

Supporting Information

Combining Neurobehavioral Analysis and *In Vivo* Photoaffinity Labeling to Understand Protein Targets of Methamphetamine in Casper Zebrafish

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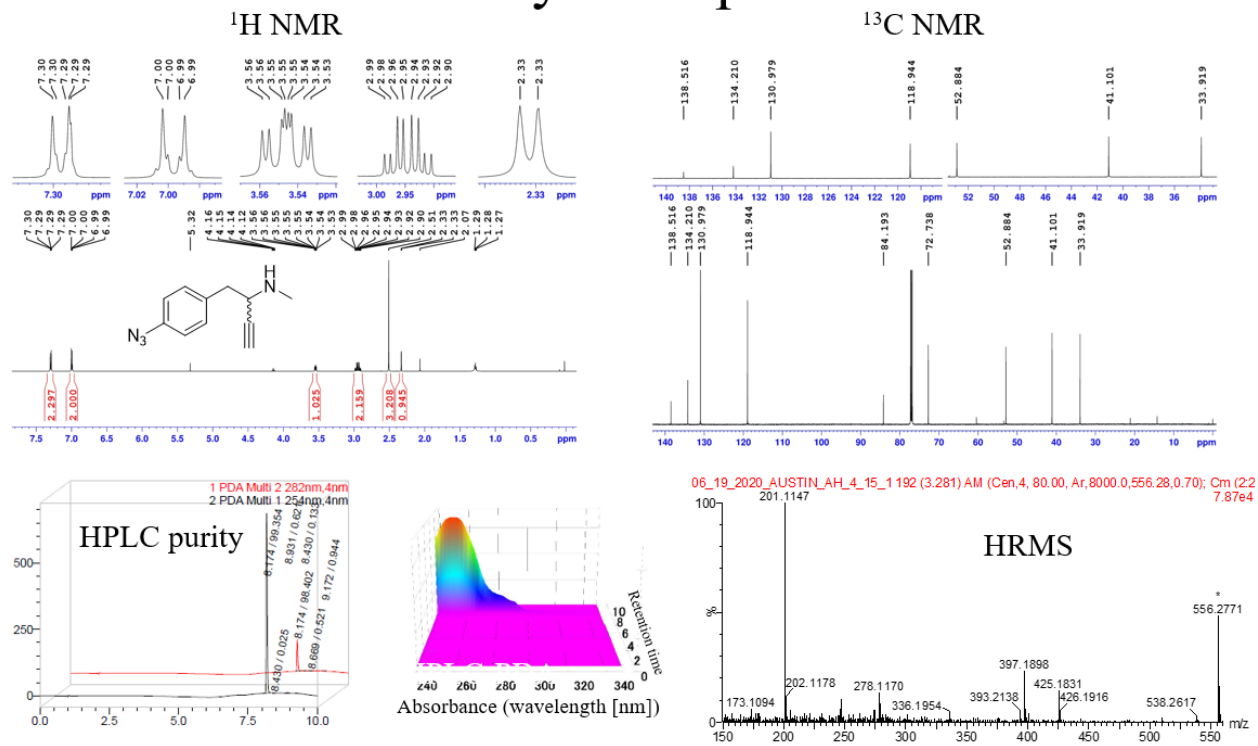
Supplement Figure S7: Result from Psychoactive Drug Screening Program

