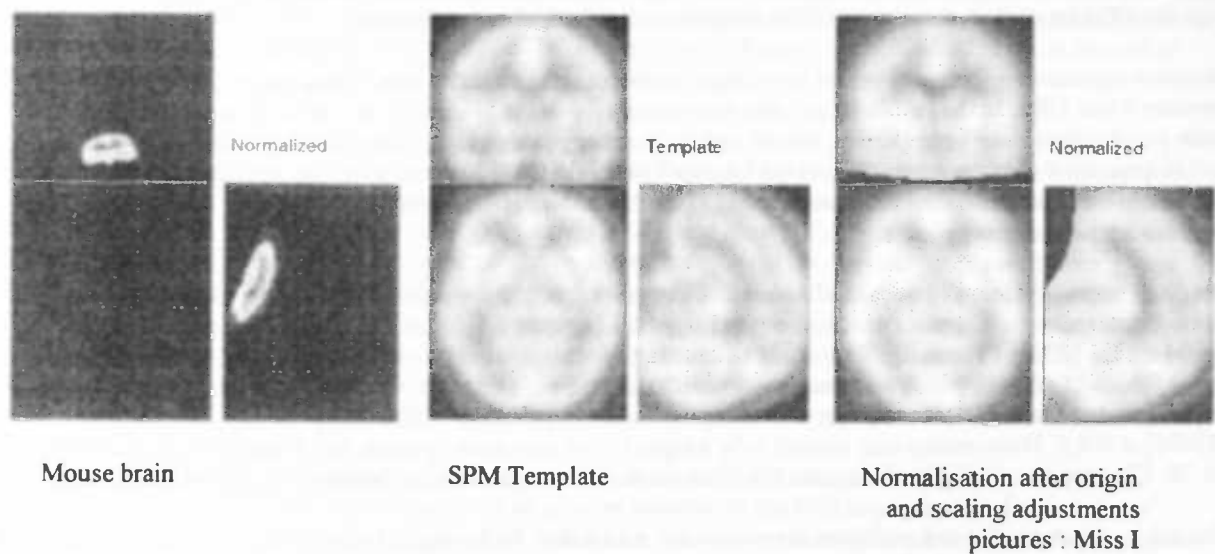
The origin values needed to be adapted in the x, y and z direction. In the sagittal displayed picture the z-axis and the y-axis could be adjusted. The z-axis was the most difficult axis to adjust. This was done with help of the Talairach and Tournoux Brain atlas which has the convention of placing the z-axis at the AC-PC line (CA-CP). The CA-CP line passes through the superior edge of the anterior commissure and the inferior edge of the posterior commissure. This AC-PC line follows a path essentially parallel to the hypothalamic sulcus, dividing the thalamic from the subthalamic region. In the displayed sagittal picture the corpus callosum was distinguishable as a black bow in the middle of the brain. The CA-CP line must be positioned to touch the frontal lower side (anterior) of the corpus callosum and nearly touch the posterior lower side of the corpus callosum. The y-axis was comparable to the Vca line. This line is a vertical traversing the posterior margin of the anterior commissure. In the sagittal displayed picture, just below the corpus callosum a white structure was distinguishable, the thalamic region, the Vca line must touch the frontal side of this structure. The x-axis was the easiest line to position. In the transverse displayed picture, the x-axis must be positioned in the middle of the two hemispheres. Displayed figures were judged and changed again until origin values were satisfactory adjusted so that the header file could be saved. The adjustments needed to be made for each individual and for each scan. After adjustments were made to the scaling factors and the origin values realignment and normalisation became possible.

The problem with these adjustments was that they were done on personal opinion. The question arose how a deviation would affect normalisation. After some investigation it was found that a deviation of +10 or -10 in the origin values did not have much effect on the normalisation. Only the fourth factor of the linear {affine} components of the spatial normalisation became larger as deviation of the ideal case increased. Concluding: fluctuations were not a problem if they ranged between +10 and -10 from the ideal axis. However, fluctuations of 20 in the origin values again resulted in the mouse brain after normalisation. On this grounds it seemed save to set the origin values to 65, 70, 20 in the *cti2spm* program (done by A.T.M.Willemsen). However, if strange normalised pictures appear it is wise to check origin values and scaling factors of the original images.

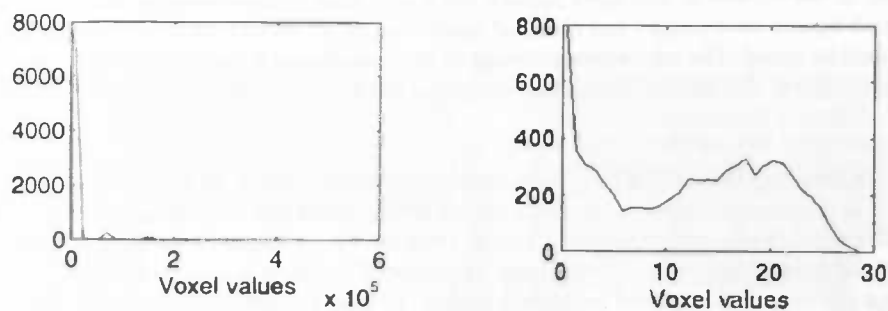
After the investigations mentioned above and value adjustments in the program *cti2spm*, users of SPM96 do not have to change origin values and scaling factors anymore. Data of the ECAT Exact HR<sup>+</sup> could now be properly analysed.

# Spatial normalisation

4x5x4 Non linear basis functions



Normalised mouse brain voxelvalues vs Normal normalisation voxel values





§5.2: Statistical comparison of SPM95 and SPM96

In the first months data could only be analysed with SPM95. Because of software restrictions data could at first not be analysed in SPM96. In the end data from the ECAT EXACT HR+ was analysed with two different statistical parametric mapping programs. SPM96 was preferred for this camera. As analysis in SPM96 was performed the output of SPM95 and SPM96 could be compared. For three patients data was smoothed with a smoothing factor of 20 FWHM. The same statistical analyses were performed in SPM95 as in SPM96. Comparisons were made on twelve items of the statistics, split up into two groups. One group was the text effects and the other group was the Identity state effects.

The search volume, number of voxels compared, for SPM96 was about a factor 10 larger in comparison to SPM95. The image size also contained more voxels to work with. One reason for this larger amount of voxels was a difference in voxel size. The voxel size in SPM95 is 2.0 2.0 4.0 but the voxel size in SPM96 is 2.0 2.0 2.0. This results in twice as much voxels in the z direction. But because of the smaller voxel size the “round” volume of the head can be better filled in. This is a reason for the larger number of voxels in the search volume and image size. The resolution{FWHM} was smaller in SPM96 than in SPM95, as shown below.

SPM95	SPM96
Search volume: 76708 voxels	Search volume: 242835 voxels
Image size: 65 87 26 voxels	Image size: 79 95 68 voxels
Voxel size 2.0 2.0 4.0 mm	Voxel size 2.0 2.0 2.0 mm
Resolution {FWHM} 19.9 19.7 19.8 mm	Resolution {FWHM} 14.8 15.5 15.6 mm

At first pictures seemed to be different. In SPM95 more small areas appeared, as dots, in the pictures. In SPM96 areas were larger and more intense, appearing less diverse. Clustering and thresholding in SPM96 might be better. SPM96 has an extent threshold of a number (X) of clusters. “Extent threshold {k} = X voxels, p = 0.299”. The p (probability) value is always around 0.299 or 0.298. X voxels must pass threshold before producing an area in the statistical picture. In SPM95 there is no cluster threshold only a value threshold for voxels. This is a possible explanation for the Dalmation-like statistical picture in SPM95, but also for better clustering in SPM96.

Cerebellar areas looked much better in SPM96. Artifacts as noticed in the condition Tt – Nt in SPM95 disappeared (no values above threshold) in SPM96. A large difference between SPM95 and SPM96 is the representation of the conditions Tn – Nn and Tt – Nt. No areas reached statistical threshold anymore in SPM96. No significant activation for texts over the two different identities. This might also be the result of different thresholding in SPM96 and SPM95, examples of statistical pictures are given in Addition V.a.

Tables of statistical analysis were compared. Lots of differences in probability values and coördinates of compared brain areas were noticed. An overview of probability values (uncorrected p-value of 0.001 and smaller, larger values or no values correspond with #) and coordinates are given in Addition V.b.

Mostly, on comparing texteffects in SPM95 and SPM96, activated areas were the same in SPM95 as in SPM96. Usually uncorrected p-values were more significant in SPM96 than in SPM95. In total four more areas were activated with SPM96 analysis (in conditons 2)1, 4a)1, 4b)1, 2)2, see Addition V.b), two parietal regions, one corpus callosum and cerebellar. Cerebellar activation might be a result of a larger calculation range in the z-direction in SPM96. Possibly the two activated parietal areas were caused by better clustering in SPM96. Threshold can be reached with more clusters which thus became significant.

On comparing SPM95 and SPM96 on identity state effects (text independent), the comparisons NnNt – TnTt, Nn – Tn, Nt – Tt showed no results after statistical analysis in SPM95, but with SPM96 all three conditions showed activated areas. Probably because of better clustering and different internal calculations activation reached threshold. On the other hand the comparison TnTt – NnNt with SPM96 showed activation patterns, but the Tn – Nn and Tt – Nt showed no result. In SPM95 some activated areas were present. On comparing uncorrected p-values from other activated areas they were seen to be just above threshold. An artifact in TnTt – NnNt in SPM95 becomes cerebellar activation in SPM96, because of a larger calculation range in SPM96.

### §5.3: Optimisation of the spatial normalisation

#### §5.3.1: Options within SPM96

Non optimal spatial normalisations could be improved by some available options within SPM96. Problems with normalisation were for example that SPM96 tries to fill up empty space in the scans. This empty space was related to the positioning of the patient in the camera. For example if the patient was positioned too low in the camera, scans contain no information of lower parts of the brain (after centering the scans by centerimage). In SPM96 the normalisation reconstructs these image parts by filling up the empty space. This was shown as stretching of the data. See figure I (12 Parameters : 4x5x4 Deformation) below. This fact may also be caused by the HR<sup>+</sup> camera. Other research groups have also experienced these kinds of problems (see §5.3.4).

The spatial normalisation consists of two steps, an affine transformation and an anatomical transformation (warping or local deformation). In both stages one has the opportunity to optimize the normalisation. In this case adjustments were made before realignment and normalisation. Steps in SPM96 are shown in the manual (written for the PET center in Groningen), which is appended in Addition A (Figures 7.2.a.I and 7.2.a.II), at the end of this graduation essay.

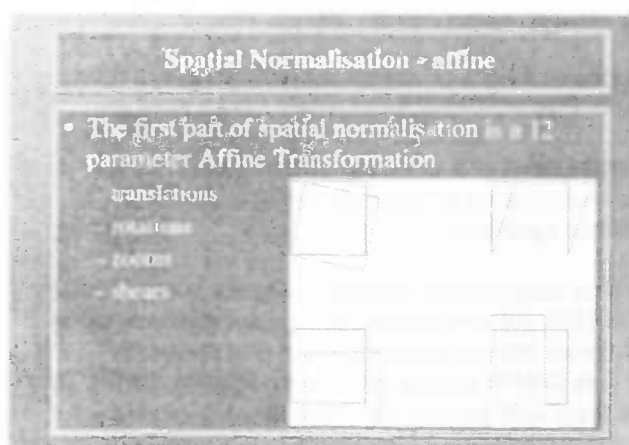
##### 1) Spatial affine transformation.

The first part of the normal normalisation concerns a 12 parameter Affine Transformation. This recommended 12 parameter spatial Affine transformation consists of translations (x,y,z-direction), rotations (roll (over the z-axis), pitch (over the y-axis) and yaw (over the x-axis)) zooms and shears. The first option to optimize the normalisation for our data, was to leave out the shears transformation option (this concerns possibly an option for fMRI data). These default values, 12 parameters for four different transformations, for spatial normalisation were set to different values. The option "Affine params" (to leave out the shears transformation) was set to 9 parameters. Only three transformation options were used to obtain the spatial normalisation. Leaving out this affine option meant leaving out the shears procedure during spatial normalisation.

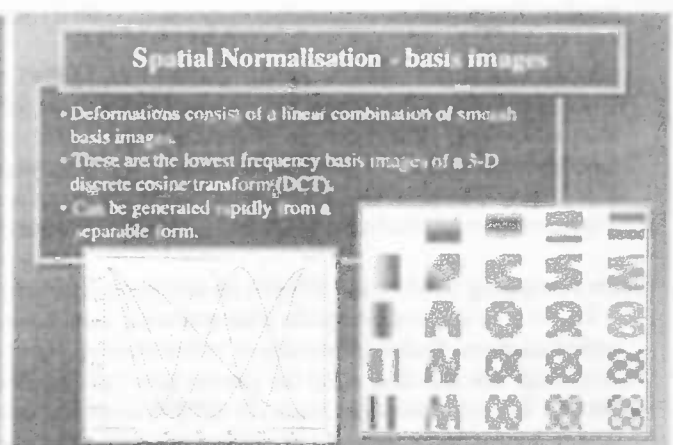
##### 2) Anatomical normalisation.

This normalisation step, the warping and deformation of structures in the head, consists of stretching and shrinking of structures in the head to map them as good as possible to the PET.img template. Following the procedure in 1), the "Nonlinear Basis Functions?" can be set. The deformation of structures in the head happens in three directions, x\*y\*z. Choices depend on personal wishes of the investigator. Standard values are 4x5x4 (see in results: figure I). For interests in small structures in the brain, one could choose larger values for the x\*y\*z. For example 5x6x5. Larger values stand for the use of more sinc functions (Fourier) to fit the patients scans to the template. Choosing larger values is only useful when the smoothing factor is small. It is of no use to use a large smoothing factor because the small structures are then not shown, because of smearing effects. For the stretching problem smaller values can be the solution, this means using less sinc functions to fit the brain onto the template, the 3x4x3 option. After adjusting these values the realignment and the normalisation can be performed.

1)



2)



White: closing of structure borders  
Black: parting of structure borders

### §5.3.2 Results

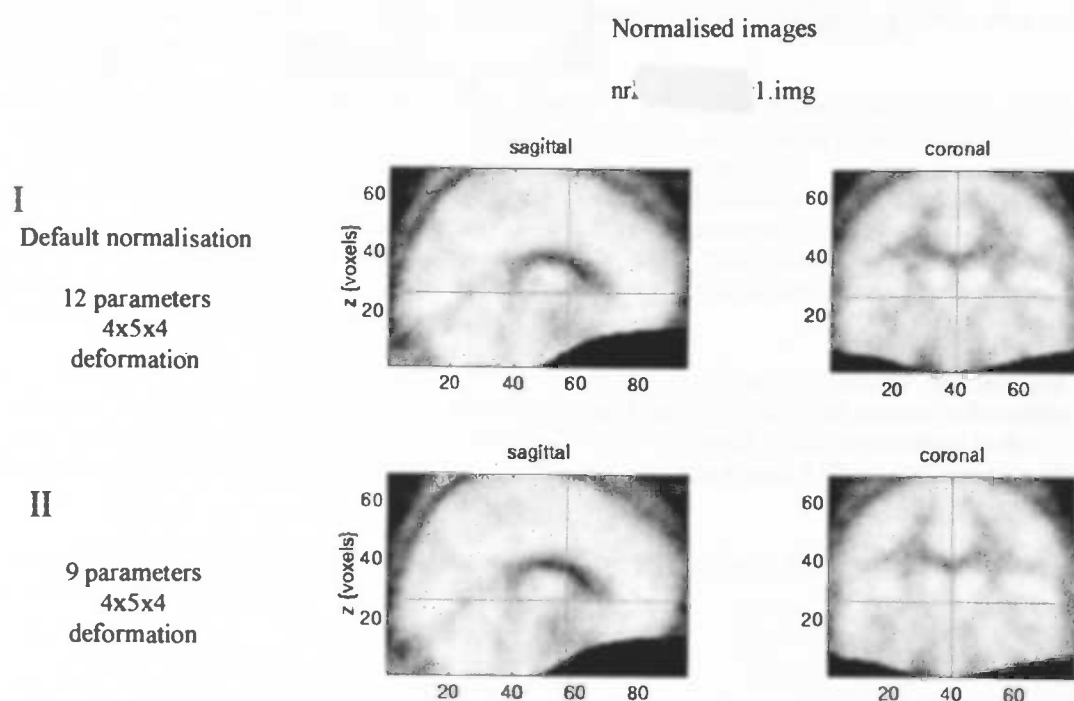
All comparisons were made with the default values, i.e. 12 parameters to normalise the head and a  $4 \times 5 \times 4$  deformation to normalise the structures in the head (figure I). I investigated different options normalising the brain. At first the affine transformation was reduced from 12 parameters to 9 parameters holding the deformation on  $4 \times 5 \times 4$  (figure II). Normalisation figures remained similar which led to the conclusion that shear transformation did not cause stretching of the brain. Anatomical normalisation probably caused the stretching problem.

Different non linear basis functions were investigated. With the  $3 \times 3 \times 3$  option no stretching appeared anymore (figures III and IV), independent of the number of parameters (9 or 12). We preferred values of the non linear basis functions as close as possible to the original deformation values ( $4 \times 5 \times 4$ ). So normalisations were done with  $3 \times 4 \times 3$  (figure V) and  $4 \times 4 \times 4$  (figure VI) deformation values, with 12 parameters. Choosing the deformation option  $3 \times 4 \times 3$  still gave a good result. However the  $4 \times 4 \times 4$  option showed stretching in the z-direction again. Adding 1 to the 3 deformation value in the z-direction gave stretching of the brain again. Conclusion: a deformation value of maximal  $3 \times 4 \times 3$  functions is a solution for the stretching problem.

In the  $3 \times 4 \times 3$  deformation normalisation pictures a visual effect appeared, compared to the  $4 \times 5 \times 4$  deformation. Intensity changes were noticed in the normalisation figures. However this was not a real intensity change in the voxels. Voxel places probably differed a little because of other deformation values. Warping and deformation of anatomical structures gave an other image of the normalisation. Voxel values stayed the same.

