DISCOS SPM course, CRC, Liège, 2009

Experimental design

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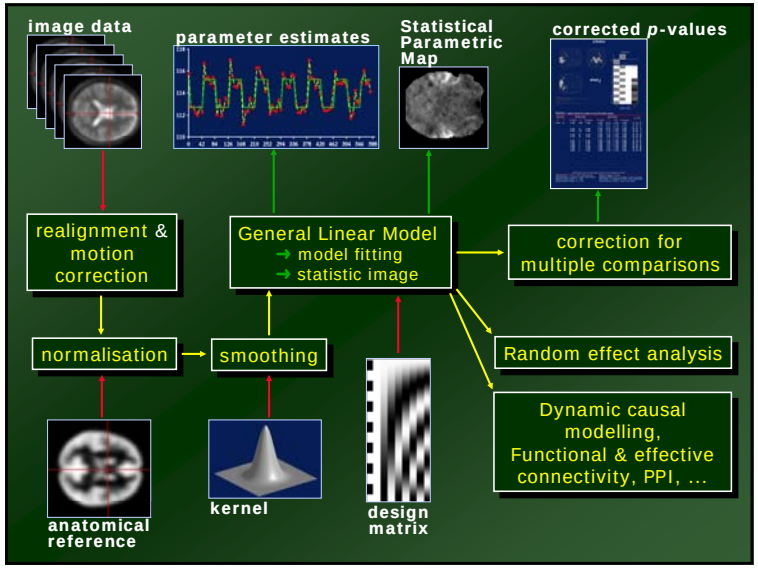
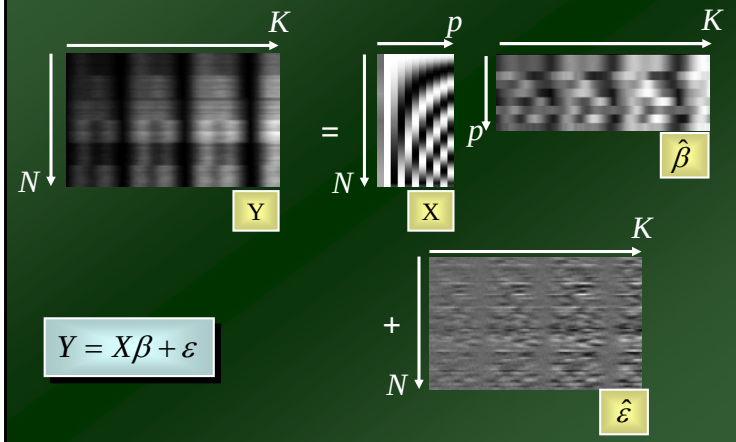
Based on slides from: C. Rush, JB. Poline

Contents

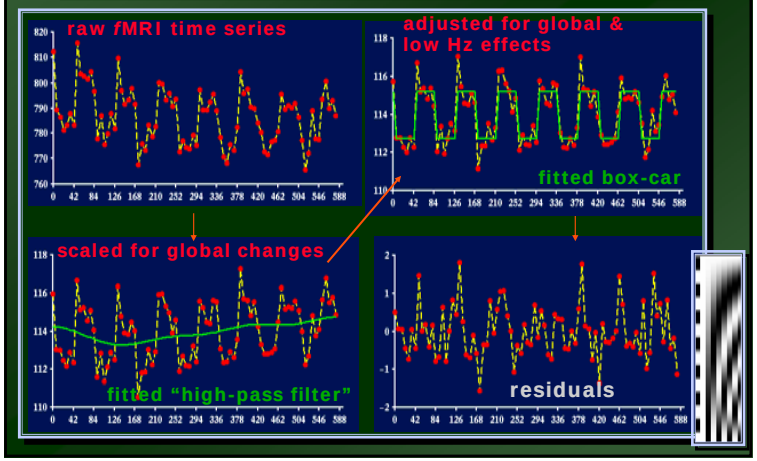
- Introduction & recap
- Experimental design
- « Take home » message



