Longitudinal Analysis of the Effect of Maternal Immune Activation on Male Offspring Rhesus Monkeys in Human Intruder Test

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Topics

- **Introduction**
- **Data**
- **Exploratory Analysis**
- **Modeling**
- **Results**
- **Conclusion**

Introduction: Maternal Immune Activation Model

- Maternal immune activation (MIA) models of rodents and nonhuman primates have shown their powerful and translational utility to extended exploration of neurobiological mechanisms underlying human neurodevelopmental disorders (Kentner, 2019).
- MIA models use the viral mimic immunostimulant, Polyinosinic: polycytidylic acid stabilized with poly-L-lysine(Poly ICLC), to elicit an immune response during gestation.
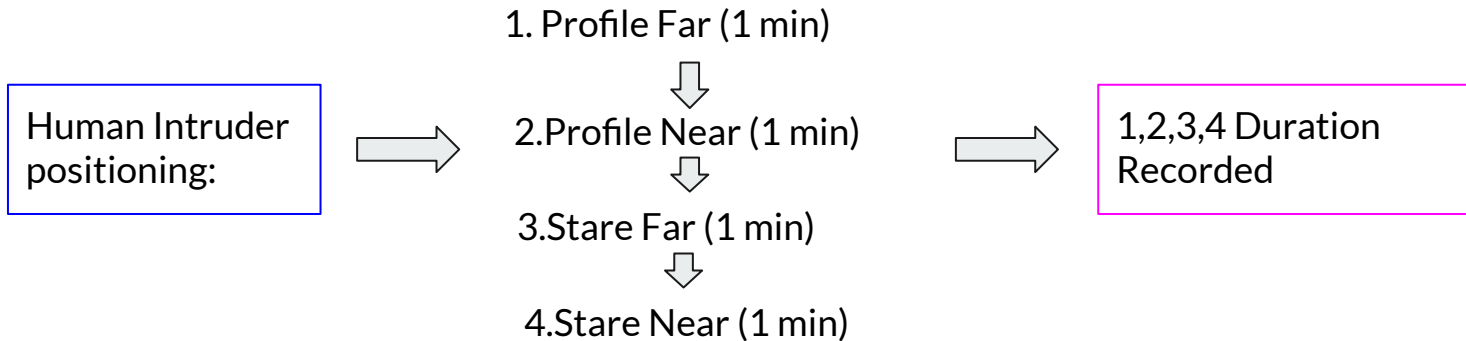
Study: MIA model on Rhesus Monkeys

- Current ongoing study on 28 male offspring of rhesus monkeys from MIA-treated (Poly ICLC treated) (n = 14), saline (n = 10), or untreated (n = 4) dams.
- Human Intruder Test (HI Test) to assess the responsiveness of male offspring rhesus monkeys to the standardized conditions of a human intruder.

Human Intruder Test (HI Test)

- Human Intruder Test
  - 14 MIA-treated and 14 control male offspring rhesus monkey tested approximately at 1, 3, 6, 23, and 35 months of age
  - A session of 4 one-min trials of a human intruder's 4 conditions:
  - Each of the 4 conditions is scored as duration.

1. Profile Far (1 min)
⇩
Human Intruder positioning: ⟹ 2.Profile Near (1 min) ⟹ 1,2,3,4 Duration Recorded
⇩
3.Stare Far (1 min)
⇩
4.Stare Near (1 min)

Study Question

- The scientific interest is to see whether there is a difference in "Front" duration under conditions: "Profile" and "Stare" between the group of male offspring rhesus monkeys of MIA-treated and control dams

Data: Human Intruder Positioning Data

- 36 total variables and attributes for 5 time points observations of 28 animals
  Defining outcome:
  - Profile Front Duration: Average of Profile Far and Near
  - Stare Front Duration: Average of Stare Far and Near
- Predictors:
  - Actual age of animals at each time point
  - Treatment group (MIA vs. Control)
- Missing Data

