Lateral line hair cells integrate mechanical and chemical cues to orient navigation

Data S2 - Statistical analysis

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Contents

1 Method

All statistical analyses were conducted using R version 3.6.1 (R Development Core Team, 2019) and plots were generated with the ggplot2 package (Wickham, 2016). Within and between group differences and changes were examined using linear mixed-effects models (LMMs). Specifically, models were built for parameters of interest including Bout frequency, Speed, Percentage of routine turns and Percentage of time swimming. For each model, zebrafish larvae with influential observations over all the parameters were identified and excluded at a Cook's distance greater than twice the amount of the cutoff value of 4/n (up to five larvae) using the R package influence.ME (v0.9-9) (Bollen and Jackman, 1985).

In the first study [effect of 5-HT], the factors Condition (5-HT versus E3), Time (t0 versus t10), and their interaction terms were regarded as fixed effects. Fish identifier was assigned as a random effect (intercept) nested within the different experiments to account for the repeated measurements by animal. All LMMs were fitted using restricted maximum-likelihood estimation (REML) with the function lmer in the lme4 package (Bates et al., 2015) (v1.1-21). Significance for the main effects and the two-way interaction of Condition and Time was assessed based on Type II Wald chi-square tests using the function Anova in the car package (v3.0-7). Post hoc pairwise comparisons were carried out on a significant interaction effect with the emmeans package (v1.4.5) to further determine where the differences occurred across the subgroups of larvae. A square root transformation was applied to the speed data prior to modeling to improve the model assumptions of linearity, normality and constant variance of residuals. P-values resulting from the post hoc tests were obtained using Kenward-Roger's approximation for degrees of freedom, and after adjustment with the Tukey's method to account for the multiplicity of contrasts. Results of the post hoc tests were also reported with the values of the estimated marginal means or estimated marginal mean differences from emmeans, with speed data back-transformed from square root to original scale.

In the second study [effect of 5-HT gradient], the same approach was used to investigate the effect of 5-HT concentration on the three parameters Bout frequency, Speed, Percentage of routine turns, by including the factors Condition (5-HT versus E3), Distance (considered as a categorical variable with seven levels: 1cm, 3cm, 5cm, 7cm, 9cm, 11cm, 13cm), and their interaction terms as fixed effects, with the same random effects as above, in the LMMs. Post hoc pairwise comparisons were carried out on a significant effect of Distance or interaction.

The aim of the last three studies [effect of 5-HT on either CuSO4-treated larvae, PLL-ablated or OMP-ablated larvae] was to investigate the additional effect of either CuSO4 treatment, PLL ablation or OMP ablation in larvae on Speed and Percentage of time swimming. In this analysis, LMMs included the fixed effects of Condition (5-HT versus EM), Treatment (for Sham ablation versus either PLL or OMP Ablation), Time (t0 versus t10), and their interaction terms as fixed effects, with the same random effects as above. Pairwise comparisons were carried out on the group estimated marginal means (emmeans) from significant higher-level interactions. The level of statistical significance was set at p or adjusted $p < 0.05$ for all tests.

References

R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Bates D, Maechler M, Bolker B, Walker S. Fitting Linear Mixed Effects Models Using lme4. J Stat Softw. 2015;67:1–48.

Bollen KA, Jackman RW. Regression Diagnostics: An Expository Treatment of Outliers and Influential Cases. Sociol Methods Res. 1985; 13: 510-542.

Wickham H. 2016. Ggplot2: Elegant Graphics for Data Analysis. Springer.

2 Effect of 5-HT (Figure 2D-G)

2.1 Check for influential observations (Cook's distance)

We excluded five larvae: FishID 40, FishID 43, FishID 62, FishID 83 and FishID 95.

Table 1: Influential observations across all the parameters

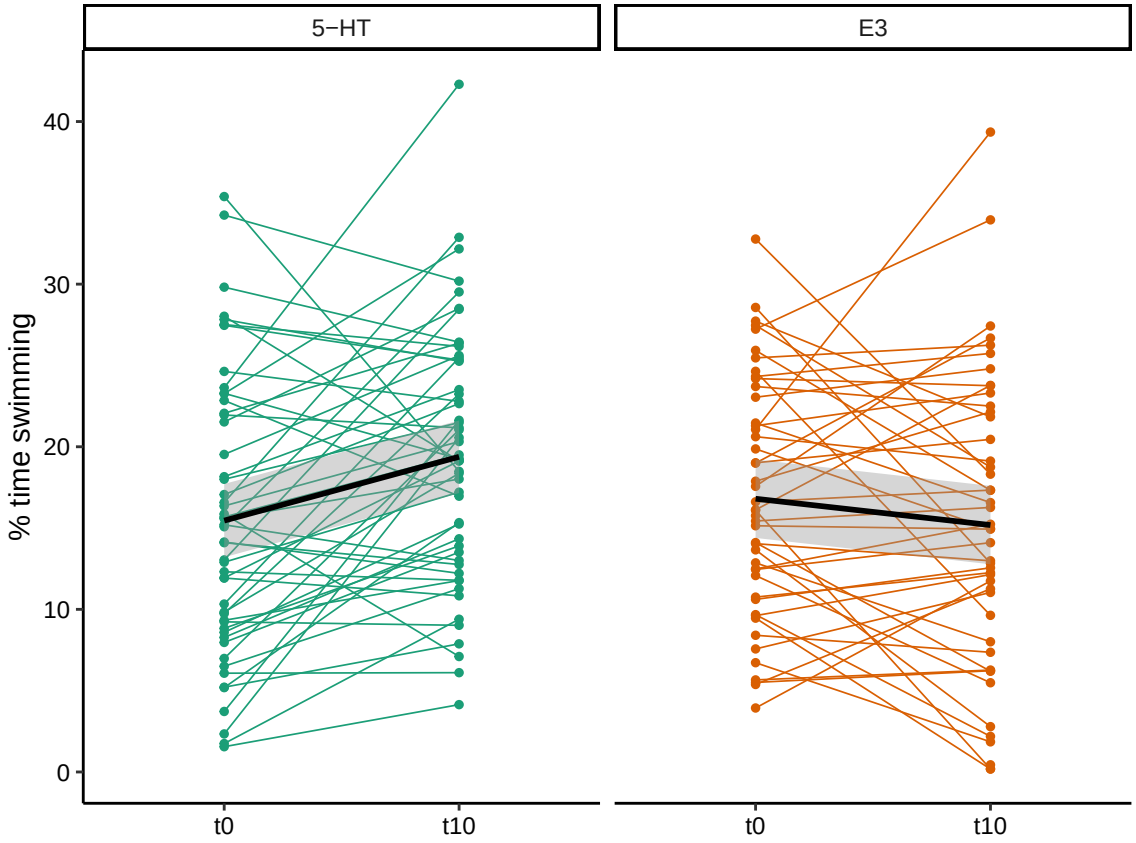
	FishID	Condition	Time	Value	CookD	Parameter
69	40	5-HT	t0	100.000	0.082	Turns
124	43	E3	t10	19.871	0.131	Speed
22	43	E3	t0	0.000	0.145	Turns
1241	43	E3	t10	100.000	0.204	Turns
183	62	5-HT	t10	43.880	0.051	FQ
1831	62	5-HT	t10	43.880	0.114	Swim
142	83	E3	t10	0.000	0.089	Turns
148	95	E3	t10	0.000	0.087	Turns

2.2 Percentage of time swimming (Figure 2D)

2.2.1 Descriptive data

Table 2: Percentage of time swimming by Condition and Time

	5-HT	E3
t0	15.46 ± 8.49 (n = 51, r = 1.55-35.39)	16.8 ± 7.29 (n = 46, r = 3.93-32.77)
t10	19.38 ± 7.73 (n = 52, r = 4.14-42.29)	15.18 ± 9.08 (n = 46, r = 0.18-39.35)



2.2.2 Linear mixed model with Wald chi-square tests

Significant interaction between Condition (5-HT, E3) and Time (t0, t10) on the percentage of time swimming (Wald $\chi^2 = 13.03$, df $= 1$, $^{***}p = 0.0003$).

```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: Swim
##                  Chisq Df Pr(>Chisq)
## Condition       3.1169  1  0.0774823 .
## Time            3.4503  1  0.0632415 .
## Condition:Time 13.0261  1  0.0003072 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

2.2.3 Tukey's post hoc tests

Table 3: Post hoc pairwise comparisons with Tukey's method - Mean differences between t0 and t10 by Condition

Time	Condition	emmean	SE	df	lower.CL	upper.CL
t0	5-HT	15.40626	1.422535	34.99121	12.51834	18.29419
t10	5-HT	19.34788	1.417498	34.52817	16.46880	22.22696
t0	E3	16.14360	1.459507	38.26464	13.18965	19.09755
t10	E3	14.66600	1.460649	38.32591	11.70990	17.62211

contrast	Condition	estimate	SE	df	t.ratio	p.value
t0 - t10	5-HT	-3.94	1.03	95.0	-3.827	0.0002326
t0 - t10	E3	1.48	1.09	95.9	1.352	0.1796042

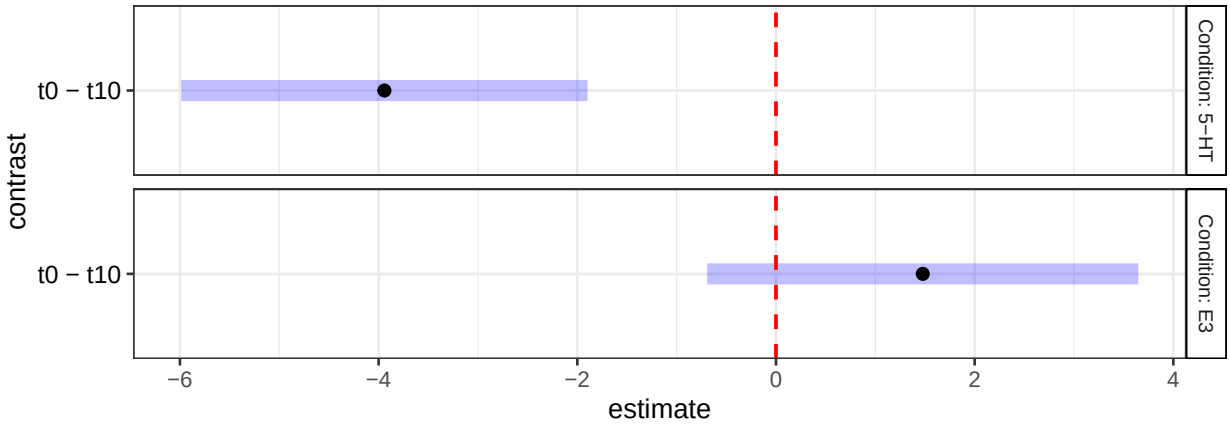
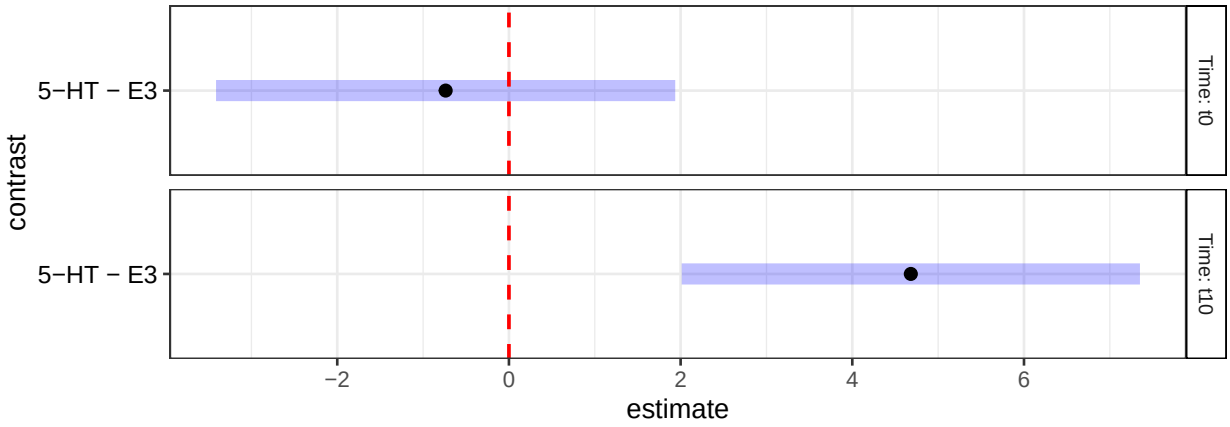


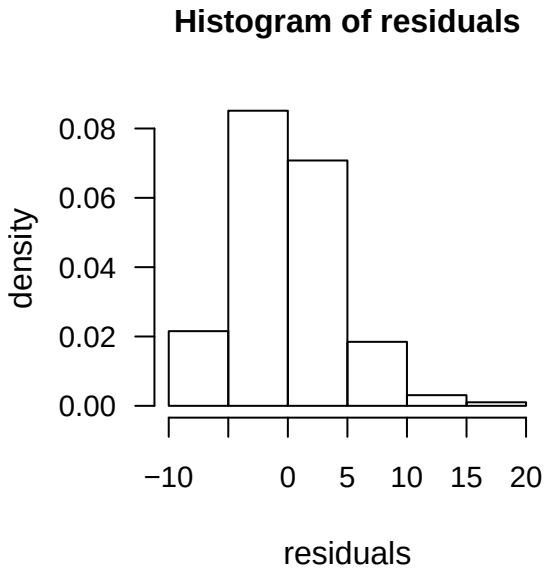
Table 5: Post hoc pairwise comparisons with Tukey's method - Mean differences between E3 and 5-HT at t0 and t10

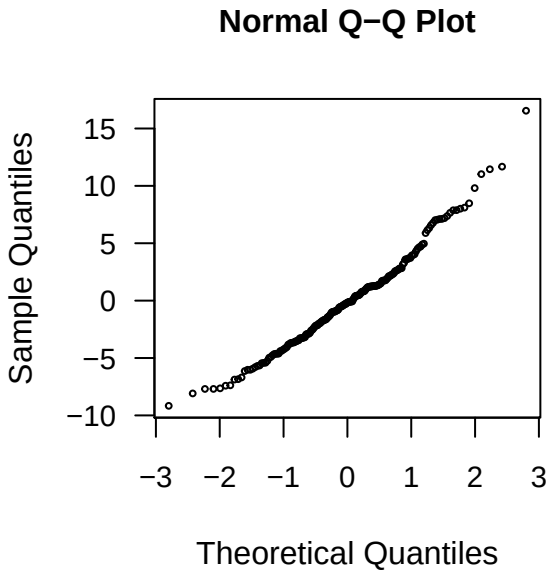
Condition	Time	emmean	SE	df	lower.CL	upper.CL
5-HT	t0	15.40626	1.422535	34.99121	12.51834	18.29419
E3	t0	16.14360	1.459507	38.26464	13.18965	19.09755
5-HT	t10	19.34788	1.417498	34.52817	16.46880	22.22696
E3	t10	14.66600	1.460649	38.32591	11.70990	17.62211

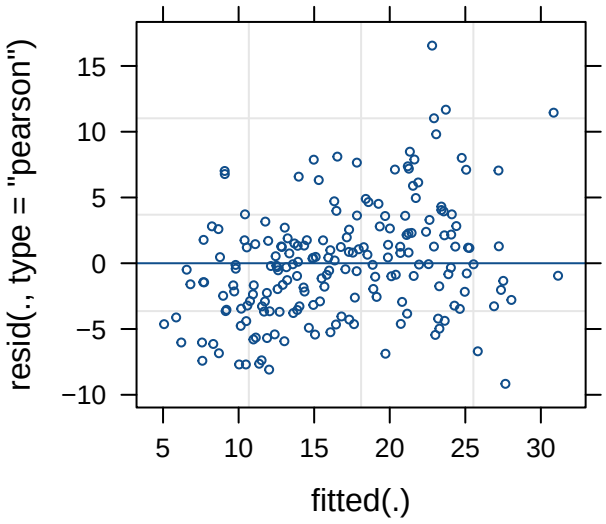
contrast	Time	estimate	SE	df	t.ratio	p.value
5-HT - E3	t0	-0.74	1.35	141.9	-0.545	0.5866251
5-HT - E3	t10	4.68	1.35	141.9	3.467	0.0006959



2.2.4 Checking model assumptions







2.2.5 Conclusion

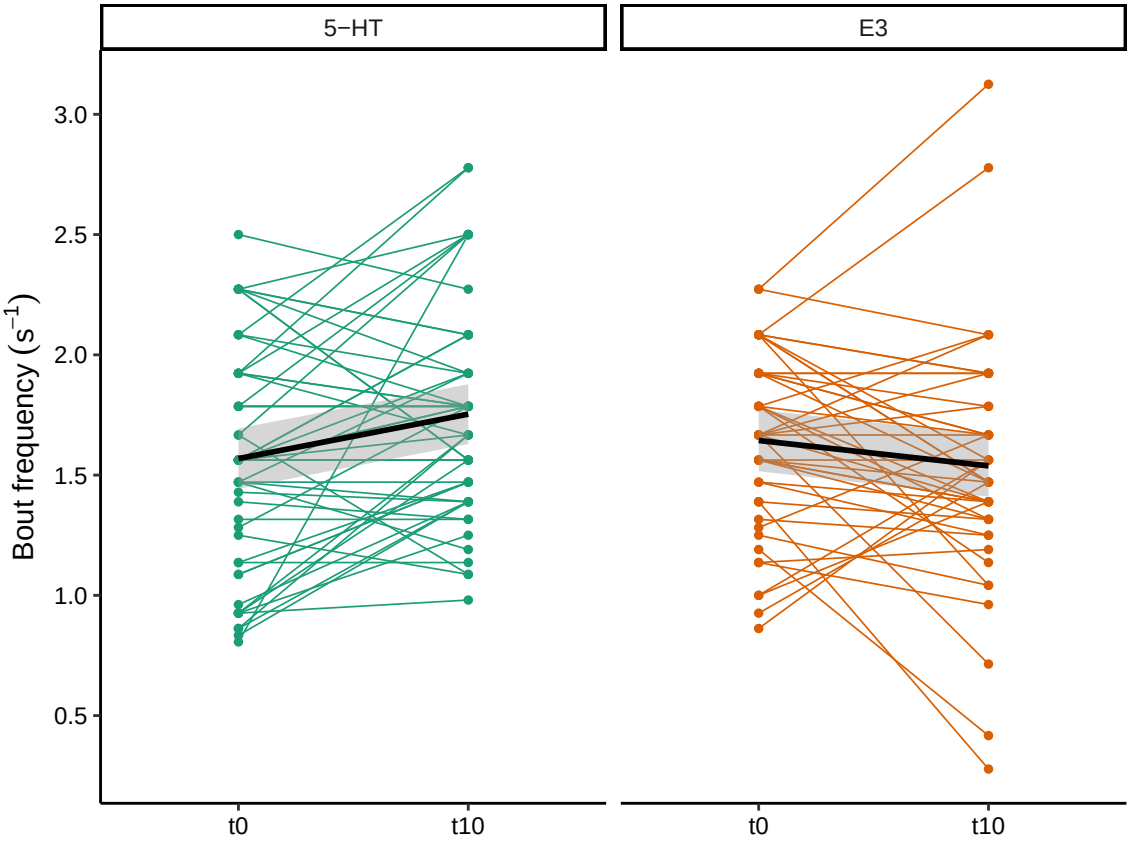
Type II Wald chi-square tests for the fitted model indicated a significant interaction effect between Condition and Time on the percentage of time swimming (Wald $\chi^2 = 13.03$, df $= 1$, $^{***}p = 0.0003$). Post hoc tests using Tukey's method showed significant differences between t0 and t10 in 5-HT ($^{***}p = 0.0002$), and between E3 and 5-HT at t10 ($^{***}p = 0.0007$), while there was no significant difference between t0 and t10 in E3 ($^{ns}p = 0.18$), and between E3 and 5-HT at t0 ($^{ns}p = 0.59$).

2.3 Bout frequency (Figure 2E)

2.3.1 Descriptive data

Table 7: Bout frequency by Condition and Time

	5-HT	E3
t0	1.57 ± 0.46 (n = 51, r = 0.81-2.5)	1.64 ± 0.37 (n = 46, r = 0.86-2.27)
t10	1.75 ± 0.44 (n = 52, r = 0.98-2.78)	1.54 ± 0.5 (n = 46, r = 0.28-3.12)



2.3.2 Linear mixed model with Wald chi-square tests

Significant interaction effect of Condition (5-HT, E3) and Time (t0, t10) on Bout frequency (Wald $\chi^2 = 10.34$, df $= 1$, $^{**}p = 0.0013$).

```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: FQ
##                   Chisq Df Pr(>Chisq)
## Condition       2.4684  1     0.1162
## Time            0.9443  1     0.3312
## Condition:Time 10.3427  1     0.0013 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

2.3.3 Tukey's post hoc tests

Table 8: Post hoc pairwise comparisons with Tukey's method - Mean differences between t0 and t10 by Condition

Time	Condition	emmean	SE	df	lower.CL	upper.CL
t0	5-HT	1.570748	0.0731355	44.81112	1.423429	1.718068
t10	5-HT	1.753085	0.0727803	44.03264	1.606409	1.899761
t0	E3	1.615176	0.0756000	49.80835	1.463315	1.767038
t10	E3	1.504089	0.0756714	49.83836	1.352086	1.656092

contrast	Condition	estimate	SE	df	t.ratio	p.value
t0 - t10	5-HT	-0.18	0.06	95	-2.913	0.0044653
t0 - t10	E3	0.11	0.07	96	1.673	0.0976346

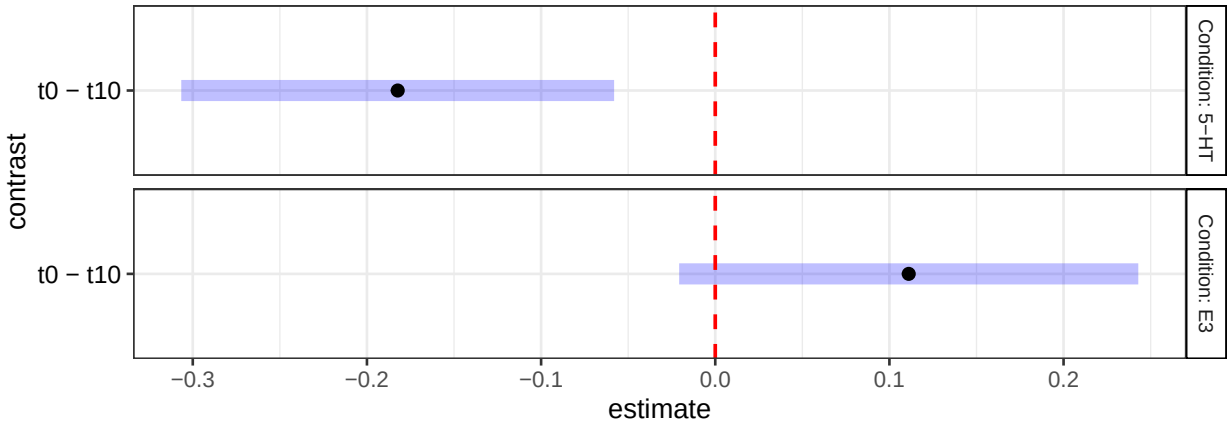
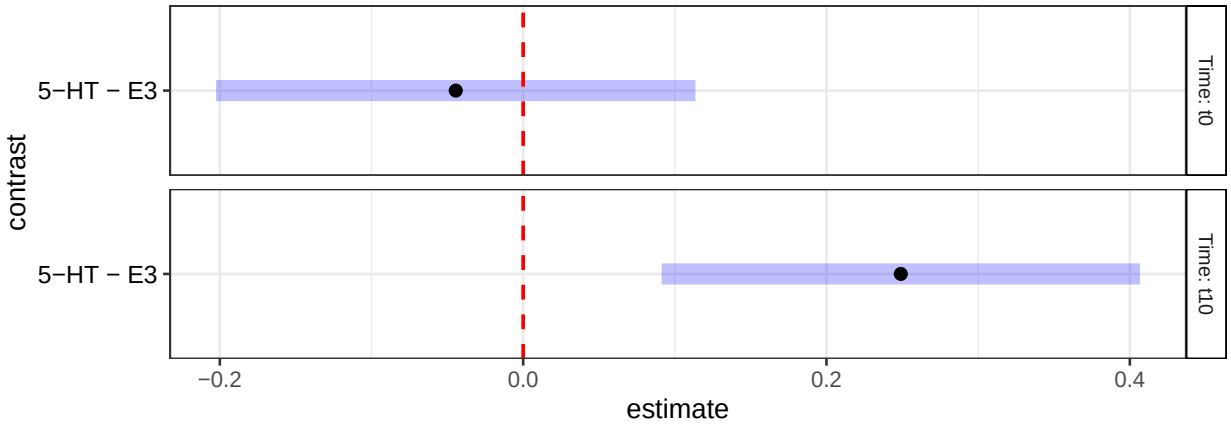


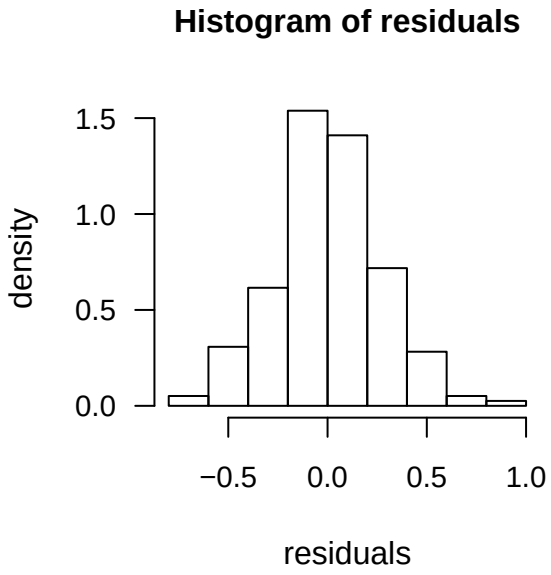
Table 10: Post hoc pairwise comparisons with Tukey's method - Mean differences between E3 and 5-HT at t0 and t10

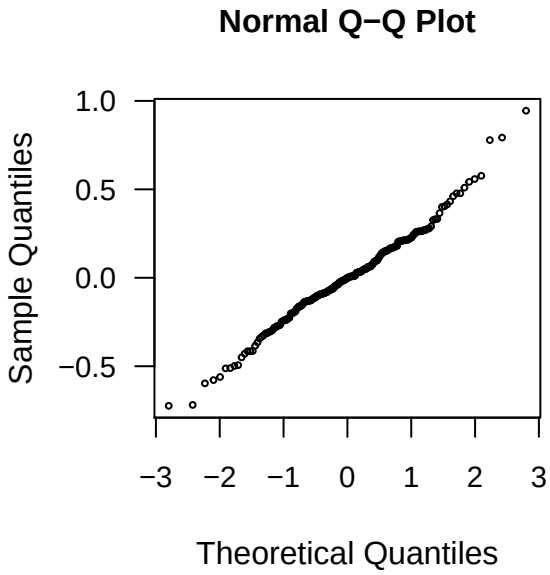
Condition	Time	emmean	SE	df	lower.CL	upper.CL
5-HT	t0	1.570748	0.0731355	44.81112	1.423429	1.718068
E3	t0	1.615176	0.0756000	49.80835	1.463315	1.767038
5-HT	t10	1.753085	0.0727803	44.03264	1.606409	1.899761
E3	t10	1.504089	0.0756714	49.83836	1.352086	1.656092

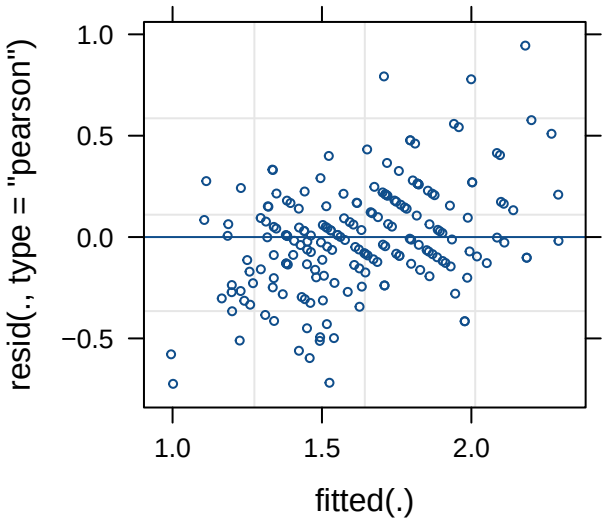
contrast	Time	estimate	SE	df	t.ratio	p.value
5-HT - E3	t0	-0.04	0.08	146.7	-0.556	0.5793661
5-HT - E3	t10	0.25	0.08	146.8	3.121	0.0021732



2.3.4 Checking model assumptions







2.3.5 Conclusion

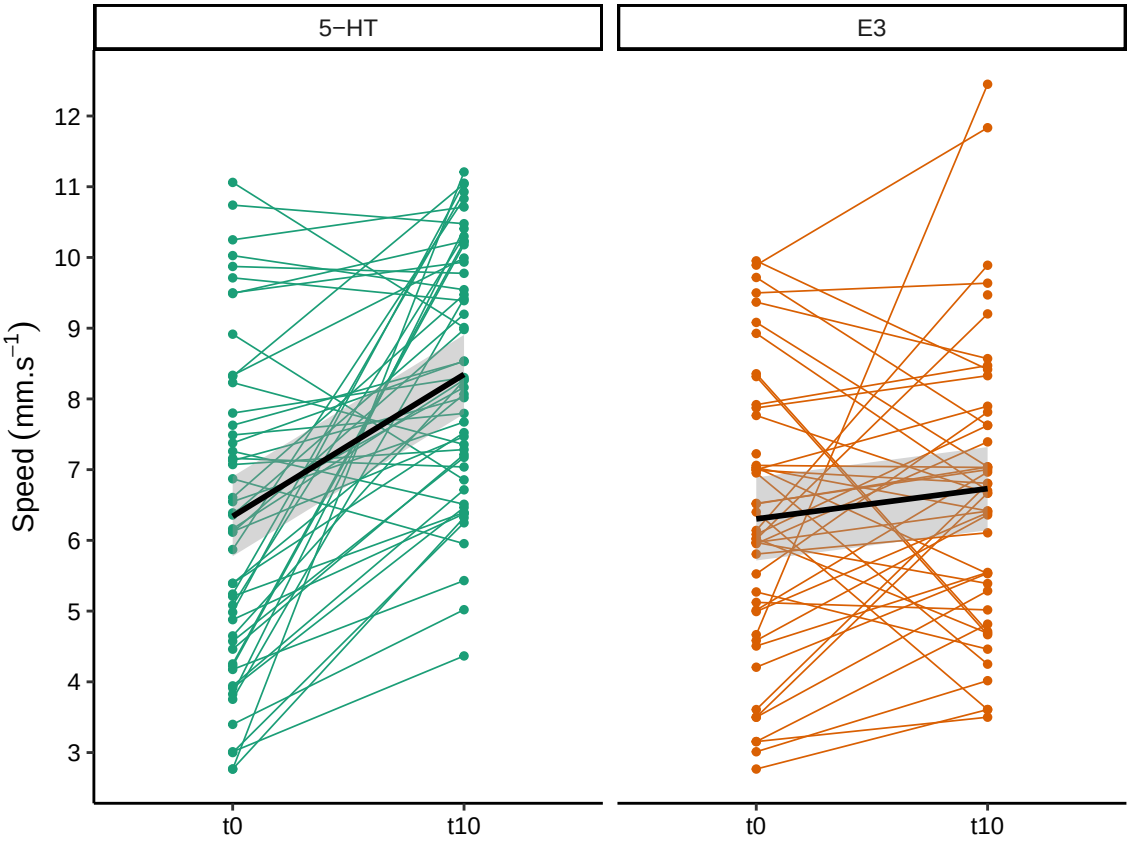
Type II Wald chi-square tests for the fitted model indicated a significant interaction effect between Condition and Time on Bout frequency (Wald $\chi^2 = 10.34$, df $= 1$, $^{**}p = 0.0013$). Post hoc tests using Tukey's method showed significant differences between E3 and 5-HT at t10 ($^{**}p = 0.002$), and between t0 and t10 in 5-HT ($^{**}p = 0.004$), while no differences were observed between t0 and t10 in E3 ($^{ns}p = 0.10$), and between E3 and 5-HT at t0 ($^{ns}p = 0.58$).

