Mixed Design Repeated Measures ANOVA & Multilevels Linear Model: Analysis of Longitudinal ERP Data

Rui QIN

Outline

- Problem description
  - What is dyslexia?
  - Design of the experiment
  - Data structure
  - Research questions and hypotheses
- Statistical analyses using mixed design repeated measures ANOVA
- Statistical analyses using multilevels linear model

Problem Background

- Dyslexia : specific reading difficulty
  - *dys (inadequate)+ lexis (word)*
  - A learning disorder: severe difficulty in recognizing and comprehending written words
  - Genetic origin of dyslexia:

  Prevalence in general population: 3 to 10% (EU HLG Literacy Report, 2012)

  Prevalence in families with a history of dyslexia: 40-60% (Grigorenko, 2001)

- The key to minimizing the detrimental effects of dyslexia:
  - Diagnosis and intervention at the youngest possible age

- In search of early precursors of dyslexia:
  - Longitudinal Dutch Dyslexia Program (Ben Maassen et al., 1997-2011)

Problem Background

- Longitudinal Dutch Dyslexia Program
  - Participants:
    - Children at familial risk of dyslexia (with at-least one dyslexic parent)
    - Control children: age controls without familial risk of dyslexia
  - Testing scheme:
    - ERP measurement at particular time points, eg., 17 month and 29 month
    - Reading test in Grade 4/5
  - Statistical analyses
    - **Difference between the ERP response of at-risk and control children**
    - Correlation between early ERP measures and reading outcome

Problem background

- ERP (Event-related potentials)
  - Brain activities time-locked to the onset of specific experimental stimuli, measured with electrodes placed on the scalp.
  - Recorded while subjects are actively processing or merely exposed to specific stimuli
  - Offline analyses of the ERPs:

    How does the brain process the stimuli,

    consciously or subconsciously?

Experiment Design

- Early indicators of dyslexia in **auditory perception**:
  - Rapid auditory processing theory (Tallal, 1980; Tallal et al., 1993)

  Deficient auditory perception is the underlying cause of dyslexia.

  Dyslexic children cannot perceive subtle phonemic differences

  e.g., bak vs. dak
- Mismatch Response (MMR):
  - An ERP component indexing the accuracy of auditory discrimination
- Paradigm: Oddball paradigm
  - bak bak bak bak dak bak bak dak bak bak bak dak bak dak bak…
  - MMR = standard (bak) – deviant (dak)
  - In dyslexic individuals, the MMR is expected to be absent or attenuated

Data structure

Status	ID	17 month				29 month			
		Left		Right		Left		Right	
		Stand.	Dev.	Stand.	Dev.	Stand.	Dev.	Stand.	Dev.
Control Group (n=28)	1								
	2								
	3								
	…								
	28								
At-risk Group (n=35)	29								
	30								
	31								
	…								
	63								

Research Questions and Hypotheses

Research Questions and Hypotheses:

1. Do at-risk children differ from control children in their mismatch response (standard vs. deviant)

   Hypothesis: At-risk: Standard – Deviant < Control: Standard – Deviant

2. Do at-risk children differ from control children in the lateralization of their ERP response

   Hypothesis: Control: Left > Right

   At-risk: Left = Right or Left < Right

3. Do at-risk children differ from control children in the development of ERP response over time?

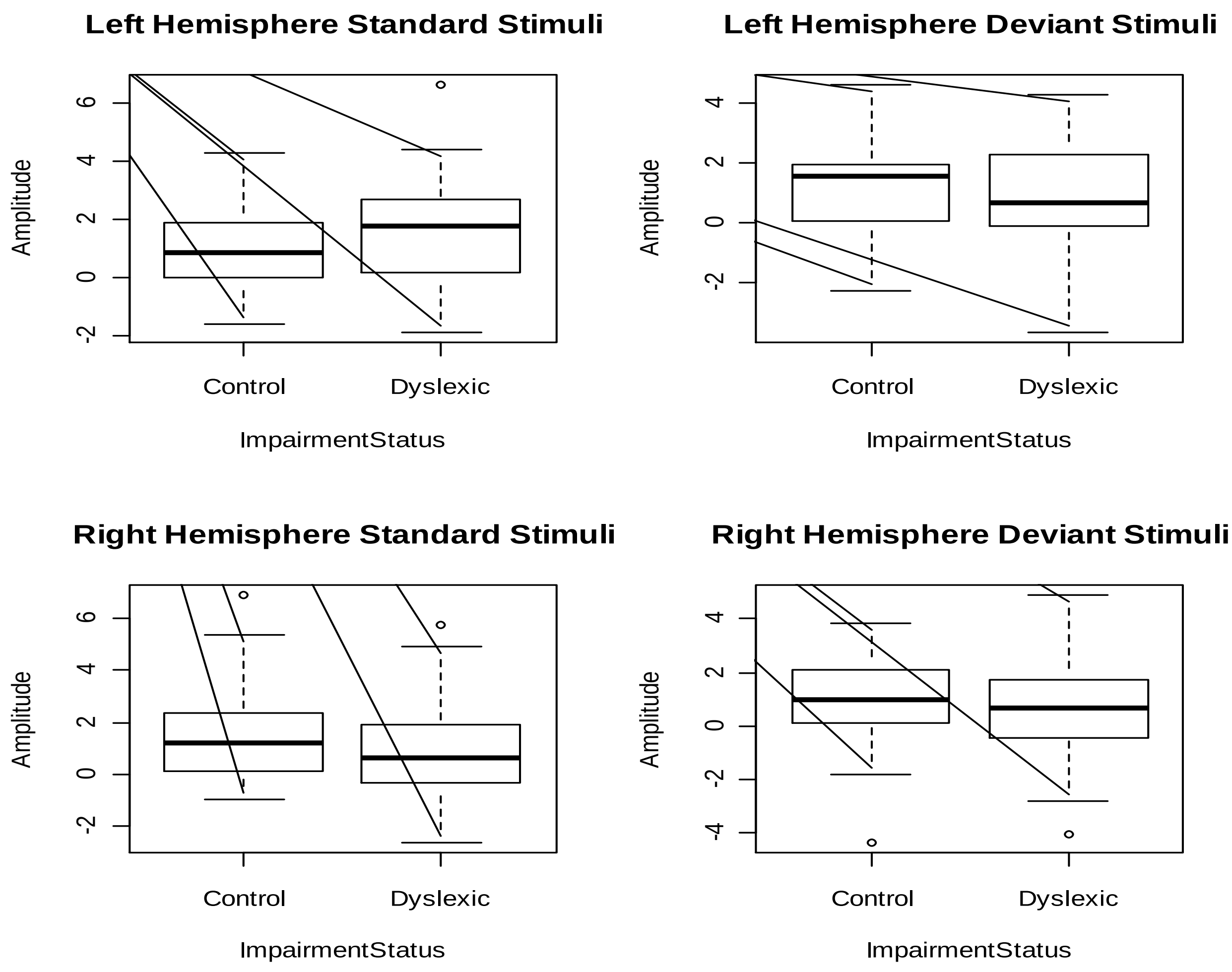
   Hypothesis: At-risk: Age 29 month – Age 17month < Control: Age 29 month – Age 17month