Descriptive Summary

|	1 Month		3 Months		6 Months		23 Months		35 Months		Overall	
Table 1. Subset Chracteristics Summary of MIA and Control Group over 1 month to 35 months												
	MIA	Control	MIA	Control	MIA	Control	MIA	Control	MIA	Control	MIA	Control
	(N=14)	(N=12)	(N=13)	(N=14)	(N=14)	(N=14)	(N=13)	(N=14)	(N=13)	(N=14)	(N=67)	(N=68)
PF FRONT DUR (In Sec)												
Mean (SD)	24.1 (18.2)	37.9 (25.6)	39.3 (17.2)	38.0 (11.9)	28.0 (18.5)	35.8 (19.3)	36.3 (15.3)	35.4 (17.5)	33.9 (14.9)	36.4 (18.0)	32.1 (17.3)	36.7 (18.2)
Median [Min, Max]	23.4 [0, 52.6]	49.2 [0, 60.0]	43.2 [0, 60.0]	38.8 [16.8, 60.0]	27.8 [0, 60.0]	35.6 [0, 60.0]	37.2 [0, 60.0]	39.4 [0, 53.9]	36.1 [8.76, 51.5]	37.3 [6.47, 60.0]	35.3 [0, 60.0]	38.8 [0, 60.0]
PN FRONT DUR (In Sec)												
Mean (SD)	23.7 (21.7)	43.6 (22.9)	41.3 (19.4)	31.3 (13.4)	31.4 (16.4)	25.9 (18.7)	34.7 (17.9)	33.6 (13.5)	30.2 (12.2)	28.5 (15.5)	32.1 (18.3)	32.3 (17.5)
Median [Min, Max]	22.7 [0, 60.0]	54.5 [0, 60.0]	45.0 [0, 60.0]	35.0 [2.56, 47.5]	30.7 [0, 60.0]	29.6 [0, 49.4]	36.5 [0, 60.0]	37.2 [0, 57.5]	34.6 [6.30, 44.7]	29.3 [6.41, 55.7]	32.8 [0, 60.0]	37.1 [0, 60.0]
SF FRONT DUR (In Sec)												
Mean (SD)	29.0 (24.4)	34.5 (22.5)	47.8 (12.7)	40.5 (18.6)	45.3 (10.9)	37.4 (18.4)	34.7 (9.24)	29.5 (12.6)	26.0 (11.6)	24.4 (14.0)	36.6 (16.9)	33.2 (17.8)
Median [Min, Max]	23.0 [0, 60.0]	38.6 [0, 60.0]	53.2 [26.8, 60.0]	43.5 [0, 60.0]	44.4 [30.1, 60.0]	42.4 [4.93, 60.0]	38.7 [16.0, 46.9]	26.4 [8.50, 54.1]	29.9 [5.53, 39.4]	21.6 [7.47, 54.1]	37.2 [0, 60.0]	34.5 [0, 60.0]
SN FRONT DUR (In Sec)												
Mean (SD)	20.1 (19.2)	42.7 (22.9)	41.1 (16.8)	31.1 (17.3)	39.3 (14.9)	34.2 (19.1)	29.1 (9.96)	23.9 (13.5)	28.8 (14.0)	28.6 (15.7)	31.6 (16.8)	31.8 (18.3)
Median [Min, Max]	14.1 [0, 60.0]	58.7 [0, 60.0]	41.1 [9.47, 60.0]	30.1 [0, 60.0]	38.3 [14.2, 60.0]	40.4 [0, 58.8]	31.7 [9.97, 46.7]	26.8 [0, 46.6]	27.1 [8.94, 47.0]	30.6 [5.34, 60.0]	32.3 [0, 60.0]	31.3 [0, 60.0]
Abbreviations: MIA, Maternal Immune Acitivation, PF, Profile Far, PN, Profile Near, SF, Stare Far, SN, Stare Near												

Table 1. Subset Chracteristics Summary of MIA and Control Group over 1 month to 35 months