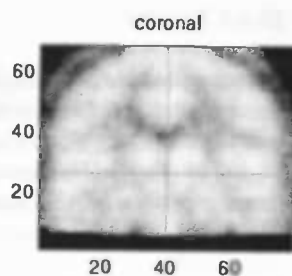
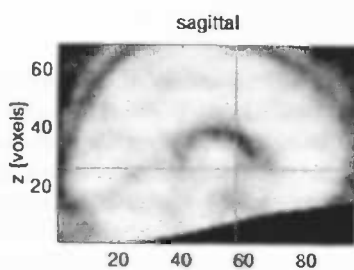
Possible effects on statistics. In the ideal case one patient is allowed to have different applied normalisation in a set of patients. But it is recommended to perform the spatial normalisation in the same manner for all the patients, the anatomical structures must be the same (matched in a similar way on the template). With deformation values of  $3 \times 4 \times 3$ , smaller structures in the head may perhaps not as precisely be formed to the PET template image as with default values, which means a loss of information. For interests in small brain structures high deformation values are recommended together with a small smoothing factor.

$3 \times 4 \times 3$  deformation is recommended if stretching of the brain appears after normalisation, with  $4 \times 5 \times 4$  deformation values. Counts remain in original place, they are not stretched to another place. It is allowed to perform the  $3 \times 4 \times 3$  deformation on one person in the group, but it is better to normalise the complete group in the same manner. However, problems of this kind appear more often with data from the ECAT Exact HR+ (see email SPM-mailing list in §5.3.4), which leads to the strong recommendation to always normalise data with  $3 \times 4 \times 3$  non linear basis functions.



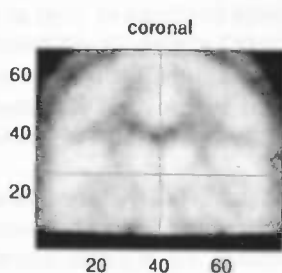
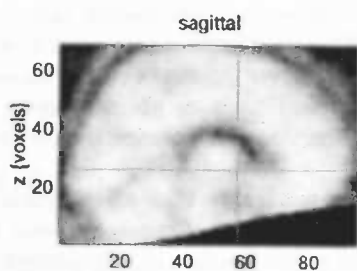
### III

9 parameters  
3x4x3  
deformation



### IV

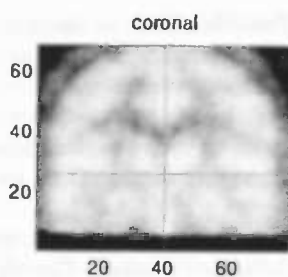
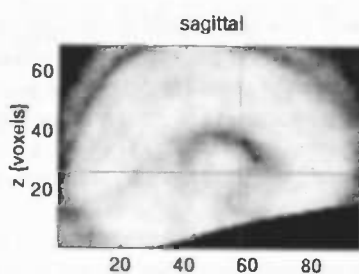
12 parameters  
3x3x3  
deformation



### V

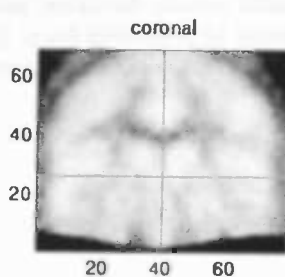
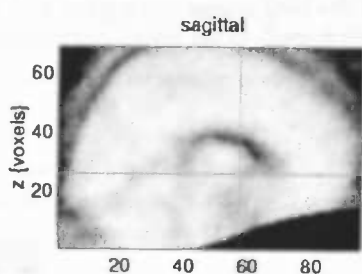
Best normalisation  
adjustment

12 parameters  
3x4x3  
deformation



### VI

12 parameters  
4x4x4  
deformation



### §5.3.3: Effects on statistical analysis using 3x4x3 deformation anatomical normalisation

To investigate results on statistical analyses, 12 parameter 4x5x4 deformation statistics were compared with 12 parameter 3x4x3 statistics. Comparisons were made between the conditions 1)NnNt vs TnTt, 2)NnNt vs TnTt, 4) Nn vs Nt or Tn vs Tt and 5)Nn vs Tn or Nt vs Tt. Statistical values are shown in Addition V.b. However, only pictures of conditions Tt -Tn /Tn -Tt (4b, identity effects) and Tt -Nt / Nt -Tt (5b, text effects) are shown as illustration in Addition V.a.

The search volume in the SPM{F} figure was smaller in 3x4x3 deformation normalisation, the difference is about 3000 (+ or - 500) voxels. Image sizes were similar. The resolution {FWHM} was about the same in both statistical analyses. Differences were about 0.1 - 0.2 mm independent of x,y or z- direction.

Statistical data was compared in the two situations, 12 parameters 4x5x4 and 12 parameters 3x4x3. Text effects compared, Nn - Nt showed no values above threshold anymore. Within traumatic identity, effects of traumatic text minus neutral texts were larger in the 3x4x3 deformation analysis. As predicted using smaller deformation values gave loss of information for smaller structures. Smaller spots (4x5x4) in for example Nn - Nt and Nt - Nn disappeared (as expected), Nn - Nt showed no more values above threshold. Examples are shown in the Addition V.a.

Comparisons of effects within Identity state in the two situations, 3x4x3 and 4x5x4 deformation anatomical normalisation, showed disappearance of two figures (NnNt - TnTt and Nt - Tt) in the 3x4x3 situation. But one figure, Tt - Tn that did not show values above threshold in the 4x5x4 situation, now showed values above threshold in the 3x4x3 situation, nearby cerebellar regions. A reason for these appearing activations was that in the 4x5x4 at least one patients showed the stretching problem. So in one out of three patients count information was smeared over a larger area, so there were less counts per mm<sup>3</sup> after smearing the area. Less counts per mm<sup>3</sup> were available for statistical analyses in that particular area. Preventing this stretching by use of 3x4x3 deformation normalisation, no smearing takes place, so counts were not smeared out over a larger area. So the counts can be numerous enough to show activation in the statistical analyses. This is possible evidence that stretching in the normalisation has effects on the statistical results in the stretched area.

Statistical values and coordinates were checked more precisely. Uncorrected p-values of 0.001 and smaller are shown in Addition V.b. for conditions Tt -Tn / Tn -Tt and Tt -Nt / Nt -Tt, pictures are shown in Addition V.a. In the Tt -Tn condition there were more areas and values above threshold for significance, Addition V.b. This could also be seen in more states, for example in states: 2)2, 4a)2, 5a)1, 1)2, 5b)2. In other states there were less areas and the values were less significant, for example in states: 2)1,4a)1,4b)1, 5b)1. Reasons for this were hard to find. We speculated that smaller deformation values gave loss of information in smaller structures so less areas were shown and shown areas were less significant. A possible reason for smaller p-values and the appearance of more areas: because of the use of less non linear basis functions, counts were not divided into smaller areas so stayed divided over larger areas and so gave, because of spreading more counts in an area and so areas (and larger areas) reached threshold values.

Areas that differed a lot were looked up in the atlas. For condition Tt - Tn, areas found in the 4x5x4 situation were also found in the 3x4x3 situation except for the cuneus, this structure is not found in the 3x4x3 situation. However in the 3x4x3 analysis the temporal lobe became more activated. In the TtNt - NnTn condition a new area also became significant, again the temporal gyrus. In the Tt - Nt condition the temporal lobe and the cerebellum showed activated areas in the 3x4x3 situation. In the 4x5x4 situation no significant areas were shown. In the 3x4x3 method shown areas were low structures of the brain. These areas show significance as a result of absence of the stretching problem. As seen above, the temporal gyrus becomes more often activated in the 3x4x3 statistical analysis than in the 4x5x4 analysis.

To draw very careful conclusions: because of smaller deformation values the smaller structures were less well adapted, so they were not reproduced as significant areas in the statistical maps. However smaller deformation values also gave spreading of counts over larger structures in the brain that could reach the threshold in this manner. However, because of filtering with 20 mm one can doubt these suggestions, a filter of 20 mm smoothes a lot.

One sure conclusion is that because of the disappearance of the stretching problem, lower structures become activated in the temporal lobe and cerebellum.

### §5.3.4: SPM-mailing list

On the SPM-mailing list (see also <http://www.fil.ion.ucl.ac.uk/spm>) problems concerning SPM are discussed. Investigators from all over the world use this list. This possibility is offered by the makers of SPM. SPM is hard to understand for users. In October, about one month after above-mentioned investigations on adjustments to the spatial normalisation were completed, an email concerning this subject appeared on the mailing list and (concerning our knowledge) was answered by A.T.M. Willemsen (PET-center, Groningen). This research group was also working with data from an HR<sup>+</sup> camera. This led to the conclusion, that data from an HR<sup>+</sup> camera needs the adjusted normalisation procedure, using 3x4x3 non linear basis functions.

E-mail as appeared on the SPM-mailing list:

Spm archives – October 1998: Re: ReSampling & Normalization

<Http://www.mailbase.ac.uk/lists/spm/1998-10/0174.html>

### Re: ReSampling & Normalization

Antoon Willemsen ([a.t.m.willemsen@pet.azg.nl](mailto:a.t.m.willemsen@pet.azg.nl))

Wed, 28 Oct 1998 16:15:01 +0000

- \* Messages sorted by: [ date ] [ thread ] [ subject ] [ author ]
- \* Next message: John Ashburner: "[Re: normalization of individual brain](#)"
- \* Previous message: John Ashburner: "[Re: ReSampling & Normalization](#)"
- \* Maybe in reply to: Steven Grant: "[ReSampling & Normalization](#)"

- > I have a question regarding resampling of high resolution PET images. We
- > have a Siemens EXACT HR<sup>+</sup> scanner. The images are reconstructed with voxel
- > sizes of 1.88 mm (X) x 1.88 (Y) x 2.42 mm (Z). There are 63 planes with
- > 128 x 128 matrix in each plane yielding a FOV of 24 cm X 24 cm in plane and
- > 15.2 cm in the z-axis.
- >
- > As a result the images include the entire brain with room to spare on all
- > sides. I have noticed that the top and bottom (brainstem) of the brain
- > appears smeared after spatial normalization. I am using the PET.img
- > template with the MNI bounding box. Spatial normalization parameters are 4
- > x 5 x 4 basis functions, 8 non-linear iterations, sinc interpolation and
- > 2.0 x 2.0 x 2.0 mm voxel size.
- >
- > I have tried changing the normalization parameters, but there is very
- > little difference to my eye. I have even changed the normalization fudge
- > factor in the spm\_defaults.m, but the 0.02 default value seems to be best.

We experienced similar problems with HR<sup>+</sup> data normalisations. We found that the number of basis functions (4 x 5 x 4) was too high, in particular in the z direction. Therefore we reduced the basis functions to (3 x 4 x 3) which solved the problem.

Hope this helps,

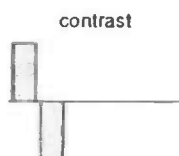
Antoon Willemsen ([a.t.m.willemsen@pet.azg.nl](mailto:a.t.m.willemsen@pet.azg.nl))  
PET Center  
Groningen University Hospital  
Groningen  
The Netherlands

%%%

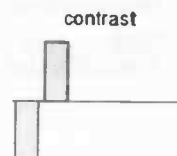
- \* Next message: John Ashburner: "[Re: normalization of individual brain](#)"
- \* Previous message: John Ashburner: "[Re: ReSampling & Normalization](#)"
- \* Maybe in reply to: Steven Grant: "[ReSampling & Normalization](#)"

# Addition V.a

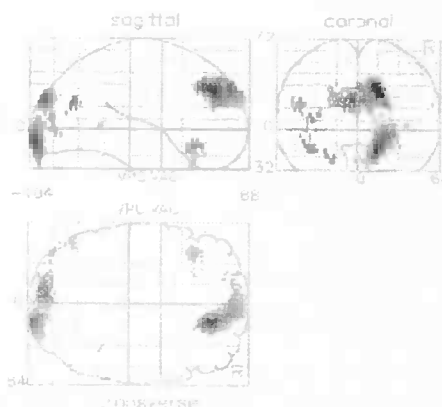
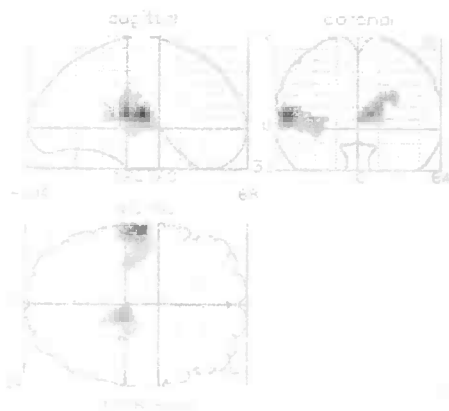
Tn - Tt  
Text effects  
in  
Traumatic identity



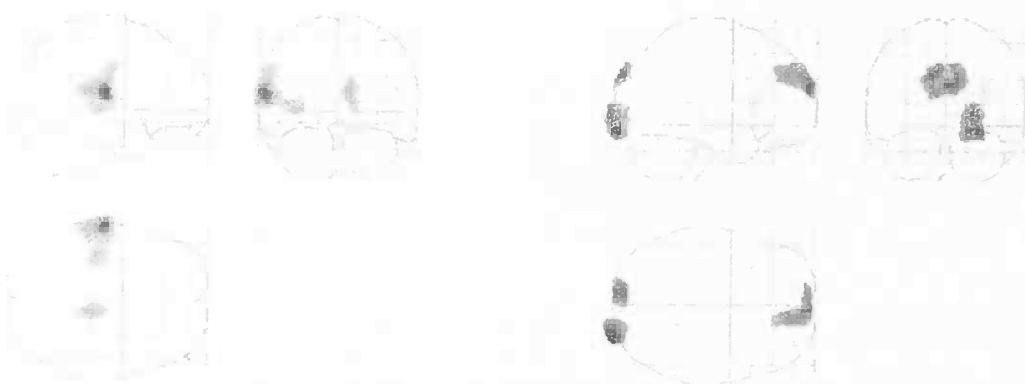
Tt - Tn  
Text effects  
in  
Traumatic identity



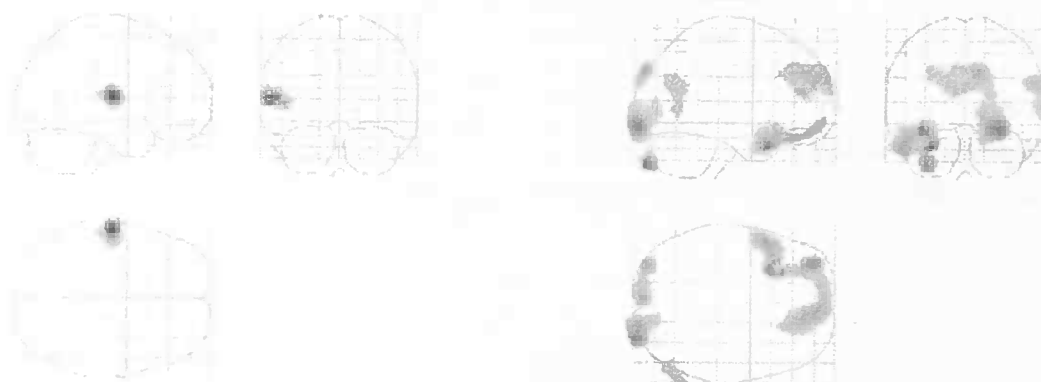
## SPM95



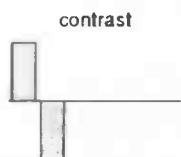
## SPM96 (4x5x4 basis functions)



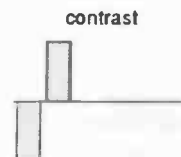
## SPM96 (3x4x3 basis functions)



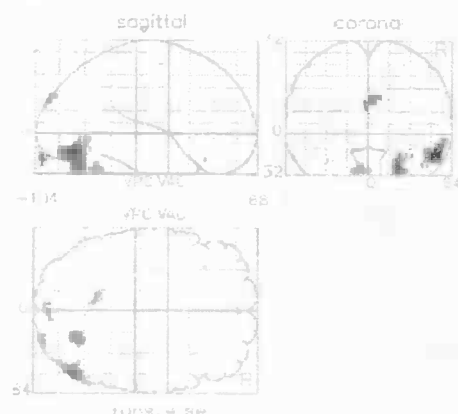
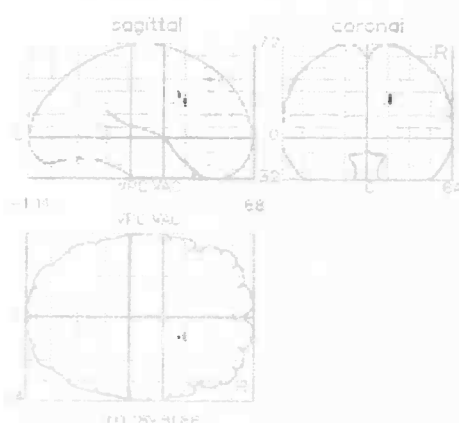
Nt - Tt  
Identity effects  
with  
Traumatic text



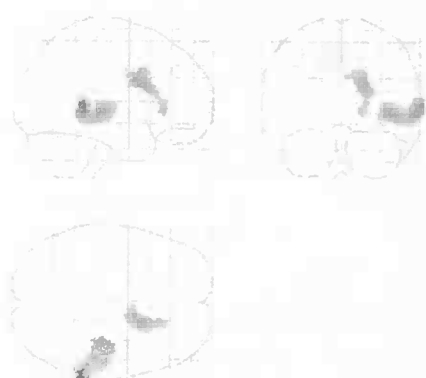
Tt - Nt  
Identity effects  
with  
Traumatic text



### SPM95



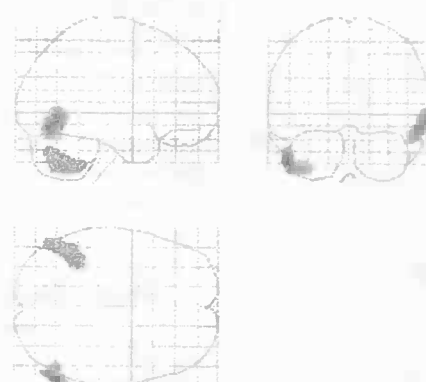
### SPM96 (4x5x4 basis functions)



No clusters above threshold

### SPM96 (3x4x3 basis functions)

No clusters above threshold





## Addition V.b

Overview of uncorrected p-values ( $p < 0.001$ ) for SPM95, SPM96 using 4x5x4 non linear basis functions and SPM96 using 3x4x3 non linear basis functions.