Mass univariate approach



GLM fitted



t-statistic - Computations

$Y = X\beta + \varepsilon$

$\hat{\beta}$: least squares estimates

c = +1 0 0 0 0 0 0 0 0 0 0

$t = \dfrac{c^T\hat{\beta}}{\hat{Std}(c^T\hat{\beta})}$

$\hat{Std}(c^T\hat{\beta}) = \sqrt{\hat{\sigma}^2 c^T X^- V X^{-T} c}$

X

$\hat{\sigma}^2 = \dfrac{\sum(y - X\hat{\beta})^2}{df}$

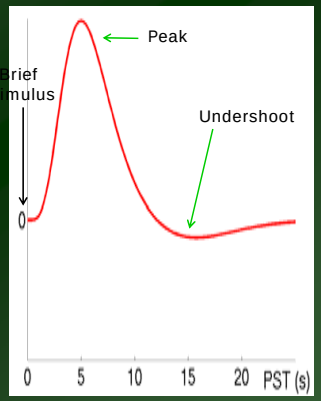
compute ***df*** using Satterthwaite approximation

V

ReML

BOLD Impulse Response

- Function of blood oxygenation, flow, volume (Buxton et al, 1998)
- Peak (max. oxygenation) 4-6s poststimulus; baseline after 20-30s
- Initial undershoot can be observed (Malonek & Grinvald, 1996)
- Similar across V1, A1, S1…
- … but differences across: other regions (Schacter et al 1997) individuals (Aguirre et al, 1998)



Advantages of Event-related fMRI

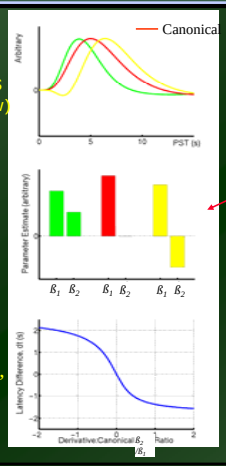
1. **Randomised trial order**
   c.f. confounds of blocked designs
2. **Post hoc / subjective classification of trials**
   e.g, according to subsequent memory
3. **Some events can only be indicated by subject** (in time)
   e.g, spontaneous perceptual changes
4. **Some trials cannot be blocked**
   e.g, "oddball" designs
5. **More accurate models even for blocked designs?**
   e.g, "state-item" interactions

BOLD Response Latency (Linear)

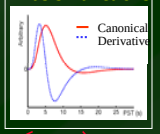
Delayed Responses (green/ yellow)

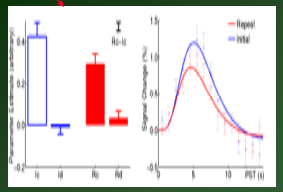
Parameter Estimates

Actual latency, *dt*, vs. β_2 / β_1



Basis Functions





Face repetition reduces latency as well as magnitude of fusiform response

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Experimental design

- Categorical designs
  - Subtraction - Pure insertion, evoked / differential responses
  - Conjunction - Testing multiple hypotheses
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- Factorial designs
  - Categorical - Interactions and pure insertion
  - Parametric - Linear and nonlinear interactions

Subtraction Logic

Cognitive subtraction originated with reaction time experiments (F. C. Donders, a Dutch physiologist).

Measure the time for a process to occur by comparing two reaction times, one which has the same components as the other + the process of interest.


Franciscus Cornelis Donders (1818-1889)

Example:

T1: Hit a button when you see a light
T2: Hit a button when the light is green but not red
T3: Hit the left button when the light is green and the right button when the light is red

T2 – T1 = time to make discrimination between light color

T3 – T2 = time to make a decision

Assumption of pure insertion: You can insert a component process into a task without disrupting the other components.
Widely criticized (we'll come back to this when we talk about parametric studies)

Activation and Baseline Conditions

Aim:

To reveal brain activation related to a cognitive or sensori-motor process of interest (PI)

Cognitive Subtraction:

Contrast Activation task (engages PI) to a Baseline task (no PI).
Difference = Brain regions associated with PI.

Example:

PI = Object recognition

Activation task: with PI *Baseline task: no PI*

Difference = Brain regions associated with Object Recognition

Cognitive Subtractions:
Stimulus or task changes

Stimulus Change

Activation condition *Baseline condition*

Task: (constant) "View picture" "View picture"

= **Object Recognition**

Stimulus (constant)

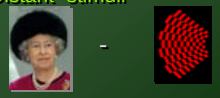
Activation condition *Baseline condition*

Task: Change: "Name Object" "Say:"Yes"

= **Name Retrieval**

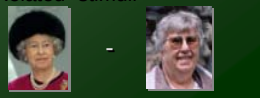
Cognitive Subtraction:
Baseline-problems

• „Distant" stimuli



➔ Several components differ!

