

Supplementary Materials: Exploring Neurobehaviour in Zebrafish Embryos as a Screening Model for Addictiveness of Substances

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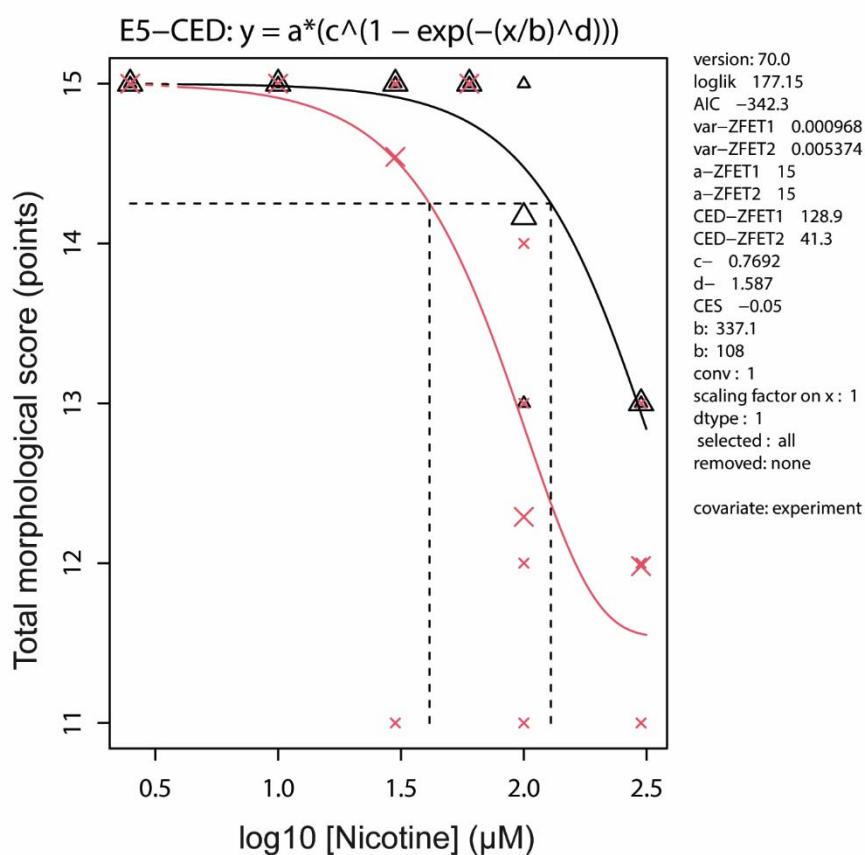


Figure S1. ZFET. A combined analysis of two independent ZFET experiments. Large symbols represent the geometric mean of single embryo data (small symbols). Horizontal dashed line represents the -5% (0.05) effect level (CES), the intersection with the fitted curve determines the CED.

Table S1. CED's and confidence bounds at CES = -0.05 (-5%).

	CED ₀₅	lower bound of 90%CI	upper bound of 90%CI
ZFET1	128.9	87.6	162
ZFET2	41.3	22.6	54.7
average	85.1		

A comparison was conducted between the full 10-minutes dark-1 block and its middle 6-minutes section of the data underlying Figure 4 (main text), i.e. fixed pre-treatment

followed by acute nicotine in a dose-range. This comparison shows that exp20201014 separates from the other two at the 10-minutes analysis, whereas there is a more gradual overlap at the 6-minutes analysis (Figure S5). Also, the ratio of the highest BMDU and the lowest BMDL of all three experiments is smaller in the 6-minutes analysis as compared to the 10-minutes analysis (Table S1), indicating a lower data variation in the former set. This analysis supports preferred use of the middle 6-minutes section.