Mixed Design ANOVA with Repeated Measures

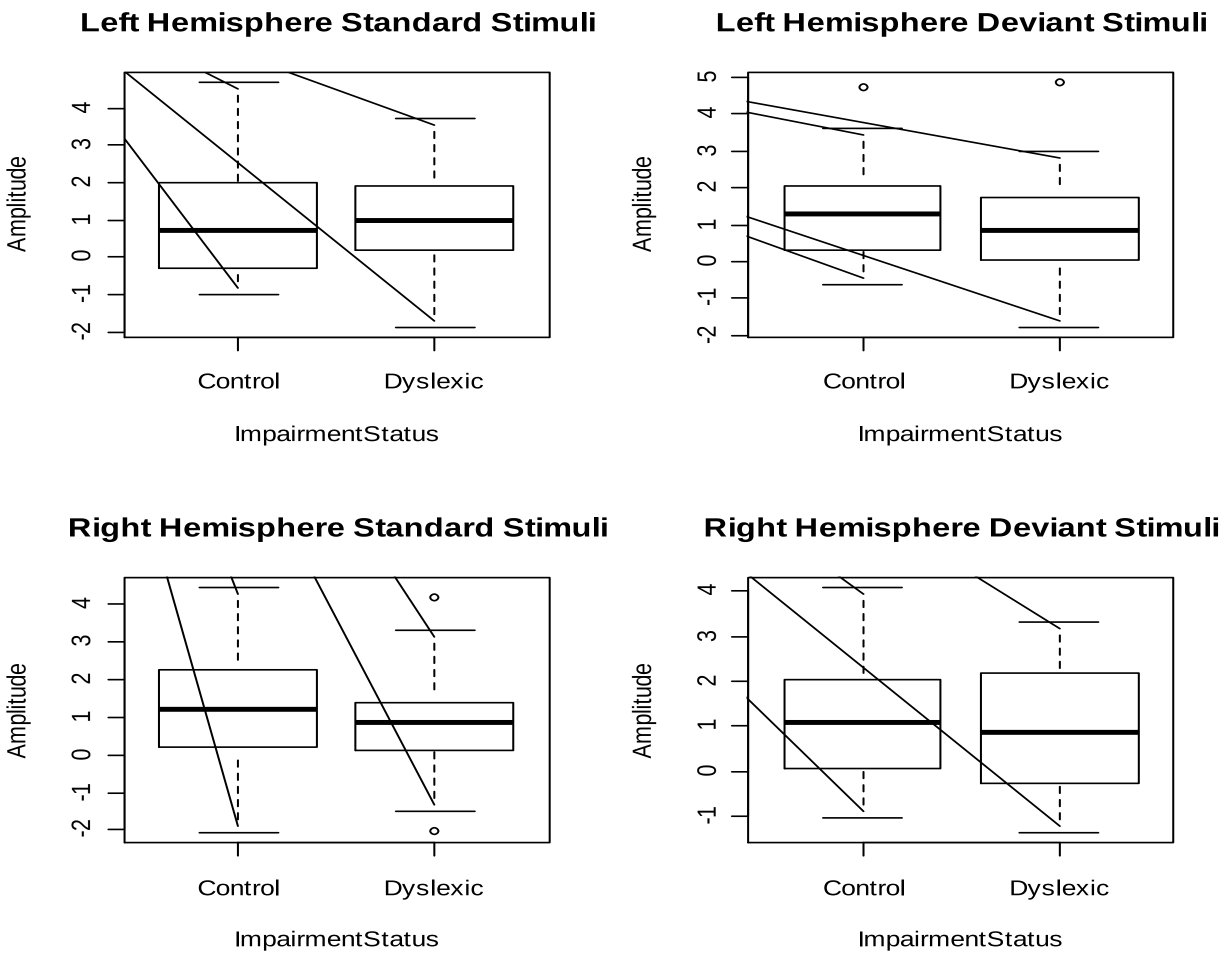
- Dependent variables:
  - Mean amplitude of ERP responses
- Independent variables:
  - Between-subject factors:
    - Impairment Status: at-risk vs. control
  - Within-subject factors:
    - Location: left – right
    - Stimulus: standard vs. deviant
    - Age: 17 month vs. 29 month