2.4 Speed (square root transformed data) (Figure 2F)

2.4.1 Descriptive data

Table 12: Speed by Condition and Time

	5-HT	E3
t0	2.48 ± 0.45 (n = 51, r = 1.66-3.33)	2.48 ± 0.41 (n = 46, r = 1.66-3.15)
t10	2.87 ± 0.31 (n = 52, r = 2.09-3.35)	2.57 ± 0.38 (n = 46, r = 1.87-3.53)



2.4.2 Linear mixed model with Wald chi-square tests

Significant interaction effect between Condition (5-HT, E3) and Time (t0, t10) on Speed (Wald $\chi^2 = 12.71$, df = 1, $^{***}p = 0.0004$).

```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: sqrt(Speed)
##                  Chisq Df Pr(>Chisq)
## Condition       9.9481  1  0.0016102 **
## Time           33.5438  1  6.968e-09 ***
## Condition:Time 12.7148  1  0.0003628 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

2.4.3 Tukey's post hoc tests

Table 13: Post hoc pairwise comparisons with Tukey's method - Mean differences between t0 and t10 by Condition (back-transformed estimate values from the sqrt scale)

Time	Condition	response	SE	df	lower.CL	upper.CL
t0	5-HT	6.063389	0.3223914	45.55588	5.414278	6.712500
t10	5-HT	8.195909	0.3729048	44.73543	7.444718	8.947101
t0	E3	5.985995	0.3306763	50.54931	5.321992	6.650000
t10	E3	6.424439	0.3429299	50.60150	5.735847	7.113031

contrast	Condition	estimate	SE	df	t.ratio	p.value
t0 - t10	5-HT	-2.13	0.32	44.7	-6.596	0.0000000
t0 - t10	E3	-0.44	0.32	50.5	-1.380	0.1737336

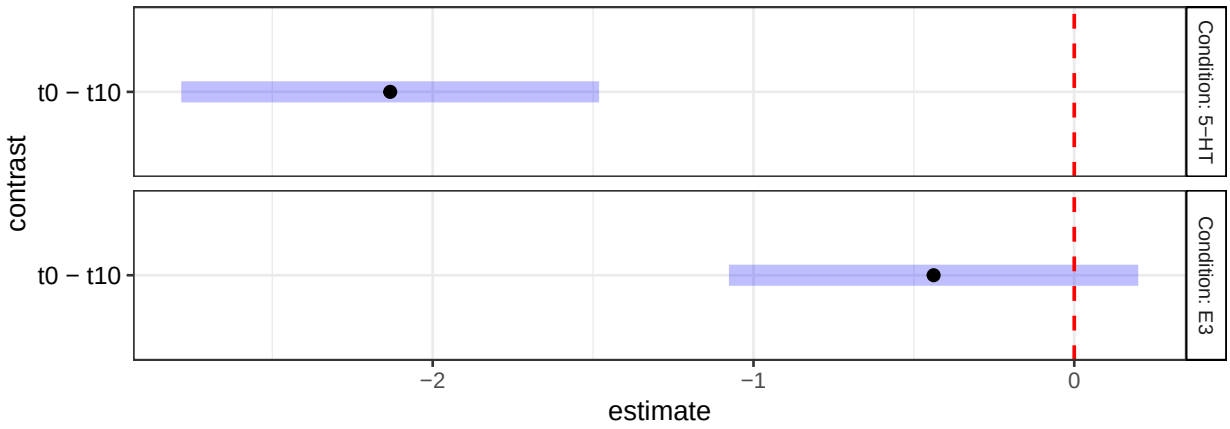
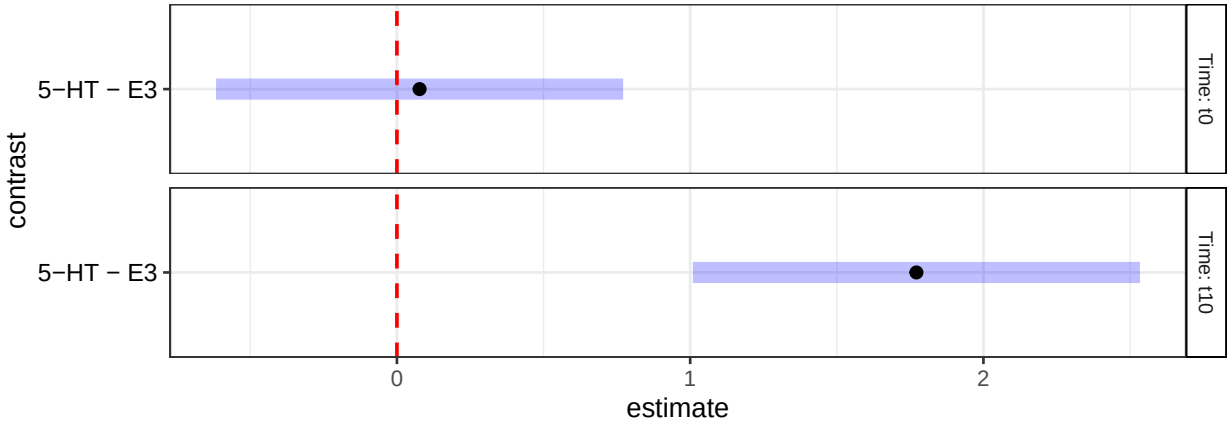


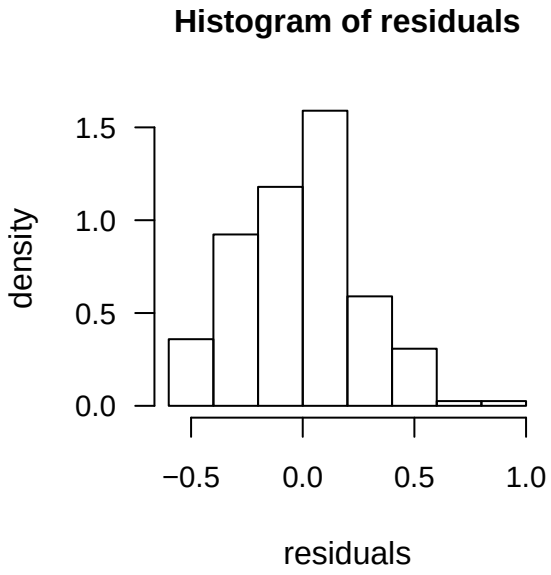
Table 15: Post hoc pairwise comparisons with Tukey's method - Mean differences between E3 and 5-HT at t0 and t10 (back-transformed estimate values from the sqrt scale)

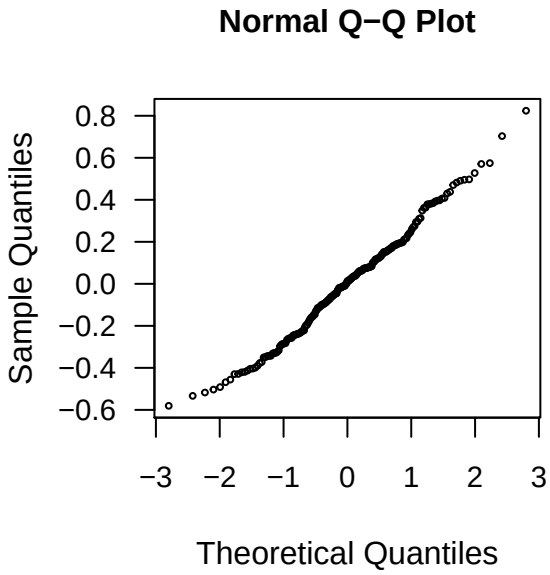
Condition	Time	response	SE	df	lower.CL	upper.CL
5-HT	t0	6.063389	0.3223914	45.55588	5.414278	6.712500
E3	t0	5.985995	0.3306763	50.54931	5.321992	6.650000
5-HT	t10	8.195909	0.3729048	44.73543	7.444718	8.947101
E3	t10	6.424439	0.3429299	50.60150	5.735847	7.113031

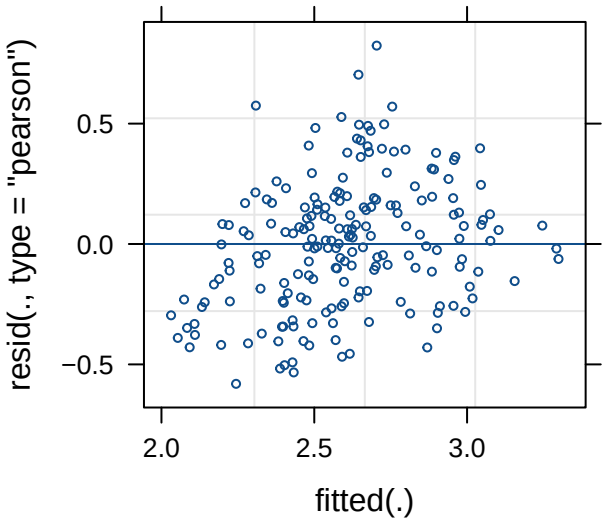
contrast	Time	estimate	SE	df	t.ratio	p.value
5-HT - E3	t0	0.08	0.34	45.6	0.225	0.8233684
5-HT - E3	t10	1.77	0.38	44.7	4.681	0.0000267



2.4.4 Checking model assumptions







2.4.5 Conclusion

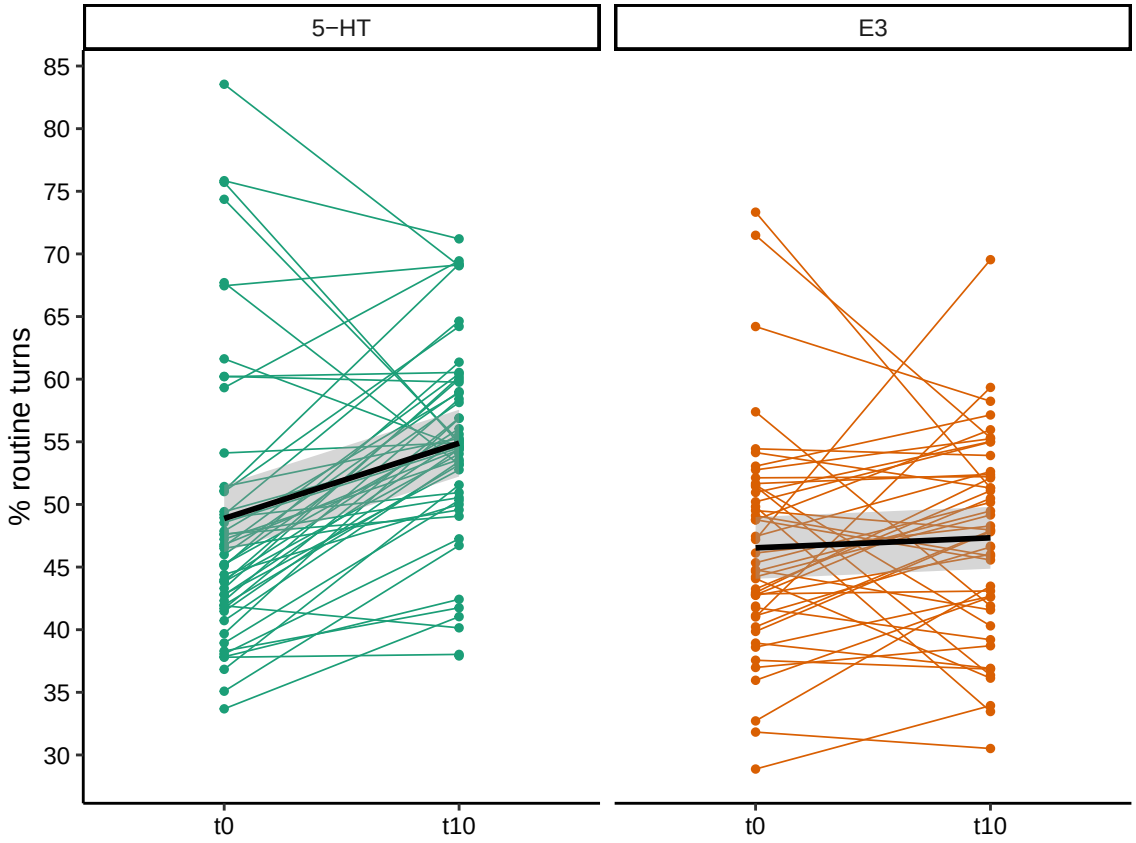
Type II Wald chi-square tests for the fitted model indicated a significant interaction effect between Condition and Time on Speed (Wald $\chi^2 = 12.71$, df $= 1$, $^{***}p = 0.0004$). Post hoc tests using Tukey's method showed a difference between t0 and t10 in 5-HT ($^{***}p =$ 4.2e-8) (faster at t10), and between E3 and 5-HT at t10 ($^{***}p =$ 2.7e-5) (faster in 5-HT), while no differences were observed between t0 and t10 in E3 ($^{ns}p = 0.17$), and between E3 and 5-HT at t0 ($^{ns}p = 0.82$).

2.5 Percentage of routine turns (Figure 2G)

2.5.1 Descriptive data

Table 17: Percentage of routine turns by Condition and Time

	5-HT	E3
t0	48.88 ± 11.29 (n = 51, r = 33.68-83.54)	46.53 ± 8.88 (n = 46, r = 28.88-73.33)
t10	54.89 ± 7.82 (n = 52, r = 37.9-71.2)	47.34 ± 7.95 (n = 46, r = 30.51-69.54)



2.5.2 Linear mixed model with Wald chi-square tests

Significant interaction between Condition (5-HT, E3) and Time (t0, t10) on the percentage of routine turns (Wald $\chi^2 = 8.89$, df $= 1$, $^{**}p = 0.003$).

```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: Turns
##                  Chisq Df Pr(>Chisq)
## Condition      13.0629  1  0.0003012 ***
## Time           15.4716  1  8.375e-05 ***
## Condition:Time  8.8854  1  0.0028746 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

2.5.3 Tukey's post hoc tests

Table 18: Post hoc pairwise comparisons with Tukey's method - Mean differences between t0 and t10 by Condition

Time	Condition	emmean	SE	df	lower.CL	upper.CL
t0	5-HT	48.62546	1.460635	48.46760	45.68939	51.56153
t10	5-HT	54.81326	1.453170	47.59040	51.89082	57.73571
t0	E3	46.33320	1.514471	54.06421	43.29695	49.36944
t10	E3	47.04067	1.515859	54.05261	44.00163	50.07972

contrast	Condition	estimate	SE	df	t.ratio	p.value
t0 - t10	5-HT	-6.19	1.26	95.0	-4.906	0.0000038
t0 - t10	E3	-0.71	1.34	95.9	-0.529	0.5982988

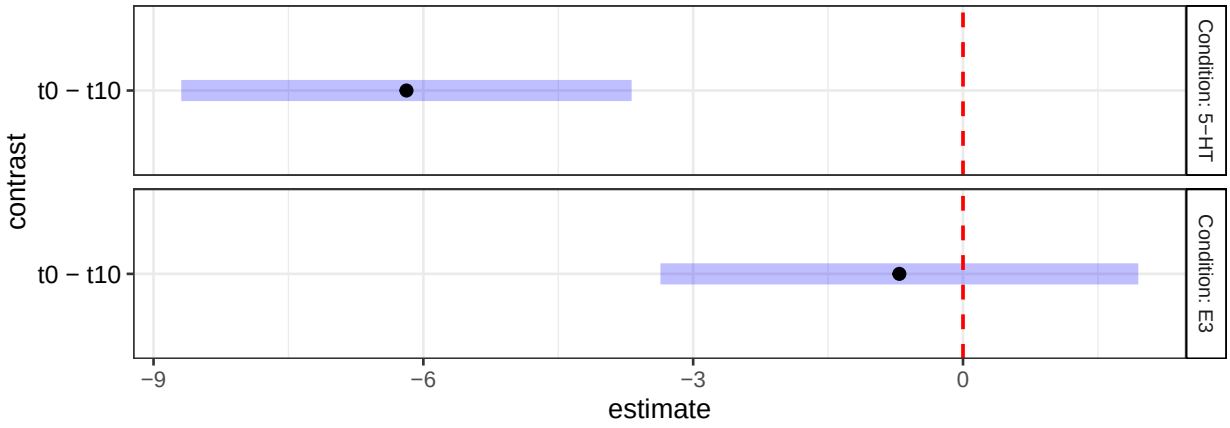
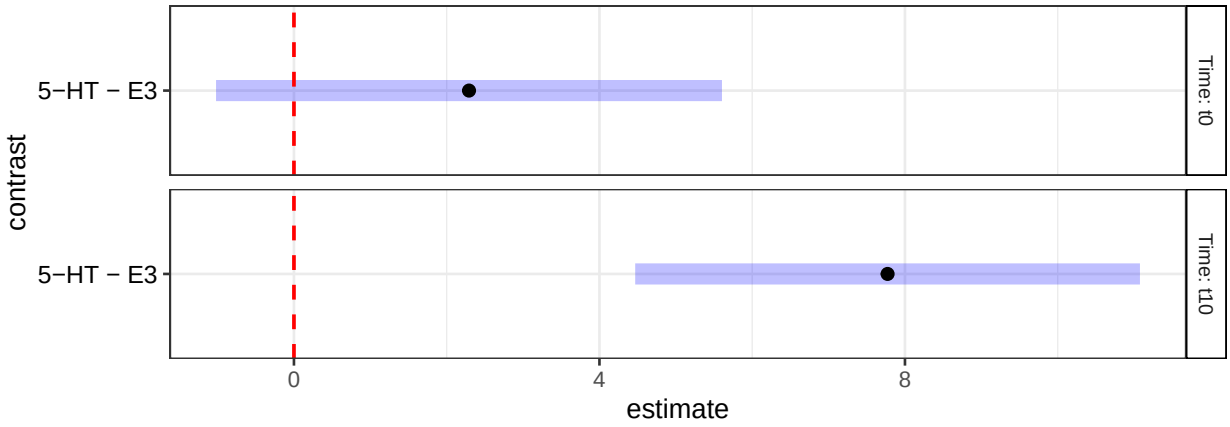


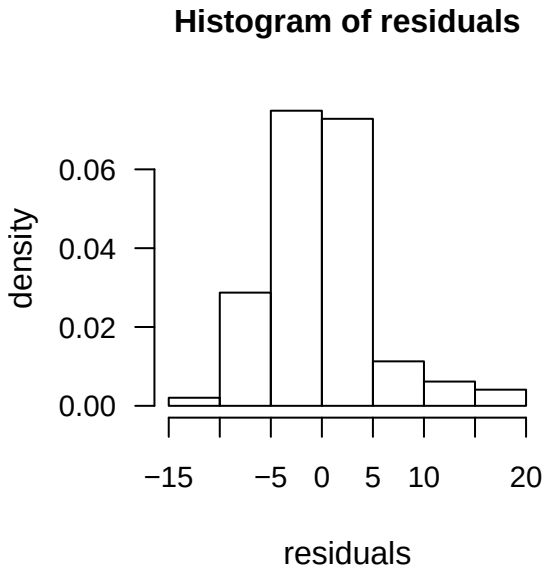
Table 20: Post hoc pairwise comparisons with Tukey's method - Mean differences between E3 and 5-HT at t0 and t10

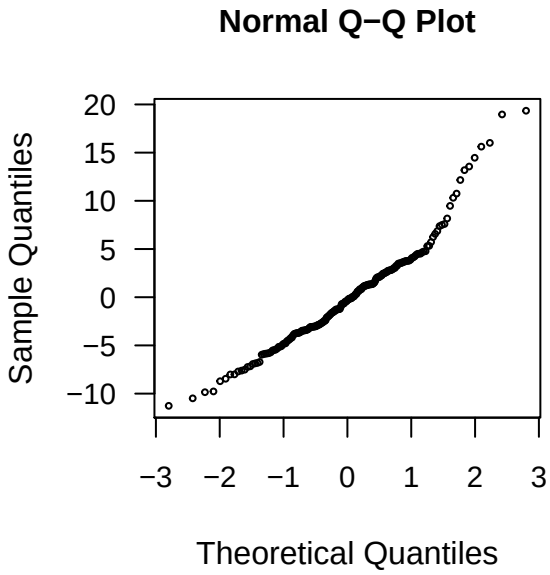
Condition	Time	emmean	SE	df	lower.CL	upper.CL
5-HT	t0	48.62546	1.460635	48.46760	45.68939	51.56153
E3	t0	46.33320	1.514471	54.06421	43.29695	49.36944
5-HT	t10	54.81326	1.453170	47.59040	51.89082	57.73571
E3	t10	47.04067	1.515859	54.05261	44.00163	50.07972

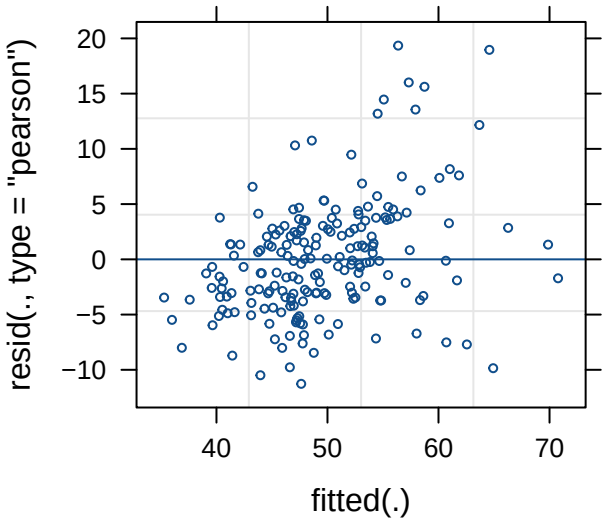
contrast	Time	estimate	SE	df	t.ratio	p.value
5-HT - E3	t0	2.29	1.68	142.1	1.368	0.1734188
5-HT - E3	t10	7.77	1.67	142.2	4.650	0.0000075



2.5.4 Checking model assumptions







2.5.5 Conclusion

Type II Wald chi-square tests for the fitted model indicated a significant interaction effect between Condition and Time on then percentage of routine turns (Wald $\chi^2 = 8.89$, df $= 1$, $^{**}p = 0.003$). Post hoc tests using Tukey's method showed significant differences between t0 and t10 in 5-HT ($^{***}p =$ 3.8e-6), and between E3 and 5-HT at t10 ($^{***}p =$ 7.5e-6), while there was no significant difference between t0 and t10 in E3 ($^{ns}p =$ 0.60), and between E3 and 5-HT at t0 ($^{ns}p = 0.17$).

3 Effect of 5-HT gradient (Figure 2I-K)

3.1 Check for influential observations (Cook's distance)

We removed three larvae: FishID 55, FishID 57 and FishID 112.

Table 22: Influential observations across all the parameters

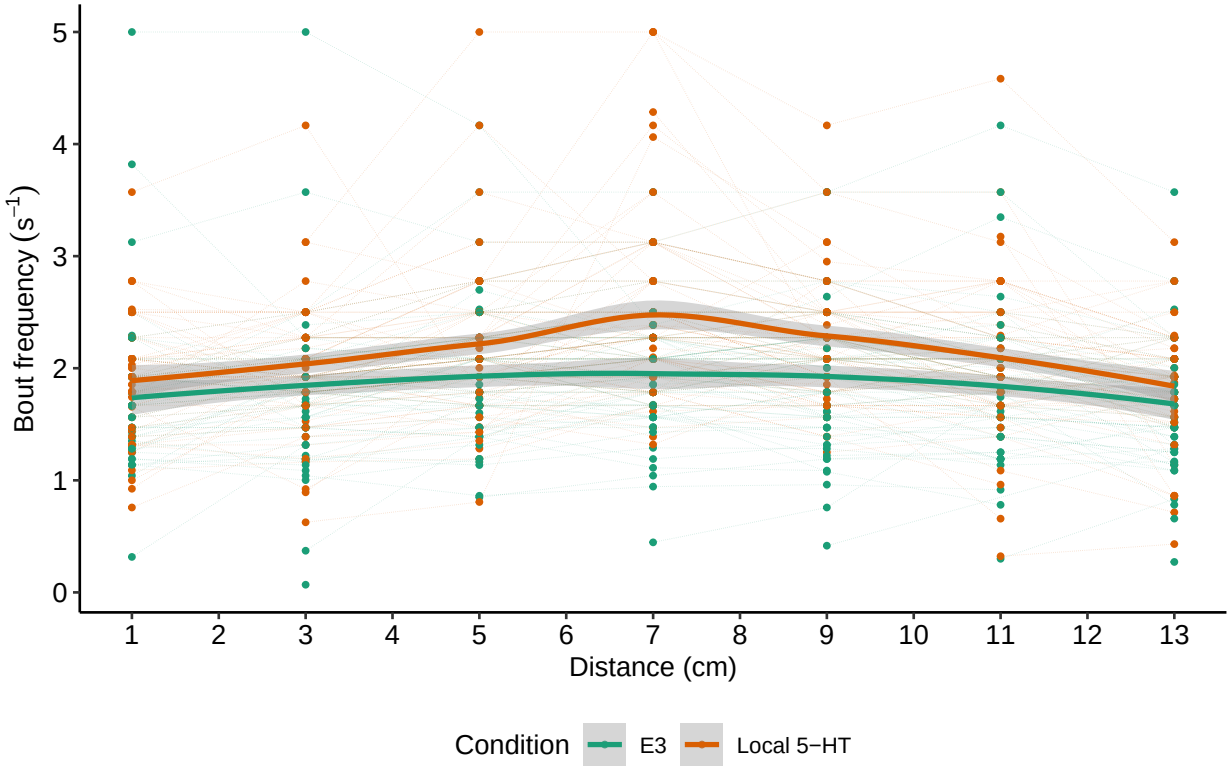
	FishID	Condition	Time	Value	CookD	Parameter
304	55	Local 5-HT	5cm	42.136	0.083	Speed
4591	57	Local 5-HT	7cm	6.250	0.065	FQ
459	57	Local 5-HT	7cm	40.910	0.070	Speed
358	112	Local 5-HT	5cm	35.102	0.080	Speed

3.2 Bout frequency (Figure 2I)

3.2.1 Descriptive data

Table 23: Bout frequency by Condition and Distance

	E3	Local.5.HT
1cm	1.76 ± 0.73 (n = 49, r = 0.32-5)	1.92 ± 0.5 (n = 61, r = 0.76-3.57)
3cm	1.79 ± 0.64 (n = 68, r = 0.07-5)	1.98 ± 0.52 (n = 76, r = 0.63-4.17)
5cm	1.97 ± 0.56 (n = 70, r = 0.85-4.17)	2.16 ± 0.62 (n = 79, r = 0.81-5)
7cm	1.95 ± 0.51 (n = 70, r = 0.45-3.57)	2.48 ± 0.78 (n = 81, r = 1.32-5)
9cm	1.91 ± 0.56 (n = 70, r = 0.42-3.57)	2.23 ± 0.51 (n = 78, r = 1.25-4.17)
11cm	1.87 ± 0.59 (n = 67, r = 0.3-4.17)	2.06 ± 0.61 (n = 75, r = 0.32-4.58)
13cm	1.67 ± 0.54 (n = 59, r = 0.27-3.57)	1.86 ± 0.5 (n = 63, r = 0.43-3.12)