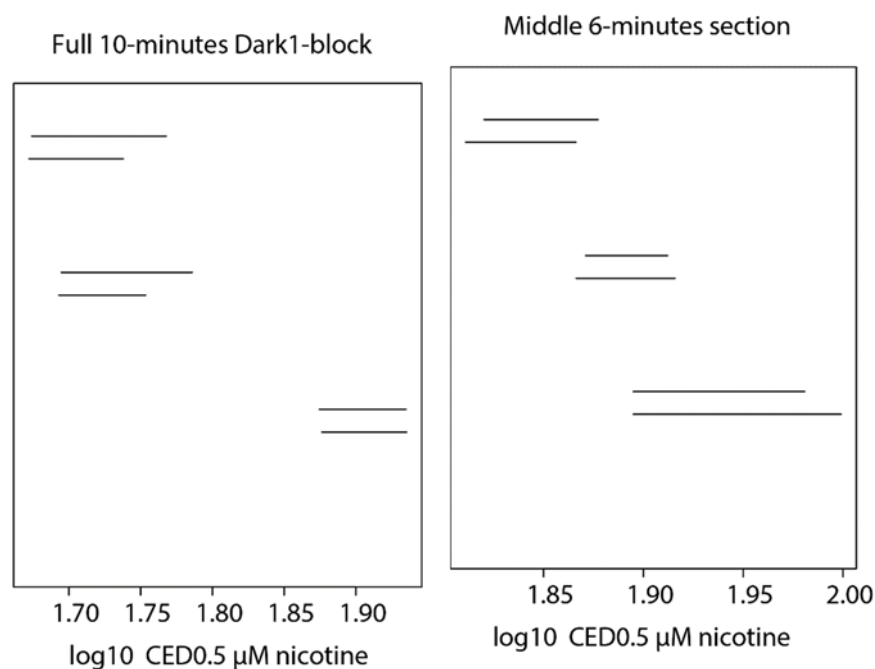


Figure S2. Confidence intervals of three experiments (20201014, 20201110, 20201114, from bottom to top), derived at a 0.5 critical effect size (CES) in a data set of the full first dark 10-minutes block (left) and its middle 6-minutes section. Each pair of bars represents the analysis with exponential (upper) and Hill (lower) models.

Table S2. BMD confidence bounds at CES = −0.5 (−50%).

experiment	full 10-minutes Dark-1 block		middle 6-minutes section	
	BMDL.lowest	BMDU.highest	BMDL.lowest	BMDU.highest
1 CED-20201014	74.9	86.2	73.5	82.4
2 CED-20201110	49.3	61.1	64.7	75.4
3 CED-20201117	47	58.6	78.5	99.8
highest ratio U/L: 86.2/47 = 1.83		highest ratio U/L: 99.8/64.7 = 1.54		

BMDL, BMDU, lower and upper bounds of the 90% confidence interval; BMDL and BMDU were derived from a combined analysis with exponential and Hill models.

