

Supplementary Materials of “Response to change in the number of visual stimuli in zebrafish: A behavioural and molecular study”

Andrea Messina^{1,*}, Davide Potrich¹, Ilaria Schiona¹, Valeria Anna Sovrano^{1,2}, Scott E. Fraser³, Caroline H. Brennan⁴, Giorgio Vallortigara¹

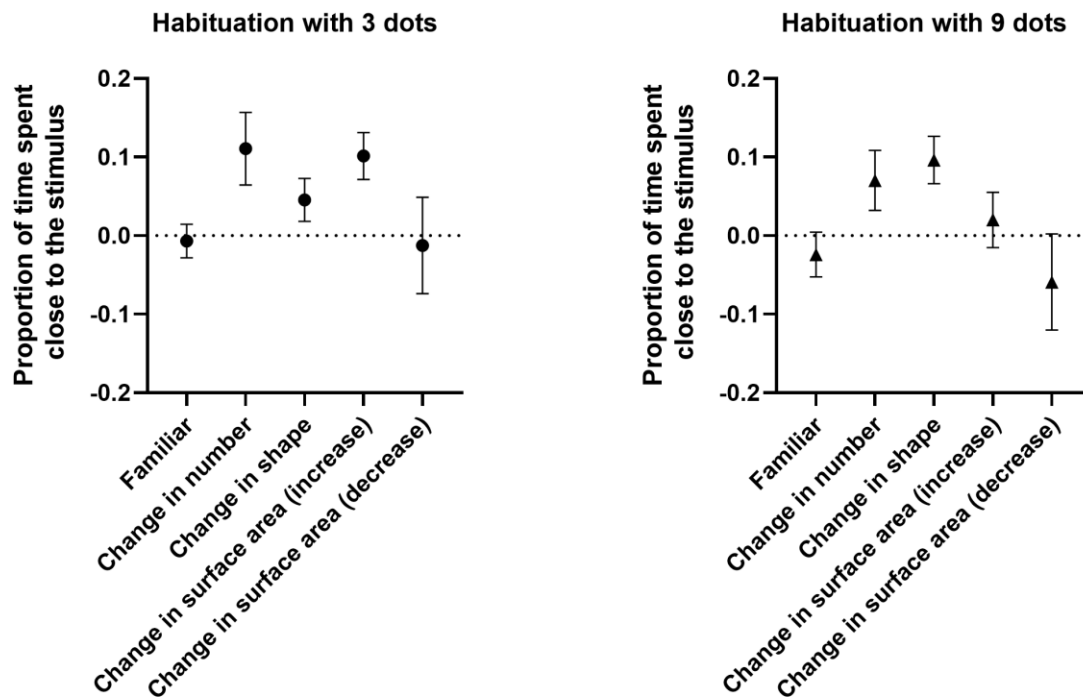
¹ Center for Mind/Brain Sciences, University of Trento, Rovereto, Italy.

² Department of Psychology and Cognitive Science, University of Trento, Rovereto, Italy.

³ Michelson Center for Convergent Bioscience, University of Southern California, Los Angeles, USA

⁴ School of Biological and Chemical Sciences, Queen Mary University London, UK.

Supplementary Materials Figure 1: Proportion of time spent near the stimulus during dishabituation (comparing the dishabituation trial with the first of the four trials previously performed during the last habituation session) as a function of habituation conditions (with 3 or 9 dots) and test conditions [no change (familiar), change in number, change in shape, change in surface area (increase), change in surface area (decrease)].



Supplementary Table 1: Overall analyses of variance (ANOVA) for *c-fos* and for *egr-1*, with habituation (habituation with 3 dots, habituation with 9 dots) and type of test (familiar, number, shape, surface area increase, surface area decrease) as between-subject factors, and brain areas (retina, optic tectum, thalamus, telencephalon, cerebellum, medulla oblongata) as a within-subject factor.

ANOVA			
c-fos	Main effect of Brain Area	$F(4.221, 253.272)=53.493$	$p=0.0001$
	Main effect of Habituation	$F(1, 60)=5.062$	$p=0.028$
	Main effect of Test	$F(4, 60)=4.655$	$p=0.002$
	Brain Area x Test interaction	$F(16.885, 253.272)=2.605$	$p=0.001$
	Habituation x Test interaction	$F(4, 60)=14.255$	$p=0.0001$
	Brain Area x Habituation interaction	$F(4.221, 253.272)=3.774$	$p=0.005$
	Brain Area x Test x Habituation interaction	$F(16.885, 253.272)=33.228$	$p=0.0001$
egr-1	Main effect of Brain Area	$F(3.334, 200.028)=205.880$	$p=0.0001$
	Main effect of Habituation	$F(1, 60)=23.106$	$p=0.0001$
	Main effect of Test	$F(4, 60)=6.211$	$p=0.0001$
	Brain Area x Test interaction	$F(13.335, 200.028)=2.915$	$p=0.001$
	Habituation x Test interaction	$F(4, 60)=5.717$	$p=0.001$
	Brain Area x Habituation interaction	$F(3.334, 200.028)=3.663$	$p=0.011$
	Brain Area x Test x Habituation interaction	$F(13.335, 200.028)=1.934$	$p=0.027$

Supplementary Table 2: Analyses of variance (ANOVA) for *c-fos* and for *egr-1* for the Retina, with habituation (habituation with 3 dots, habituation with 9 dots) and type of test (familiar, number, shape, surface area increase, surface area decrease) as between-subject factors.