3.2.2 Linear mixed model with Wald chi-square tests

Significant interaction effect between Condition (Local 5-HT, E3) and Distance (as factor) on Bout frequency (Wald $\chi^2 = 27.28$, df = 6, $^{***}p = 0.00013$).

```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: FQ
##                        Chisq Df Pr(>Chisq)
## Condition             17.588  1  2.743e-05 ***
## Distance2            138.221  6  < 2.2e-16 ***
## Condition:Distance2   27.281  6  0.0001283 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

3.2.3 Tukey's post hoc tests

Table 24: Post hoc pairwise comparisons with Tukey's method - Mean differences between E3 and 5-HT at each distance

Condition	Distance2	emmean	SE	df	lower.CL	upper.CL
E3	1cm	1.675611	0.0768101	527.7233	1.524720	1.826502
Local 5-HT	1cm	1.913712	0.0715047	451.9088	1.773189	2.054235
E3	3cm	1.730814	0.0700039	406.9619	1.593199	1.868428
Local 5-HT	3cm	1.946672	0.0675162	378.9873	1.813919	2.079426
E3	5cm	1.874542	0.0696013	398.1918	1.737710	2.011374
Local 5-HT	5cm	2.146383	0.0668698	367.4624	2.014888	2.277879
E3	7cm	1.850350	0.0696438	398.5137	1.713435	1.987265
Local 5-HT	7cm	2.453160	0.0664493	360.1172	2.322483	2.583838
E3	9cm	1.825247	0.0696112	398.2744	1.688396	1.962098
Local 5-HT	9cm	2.207576	0.0670759	371.2324	2.075679	2.339472
E3	11cm	1.768141	0.0704081	412.4944	1.629738	1.906545
Local 5-HT	11cm	2.036139	0.0676871	382.7949	1.903054	2.169224
E3	13cm	1.569411	0.0727323	456.1464	1.426479	1.712343
Local 5-HT	13cm	1.810336	0.0707641	439.9872	1.671258	1.949414

contrast	Distance2	estimate	SE	df	t.ratio	p.value
E3 - Local 5-HT	1cm	-0.24	0.1	491.1	-2.269	0.0237083
E3 - Local 5-HT	3cm	-0.22	0.1	393.2	-2.219	0.0270254
E3 - Local 5-HT	5cm	-0.27	0.1	383.1	-2.816	0.0051069
E3 - Local 5-HT	7cm	-0.60	0.1	379.6	-6.262	0.0000000
E3 - Local 5-HT	9cm	-0.38	0.1	385.0	-3.955	0.0000911
E3 - Local 5-HT	11cm	-0.27	0.1	397.9	-2.744	0.0063443
E3 - Local 5-HT	13cm	-0.24	0.1	448.2	-2.374	0.0180077

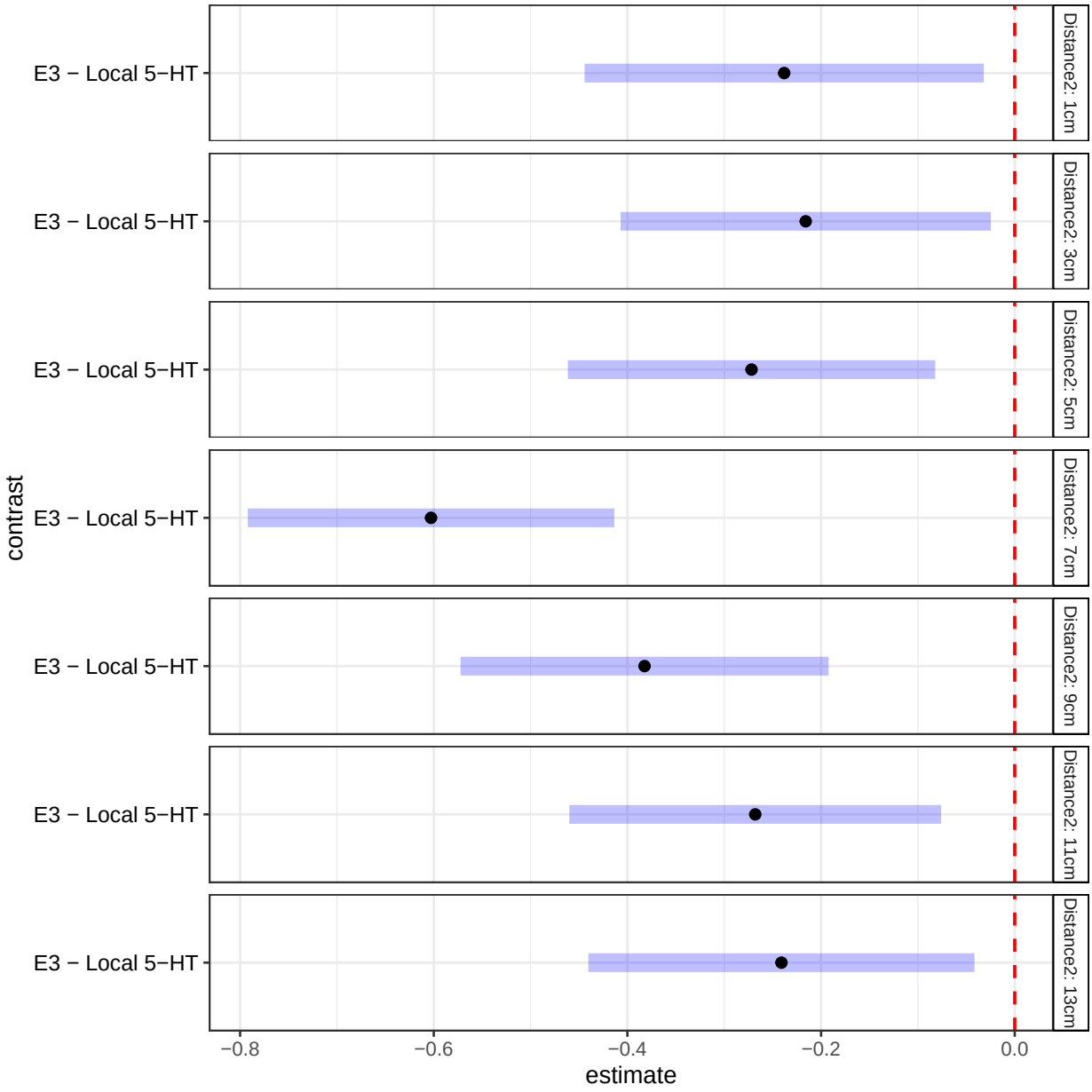

contrast
E3 − Local 5−HT
Distance2: 1cm
Distance2: 3cm
Distance2: 5cm
Distance2: 7cm
Distance2: 9cm
Distance2: 11cm
Distance2: 13cm
−0.8
−0.6
−0.4
−0.2
0.0
estimate

Table 26: Post hoc pairwise comparisons with Tukey's method - Mean differences between the successive distance for E3 and 5-HT

Distance2	Condition	emmean	SE	df	lower.CL	upper.CL
1cm	E3	1.675611	0.0768101	527.7233	1.524720	1.826502
3cm	E3	1.730814	0.0700039	406.9619	1.593199	1.868428
5cm	E3	1.874542	0.0696013	398.1918	1.737710	2.011374
7cm	E3	1.850350	0.0696438	398.5137	1.713435	1.987265
9cm	E3	1.825247	0.0696112	398.2744	1.688396	1.962098
11cm	E3	1.768141	0.0704081	412.4944	1.629738	1.906545
13cm	E3	1.569411	0.0727323	456.1464	1.426479	1.712343
1cm	Local 5-HT	1.913712	0.0715047	451.9088	1.773189	2.054235
3cm	Local 5-HT	1.946672	0.0675162	378.9873	1.813919	2.079426
5cm	Local 5-HT	2.146383	0.0668698	367.4624	2.014888	2.277879
7cm	Local 5-HT	2.453160	0.0664493	360.1172	2.322483	2.583838
9cm	Local 5-HT	2.207576	0.0670759	371.2324	2.075679	2.339472
11cm	Local 5-HT	2.036139	0.0676871	382.7949	1.903054	2.169224
13cm	Local 5-HT	1.810336	0.0707641	439.9872	1.671258	1.949414

contrast	Condition	estimate	SE	df	t.ratio	p.value
3cm - 1cm	E3	0.06	0.07	795.5	0.757	0.9536141
5cm - 3cm	E3	0.14	0.07	796.2	2.178	0.1505852
7cm - 5cm	E3	-0.02	0.07	790.6	-0.371	0.9986727
9cm - 7cm	E3	-0.03	0.07	790.6	-0.385	0.9983771
11cm - 9cm	E3	-0.06	0.07	790.1	-0.868	0.9176735
13cm - 11cm	E3	-0.20	0.07	799.6	-2.862	0.0250060
3cm - 1cm	Local 5-HT	0.03	0.07	790.4	0.496	0.9937302
5cm - 3cm	Local 5-HT	0.20	0.06	787.5	3.237	0.0074050
7cm - 5cm	Local 5-HT	0.31	0.06	787.4	5.055	0.0000034
9cm - 7cm	Local 5-HT	-0.25	0.06	789.0	-4.024	0.0003588
11cm - 9cm	Local 5-HT	-0.17	0.06	790.6	-2.750	0.0340057
13cm - 11cm	Local 5-HT	-0.23	0.07	793.3	-3.413	0.0038139

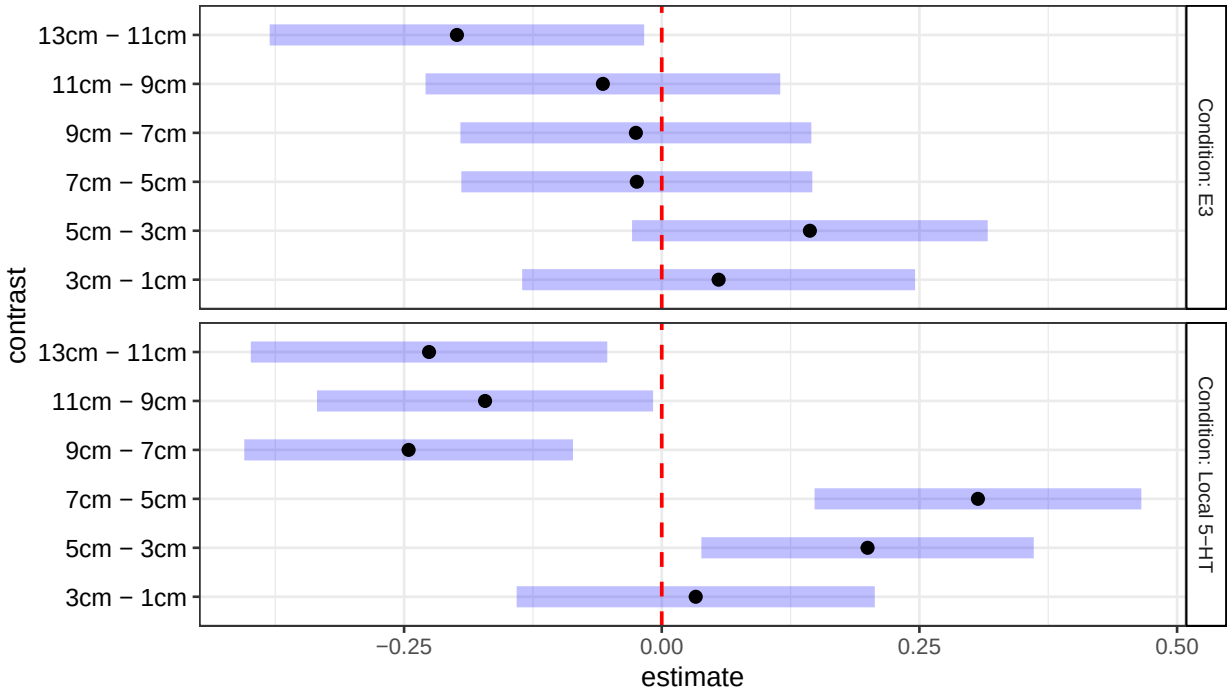
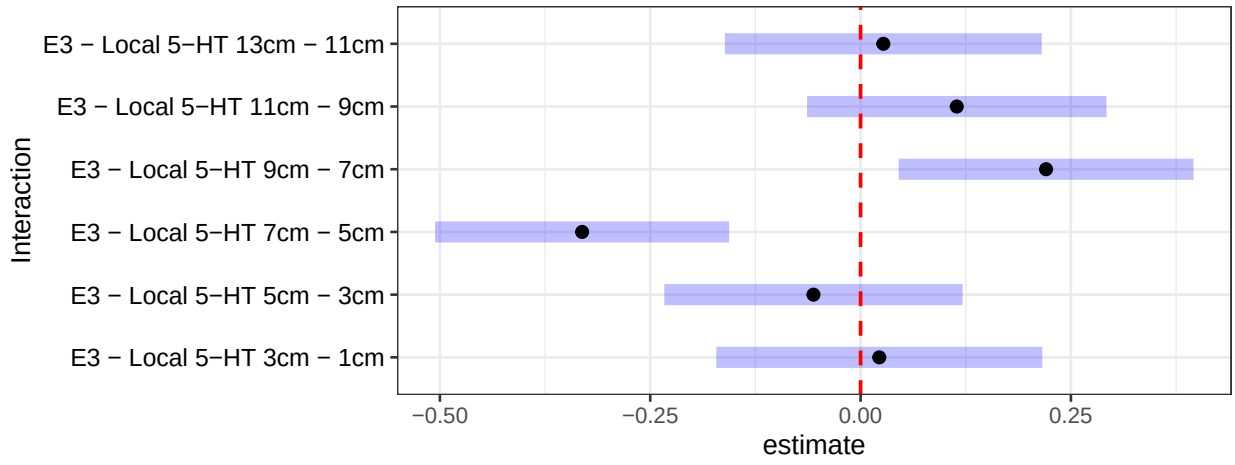

13cm − 11cm
11cm − 9cm
9cm − 7cm
7cm − 5cm
5cm − 3cm
3cm − 1cm
contrast
Condition: E3
Condition: Local 5−HT
−0.25
0.00
0.25
0.50
estimate

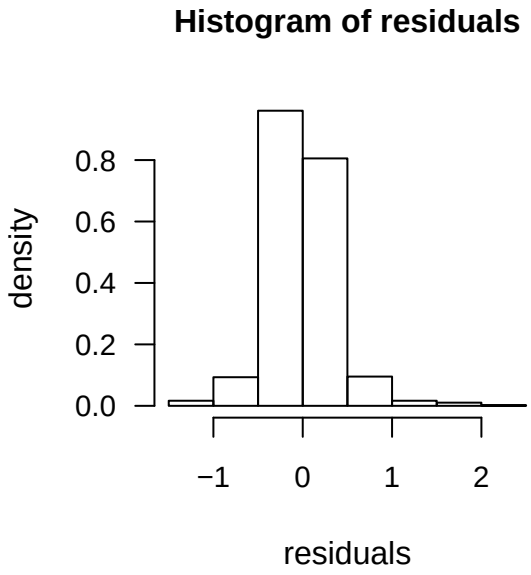
Table 28: Post hoc pairwise comparisons with Tukey's method - Changes in condition differences by concentration change

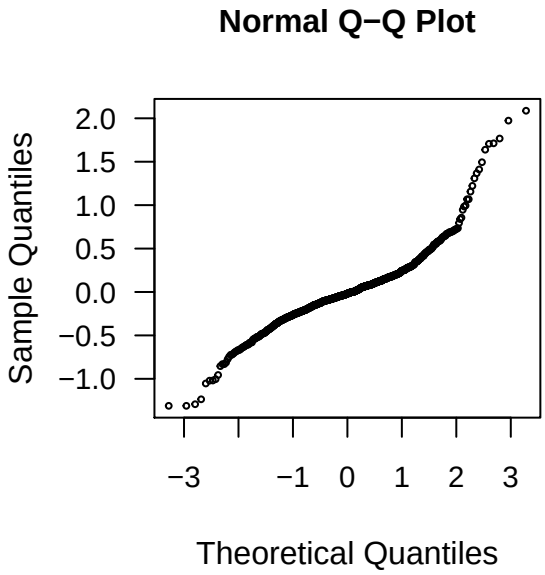
Distance2	Condition	emmean	SE	df	lower.CL	upper.CL
1cm	E3	1.675611	0.0768101	527.7233	1.524720	1.826502
3cm	E3	1.730814	0.0700039	406.9619	1.593199	1.868428
5cm	E3	1.874542	0.0696013	398.1918	1.737710	2.011374
7cm	E3	1.850350	0.0696438	398.5137	1.713435	1.987265
9cm	E3	1.825247	0.0696112	398.2744	1.688396	1.962098
11cm	E3	1.768141	0.0704081	412.4944	1.629738	1.906545
13cm	E3	1.569411	0.0727323	456.1464	1.426479	1.712343
1cm	Local 5-HT	1.913712	0.0715047	451.9088	1.773189	2.054235
3cm	Local 5-HT	1.946672	0.0675162	378.9873	1.813919	2.079426
5cm	Local 5-HT	2.146383	0.0668698	367.4624	2.014888	2.277879
7cm	Local 5-HT	2.453160	0.0664493	360.1172	2.322483	2.583838
9cm	Local 5-HT	2.207576	0.0670759	371.2324	2.075679	2.339472
11cm	Local 5-HT	2.036139	0.0676871	382.7949	1.903054	2.169224
13cm	Local 5-HT	1.810336	0.0707641	439.9872	1.671258	1.949414

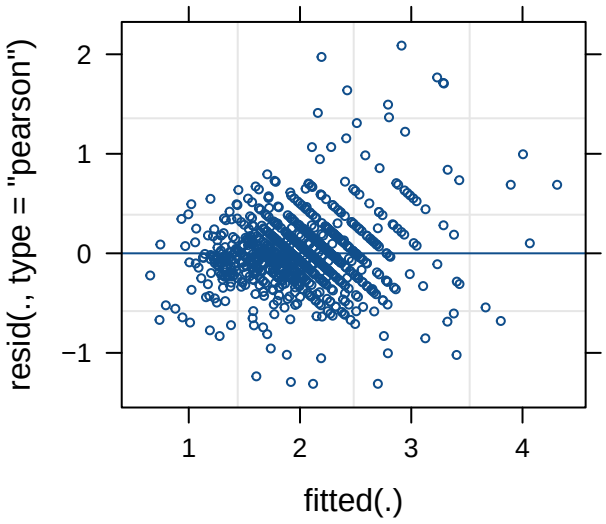
Condition_pairwise	Distance2_consec	estimate	SE	df	t.ratio	p.value
E3 - Local 5-HT	3cm - 1cm	0.02	0.10	793.2	0.225	0.8217545
E3 - Local 5-HT	5cm - 3cm	-0.06	0.09	792.1	-0.620	0.5355929
E3 - Local 5-HT	7cm - 5cm	-0.33	0.09	789.1	-3.717	0.0002160
E3 - Local 5-HT	9cm - 7cm	0.22	0.09	789.9	2.469	0.0137434
E3 - Local 5-HT	11cm - 9cm	0.11	0.09	790.3	1.261	0.2076414
E3 - Local 5-HT	13cm - 11cm	0.03	0.10	796.7	0.282	0.7778215



3.2.4 Checking model assumptions







3.2.5 Conclusion

Type II Wald chi-square tests for the fitted model indicated a significant interaction effect between Condition and Distance on Bout frequency (Wald $\chi^2 = 27.28$, df = 6, $^{***}p = 0.00013$). Post hoc tests using Tukey's method showed significant differences of Bout frequency between E3 and 5-HT at 1cm ($^{*}p = 0.02$), 3cm ($^{*}p = 0.03$), 5cm ($^{**}p = 0.005$), 7cm ($^{***}p$ = 1.0e-9), 9cm ($^{***}p$ = 9.1e-5), 11cm ($^{**}p = 0.006$) and 13cm ($^{*}p = 0.018$) (faster in 5-HT); in E3 between 11cm and 13cm ($^{*}p = 0.02$), and in Local 5-HT: between 3cm and 5cm ($^{**}p = 0.007$), between 5cm and 7cm ($^{***}p$ = 2.4e-6), 7cm and 9cm ($^{***}p = 0.0004$), 9cm and 11cm ($^{*}p$

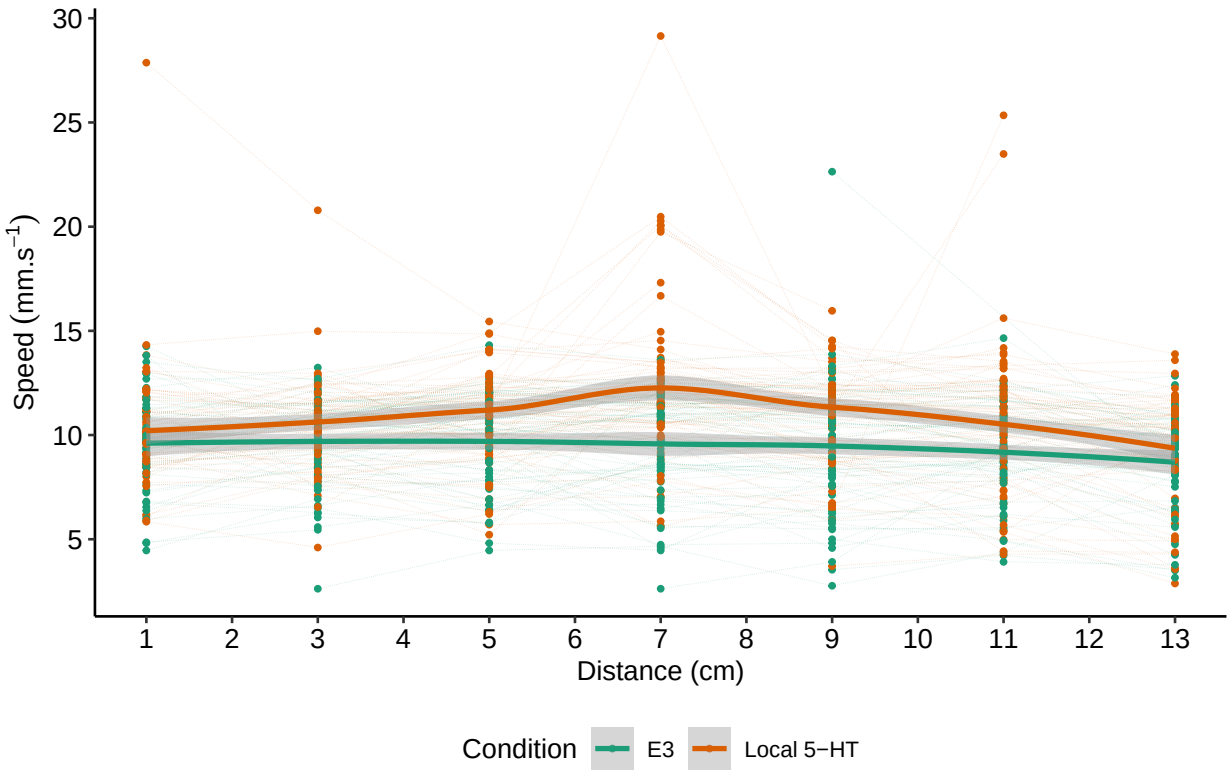
= 0.03) and 11cm and 13cm ($^{**}p = 0.004$). Moreover the difference (E3)-(5-HT) was significantly increased from 5cm to 7cm ($^{***}p = 0.0002$) and significantly decreased from 7cm to 9cm ($^{*}p = 0.013$).

3.3 Speed (square root transformed data) (Figure 2J)

3.3.1 Descriptive data

Table 30: Speed by Condition and Distance

	E3	Local.5.HT
1cm	9.62 ± 2.37 (n = 49, r = 4.46-14.26)	10.33 ± 2.95 (n = 61, r = 5.84-27.87)
3cm	9.65 ± 2.09 (n = 68, r = 2.62-13.24)	10.46 ± 2.1 (n = 76, r = 4.6-20.79)
5cm	9.75 ± 2.16 (n = 70, r = 4.46-14.31)	10.8 ± 2.06 (n = 79, r = 5.22-15.45)
7cm	9.57 ± 2.45 (n = 70, r = 2.62-13.61)	12.26 ± 3.58 (n = 81, r = 5.6-29.15)
9cm	9.49 ± 3.06 (n = 70, r = 2.77-22.64)	10.78 ± 2.21 (n = 78, r = 3.71-15.96)
11cm	9.22 ± 2.34 (n = 67, r = 3.91-14.65)	10.71 ± 3.4 (n = 75, r = 4.28-25.34)
13cm	8.68 ± 2.33 (n = 59, r = 3.15-12.84)	9.32 ± 2.58 (n = 63, r = 2.88-13.89)



3.3.2 Linear mixed model with Wald chi-square tests

Significant interaction effect between Condition (Local 5-HT, E3) and Distance (as factor) on Speed (Wald $\chi^2 = 22.12$, df = 6, $^{**}p = 0.0012$)

```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: sqrt(Speed)
##                      Chisq Df Pr(>Chisq)
## Condition           16.649  1  4.498e-05 ***
## Distance2           75.820  6  2.602e-14 ***
## Condition:Distance2 22.119  6   0.001152 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

3.3.3 Tukey's post hoc tests

Table 31: Post hoc pairwise comparisons with Tukey's method - Mean differences between E3 and Local 5-HT at each distance point (back-transformed estimate values from the sqrt scale)

Condition	Distance2	response	SE	df	lower.CL	upper.CL
E3	1cm	8.846998	0.3279269	533.6413	8.202812	9.491184
Local 5-HT	1cm	9.863040	0.3221579	457.0821	9.229946	10.496135
E3	3cm	9.228091	0.3049647	411.4264	8.628608	9.827574
Local 5-HT	3cm	10.156065	0.3084849	383.0590	9.549529	10.762600
E3	5cm	9.352771	0.3052291	402.4767	8.752729	9.952813
Local 5-HT	5cm	10.514357	0.3108401	371.3431	9.903129	11.125585
E3	7cm	9.161635	0.3022794	402.7894	8.567393	9.755878
Local 5-HT	7cm	11.791749	0.3270878	363.8772	11.148529	12.434969
E3	9cm	9.064455	0.3005305	402.5570	8.473649	9.655260
Local 5-HT	9cm	10.552034	0.3123679	375.1786	9.937823	11.166246
E3	11cm	9.006930	0.3030420	416.9927	8.411250	9.602611
Local 5-HT	11cm	10.472984	0.3140648	386.9497	9.855497	11.090471
E3	13cm	8.254872	0.2997958	461.3056	7.665738	8.844007
Local 5-HT	13cm	9.233469	0.3084496	445.0427	8.627271	9.839668

contrast	Distance2	estimate	SE	df	t.ratio	p.value
E3 - Local 5-HT	1cm	-1.02	0.46	457.1	-2.210	0.0275831
E3 - Local 5-HT	3cm	-0.93	0.43	383.1	-2.139	0.0330458
E3 - Local 5-HT	5cm	-1.16	0.44	371.3	-2.666	0.0080029
E3 - Local 5-HT	7cm	-2.63	0.45	363.9	-5.905	0.0000000
E3 - Local 5-HT	9cm	-1.49	0.43	375.2	-3.432	0.0006663
E3 - Local 5-HT	11cm	-1.47	0.44	386.9	-3.359	0.0008593
E3 - Local 5-HT	13cm	-0.98	0.43	445.0	-2.275	0.0233761

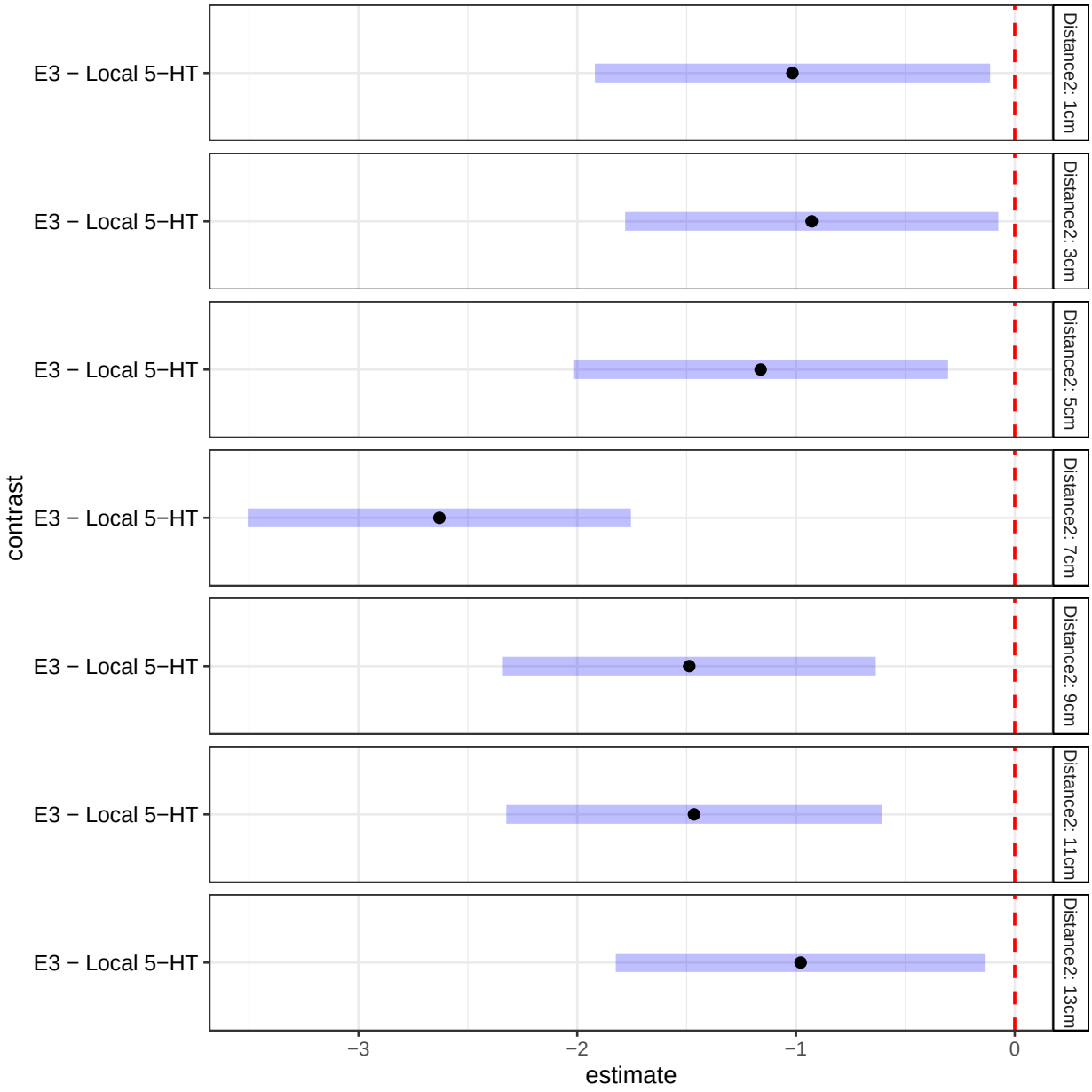

contrast
E3 − Local 5−HT
Distance2: 1cm
Distance2: 3cm
Distance2: 5cm
Distance2: 7cm
Distance2: 9cm
Distance2: 11cm
Distance2: 13cm
−3
−2
−1
0
estimate

Table 33: Post hoc pairwise comparisons with Tukey's method - Mean differences between the successive distance points for E3 and Local 5-HT (back-transformed estimate values from the sqrt scale)

Distance2	Condition	response	SE	df	lower.CL	upper.CL
1cm	E3	8.846998	0.3279269	533.6413	8.202812	9.491184
3cm	E3	9.228091	0.3049647	411.4264	8.628608	9.827574
5cm	E3	9.352771	0.3052291	402.4767	8.752729	9.952813
7cm	E3	9.161635	0.3022794	402.7894	8.567393	9.755878
9cm	E3	9.064455	0.3005305	402.5570	8.473649	9.655260
11cm	E3	9.006930	0.3030420	416.9927	8.411250	9.602611
13cm	E3	8.254872	0.2997958	461.3056	7.665738	8.844007
1cm	Local 5-HT	9.863040	0.3221579	457.0821	9.229946	10.496135
3cm	Local 5-HT	10.156065	0.3084849	383.0590	9.549529	10.762600
5cm	Local 5-HT	10.514357	0.3108401	371.3431	9.903129	11.125585
7cm	Local 5-HT	11.791749	0.3270878	363.8772	11.148529	12.434969
9cm	Local 5-HT	10.552034	0.3123679	375.1786	9.937823	11.166246
11cm	Local 5-HT	10.472984	0.3140648	386.9497	9.855497	11.090471
13cm	Local 5-HT	9.233469	0.3084496	445.0427	8.627271	9.839668

contrast	Condition	estimate	SE	df	t.ratio	p.value
3cm - 1cm	E3	0.38	0.32	411.4	1.208	0.7321284
5cm - 3cm	E3	0.12	0.29	402.5	0.430	0.9970145
7cm - 5cm	E3	-0.19	0.29	402.5	-0.669	0.9731000
9cm - 7cm	E3	-0.10	0.28	402.6	-0.343	0.9991269
11cm - 9cm	E3	-0.06	0.29	402.6	-0.202	0.9999572
13cm - 11cm	E3	-0.75	0.29	417.0	-2.559	0.0587454
3cm - 1cm	Local 5-HT	0.29	0.30	383.1	0.967	0.8726195
5cm - 3cm	Local 5-HT	0.36	0.29	371.3	1.253	0.6998258
7cm - 5cm	Local 5-HT	1.28	0.29	363.9	4.368	0.0001093
9cm - 7cm	Local 5-HT	-1.24	0.29	363.9	-4.212	0.0001705
11cm - 9cm	Local 5-HT	-0.08	0.29	375.2	-0.271	0.9997550
13cm - 11cm	Local 5-HT	-1.24	0.30	386.9	-4.147	0.0002606

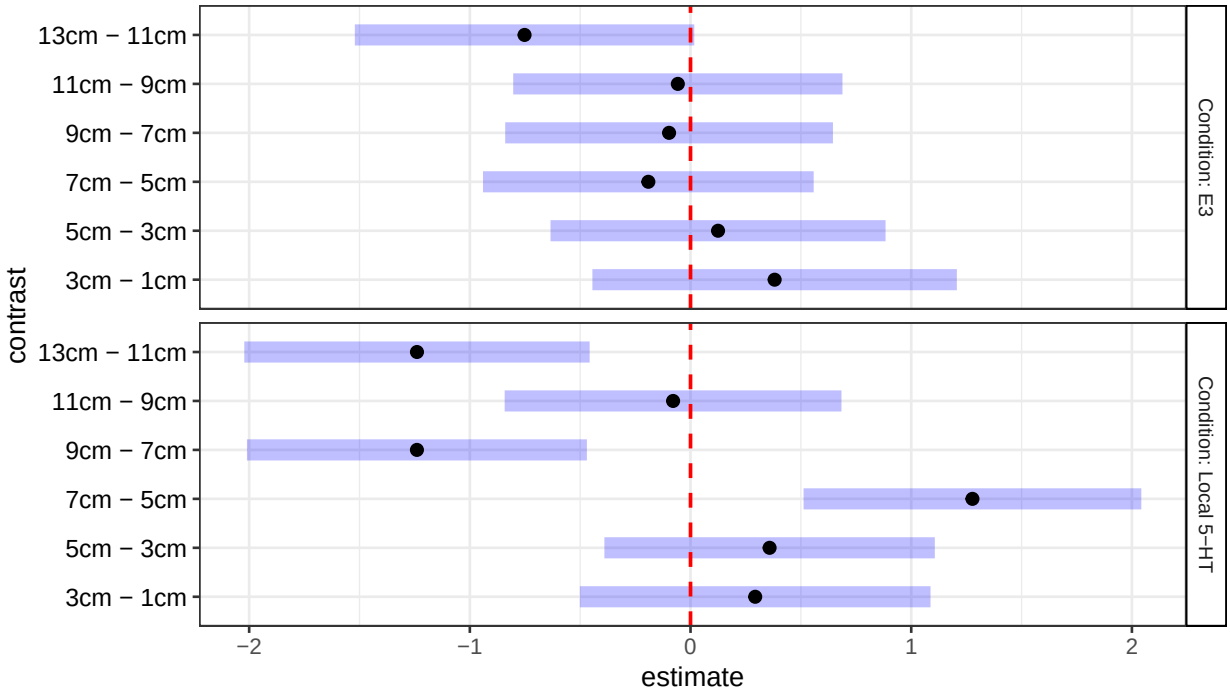
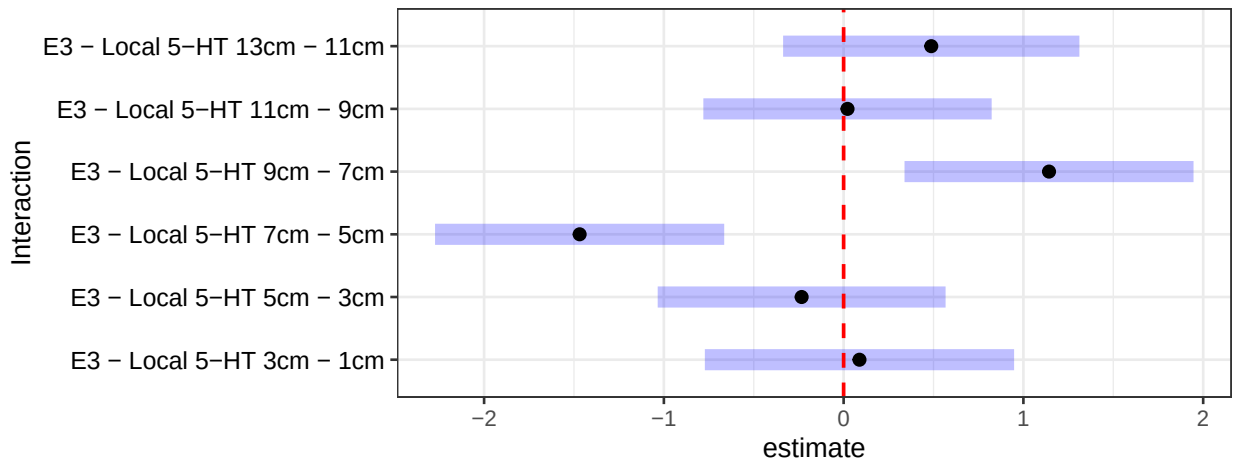

13cm − 11cm
11cm − 9cm
9cm − 7cm
7cm − 5cm
5cm − 3cm
3cm − 1cm
contrast
Condition: E3
Condition: Local 5−HT
−2
−1
0
1
2
estimate

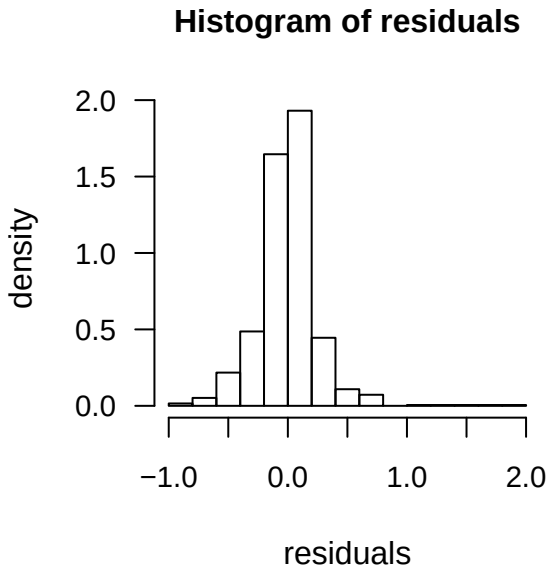
Table 35: Post hoc pairwise comparisons with Tukey's method - Changes in condition differences by concentration change (back-transformed estimate values from the sqrt scale)

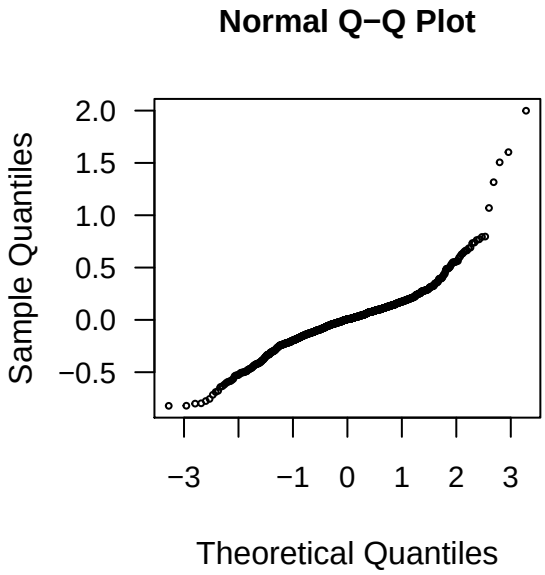
Condition	Distance2	response	SE	df	lower.CL	upper.CL
E3	1cm	8.846998	0.3279269	533.6413	8.202812	9.491184
Local 5-HT	1cm	9.863040	0.3221579	457.0821	9.229946	10.496135
E3	3cm	9.228091	0.3049647	411.4264	8.628608	9.827574
Local 5-HT	3cm	10.156065	0.3084849	383.0590	9.549529	10.762600
E3	5cm	9.352771	0.3052291	402.4767	8.752729	9.952813
Local 5-HT	5cm	10.514357	0.3108401	371.3431	9.903129	11.125585
E3	7cm	9.161635	0.3022794	402.7894	8.567393	9.755878
Local 5-HT	7cm	11.791749	0.3270878	363.8772	11.148529	12.434969
E3	9cm	9.064455	0.3005305	402.5570	8.473649	9.655260
Local 5-HT	9cm	10.552034	0.3123679	375.1786	9.937823	11.166246
E3	11cm	9.006930	0.3030420	416.9927	8.411250	9.602611
Local 5-HT	11cm	10.472984	0.3140648	386.9497	9.855497	11.090471
E3	13cm	8.254872	0.2997958	461.3056	7.665738	8.844007
Local 5-HT	13cm	9.233469	0.3084496	445.0427	8.627271	9.839668

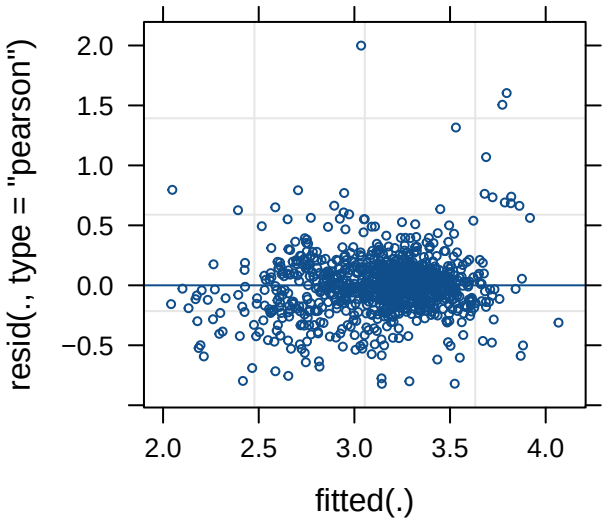
Condition_pairwise	Distance2_consec	estimate	SE	df	t.ratio	p.value
E3 - Local 5-HT	3cm - 1cm	0.09	0.44	383.1	0.201	0.8405832
E3 - Local 5-HT	5cm - 3cm	-0.23	0.41	371.3	-0.574	0.5665808
E3 - Local 5-HT	7cm - 5cm	-1.47	0.41	363.9	-3.591	0.0003753
E3 - Local 5-HT	9cm - 7cm	1.14	0.41	363.9	2.795	0.0054629
E3 - Local 5-HT	11cm - 9cm	0.02	0.41	375.2	0.053	0.9579359
E3 - Local 5-HT	13cm - 11cm	0.49	0.42	386.9	1.163	0.2455793



3.3.4 Checking model assumptions







3.3.5 Conclusion

Type II Wald chi-square tests for the fitted model indicated a significant interaction effect between Condition and Distance on Speed (Wald $\chi^2 = 22.12$, df $= 6$, $^{**}p = 0.0012$). Post hoc tests using Tukey's method showed significant differences of speed between E3 and 5-HT at 1cm ($^{*}p = 0.03$), 3cm ($^{*}p = 0.03$), 5cm ($^{**}p = 0.008$), 7cm ($^{***}p =$ 8.1e-9), 9cm ($^{***}p = 0.0007$), 11cm ($^{***}p = 0.0009$), and 13cm ($^{*}p = 0.02$) (faster in 5-HT); and in 5-HT: between 5cm and 7cm ($^{***}p =$ 9.4e-5), 7cm and 9cm ($^{***}p = 0.0002$) and between 11cm and 13cm ($^{***}p = 0.0003$), and in Local 5-HT: between 5cm and 7cm ($^{*}p = 0.02$), 7cm and 9cm ($^{***}p =$ 2.4e-5),

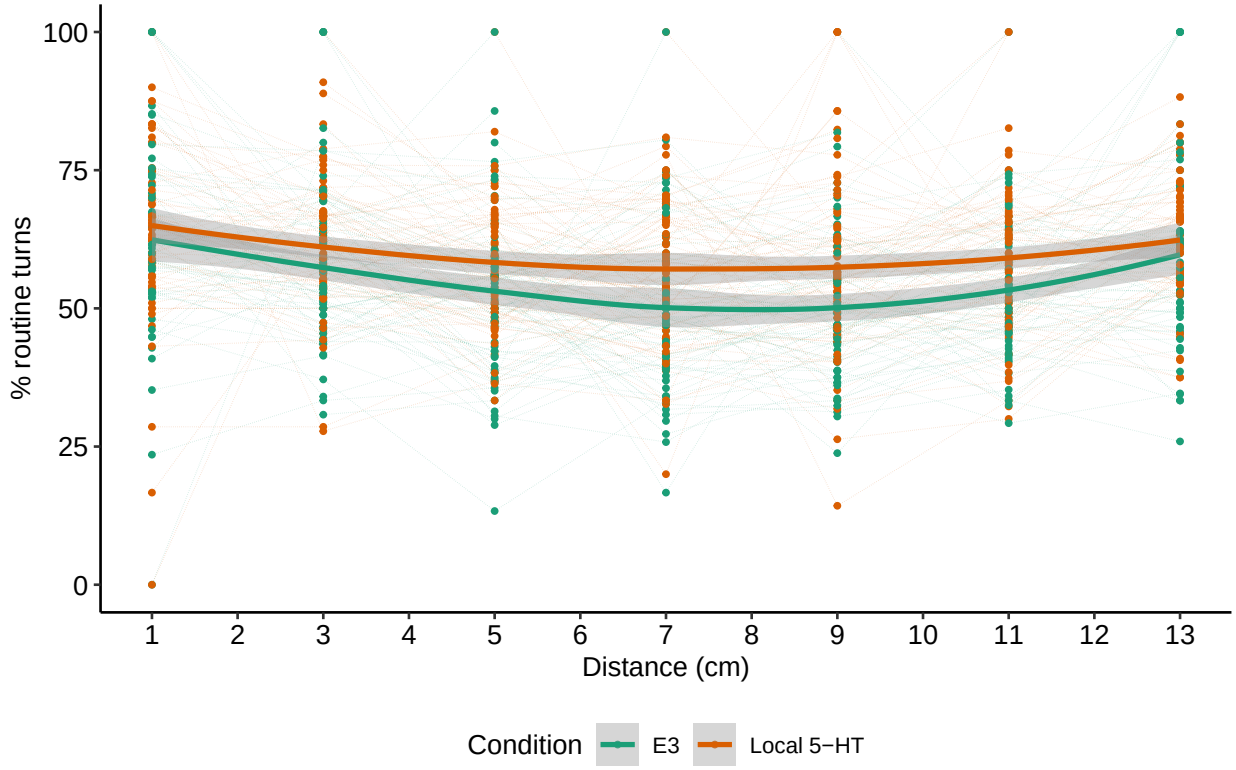
and 11cm and 13cm ($^{***}p = 0.0009$). Moreover the difference (E3)-(5-HT) was significantly increased from 5cm to 7cm ($^{***}p = 0.0004$) and significantly decreased from 7cm to 9cm ($^{**}p = 0.005$).