### Text Effects:

condition	probability (uncorrected)	coördinates	area
Neutral Text-Traumatic Text			
2)1 NNTN-NTTT			
SPM95	0.001/0.005/0.008	58,-34,20/46,-32,20	42(22) = GTs/sl
SPM96 (4x5x4)	0.000/0.001/0.001	62,-38,22/70,-36,18/50,-36,28	22 = GTs/sl
	0.000/0.001/0.001	14,-48,64/14,-56,62/6,-50,60	7 = LPs/Pcu
SPM96 (3x4x3)	0.001/0.002/0.004	62,-34,22/56,-34,22/68,-34,16	22(42) = GTs
4a)1 NN-NT			
SPM95	#		
SPM96 (4x5x4)	0.001/0.005	-42,-34,46/-40,-42,52	40(7) = Lpi
SPM96 (3x4x3)	#		
4b)1 TN-TT			
SPM95	0.000/0.000/0.001	-56,-14,12/-56,-24,12/-34,-20,4	42 = GTs
	0.000/0.000/0.001	10,-30,12/2,-26,12/16,-20,24	Th/CC
SPM96 (4x5x4)	0.000/0.000/0.000	-64,-18,14/-64,-30,18/-68,-16,6	42(22) = GTs
	0.001/0.001/0.001	8,-22,18/10,-26,8/10,-30,20	P/CC
SPM96 (3x4x3)	0.000/0.000/0.001	-62,-12,10/-54,-10,10/-60,-18,16	42(22) = GTs/sl
Traumatic Text-Neutral Text			
2)2 NTTT-NNTN			
SPM95	0.000/0.000/0.000	4,62,24/10,56,28/-6,62,24	9(10) = GFs/GFd
SPM96 (4x5x4)	0.000/0.000/0.000	4,68,26/8,60,24/-6,66,20	9(10) = GFs/GFd
	0.001/0.002/0.002	4,-84,-30/4,-82,-38/20,-94,-24	cerebellum
SPM96 (3x4x3)	0.000/0.000/0.000	10,56,26/-6,68,18/6,62,30	10(9) = GFs/GFd
	0.000/0.001/0.001	-36,16,-26/-36,24,-18/-44,20,-18	38 = GTs
	0.000/0.001/0.002	2,-84,-26/0,-82,-34/12,-84,-40	cerebellum
4a)2 NT-NN			
SPM95	0.000/0.003/0.009	#	
SPM96 (4x5x4)	0.000/0.002/0.003	#	
SPM96 (3x4x3)	0.000/0.000/0.006	-62,-6,36/-62,-16,-38/-62,6,34	6(4) = GPrC
4b)2 TT-TN			
SPM95	0.000/0.000/0.001	14,40,32/16,30,32/10,50,24	9(8) = GFs/GFm
	0.000/0.001/0.001	14,-100,-16/18,-98,-8/28,-98,-4	18(17) = GL/GOi
	0.000/0.002/0.005	-6,-90,24/-20,-92,20/0,-86,28	Cu/GOm
SPM96 (4x5x4)	0.001/0.002/0.002	4,68,26/-10,66,18/10,64,30	10 = GFs/GFd
	0.001/0.001/0.002	28,-98,-14/24,-104,0/20,-96,-12	18(17) = GL/GOi
	0.001/0.001/0.003	-4,-94,32/-12,-96,30/-2,-90,40	18(19) = Cuneus
SPM96 (3x4x3)	0.000/0.000/0.000	-28,14,-30/-34,50,-18/-26,30,-24	38 = GTs/ 11 = GFm
	0.000/0.000/0.001	32,-98,-12/24,-94,-14/24,-104,-2	18 = GF/GOi/m
	0.000/0.001/0.001	-30,52,38/14,46,28/14,38,34	9 = GFd/m/s
	0.000/0.001/0.001	62,-66,28/64,-60,20/64,-66,0	39(37) = GTs/m/GTi

## Identity Effects

condition	probability (uncorrected)	coördinates	area
<b>Neutral Identity-Traumatic Identity</b>			
<b>1)1 NNNT-TNTT</b>			
SPM95	#		
SPM96 (4x5x4)	0.000/0.008	6,-22,2/4,-24,10	P(Thalamus)
	0.001/0.001/0.002	14,24,34/12,8,28/6,30,24	32 = GC/CC
SPM96 (3x4x3)	#		
<b>5a)1 NN-TN</b>			
SPM95	#		
SPM96 (4x5x4)	0.001/0.001/0.001	-6,-60,2/-2,-56,16/8,-62,22	18(19) = GL, 23(31) = GC, 31 = PCu
	0.001/0.002/0.002	2,-24,0/2,-10,2/-6,-20,-2	Th
SPM96 (3x4x3)	0.000/0.001/0.001	-6,-16,0/-2,-8,0/2,-24,-2	Thalamus
	0.000/0.000/0.001	-16,-48,24/-10,-56,0/-4,-58,16	19 = GL, 31 = PCu/GC
<b>5b)1 NT-TT</b>			
SPM95	#		
SPM96 (4x5x4)	0.000/0.000/0.000	68,-40,8/64,-40,-2/38,-22,-2	22(21) = GTs/GTm
	0.001/0.001/0.001	14,6,28/18,14,28/18,30,2	CC
SPM95 (3x4x3)	#		
<b>Traumatic Identity-Neutral Identity</b>			
<b>1)2 TNTT-NNNT</b>			
SPM95	0.001/0.002/0.003	48,-78,-12/54,-66,-8/42,-86,-16	artefact (border)
	0.002/0.004/0.005	30,-60,-24/24,-66,-24/36,-54,-28	cerebellum
SPM96 (4x5x4)	0.000/0.001/0.001	50,-44,-46/54,-54,-46/62,-64,-16	cerebellum
SPM96 (3x4x3)	0.000/0.000/0.001	50,-46,-46/56,-52,-42/62,-66,-14	cerebellum
	0.001/0.001/0.001	-38,-50,-50/-50,-56,-46/-30,-42,-48	cerebellum
<b>5a)2 TN-NN</b>			
SPM95	0.000/0.002/0.003	60,-16,-24/62,-24,-20/54,-24,-16	20(21) = GTi/GTm
SPM96 (4x5x4)	#		
SPM96 (3x4x3)	#		
<b>5b)2 TT-NT</b>			
SPM95	0.000/0.004/0.004	52,-74,-16/54,-66,-12/44,-84,-12	artefact (border)
	0.001	24,-68,-28	cerebellum
SPM96 (4x5x4)	#		
SPM96 (3x4x3)	0.000/0.000/0.003	64,-60,-4/54,-62,-14/54,-62,-14	37 = GTm/GTi
	0.000/0.003/0.003	-52,-68,-42/-54,-70,-32/-42,-52,-48	cerebellum

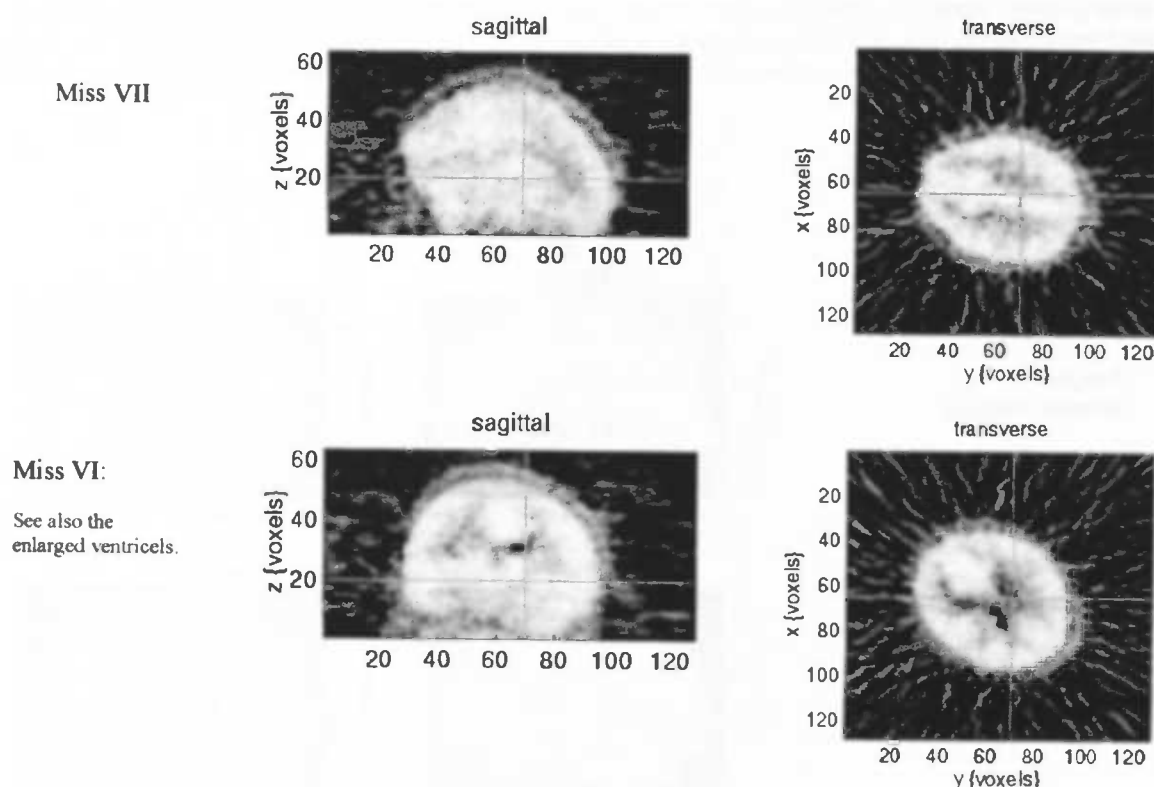
### Overview of abbreviations:

CC	= Corpus Callosum	GPrC	= Gyrus Precentralis
Cu	= Cuneus	GTi	= Gyrus Temporalis inferior
GC	= Gyrus Cinguli	GTm	= Gyrus Temporalis medius
GF	= Gyrus Fusiformis	GTs	= Gyrus Temporalis superior
GFd	= Gyrus Frontalis medialis	LPI	= Lobulus parietalis inferior
GFm	= Gyrus Frontalis medius	LPs	= Lobulus paracentralis
GFs	= Gyrus Frontalis superieur	P	= Pulvinar
GL	= Gyrus Lingualis	PCu	= Precuneus
GOi	= Gyrus Occipitalis inferior	sl	= sulcus lateralis cerebri
GOM	= Gyrus Occipitalis medius	Th	= Thalamus

## §5.4: Rotations

Two out of nine patients were difficult to position correctly into the camera. These two patients were afraid to wear the band around their head to keep their head in the correct position during the scanning procedure. This resulted in rotations over the three axis of the head. To position the images to the point of departure the required rotations over the z-axis were maximally 20° for Miss VII and maximally 45° for Miss VI.

Displayed images before spatial normalisation, without rotations



In the PET-center it was known that SPM95 has difficulty finding the AC-PC-line correctly if the head was rotated over 6° or more over the z-axis. Some people even rotated with head rotations over 3°. These rotations were performed in the program Imagetool. Imagetool could only perform these rotations over the z-axis, not over the x- and y-axis. However a new program was available, called CAPP5. This program did give the opportunity to rotate over the x- and y-axis. Some of the patients, especially Miss VII and Miss VI, were also rotated over these axis with 10°-20°. So if I wished to rotate over the z-axis, why not over the x- and y-axis? Because of the amount of degrees this reasoning seemed logical. So if I decided to perform rotations on the images, then I wanted to perform them over three directions.

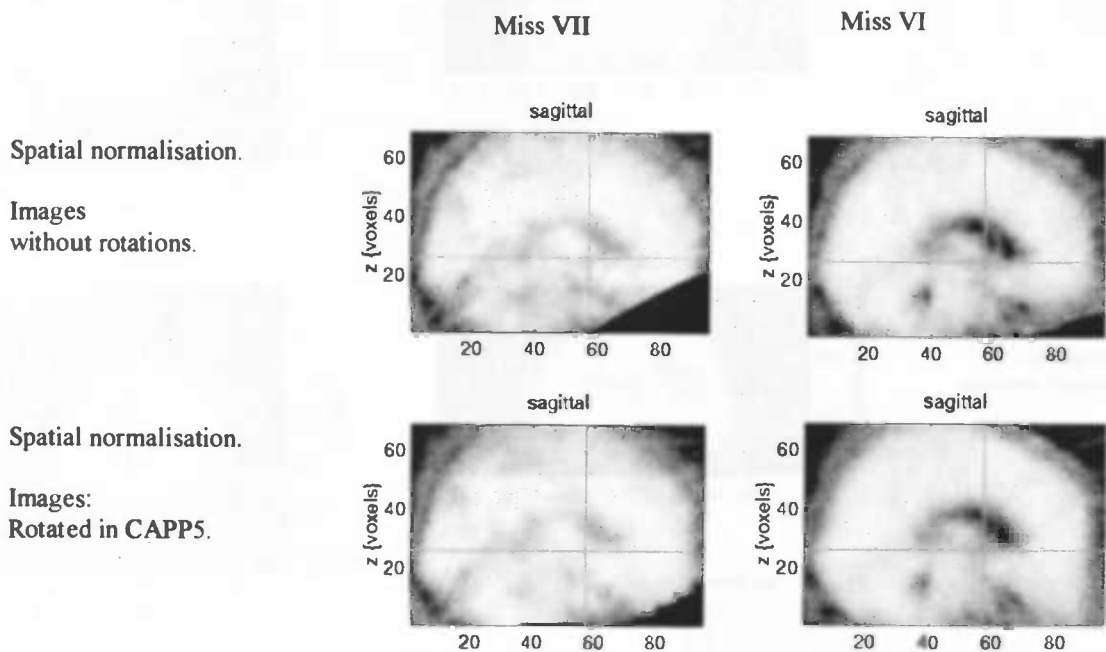
But were rotations needed for SPM96, or could the Spatial Normalisation handle rotations up to 45°? First I performed the normalisation (3x4x3 non linear basis functions) without any rotations. The normalisations looked surprisingly well. Mr. P. Maguire told me in a meeting that the normalisation seemed okay, just (and only) by looking at it. For Miss VII I rotated the images in Imagetool over the z-axis and obtained the normalisation. The normalisations without and with rotations were subtracted from each other in Imagetool. Differences were very small, which led to the conclusion that rotations were not needed. But here it only concerned rotations over the z-axis and over 20° instead of 45° for Miss VI. It still was not convincing enough for me. After conference with A.M.J. Paans we decided to check the rotations in CAPP5, in three directions and the outcome of the statistics in SPM96 without and with rotations. This was also to investigate for the PET-center if CAPP5 was suitable to perform rotations on images which have to be entered in SPM96.

The rotations were performed with the re-slicer in CAPP5. Here the first problem appeared. CAPP5 works with cubic voxels. The voxels in the data from camera II are not cubic but rectangular. The maximum number of slices returned by CAPP5 was 45. The number of slices entered in CAPP5 amounts to 63. So after rotations (see rotationlist, and rotated pictures in Addition V.e) and re-slicing, the number of slices were 45 instead of 63,

which need to be entered into SPM96. An adjustment in the program citospm (by A.T.M. Willemsen) brought the number of slices back to 62, well enough for SPM96.

However, re-slicing the number of slices to 45 and after that increasing the number of slices up to 62, brought down the resolution. The normalisation looked smoother, the images after the normalisation were less sharp. This loss of resolution is not a problem if the smoothing factor in SPM96 is chosen 12mm or larger. If one wishes to choose a smaller filter, interest in smaller structures of the brain, than this resolution loss might be a problem.

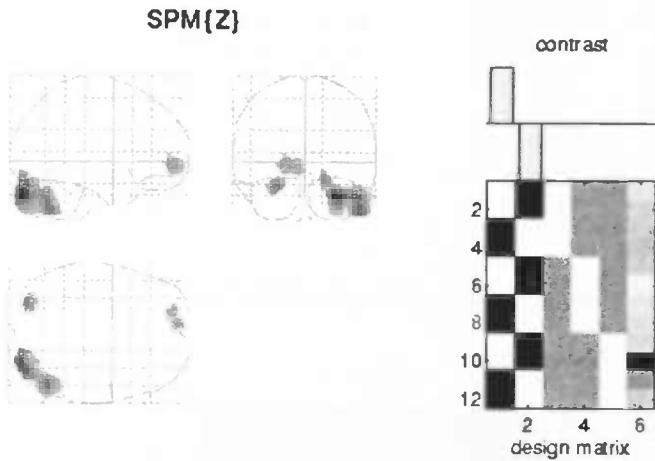
Another problem seemed to appear (as statistical analyses were already performed), after a closer look at the rotated normalisation images. The frontal and back side seemed to be flattened. During rotation the head became rotated outside the defined area (representing the image) of CAPP5, cutting of pieces of the front area and back area of the head.



Statistical analyses were performed in SPM96 with the rotated images and the images without rotations for the condition TN-TT. Rotations were performed on Miss VII and Miss VI, by whom the largest rotations were needed, and Miss VIII. Three patients were needed for larger statistical power. Results were astonishing. With rotations the condition Tn-Tt showed no significant areas. Without rotations there were significant areas. These areas were located mostly cerebellar at the back of the head. Disappearance of these areas could be the result of rotation outside the defined area in CAPP5. Results without rotations are presented in Addition V.c, with rotations in Addition V.d.

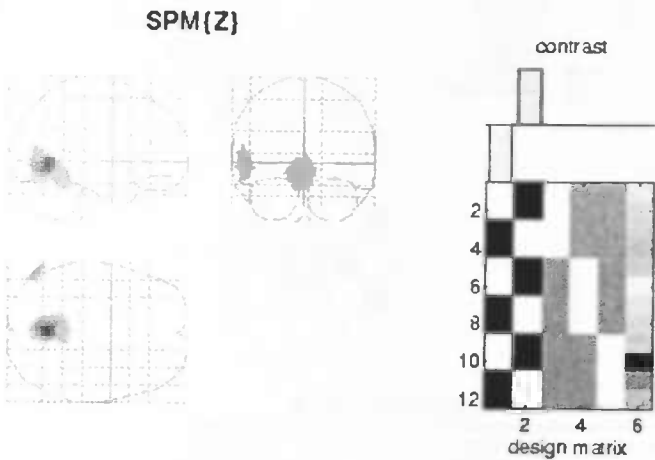
In the other contrast Tt-Tn, a large area showed in both cases, almost at the same location. However, voxel-level {Z}-scores differ between the two analyses. Without rotation, Z-scores up to 4.66 were reached (corrected significant). With rotations Z-scores of 3.44 (corrected not significant) were received. Also statistical cluster-level values and uncorrected p-values were worse after rotations performed in CAPP5.

Statistical analyses and spatial normalisation checks led to the conclusion that CAPP5 is not suitable to perform rotations on images, which have to enter SPM96. Considering similar normalisations after z-axis rotation in Imagetool compared with normalisation without rotations, no rotations need to be performed at all. SPM96 is able to find the AC-PC-line very well during spatial normalisation. Conclusion: SPM96 does not need pre-rotated images, it is able to perform rotations up to 45° by itself.