	1 Month		3 Months		6 Months		23 Months		35 Months		Overall	
	MIA	Control	MIA	Control	MIA	Control	MIA	Control	MIA	Control	MIA	Control
	(N=14)	(N=12)	(N=13)	(N=14)	(N=14)	(N=14)	(N=13)	(N=14)	(N=13)	(N=14)	(N=67)	(N=68)
Accurate Age (In Days)												
Mean (SD)	30.1 (1.21)	30.3 (0.965)	91.3 (1.93)	90.5 (1.22)	180 (2.04)	179 (3.51)	705 (15.1)	695 (9.93)	1070 (13.2)	1060 (10.7)	406 (406)	423 (406)
Median [Min, Max]	30.0 [28.0, 32.0]	30.0 [29.0, 32.0]	91.0 [88.0, 94.0]	91.0 [88.0, 92.0]	180 [176, 184]	180 [171, 185]	706 [677, 725]	695 [678, 709]	1070 [1040, 1090]	1060 [1050, 1090]	180 [28.0, 1090]	180 [29.0, 1090]
Accurate Age (In Months)												
Mean (SD)	0.988 (0.0396)	0.994 (0.0317)	3.00 (0.0635)	2.97 (0.0402)	5.91 (0.0670)	5.88 (0.115)	23.2 (0.498)	22.8 (0.326)	35.1 (0.433)	35.0 (0.351)	13.3 (13.3)	13.9 (13.3)
Median [Min, Max]	0.986 [0.920, 1.05]	0.986 [0.953, 1.05]	2.99 [2.89, 3.09]	2.99 [2.89, 3.02]	5.91 [5.78, 6.05]	5.91 [5.62, 6.08]	23.2 [22.2, 23.8]	22.8 [22.3, 23.3]	35.1 [34.2, 35.7]	34.8 [34.5, 35.7]	5.91 [0.920, 35.7]	5.91 [0.953, 35.7]
Profile Front (PF + PN)/2												
Mean (SD)	23.9 (18.8)	40.8 (22.8)	40.3 (17.6)	34.7 (9.44)	29.7 (16.3)	30.8 (16.2)	35.5 (15.6)	34.5 (13.8)	32.1 (12.3)	32.5 (14.3)	32.1 (16.8)	34.5 (15.5)
Median [Min, Max]	24.6 [0, 55.6]	51.8 [0, 60.0]	42.6 [0, 60.0]	36.0 [15.8, 46.2]	27.6 [0, 60.0]	32.8 [0, 50.0]	38.7 [0, 59.8]	36.8 [0, 55.7]	37.9 [7.53, 45.8]	35.0 [6.44, 57.8]	33.2 [0, 60.0]	36.6 [0, 60.0]
Stare Front (SF + SN)/2												
Mean (SD)	24.6 (20.8)	38.6 (20.5)	44.5 (13.7)	35.8 (16.5)	42.3 (11.7)	35.8 (15.9)	31.9 (8.74)	26.7 (12.7)	27.4 (11.2)	26.5 (12.9)	34.1 (15.7)	32.5 (16.1)
Median [Min, Max]	18.9 [0, 60.0]	42.3 [0, 60.0]	47.2 [21.4, 60.0]	37.0 [0, 55.5]	43.3 [23.8, 60.0]	38.3 [2.47, 55.9]	34.8 [17.0, 42.7]	25.8 [4.25, 50.4]	32.1 [7.24, 39.5]	27.5 [6.90, 46.9]	34.6 [0, 60.0]	33.1 [0, 60.0]

Abbreviations: MIA, Maternal Immune Acitivation, PF, Profile Far, PN, Profile Near, SF, Stare Far, SN, Stare Near

Outcome 1 (Profile Front (PF + PN)/2)

Outcome 2 (Stare Front (SF + SN)/2)

Exploratory Analysis
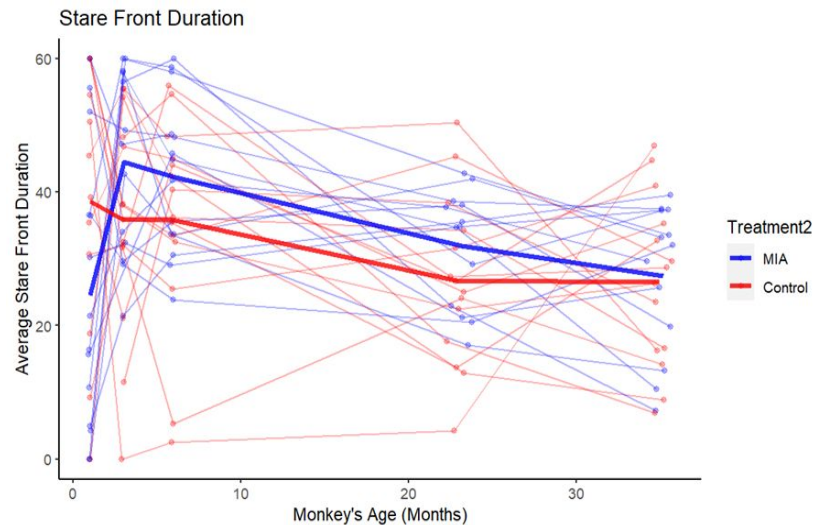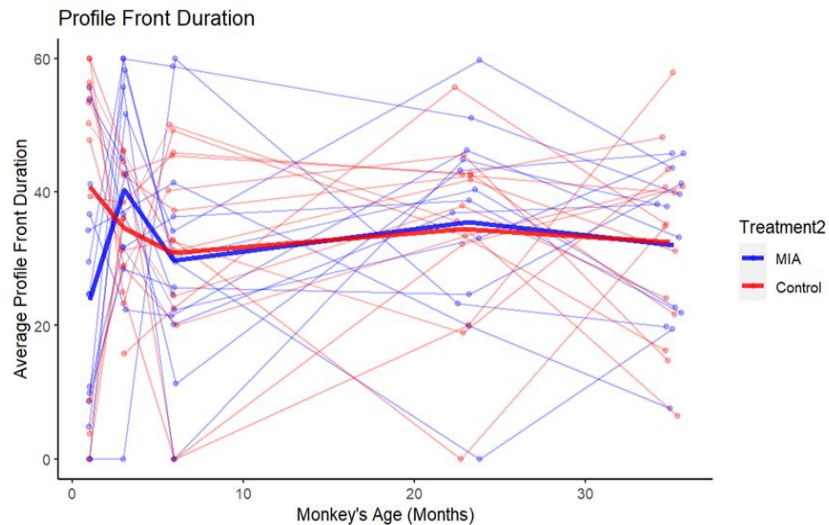
Method