3.4 Percentage of routine turns (Figure 2K)

3.4.1 Descriptive data

Table 37: Turns by Condition and Distance

	E3	Local.5.HT
1cm	61.68 ± 18.35 (n = 49, r = 0-100)	64.51 ± 16.98 (n = 61, r = 0-100)
3cm	58.86 ± 14.58 (n = 68, r = 30.77-100)	62.2 ± 12.02 (n = 76, r = 27.78-100)
5cm	52.42 ± 14.5 (n = 70, r = 13.33-100)	57.62 ± 11.54 (n = 79, r = 33.33-100)
7cm	50.13 ± 14.7 (n = 70, r = 16.67-100)	57.12 ± 13.03 (n = 81, r = 20-100)
9cm	50.13 ± 13.22 (n = 70, r = 23.81-100)	57.74 ± 15.36 (n = 78, r = 14.29-100)
11cm	53.07 ± 12.24 (n = 67, r = 29.23-100)	58.54 ± 13.22 (n = 75, r = 30-100)
13cm	59.8 ± 17.61 (n = 59, r = 25.93-100)	62.61 ± 11.13 (n = 63, r = 37.5-88.24)



3.4.2 Linear mixed model with Wald chi-square tests

Significant effects of Condition (Local 5-HT, E3) (Wald $\chi^2 = 13.78$, df = 1, $^{***}p = 0.0002$) and Distance (as factor) (Wald $\chi^2 = 68.78$, df = 6, $^{***}p = 7.3\text{e-}13$) on the percentage of routine turns, without effect of interaction (Wald $\chi^2 = 3.41$, df = 6, $^{ns}p = 0.76$).

```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: df$Turns
##                       Chisq Df Pr(>Chisq)
## Condition           13.7812  1  0.0002054 ***
## Distance2           68.7839  6  7.259e-13 ***
## Condition:Distance2  3.4057  6  0.7564752
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

3.4.3 Tukey's post hoc tests

Table 38: Post hoc pairwise comparisons with Tukey's method - Mean differences between the consecutive distance points

Distance2	emmean	SE	df	lower.CL	upper.CL
1cm	63.25693	1.338719	912.3183	60.62961	65.88426
3cm	60.67557	1.177025	854.2447	58.36537	62.98577
5cm	55.18689	1.159531	842.8502	52.91099	57.46280
7cm	53.87900	1.153490	838.8771	51.61494	56.14307
9cm	54.32014	1.162761	844.4332	52.03790	56.60238
11cm	56.39729	1.184989	856.6655	54.07147	58.72311
13cm	61.90617	1.268742	894.3052	59.41611	64.39623

contrast	estimate	SE	df	t.ratio	p.value
3cm - 1cm	-2.58	1.64	810.0	-1.571	0.4729959
5cm - 3cm	-5.49	1.51	801.2	-3.644	0.0016303
7cm - 5cm	-1.31	1.49	797.0	-0.879	0.9121763
9cm - 7cm	0.44	1.49	798.9	0.296	0.9996108
11cm - 9cm	2.08	1.51	799.3	1.373	0.6151996
13cm - 11cm	5.51	1.59	809.4	3.454	0.0035703

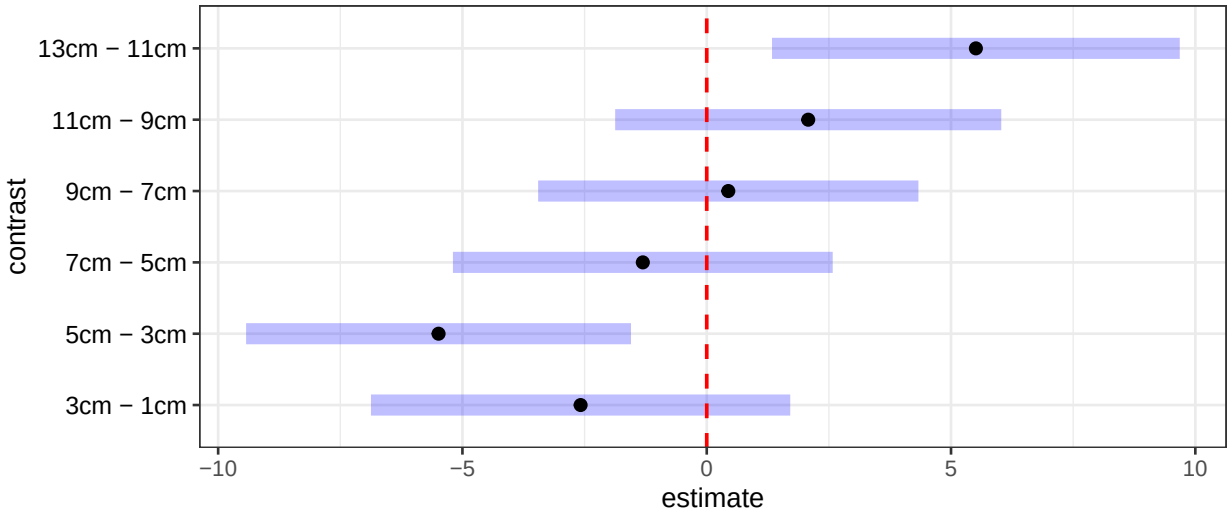

13cm − 11cm
11cm − 9cm
9cm − 7cm
7cm − 5cm
5cm − 3cm
3cm − 1cm
contrast
−10
−5
0
5
10
estimate

Table 40: Post hoc pairwise comparisons with Tukey's method - Mean differences between E3 and Local 5-HT at each distance point

Condition	Distance2	emmean	SE	df	lower.CL	upper.CL
E3	1cm	61.72245	1.989206	922.9173	57.81856	65.62634
Local 5-HT	1cm	64.79142	1.792130	897.3479	61.27416	68.30867
E3	3cm	59.08956	1.707902	863.3991	55.73743	62.44168
Local 5-HT	3cm	62.26159	1.620069	843.7325	59.08175	65.44143
E3	5cm	52.68248	1.686597	852.0389	49.37211	55.99285
Local 5-HT	5cm	57.69130	1.591678	832.2464	54.56713	60.81548
E3	7cm	50.67256	1.687123	850.5513	47.36115	53.98397
Local 5-HT	7cm	57.08544	1.573459	825.0038	53.99699	60.17390
E3	9cm	50.86572	1.686712	851.6895	47.55512	54.17632
Local 5-HT	9cm	57.77457	1.600955	836.1998	54.63220	60.91693
E3	11cm	54.03056	1.721005	863.1427	50.65271	57.40840
Local 5-HT	11cm	58.76403	1.629398	849.2642	55.56591	61.96215
E3	13cm	60.54972	1.823648	894.9052	56.97059	64.12884
Local 5-HT	13cm	63.26263	1.764408	893.6615	59.79976	66.72550

contrast	Distance2	estimate	SE	df	t.ratio	p.value
E3 - Local 5-HT	1cm	-3.07	2.68	912.3	-1.146	0.2519985
E3 - Local 5-HT	3cm	-3.17	2.35	854.2	-1.347	0.1781838
E3 - Local 5-HT	5cm	-5.01	2.32	842.9	-2.160	0.0310658
E3 - Local 5-HT	7cm	-6.41	2.31	838.9	-2.780	0.0055614
E3 - Local 5-HT	9cm	-6.91	2.33	844.4	-2.971	0.0030537
E3 - Local 5-HT	11cm	-4.73	2.37	856.7	-1.997	0.0461124
E3 - Local 5-HT	13cm	-2.71	2.54	894.3	-1.069	0.2852972

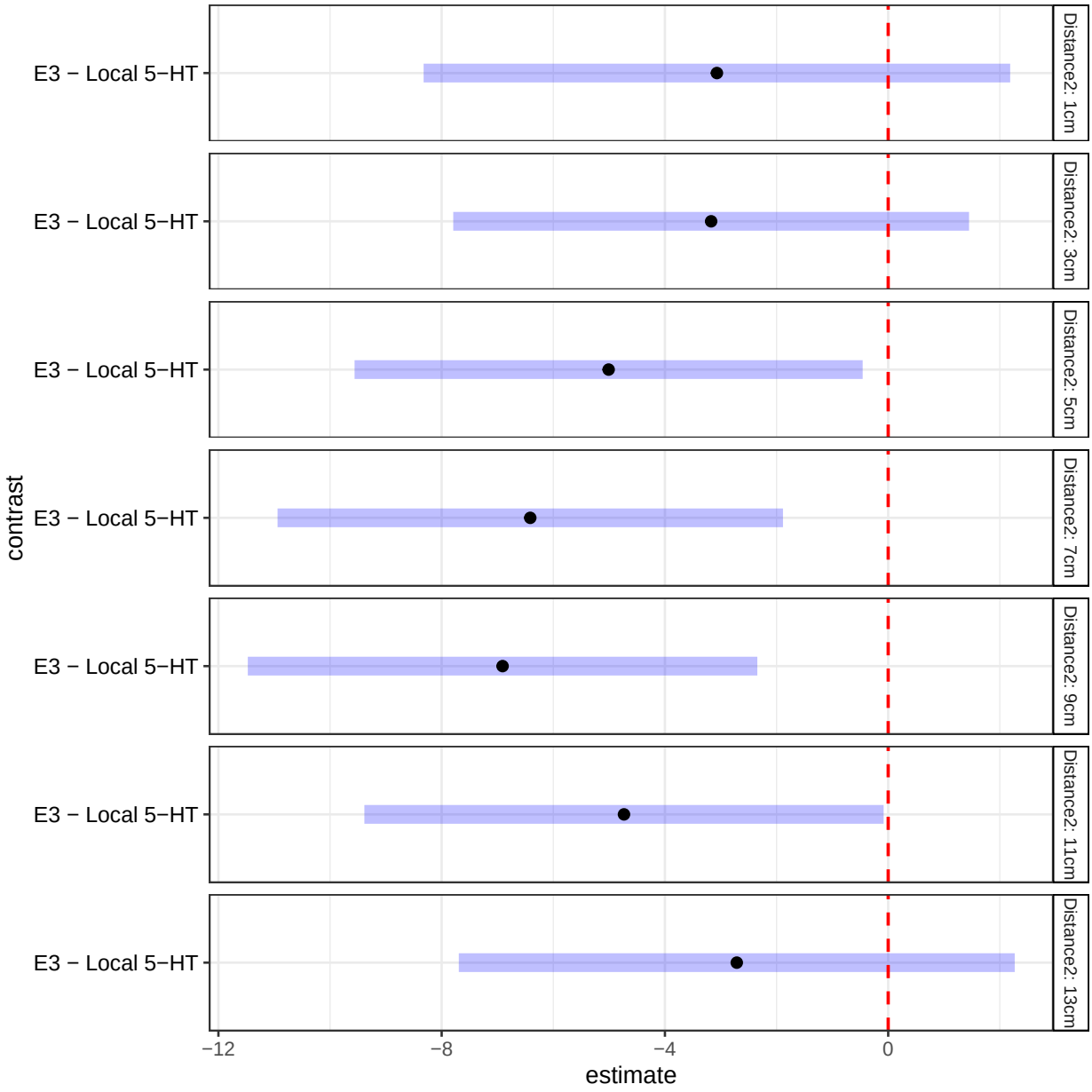

contrast
E3 − Local 5−HT
Distance2: 1cm
Distance2: 3cm
Distance2: 5cm
Distance2: 7cm
Distance2: 9cm
Distance2: 11cm
Distance2: 13cm
−12
−8
−4
0
estimate

Table 42: Post hoc pairwise comparisons with Tukey's method - Mean differences between the successive distance points for E3 and Local 5-HT

Distance2	Condition	emmean	SE	df	lower.CL	upper.CL
1cm	E3	61.72245	1.989206	922.9173	57.81856	65.62634
3cm	E3	59.08956	1.707902	863.3991	55.73743	62.44168
5cm	E3	52.68248	1.686597	852.0389	49.37211	55.99285
7cm	E3	50.67256	1.687123	850.5513	47.36115	53.98397
9cm	E3	50.86572	1.686712	851.6895	47.55512	54.17632
11cm	E3	54.03056	1.721005	863.1427	50.65271	57.40840
13cm	E3	60.54972	1.823648	894.9052	56.97059	64.12884
1cm	Local 5-HT	64.79142	1.792130	897.3479	61.27416	68.30867
3cm	Local 5-HT	62.26159	1.620069	843.7325	59.08175	65.44143
5cm	Local 5-HT	57.69130	1.591678	832.2464	54.56713	60.81548
7cm	Local 5-HT	57.08544	1.573459	825.0038	53.99699	60.17390
9cm	Local 5-HT	57.77457	1.600955	836.1998	54.63220	60.91693
11cm	Local 5-HT	58.76403	1.629398	849.2642	55.56591	61.96215
13cm	Local 5-HT	63.26263	1.764408	893.6615	59.79976	66.72550

contrast	Condition	estimate	SE	df	t.ratio	p.value
3cm - 1cm	E3	-2.63	2.42	814.1	-1.086	0.8092783
5cm - 3cm	E3	-6.41	2.19	807.8	-2.919	0.0205818
7cm - 5cm	E3	-2.01	2.17	800.3	-0.924	0.8934893
9cm - 7cm	E3	0.19	2.17	800.4	0.089	0.9999997
11cm - 9cm	E3	3.16	2.20	798.5	1.440	0.5667250
13cm - 11cm	E3	6.52	2.31	812.8	2.827	0.0271771
3cm - 1cm	Local 5-HT	-2.53	2.22	804.9	-1.141	0.7749260
5cm - 3cm	Local 5-HT	-4.57	2.06	793.7	-2.215	0.1379349
7cm - 5cm	Local 5-HT	-0.61	2.03	793.2	-0.299	0.9995887
9cm - 7cm	Local 5-HT	0.69	2.04	797.2	0.338	0.9991780
11cm - 9cm	Local 5-HT	0.99	2.08	800.2	0.476	0.9948800
13cm - 11cm	Local 5-HT	4.50	2.20	805.6	2.041	0.2024972

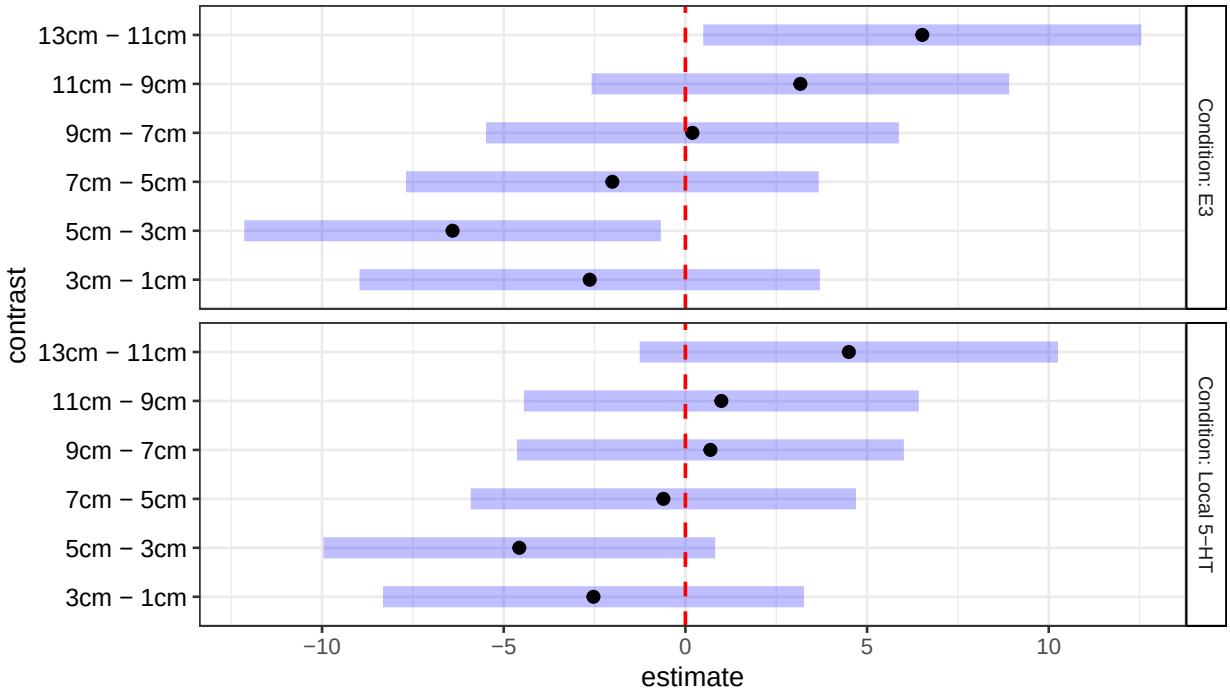
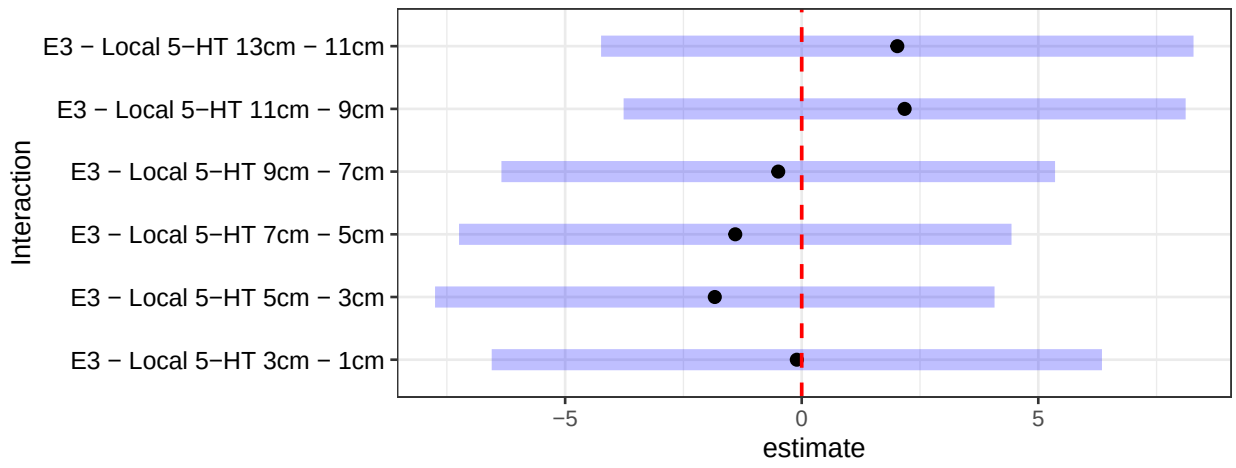

13cm − 11cm
11cm − 9cm
9cm − 7cm
7cm − 5cm
5cm − 3cm
3cm − 1cm
contrast
Condition: E3
Condition: Local 5−HT
−10
−5
0
5
10
estimate

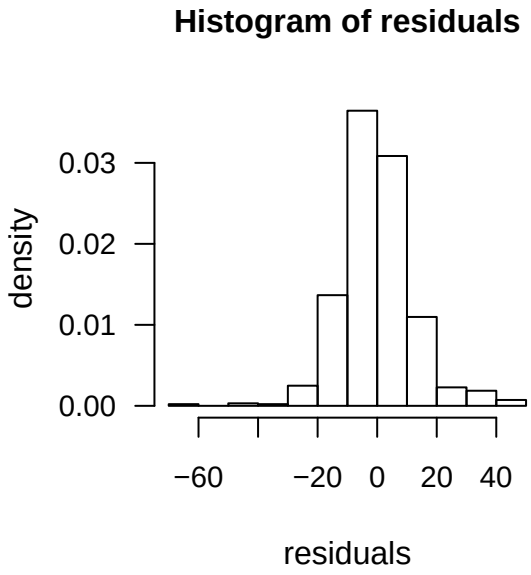
Table 44: Post hoc pairwise comparisons with Tukey's method - Changes in condition differences by concentration change (back-transformed estimate values from the sqrt scale)

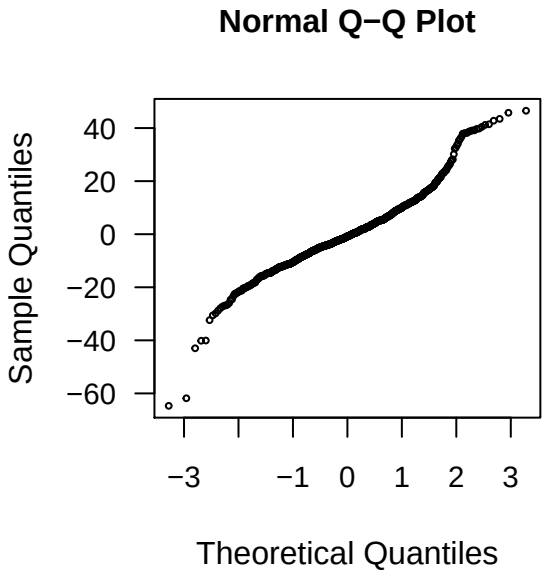
Condition	Distance2	emmean	SE	df	lower.CL	upper.CL
E3	1cm	61.72245	1.989206	922.9173	57.81856	65.62634
Local 5-HT	1cm	64.79142	1.792130	897.3479	61.27416	68.30867
E3	3cm	59.08956	1.707902	863.3991	55.73743	62.44168
Local 5-HT	3cm	62.26159	1.620069	843.7325	59.08175	65.44143
E3	5cm	52.68248	1.686597	852.0389	49.37211	55.99285
Local 5-HT	5cm	57.69130	1.591678	832.2464	54.56713	60.81548
E3	7cm	50.67256	1.687123	850.5513	47.36115	53.98397
Local 5-HT	7cm	57.08544	1.573459	825.0038	53.99699	60.17390
E3	9cm	50.86572	1.686712	851.6895	47.55512	54.17632
Local 5-HT	9cm	57.77457	1.600955	836.1998	54.63220	60.91693
E3	11cm	54.03056	1.721005	863.1427	50.65271	57.40840
Local 5-HT	11cm	58.76403	1.629398	849.2642	55.56591	61.96215
E3	13cm	60.54972	1.823648	894.9052	56.97059	64.12884
Local 5-HT	13cm	63.26263	1.764408	893.6615	59.79976	66.72550

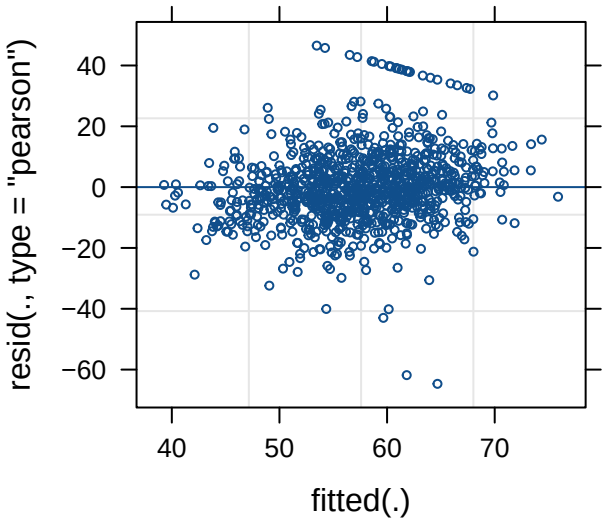
Condition_pairwise	Distance2_consec	estimate	SE	df	t.ratio	p.value
E3 - Local 5-HT	3cm - 1cm	-0.10	3.29	810.0	-0.031	0.9749841
E3 - Local 5-HT	5cm - 3cm	-1.84	3.01	801.2	-0.610	0.5421619
E3 - Local 5-HT	7cm - 5cm	-1.40	2.97	797.0	-0.472	0.6370209
E3 - Local 5-HT	9cm - 7cm	-0.50	2.98	798.9	-0.166	0.8678879
E3 - Local 5-HT	11cm - 9cm	2.18	3.03	799.3	0.719	0.4723938
E3 - Local 5-HT	13cm - 11cm	2.02	3.19	809.4	0.633	0.5265891



3.4.4 Checking model assumptions







3.4.5 Conclusion

Type II Wald chi-square tests for the fitted model indicated a significant interaction effects of Condition (Local 5-HT, E3) (Wald $\chi^2 = 13.78$, df = 1, $^{***}p = 0.0002$) and Distance (as factor) (Wald $\chi^2 = 68.78$, df = 6, $^{***}p = 7.3\text{e-}13$) on the percentage of routine turns, without interaction effect (Wald $\chi^2 = 3.41$, df = 6, $^{ns}p = 0.76$). Post hoc tests using Tukey's method only showed significant differences of the percentage of routine turns between 3cm and 5cm ($^{**}p = 0.002$) and between 11cm and 13cm ($^{**}p = 0.003$).

4 Effect of 5-HT on CuSO4-treated larvae (Figure 5A-B)

4.1 Check for influential observations (Cook's distance)

We excluded two larvae: FishID 26 and FishID 179.

Table 46: Influential observations across all the parameters

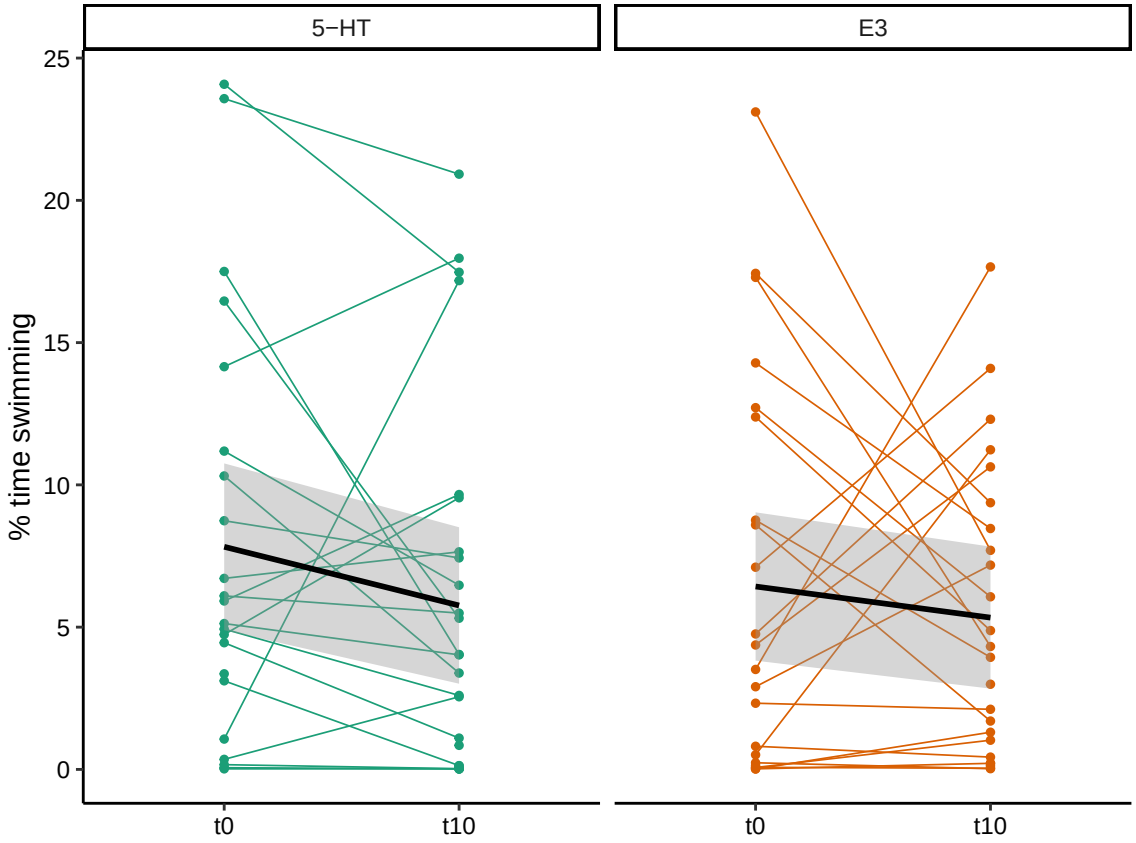
	FishID	Condition	Time	Value	CookD	Parameter
78	26	5-HT	t10	12.943	0.177	Speed
14	179	E3	t0	2.807	0.149	FQ

4.2 Percentage of time swimming (Figure 5A)

4.2.1 Descriptive data

Table 47: Percentage of time swimming by Condition and Time

	5-HT	E3
t0	7.82 ± 7.26 (n = 22, r = 0.02-24.08)	6.43 ± 6.96 (n = 22, r = 0.01-23.11)
t10	5.76 ± 6.42 (n = 25, r = 0.01-20.92)	5.34 ± 5.12 (n = 24, r = 0.03-17.66)



4.2.2 Linear mixed model with Wald chi-square tests

No significant effects of Condition and Time factors or their interaction were found on the percentage of time swimming.