• „Related" stimuli



„Queen!" „Aunt Jenny?"

➔ *P* implicit in control task ?

• Same stimuli, different task

Name Person! Name Gender!

➔ Interaction of process and task ?

Cognitive Subtractions:
Serial subtraction

Baseline condition for one contrast acts as activation condition for another contrast

Example:

Condition A. *Condition B.* *Condition C.*

Stimulus:

Task: **Name Object** **Say:"Yes"** **Say:"Yes"**

A-B = Name Retrieval
B-C = Object Recognition

Very limited...

Problems with Serial Subtractions

	Condition A	Condition B	Condition C
Stimulus:			
Task:	Say: Name of Object	Say: "Yes"	Say: "Yes"

Assumptions:

A - B = only changes processing associated with Name Retrieval

B - C = only changes processing associated with Object Recognition

BUT

1. There may be *implicit* naming in condition B. In which case: naming component is removed from A-B and introduced into B-C.
2. Name Retrieval may increase the demands on object recognition (*Interactions*).

i.e A - B : May reveal Object recognition NOT Name retrieval.

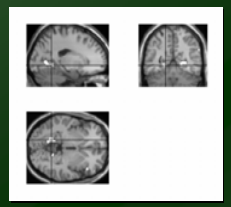
B - C : May reveal Object Recognition AND Name Retrieval

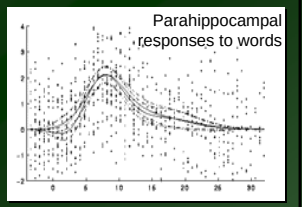
Implicit processing and interactions between processing components make it difficult to find baseline tasks that control for all but the process of interest.

Evoked responses

Differential event-related fMRI

SPM{F} testing for evoked responses





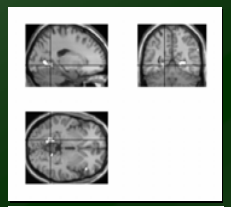
BOLD EPI fMRI at 2T, TR 3.2sec. Words presented every 16 secs; (i) studied words or (ii) new words

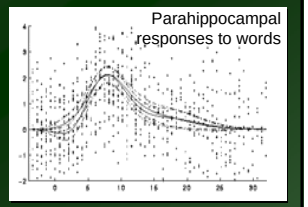
- "Baseline" here corresponds to session mean (and thus processing during "rest")
- Null events or long SOAs essential for estimation
- "Cognitive" interpretation hardly possible, but useful to define regions generally involved in the task

Differential responses

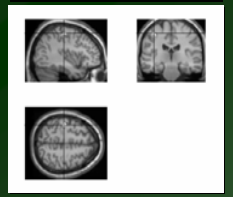
Differential event-related fMRI

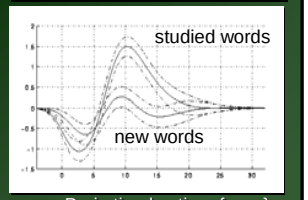
SPM{F} testing for evoked responses





SPM{F} testing for differences





Peri-stimulus time {secs}

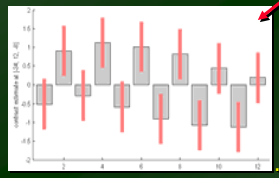
BOLD EPI fMRI at 2T, TR 3.2sec. Words presented every 16 secs; (i) studied words or (ii) new words

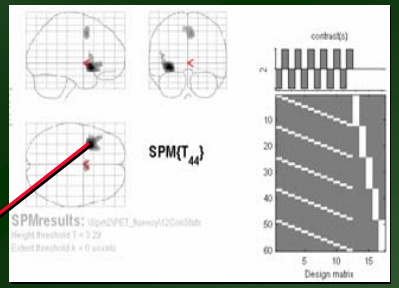
A categorical analysis

Experimental design

Word generation	G
Word repetition	R

R G R G R G R G R G





G - R = Intrinsic word generation

...under assumption of pure insertion

Overview

- Categorical designs
  - Subtraction - Pure insertion, evoked / differential responses
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Conjunctions

- One way to minimise the baseline/pure insertion problem is to isolate the same process by two or more separate comparisons, and inspect the resulting simple effects for commonalities
- A test for such activation common to several independent contrasts is called "Conjunction"
- Conjunctions can be conducted across a whole variety of different contexts:
  - tasks
  - stimuli
  - senses (vision, audition)
  - etc.
- But the contrasts entering a conjunction have to be truly independent!