    => mixed ANOVA with repeated measures

Visualize the data-17 month



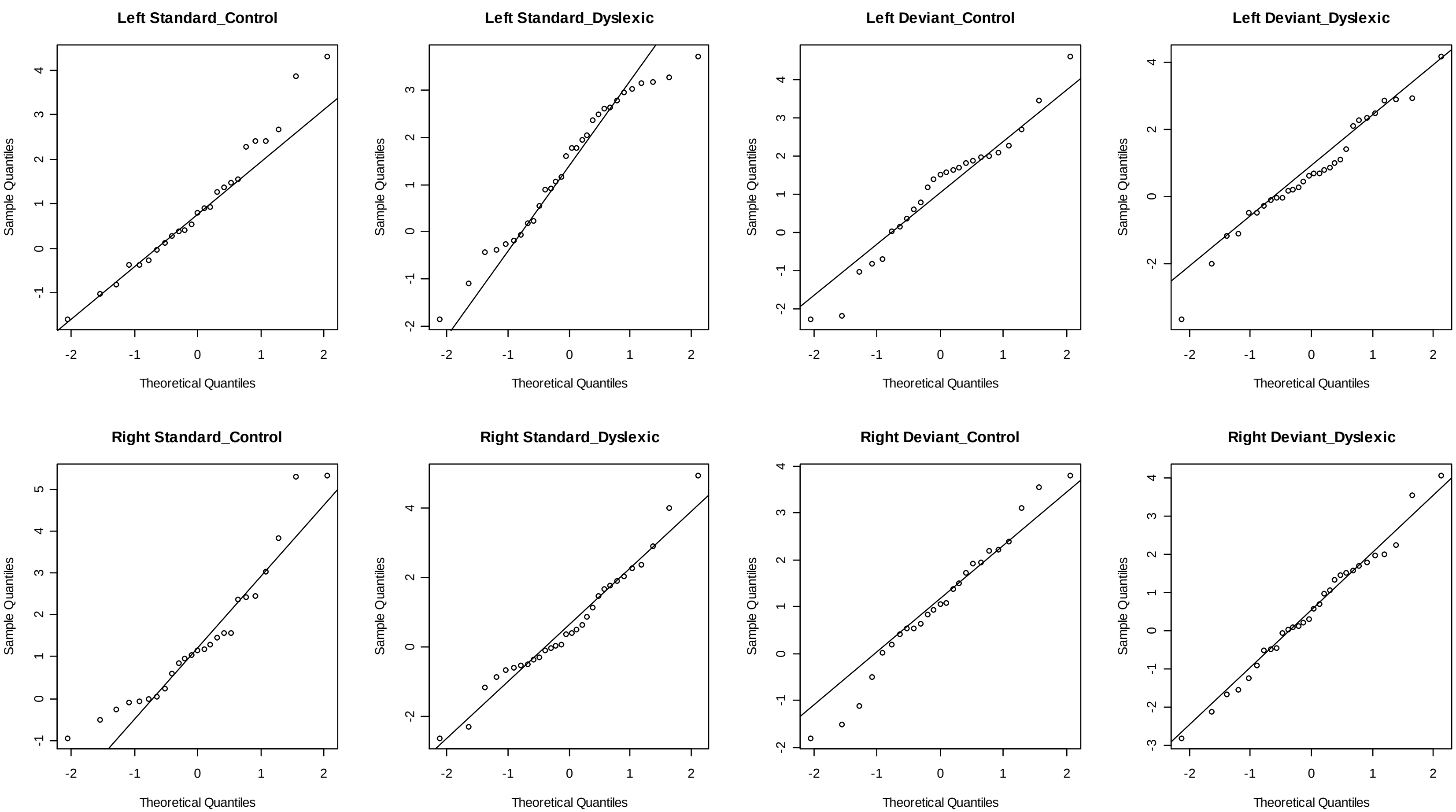
Visualize the data-29 month



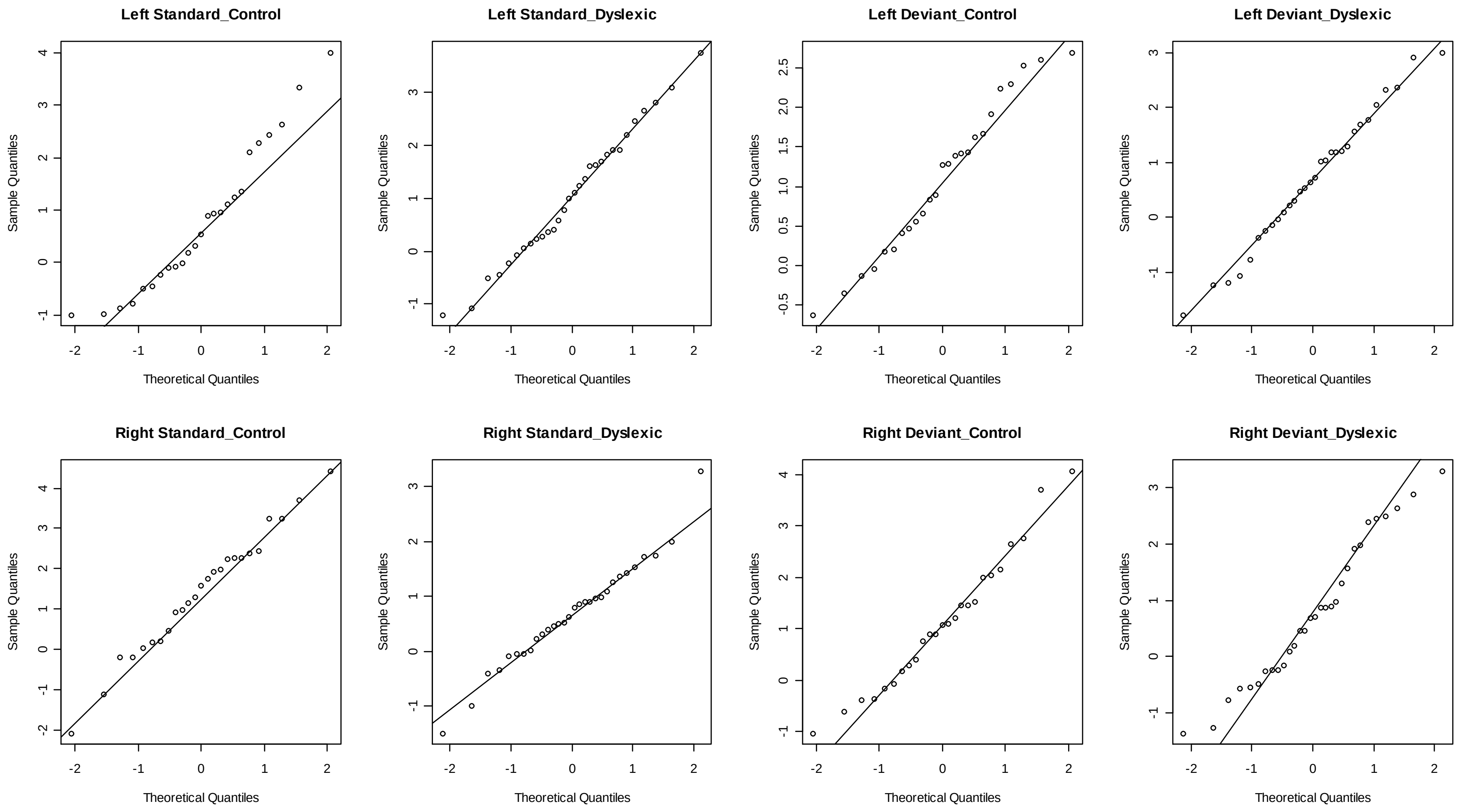
Mixed Design Repeated Measures ANOVA

- Testing the assumptions of ANOVA
  - **Independent** observations -> Repeated measures
  - **Sphericity** for within-subject factors that have more than two levels
  - **Normal distribution** in each condition
  - **Homogeneity of variances** in each condition

Testing assumptions of ANOVA: Normality QQ plot—17 month



Testing assumptions of ANOVA: Normality
QQ plot—29 month



Testing assumptions of ANOVA: Normality Shapiro Test—17 month

- Normal distribution in each subgroup: 17 month

Condition	Impairment Status	Sample Size	Shapiro -Wilk	P-value
Left-Standard	Control	25	0.968	0.587
	Dyslexic	30	0.957	0.260
Left-Deviant	Control	25	0.961	0.433
	Dyslexic	30	0.972	0.608