```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: Swim
##                  Chisq Df Pr(>Chisq)
## Condition      0.1652  1     0.6844
## Time           1.4170  1     0.2339
## Condition:Time 0.1280  1     0.7205
```

4.2.3 Tukey's post hoc tests

Table 48: Post hoc pairwise comparisons with Tukey's method - Mean differences between t0 and t10 by Condition

Time	Condition	emmean	SE	df	lower.CL	upper.CL
t0	5-HT	6.404382	1.614582	24.47995	3.075502	9.733262
t10	5-HT	4.863482	1.555066	21.76699	1.636470	8.090495
t0	E3	5.538626	1.590318	24.66185	2.261027	8.816225
t10	E3	4.708443	1.564694	22.57647	1.468265	7.948622

contrast	Condition	estimate	SE	df	t.ratio	p.value
t0 - t10	5-HT	1.54	1.41	45.4	1.094	0.2798104
t0 - t10	E3	0.83	1.42	44.5	0.586	0.5611248

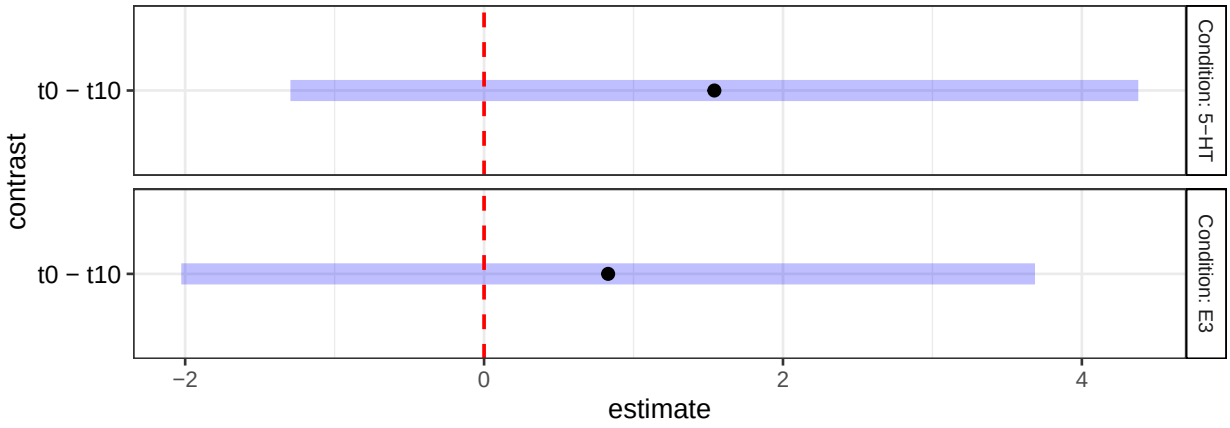
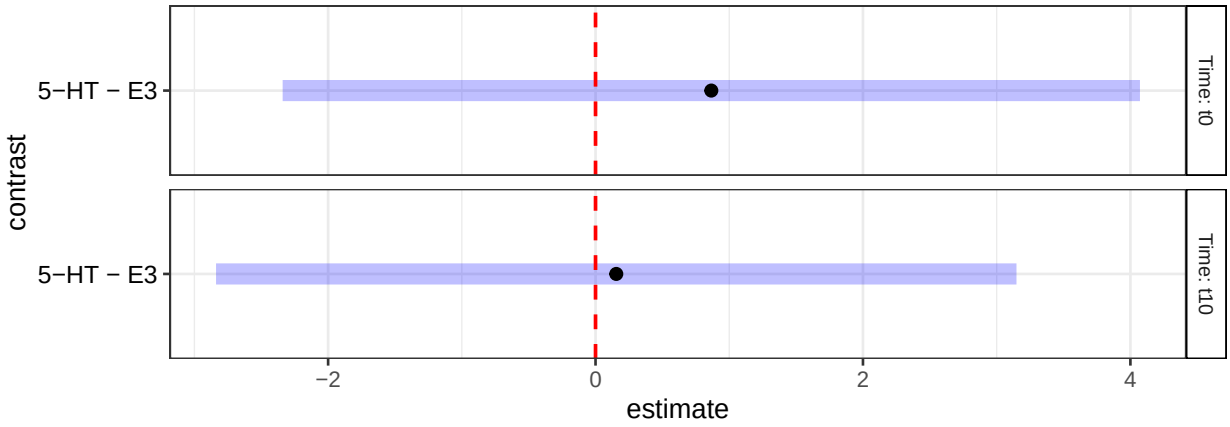


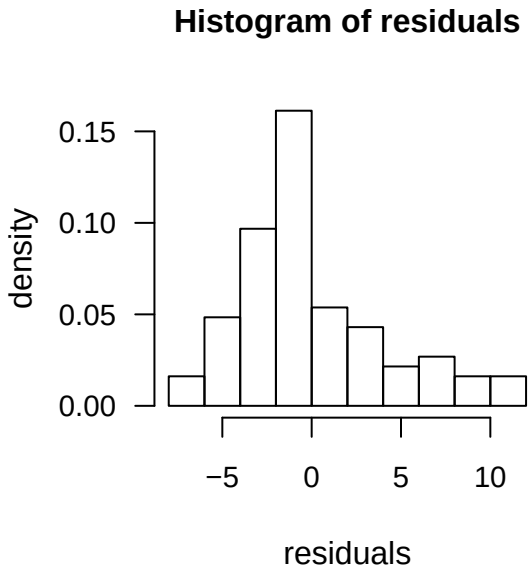
Table 50: Post hoc pairwise comparisons with Tukey's method - Mean differences between E3 and 5-HT at t0 and t10

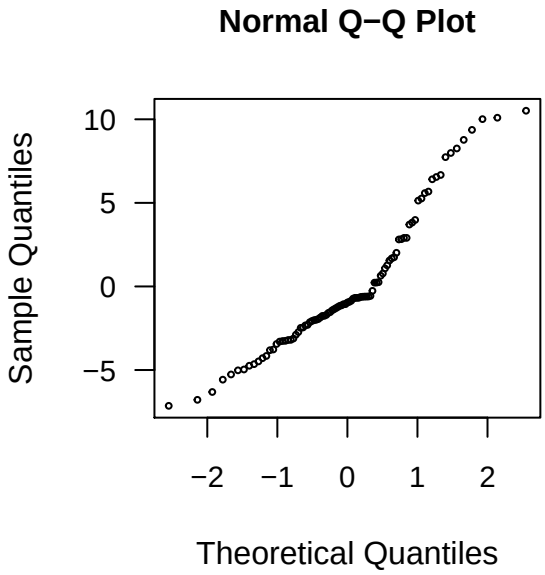
Condition	Time	emmean	SE	df	lower.CL	upper.CL
5-HT	t0	6.404382	1.614582	24.47995	3.075502	9.733262
E3	t0	5.538626	1.590318	24.66185	2.261027	8.816225
5-HT	t10	4.863482	1.555066	21.76699	1.636470	8.090495
E3	t10	4.708443	1.564694	22.57647	1.468265	7.948622

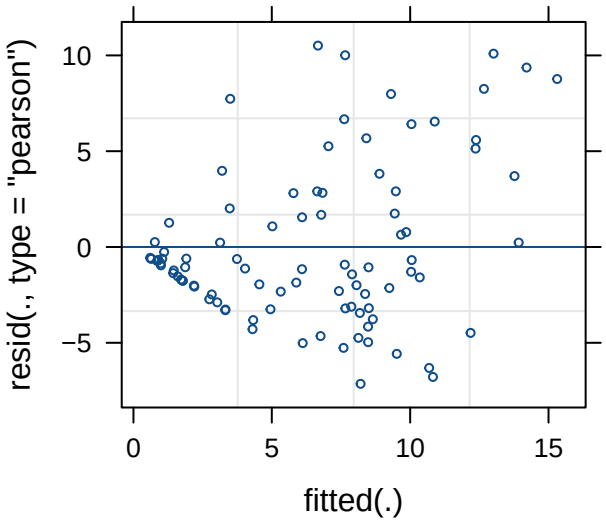
contrast	Time	estimate	SE	df	t.ratio	p.value
5-HT - E3	t0	0.87	1.61	77.8	0.538	0.5923423
5-HT - E3	t10	0.16	1.50	73.8	0.103	0.9180706



4.2.4 Checking model assumptions







4.2.5 Conclusion

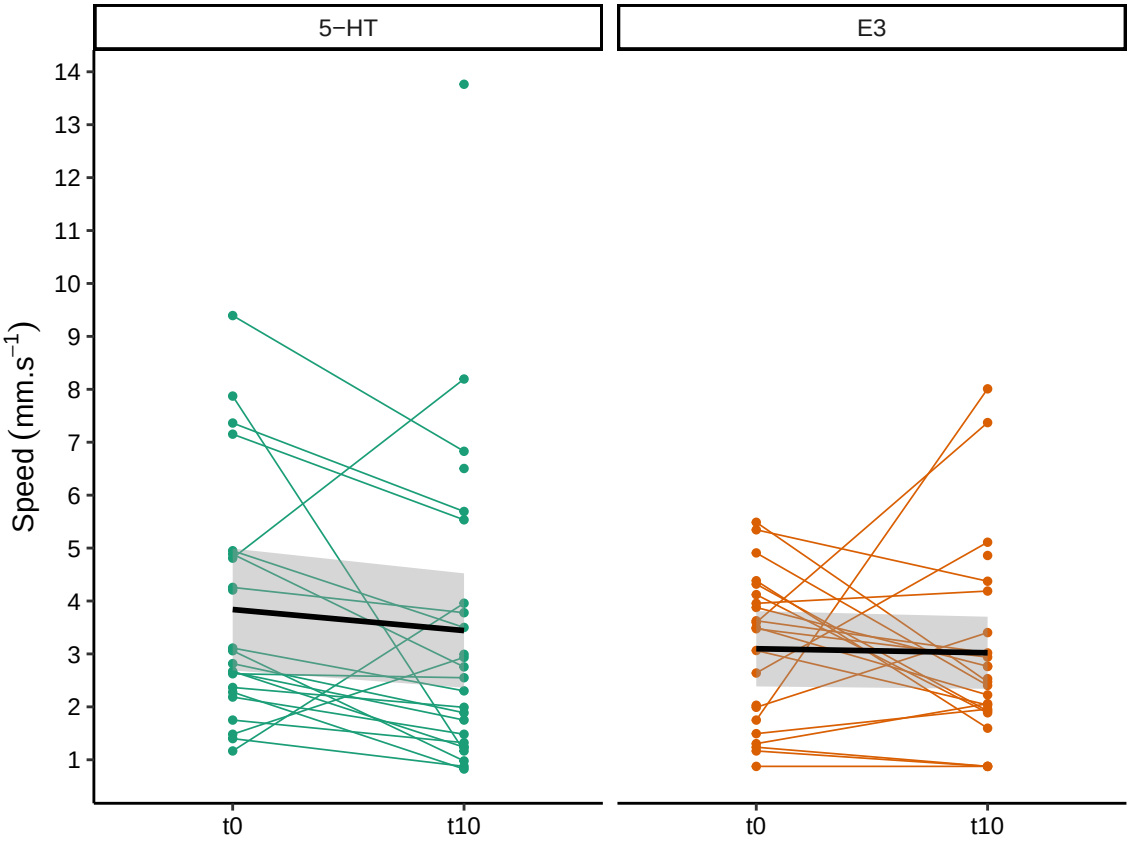
Type II Wald chi-square tests for the fitted model indicated did not show any effect of Condition and Time on the percentage of time swimming.

4.3 Speed (square root transformed data) (Figure 5B)

4.3.1 Descriptive data

Table 52: Speed by Condition and Time

	5-HT	E3
t0	1.88 ± 0.56 (n = 22, r = 1.08-3.06)	1.71 ± 0.43 (n = 22, r = 0.94-2.34)
t10	1.72 ± 0.7 (n = 25, r = 0.91-3.71)	1.67 ± 0.49 (n = 24, r = 0.94-2.83)



4.3.2 Linear mixed model with Wald chi-square tests

No significant main effects or interaction of Condition and Time were found on Speed.

```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: sqrt(Speed)
##                 Chisq Df Pr(>Chisq)
## Condition      0.8918  1     0.3450
## Time           2.3154  1     0.1281
## Condition:Time 0.7518  1     0.3859
```

4.3.3 Tukey's post hoc tests

Table 53: Post hoc pairwise comparisons with Tukey's method - Mean differences between t0 and t10 by Condition (back-transformed estimate values from the sqrt scale)

Time	Condition	response	SE	df	lower.CL	upper.CL
t0	5-HT	3.729701	0.4637449	48.45013	2.797503	4.661899
t10	5-HT	2.981580	0.3925651	45.26947	2.191043	3.772116
t0	E3	2.955420	0.4137529	52.64515	2.125406	3.785434
t10	E3	2.766732	0.3861277	48.53605	1.990591	3.542872

contrast	Condition	estimate	SE	df	t.ratio	p.value
t0 - t10	5-HT	0.75	0.45	45.3	1.665	0.1027671
t0 - t10	E3	0.19	0.41	48.5	0.457	0.6499668

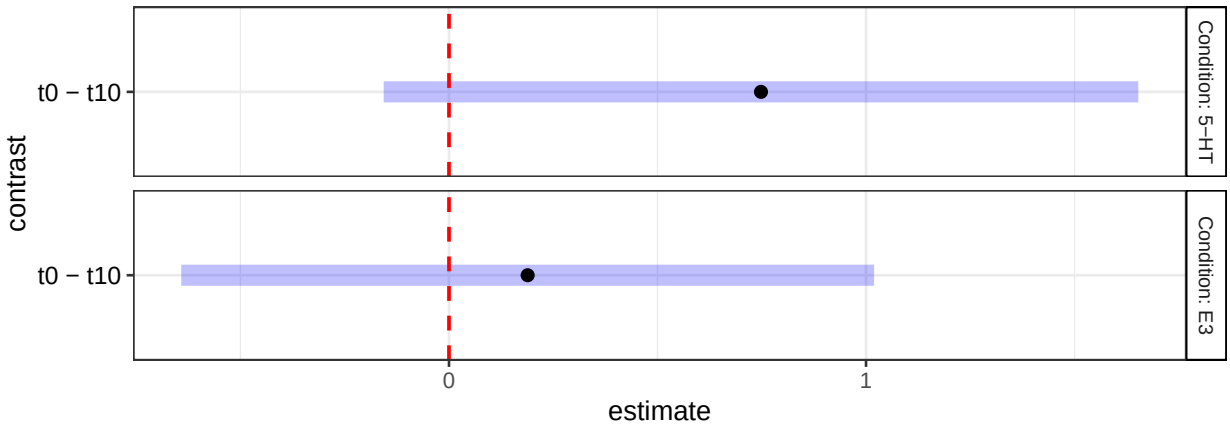
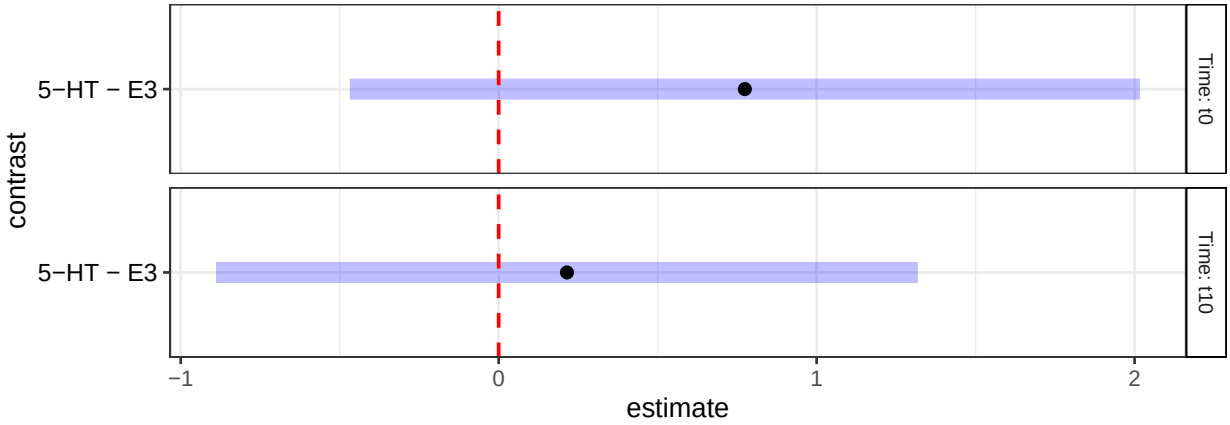


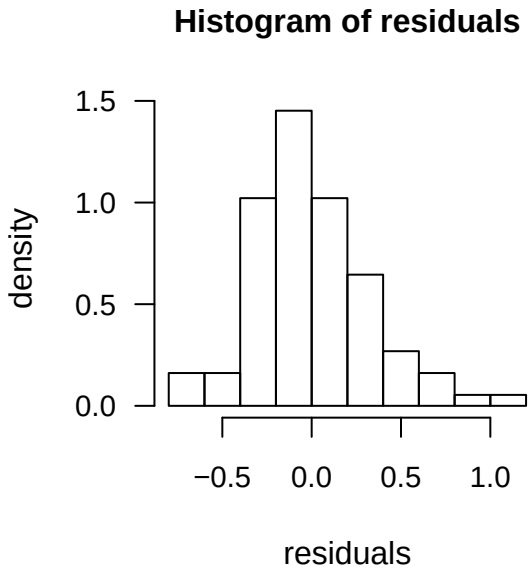
Table 55: Post hoc pairwise comparisons with Tukey's method - Mean differences between E3 and 5-HT at t0 and t10 (back-transformed estimate values from the sqrt scale)

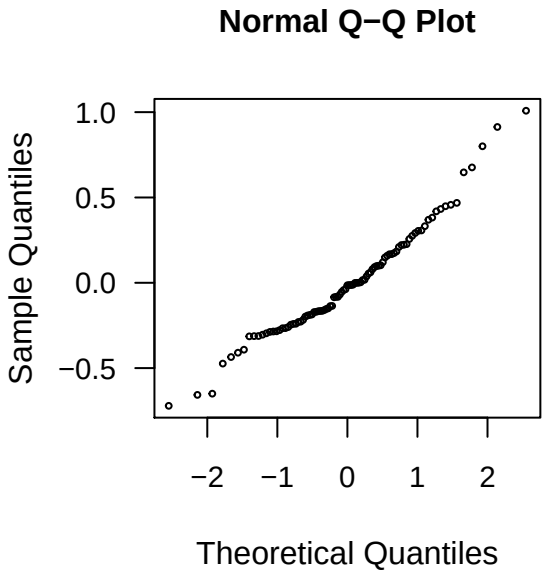
Condition	Time	response	SE	df	lower.CL	upper.CL
5-HT	t0	3.729701	0.4637449	48.45013	2.797503	4.661899
E3	t0	2.955420	0.4137529	52.64515	2.125406	3.785434
5-HT	t10	2.981580	0.3925651	45.26947	2.191043	3.772116
E3	t10	2.766732	0.3861277	48.53605	1.990591	3.542872

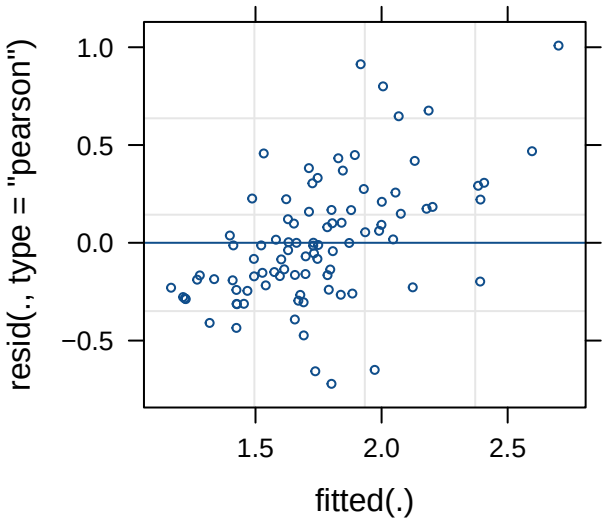
contrast	Time	estimate	SE	df	t.ratio	p.value
5-HT - E3	t0	0.77	0.62	48.5	1.252	0.2164125
5-HT - E3	t10	0.21	0.55	45.3	0.392	0.6968872



4.3.4 Checking model assumptions







4.3.5 Conclusion

Type II Wald chi-square tests for the fitted model indicated did not show any effect of Condition and Time on Speed.

5 Effect of 5-HT on PLL-ablated larvae (Figure 5D-E)

5.1 Check for influential observations (Cook's distance)

We excluded five larvae: FishID 14, FishID 29, FishID 56, FishID 68 and FishID 84.

Table 57: Influential observations across all the parameters

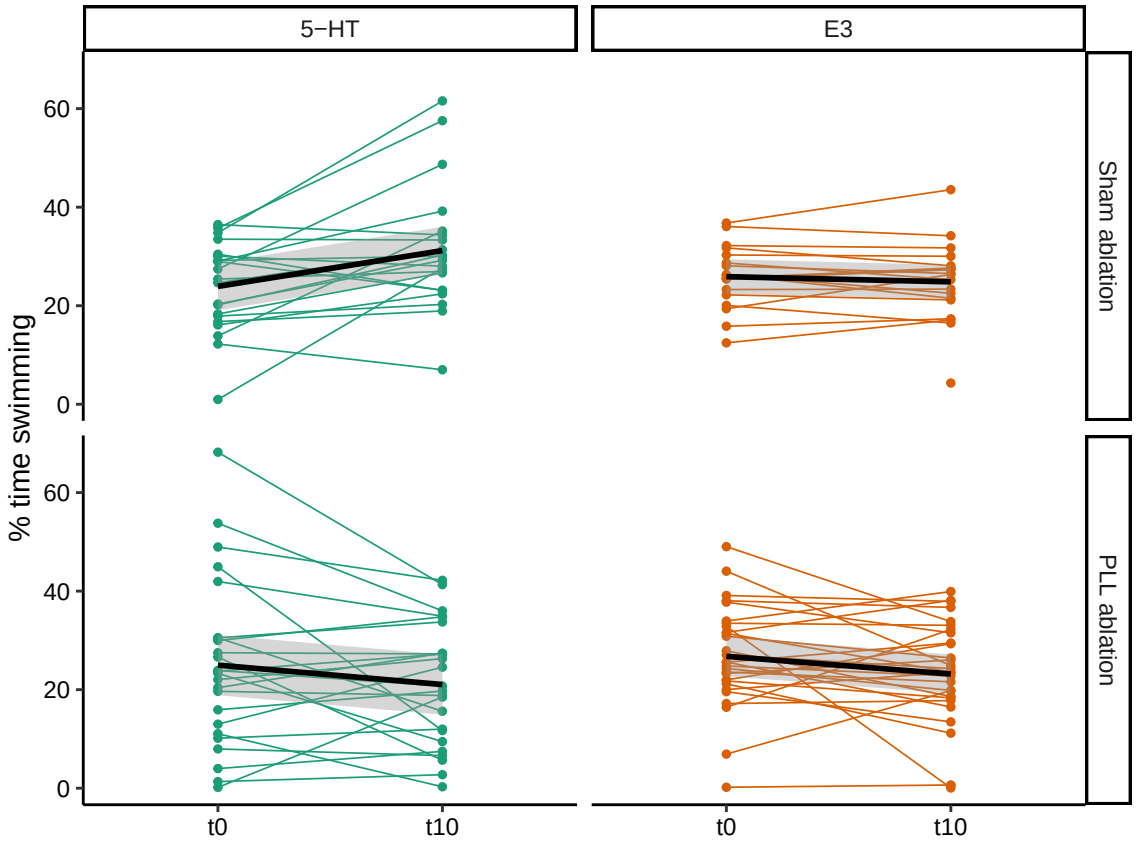
	FishID	Condition	Time	Value	CookD	Parameter
75	14	5-HT	t0	0.000	0.104	Turns
8	29	E3	t0	0.000	0.161	Turns
104	29	E3	t10	100.000	0.156	Turns
12	56	E3	t0	51.087	0.139	Swim
1091	56	E3	t10	0.067	0.125	Swim
109	56	E3	t10	100.000	0.110	Turns
113	68	E3	t10	0.000	0.252	Speed
189	84	5-HT	t10	14.579	0.090	Speed

5.2 Percentage of time swimming (Figure 5D)

5.2.1 Descriptive data

Table 58: Percentage of time swimming by Treatment, Condition and Time

	Sham ablation	PLL ablation
5.HT.t0	23.92 ± 9.09 (n = 21, r = 0.97-36.47)	24.98 ± 17.02 (n = 24, r = 0.17-68.19)
E3.t0	25.9 ± 6.45 (n = 18, r = 12.47-36.79)	26.77 ± 10.37 (n = 28, r = 0.19-49.03)
5.HT.t10	31.19 ± 12.54 (n = 21, r = 7.01-61.57)	21.04 ± 12.37 (n = 24, r = 0.29-42.19)
E3.t10	24.85 ± 8.12 (n = 19, r = 4.31-43.55)	23.17 ± 10.86 (n = 29, r = 0.05-39.91)



5.2.2 Linear mixed model with Wald chi-square tests

Significant three way interaction effect of Treatment (Sham ablation, PLL ablation), Condition (E3, 5-HT) and Time (t0, t10) (Wald $\chi^2 = 4.05$, df = 1, $^*p = 0.044$) on the percentage of time swimming.

```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: Swim
##                             Chisq Df Pr(>Chisq)
## Condition                  0.0054  1  0.9416892
## Treatment                  1.3391  1  0.2471968
## Time                       0.1490  1  0.6995182
## Condition:Treatment        0.4570  1  0.4990177
## Condition:Time             1.8125  1  0.1782104
## Treatment:Time            11.0334  1  0.0008948 ***
## Condition:Treatment:Time   4.0502  1  0.0441665 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

5.2.3 Tukey's post hoc tests

Table 59: Post hoc pairwise comparisons with Tukey's method - Mean differences between t0 and t10 by Condition and Treatment

Time	Condition	Treatment	emmean	SE	df	lower.CL	upper.CL
t0	5-HT	Sham ablation	23.04931	2.802959	49.54997	17.41814	28.68049
t10	5-HT	Sham ablation	30.31280	2.802959	49.54997	24.68163	35.94398
t0	E3	Sham ablation	25.53070	2.950601	57.66035	19.62369	31.43771
t10	E3	Sham ablation	25.14998	2.908909	55.10236	19.32064	30.97932
t0	5-HT	PLL ablation	24.91076	2.637419	44.65140	19.59758	30.22394
t10	5-HT	PLL ablation	20.96965	2.637419	44.65140	15.65647	26.28283
t0	E3	PLL ablation	25.86801	2.471030	36.68495	20.85978	30.87625
t10	E3	PLL ablation	22.75729	2.450976	35.63377	17.78471	27.72988

contrast	Condition	Treatment	estimate	SE	df	t.ratio	p.value
t0 - t10	5-HT	Sham ablation	-7.26	2.17	87.1	-3.351	0.0011937
t0 - t10	E3	Sham ablation	0.38	2.33	88.6	0.163	0.8705792
t0 - t10	5-HT	PLL ablation	3.94	2.03	87.1	1.944	0.0551778
t0 - t10	E3	PLL ablation	3.11	1.87	88.1	1.662	0.1000092

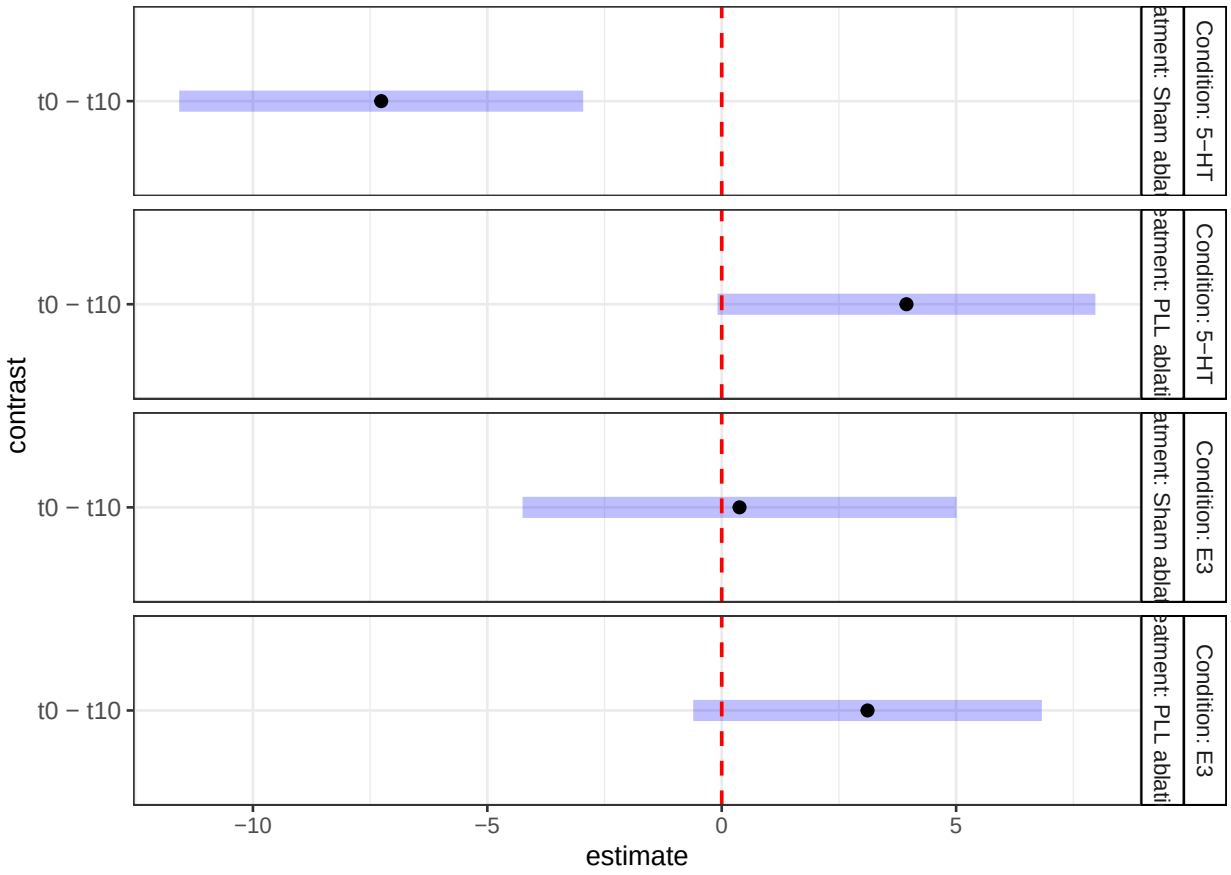
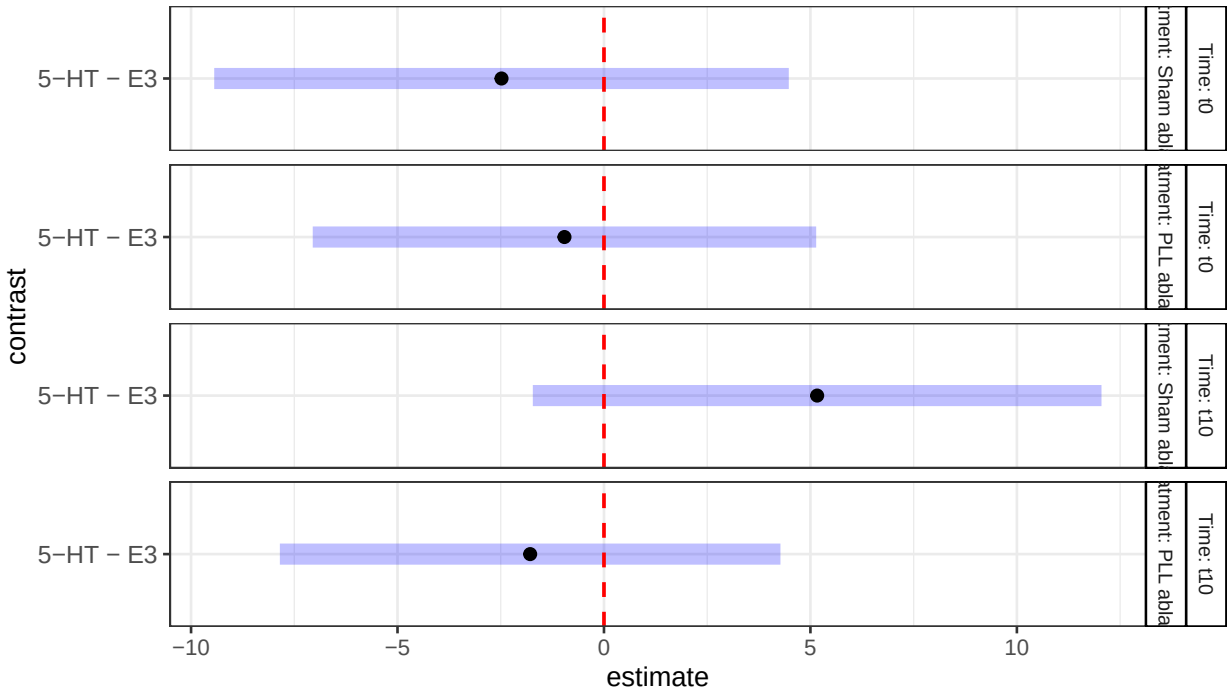


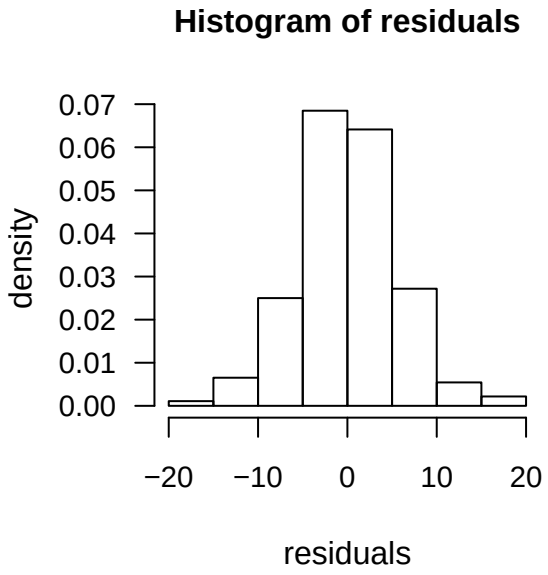
Table 61: Post hoc pairwise comparisons with Tukey's method - Mean differences between E3 and 5-HT at t0 and t10 by treatment group

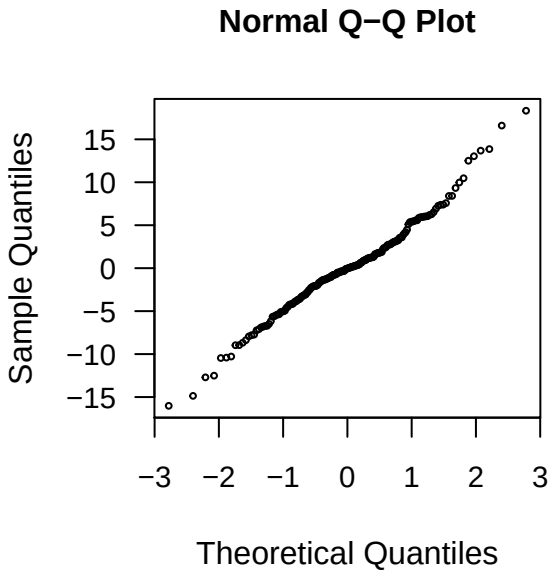
Condition	Time	Treatment	emmean	SE	df	lower.CL	upper.CL
5-HT	t0	Sham ablation	23.04931	2.802959	49.54997	17.41814	28.68049
E3	t0	Sham ablation	25.53070	2.950601	57.66035	19.62369	31.43771
5-HT	t10	Sham ablation	30.31280	2.802959	49.54997	24.68163	35.94398
E3	t10	Sham ablation	25.14998	2.908909	55.10236	19.32064	30.97932
5-HT	t0	PLL ablation	24.91076	2.637419	44.65140	19.59758	30.22394
E3	t0	PLL ablation	25.86801	2.471030	36.68495	20.85978	30.87625
5-HT	t10	PLL ablation	20.96965	2.637419	44.65140	15.65647	26.28283
E3	t10	PLL ablation	22.75729	2.450976	35.63377	17.78471	27.72988

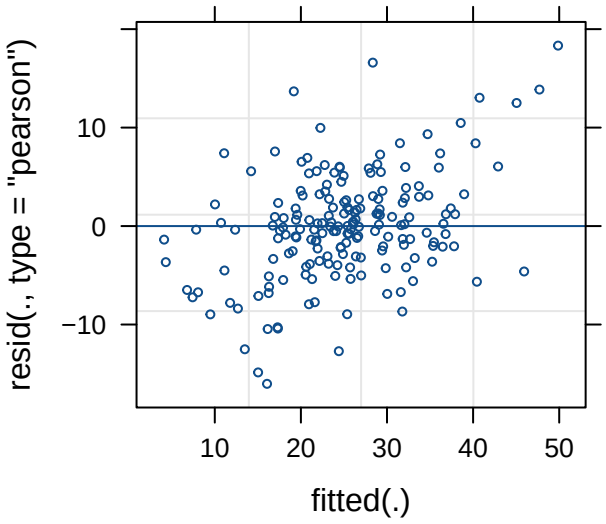
contrast	Time	Treatment	estimate	SE	df	t.ratio	p.value
5-HT - E3	t0	Sham ablation	-2.48	3.52	124.4	-0.706	0.4815850
5-HT - E3	t10	Sham ablation	5.16	3.48	121.5	1.484	0.1403542
5-HT - E3	t0	PLL ablation	-0.96	3.08	127.3	-0.311	0.7565337
5-HT - E3	t10	PLL ablation	-1.79	3.06	125.7	-0.584	0.5605283



5.2.4 Checking model assumptions







5.2.5 Conclusion

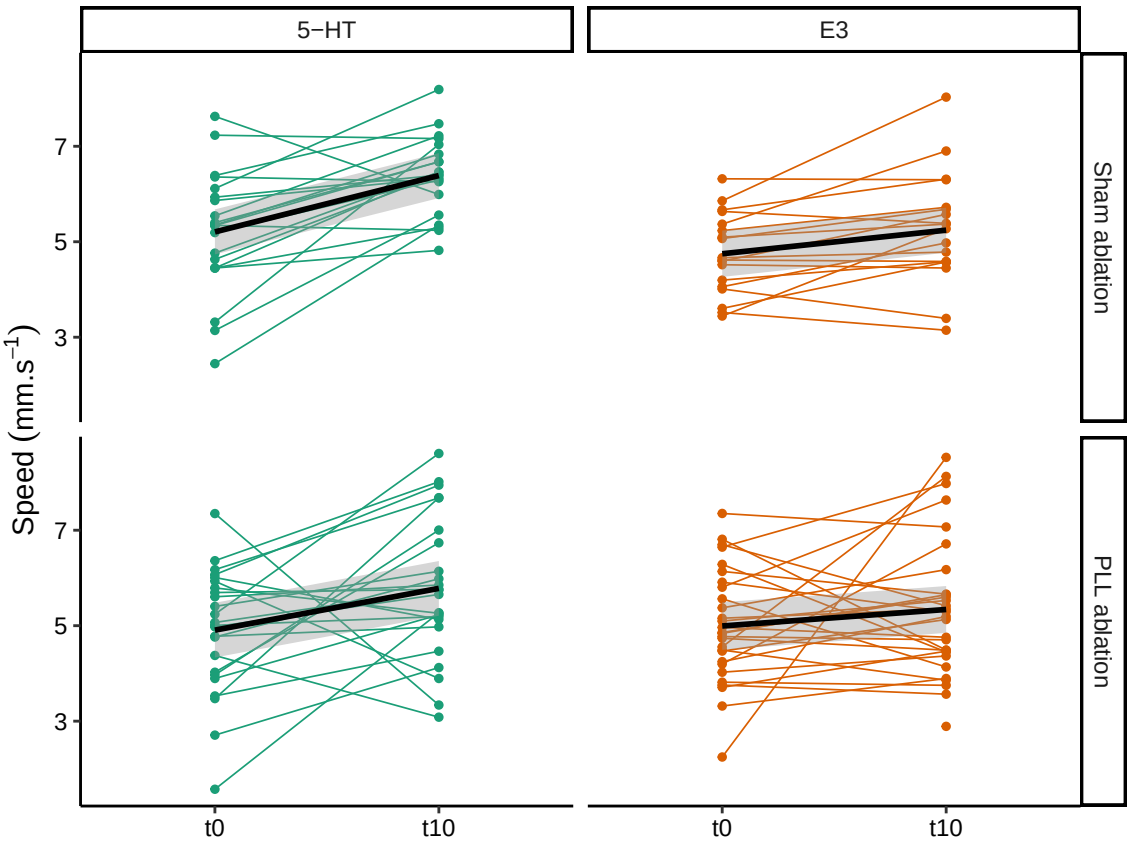
Type II Wald chi-square tests for the fitted model indicated three way interaction effect of Treatment (Sham ablation, PLL ablation), Condition (E3, 5-HT) and Time (t0, t10) (Wald $\chi^2 = 4.05$, df $= 1$, $^{*}p = 0.044$) on the percentage of time swimming. Post hoc tests using Tukey's method indicated a significant difference between t0 and t10 in 5-HT with Sham ablation ($^{**}p = 0.0012$).