CONJUNCTION DESIGNS

Conjoint effects from multiple contrasts.

	A. Name Object	B. View Nonobject	
Contrast 1	 —		**A - B =** **Name retrieval** *confounded with* ***object recognition***
	C. Name Colour	D. View Nonobject	
Contrast 2	 —		**C - D =** **Name retrieval** *confounded with* ***colour processing***
	E. Name Word	F. View XXXs	
Contrast 3	BUS —	XXX	**E - F =** **Name retrieval** *confounded with* ***word recognition.***

Name Retrieval = Areas activated by Conjunction of A-B and C-D and E-F

Conjunctions

Example:

Which neural structures support object recognition, independent of task (naming vs viewing)?

Visual Processing	**V**
Object Recognition	**R**
Phonological Retrieval	**P**

Task (1/2)

Stimuli (A/B)	Viewing	Naming
Colours	A1	A2
Objects	B1	B2

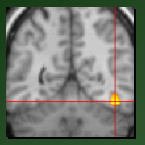
(Object - Colour viewing) & (Object - Colour naming)

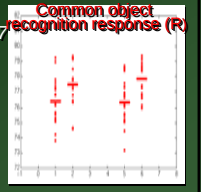
➔ [1 -1 0 0] & [0 0 1 -1]

➔ **[R,V - V] & [P,R,V - P,V]**

= R & R = R

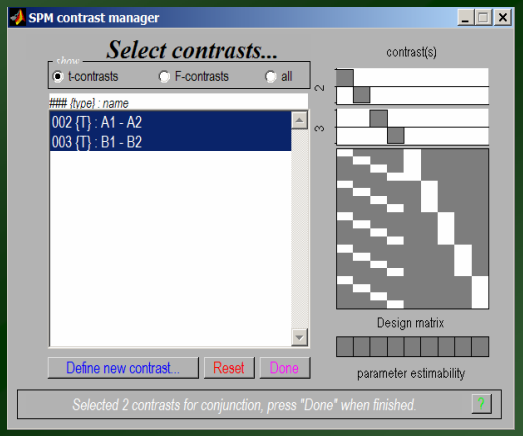
Price et al, 1997





(assuming no interaction RxP; see later)

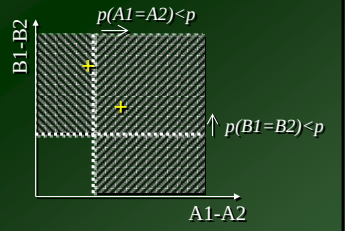
Conjunctions



Two flavours of inference about conjunctions

SPM5/8 offers two general ways to test the significance of conjunctions:

- Test of global null hypothesis: Significant set of consistent effects
  - ➔ *"which voxels show effects of similar direction (but not necessarily individual significance) across contrasts?"*
- Test of conjunction null hypothesis: Set of consistently significant effects
  - ➔ *"which voxels show, for each specified contrast, effects > threshold?"*
- Choice of test depends on hypothesis and congruence of contrasts; the global null test is more sensitive (i.e., when direction of effects hypothesised)



Friston et al. (2005). *Neuroimage*, 25:661-7.

Nichols et al. (2005). *Neuroimage*, 25:653-6

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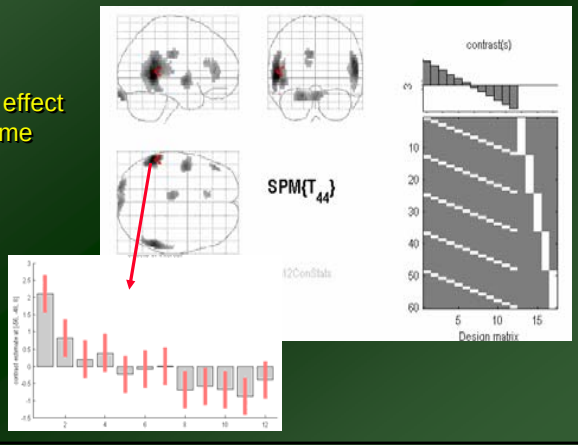
Parametric Designs: General Approach