Right-Standard	Control	25	0.909	0.028*
	Dyslexic	30	0.969	0.522
Right-Deviant	Control	25	0.981	0.906
	Dyslexic	30	0.987	0.966

Testing assumptions of ANOVA: Normality Shapiro Test—29 month

- Normal distribution in each subgroup: 29 month

Condition	Impairment Status	Sample Size	Shapiro -Wilk	P-value
Left-Standard	Control	25	0.937	0.127
	Dyslexic	30	0.984	0.927
Left-Deviant	Control	25	0.968	0.584
	Dyslexic	30	0.985	0.934
Right-Standard	Control	25	0.987	0.980
	Dyslexic	30	0.979	0.795
Right-Deviant	Control	25	0.969	0.612
	Dyslexic	30	0.962	0.339

Testing assumptions of ANOVA: Homogeneity of Variances

- Levene's test

Age	Condition	F- value	Df1	Df2	P
17 month	Left-Standard	0.279	1	53	0.599
	Left-Deviant	4e-o4	1	53	0.984
	Right-Standard	0.07	1	53	0.792
	Right-Deviant	0.346	1	53	0.559
29 month	Left-Standard	0.172	1	53	0.680
	Left-Deviant	1.277	1	53	0.264
	Right-Standard	5.864	1	53	0.019*
	Right-Deviant	0.024	1	53	0.877