5.3 Speed (square root transformed data) (Figure 5E)

5.3.1 Descriptive data

Table 63: Speed by Treatment, Condition and Time

	Sham ablation	PLL ablation
5.HT.t0	5.21 ± 1.27 (n = 21, r = 2.45-7.63)	4.91 ± 1.29 (n = 24, r = 1.57-7.35)
E3.t0	4.75 ± 0.84 (n = 18, r = 3.44-6.32)	4.99 ± 1.18 (n = 28, r = 2.25-7.35)
5.HT.t10	6.39 ± 0.82 (n = 21, r = 4.82-8.19)	5.78 ± 1.49 (n = 24, r = 3.08-8.61)
E3.t10	5.24 ± 1.15 (n = 19, r = 3.15-8.03)	5.34 ± 1.44 (n = 29, r = 2.89-8.53)



5.3.2 Linear mixed model with Wald chi-square tests

Trend for a non significant interaction effect of Condition (5-HT, E3) and Time (t0, t10) (Wald $\chi^2 = 3.42$, df $= 1$, $^{ns}p = 0.065$) on Speed. No differences were observed between the treatment groups (Sham ablation, PLL ablation) (Wald $\chi^2 = 0.66$, df $= 1$, $^{ns}p = 0.42$).

```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: sqrt(Speed)
##                             Chisq Df Pr(>Chisq)
## Condition                  4.4133  1    0.03566 *
## Treatment                  0.6609  1    0.41623
## Time                      19.5918  1  9.588e-06 ***
## Condition:Treatment        2.1526  1    0.14233
## Condition:Time             3.4175  1    0.06451 .
## Treatment:Time             0.4644  1    0.49557
## Condition:Treatment:Time   0.0662  1    0.79699
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

5.3.3 Tukey's post hoc tests

Table 64: Post hoc pairwise comparisons with Tukey's method - Mean differences between t0 and t10 by Condition (back-transformed estimate values from the sqrt scale)

Time	Condition	response	SE	df	lower.CL	upper.CL
t0	5-HT	4.958577	0.2003071	41.77526	4.554277	5.362878
t10	5-HT	6.008198	0.2204908	41.77647	5.563159	6.453237
t0	E3	4.792790	0.1981221	41.81048	4.392910	5.192670
t10	E3	5.217818	0.2029773	39.57353	4.807447	5.628188

contrast	Condition	estimate	SE	df	t.ratio	p.value
t0 - t10	5-HT	-1.05	0.23	41.8	-4.499	0.0000536
t0 - t10	E3	-0.43	0.22	39.6	-1.925	0.0614729

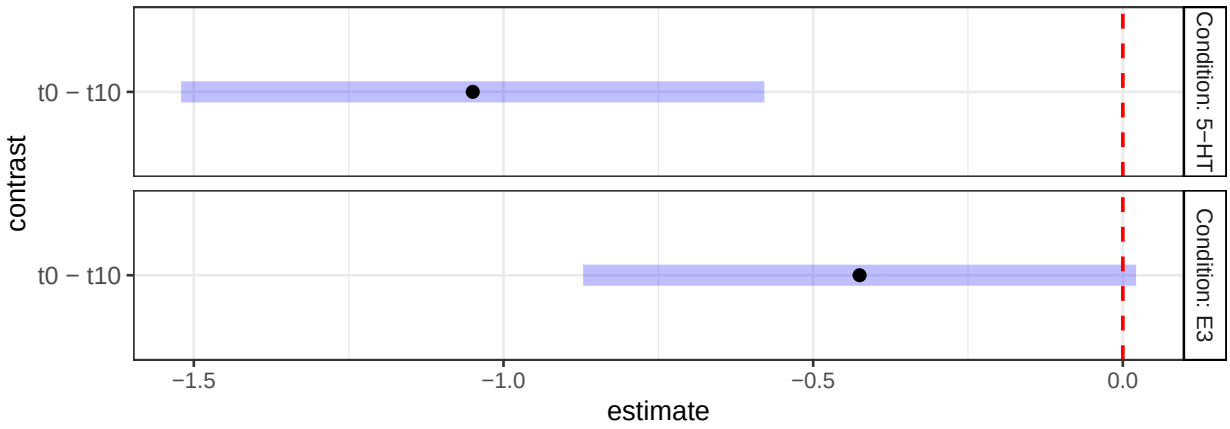


Table 66: Post hoc pairwise comparisons with Tukey's method - Mean differences between EM and 5-HT at t0 and t10 (back-transformed estimate values from the sqrt scale)

Condition	Time	response	SE	df	lower.CL	upper.CL
5-HT	t0	4.958577	0.2003071	41.77526	4.554277	5.362878
E3	t0	4.792790	0.1981221	41.81048	4.392910	5.192670
5-HT	t10	6.008198	0.2204908	41.77647	5.563159	6.453237
E3	t10	5.217818	0.2029773	39.57353	4.807447	5.628188

contrast	Time	estimate	SE	df	t.ratio	p.value
5-HT - E3	t0	0.17	0.25	41.8	0.658	0.5141942
5-HT - E3	t10	0.79	0.27	39.6	2.958	0.0052100

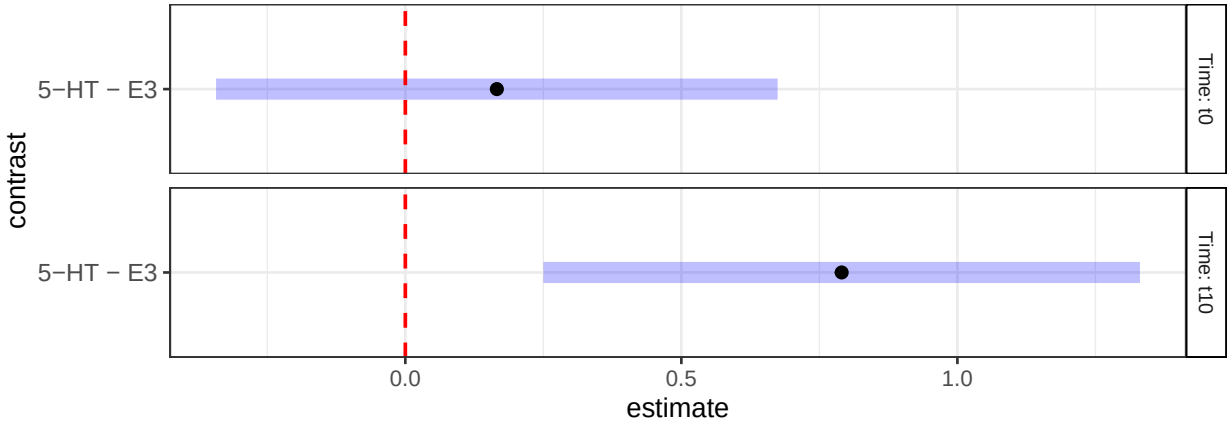


Table 68: Post hoc pairwise comparisons with Tukey's method - Mean differences between t0 and t10 by Treatment (back-transformed estimate values from the sqrt scale)

Time	Treatment	response	SE	df	lower.CL	upper.CL
t0	Sham ablation	4.908695	0.2130377	47.02624	4.480125	5.337265
t10	Sham ablation	5.758260	0.2282663	45.40102	5.298620	6.217899
t0	PLL ablation	4.842081	0.1849805	36.77802	4.467199	5.216964
t10	PLL ablation	5.455866	0.1952016	35.91236	5.059945	5.851787

contrast	Treatment	estimate	SE	df	t.ratio	p.value
t0 - t10	Sham ablation	-0.85	0.25	45.4	-3.457	0.0011955
t0 - t10	PLL ablation	-0.61	0.21	35.9	-2.930	0.0058654

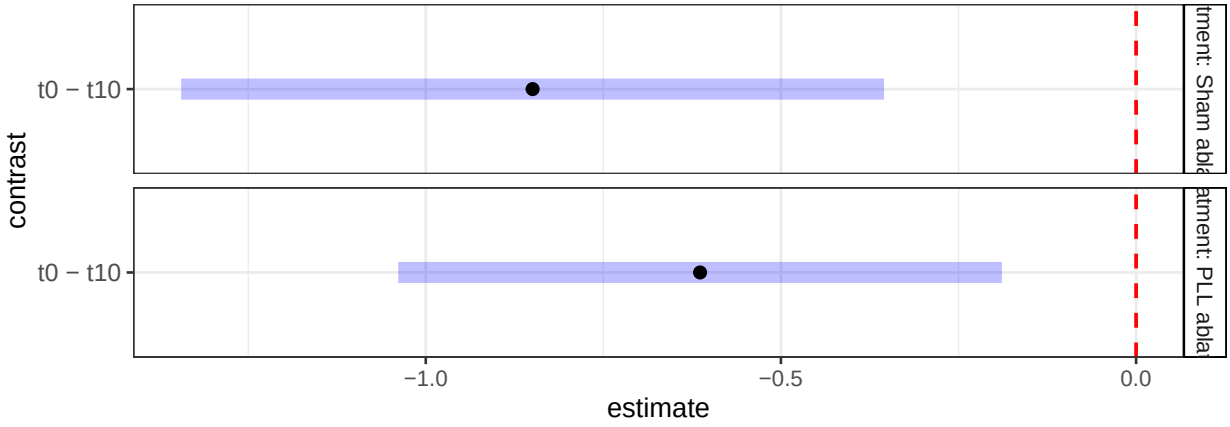


Table 70: Post hoc pairwise comparisons with Tukey's method - Mean differences between Sham ablation and PLL ablation at t0 and t10 (back-transformed estimate values from the sqrt scale)

Treatment	Time	response	SE	df	lower.CL	upper.CL
Sham ablation	t0	4.908695	0.2130377	47.02624	4.480125	5.337265
PLL ablation	t0	4.842081	0.1849805	36.77802	4.467199	5.216964
Sham ablation	t10	5.758260	0.2282663	45.40102	5.298620	6.217899
PLL ablation	t10	5.455866	0.1952016	35.91236	5.059945	5.851787

contrast	Time	estimate	SE	df	t.ratio	p.value
Sham ablation - PLL ablation	t0	0.07	0.25	36.8	0.264	0.7935800
Sham ablation - PLL ablation	t10	0.30	0.27	35.9	1.127	0.2673872

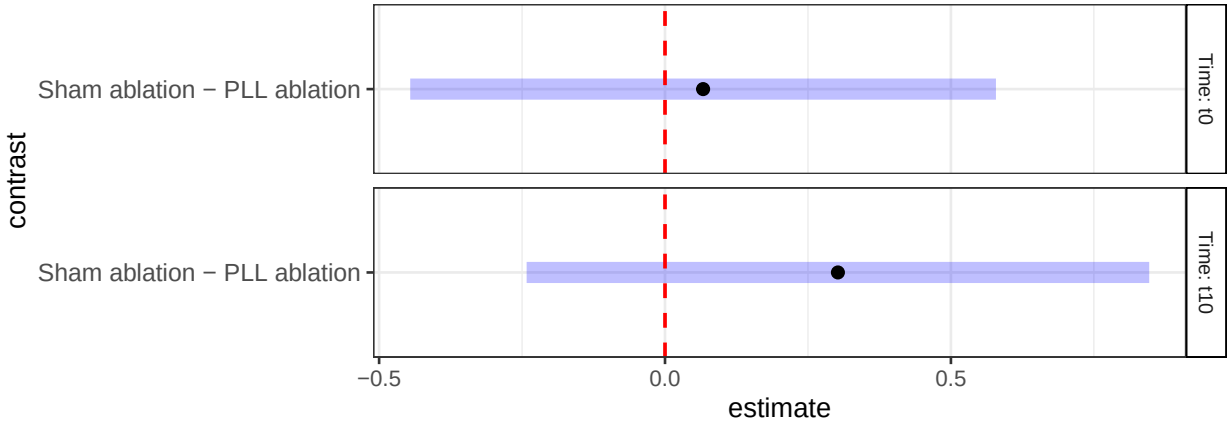


Table 72: Post hoc pairwise comparisons with Tukey's method - Mean differences between t0 and t10 by Condition and Treatment

Time	Condition	Treatment	emmean	SE	df	lower.CL	upper.CL
t0	5-HT	Sham ablation	2.266177	0.0625365	96.06807	2.142044	2.390309
t10	5-HT	Sham ablation	2.524389	0.0625365	96.06807	2.400256	2.648522
t0	E3	Sham ablation	2.165825	0.0670103	109.18268	2.033016	2.298635
t10	E3	Sham ablation	2.274387	0.0653754	104.87960	2.144758	2.404016
t0	5-HT	PLL ablation	2.186802	0.0585366	90.48954	2.070518	2.303087
t10	5-HT	PLL ablation	2.378523	0.0585366	90.48954	2.262239	2.494808
t0	E3	PLL ablation	2.214814	0.0543412	78.83770	2.106647	2.322981
t10	E3	PLL ablation	2.292325	0.0535290	75.93899	2.185712	2.398939

contrast	Condition	Treatment	estimate	SE	df	t.ratio	p.value
t0 - t10	5-HT	Sham ablation	-0.26	0.07	87.3	-3.532	0.0006620
t0 - t10	E3	Sham ablation	-0.11	0.08	89.5	-1.388	0.1685719
t0 - t10	5-HT	PLL ablation	-0.19	0.07	87.3	-2.803	0.0062308
t0 - t10	E3	PLL ablation	-0.08	0.06	88.8	-1.232	0.2212327

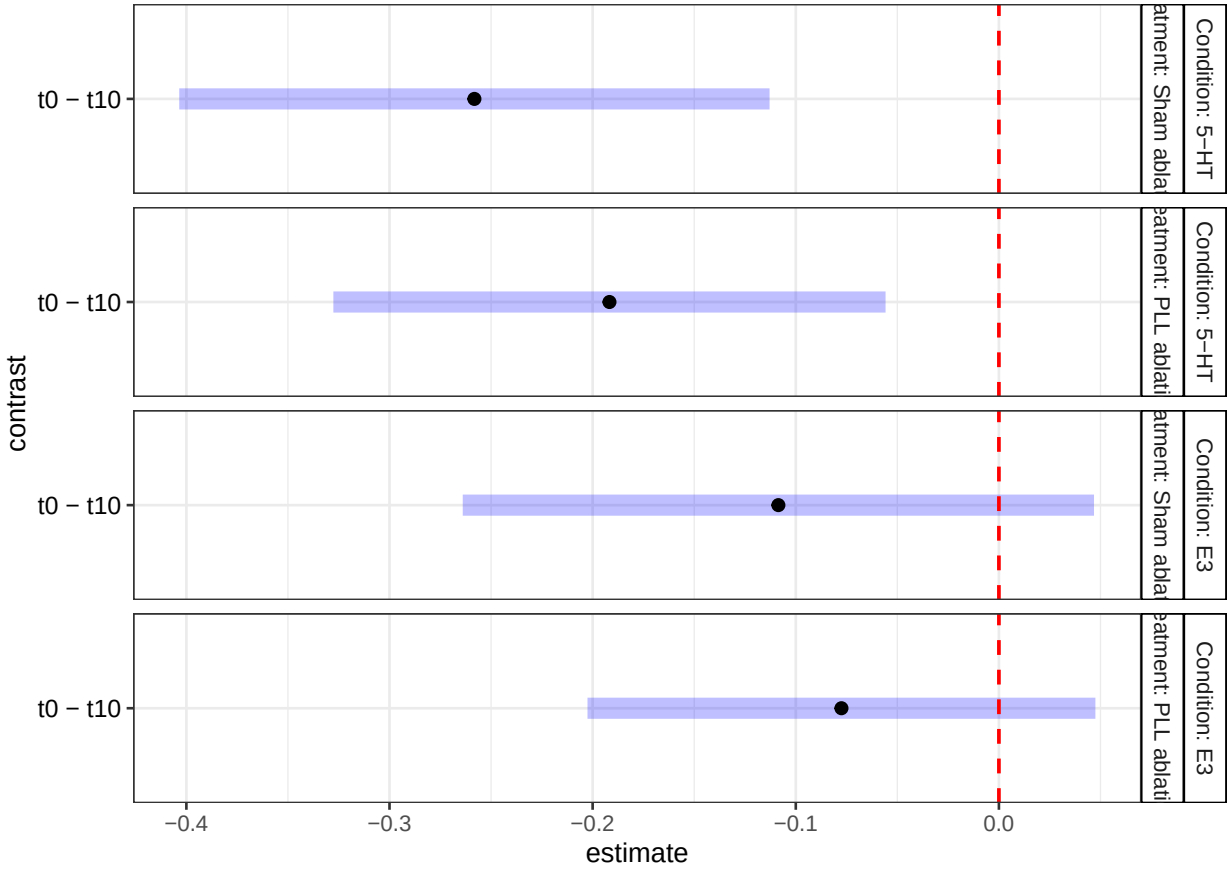


Table 74: Post hoc pairwise comparisons with Tukey's method - Mean differences between E3 and 5-HT at t0 and t10 by treatment group

Condition	Time	Treatment	emmean	SE	df	lower.CL	upper.CL
5-HT	t0	Sham ablation	2.266177	0.0625365	96.06807	2.142044	2.390309
E3	t0	Sham ablation	2.165825	0.0670103	109.18268	2.033016	2.298635
5-HT	t10	Sham ablation	2.524389	0.0625365	96.06807	2.400256	2.648522
E3	t10	Sham ablation	2.274387	0.0653754	104.87960	2.144758	2.404016
5-HT	t0	PLL ablation	2.186802	0.0585366	90.48954	2.070518	2.303087
E3	t0	PLL ablation	2.214814	0.0543412	78.83770	2.106647	2.322981
5-HT	t10	PLL ablation	2.378523	0.0585366	90.48954	2.262239	2.494808
E3	t10	PLL ablation	2.292325	0.0535290	75.93899	2.185712	2.398939

contrast	Time	Treatment	estimate	SE	df	t.ratio	p.value
5-HT - E3	t0	Sham ablation	0.10	0.09	161.8	1.158	0.2487553
5-HT - E3	t10	Sham ablation	0.25	0.09	160.3	2.928	0.0039062
5-HT - E3	t0	PLL ablation	-0.03	0.08	164.3	-0.372	0.7102717
5-HT - E3	t10	PLL ablation	0.09	0.07	163.5	1.155	0.2498410

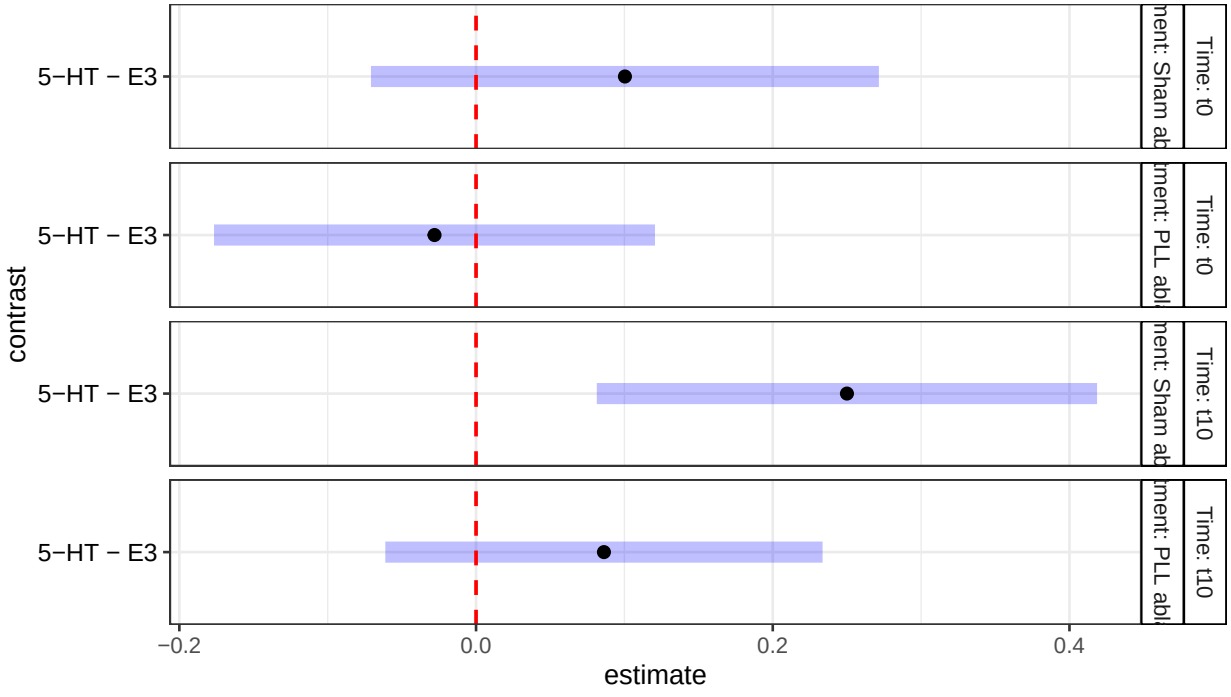


Table 76: Post hoc pairwise comparisons with Tukey's method - Mean differences between Sham and Ablation by Condition (back-transformed estimate values from the sqrt scale)

contrast	Condition	estimate	SE	df	t.ratio	p.value
Sham ablation - PLL ablation	5-HT	0.53	0.30	44.2	1.766	0.0842207
Sham ablation - PLL ablation	E3	-0.15	0.28	37.5	-0.538	0.5939003

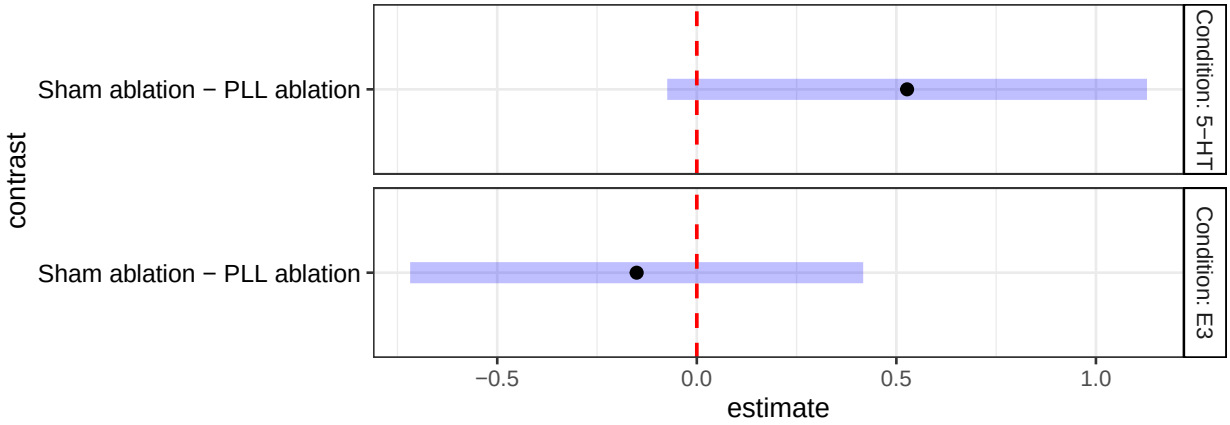
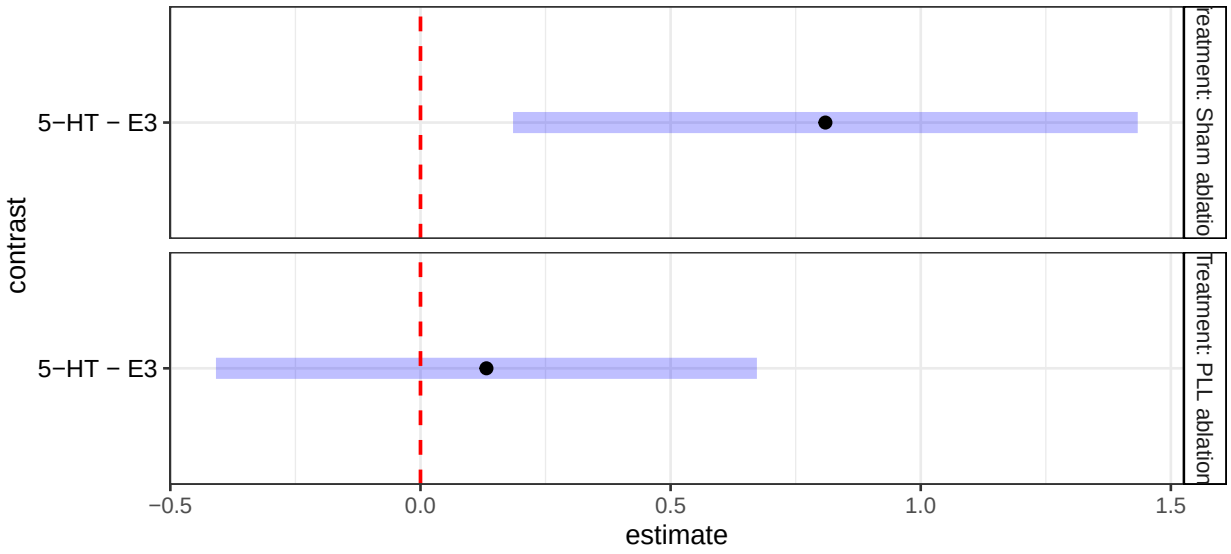


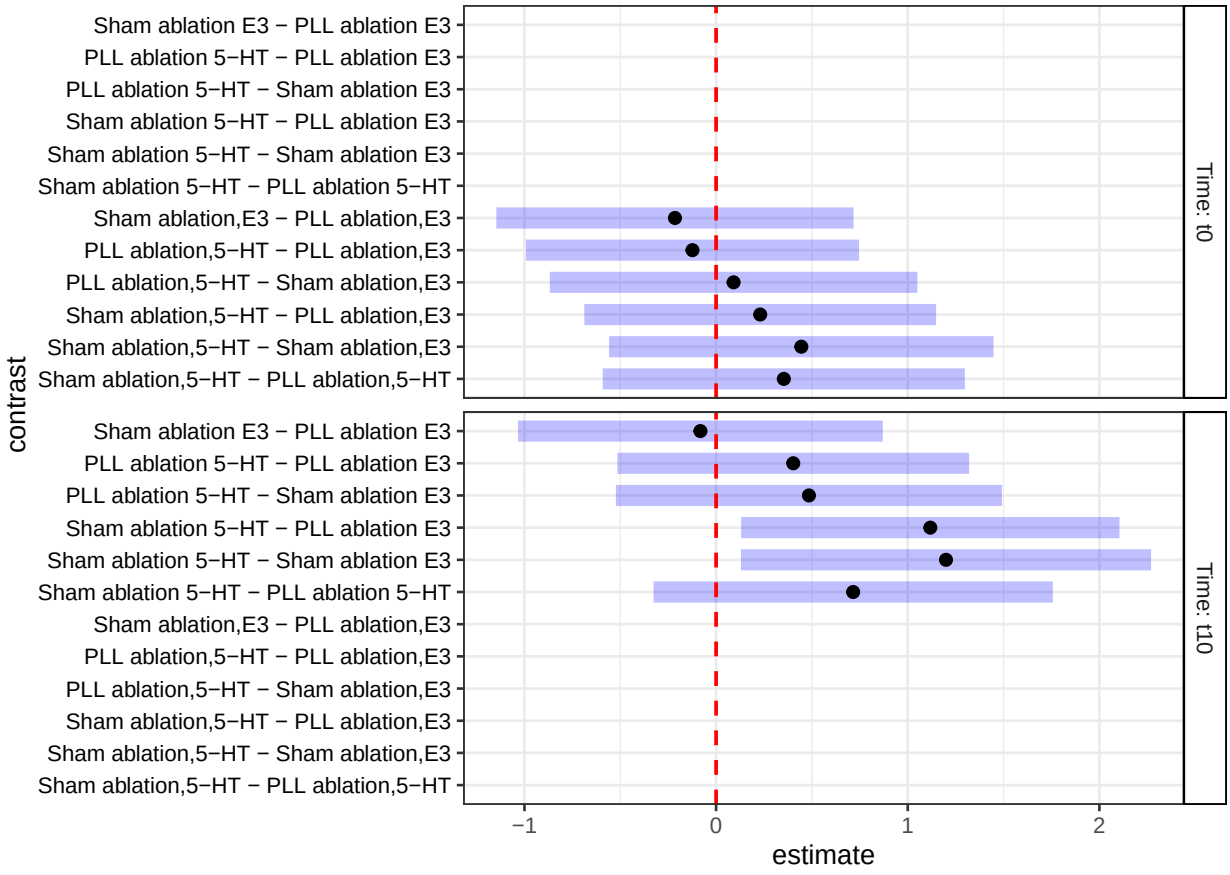
Table 77: Post hoc pairwise comparisons with Tukey's method - Mean differences between EM and 5-HT by Treatment (back-transformed estimate values from the sqrt scale)

contrast	Treatment	estimate	SE	df	t.ratio	p.value
5-HT - E3	Sham ablation	0.81	0.31	47.2	2.608	0.0121646
5-HT - E3	PLL ablation	0.13	0.27	37.5	0.494	0.6242758

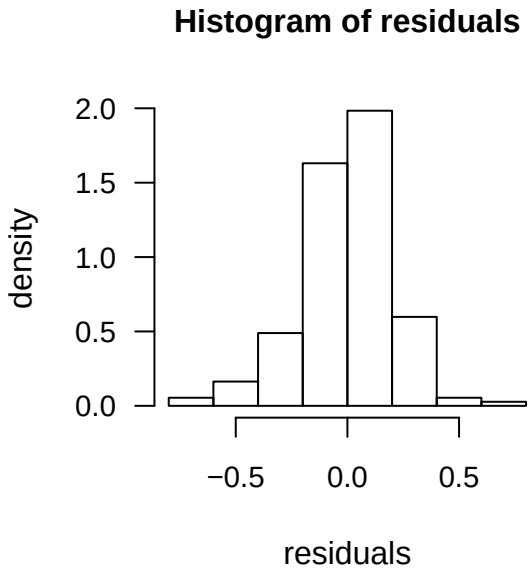


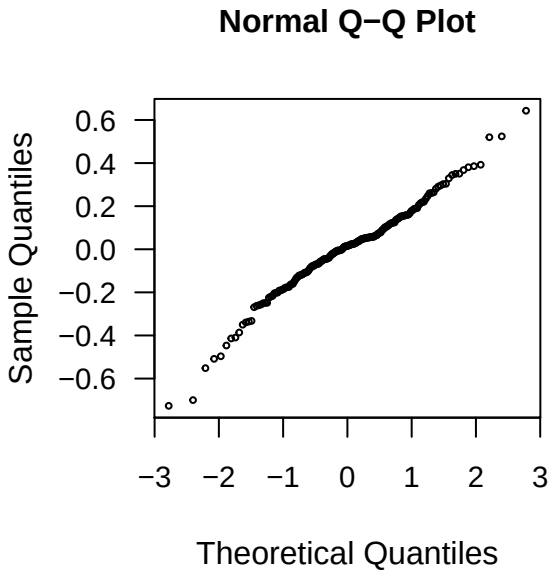
Treatment	Condition	Time	response	SE	df	lower.CL	upper.CL
Sham ablation	5-HT	t0	5.135557	0.2834374	96.06807	4.572944	5.698171
PLL ablation	5-HT	t0	4.782104	0.2560158	90.48954	4.273522	5.290687
Sham ablation	E3	t0	4.690800	0.2902654	109.18268	4.115514	5.266086
PLL ablation	E3	t0	4.905402	0.2407115	78.83770	4.426263	5.384542
Sham ablation	5-HT	t10	6.372541	0.3157327	96.06807	5.745822	6.999260
PLL ablation	5-HT	t10	5.657373	0.2784612	90.48954	5.104202	6.210545
Sham ablation	E3	t10	5.172837	0.2973781	104.87960	4.583184	5.762491
PLL ablation	E3	t10	5.254756	0.2454116	75.93899	4.765970	5.743542

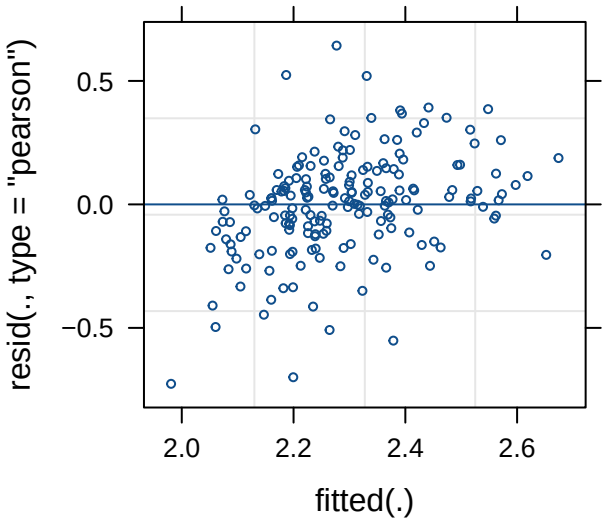
contrast	Time	estimate	SE	df	t.ratio	p.value
Sham ablation,5-HT - PLL ablation,5-HT	t0	0.35	0.36	90.5	0.979	0.7617118
Sham ablation,5-HT - Sham ablation,E3	t0	0.44	0.38	96.1	1.159	0.6538808
Sham ablation,5-HT - PLL ablation,E3	t0	0.23	0.35	78.8	0.658	0.9123566
PLL ablation,5-HT - Sham ablation,E3	t0	0.09	0.37	90.5	0.249	0.9945317
PLL ablation,5-HT - PLL ablation,E3	t0	-0.12	0.33	78.8	-0.372	0.9822629
Sham ablation,E3 - PLL ablation,E3	t0	-0.21	0.36	78.8	-0.604	0.9304369
Sham ablation 5-HT - PLL ablation 5-HT	t10	0.72	0.40	90.5	1.797	0.2815130
Sham ablation 5-HT - Sham ablation E3	t10	1.20	0.41	96.1	2.931	0.0216125
Sham ablation 5-HT - PLL ablation E3	t10	1.12	0.38	75.9	2.974	0.0201350
PLL ablation 5-HT - Sham ablation E3	t10	0.48	0.38	90.5	1.259	0.5912378
PLL ablation 5-HT - PLL ablation E3	t10	0.40	0.35	75.9	1.152	0.6583926
Sham ablation E3 - PLL ablation E3	t10	-0.08	0.36	75.9	-0.226	0.9958930


contrast
Sham ablation E3 − PLL ablation E3
PLL ablation 5−HT − PLL ablation E3
PLL ablation 5−HT − Sham ablation E3
Sham ablation 5−HT − PLL ablation E3
Sham ablation 5−HT − Sham ablation E3
Sham ablation 5−HT − PLL ablation 5−HT
Sham ablation,E3 − PLL ablation,E3
PLL ablation,5−HT − PLL ablation,E3
PLL ablation,5−HT − Sham ablation,E3
Sham ablation,5−HT − PLL ablation,E3
Sham ablation,5−HT − Sham ablation,E3
Sham ablation,5−HT − PLL ablation,5−HT
Time: t0
Time: t10
−1
0
1
2
estimate

5.3.4 Checking model assumptions







5.3.5 Conclusion

Type II Wald chi-square tests for the fitted model indicated a trend for a non significant interaction effect of Condition and Time (Wald $\chi^2 = 3.42$, df $= 1$, $^{ns}p = 0.065$) on Speed. Post hoc tests using Tukey's method showed differences between t0 and t10 in 5-HT (faster at t10) ($^{***}p =$ 5.4e-5), between E3 and 5-HT at t10 (faster in 5-HT) ($^{**}p = 0.005$); while no differences were observed between t0 and t10 in E3 ($^{ns}p = 0.061$), and between E3 and 5-HT at t0 ($^{ns}p = 0.51$). No differences were observed between the treatment groups (Sham ablation, PLL ablation) (Wald $\chi^2 = 0.66$, df $= 1$, $^{ns}p = 0.42$).

6 Effect of 5-HT on OMP-ablated larvae (Figures 5F-G)

6.1 Check for influential observations (Cook's distance)

We removed two larvae: FishID 83 and FishID 119.

Table 80: Influential observations across all the parameters

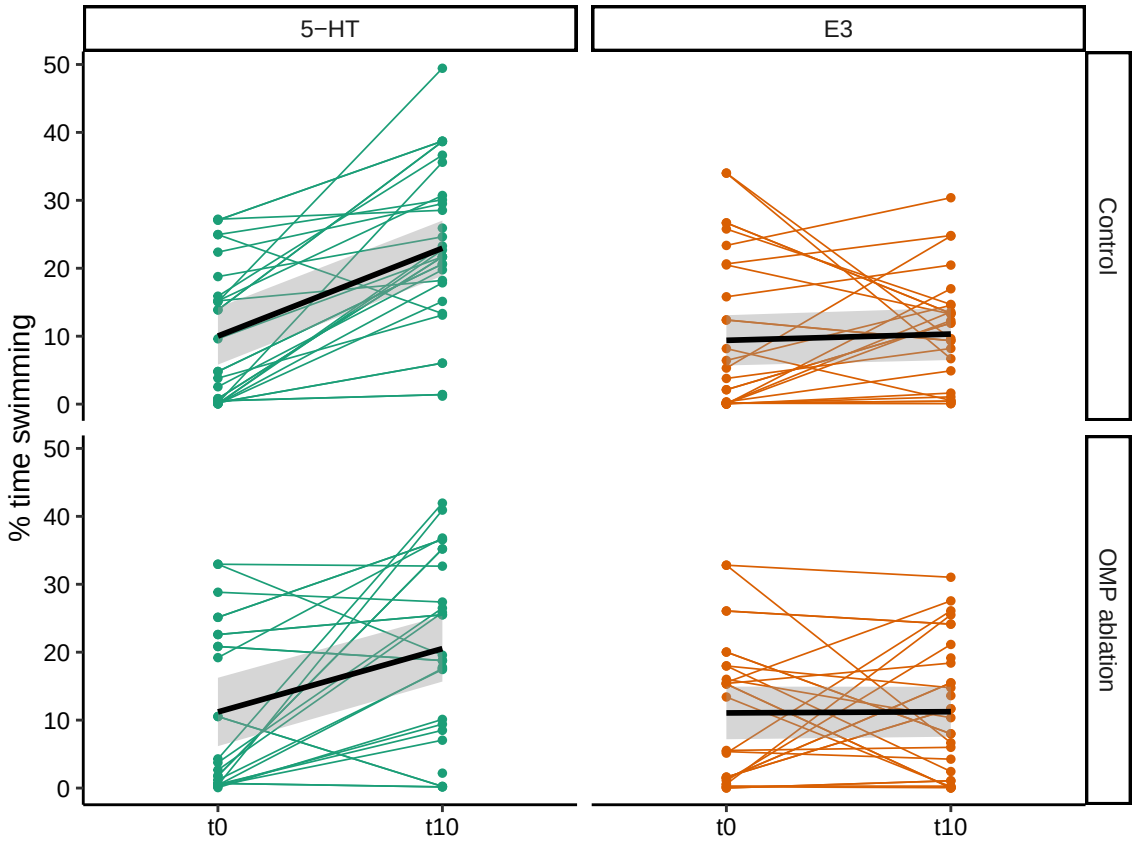
	FishID	Condition	Time	Value	CookD	Parameter	NA.
104	83	5-HT	OMP ablation	t0	0	0.069	Turns
233	119	5-HT	OMP ablation	t10	5	0.064	FQ

6.2 Percentage of time swimming (Figure 5F)

6.2.1 Descriptive data

Table 81: Percentage of time swimming by Treatment, Condition and Time

	Control	OMP ablation
5.HT.t0	10.02 ± 10.15 (n = 29, r = 0.03-27.22)	11.2 ± 11.89 (n = 26, r = 0.07-32.95)
E3.t0	9.39 ± 11.54 (n = 30, r = 0.01-34.01)	11.06 ± 10.57 (n = 28, r = 0.01-32.81)
5.HT.t10	22.95 ± 12.3 (n = 31, r = 1.17-49.44)	20.52 ± 13.57 (n = 28, r = 0.17-41.95)
E3.t10	10.32 ± 8.31 (n = 28, r = 0.07-30.38)	11.24 ± 9.92 (n = 31, r = 0.01-31.03)



6.2.2 Linear mixed model with Wald chi-square tests

Significant interaction between Condition (5-HT, E3) and Time (t0, t10) on the percentage of time swimming (Wald $\chi^2 = 22.02$, df = 1, $^{***}p = 2.7\text{e-}6$). No differences were observed between the treatment groups (Control, OMP ablation) (Wald $\chi^2 = 0.06$, df = 1, $^{ns}p = 0.81$).