Supplement Table S1: Sex of each zebrafish cohort

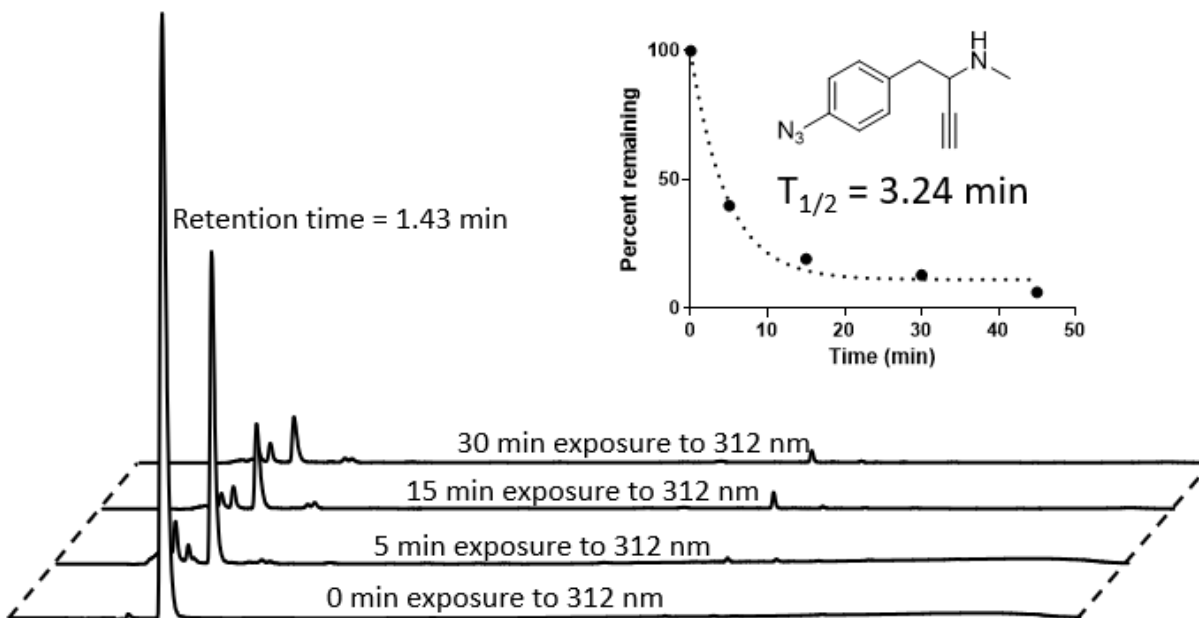
Provided as other attachments (not found in this document):