- Parametric designs approach the baseline problem by:
  - Varying the stimulus-parameter of interest on a continuum, in multiple (n>2) steps...
  - ... and relating blood-flow to this parameter
- Possible tests for such relations are manifold:
  - Linear
  - Nonlinear: Quadratic/cubic/etc.
  - „Data-driven" (e.g., neurometric functions)

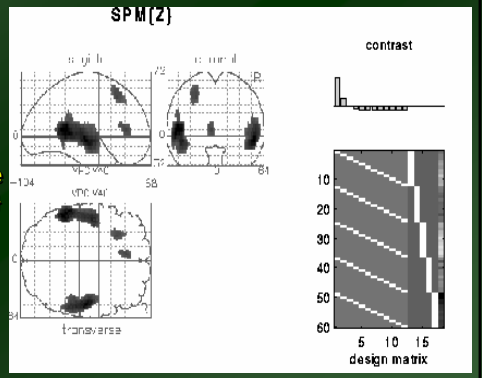
A linear parametric contrast

Linear effect of time

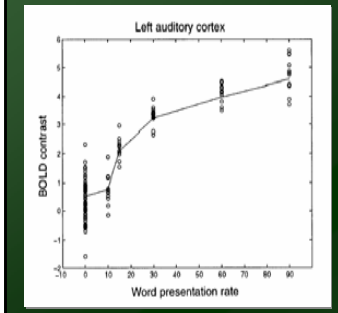


A nonlinear parametric contrast

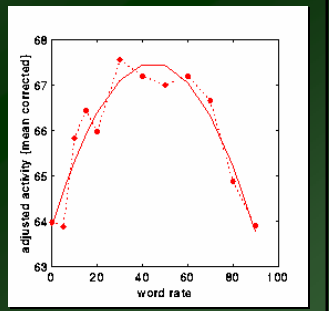
The nonlinear effect of time assessed with the SPM{T}



Nonlinear parametric design matrix



versus



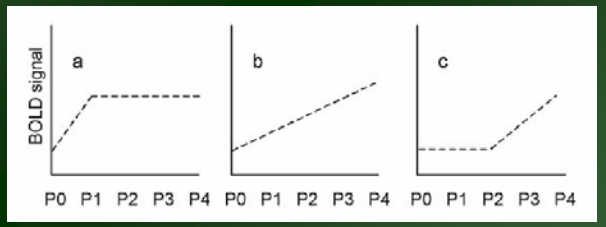
Inverted 'U' response to increasing word presentation rate in the DLPFC

Rees, G., et al. (1997). *Neuroimage, 6*: 27-78

Parametric Designs: Neurometric functions

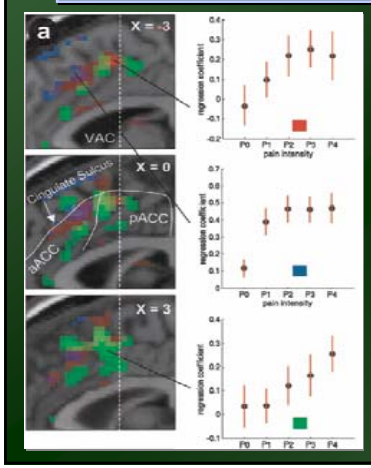
Coding of tactile stimuli in Anterior Cingulate Cortex:
Stimulus (a) presence, (b) intensity, and (c) pain intensity

- **Variation of intensity of a heat stimulus applied to the right hand (300, 400, 500, and 600 mJ)**
- **Assumptions:**

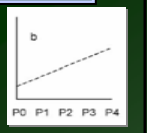


Büchel et al. (2002). *The Journal of Neuroscience, 22*: 970-6

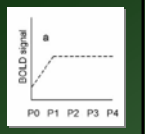
Parametric Designs: Neurometric functions



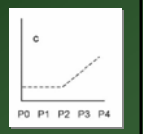
→ Stimulus intensity



→ Stimulus presence



→ Pain intensity

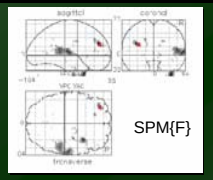


Büchel et al. (2002). *The Journal of Neuroscience*, 22: 970-6

Nonlinear parametric design matrix

E.g, F-contrast [0 1 0] on Quadratic Parameter =>

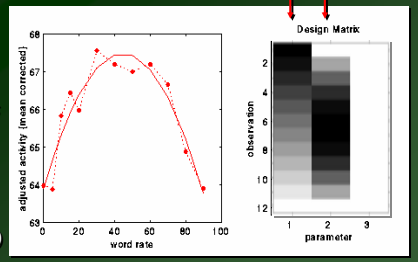
Inverted 'U' response to increasing word presentation rate in the DLPFC