- Modeling for outcome: Front duration in 2 conditions: Profile and Stare
  - Linear Mixed Effect Model
- Model Selection
- Assessment of Treatment Effect

Initial Model: Linear Mixed-Effect Model (ReML)

- $Y_{\{ij\}}$: front duration for subject i for either profile or stare
- $Age_{\{ij\}}$ - 1: subject's age centered at 1 month, for $i = \{1, \dots, n_i\}$
- Time point $j = \{1, \dots, n_j\}$
- $Treatment_{\{i\}}$: indicator variable for $Treatment_{\{i\}}$ = 1 for MIA, and $Treatment_{\{i\}}$ = 0 for control group.

Consider the full model

$$Y_{\{ij\}} = \beta_0 + \beta_1\{Treatment_{\{i\}}\} + \beta_2\{Age_{\{ij\}} - 1\} + \beta_3\{Age_{\{ij\}} - 1\}^2 + \beta_4\{Age_{\{ij\}} - 1\}^3$$
$$+ \beta_5\{Treatment_i\} * \{Age_{\{ij\}} - 1\} + \beta_6\{Treatment_i\} * \{Age_{\{ij\}} - 1\}^2$$
$$+ \beta_7\{Treatment_i\} * \{Age_{\{ij\}} - 1\}^3 + e_{\{ij\}},$$

$$e_{\{ij\}} = U_{0i} + Z_{ij}$$

with i.i.d. measurement error Z_{ij}, and U_{0i} is random intercept
$$Z_{ij} \sim N(O, \tau^2)$$

$$(U_{0i}) \sim N(0, G), \text{E}(U_{0i}) = (0), \text{Var}(U_{0i}) = G = (\sigma_{0i}^2).$$

Modeling: Profile Front Duration

Covariance Model for Var $(e_{\{ij\}})$

1. Exchangeable (Compound Symmetry) Covariance Model: $e_{\{ij\}} = U_{0i} + Z_{ij}$
2. Random Intercept + Random slope Model: $e_{\{ij\}} = U_{0i} + U_{1i}\{Age_{\{ij\}} - 1\} + Z_{ij}$

Covariance Model Comparison:

Model	BIC	AIC	Log Likelihood
1	1206.569	1178.127	-579.063
2	1216.258	1182.127	-579.063

Final Covariance Model: Exchangeable (Compound Symmetry) Covariance Model

Modeling Mean Model: Profile Front Duration

- Initial fitting of mean model with exchangeable covariance model
- Multiple testing for all interaction terms

Testing for multiple coefficients jointly:

$$H_o: \beta_5 = \beta_6 = \beta_7 = 0$$

$$H_a: \text{At least one of } \beta_5, \beta_6, \beta_7 \text{ is not equal to } 0$$

- Sequentially test higher order "Age" effects while always keep treatment and linear age effect in the model (t-test)
- Decide on final model