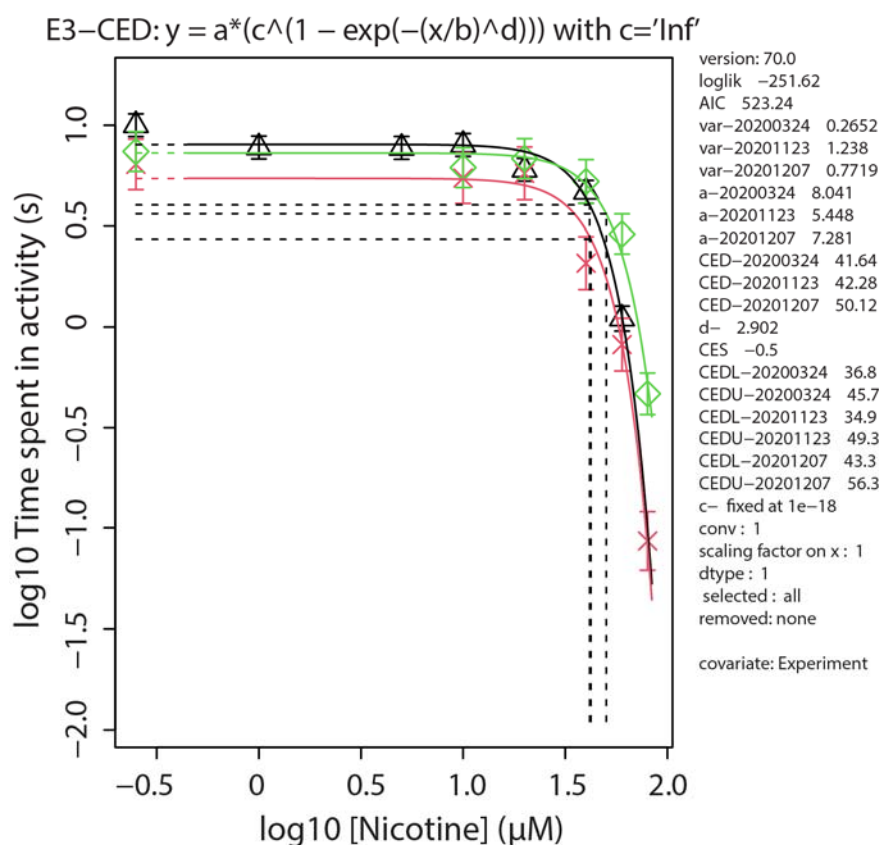


Figure S3. Concentration-response analysis showing fitted concentration-response curves for the effect of exposure to nicotine on locomotion. Different colors of curves and symbols represent independent experiments. Symbols represent the geometric mean of $n = 12$ single embryo data; error bars are 90% confidence intervals. CEDs were measured at the 5% effect level (CES). Concentration-response data was fitted using the E3-CED model [equation: $y = a \cdot (c^{(1 - \exp(-(x/b)^d)))}$ with $c = \text{Inf}$].

Output variables:

Loglik: log likelihood

var: within group variance

a: background response according to the fitted model

d: steepness of the fit

c: maximum response

conv: did the fit algorithm converge? Yes if 1, no if 0.