Polynomial expansion:
$f(x) \sim b_1 x + b_2 x^2 + ...$

...up to (N-1)th order for N levels

(SPM8 GUI offers polynomial expansion as option during creation of parametric modulation regressors)



Correlation design

- No need to find baseline that controls for all but the process of interest
- Segregates areas showing differential effects (linear and nonlinear effects)

But:

- Common effects can not be revealed without a baseline.
- Limited to continuous variables (e.g. duration, frequency, word length, R.T.s etc)

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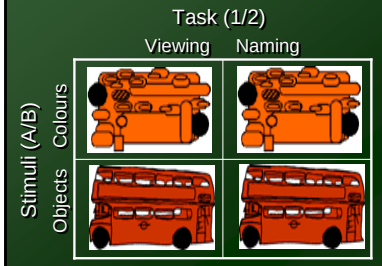
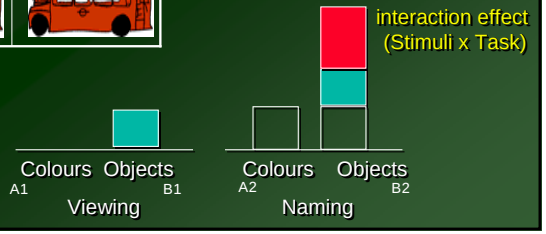
Factorial designs: Main effects and Interactions



- **Main effect of task:**
  (A1 + B1) – (A2 + B2)
- **Main effect of stimuli:**
  (A1 + A2) – (B1 + B2)
- **Interaction of task and stimuli:**
  Can show a failure of pure insertion
  (A1 – B1) – (A2 – B2)

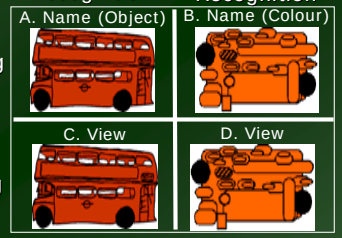


Factorial Designs

dissociate interactions

B2 – B1 =
Object Recognition
during naming

A2 – A1 =
Object Recognition
during viewing



The Interaction effect
(B2-B1) - (A2-A1)
i.e. The effect of Naming on Object recognition
(B2-A2) - (B1-A1)
i.e. The effect of object recognition on Naming.

Conjunction of B2-B1 and A2-A1 reveals areas involved in object recognition irrespective of naming.
Conjunction of B2-A2and B1-A1 reveals areas involved in naming irrespective of object recognition.

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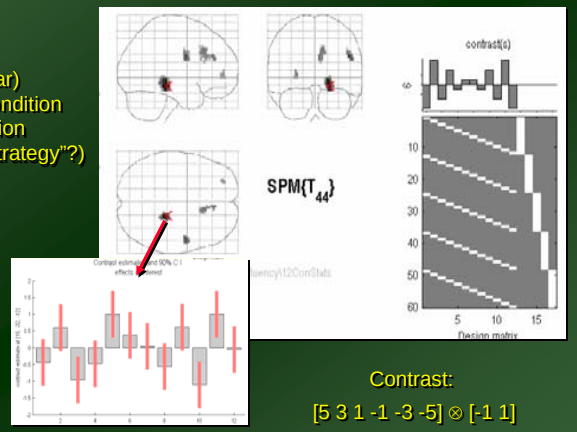
Linear Parametric Interaction

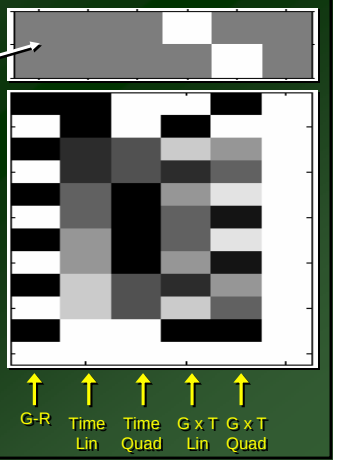
A (Linear)
Time-by-Condition
Interaction
("Generation strategy"?)