Modeling Stare Front Duration

Covariance Model for Var $(e_{\{ij\}})$

1. Exchangeable (Compound Symmetry) Covariance Model: $e_{\{ij\}} = U_{0i} + Z_{ij}$
2. Random Intercept + Random slope Model: $e_{\{ij\}} = U_{0i} + U_{1i}\{Age_{\{ij\}} - 1\} + Z_{ij}$

Covariance Model Comparison:

Model	BIC	AIC	Log Likelihood
1	1186.714	1158.272	-569.136
2	1196.510	1162.380	-569.190

Final Covariance Model: Exchangeable (Compound Symmetry) Covariance Model

Modeling Mean Model: Stare Front Duration

- Initial fitting of mean model with exchangeable covariance model
- Multiple testing for all interaction terms

Testing for multiple coefficients jointly:

$$H_o: \beta_5 = \beta_6 = \beta_7 = 0$$

$$H_a: At\ least\ one\ of\ \beta_5, \beta_6, \beta_7\ is\ not\ equal\ to\ 0$$

- Sequentially test higher order "Age" effects: while always keep treatment and linear age effect in the model (t-test)
- Decide on final model

Model Selection

- Both Profile and Stare Front duration:
  - All age interaction terms are not significant

Full Model vs. Model without all interaction terms	Profile Model	Stare Model
F-Statistic	1.319	1.860
P-value	0.272	0.073

- Profile: cubic, quadratic age are dropped by t-test
- Stare: cubic, quadratic age are not dropped by t-test

Results: Final Model

- **Profile Front Duration**

$$Y_{\{ij\}} = \beta_0 + \beta_1\{Treatment_{\{i\}}\} + \beta_2\{Age_{\{ij\}} - 1\} + U_{\{0i\}} + Z_{\{ij\}}$$

- **Stare Front Duration**

$$Y_{\{ij\}} = \beta_0 + \beta_1\{Treatment_{\{i\}}\} + \beta_2\{Age_{\{ij\}} - 1\} + \beta_3\{Age_{\{ij\}} - 1\}^2 + \beta_4\{Age_{\{ij\}} - 1\}^3 + U_{\{0i\}} + Z_{\{ij\}}$$

Results: Final Model

Table 2: Fixed-Effect Parameter Estimates (SE) for the linear mixed-effects models predicting Profile and Stare Front Duration

Model Term	*Dependent variable:*	
	Profile Front Duration Estimate (SE) p-value	Stare Front Duration Estimate (SE) p-value
Intercept	$34.585^{***}(2.393)$ $p = 0.000$	$31.811^{***}(3.05)$ $p = 0.000$
Treatment: MIA vs. Control	$-2.353 (2.793)$ $p = 0.408$	$1.514 (2.633)$ $p = 0.571$
Linear Age	$-0.008 (0.105)$ $p = 0.937$	$2.559^{**} (1.216)$ $p = 0.038$
Quadratic Age		$-0.203^{**} (0.086)$ $p = 0.021$
Cubic Age		$0.004^{**}(0.020)$ $p = 0.024$
Observations	135	135

$^{*}p<0.01$ $^{**}p<0.05$ $^{***}p<0.01$

Results: Final Model (continued)

- No interaction of treatment and age are significant (profile: p= 0.2722758, stare: p = 0.0728028)
  - Effect of treatment on front duration is not significantly different over time
    - Profile (est. = -2.353, p = 0.408), Stare (est. = 1.514, p= 0.571)
  - Effect of age on front duration is not significantly different across MIA and control group with linear age not significant for profile front duration (est. = -0.008, p = 0.937) and linear, quadratic, cubic age significant for stare front duration (est. = 2.559, p = 0.038, est = -0.203, = 0.021, est = 0.004, p = 0.024, respectively).