P values & statistics: /home/simone/DFS/Patienten/Rotale

set-level (c)	cluster-level (k,Z)	voxel-level (Z)	uncorrected k & Z		x,y,z (mm)		
0.981 (3)	0.049 (132, 3.59)	0.761 (3.59)	0.000	0.000	30	-48	-32
		0.946 (3.39)			38	-80	-32
		0.957 (3.32)			22	-64	-22
	0.963 (152, 3.39)	0.952 (3.33)	0.195	0.000	-24	-60	-22
		1.000 (2.67)			-32	-76	-24
	0.982 (192, 2.81)	1.000 (2.81)	0.148	0.002	-22	54	-2
		1.000 (2.63)			-8	64	-6
		1.000 (2.52)			-18	58	-8



P values & statistics: /home/simone/DFS/Patienten/Rotale

set-level (c)	cluster-level (k,Z)	voxel-level (Z)	uncorrected k & Z		x,y,z (mm)		
0.996 (2)	0.073 (1330, 4.66)	0.029 (4.66)	0.001	0.000	0	-62	-4
		0.428 (3.89)			-6	-66	-8
		0.842 (3.51)			-8	-56	-4
	0.854 (205, 3.54)	0.813 (3.54)	0.136	0.000	-68	-78	6
		0.929 (3.38)			-52	-74	0
		0.994 (3.13)			-58	-70	-8

Addition V.d

Statistical analysis:

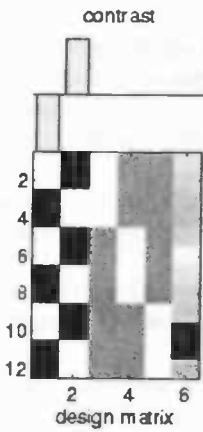
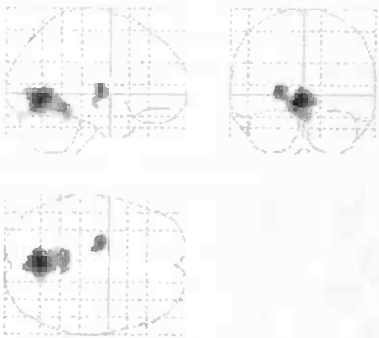
TT-TN

Images rotated in CAPP5

TN-TT:

No clusters above threshold

SPM{Z}



P values & statistics: /home/simone/DIS/Patienten/Rotate

set-level (c)	cluster-level (k, Z)	voxel-level (Z)	uncorrected k & Z	x, y, z (mm)
0.997 (2)	0.174 (926, 3.44)	0.908 (3.44)	0.004	0.000 -4 -68 -8
		0.913 (3.45)		0.000 0 -60 0
		0.943 (3.38)		0.000 -2 -74 2
		0.980 (3.26)		0.001 -22 -10 4
	0.985 (104, 3.26)	1.000 (2.42)	0.008	-14 -16 -6

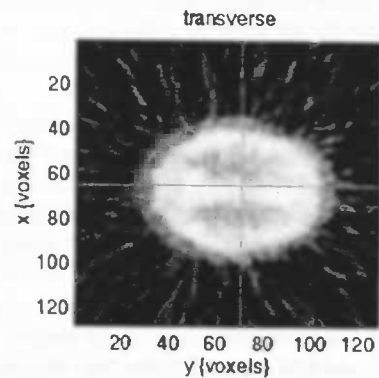
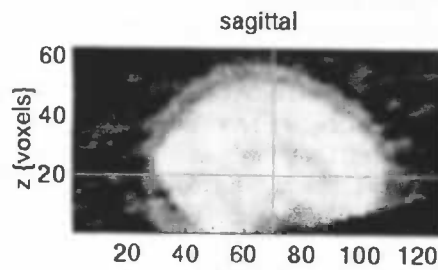
# Addition V.e

Rotation list:

	X-axis	Y-axis	Z-axis		X-axis	Y-axis	Z-axis
Miss VII1:	-15.07	5.36	13.09	Miss VI1:	-13.77	9.56	40.75
Miss VII2:	-15.20	6.67	12.78	Miss VI2:	- 8.13	6.77	40.51
Miss VII3:	-16.17	4.13	16.12	Miss VI3:	-10.65	7.10	43.80
Miss VII4:	-16.54	5.83	16.71	Miss VI4:	-11.20	7.13	41.19
Miss VII5:	-13.51	7.52	19.88	Miss VI5:	-10.78	6.84	42.33
Miss VII6:	-14.72	5.83	15.71	Miss VI6:	-10.78	9.03	40.41
Miss VII7:	-14.57	7.19	19.45	Miss VI7:	- 9.55	8.97	41.71
Miss VII8:	-12.43	6.98	18.71	Miss VI8:	-12.34	8.04	40.79

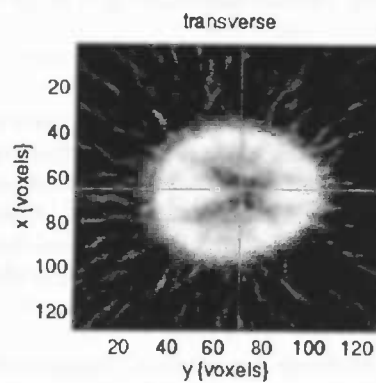
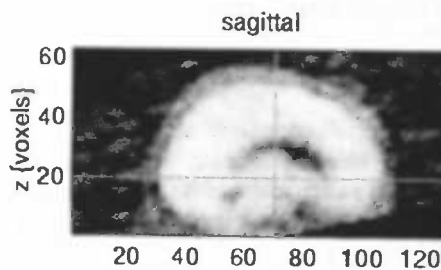
Displayed images before spatial normalistaion , with rotations performed in CAPP5

Miss VII



Miss VI:

See also the enlarged ventricels.



# Chapter VI: Results

## §6.1: Results

Results could be split up in two major groups: text effects and Identity state effects (text minor, Identity state capital letter). Pictures of results are shown in the Additions VI.a and b. P values and Z scores are shown in Addition VI.e.

### §6.1.1: Text effects

Pictures concerning text effects are shown in Addition VI.a. Conditions concerning text effects involved activation and deactivation caused by texts, within same Identity states. Brain activation effects were defined as activation in Tt or Nt minus Tn or Nn and deactivation by the reverse subtraction. Text effects could be shown within Traumatic and Neutral identity state. Interest concerned the subtractions:

- 1) Tt – Tn (brain activation caused by traumatic text compared to neutral text in Traumatic identity),
- 2) Tn – Tt (brain deactivation by traumatic text compared to the neutral text in Traumatic identity),
- 3) Nt – Nn (brain activation by traumatic text compared to the neutral text in Neutral identity) and
- 4) Nn – Nt (brain deactivation of the traumatic text compared to the neutral text in Neutral identity).

Within the Traumatic identity state three activated brain areas showed due to traumatic text as compared to neutral text as shown in picture 1). Deactivation picture 2), showed no values above threshold. Areas were only activated in the Traumatic identity due to the traumatic text, not deactivated. The most important area was left temporal superior, area 38. Also cerebellar activation was present. The most important results in this condition was the finding of activation in the left temporal cortex and the absence of deactivation patterns.

Same comparisons were made for Neutral identity (pictures 3) and 4)). The most remarkable finding was absence of temporal activation. Only cerebellar and frontal activations, area 9, were recognisable. The frontal activation could be due to judging and making sense about the traumatic text compared to neutral text. A parietal inferior area, area 40, was deactivated as the Neutral identity state listened to the traumatic text.

### §6.1.2: Identity state effects

The other group of results was Identity state effects. Pictures concerning Identity state effects are shown in Addition VI.b. Conditions concerning Identity state effect also involved brain activation and brain deactivation caused by Identity states. Activation effects were defined as activations in Tt or Tn minus Nt or Nn and deactivation by the reverse subtraction. Identity state effects could be shown with traumatic and the neutral text between two identity states. Interest concerned the subtractions:

- 5) Tt – Nt (brain activation of Traumatic identity state compared to Neutral identity state with traumatic text),
- 6) Nt – Tt (brain deactivation of Traumatic identity state compared to Neutral identity state with traumatic text),
- 7) Tn – Nn (brain activation of Traumatic identity state compared to Neutral identity state with neutral text),
- 8) Nn – Tn (brain deactivation of Traumatic identity state compared to Neutral identity state with neutral text).

As can be seen in picture 5) below, three brain areas were activated. Two temporal areas were activated. One right temporal inferior area, area 38, which is posterior located and one left temporal superior area, area 37. The third area was right parietal located, area 40. This means that traumatic text shows three activated areas in Traumatic identity state as compared to Neutral identity state listening to the same text. Deactivated areas were present as shown in picture 6). One area was area 19, occipital located. The other area was not defined according to the Talairach and Tournoux atlas. Summarising: listening to traumatic text, right temporal cortex, right parietal cortex and left temporal cortex were activated. One area, occipital, was deactivated in Traumatic identity state compared to Neutral identity state.

Neutral text gave no locatable (in the brain atlas) activations or deactivations between identities (pictures 7) and 8)). This means that no brain areas were activated as the Traumatic identity state listened to the neutral text compared to the Neutral identity state listening to the neutral text. This could be interpreted as there being no different information processing between Traumatic and Neutral identity state while listening to an emotionless text.



### §6.1.3: Grouped comparisons

Pictures concerning grouped comparisons are shown in Addition VI.c. In keeping the text combination constant but varying identity state re-activity of the brain could be shown (this conditions consist of above mentioned comparisons, but are represented in this manner for clearness):

- a) Tt-Tn versus Nt-Nn (brain activation by traumatic text over different identity states),
- b) Nt-Nn versus Tt-Tn (brain deactivation by traumatic text over different identity states).

These comparisons could also be done keeping the identity as a constant factor, which resulted in:

- c) Tt-Nt versus Tn-Nn (brain activation by traumatic text over different identity states versus brain activation by neutral text over different identities),
- d) Tn-Nn versus Tt-Nt (brain deactivation by traumatic text over different identities versus brain activation by neutral text over different identities).

On comparing a1) and a2) the most important differences were in the left temporal cortex and prefrontal cortex. Traumatic text compared to neutral text, gave temporal activation in the Traumatic identity state but not in the Neutral identity state. The Neutral identity state shows prefrontal cortex activation which is not present in Traumatic identity. On comparing c1) and c2) traumatic text effects over identities as compared to neutral text effects over identities large differences were found. In condition c2) no values were above threshold. This could mean, as stated above, that there is no different information processing between Traumatic and Neutral identity state while listening to an emotion-less text. In condition c1) two temporal areas were activated and a parietal area. These findings gave indications that traumatic emotional texts, were processed differently in different identities. With traumatic text three areas were activated in the Traumatic identity state as compared to Neutral identity state. Also one deactivated area, an occipital area, was found while comparing the emotional condition versus emotion-less between identity state.

One of the hypotheses stated that Traumatic state exposed to traumatic text shows different or no brain activations in comparison with neutral text or Neutral state. This hypothesis was proven by comparing the processing of the different texts between identities (Tt-Nt versus Tn-Nn), and by the Tt- Nt condition alone.

The hypothesis stated that the Tt condition would show different brain activation patterns compared to all the other conditions: Tn, Nt and Nn. This hypothesis was tested in SPM, after the above findings. The four conditions could be scaled on the basis of this hypothesis, with different contrast values within SPM. The Tt condition was given contrast value three (because Tt was suspected to be different), the other conditions received contrast value -1. In this manner it could be tested if the Tt condition differed from the other conditions according to the hypothesis. The other contrast investigated Nt compared to Tt, Tn and Nn. Results are given in Addition VI.d. For the contrast Nt -{Tt, Tn, Nn} a prefrontal cortex activation was shown, area 9. This could mean that the Neutral identity state with traumatic text, compared to all other conditions, showed activity which could be explained by the personality state trying to make sense of the text and judging the text. For the contrast Tt -{Tn, Nt, Nn} left superior temporal cortex, area 38, showed activation. The Z score was  $Z = 3.95$  with a p value of 1.88 corrected for multiple comparisons. A corrected p value of 2.00 or smaller means that no false positives are shown. It can be stated that this result confirms the hypothesis.

Concluded: Traumatic identity state exposed to traumatic text showed different or no brain activation compared with neutral text or in Neutral state. Differential dissociation, thus state dependent brain (re-)activity was found. These findings contribute to the scientific objectivity on the subjective experience of patients with DID.

### §6.2: Discussion

Hypotheses were that DID patients, which are in a personality state aware of traumatic memories, will show brain activations corresponding to those of PTSD patients exposed to their traumatic memories. These brain activations will not be shown being exposed to neutral texts or in the neutral identity, which is not familiar with, or affected by, the traumatic memories. Probably the neutral text in traumatic identity and text effects in neutral identity show different state dependent activated brain areas.

PET investigation on PTSD patients <sup>A17</sup> indicates that texts concerning autobiographic traumatic memory give cause to activation in different brain areas compared to texts referring to neutral autobiographical life events. At this point DID-PET findings are in agreement with the PTSD-PET investigation. In both Neutral and Traumatic identity state traumatic text showed increase in normalised blood flow compared with Neutral text.

However, increase for the traumatic text condition compared with control conditions right-sided limbic and paralimbic territories, including anterior cingulate cortex, right/posterior orbitofrontal found in the PTSD-PET

study <sup>A17</sup>, were not found in the DID-PET study with only seven patients. Right temporal cortex activation was found in accordance with the PTSD-PET investigation. Right temporal cortex activation was found comparing the Traumatic identity state with Neutral state with traumatic text. Traumatic identity state was emotionally more affected than Neutral identity state with traumatic text. The right hemisphere is more related to experience of emotions and is associated with memory processes.

Shin et al <sup>A20</sup> used 15 adult victims of childhood sexual abuse, 8 with PTSD and 7 without PTSD (to serve as a traumatic exposed control group). PET data of the traumatic vs control condition, showed significantly larger rCBF increases in (among other areas) anterior temporal cortex for the subjects with PTSD in comparison with the non-PTSD group. Consistent with this study <sup>A17</sup>, in the DID-PET study temporal cortex activation was found in two conditions: Tt – Tn and Tt – Nt. The hypothesis that DID patients show brain activations corresponding to PTSD patients is partially supported. Not all areas were found but overlap between these psychiatric syndromes is possibly present.

Previous studies have also reported fluctuations in cerebral blood flow in patients with DID concerning the temporal lobe <sup>A9,24</sup>. In the SPECT study <sup>A24</sup> significant blood flow increase was shown in four different alter personalities. Significant regional cerebral blood flow changes were observed in the left temporal lobe. The mean increase in activation in the left temporal lobe in the four personality states was 10.7%. The authors of this article stated that these findings suggests that the temporal lobe may be a mediating factor in the psycho-physiology of MPD. This statement was confirmed in the DID-PET investigation. Another article <sup>A9</sup> has also reported fluctuations in cerebral blood flow between personality states in patients with MPD. The right-sided temporal showed increased blood flow. Both sided parietal regions also showed increased blood flow. This increase in temporal lobe blood flow is indicative of functional over-activity.

Results from the DID-PET investigation are consistent with these findings. Left parietal and left and right temporal lobe activation was found between alter personality states listening to the traumatic text, as in the condition Tt – Nt. Comparing Tt with all other conditions significant left temporal cortex activation in accordance with findings in literature <sup>A24</sup> was shown. This left temporal lobe activation is indicative of functional over activity in a Traumatic identity state compared to Neutral identity state.

Brain regions probably involved with DID according to electric evoked responses studies were: left hemisphere (temporal,hippocampal) <sup>A4</sup> and left temporal and right posterior regions <sup>A7</sup>. These brain areas were also found in the DID-PET study, so the temporal cortex plays an important role in patients with Dissociative Identity Syndrom.

The conclusion from a paper <sup>A23</sup> that there is a correlation between cerebral blood flow pattern and different personality states in DID, can be supported by this DID-PET investigation. However, these results are preliminary after study of seven patients. More patients will be investigated to give more power to statistics and perhaps find more brain areas in accordance with PTSD patients.

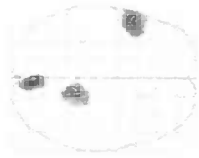
Conclusions: The temporal lobe may be a mediating factor in the psycho-physiology of MPD. Traumatic state exposed to traumatic text shows different brain activation patterns compared to neutral text or neutral state. Brain activation in patients with DID is state dependent. Identity state dependent information processing is shown in an objective way.