CED - x: Critical effect dose - experiment identifier

CES: Critical effect size

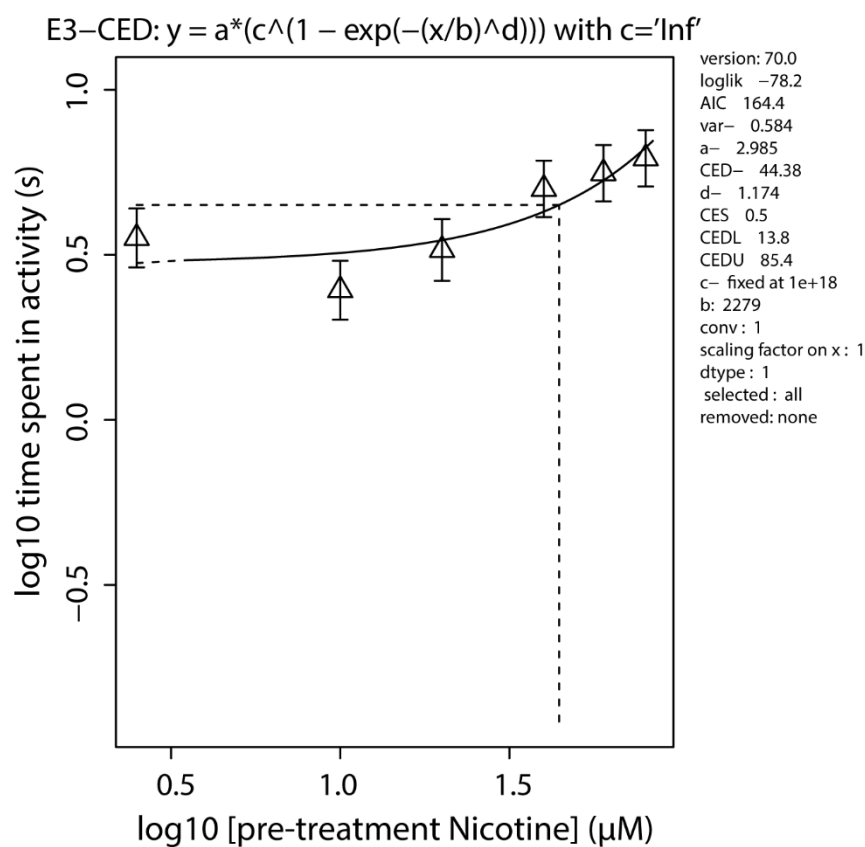


Figure S4. The effect of a concentration-range nicotine pre-treatment on the effectivity of acute exposure to nicotine. The effect of a concentration-range nicotine pre-treatment (96–104hpf) on the effectivity of acute exposure to nicotine (40 μM , 118–120hpf). The graph indicates that with increasing concentration pre-treatment (x -axis), the level of activity increases demonstrating the decreased potency of acute exposure to nicotine. Symbols represent the geometric mean of $n = 12$ single embryo data; error bars are 90% confidence intervals. CED (intersection dashed lines) is measured at the 5% effect level (CES). Locomotion was analysed directly after acute exposure. Data was fitted using the E5-CED model following the equation: $y = a \cdot (c^{1 - \exp(-(x/b)^d)})$.