Supplement Table S2: Proteomics identified the selective pulldown

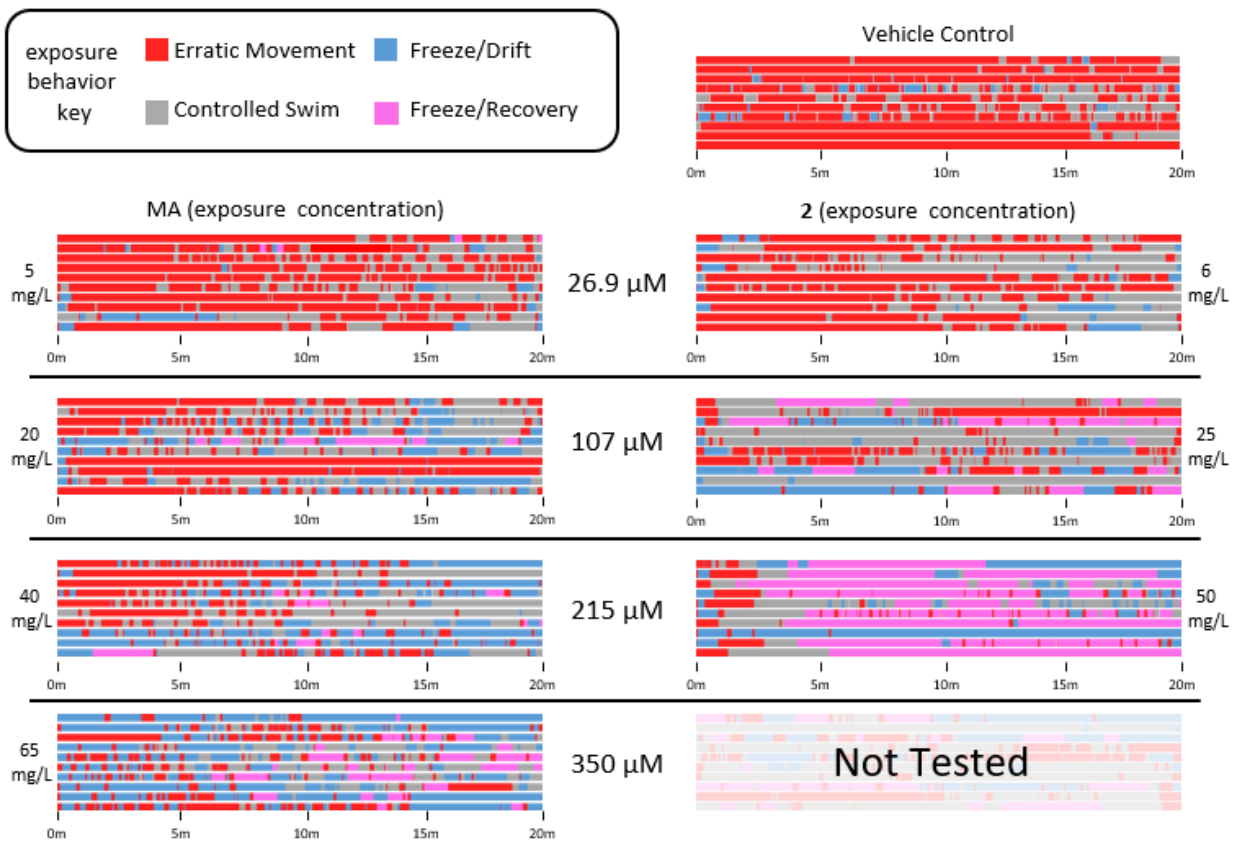
Analysis Report



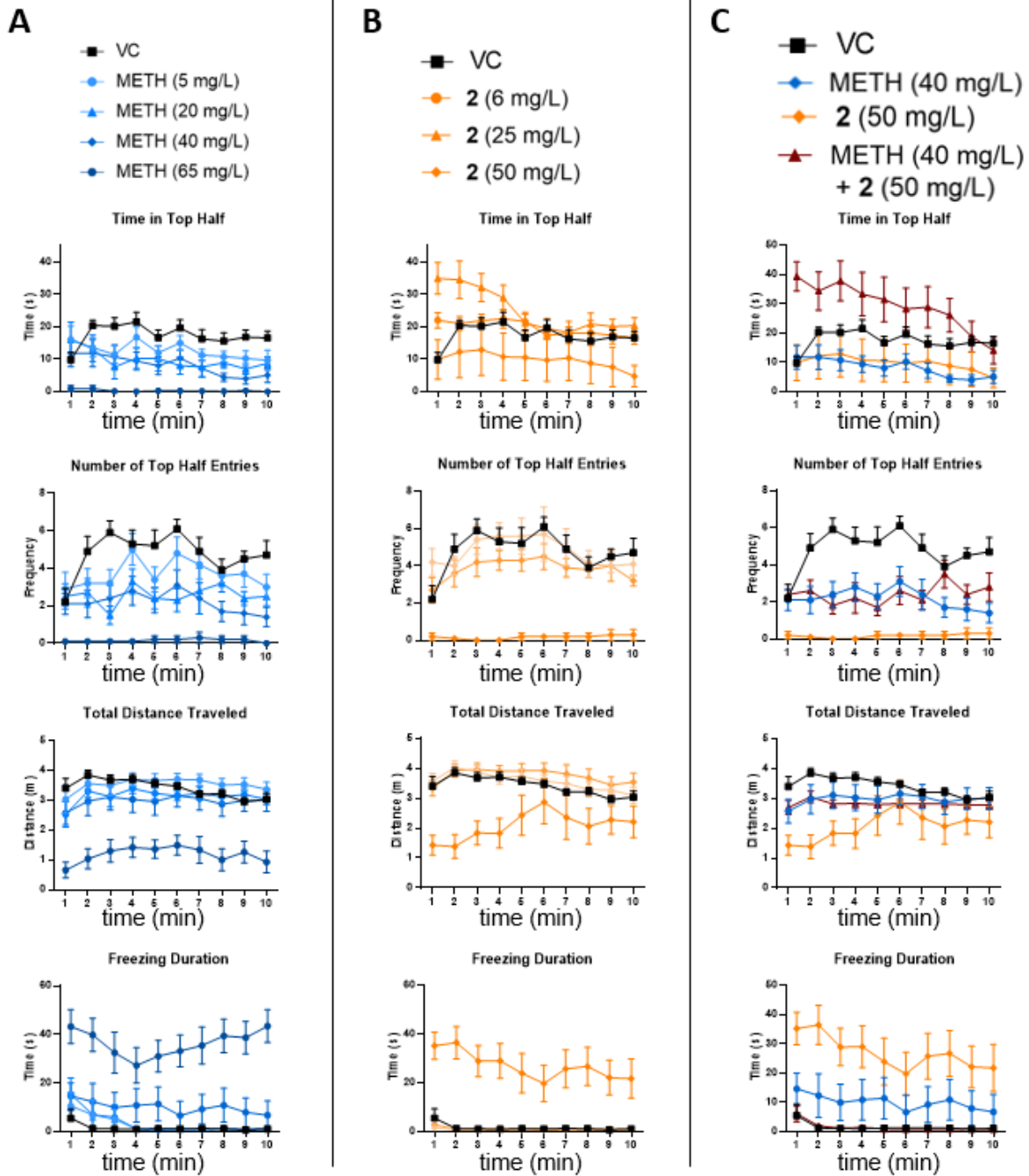
Supplement Figure S1: Characterization data for the phenethylamine probe, **2**.