### Addition VI.a

Brain activation condition ↓



- 1)  $Tt - Tn$   
Text effect  
in  
Traumatic identity



No clusters above  
threshold

- 2)  $Tn - Tt$   
Text effect  
in  
Traumatic identity



- 3)  $Nt - Nn$   
Text effect  
in  
Neutral identity



- 4)  $Nn - Nt$   
Text effect  
in  
Neutral identity



### Addition VI.b



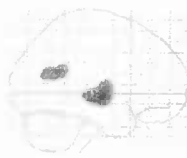
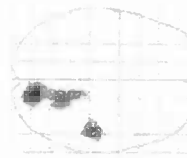
- 5)  $Tt - Nt$   
Identity effect  
with  
Traumatic text

No clusters above  
threshold

- 7)  $Tn - Nn$   
Identity effect  
with  
Neutral text



- 6)  $Nt - Tt$   
Identity effect  
with  
Traumatic text

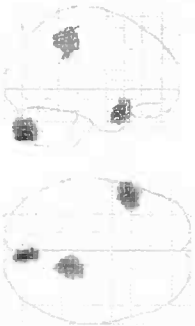


- 8)  $Nn - Tn$   
Identity effect  
with  
Neutral text



Addition VI.c

Activation



a1)  $Tt - Tn$   
Text effect  
in  
Traumatic identity



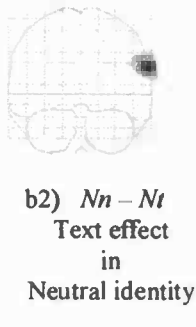
a2)  $Nt - Nn$   
Text effect  
in  
Neutral identity

Deactivation

No values above threshold

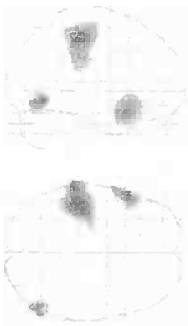


b1)  $Tn - Tt$   
Text effect  
in  
Traumatic identity



b2)  $Nn - Nt$   
Text effect  
in  
Neutral identity

Activation



c1)  $Tt - Nt$   
Identity effect  
with  
Traumatic text



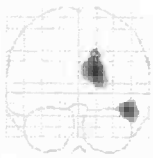
c2)  $Tn - Nn$   
Identity effect  
with  
Neutral text

No values above threshold

Deactivation

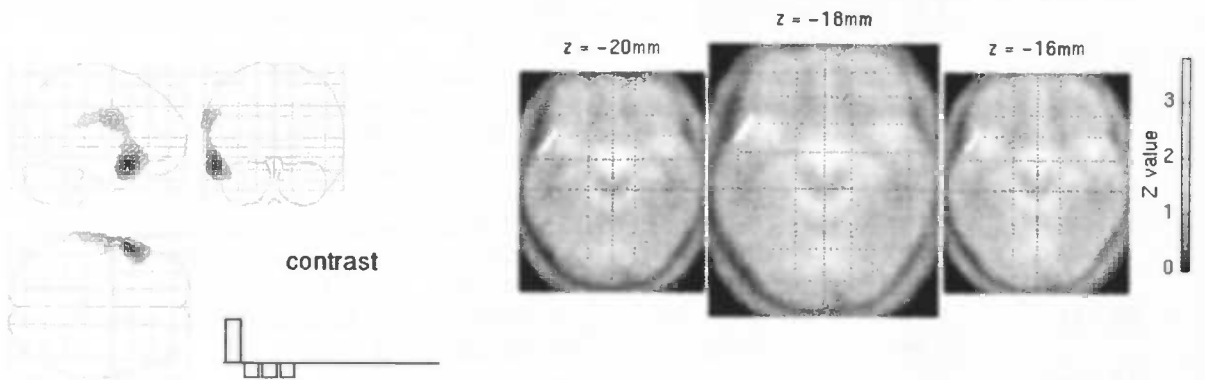


d1)  $Nt - Tt$   
Identity effect  
with  
Traumatic text

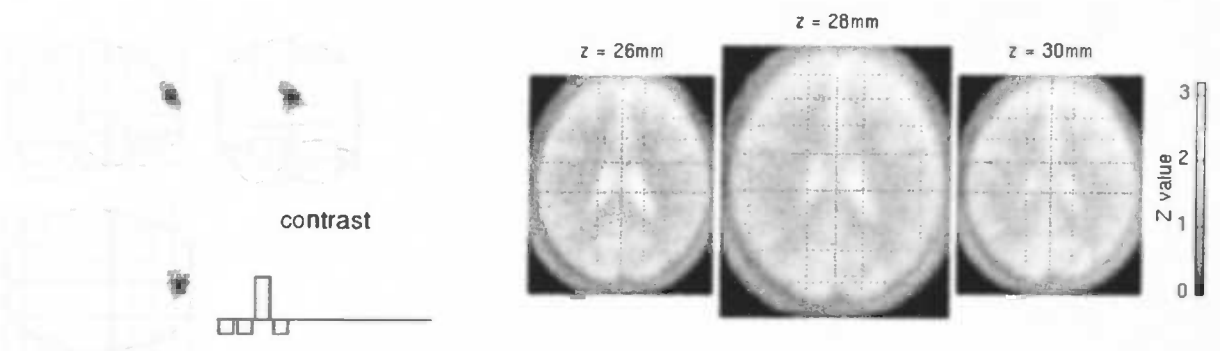


d2)  $Nn - Tn$   
Identity effect  
with  
Neutral text

Tt versus {Tn, Nt, Nn}



Nt versus {Tt, Tn, Nn}



**Addition VI.e: Z scores and p values**

Condition	Z-score (uncorrected p value)		coordinates	Brain region (Brodmann number)
Tt – Tn	3.21	0.001	6,-82,-44	≈ cerebellum
	2.97	0.001	-52,12,-26	Left Gyrus temporalis superior (38)
	2.98	0.002	14,-36,50	/
Tn – Tt	—	—	—	—
Nt – Nn	3.87	0.000	-64,-12,34	≈ Gyrus precentralis (4)
	3.32	0.000	4,66,28	Gyrus frontalis superior (9)
	3.05	0.001	42,-84,-48	/
Nn – Nt	3.60	0.000	62,-38,26	Right Lobulus parietalis inferior (40)
Tt – Nt	3.77	0.000	54,-62,-10	Right Gyrus temporalis inferior (37)
	3.42	0.000	-46,-28,50	Left Lobulus parietalis inferior (40)
	3.05	0.001	-58,16,-18	Left Gyrus temporalis superior (38)
Nt – Tt	3.11	0.001	14,-82,24	≈ Right Gyrus occipitalis superior (9)
	2.96	0.002	48,-24,-16	/
Tn – Nn	—	—	—	—
Nn – Tn	3.26	0.001	28,-48,22	/
	3.10	0.001	-6,-14,-8	/
Tt – {Tn,Nt,Nn}	3.95	0.000	-58,16,-18	Left Gyrus temporalis superior (38)
Nt – {Tt,Tn,Nn}	3.20	0.001	2,62,28	Gyrus frontalis superior (9)

## Acknowledgements

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Last but not least I wish to thank Johan for all his corrections, tea and mental support.

# **Appendix A**

## **Manual**

# **MANUAL**

## **H<sub>2</sub><sup>15</sup>O-PET data analysis**

- Preparation of the raw data for statistical analysis in SPM.
- Spm.ps explanation

**Written by: A.A.T.S.Reinders**

**Corrections: A.M.J.Paans, J.Quak.**

**February 1999**



## **Preparation of the raw data, from camer I or II, for statistical analysis in SPM.**

### **Checklist:**

- 1) Copy the necessary files from the Petsun to own directory. There are two ways:
  - a) From /data/camera II (or /data/camera I)
  - b) From a CD or optical disk (OD)
- 2) \* If the Ecat 951/31 is used for the experiment (also called camera I), and with the ten minutes protocol, background correction is needed. The files can be renamed in the same step.  
\* If the Ecat Exact HR<sup>+</sup> is used for the experiment (also called camera II), with the ten minutes protocol background correction and a the data has to be transformed from Ecat 7 to Ecat 6 data format is needed.
- 3) Center the images, and rename file.
- 4) Check the pictures for translation or rotation.
  - a) If the pictures are checked in Imagetool, transfer the images first from Ecat7 to Ecat6 format for data originating from camera II.
  - b) If the pictures are checked in CAPP5 the data format is Ecat7 and after editing the files transform the data from Ecat7 to Ecat6 format for data originating from camera II.
- 5) Transform the data from cti format to SPM format.

### ***In the SPM program.***

- 6) Realign.
- 7) Normalize.
- 8) Smooth.

It is recommended to print the results after each step. For steps 1) through 5) just open a tekst editor and copy the commands and output of the command tool in it, so it is clear which steps are made. This text file can also be used to make notes.

For steps 6) and 7) print the spm.ps file after each step, i.e. after the realignment and after the normalisation. One can remove the spm.ps file after printing, or rename the file to for example stereo.ps if you desire to keep this file. New spm.ps files are else add to old ones. Put al this paperwork in a map to keep the overview.

For the steps 1) through 5) the commandos can be written in a text file. This text file containing all of the commando's has to be made an executable script with `chmod +x "filename"`. Just type the filename in the commandtool and the commando's are executed. In the directory "/home/simone/manual" some of these files are placed. One can read and/or copy these files to the own home directory and use them files.

## Transform raw data to an useable SPM form: explanation.

### § 1 : Data from camera I or II.

The numbers of the scans of the experiment are noted in the logbook in the control room. If the data is recent it can be copied (use *cp* "filename") from /data/camera2 (or camera1) on the Xpet computers. The files from the latest period (several weeks) are registered in the directory /data/camera2. Copy the image files (.v or .img files) to your own home directory (/home/'username').

Use the Petsun computers to look up the necessary files from the OD or CD if the files are older because older data has been written to an optical disk (OD) or CD. All scan data is stored there permanently. Use the "eod2here" command if the information is written on OD. If the information is written on CD use the command "cdrom12here" in the directory /home/data.

Now go to the other side, the Xpet terminals.

#### § 2.1: Data from camera I.

!!Watch out; always backup the original files.

If the datafile from camera I contains two frames background correction is needed. In the first frame the background radiation (radiation left from the previous injection) is measured and the second frame has the information needed. The command "*backgroundcorr*" is the correction for radiation remaining from the previous injection and is used like:

- *backgroundcorr -i "nameold.img" -o "namenew\_b.img"*.

Two frames become one. Rename the file for example with a *\_b* behind the name, so it is clear that the image is already corrected. It is necessary integrate the two files to one file because the statistics in SPM one needs one file per scan.

Watch out!! The first dynamic scan does not need a background correction. This file only needs a new name (with the commando "*mv* "name-old" name-new") because this scan has only one frame. To keep the original dynamical scan it is necessary to copy the file first to another directory.

#### § 2.2: Data from camera II.

Data obtained with camera II also need a background correction. But this is another command, because it is another program for different data. The command is:

- *BackgroundCorr -i "nameold.v" -o "namenew\_b.v"*.

Watch out!! If during the experiment a long time (about 30 minutes) passes between one scan and the other, there is hardly any background radiation left. The first frame is then empty, but the image file has two frames. If this is the case during the experiment an error is given on the computers in the control room. The computer can not create the frame for background correction. To note this in the filelist (*ls*), the number of contents (number of bytes) is just as big as the first scan. The program BackgroundCorr can not be used under these circumstances. Use the program SelectMatrix. This program turns two frames into one frame. This is the first number 2 that becomes 1, after the command.

- *SelectMatrix -i "nameold.v",2,1,1,0,0 -o "namenew\_b.v",1,1,1,0,0*

Concluding: two frames have to be corrected to one frame, either by backgroundcorrection or, when there is no backgroundactivity left, by SelectMatrix.

### § 3: Center images.

Images need to be centered to the same point of departure. All images, also from other experiments were centered to the same location in the defined plane. In one experiment the head could be at a position for example more left in the plane than in another experiment. To center the images, use:

For camera I data (the names of the executable files start with lower case):

- `(/home/appl/woods/Solaris2/centerimage "name_b.img" "name_c.img")`.

For camera II data (the names of the executable files start with Capital letters):

- `CenterImage -i "name_b.v" -o "name_c.v"`.

All the images are now centered in the same way and same location in the defined plane. It is important that the images are centered to the same basis for normalisation and statistical analyses.

### § 4: Picture checks and Rotation.

#### § 4.1: Imagetool.

The format of the data from camera II can not be read by all of the programs used (for example: Imagetool). The data has to be transformed from Ecat7 to Ecat6 format, using:

- `Ecat7to6 -i "oldname_c.v" -o "newname_c6.img"`.

Data always has to be transformed into Ecat6 format. The program `cti2spm` cannot read Ecat7 format data.

With the program 'Imagetool' on the Petsun terminals, the images can be checked for translations and rotations. According to old opinions the maximum rotation that can be corrected by SPM95 itself with the realignment and normalisation is about 6-7 degrees. If the pictures are rotated more than 7 degrees SPM95 is not able to handle this anymore and a rotation program is needed.

However according to new opinions (A.Paans, P.Maguire and myself: see also my graduation essay) SPM96 can find the ACPC line very well. This means that no rotations have to be performed. The normalisation(!) in SPM96 can handle rotations up to 45 degrees. So, if rotations less than 45 degrees are seen, rotating your data is not necessary.

If one stills wants to perform rotations in imagetool, steps are given in the Appendix.

#### § 4.2: CAPP5.

CAPP5 is a program to check on the images. Use Ecat7 format files to check the pictures in CAPP5. To use CAPP5 make a new directory called ECAT7. CAPP5 can only read the files from this directory; subdirectories can not be read. Copy the files you want to use to look at to this directory. All steps before SPM can be checked in CAPP5 (also Ecat6 format files). When files are saved in CAPP5 they are always in ECAT7 format.

To start CAPP5 type CAPP5 after the prompt in the directory ECAT7. After a while (reading in the database), click 'Launcher' in the upper left corner of the screen.

With 'Slice Viewer' (comparable with imagetool), different files can be checked on position changes and rotations. Select 'Slice viewer', choose a scan file, click OK (three times). One can click on the brain to see what the pixel values are in the horizontal and vertical direction. In the left window one can move the axis to a specific position.

With 'Reslicer', rotations can be performed. The rotations can be done in the three planes:

- 1) Coronal : rotations over the y-axis.
- 2) Transaxial : rotations over the z-axis.
- 3) Sagittal : rotations over the x-axis.

To rotate in sagittal plane, the AC-PC line has to be placed in such order that it touches the frontal lower side (anterior) of the corpus callosum and nearly touch the posterior lower side of the corpus callosum. The thalamic region can also be a marker, which appears as a bright colored dot. The line has to pass through theinion at the

back of the head. To rotate click the mouse at the starting point, a line pops up and end (release mouse button) at the chosen end point.

To enlarge the pictures for a better view choose from the menu bar: 'Edit', 'defaults', 'zoom3'. To keep an eye on the degrees, choose from the menu bar: 'View', 'rotationlist'. Place the little window outside the CAPP5 window, to be free of overlap.

To save the rotated pictures choose from the menu bar: 'Edit', 'Apply to', now choose the file that needs the rotation, 'OK', give pwd and filename (the filename has to be different each time, the files can not be overwritten).

**!!Watch out!!** Rotated image files that are saved in CAPP5 can not be used any more in SPM96 because of resolution loss (63 slices to 45 slices and again to 63 slices) and rotations outside the defined areas of CAPP5. Statistical calculations are very inaccurate (see also my graduation essay).

If the pictures are checked in CAPP5 the data format is Ecat7. And after editing the files transform the data from Ecat7 to Ecat6 format, to be able to transform the data from cti to spm format. The data is in Ecat7 format and has to be transformed to Ecat6 format, using:

- `Ecat7to6 -i "oldname_c.v" -o "newname_c6.img"`

Data always has to be transformed into Ecat6 format. The program cti2spm cannot read Ecat7 format data.

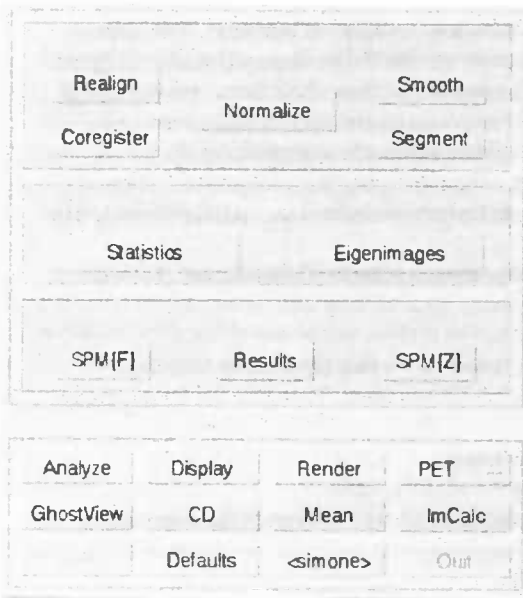
## § 5: Transformation.

Transformation from cti to SPM format has to be done at a XPET-terminal, using Ecat6 data format.

- `/home/appl/woods/Solaris2/cti2spm "name_c6.img" "name_c_t.img"`

Together with the image (.img) files are now also the header files (.hdr) created which are needed in SPM96.

## The SPM96 interface



### Select design type.....

SPM94 style questioning  
 Single-subject: replication of conditions  
 Single-subject: conditions & covariates  
 Single-Subject: covariates only  
 Multi-subject: different conditions  
 Multi-subject: with replications  
 Multi-subject: condition & covariates  
 Multi-subject: with replications & covariates  
 Multi-subject: covariates only  
 Multi-study: different conditions  
 Multi-study: with replications  
 Multi-study: condition & covariates  
 Multi-study: with replications & covariates  
 Multi-study: covariates only  
 Compare-groups: 1 scan per subject

Print Clear ColorMap Effects out move size text

### SPM96

#### This is SPM96

This is SPM96, the public edition of SPM'96 - released 09/04/1997. Please refer to this version as "SPM96" in papers and communications, citing the "Wellcome Department of Cognitive Neurology".

SPM96 is backward compatible, in that each module will read files from previous versions. (E.g. SPM96 statistics will read normalised images from SPM95) However, it is recommended that you don't mix versions of the same module in a single analysis. (E.g. Don't mix SPM96 and SPM95 spatial normalisations in the same study group - the templates are different!) You should identify which versions of SPM were used for which stages of analysis in any publications.

A version choosing routine, `spm_choose`, is provided in the distribution. This presents a graphical menu of versions, which set the Matlab path and start the appropriate `spm`. Type '`spm_choose`' to use this facility. (system administrators need to edit the version and path definitions in `spm_choose.m` for this to work.)

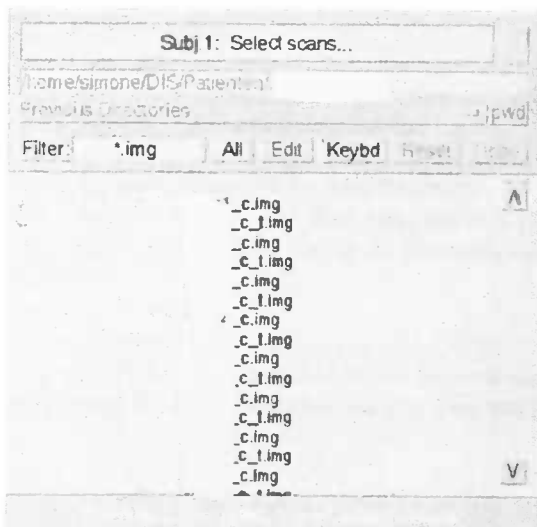
Please take time to review the the help system, in particular the "About SPM" topic (`spm.man`).

SPM96 is SPM96b with the public patches applied, together with some additional (cosmetic) improvements. The `Contents.m` file contains a list of the differences. Details of the SPM96b updates are on the SPMweb site.

Further information may be found at the SPMweb site:  
<http://www.fil.ion.ucl.ac.uk/spm>  
 ...where details of the SPM email discussion list can be found:  
<http://www.fil.ion.ucl.ac.uk/spm/help>

Warmest regards,  
 the authors. spm\_notd.man

The FIL methods group : The Wellcome Department of Cognitive Neurology



This 'Pop-up screen' appears after selecting a statistical "design type".

Blue *Filter* box: letters can be typed included in the wanted file names (here all the \*.img files are represented)  
*All*: selects all files  
*Reset*: clears selected files  
*Done*: confirm selected files, after clicking *done* there is no way back.