```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: Swim
##                             Chisq Df Pr(>Chisq)
## Condition                12.2215  1  0.0004724 ***
## Treatment                 0.0603  1  0.8060659
## Time                     25.9578  1  3.490e-07 ***
## Condition:Treatment       0.4236  1  0.5151232
## Condition:Time           22.0194  1  2.699e-06 ***
## Treatment:Time            0.7354  1  0.3911308
## Condition:Treatment:Time  0.4315  1  0.5112606
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

6.2.3 Tukey's post hoc tests

Table 82: Post hoc pairwise comparisons with Tukey's method - Mean differences between t0 and t10 by Condition (E3, 5-HT)

Time	Condition	emmean	SE	df	lower.CL	upper.CL
t0	5-HT	10.30410	1.489840	73.14845	7.334956	13.27325
t10	5-HT	21.79785	1.443509	68.61765	18.917845	24.67786
t0	E3	10.00351	1.445804	71.79899	7.121214	12.88580
t10	E3	10.48872	1.434315	71.25996	7.628962	13.34849

contrast	Condition	estimate	SE	df	t.ratio	p.value
t0 - t10	5-HT	-11.49	1.65	110.7	-6.959	0.0000000
t0 - t10	E3	-0.49	1.65	117.0	-0.294	0.7692128

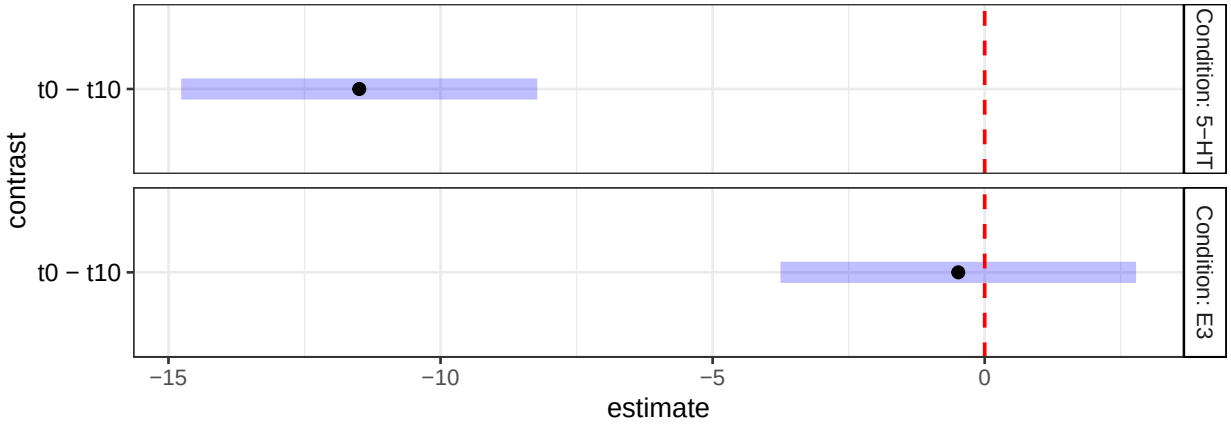


Table 84: Post hoc pairwise comparisons with Tukey's method - Mean differences between E3 and 5-HT at t0 and t10

Condition	Time	emmean	SE	df	lower.CL	upper.CL
5-HT	t0	10.30410	1.489840	73.14845	7.334956	13.27325
E3	t0	10.00351	1.445804	71.79899	7.121214	12.88580
5-HT	t10	21.79785	1.443509	68.61765	18.917845	24.67786
E3	t10	10.48872	1.434315	71.25996	7.628962	13.34849

contrast	Time	estimate	SE	df	t.ratio	p.value
5-HT - E3	t0	0.30	2.07	195.8	0.145	0.8848984
5-HT - E3	t10	11.31	2.03	192.4	5.560	0.0000001

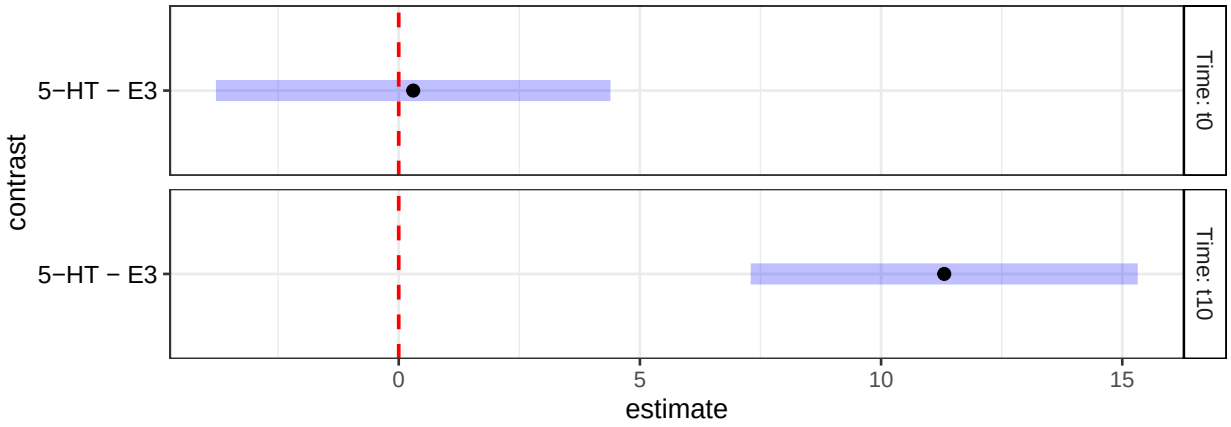


Table 86: Post hoc pairwise comparisons with Tukey's method - Mean differences between t0 and t10 by Condition and Treatment

Time	Condition	Treatment	emmean	SE	df	lower.CL	upper.CL
t0	5-HT	Control	9.771781	2.061316	150.5973	5.698949	13.84461
t10	5-HT	Control	22.949462	2.000712	146.3431	18.995441	26.90348
t0	E3	Control	9.146787	2.025238	151.9462	5.145525	13.14805
t10	E3	Control	9.829060	2.087774	161.4171	5.706188	13.95193
t0	5-HT	OMP ablation	10.896627	2.177571	161.8207	6.596508	15.19675
t10	5-HT	OMP ablation	20.522857	2.107005	154.6551	16.360634	24.68508
t0	E3	OMP ablation	10.902356	2.087668	161.4193	6.779693	15.02502
t10	E3	OMP ablation	11.110467	1.995694	149.8640	7.167135	15.05380

contrast	Condition	Treatment	estimate	SE	df	t.ratio	p.value
t0 - t10	5-HT	Control	-13.18	2.28	108.5	-5.772	0.0000001
t0 - t10	E3	Control	-0.68	2.36	115.8	-0.289	0.7727554
t0 - t10	5-HT	OMP ablation	-9.63	2.41	108.8	-3.996	0.0001175
t0 - t10	E3	OMP ablation	-0.21	2.33	113.6	-0.089	0.9289437

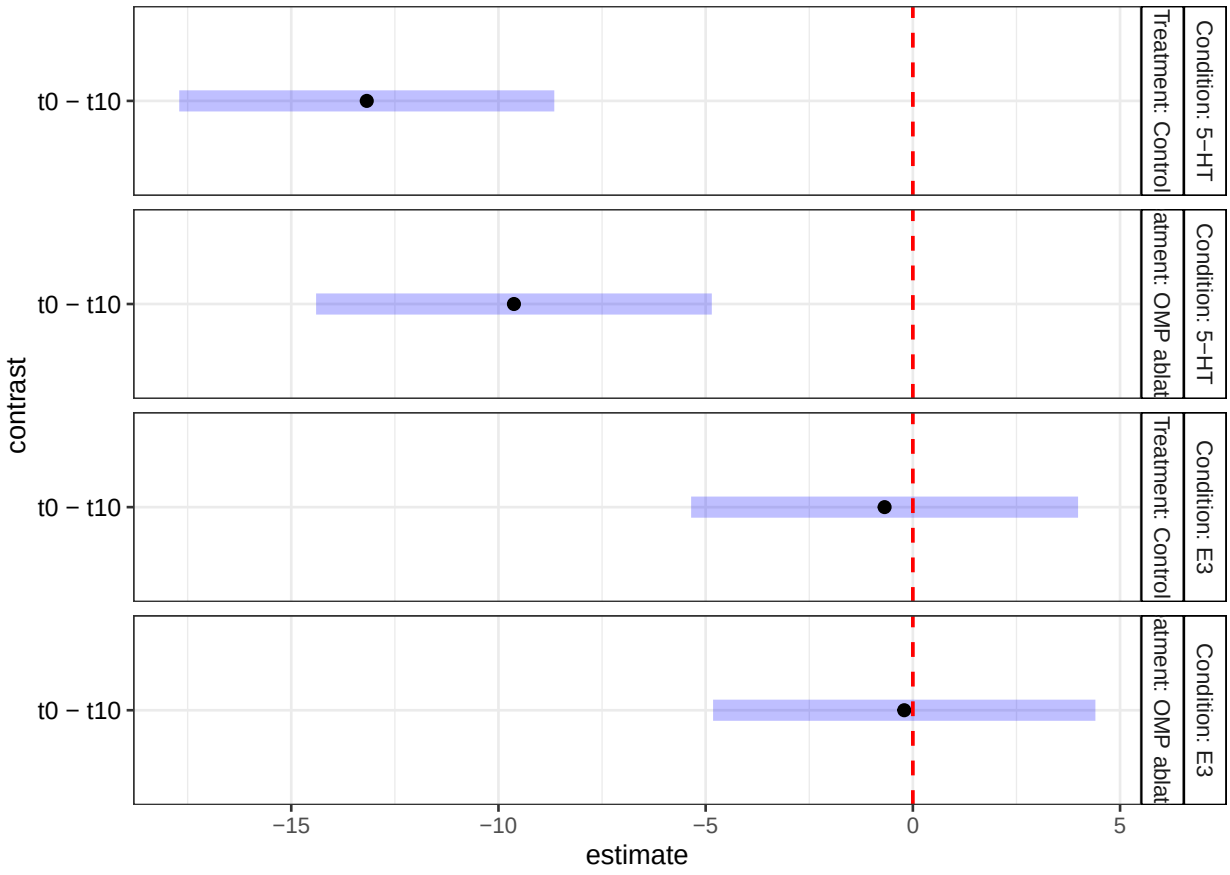


Table 88: Post hoc pairwise comparisons with Tukey's method - Mean differences between E3 and 5-HT at t0 and t10 by treatment group

Condition	Time	Treatment	emmean	SE	df	lower.CL	upper.CL
5-HT	t0	Control	9.771781	2.061316	150.5973	5.698949	13.84461
E3	t0	Control	9.146787	2.025238	151.9462	5.145525	13.14805
5-HT	t10	Control	22.949462	2.000712	146.3431	18.995441	26.90348
E3	t10	Control	9.829060	2.087774	161.4171	5.706188	13.95193
5-HT	t0	OMP ablation	10.896627	2.177571	161.8207	6.596508	15.19675
E3	t0	OMP ablation	10.902356	2.087668	161.4193	6.779693	15.02502
5-HT	t10	OMP ablation	20.522857	2.107005	154.6551	16.360634	24.68508
E3	t10	OMP ablation	11.110467	1.995694	149.8640	7.167135	15.05380

contrast	Time	Treatment	estimate	SE	df	t.ratio	p.value
5-HT - E3	t0	Control	0.62	2.89	189.2	0.216	0.8288705
5-HT - E3	t10	Control	13.12	2.89	189.7	4.539	0.0000100
5-HT - E3	t0	OMP ablation	-0.01	3.02	193.5	-0.002	0.9984861
5-HT - E3	t10	OMP ablation	9.41	2.90	186.1	3.244	0.0013966

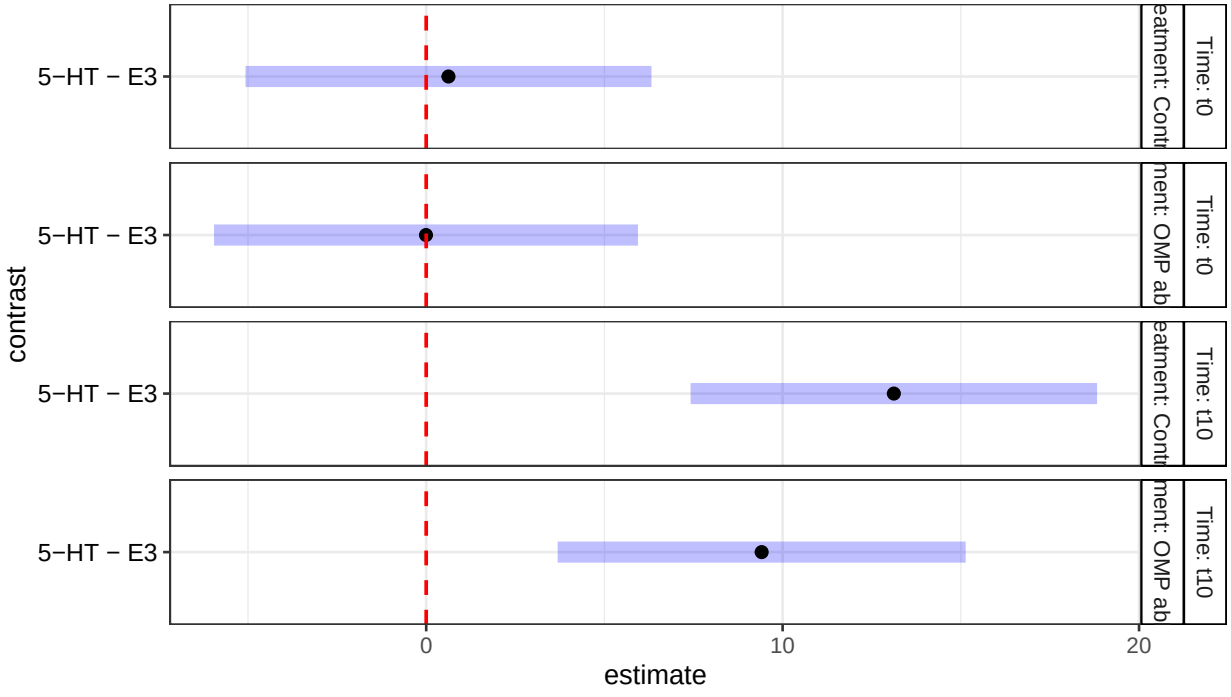


Table 90: Post hoc pairwise comparisons with Tukey's method - Mean differences between Control and OMP ablation by Condition

Treatment	Condition	emmean	SE	df	lower.CL	upper.CL
Control	5-HT	16.376656	1.679327	80.41977	13.034957	19.71836
OMP ablation	5-HT	15.688576	1.771058	86.69799	12.168234	19.20892
Control	E3	9.504112	1.684687	86.61203	6.155403	12.85282
OMP ablation	E3	11.031581	1.676693	85.51085	7.698154	14.36501

contrast	Condition	estimate	SE	df	t.ratio	p.value
Control - OMP ablation	5-HT	0.69	2.44	106.1	0.282	0.7782611
Control - OMP ablation	E3	-1.53	2.38	110.9	-0.643	0.5217833

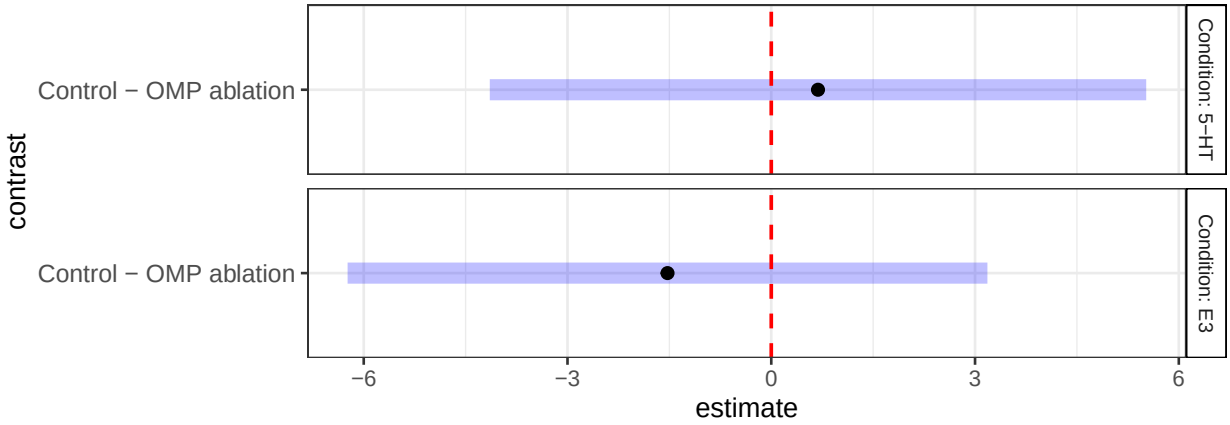
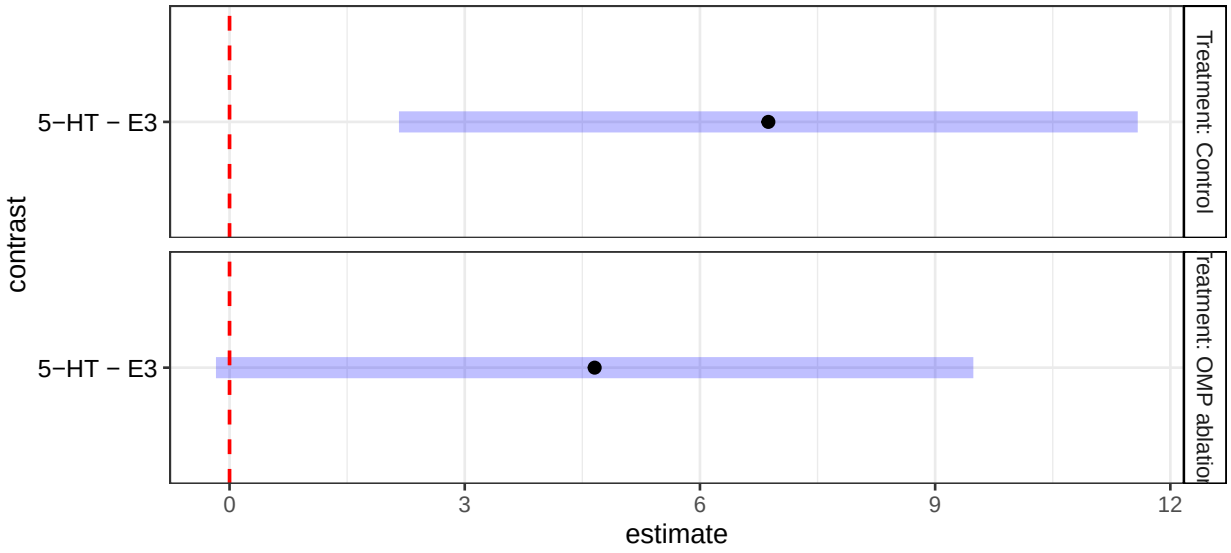


Table: Post hoc pairwise comparisons with Tukey's method - Mean differences between E3 and 5-HT by Treatment

```
## boundary (singular) fit: see ?isSingular
```

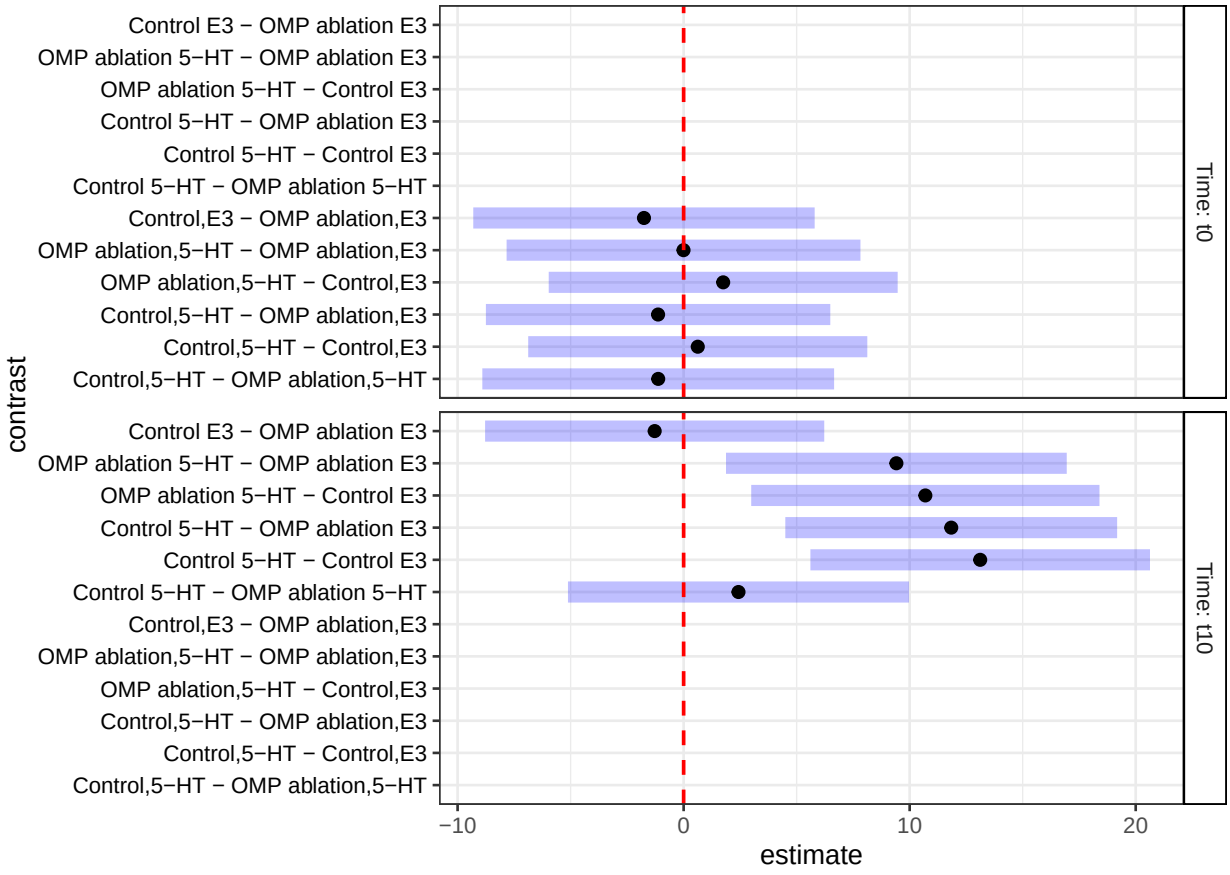
Condition	Treatment	emmean	SE	df	lower.CL	upper.CL
5-HT	Control	16.376656	1.679327	80.41977	13.034957	19.71836
E3	Control	9.504112	1.684687	86.61203	6.155403	12.85282
5-HT	OMP ablation	15.688576	1.771058	86.69799	12.168234	19.20892
E3	OMP ablation	11.031581	1.676693	85.51085	7.698154	14.36501

contrast	Treatment	estimate	SE	df	t.ratio	p.value
5-HT - E3	Control	6.87	2.38	108.6	2.891	0.0046389
5-HT - E3	OMP ablation	4.66	2.44	108.6	1.911	0.0586538

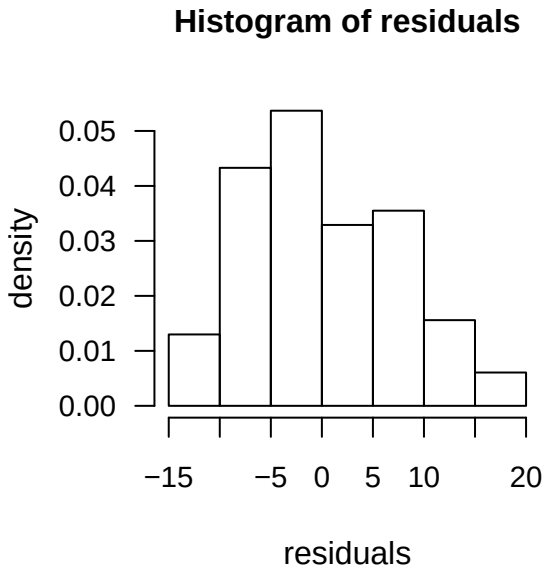


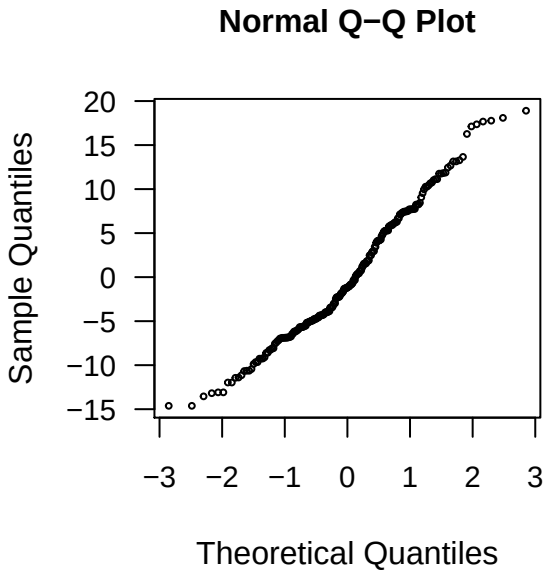
Treatment	Condition	Time	emmean	SE	df	lower.CL	upper.CL
Control	5-HT	t0	9.771781	2.061316	150.5973	5.698949	13.84461
OMP ablation	5-HT	t0	10.896627	2.177571	161.8207	6.596508	15.19675
Control	E3	t0	9.146787	2.025238	151.9462	5.145525	13.14805
OMP ablation	E3	t0	10.902356	2.087668	161.4193	6.779693	15.02502
Control	5-HT	t10	22.949462	2.000712	146.3431	18.995441	26.90348
OMP ablation	5-HT	t10	20.522857	2.107005	154.6551	16.360634	24.68508
Control	E3	t10	9.829060	2.087774	161.4171	5.706188	13.95193
OMP ablation	E3	t10	11.110467	1.995694	149.8640	7.167135	15.05380

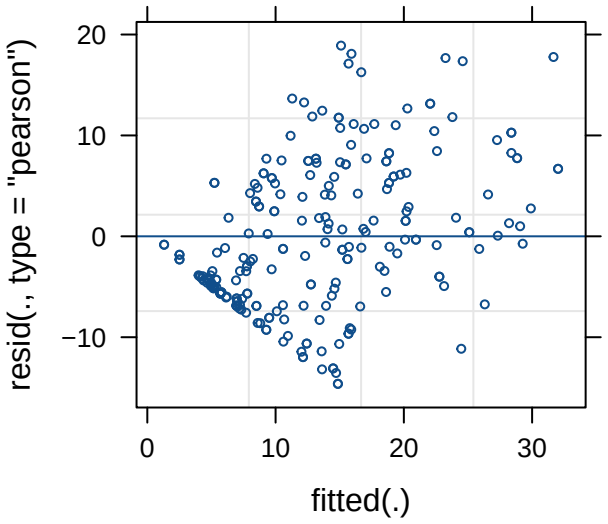
contrast	Time	estimate	SE	df	t.ratio	p.value
Control,5-HT - OMP ablation,5-HT	t0	-1.12	3.00	150.6	-0.375	0.9818863
Control,5-HT - Control,E3	t0	0.62	2.89	150.6	0.216	0.9964045
Control,5-HT - OMP ablation,E3	t0	-1.13	2.93	150.6	-0.385	0.9804578
OMP ablation,5-HT - Control,E3	t0	1.75	2.97	151.9	0.589	0.9353271
OMP ablation,5-HT - OMP ablation,E3	t0	-0.01	3.02	161.4	-0.002	1.0000000
Control,E3 - OMP ablation,E3	t0	-1.76	2.91	151.9	-0.604	0.9307338
Control 5-HT - OMP ablation 5-HT	t10	2.43	2.90	146.3	0.836	0.8373437
Control 5-HT - Control E3	t10	13.12	2.89	146.3	4.539	0.0000687
Control 5-HT - OMP ablation E3	t10	11.84	2.82	146.3	4.191	0.0002771
OMP ablation 5-HT - Control E3	t10	10.69	2.97	154.7	3.605	0.0023550
OMP ablation 5-HT - OMP ablation E3	t10	9.41	2.90	149.9	3.244	0.0078432
Control E3 - OMP ablation E3	t10	-1.28	2.89	149.9	-0.444	0.9707360


Control E3 − OMP ablation E3
OMP ablation 5−HT − OMP ablation E3
OMP ablation 5−HT − Control E3
Control 5−HT − OMP ablation E3
Control 5−HT − Control E3
Control 5−HT − OMP ablation 5−HT
Control,E3 − OMP ablation,E3
OMP ablation,5−HT − OMP ablation,E3
OMP ablation,5−HT − Control,E3
Control,5−HT − OMP ablation,E3
Control,5−HT − Control,E3
Control,5−HT − OMP ablation,5−HT
contrast
Time: t0
Time: t10
−10
0
10
20
estimate

6.2.4 Checking model assumptions







6.2.5 Conclusion

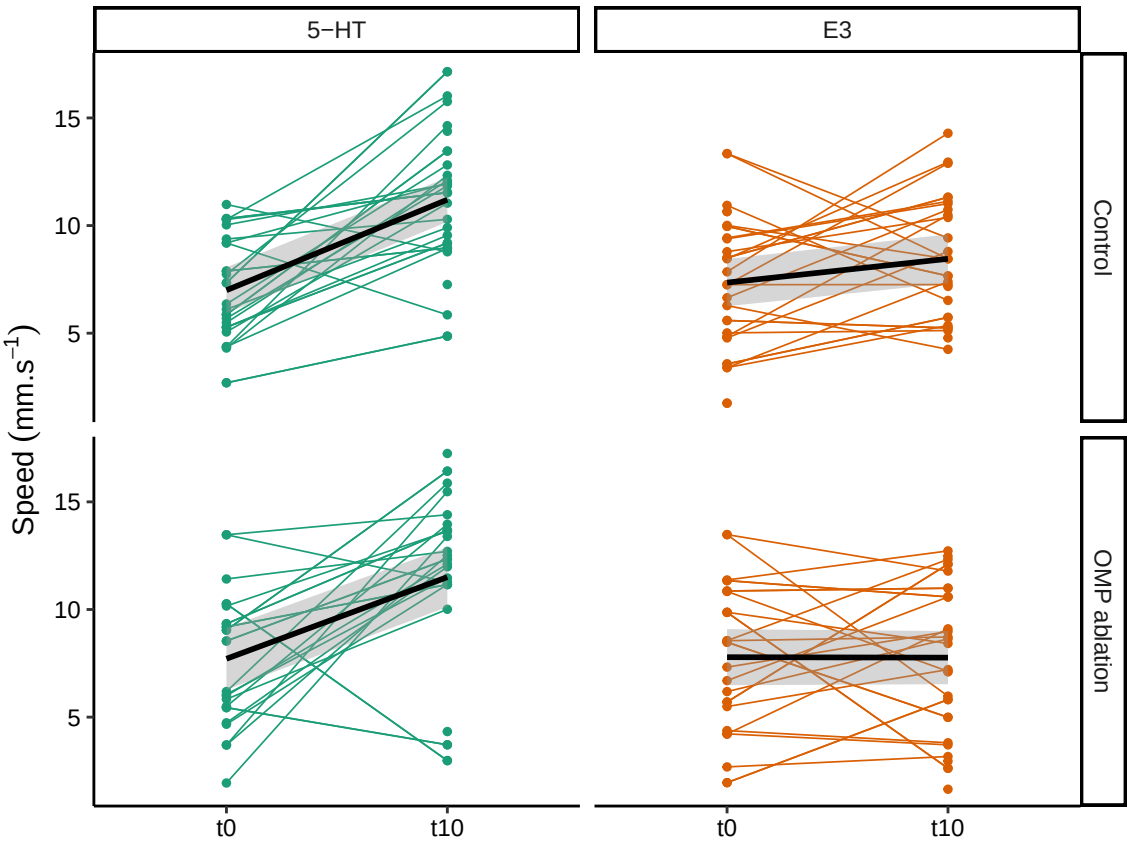
Type II Wald chi-square tests for the fitted model indicated a significant interaction effect between Condition (E3, 5-HT) and Time (t0, t10) (Wald $\chi^2 = 22.02$, df $= 1$, $^{***}p = 2.7\text{e-}6$) on the percentage of time swimming. Post hoc tests using Tukey's method only indicated significant differences between t0 and t10 in 5-HT ($^{***}p = 2.6\text{e-}10$), and between E3 and 5-HT at t10 ($^{**}p = 8.9\text{e-}8$); while no differences were observed between t0 and t10 in E3 ($^{ns}p = 0.77$), and between E3 and 5-HT at t0 ($^{ns}p = 0.88$). No differences were observed between the treatment groups (Control, OMP ablation) (Wald $\chi^2 = 0.06$, df $= 1$, $^{ns}p = 0.86$).