Model Diagnostics

Fitted Coefficient Mean Functions: Profile
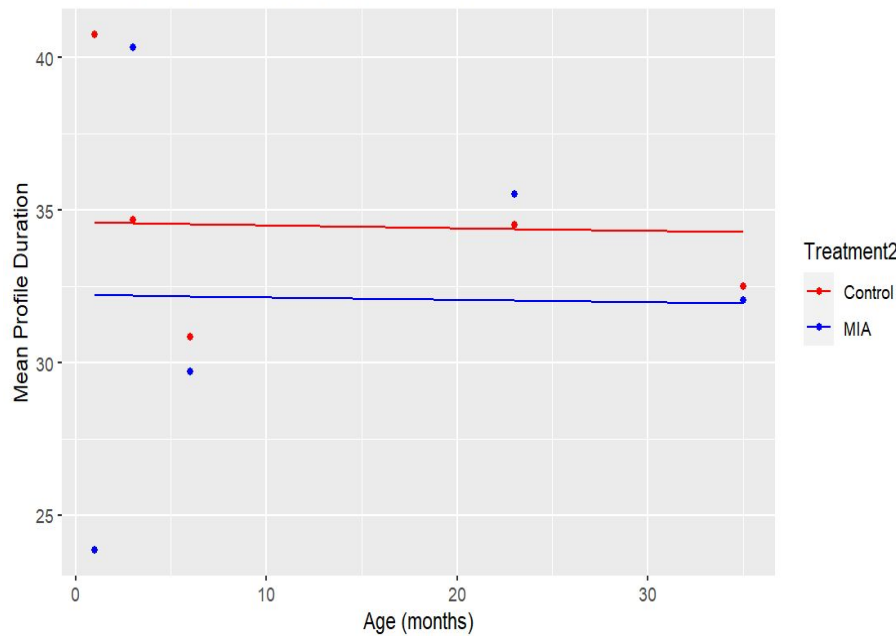
Fitted Coefficient Mean Functions: Stare