### SPM96 interface:

The “Spatial” box contains options to adapt spatial properties (place and form) of images. The three most important items are:

- 4) “Realign”: Realignment is a transfer of coordinate system from the scans to those of one scan. This scan is the first scan to enter the realignment and functions as reference point to which the other scans are realigned. The movements can take place in the x,y,z-direction. Rotation also occurs in three directions: movement of the nose towards the ears (roll), towards the chin/forehead (pitch) or rotation towards the shoulders (yaw).
- 5) “Normalize”: Normalisation concerns a mapping of scanned individual brains to a template brain. Parameters needed to do this were obtained from the mean image, created during the realignment. The template contains a standard brain. The brains of the subjects from the experiments were standardized to this standard brain.
- 6) “Smoothing”: With smoothing local contrasts will be reduced, this means noise will be reduced. However, the signal to noise ratio will increase.

The “Analysis” box contains two options to perform analysis. If the “Statistics” option is chosen the blue window will pop up with the title “Select design type”. A design type can be selected. From this list three groups are the most important:

- 4) “Single-subject” (a statistical analysis which concerns only one subject),
- 5) “Multi-subject” (a statistical analysis which concerns more than one subjects) and
- 6) “Multi-study” (for group comparisons, e.g. a patient group and a control group). Different choices can be made per group. Selections can be made from
  - d) “different conditions” (different conditions e.g. listening and rest),
  - e) “replication” (if conditions are replicated),
  - f) “covariates” (a covariate is a factor that can be of influence on rCBF, e.g. task difficulty).

With the “Results” box, statistical results can be revised and manipulated, e.g. show results at a different threshold with the SPM{Z} option, to see more (lower Z score/p value threshold) or less (higher Z score/p value threshold) activation patterns.

In the box below “SPM for PET/SPECT” different options are available. For example a button “Ghostview” to view postscript files (e.g. the spm.ps file). With the button “Display”, files can be displayed e.g. original files, or normalised files. With the “Default” button, default values within SPM can be set to other values, e.g. the number of non linear basis functions (for the anatomical normalisation) can be set from 4x5x4 to 3x4x3. To leave the SPM program the button “Quit” is available.

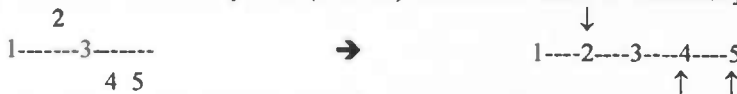
In the window on the left side results are presented after execution of a SPM function, e.g. realignment, normalisation, statistical analysis, manipulation of thresholds, etc. At the top of the window some buttons are present, e.g. “print” (prints the content of the window to the spm.ps file), “clear” (removes the content of the window or clears a SPM function). The other buttons are used to manipulate the content of the window. The button on the right is sometimes useful to receive help.

## Within SPM.

### § 6: Realignment.

Now start the program SPM(95/96) for the realignment. There are three boxes at the left upper window are: Spatial, Statistics and Results. In the Spatial Box are three items: "Realign", "Normalize" and "Smooth". These three items are explained in this paragraph. During these steps files need to be selected. This can be done by clicking the mouse button and then pressing "Done", or by selecting "All" and then pressing "Done". In the type window next to these buttons, letters can be typed that are included in the file names needed. Press enter and the selected files are represented alone in the file name list.

Realignment is a transfer from the scans to (for example) the middle scan. The middle scan is the first scan to enter the realignment. The middle scan functions as reference point, to which the other scans are realigned. The middle scan usually lies in the middle of movement. During the scanning procedure the head may shift a little bit. The order of scans imported (in SPM) must be the same for each subject.



For example in the realignment procedure scan 2 is shifted down a little and scans 4 and 5 are shifted up. The movements can take place in the x,y,z-direction. Rotation also occurs in three directions: movement of the nose towards the ears (roll), towards the chin/forehead (pitch) or a rotation towards the shoulders (yaw).

#### § 6.1: SPM95.

Type 'SPM95' in the command tool and 'spm' in matlab. The realignment in SPM95 will be explained. Watch out!! After a mistake occurs delete the .mat files, because they are used again in calculations, and the spm.ps file because new information is added. (My personal opinion is: do the realignment and the normalisation immediately after each other in SPM95 for one subject at a time.)

- Choose "realign" from the Spatial box. The first scan chosen is the scan to which the other scans are realigned. It is recommended to select the middle scan as the first scan. Use the "\*\_c\_t.img" files.
- Choose "realign" again. But now the number of scans to work with is n+1. Because the mean scan is the scan to realign to, in this second step. Select the mean\* scan as the first scan, the other scans are the scans starting with a "r".

The graphics present the translation and rotation of the head during the experiment.

#### § 6.2: SPM96.

Type 'SPM96' in the command tool and 'spm' in matlab.

Watch out!! After a mistake occurs delete the .mat files, because they are used again in calculations, and the spm.ps file because new information is added.

- Choose "realign". The first scan chosen is the scan to which the other scans are realigned. It is recommended to select the middle scan as the first scan. Use the "\*\_c\_t.img" files.
- choose "coregister & reslice"
- "bilinear interpolation"
- "meanimage only"

The graphics present the translation and rotation of the head after the procedure. The brain is also resliced to 43 slices. Print the spm.ps file and delete the file.

A second realignment is needed for a better result.

- Again, choose "realign". The first scan clicked is the "mean\*.img" created in the first realignment. The next scans are the same scans as used in the first realignment. One scan more is selected than in the first realignment.
- "coregister & reslice"
- "bilinear interpolation"
- "images 2\*n", for the scan 2 and higher because the first scan is the mean.

Again print the spm.ps file and delete the file. The resulting files are the files starting with a "r".

## § 7: Spatial normalisation.

Normalisation concerned a mapping of scanned individual brains to a template brain. Parameters needed to do this were obtained from the meanimage created during the realignment. The template concerns a standard brain. The brains of the subjects from the experiments were standardized to this standard brain.

### § 7.1: Normalisation in SPM95.

- choose “normalize” from the Spatial box.
- working with PET data choose the “PET: option., and the number of scans plus 1 for the mean scan.
- choose the “mean\*” file (same as with the realignment) and the “rr\*” file.
- “number of other images =” 0.
- leave all the numbers the way they are. Click OK.

To print a picture of the normalisation for each scan: the following steps are taken the number of scans times, per scan one time.

- Choose “display” (in the SPM for PET/SPECT box)
- Select one scan (first, etc) starting “nrr\*.img”. In the popping window, choose “display” again. Click the “print” button in the top of the right window. The result is a picture for each normalized scan.

### § 7.2: Normalisation in SPM96.

#### § 7.2.a: Optimization of the spatial normalisation.

!!Watch out!! This procedure is always needed if one works with data from camera II (HR+). One must always check the normalisation afterwards. If one wants to see the normalisation before adjustments, first perform step § 7.2.b. Perhaps backups of the files are needed.

If the stereotactic normalisation is not satisfactory then there are some options to improve it. Problems with the normalisation occur for example if SPM96 tries to fill in the empty space in the scans. This empty space is related to the positioning of the subject in the camera. For example if the subject is positioned too low in the camera, or with data from camera I (smaller axial length) the problem becomes obvious. The scans contain no information of the lower parts of the brain because this parts are not included in the defined area. In SPM96 the normalisation procedure tries to fill in this empty space. This is shown as stretching of the data.

The stereotactic normalisation consists of two steps, an linear affine transformation and a plastic anatomical transformation (warping or local deformation). In both stages one has the opportunity to optimize the normalisation. However, the linear affine transformation does not contribute to stretching of the brain. In this case, default value adjustments are made before realignment and normalisation.

#### Anatomical normalisation.

This is the deformation of the structures in the head consisting of stretching and shrinking of the structures in the head to map them as precisely as possible to the PET.img template.

To do this in SPM96, take the following steps:

- click “Defaults” (in the SPM for PET/SPECT box)
- “Defaults area?” choose the “Spatial Normalisation” option (see below Fig:7.2.a.I).
- “Affine Starting Estimates” choose the recommended “\*” option (“Disallow Customized”)
- “Affine Params?” choose the recommended (\*12 parameters) option, in this step the affine transformation can be manipulated. However no effects were found on the stretching phenomenon (see also graduation essay).
- “Nonlinear Basis Functions?”, different settings can be choosen. This deformation of structures in the head occurs in three directions,  $x*y*z$ , with different numbers as shown in the menu.

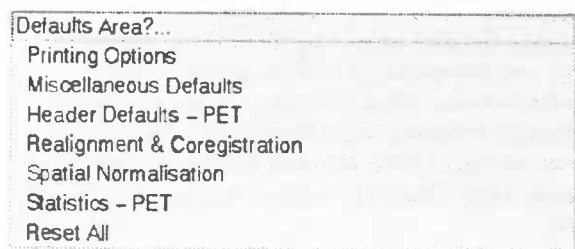
The choice depends on the purpose for which structures the deformation is needed. The standard values are 4x5x4. When one is interested in small structures in the brain, one can choose larger values for the  $x*y*z$ . For example 5x6x5. Larger values involve the use of more sinus and/or cosinus functions (sinc functions) (Fourier) to fit the subjects scans to the template. Choosing larger values is only useful when the smoothing factor is small.



It is of no use to use a large smoothing factor because the small structures are not shown then. For the stretching problem, smaller values can be the solution. For example the 3x4x3 option.

- In the popped up window choose the 3x4x3 option (see below Fig:7.2.a.II).
- “Nonlinear Iterations” choose the “\*” (“8 Nonlinear iterations”) option, if in Matlab the values of the following iterations differ much, more iterations are needed.
- Choose all of the “\*” options
  - “Bounding Box” = “-78:78 -112:76 -50:85 (MNI)”
  - “Voxel Sizes?” = “2 2 2”

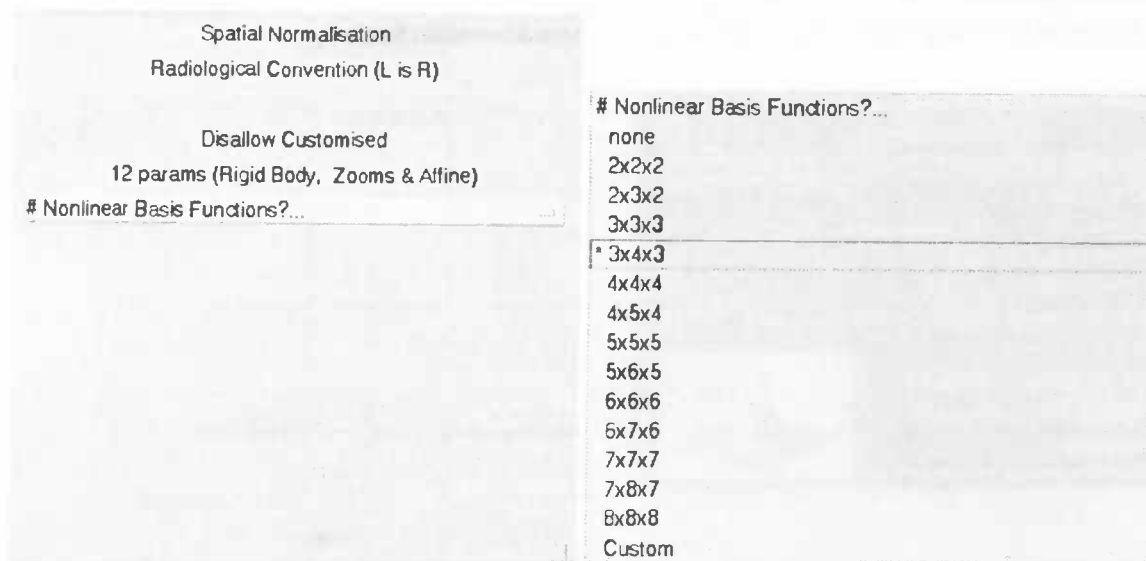
After adjusting these values the normalisation can be performed.



← Fig:7.2.a.I

The fifth option is the needed option to adjust the spatial normalisation.

↓ Fig:7.2.a.II



## § 7.2.b: The normalisation.

- choose “normalize”
  - “determine parameter & write normalized”
  - “image to determine parameters from”, choose the ‘mean\*.img’ scan formed in the realignment.
  - “image to write normalised”, choose the scans starting with an “r”, the end result of the realignment.
- Watch out!! Be sure to choose the files starting with a “r”.

- The template to choose is the “PET .img”
- “bilinear interpolation”

The result are files named as “nr\*.img”, normalised and realigned.

To print a picture of the normalisation for each scan: the following steps are taking the number of scans times, per scan one time.

- Choose “display” (in the SPM for PET/SPECT box)

- Select one scan (first, etc) starting “nr\*.img”. In the popping window, choose display again. Click the “print” button at the top of the right window. The result is a picture for each normalized scan.

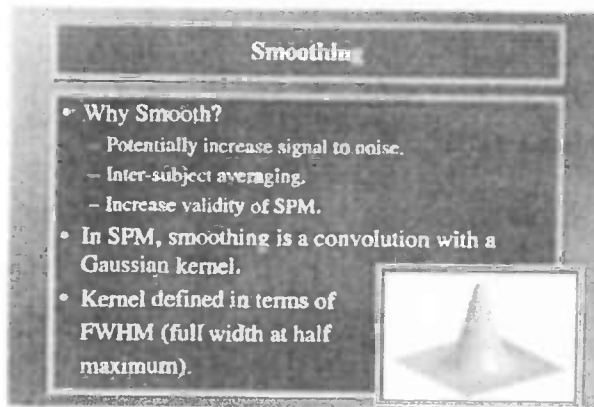
## § 8: Smoothing.

With smoothing local contrasts will be reduced, this means noise will be reduced. However, the signal to noise ratio will increase. Details on smaller scale (i.e. noise) will disappear also variations will disappear, within this smaller scale some information will also be lost. However, on a larger scale there is no information loss, details will stay visible. With smoothing the number of pixels stays the same, so resolution will stay the same. In SPM smoothing is a convolution with a Gaussian Kernel.

In the smoothing procedure a smoothing number has to be chosen. Regular smoothing filter width of FWHM (Full Width Half Maximum) are 12mm or 20mm (this is a large smoothing factor). The Exact HR<sup>+</sup> has a resolution of 5mm so, a smaller filter (for example 8mm) can also be used. When choosing a larger number to smooth with, sharpness becomes smaller. The localisation (accuracy) becomes worse because a larger area is taken into account to obtain significance. So the sensitivity becomes better when you take little areas together. With a smoothing factor of 12, the smearing is less. So if an area is significant it is sharper. A smoothing factor of 12 is recommended to look at small regions with a lot of data.

- choose “smooth” from the spatial box.
- “number of scans to smooth”, all the scans can be smoothed simultaneously.
- “smoothing {FWHM in mm}” type 12 or 20 or some other number if desired.
- Choose the files starting with “nr\*.img”

Now it is recommended to copy all the “snr\*” files to your own directory for the use in the statistical procedure. Mistakes to choose the wrong files during statistic analysis are made smaller that way.



# Image realignment

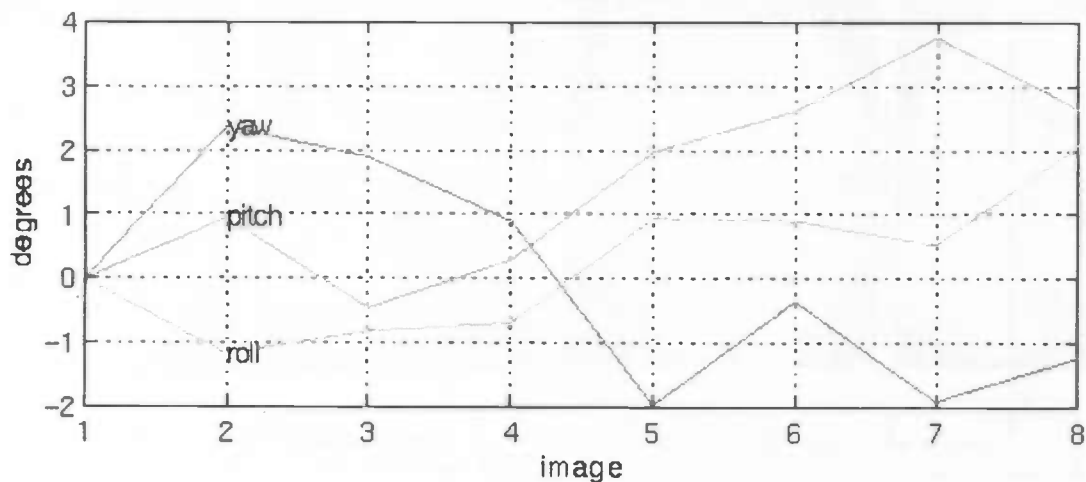
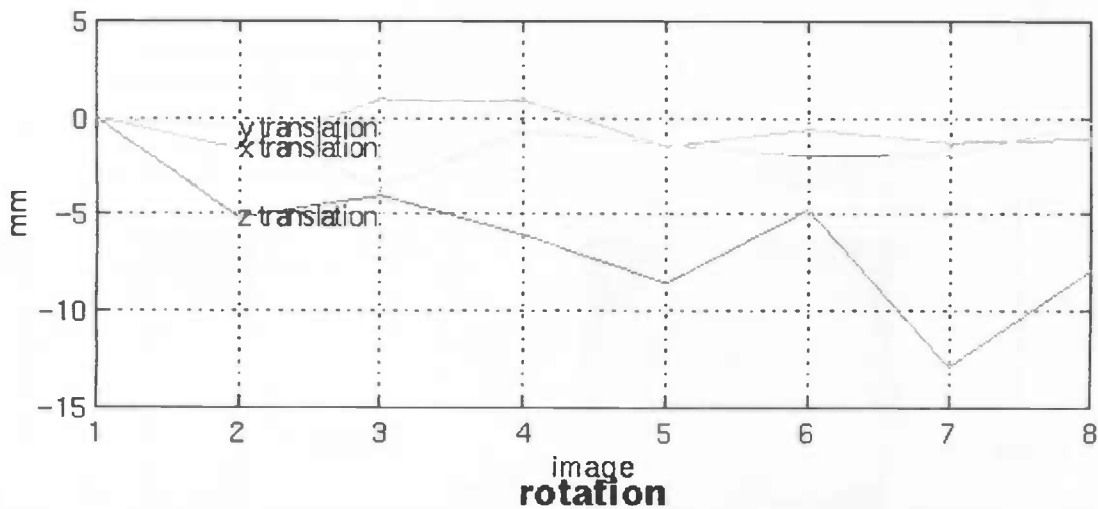
```
1 /home/simone/DIS/ 4_c_timg
2 /home/simone/DIS/ 1_c_timg
3 /home/simone/DIS/ 2_c_timg
4 /home/simone/DIS/ 3_c_timg
5 /home/simone/DIS/ 5_c_timg
6 /home/simone/DIS/ 6_c_timg
7 /home/simone/DIS/ 7_c_timg
8 /home/simone/DIS/ 8_c_timg
```

The first scan selected is the middle scan in time.  
\_c means centered image, \_t means cti2spm tranformed

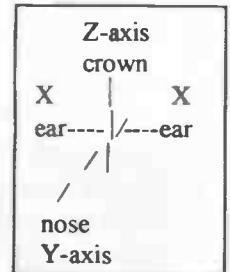
Translation of images to the middle scan:  
Red = x-axis, Green = y-axis, Blue = z-axis  
Horizontal: imagenumber  
Vertical: translation in mm, usually between +10 and -10

One can read for every scan the translation over the three axis copared to the previous scan.