Apply Mixed Design ANOVA with Repeated Measures

```
> library(ez)
> m<-ezANOVA(data=dataLR,dv=.(Amplitude),wid=.(SubjectID),between=.(ImpairmentStatus),within=.(Age,Location,Stimulus),type=3)
Warning: Data is unbalanced (unequal N per group). Make sure you specified a well-considered value for the type argument to ezANOVA().
> print(m)
$ANOVA
                                Effect DFn DFd            F          p p<.05          ges
2                     ImpairmentStatus   1  53 2.5249949287 0.11800342       1.189508e-02
3                                  Age   1  53 0.0134218176 0.90820724       4.598036e-05
4                 ImpairmentStatus:Age   1  53 0.0022900933 0.96201174       7.845683e-06
5                             Location   1  53 0.1337295724 0.71605155       2.401529e-04
6            ImpairmentStatus:Location   1  53 6.2068251761 0.01589112     * 1.102603e-02
7                             Stimulus   1  53 1.2006981441 0.27813654       3.345912e-03
8            ImpairmentStatus:Stimulus   1  53 0.6617626579 0.41957801       1.846867e-03
9                         Age:Location   1  53 0.9673442904 0.32981211       1.714537e-03
10       ImpairmentStatus:Age:Location   1  53 0.2444573211 0.62304842       4.338362e-04
11                        Age:Stimulus   1  53 0.8889462006 0.35004115       1.291416e-03
12       ImpairmentStatus:Age:Stimulus   1  53 0.1485492856 0.70146819       2.160373e-04
13                   Location:Stimulus   1  53 0.0357160415 0.85082495       5.221680e-05
14  ImpairmentStatus:Location:Stimulus   1  53 5.9287955037 0.01829464     * 8.593848e-03
15               Age:Location:Stimulus   1  53 0.1307463609 0.71909702       1.816956e-04
16 ImpairmentStatus:Age:Location:Stimulus   1  53 0.0002003407 0.98876013       2.784600e-07
```

ANOVA Output

- Main effect:

Effect	DFn	DFd	F-value	P-value
ImpairmentStatus	1	53	2.525	0.118
Age	1	53	0.013	0.908
Location	1	53	0.134	0.716
Stimulus	1	53	1.201	0.278

ANOVA Output

- 2-way interaction

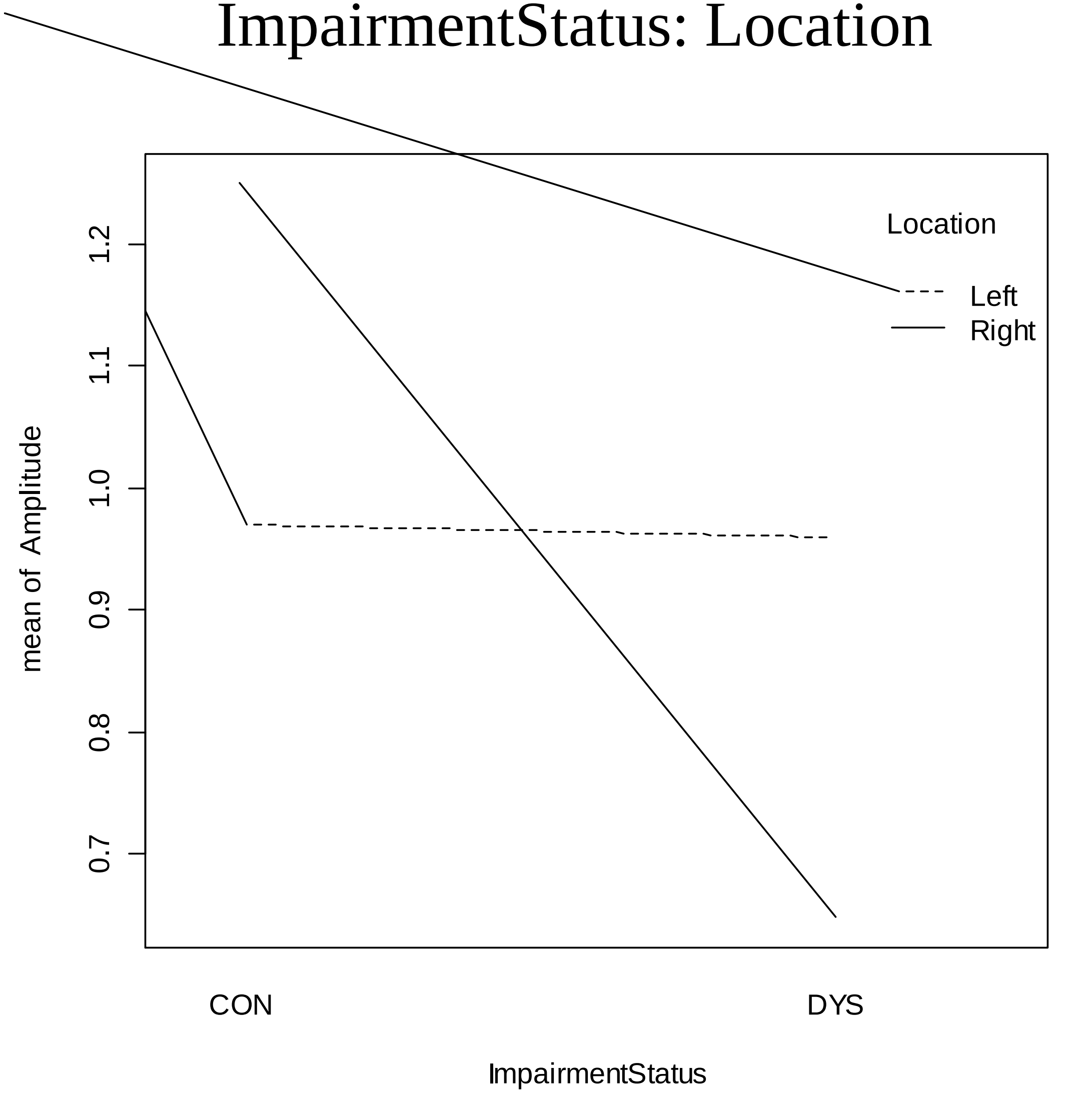
Effect	DFn	DFd	F-value	P-value
ImpairmentStatus: Age	1	53	0.002	0.962
ImpairmentStatus: Location	1	53	6.207	0.016*
ImpairmentStatus: Stimulus	1	53	0.662	0.420
Age: Location	1	53	0.967	0.330
Age: Stimulus	1	53	0.889	0.350
Location: Stimulus	1	53	0.035	0.851