RETINA			
c-fos	Main effect of Habituation	$F(1, 60)=0.077$	$p=0.783$
	Main effect of Test	$F(4, 60)=1.643$	$p=0.0175$
	Habituation x Test interaction	$F(4, 60)=7.929$	$p=0.0001$
egr-1	Main effect of Habituation	$F(1, 60)=0.126$	$p=0.723$
	Main effect of Test	$F(4, 60)=2.981$	$p=0.026$
	Habituation x Test interaction	$F(4, 60)=13.541$	$p=0.0001$

Supplementary Table 3: Analyses of variance (ANOVA) for *c-fos* and for *egr-1* for the Optic Tectum, with habituation (habituation with 3 dots, habituation with 9 dots) and type of test (familiar, number, shape, surface area increase, surface area decrease) as between-subject factors.

OPTIC TECTUM			
c-fos	Main effect of Habituation	$F(1, 60)=0.852$	$p=0.360$
	Main effect of Test	$F(4, 60)=8.410$	$p=0.0001$
	Habituation x Test interaction	$F(4, 60)=2.146$	$p=0.086$
egr-1	Main effect of Habituation	$F(1, 60)=20.291$	$p=0.0001$
	Main effect of Test	$F(4, 60)=6.948$	$p=0.0001$
	Habituation x Test interaction	$F(4, 60)=4.243$	$p=0.004$

Supplementary Table 4: Analyses of variance (ANOVA) for *c-fos* and for *egr-1* for the Thalamus, with habituation (habituation with 3 dots, habituation with 9 dots) and type of test (familiar, number, shape, surface area increase, surface area decrease) as between-subject factors.

THALAMUS			
c-fos	Main effect of Habituation	$F(1, 60)=1.141$	$p=0.290$
	Main effect of Test	$F(4, 60)=6.329$	$p=0.0001$
	Habituation x Test interaction	$F(4, 60)=8.629$	$p=0.0001$
egr-1	Main effect of Habituation	$F(1, 60)=11.356$	$p=0.001$
	Main effect of Test	$F(4, 60)=4.369$	$p=0.004$
	Habituation x Test interaction	$F(4, 60)=2.190$	$p=0.081$

Supplementary Table 5: Analyses of variance (ANOVA) for *c-fos* and for *egr-1* for the Telencephalon, with habituation (habituation with 3 dots, habituation with 9 dots) and type

of test (familiar, number, shape, surface area increase, surface area decrease) as between-subject factors.

TELENCEPHALON			
c-fos	Main effect of Habituation	$F(1, 60)=7.930$	$p=0.007$
	Main effect of Test	$F(4, 60)=2.065$	$p=0.097$
	Habituation x Test interaction	$F(4, 60)=5.588$	$p=0.001$
egr-1	Main effect of Habituation	$F(1, 60)=15.969$	$p=0.0001$
	Main effect of Test	$F(4, 60)=2.124$	$p=0.089$
	Habituation x Test interaction	$F(4, 60)=6.144$	$p=0.0001$

Supplementary Table 6: Analyses of variance (ANOVA) for *c-fos* and for *egr-1* for the Cerebellum, with habituation (habituation with 3 dots, habituation with 9 dots) and type of test (familiar, number, shape, surface area increase, surface area decrease) as between-subject factors.

CEREBELLUM			
c-fos	Main effect of Habituation	$F(1, 60)=8.713$	$p=0.005$
	Main effect of Test	$F(4, 60)=1.025$	$p=0.402$
	Habituation x Test interaction	$F(4, 60)=4.037$	$p=0.006$
egr-1	Main effect of Habituation	$F(1, 60)=3.405$	$p=0.070$
	Main effect of Test	$F(4, 60)=3.616$	$p=0.010$
	Habituation x Test interaction	$F(4, 60)=1.726$	$p=0.156$

Supplementary Table 7: Analyses of variance (ANOVA) for *c-fos* and for *egr-1* for the Medulla Oblongata, with habituation (habituation with 3 dots, habituation with 9 dots) and type of test (familiar, number, shape, surface area increase, surface area decrease) as between-subject factors.

MEDULLA OBLONGATA			
c-fos	Main effect of Habituation	F(1, 60)=0.090	p=0.765
	Main effect of Test	F(4, 60)=3.956	p=0.006
	Habituation x Test interaction	F(4, 60)=10.348	p=0.0001
egr-1	Main effect of Habituation	F(1, 60)=5.657	p=0.021
	Main effect of Test	F(4, 60)=0.561	p=0.692
	Habituation x Test interaction	F(4, 60)=2.440	p=0.056