Model Diagnostics: Conditional Residual Analysis
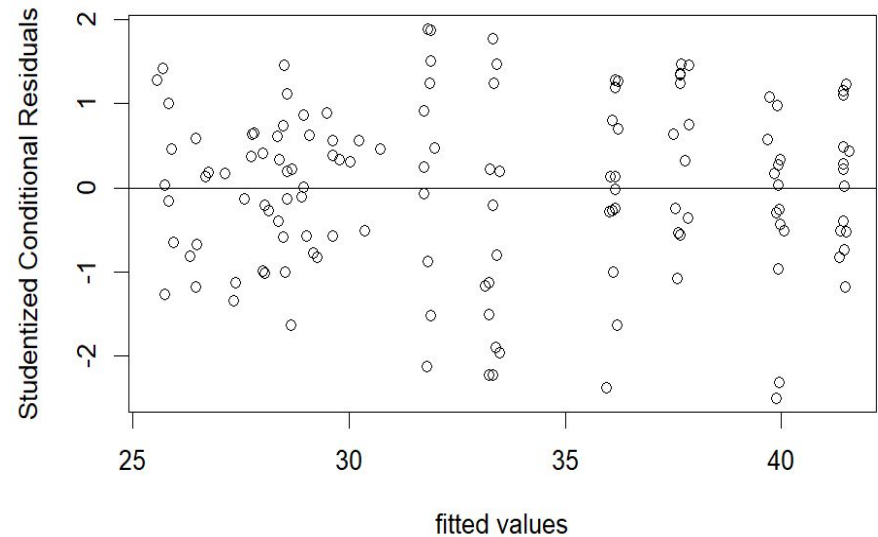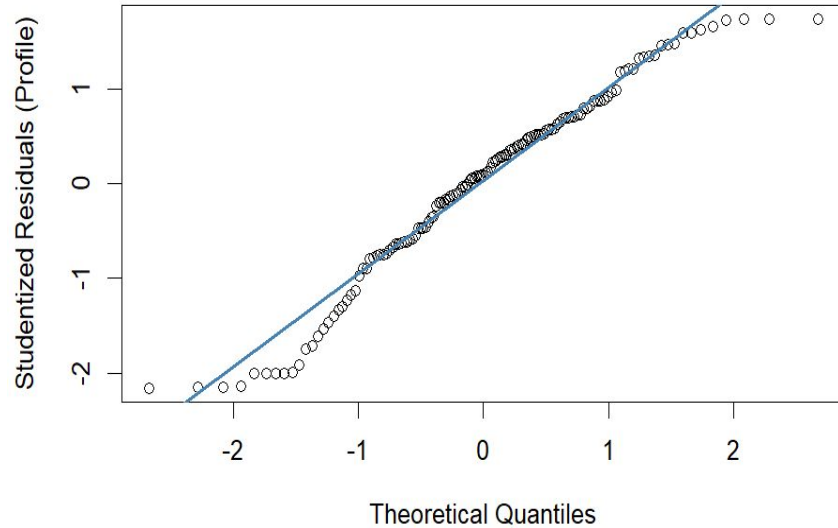
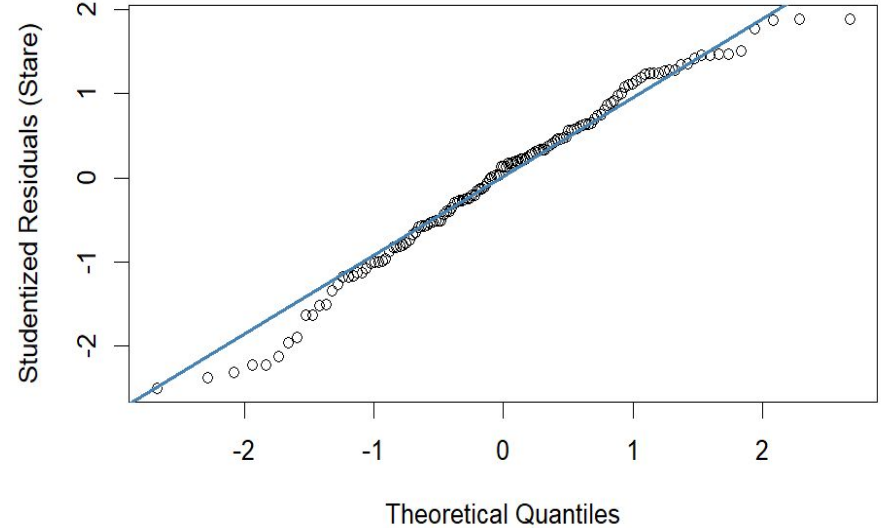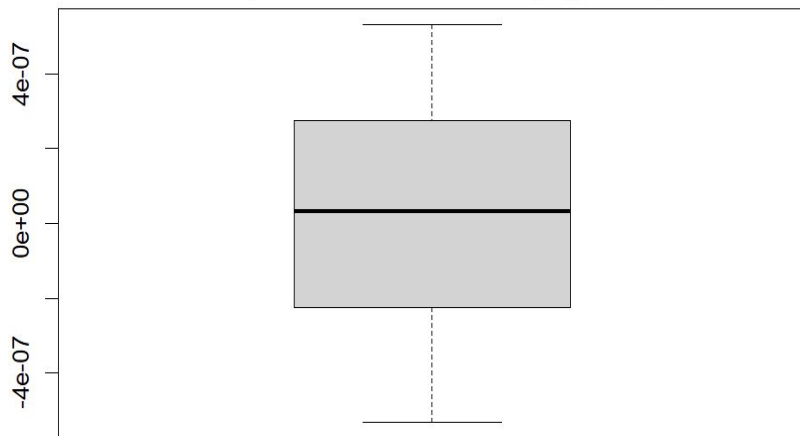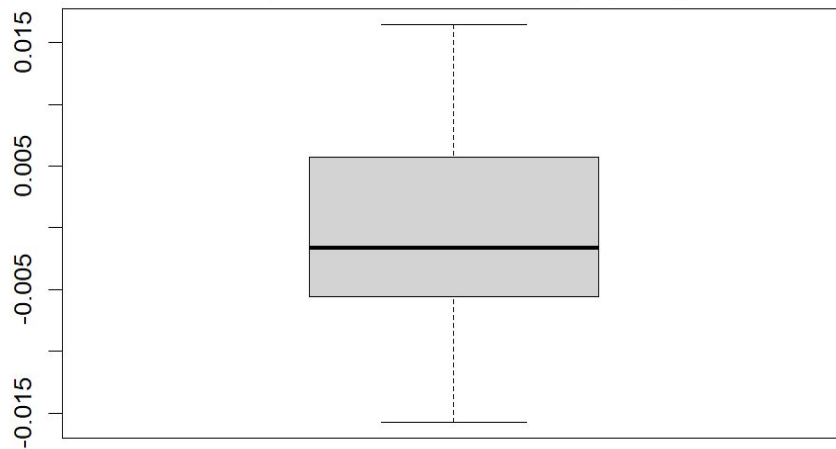# Model Diagnostics: Conditional Residual Analysis

Model Diagnostics: Random Intercept

Boxplot of Random Intercept (Profile)

Boxplot of Random Intercept (stare)

Conclusion

- Treatment effect is not different over time for profile and stare conditions.
- Age effect is not different across treatment group for profile and stare conditions.
- MIA and control group perform similarly on "Front" duration on human intruder's conditions.
- Possible future investigation on other outcome measures of the HI test
  - Frequency of position change
  - Frequency of various types of behaviors

References

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