Contrast:
[5 3 1 -1 -3 -5] ⊗ [-1 1]
= [-5 5 -3 3 -1 1 1 -1 3 -3 5 -5]

Nonlinear Parametric Interaction

F-contrast tests for nonlinear Generation-by-Time interaction (including both linear and Quadratic components)



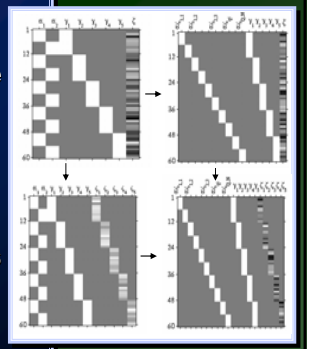
Factorial Design with 2 factors:

1. Gen/Rep (Categorical, 2 levels)
2. Time (Parametric, 6 levels)

Time effects modelled with both linear and quadratic components…

Model selection

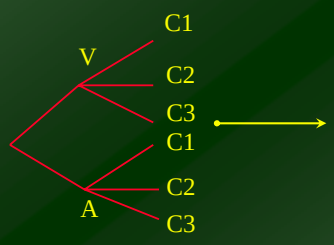
- **Model must fit** *i.e. model assumptions met*
  - **at *every* voxel**
- **Omitting relevant effects**
  - **effects contribute to variance**
    - ⇒ **residuals not *iid*. Normal**
    - ☹ **model not valid**
  - **outcomes?**
    - **variance ↑** *(usually, but can ↓)*
    - **increased residual *d.f.***
    - **invalid inference**
- **Including irrelevant effects**
  - 😐 **"waste" degrees of freedom**
  - 😐 **conservative tests**
  - ✪ **but safest!**

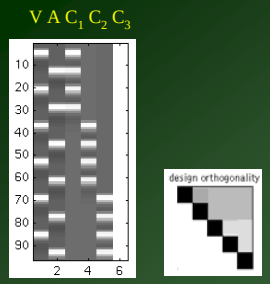


A real example (almost !)

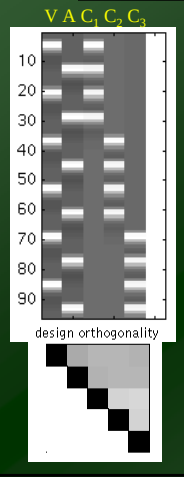
Experimental Design ⟶ Design Matrix

Factorial design with 2 factors : modality and category
2 levels for modality (eg. Visual/Auditory)
3 levels for category (eg. 3 categories of words)





Asking ourselves some questions ...



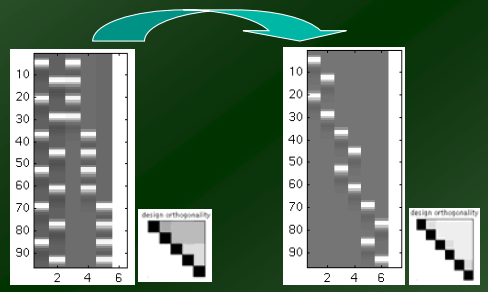
Test C1 > C2 : c = [0 0 1 -1 0 0]
Test V > A : c = [1 -1 0 0 0 0]

Test C1,C2,C3 ? (F) c = [0 0 1 0 0 0]
[0 0 0 1 0 0]
[0 0 0 0 1 0]

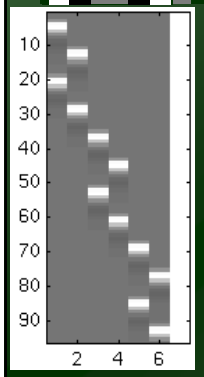
Test the interaction MxC ?

- Design Matrix not orthogonal
- Many contrasts are non estimable
- Interactions MxC are not modelled

Modelling the interactions



Asking ourselves some questions ...



Test C1 > C2 : c = [1 1 -1 -1 0 0 0]

Test V > A : c = [1 -1 1 -1 1 -1 0]

Test the category effect :

c = [1 1 -1 -1 0 0 0]
[0 0 1 1 -1 -1 0]
[1 1 0 0 -1 -1 0]

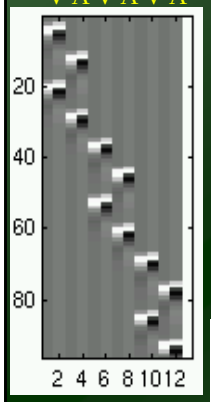
Test the interaction MxC :

c = [1 -1 -1 1 0 0 0]
[0 0 1 -1 -1 1 0]
[1 -1 0 0 -1 1 0]

- Design Matrix orthogonal
- All contrasts are estimable
- Interactions MxC modelled
- If no interaction ... ? Model is too “big” !