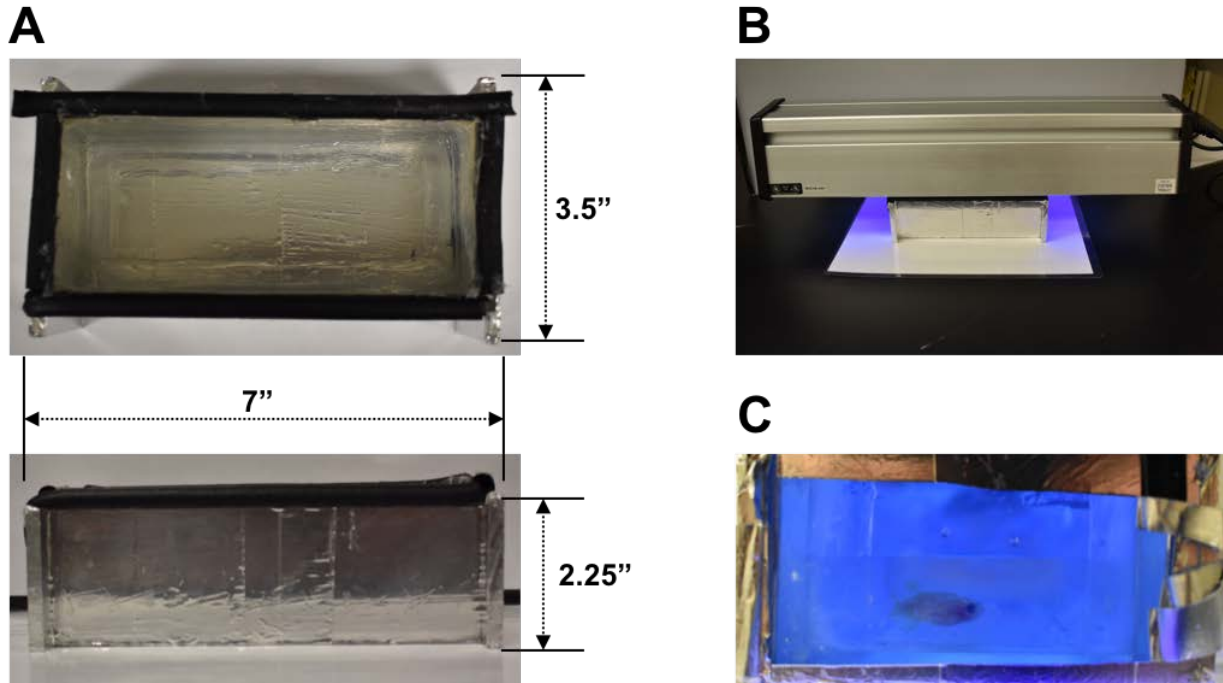
Supplemental Figure S2. Chromatograms of photolytic breakdown of **2** activated upon irradiation with 312 nm. Percent remaining was based on area under the curve (AUC) of UV absorption at 254 nm. Inset shows plot of changes in AUC with time and dotted line represents one-phase decay line of best fit ($r^2 = 0.9916$); $t_{1/2} = 3.24$ min. Less than 10 % breakdown was observed after 30 min with 365 nm light (not shown).