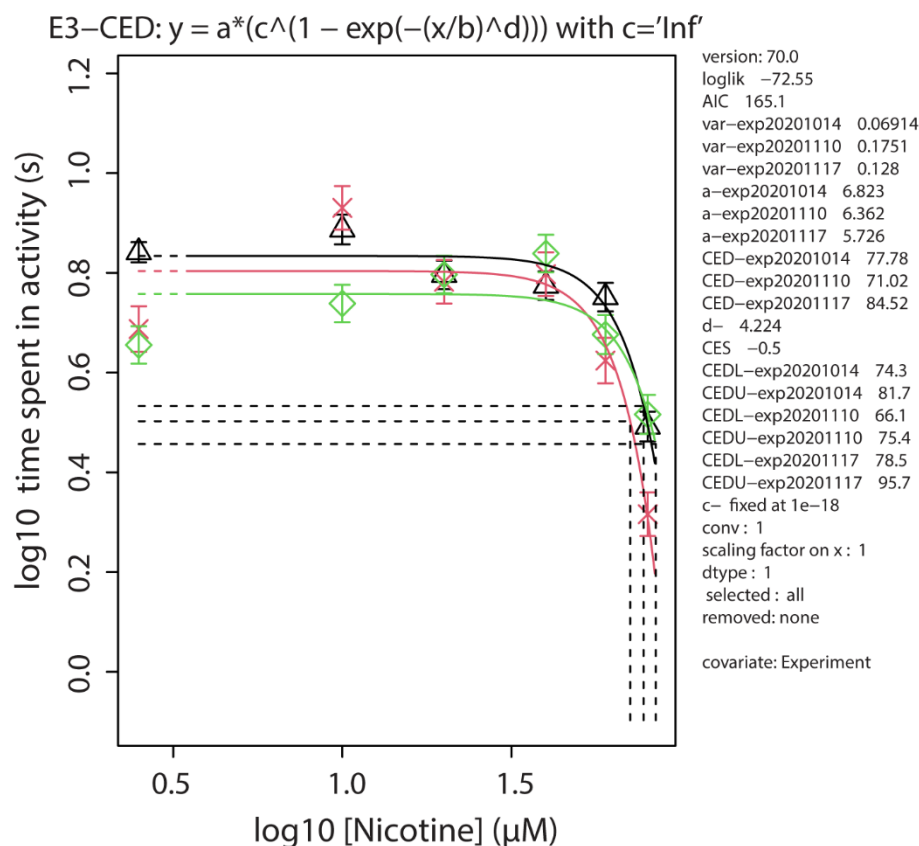


Figure S5. Nicotine pre-treatment reduces the inhibitory effect of acute nicotine exposure on locomotion. Combined analysis of acute exposure (118–120hpf) concentration-response data from three independent experiments following pre-treatment (96–104hpf) to a single concentration. Proast analysis of the data results in a single fit, indicating that there are no statistically significant differences between the individual data sets. Symbols represent the geometric mean of $n = 12$ single embryo data; error bars are 90% confidence intervals. Data was fitted using the E3-CED model [equation: $y = a \cdot (c^{1 - \exp(-(x/b)^d)})$ with $c = \text{Inf}$].

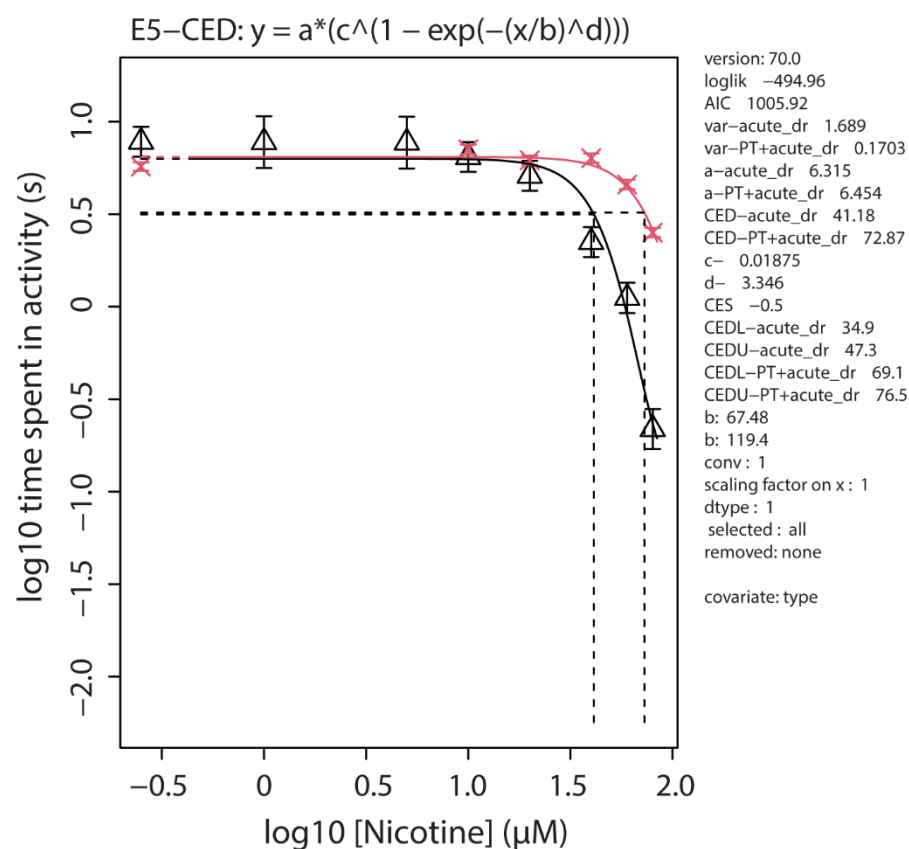


Figure S6. Combined analysis of concentration-response data upon acute exposure to nicotine without and with pre-treatment. Black, acute exposure to nicotine without pre-treatment; red, same with pre-treatment (30 μM ; 96–104hpf). Symbols represent the geometric mean of $n = 12$ single embryo data; error bars are 90% confidence intervals. CED is measured at the 5% effect level (CES). Data was fitted using the E3-CED model [equation: $y = a \cdot (c^{1 - \exp(-(x/b)^d)})$] with $c = \text{Inf}$].