## translation



# Image realignment



```

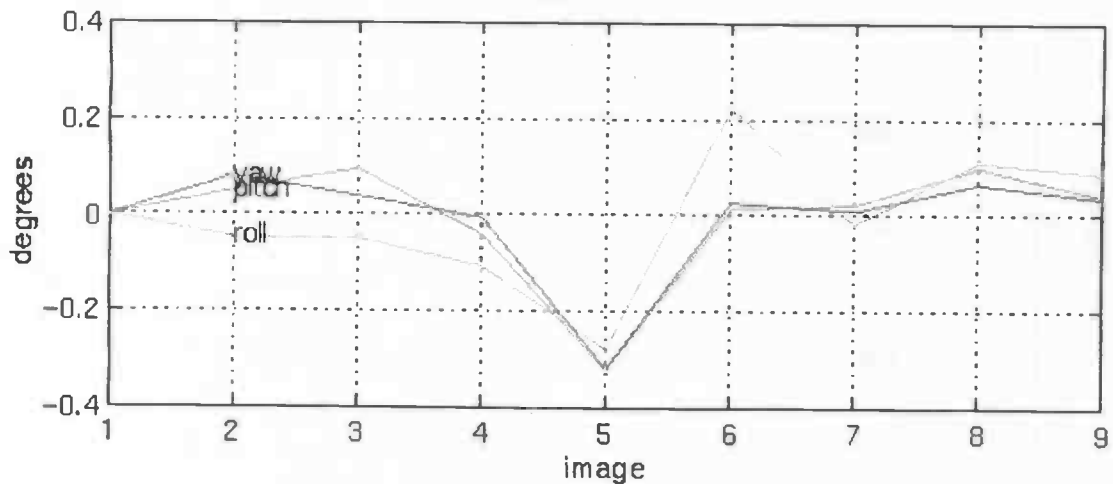
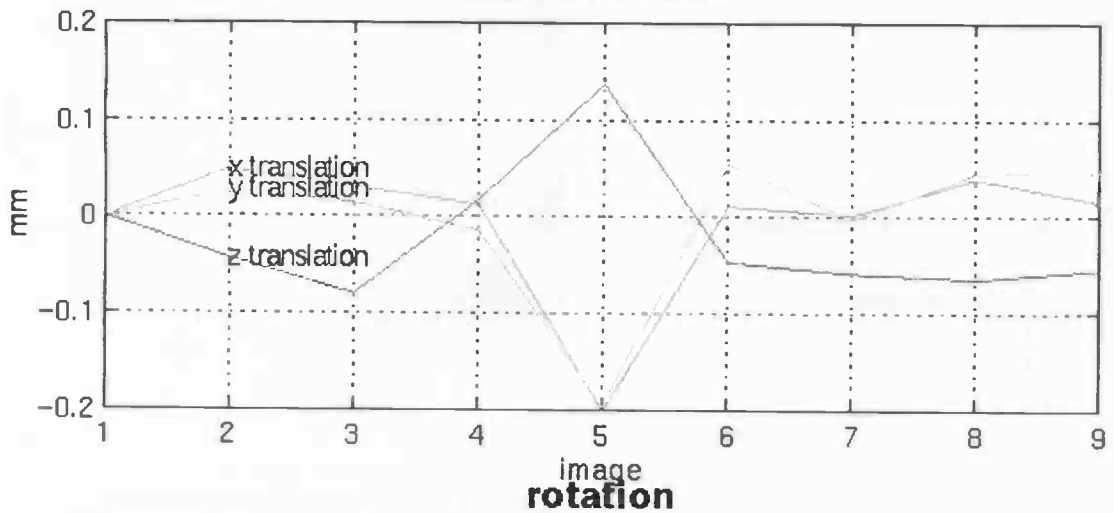
1 /home/simone/DIS/me: 4_c_timg
2 /home/simone/DIS/ 1_c_timg
3 /home/simone/DIS/ 2_c_timg
4 /home/simone/DIS/ 3_c_timg
5 /home/simone/DIS/ 4_c_timg
6 /home/simone/DIS/ 5_c_timg
7 /home/simone/DIS/ 6_c_timg
8 /home/simone/DIS/ 7_c_timg
9 /home/simone/DIS/ 8_c_timg
    
```

Second realignment:  
Realignment to the meanimage, created in  
the first realignment.

Rotation occurs in three directions:

- 4) movement of the nose towards the ears (roll): rotation about the z-axis.
- 5) movement of the nose towards the chin/forehead (pitch): rotation about the x-axis.
- 6) movement of the head towards the shoulders (yaw): rotation about the y-axis.

## translation



## Spatial Normalisation

Image : /home/simone/DIS/mean/ \_c\_t.img

Parameters : /home/simone/DIS/mean/ \_c\_t\_sn3d.mat

### Linear {affine} component

$$X1 = -1.09 \cdot X + 0.31 \cdot Y + -0.11 \cdot Z + 9.93$$

$$Y1 = 0.31 \cdot X + 0.92 \cdot Y + -0.41 \cdot Z + -8.71$$

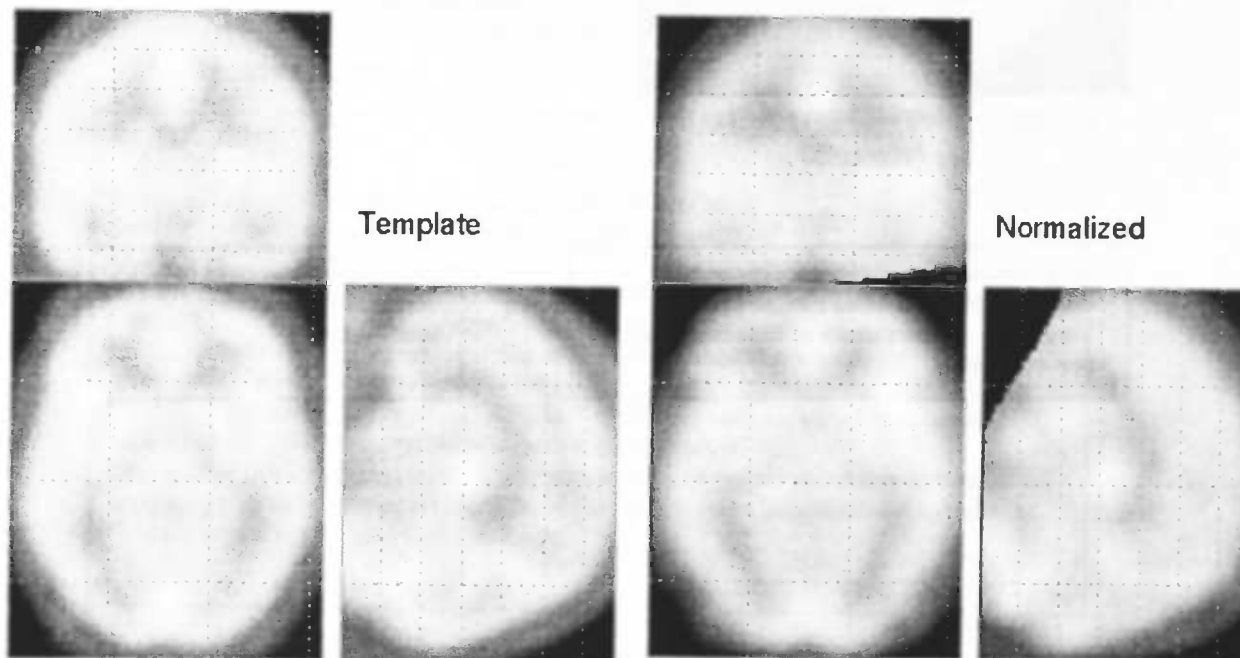
$$Z1 = 0.03 \cdot X + 0.47 \cdot Y + 1.09 \cdot Z + 8.70$$

This is the normalisation matrix.  
The given parameters are specific  
for this subject.

8 nonlinear iterations

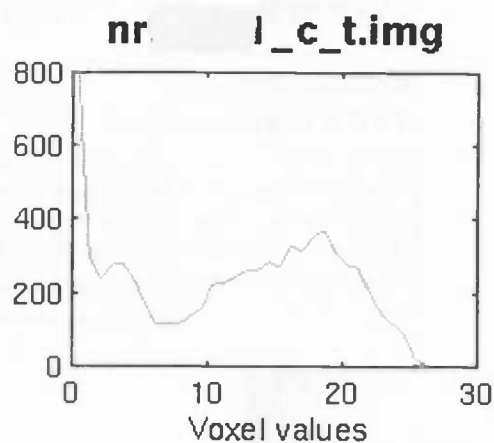
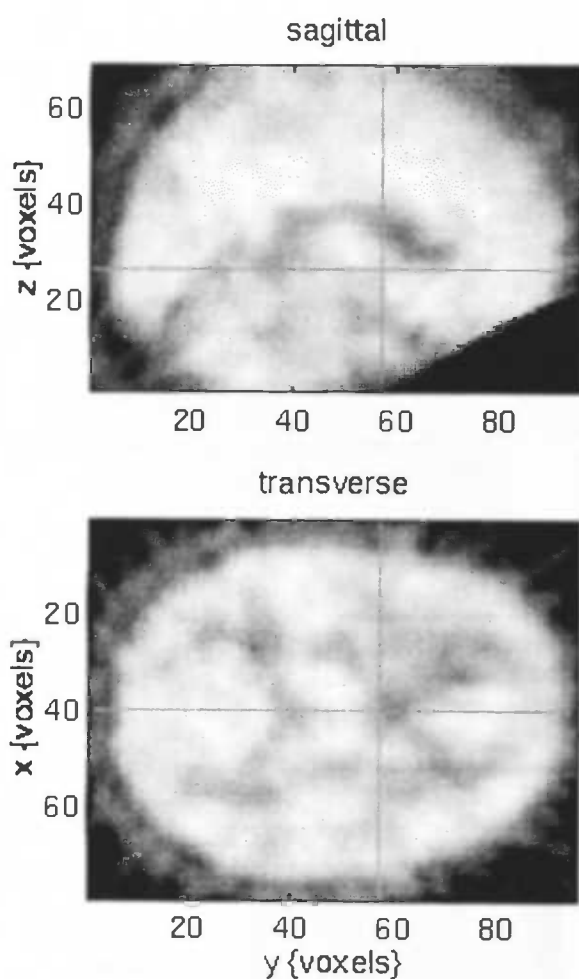
3 x 4 x 3 basis functions

These are the non-default values for the anatomical deformation, using 3x4x3 non-linear basis functions as adjusted before the normalisation. The 4x5x4 basisfunctions cause overstretched normalisation using HR<sup>+</sup> data.



Template: standard normalised brain

Normalised brain of the subject



These normalised pictures are obtained by displaying the normalised images per scan. This gives a clearer picture of the normalised brain per scan.

Histogram presentation of voxels with respective voxel values.

## Statistical analysis

Scan Index	Subject	Condition	Replication	Global	Base directory: /home/simone/DIS/Patienten/S20 File name Tails
01 :	1	1	1	39.7471 /sr	NT1_c_timg
02 :	1	1	2	38.7996 /sr	NT2_c_timg
03 :	1	2	1	35.7027 /sr	TT1_c_timg
04 :	1	2	2	38.5978 /sr	TT2_c_timg
05 :	2	1	1	48.1865 /snr	c_timg
06 :	2	1	2	50.5257 /snr	VT2_c_timg
07 :	2	2	1	51.2212 /snr	TT1_c_timg
08 :	2	2	2	52.6348 /snr	TT2_c_timg
09 :	3	1	1	41.5764 /snr	1_c_timg
10 :	3	1	2	38.8933 /snr	2_c_timg
11 :	3	2	1	48.8658 /snr	2_c_timg
12 :	4	1	1	50.1338 /snr	TT1_c_timg
13 :	4	2	1	59.2633 /snr	TT1_c_timg
14 :	4	2	2	54.3018 /snr	TT2_c_timg
15 :	5	1	1	62.3433 /snr	1_c_timg
16 :	5	1	2	68.0882 /snr	2_c_timg
17 :	5	2	1	55.3659 /snr	1_c_timg
18 :	5	2	2	53.7178 /sr	2_c_timg
19 :	6	1	1	61.6306 /snr	c_timg
20 :	6	2	1	45.1677 /snr	c_timg
21 :	6	2	2	52.9196 /snr	c_timg
22 :	7	1	1	48.128 /snr	NT2_c_timg
23 :	7	2	1	54.1892 /snr	TT2_c_timg

Images scaled to an overall grand mean of 50

Gray matter threshold is 80% of the whole brain mean

Covariates are centered before inclusion in design matrix

### Explanation:

Scan index = Number of scans for analyses in SPM

Subject = Number of subjects used for analyses

Conditions = Number of conditions

Replication = Number of replications of conditions

Global = Relative cerebral bloodflow during the scan

Filename Tails = Filenames imported in SPM for statistical analyses.

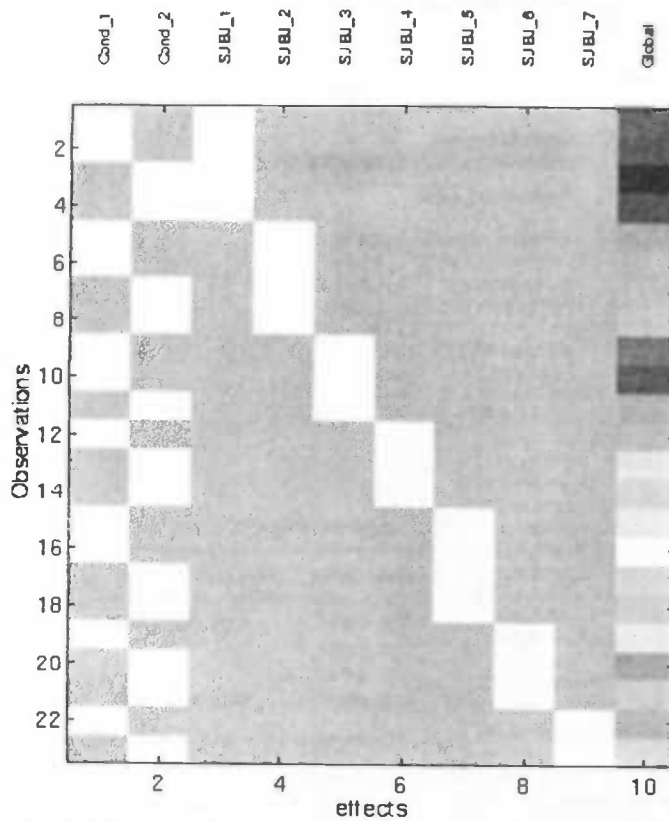
The above list shows a study with 7 subjects with 2 conditions, with and without replications. For example: subject 7 => two conditions, only 1 replication

Scaling of the global cerebral bloodflow to a grand mean of 50.

Gray matter threshold is set to 80%, this means 80%, all voxels above this 80% threshold are considered to be graymatter, so 20% of the brain is taken into statistical calculation.

Covariates: are not defined in this analysis.

## Design Matrix



Design: Multi-subject: with replications

Global normalisation: AnCova

### Parameters:

2 Condition + 0 Covariate + 7 Block + 1 Confound

= 10 parameters, having 9 degrees of freedom, giving 14 residual df (23 scans).

### Explanation:

The Design Matrix: In the subj\_number column, one white block is present, from here, going to the left, one can perceive the corresponding observation with the conditions.

The "Global" column gives information about the cerebral bloodflow. A lighter square stands for more global cerebral bloodflow.

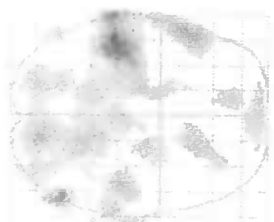
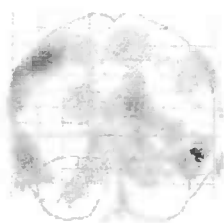
In this case the design is Multi-subject (here 7) with replications (some conditions were repeated)

Parameters: In this case analyses were made with 2 conditions, 0 covariates, 7 blocks (=7 subjects) and 1 confounded covariate (= global cerebral bloodflow)

df means degrees of freedom: in this case are 10 parameters involved ( $2+0+7+1=10$ ) this results in  $10 - 1$  (1 confound variable = gCBF) = 9 degrees of freedom, giving the number of scans (23) - 9 = 14 remaining degrees of freedom. So,  $df=14$ .



SPM{F}  $p < 0.05$ , df. 1.0,14.0



Search volume: 233425 voxels  
 Image size: 79 95 68 voxels  
 Voxel size 2.0 2.0 2.0 mm  
 Resolution {FWHM} 16.4 17.4 17.4 mm

Results directory:/home/sim one/DIS/Patienten

Contrasts	1	2
Cond_1	1	-1
Cond_2	-1	1

#### Explanation:

SPM{F} means a statistical parametric map computed with F statistical tests, areas represented have chance values from  $p < 0.05$  or smaller.

Resolution {FWHM} = Full Width Half Maximum

Smoothing is a convolution with a Gaussian kernel.