6.3 Speed (square root transformed data) (Figure 5G)

6.3.1 Descriptive data

Table 96: Speed by Treatment, Condition and Time

	Control	OMP ablation
5.HT.t0	7 ± 2.43 (n = 29, r = 2.7-10.98)	7.71 ± 2.99 (n = 26, r = 1.94-13.47)
E3.t0	7.35 ± 3.16 (n = 30, r = 1.75-13.34)	7.78 ± 3.38 (n = 28, r = 1.96-13.48)
5.HT.t10	11.2 ± 3.23 (n = 31, r = 4.86-17.15)	11.51 ± 4.19 (n = 28, r = 2.98-17.25)
E3.t10	8.46 ± 2.82 (n = 28, r = 4.25-14.29)	7.76 ± 3.49 (n = 31, r = 1.65-12.72)



6.3.2 Linear mixed model with Wald chi-square tests

Significant interaction between Condition (5-HT, E3) and Time (t0, t10) on Speed (Wald $\chi^2 = 15.66$, df = 1, $^{***}p = 7.6\text{e-}5$). No differences were observed between the treatment groups (Control, OMP ablation) (Wald $\chi^2 = 0.0023$, df = 1, $^{ns}p = 0.96$).

```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: sqrt(Speed)
##                             Chisq Df Pr(>Chisq)
## Condition                  9.8343  1   0.001713 **
## Treatment                  0.0023  1   0.962010
## Time                      31.1963  1  2.332e-08 ***
## Condition:Treatment        0.3410  1   0.559277
## Condition:Time            15.6585  1  7.587e-05 ***
## Treatment:Time             1.4659  1   0.225996
## Condition:Treatment:Time   0.1777  1   0.673361
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

6.3.3 Tukey's post hoc tests

Table 97: Post hoc pairwise comparisons with Tukey's method - Mean differences between t0 and t10 by Condition (back-transformed estimate values from the sqrt scale)

Time	Condition	response	SE	df	lower.CL	upper.CL
t0	5-HT	7.090776	0.4508250	60.43684	6.189126	7.992426
t10	5-HT	10.971393	0.5432982	56.06899	9.883066	12.059720
t0	E3	7.111530	0.4387947	58.11158	6.233223	7.989837
t10	E3	7.712500	0.4533960	57.47643	6.804754	8.620247

contrast	Condition	estimate	SE	df	t.ratio	p.value
t0 - t10	5-HT	-3.88	0.58	56.1	-6.705	0.000000
t0 - t10	E3	-0.60	0.52	57.5	-1.152	0.254036

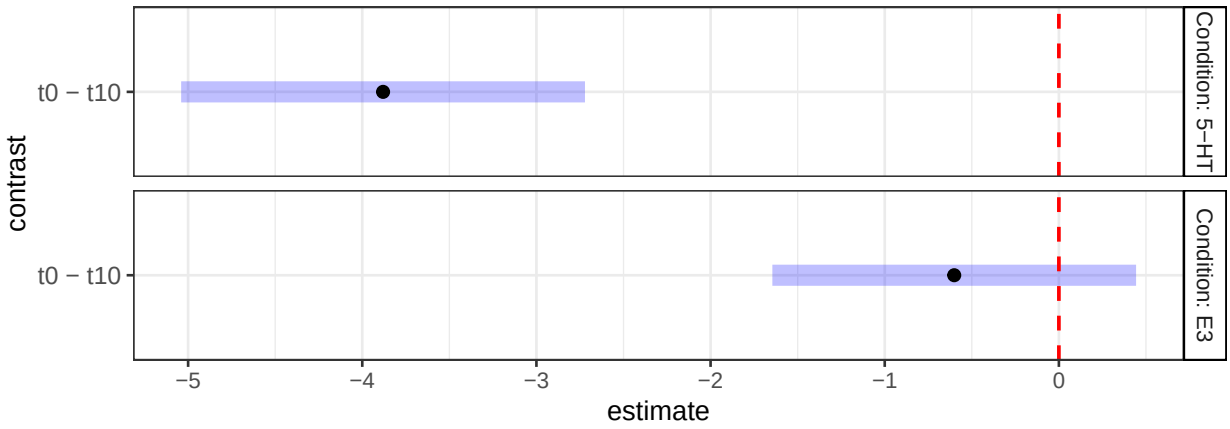


Table 99: Post hoc pairwise comparisons with Tukey's method - Mean differences between E3 and 5-HT at t0 and t10 (back-transformed estimate values from the sqrt scale)

Condition	Time	response	SE	df	lower.CL	upper.CL
5-HT	t0	7.090776	0.4508250	60.43684	6.189126	7.992426
E3	t0	7.111530	0.4387947	58.11158	6.233223	7.989837
5-HT	t10	10.971393	0.5432982	56.06899	9.883066	12.059720
E3	t10	7.712500	0.4533960	57.47643	6.804754	8.620247

contrast	Time	estimate	SE	df	t.ratio	p.value
5-HT - E3	t0	-0.02	0.59	58.1	-0.035	0.9721889
5-HT - E3	t10	3.26	0.67	56.1	4.885	0.0000090

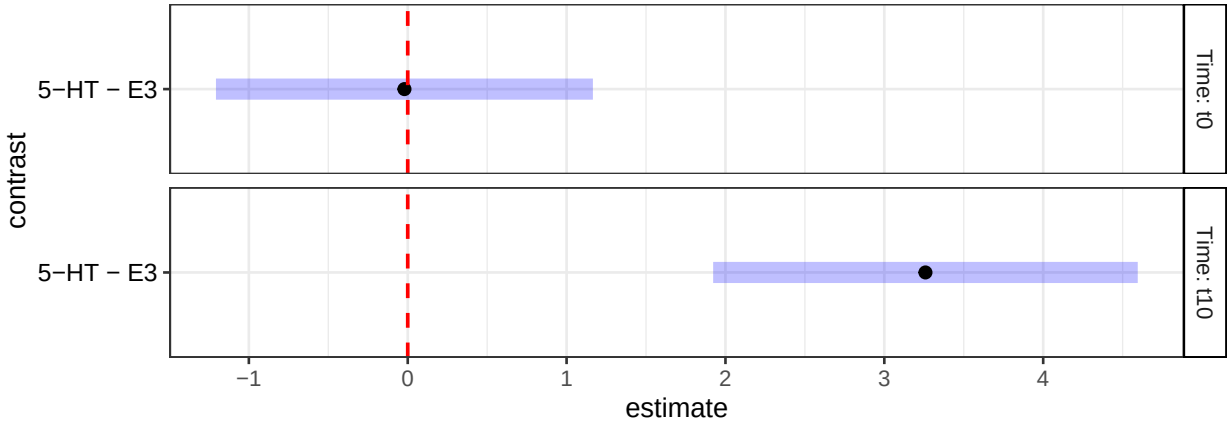


Table 101: Post hoc pairwise comparisons with Tukey's method - Mean differences between t0 and t10 by Condition and Treatment

Time	Condition	Treatment	response	SE	df	lower.CL	upper.CL
t0	5-HT	Control	6.796372	0.5915423	131.6972	5.626218	7.966526
t10	5-HT	Control	10.966040	0.7278573	127.1805	9.525761	12.406318
t0	E3	Control	6.935161	0.5868734	131.7265	5.774245	8.096077
t10	E3	Control	8.151696	0.6560327	141.4784	6.854802	9.448590
t0	5-HT	OMP ablation	7.382828	0.6489355	145.9022	6.100300	8.665356
t10	5-HT	OMP ablation	10.985950	0.7649171	138.2217	9.473498	12.498402
t0	E3	OMP ablation	7.298733	0.6207514	141.4659	6.071586	8.525881
t10	E3	OMP ablation	7.296886	0.5926200	129.5460	6.124419	8.469352

contrast	Condition	Treatment	estimate	SE	df	t.ratio	p.value
t0 - t10	5-HT	Control	-4.17	0.79	127.2	-5.255	0.0000006
t0 - t10	E3	Control	-1.22	0.75	131.7	-1.616	0.1085761
t0 - t10	5-HT	OMP ablation	-3.60	0.85	138.2	-4.239	0.0000408
t0 - t10	E3	OMP ablation	0.00	0.73	129.5	0.003	0.9979860

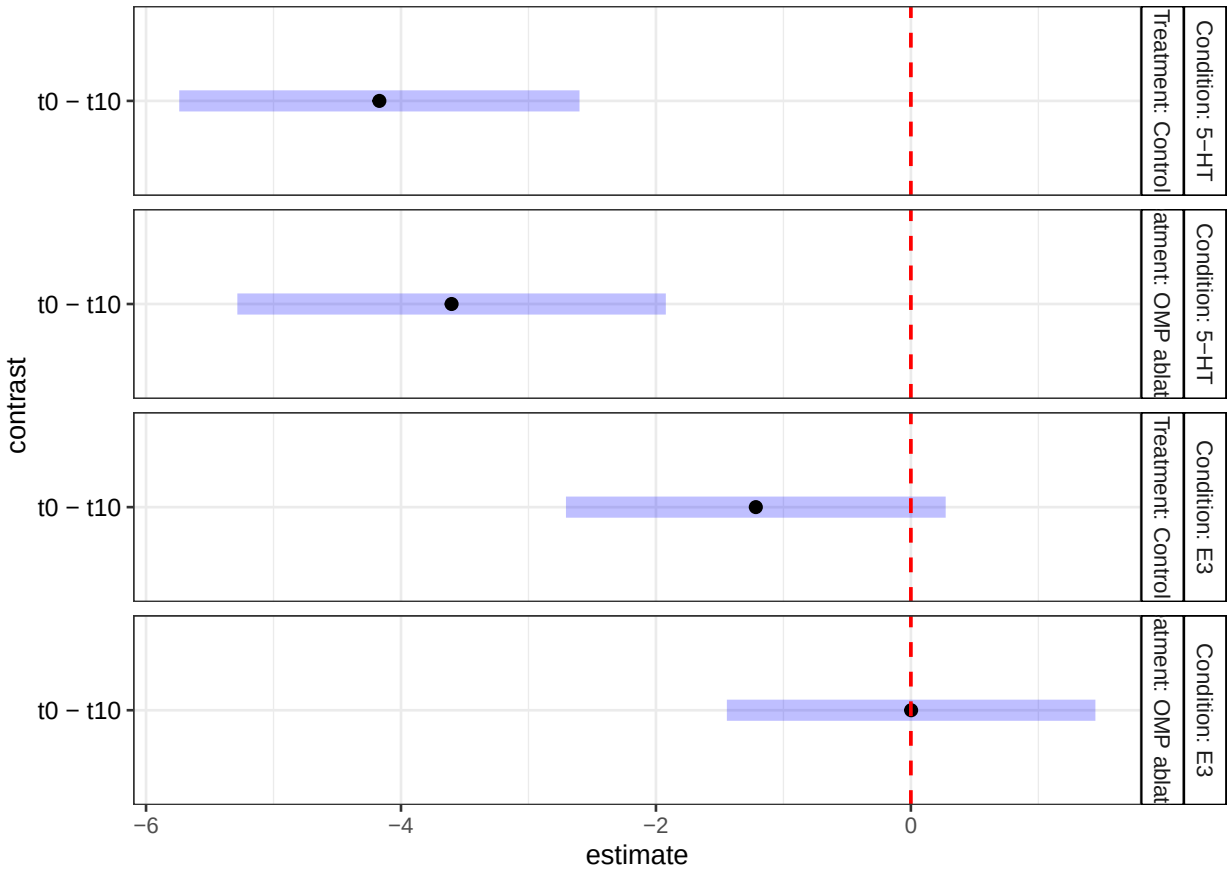


Table 103: Post hoc pairwise comparisons with Tukey's method - Mean differences between E3 and 5-HT at t0 and t10 by treatment group

Condition	Time	Treatment	response	SE	df	lower.CL	upper.CL
5-HT	t0	Control	6.796372	0.5915423	131.6972	5.626218	7.966526
E3	t0	Control	6.935161	0.5868734	131.7265	5.774245	8.096077
5-HT	t10	Control	10.966040	0.7278573	127.1805	9.525761	12.406318
E3	t10	Control	8.151696	0.6560327	141.4784	6.854802	9.448590
5-HT	t0	OMP ablation	7.382828	0.6489355	145.9022	6.100300	8.665356
E3	t0	OMP ablation	7.298733	0.6207514	141.4659	6.071586	8.525881
5-HT	t10	OMP ablation	10.985950	0.7649171	138.2217	9.473498	12.498402
E3	t10	OMP ablation	7.296886	0.5926200	129.5460	6.124419	8.469352

contrast	Time	Treatment	estimate	SE	df	t.ratio	p.value
5-HT - E3	t0	Control	-0.14	0.81	131.7	-0.172	0.8636399
5-HT - E3	t10	Control	2.81	0.95	127.2	2.962	0.0036446
5-HT - E3	t0	OMP ablation	0.08	0.87	141.5	0.096	0.9233710
5-HT - E3	t10	OMP ablation	3.69	0.94	129.5	3.929	0.0001382

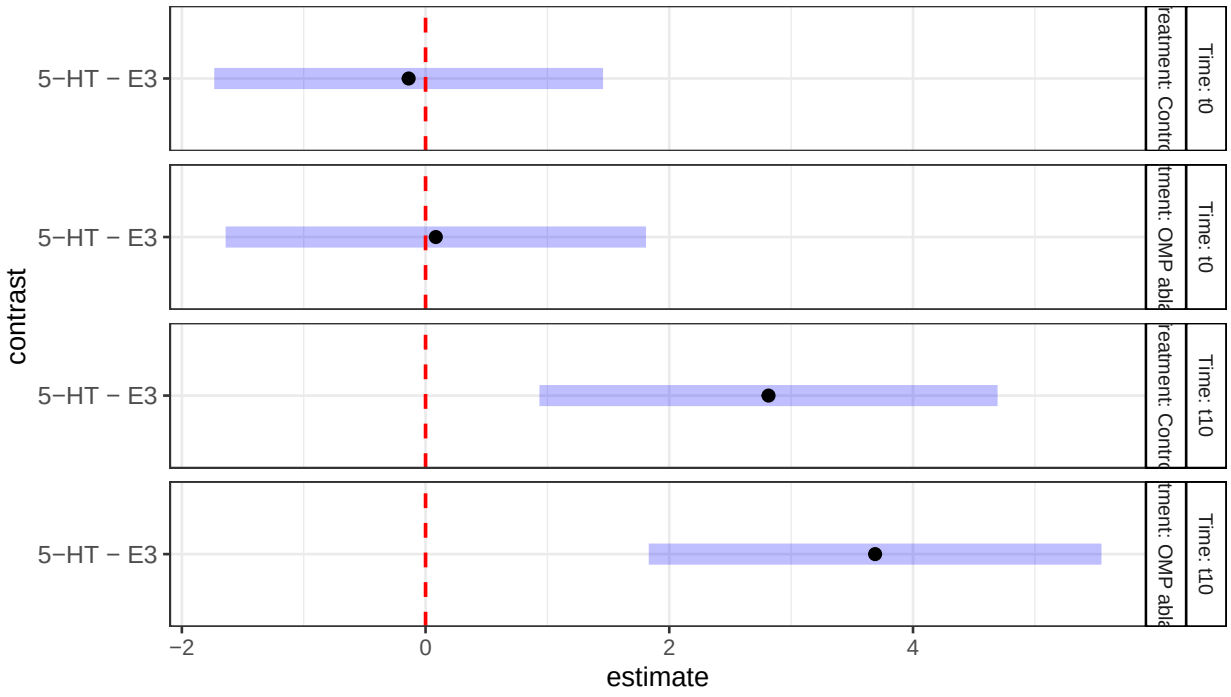


Table 105: Post hoc pairwise comparisons with Tukey's method - Mean differences between Control and OMP ablation by Condition (back-transformed estimate values from the sqrt scale)

Treatment	Condition	response	SE	df	lower.CL	upper.CL
Control	5-HT	8.753671	0.5311205	63.29693	7.692409	9.814934
OMP ablation	5-HT	9.099272	0.5681997	70.39035	7.966144	10.232400
Control	E3	7.526833	0.4949424	68.00026	6.539190	8.514475
OMP ablation	E3	7.292034	0.4844582	67.02257	6.325057	8.259011

contrast	Condition	estimate	SE	df	t.ratio	p.value
Control - OMP ablation	5-HT	-0.35	0.74	63.3	-0.467	0.6421769
Control - OMP ablation	E3	0.23	0.66	67.0	0.356	0.7229940

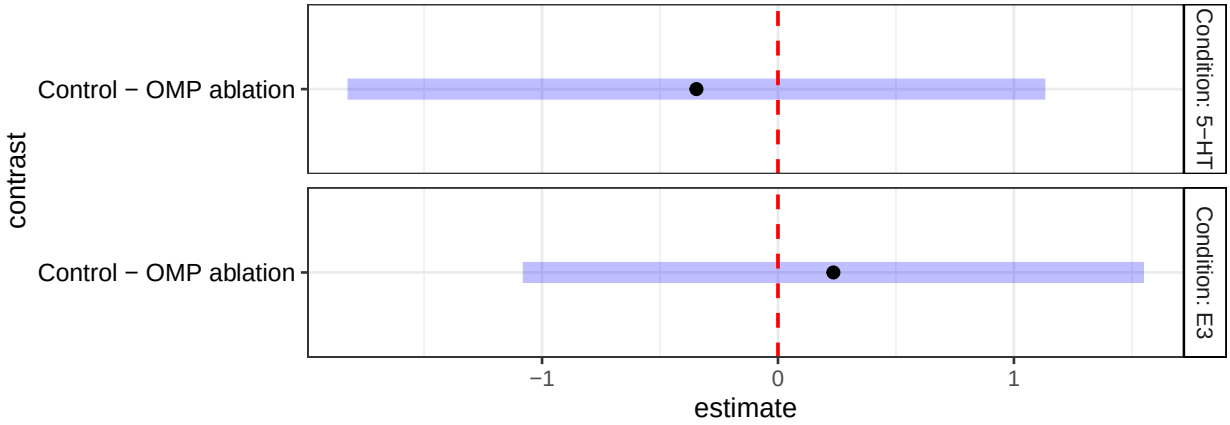
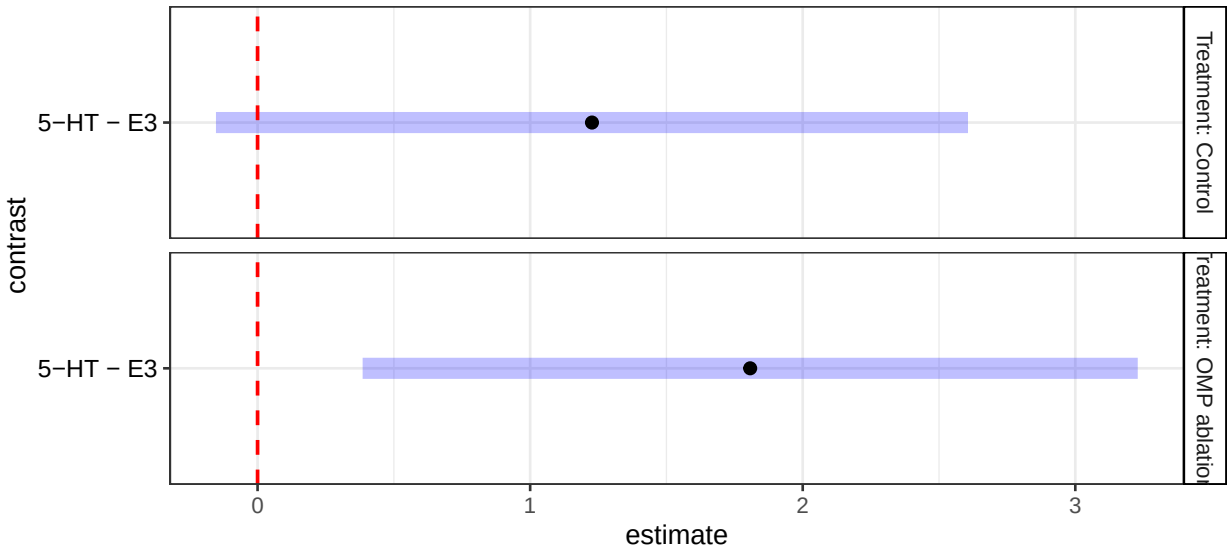


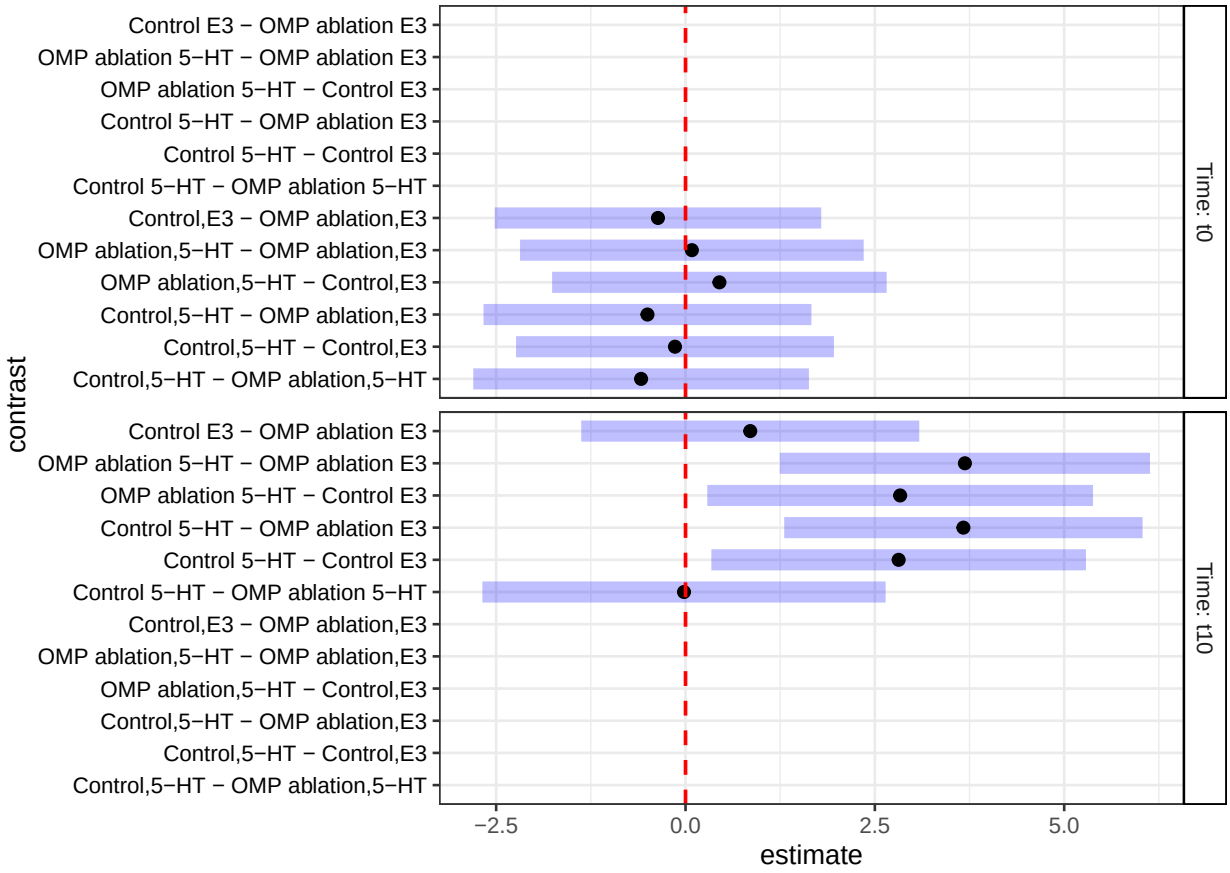
Table 107: Post hoc pairwise comparisons with Tukey's method - Mean differences between E3 and 5-HT by Treatment (back-transformed estimate values from the sqrt scale)

contrast	Treatment	estimate	SE	df	t.ratio	p.value
5-HT - E3	Control	1.23	0.69	63.3	1.777	0.0803765
5-HT - E3	OMP ablation	1.81	0.71	67.0	2.537	0.0135167



Treatment	Condition	Time	response	SE	df	lower.CL	upper.CL
Control	5-HT	t0	6.796372	0.5915423	131.6972	5.626218	7.966526
OMP ablation	5-HT	t0	7.382828	0.6489355	145.9022	6.100300	8.665356
Control	E3	t0	6.935161	0.5868734	131.7265	5.774245	8.096077
OMP ablation	E3	t0	7.298733	0.6207514	141.4659	6.071586	8.525881
Control	5-HT	t10	10.966040	0.7278573	127.1805	9.525761	12.406318
OMP ablation	5-HT	t10	10.985950	0.7649171	138.2217	9.473498	12.498402
Control	E3	t10	8.151696	0.6560327	141.4784	6.854802	9.448590
OMP ablation	E3	t10	7.296886	0.5926200	129.5460	6.124419	8.469352

contrast	Time	estimate	SE	df	t.ratio	p.value
Control,5-HT - OMP ablation,5-HT	t0	-0.59	0.85	131.7	-0.689	0.9012997
Control,5-HT - Control,E3	t0	-0.14	0.81	131.7	-0.172	0.9981802
Control,5-HT - OMP ablation,E3	t0	-0.50	0.83	131.7	-0.604	0.9306894
OMP ablation,5-HT - Control,E3	t0	0.45	0.85	131.7	0.527	0.9523113
OMP ablation,5-HT - OMP ablation,E3	t0	0.08	0.87	141.5	0.096	0.9996781
Control,E3 - OMP ablation,E3	t0	-0.36	0.83	131.7	-0.439	0.9716198
Control 5-HT - OMP ablation 5-HT	t10	-0.02	1.02	127.2	-0.019	0.9999973
Control 5-HT - Control E3	t10	2.81	0.95	127.2	2.962	0.0188705
Control 5-HT - OMP ablation E3	t10	3.67	0.91	127.2	4.037	0.0005340
OMP ablation 5-HT - Control E3	t10	2.83	0.98	138.2	2.894	0.0226621
OMP ablation 5-HT - OMP ablation E3	t10	3.69	0.94	129.5	3.929	0.0007890
Control E3 - OMP ablation E3	t10	0.85	0.86	129.5	0.997	0.7514598


Control E3 − OMP ablation E3
OMP ablation 5−HT − OMP ablation E3
OMP ablation 5−HT − Control E3
Control 5−HT − OMP ablation E3
Control 5−HT − Control E3
Control 5−HT − OMP ablation 5−HT
Control,E3 − OMP ablation,E3
OMP ablation,5−HT − OMP ablation,E3
OMP ablation,5−HT − Control,E3
Control,5−HT − OMP ablation,E3
Control,5−HT − Control,E3
Control,5−HT − OMP ablation,5−HT
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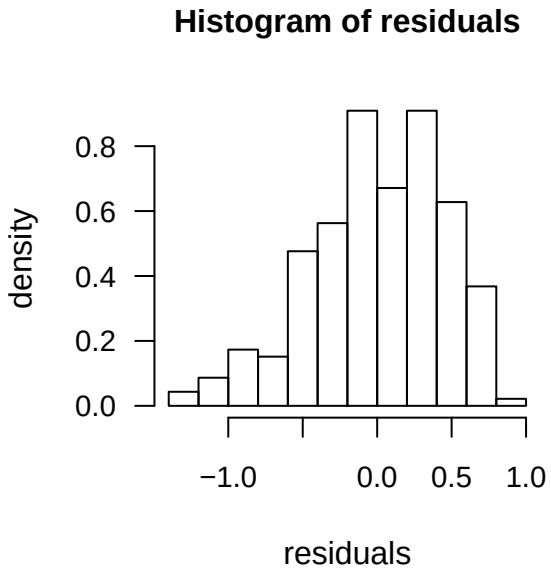
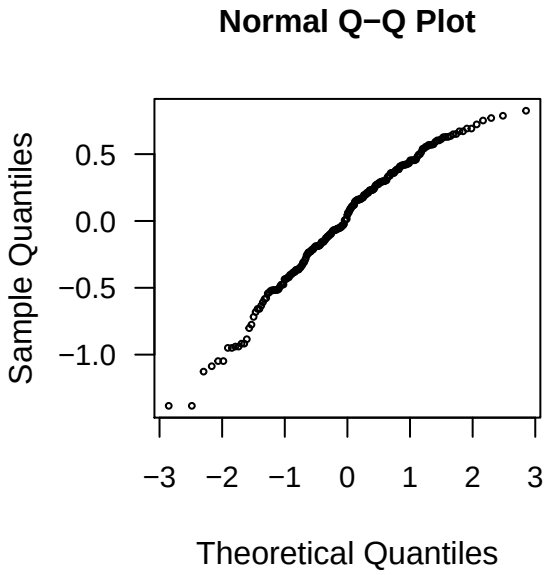
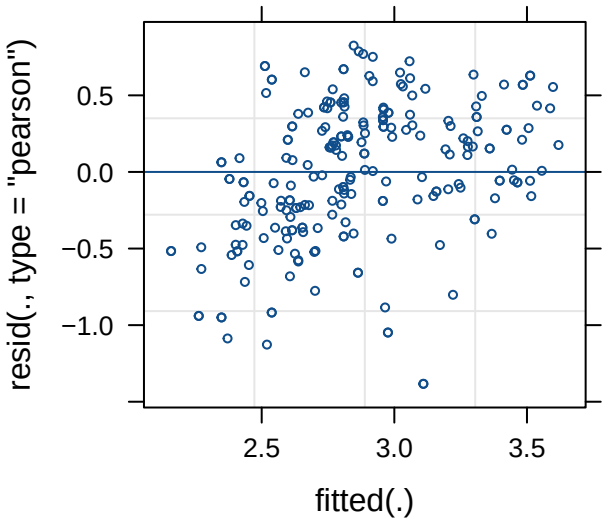
Time: t0
Time: t10
−2.5
0.0
2.5
5.0
estimate

6.3.4 Checking model assumptions







6.3.5 Conclusion

Type II Wald chi-square tests for the fitted model indicated a significant interaction effect between Condition and Time on Speed (Wald $\chi^2 = 15.66$, df $= 1$, $^{***}p = 7.6\text{e-}5$). Post hoc tests using Tukey's method showed a difference between t0 and t10 in 5-HT ($^{***}p = 1.1\text{e-}8$), and between E3 and 5-HT at t10 ($^{***}p = 9.0\text{e-}6$), while no differences were observed between t0 and t10 in E3 ($^{ns}p = 0.25$), and between E3 and 5-HT at t0 ($^{ns}p = 0.97$). No differences were observed between the treatment groups (Control, OMP ablation) (Wald $\chi^2 = 0.0023$, df $= 1$, $^{ns}p = 0.96$).

7 R session information

```
## R version 3.6.1 (2019-07-05)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 10 x64 (build 17134)
##
## Matrix products: default
##
## locale:
## [1] LC_COLLATE=French_France.1252  LC_CTYPE=French_France.1252
## [3] LC_MONETARY=French_France.1252 LC_NUMERIC=C
## [5] LC_TIME=French_France.1252
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
##  [1] knitr_1.28         emmeans_1.4.5      car_3.0-7          carData_3.0-3
##  [5] influence.ME_0.9-9 lme4_1.1-26        Matrix_1.2-18      RColorBrewer_1.1-2
##  [9] ggpubr_0.2.5       magrittr_1.5       ggplot2_3.3.3      reshape2_1.4.3
## [13] openxlsx_4.1.4
##
## loaded via a namespace (and not attached):
##  [1] Rcpp_1.0.4        mvtnorm_1.1-0     lattice_0.20-41   zoo_1.8-8
##  [5] digest_0.6.25     R6_2.4.1          cellranger_1.1.0  plyr_1.8.6
##  [9] evaluate_0.14     coda_0.19-4       highr_0.8         pillar_1.4.3
## [13] rlang_0.4.10      curl_4.3          multcomp_1.4-13   readxl_1.3.1
## [17] minqa_1.2.4       data.table_1.12.8 nloptr_1.2.2.1    rmarkdown_2.1
## [21] labeling_0.3      splines_3.6.1     statmod_1.4.35    stringr_1.4.0
## [25] foreign_0.8-76    munsell_0.5.0     compiler_3.6.1    xfun_0.22
## [29] pkgconfig_2.0.3   mgcv_1.8-31       htmltools_0.4.0   tidyselect_1.1.0
## [33] tibble_3.0.0      codetools_0.2-16  rio_0.5.16        crayon_1.3.4
## [37] dplyr_1.0.5       withr_2.4.2       MASS_7.3-51.5     grid_3.6.1
## [41] nlme_3.1-145      xtable_1.8-4      gtable_0.3.0      lifecycle_1.0.0
## [45] DBI_1.1.0         scales_1.1.1      zip_2.1.1         estimability_1.3
## [49] cli_2.5.0         stringi_1.4.6     farver_2.0.3      ggsignif_0.6.0
## [53] ellipsis_0.3.0    generics_0.0.2    vctrs_0.3.6       sandwich_2.5-1
## [57] boot_1.3-24       TH.data_1.0-10    tools_3.6.1       forcats_0.5.0
## [61] glue_1.3.2        purrr_0.3.3       hms_0.5.3         parallel_3.6.1
## [65] pbkrtest_0.4-8.6  survival_3.1-11   abind_1.4-5       yaml_2.2.1
## [69] colorspace_1.4-1  haven_2.2.0
```