ANOVA Output

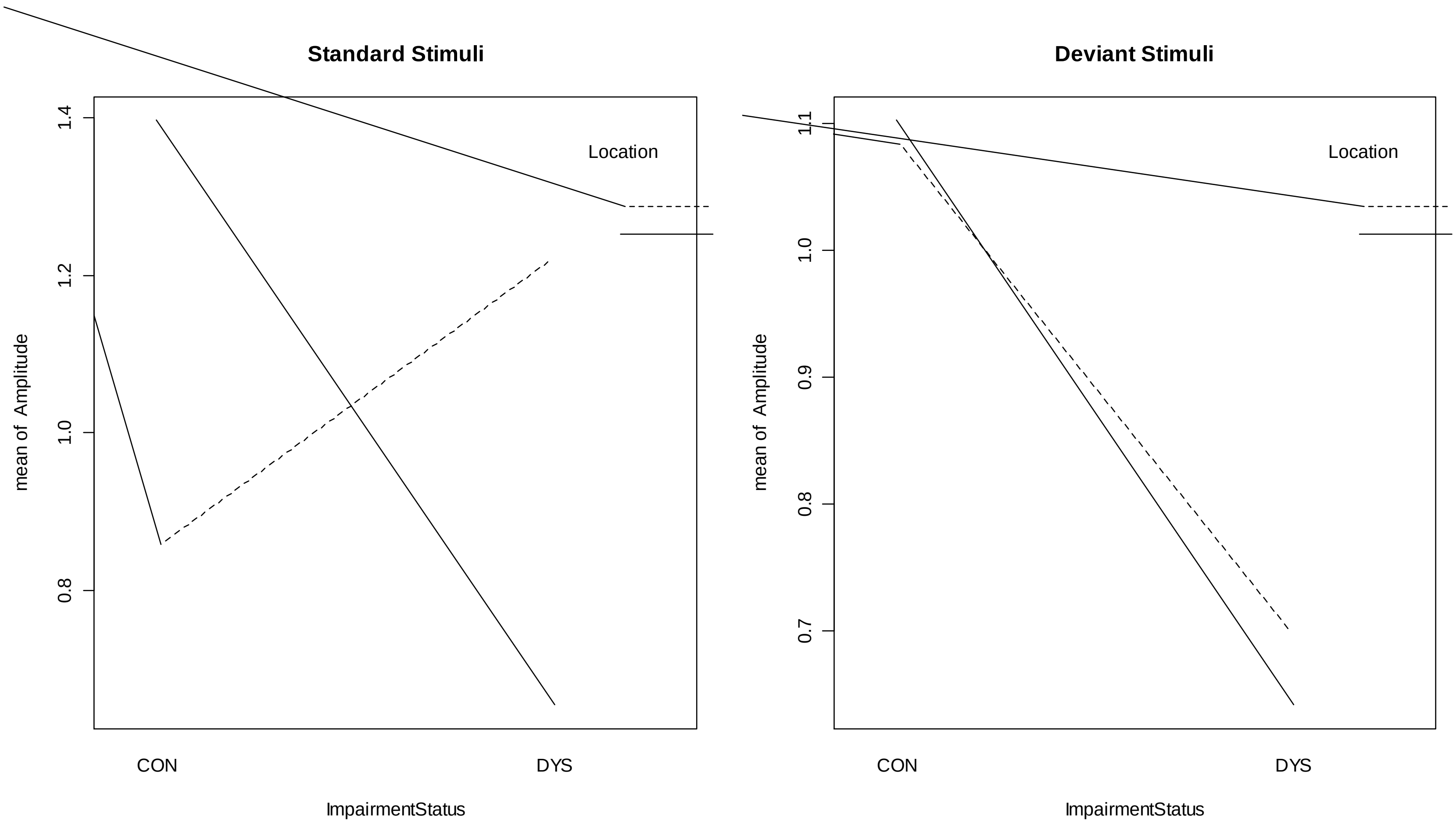
- 3-way & 4-way interaction

Effect	DFn	DFd	F-value	P-value
ImpairmentStatus: Age: Location	1	53	0.244	0.623
ImpairmentStatus: Age: Stimulus	1	53	0.149	0.701
ImpairmentStatus: Location: Stimulus	1	53	5.929	0.018*
Age: Location: Stimulus	1	53	0.131	0.719
ImpairmentStatus: Age: Location: Stimulus	1	53	0.0002	0.989

Visualize two-way interaction ImpairmentStatus: Location



Visualize three-way interaction
ImpairmentStatus: Location: Stimulus



Speculations based on visual inspection of interaction plots

- In the right hemisphere:
  - Control > At-risk (main effect of ImpairmentStatus)

- In the left hemisphere:
  - Control ≈ children (no main effect of ImpairmentStatus)

- In the left hemisphere:
  - Standard stimuli: Control < At-risk
  - Deviant stimuli: Control > At-risk
  - Interaction: ImpairmentStatus ×Stimulus

Post-hoc comparisons

- ANOVAs in right and left hemisphere, separately:
  - In the right hemisphere:
    - Main effect of ImpairmentStatus: $F(1,53) = 6.423$, $p = 0.014$*
    - ImpairmentStatus × Stimulus: $F(1,53) = 0.606$, $p = 0.440$
  - In the left hemisphere:
    - Main effect of ImpairmentStatus: $F(1,53) = 0.003$, $p = 0.958$
    - ImpairmentStatus × Stimulus: $F(1,53) = 4.232$, $p = 0.045$*
- Additional t-tests:
  - Lateralization:
    - Control: Left < Right, $p = 0.166$
    - At-risk: Left > Right, $p = 0.087$
  - Longitudinal development
    - Control: 17 month > 29 month, $p = 0.892$
    - At-risk: 17 month > 29 month, $p = 0.949$

Hypotheses vs. Results

Research Questions and Hypotheses:

1. Group difference:

   Hypothesis: At-risk: Standard – Deviant < Control: Standard – Deviant

   Result: Right hemisphere: Control > At-risk*

   Left hemisphere: ImpairmentStatus ×Stimulus*

   Standard: Control < At-risk

   Deviant: Control > At-risk

2. Lateralization:

   Hypothesis: Control: Left > Right; At-risk: Left = Right or Left < Right

   Result: Control: Left < Right; At-risk: Left > Right

3. Longitudinal difference:

   Hypothesis: At-risk: 29 month – 17month < Control: 29 month – 17month

   Result: in both groups: 17month > 29 month

Fitting multilevels linear model

- Trying out different models

```
L1<lmer(Amplitude~Stimulus+Age+Location+ImpairmentStatus+(1|SubjectID),data=dataLR)
L2 <-lmer(Amplitude~Stimulus+Location+ImpairmentStatus+(1|SubjectID),data=dataLR)
L3<-lmer(Amplitude~Stimulus*Location*ImpairmentStatus+(1|SubjectID),data=dataLR)
```

- Model Comparison
  - L3 fits significantly better than L1 (p = 0.01**)
  - L3 fits significantly better than L2 (p = 0.02*)

Thank you for your attention!
Questions?