Asking ourselves some questions ... With a more flexible model

$C_1\ C_1\ C_2\ C_2\ C_3\ C_3$
V A V A V A



Test C1 > C2 ?
Test C1 different from C2 ?
from

c = [1 1 -1 -1 0 0 0]

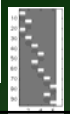
to

c = [1 0 1 0 -1 0 -1 0 0 0 0 0 0]
[0 1 0 1 0 -1 0 -1 0 0 0 0 0]

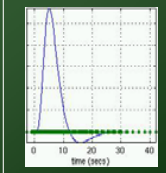
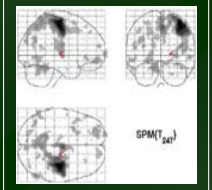
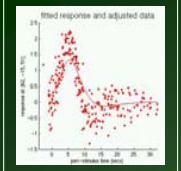
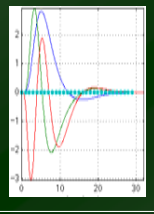
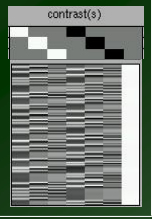
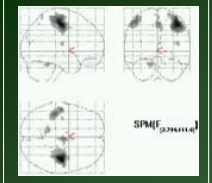
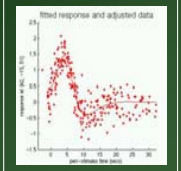
becomes an F test!

Test V > A ?

c = [1 0 -1 0 1 0 -1 0 1 0 -1 0 0]



is possible, but is OK only if the regressors coding for the delay are all equal

Convolution model	Design and contrast	SPM(t) or SPM(F)	Fitted and adjusted data
			
			

Toy example: take home ...

- *F tests have to be used when*
  - *- Testing for >0 and <0 effects*
  - *- Testing for more than 2 levels*
  - *- Conditions are modelled with more than one regressor*
- *F tests can be viewed as testing for*
  - *- the additional variance explained by a larger model wrt a simpler model, or*
  - *- the sum of the squares of one or several combinations of the betas (here the F test b1 – b2 is the same as b2 – b1, but two tailed compared to a t-test).*

Contents

Conclusions...

- **General Linear Model**
  - **(simple) standard statistical technique**
    - **temporal autocorrelation – *a Generalised Linear Model***
  - **single general framework for many statistical analyses**
    - **flexible modelling ⇐ basis functions**
  - **design matrix visually characterizes model**
    - **fit data with combinations of columns of design matrix**
  - **statistical inference: *contrasts*...**
    - ***t*–tests: planned comparisons of the parameters**
    - ***F*–tests: general linear hypotheses, model comparison**

Way to proceed

Prepare your questions.
ALL the questions !

Find a model which
- allows contrasts that translates these questions.
- takes into account ALL the effects (interaction, sessions,etc)

Devise task & stimulus presentation.

Acquire the data & analyse.

Not the other way round!!!

Three Stages of an Experiment

1. Sledgehammer Approach
   - brute force experiment : powerful stimulus & don't try to control for everything
   - look at was done before or by others
   - run a couple of subjects -- see if it looks promising
   - if it doesn't look great, tweak the stimulus or task
   - try to be a subject yourself so you can notice any problems with stimuli or subject strategies

Three Stages of an Experiment

1. Sledgehammer Approach
2. Real Experiment
   - at some point, you have to stop changing things and collect enough subjects run with the same conditions to publish it
   - how many subjects do you need
     - some psychophysical studies test two or three subjects, many studies test 6-10 subjects, random effects analysis requires at least 15 subjects,...
     - some subjects WILL be rejected, so acquire more than the minimum !
   - can run all subjects in one or two days
     - pro: minimize setup and variability
     - con: "bad magnet day" means a lot of wasted time
     - make sure all the data are treated the "same way". (script)

Three Stages of an Experiment

1. Sledgehammer Approach
2. Real Experiment
3. "Whipped Cream" experiment
   - after the real experiment works, then think about a "whipped cream" version
   - going straight to whipped cream is a huge endeavor, especially if you're new to imaging
   - and it gives you a second paper !