Two contrasts:

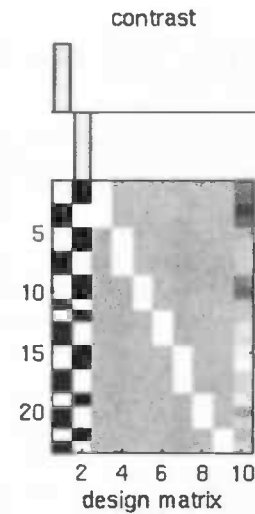
3)  $1 \cdot \text{condition\_1} + (-1 \cdot \text{condition\_2})$

4)  $-1 \cdot \text{condition\_1} + (1 \cdot \text{condition\_2})$

In this case this means:

3) NT -TT (See file list: condition1 = NT)  
 4) TT -NT ( condition2 = TT)

## SPM{Z}



### P values & statistics: /home/simone/DIS/Patienten

set-level {c}	cluster-level {k,Z}	voxel-level {Z}	uncorrected k & Z		x,y,z {mm}		
0.950 (2)	0.457 (719, 3.11)	0.967 (3.11)	0.030	0.001	14	-82	24
		0.993 (2.94)		0.002	18	-48	16
		0.996 (2.90)		0.002	16	-56	12
	0.962 (229, 2.96)	0.992 (2.96)	0.196	0.002	48	-24	-16

### Explanation P values & statistics:

The table exist of 5 columns:

#### 1) set-level {c}:

The probability of getting (2) or more clusters by chance 0.950 (small values wished). {c} is the number of clusters bigger than the given Extent threshold {k} for the given Height threshold {u} (Height and Extent threshold at the bottom of the page).

#### 2) cluster-level {k,Z}:

Probability (0.457) of obtaining a cluster size of k (for example: k = 719 cluster in voxels). The voxel Z-value of 3.11, is the maximum Z-value within this cluster of connected voxels. In this column are {c} clusters.

#### 3) Voxel-level {Z}:

It is the probability 0.967 of obtaining a Z-score of 3.11 (same Z score as in 2). This Z-score is the straight Z-score, only the neighbouring p-value (0.967) is corrected for multiple comparisons. The other two Z-scores are the two other highest maxima in the cluster that are at least 8 mm apart. Corrected (for the volume of the brain, i.e. for multiple comparisons):  $p < 0.05$  for significance. A  $p < 2.00$  means that no false positives are shown.

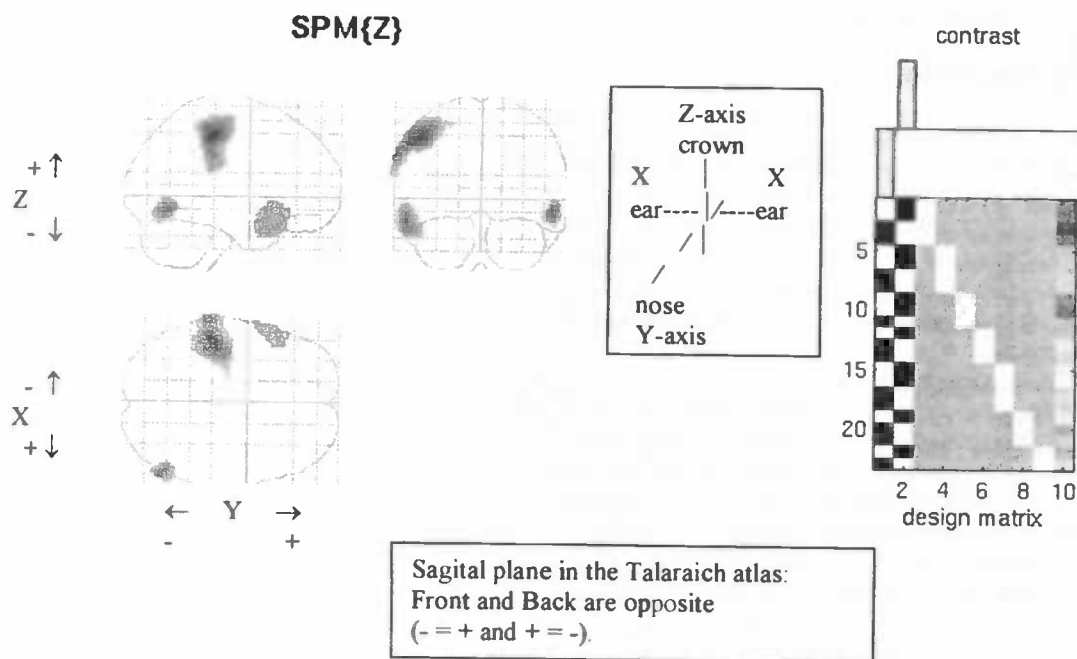
#### 4) Uncorrected k & Z:

Uncorrected probabilities, looking at cluster/voxel in isolation, i.e. without a correction for the volume of brain analysed. The first p-value is the extent of the cluster (suprathreshold cluster size), the second p-value is for the height within the extent of the cluster (local maximum within the cluster). Both p-values are uncorrected.

So a significant cluster must have significant extent and height of voxels in isolation. Significant uncorrected p-values for the height:  $p < 0.001$ .

Height threshold {u} = 2.33,  $p = 0.010$   
Extent threshold {k} = 147 voxels,  $p = 0.298$   
Expected voxels per cluster,  $E(n) = 146.6$   
Expected number of clusters,  $E(m) = 4.7$

Volume {S} = 233425 voxels or 376.6 Resels  
Degrees of freedom due to error = 14.0  
Smoothness FWHM {mm} = 16.4 17.4 17.4  
{voxels} = 82 8.78.7



**P values & statistics: /home/simone/DIS/Patienten**

set-level {c}	cluster-level {k,Z}	voxel-level {Z}	uncorrected k & Z		x,y,z {mm}		
0.052 (3)	0.473 (177, 3.77)	0.401 (3.77)	0.254	0.000	54	-62	-10
		0.976 (3.07)		0.001	58	-72	-12
		0.997 (2.87)		0.002	58	-68	-4
	0.095 (1541, 3.42)	0.770 (3.42)	0.003	0.000	-46	-28	50
		0.792 (3.40)		0.000	-66	-34	40
		0.798 (3.39)		0.000	-52	-32	46
	0.037 (376, 3.05)	0.981 (3.05)	0.104	0.001	-58	16	-18
		0.998 (2.86)		0.002	-58	18	-10
		0.999 (2.75)		0.003	-50	24	-24

p-values in the columns 1 to 4 must be as small as possible.  
Then chances are smaller that clusters found, are based on coincidence.

Height threshold {u} = 2.33, p = 0.010  
Extent threshold {k} = 147 voxels, p = 0.298  
Expected voxels per cluster, E(n) = 146.6  
Expected number of clusters, E(m) = 4.7

Volume {S} = 233425 voxels or 376.6 Resels  
Degrees of freedom due to error = 14.0  
Smoothness FWHM (mm) = 16.4 17.4 17.4  
{voxels} = 82 8.7 8.7

SPM96 (simone) - 04/12/1998 (12:18)

E{m} = number of clusters expected on average  
Extend threshold {k} = spatial extent threshold, suprathreshold clusters are only shown if they exceed this size.

## Appendix Imagetool rotations:

But if one still wants to perform the rotations, a description is given here. You can find the program 'imagetool' via the 'ecatilities'. Click "study1" to select a file, called "name\_c.img". Then click "display" to show the picture. Write down remarks.

Calculation of the angle: if the head is rotated more than 6-7 degrees a rotation is needed. To calculate the rotation angle click on a head, hold down the mouse button and select zoom4. Now, hold down the right mouse button (rm) and select 'Showvalues' and then 'Measuredistance'. Now click (with the rm button) at the middle (!) of the front part of the brain, hold down and release the button at the middle (!) at occipital part of the brain. Coordinates are given:  $x='a1'$   $endx='a2'$ ,  $y='b1'$  and  $endy='b2'$ . Calculate the difference in x and y replacement,  $xr$  and  $yr$ . Now calculate the angle using  $\tan\alpha = \text{overlying side}/\text{adjacent side} = xr/yr$ . Calculate the angle using  $\tan^{-1} xr/yr = \text{angle}$ . It is recommended to repeat this procedure for another slice of the head to see if the angle you calculated is correct.

After calculation of the angle the rotation can be performed. In 'imagetool' choose 'options' and select 'imagecalc'. First, store the file to rotate under a memory button to work with it. Choose 'files', select the file to rotate, 'files', 'store', click with the middle (!) mouse button on a memory button (for example 'A'), check the button with the rm button on, 'clear', click used memory button (A). The file is now ready to work with. Choose 'filters' (hold down mouse button), 'move, rotate & reslice', 'rotate image'. We want to rotate over the axis perpendicular to the slices, the z-axis. In the first column x and y values are zero and the z value is the calculated angle. In the second column the z value is zero. Calculate the distance between  $a1$  and  $b1$  ( $=c1$ ), and between  $a2$  and  $b2$  ( $=c2$ ) using the coordinates:  $(x,y)=(a1,a2)$  and  $(endx,endy)=(b1,b2)$ . Now divide  $c1$  and  $c2$  by two and the results are  $d1$  and  $d2$ . If the rotation is done clockwise use  $-d1$ , if it is done counter-clockwise use  $+d1$ . The x value is now  $d1$  and the y value is  $d2$ . Now click 'proceed'. The rotated file has to be saved. Click 'save', 'save results as' type '/home/username/newname\_c\_rot.img'. Now the new file is ready to be used.

However according to new opinions (A.Paans, P.Maguire and myself) SPM96 can find the ACPC line very well. This means that no rotations have to be performed. The normalisation in SPM96 can handle rotations up to 45 degrees.

## Appendix B: Premature article

### Identity state-dependent processing of neutral and traumatic scripts in Dissociative Identity Disorder as assessed by PET (Positron Emission Tomography)

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E.R.S.Nijenhuis: Cats\_Polm institute, Bilthoven.

H.P.J.Vos: Dr. Kuno van Dijk foundation, Groningen.

A.M.J.Paans, A.T.M.Willemsen: PET-center, Academic Hospital Groningen.

#### **Abstract**

**Methods:**Seven women, meeting DSM-IV criteria for DID, were, in two different identities, exposed to two auditory autobiographical texts while undergoing 3D PET imaging. Regional cerebral bloodflow (rCBF) was measured with the Siemens EXACT HR+ after intravenous injection of  $H_2^{15}O$  (500 MBq), obtaining 120-sec scans. Four different conditions were obtained twice, resulting in eight scans. The complete scanning sequence was Nn, Nt, Tn, Tt, Tn, Tt, Nn and Nt. The first character indicating the personality state (Neutral or Traumatic) and the second character the content of the text (neutral or traumatic). Data was analysed with statistical parametric mapping using SPM96 (multisubject different conditions with replications), after realignment and spatial normalisation.

#### **Introduction**

Dissociation involves failure to associate information and experience in ways that would be normally expected. In dissociative identity disorder this failure is extreme. Apart from amnesia and depersonalisation DID is characterised by identity fragmentation, related to the existence of dissociative identities.

Patients with DID usually have two or more distinct identities also called personalities, alters, parts or aspects. Each of the identities has its own pattern of perception, reaction and thinking about itself. Switches between states are manifest by nonlinear changes in a number of variables, including access to memory, attention and cognition, regulatory physiology, and a sense of self. These fluctuating states of consciousness result in changing identity notion and changing access to autobiographic memory.

DID can be a consequence of severe and chronic traumatisatisation starting at an early age (childhood trauma). This traumatisatisation can evoke dissociative identity formation resulting in "traumatic" identities with their own particular reaction to, recollection of, or emotional responses to the traumatic memories. Furthermore "neutral" identities exist with no, or in any case less, recollection of these memories. These defensive (neutral) states will be activated by traumatic labeled stimuli.

Clinical observations are that reactions of patients with DID on identical stimulations are dependent on the identity they experience at that moment. This is specific for stimulations that are associated with traumatic memory as reported by the patients. Clinical observations lead also to the notion that if the patient is in an identity which is familiar with traumatic memory and the patient is exposed to the traumatic memory, a higher level of arousal (fear, tension) is present than when the patient is in a identity wherein he or she is not aware of the traumatisatisation.

In "apparently normal" identity states patients can perform functions in daily life. In these states access to traumatic memories is restricted, or totally inhibited, as is emotional responsiveness. Thus, these neutral identities are depersonalised, numbed and amnesic with respect to trauma.

### Patients

Seven female volunteers, between 26 and 60, took part in the study which was approved by the academic hospital ethics committee, Groningen, the Netherlands. Subjects were free of neurologic illness and gave written informed consent to participate in the study after the experimental procedure and radiation risks were explained. Other alter personalities were informed and inquired during a session with the therapist and gave oral agreement to participate.

Entry criteria included Dissociative Identity Disorder according to DSM-IV criteria (American Psychiatric Association, 1994) and determined in the Structured Clinical Interview for Dissociative Disorders (SCID-D, Steinberg; Steinberg, Cichetti, Buchanan, Hall, Rounsaville, 1993). Patients were only asked to participate if they had successfully went through treatment on stabilization and symptomreduction and treatment had progressed to the phase which involves exposure to traumatic memories.

Inclusion criteria required presence of at least two dissociative identities, a trauma related and a non trauma related one, capable of being activated in co-operation with their therapist, who was present at the subject's bedside during the study. Subjects were able to switch between personality states during the investigation. Two personalities participated in the investigation. Both identities subjective aged 10 years or older. The so called traumatic identity, (re)experienced the traumatic event, which occurred at age of 10 or later, and was emotionally affected by the traumatic text. The second identity was emotionally unresponsive to the traumatic text, because of amnesia or depersonalisation. Interference of other identities was absent if one of the participating identities was activated.

Exclusion criteria were pregnancy, traumatic experiences considering a hospital, systemic or neurological illness and not mastering the Dutch language.

### Scripts

Scripts of patients' past personal events were prepared by patient and therapist. One traumatic and one neutral text were developed. The traumatic text described a traumatic event which occurred at age 10 or later in the patients life. The neutral text described an autobiographic neutral event. Each script was audiotaped in a neutral voice by the therapist for playback in the laboratory setting. Both scripts were 120 seconds of duration.

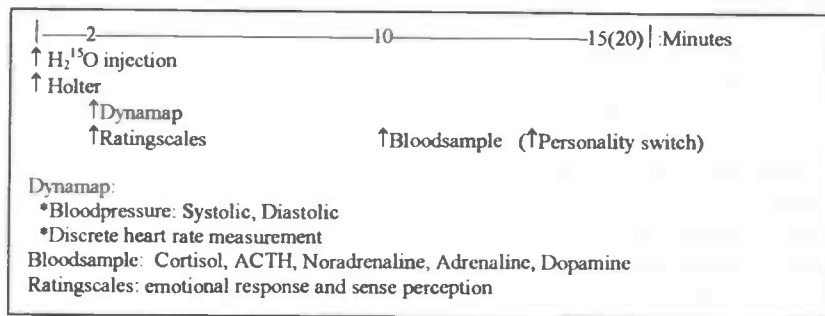
### Emotional ratingscales

Each patient rated the severity of various emotional dimensions. This emotional dimensions consisted of fear, sorrow, sombreness, anger, shame and disgust. The questionnaire was taken for each condition on separate subjective 0- to 10-point analog scales, by the therapist. Anchor points were provided by the therapist, where 0 indicated complete absence and 10 maximum possible for the specified emotion. With regard to reexperiencing phenomena during the study, each patient was asked which sensory modalities were involved, also in a 0- to 10-point analog scale. The questioned sensory modalities consists of images, touch, sounds, smells, tastes, pain, physical deafening, physical stiffening, physical paralysis and physical restlessness.

### PET symptom provocation paradigm

After a first visit, a few days or hours before the PET investigation to habituate to the PET environment, patients with DID were scanned 8 times. After a transmission scan, four different conditions were obtained twice, resulting in eight scans. The complete scanning sequence was Nn, Nt, Tn, Tt, Tn, Tt, Nn and Nt. The first character, capital, indicating the personality state (Neutral or Traumatic) and the second character, minor, the content of the text (neutral or traumatic). Four scans corresponded with the traumatic personality state and four scans corresponded with the neutral personality state. During the four scans each personality state listened to a neutral autobiographic text (two times) and to a traumatic autobiographic text (two times). Time between each text was 15 or 20 minutes, as shown in the time scanning sequence below. Time between scans was 15 minutes. If a personality switch needed to be acquired, 5 more minutes were needed for the switching procedure, time between scans was 20 minutes.

N		T		N		Identity	
n	t	n	t	n	t	n	t
15	20	15	15	15	20	15	15
						:Text	
						:Minutes	



Two frames were obtained. The first frame contained information about radiation remained from the previous scan, the second scan contained count data of patients listening to autobiographic texts. The first frame was used for background radiation correction for the second frame. The first frame started 33 seconds before injection of the radiotracer. As the tracer was injected the second frame started. At the same time the therapist received a sign to start the audiotape and mark the Holter by pressing a button. The audiotape and the second frame had both a duration of 120 seconds. Immediately following the end of the script, the researcher received a sign of the therapist to serve the dynamap. Discrete heart rate frequency, systolic and diastolic blood pressure were measured by means of the Dynamap. At the same time the therapist debriefed the patient regarding her subjective experiences by means of the ratingscales. Blood was drawn by a venous catheter, ten minutes after the start of the scan (audiotape). A personality switch was obtained if the next scan was the third or seventh in order.

### PET Camera

Subjects underwent PET scanning in a Siemens Ecat Exact HR<sup>+</sup> PET scanner, which acquires 63 slices over an axial length of 15.5 cm. The 32 ring BGO detectors returned after reconstruction 32 straight planes and 31 crossplanes over an axial length of 15.5 cm resulting in 63 slices. Before emission scanning, subjects had an attenuation scan (transmission scan) measured by three internal rotating  $^{68}\text{Ge}/^{68}\text{Ga}$  block sources. Each PET data acquisition run consisted of 2 frames, the first of 33 seconds and the second 120 seconds duration. The second scan contained the information to form images of relative bloodflow after background radiation correction using frame one. Data were acquired in the three-dimensional mode.

At the moment of intravenous injection of  $\text{H}_2^{15}\text{O}$  (a dose of 500 MBq), the 120 seconds scan acquisition started. After 23 seconds maximum count rate in the brain was achieved. Images were reconstructed with a 3D reconstruction algorithm using a Hanning filter of cut-off frequency 0.5 cycles-per-pixel and attenuation corrected by means of the transmission scan. Measured coincidences were globally corrected for count loss caused by electronic dead time, not for scattered radiation or random coincidence. The resolution of the raw images was 5 mm. Following reconstruction, images were visually inspected on translations and rotations in Imagetool or CAPP5. The raw PET images were realigned to correct for translation and rotation motions between scans. After realignment the images were transformed into a standard stereotaxic anatomical space (SPM96, MNI template) by use of 3x4x3 non linear basis functions during the normalisation procedure. Signal-to-noise ratio was improved by smoothing with a 2-dimensional gaussian filter of 20 mm in width (full-width half maximum). The effects of the four conditions were estimated on a pixel-by-pixel basis using parametric maps. Global differences were first covariates out, and comparisons of the conditions (multi subject different conditions with replications) were then made using t-statistics. Then computed paired t values were converted into z scores, using Z-statistics, producing statistical parametric maps.

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