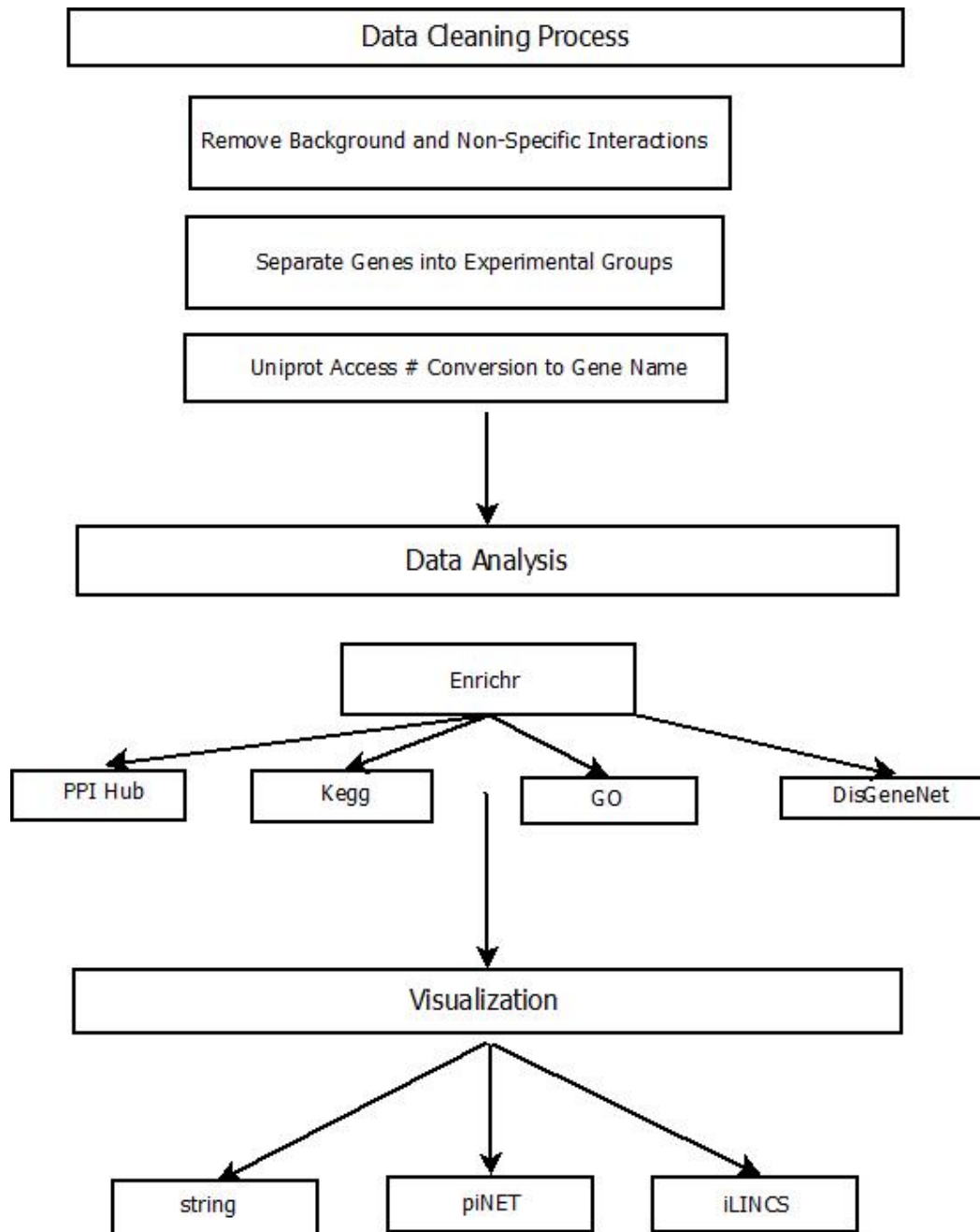
Supplemental Figure S3. Exposure Behavior Time Course. Each line indicates swimming behavior of an individual fish over the 20 min exposure period based on the color key shown above.



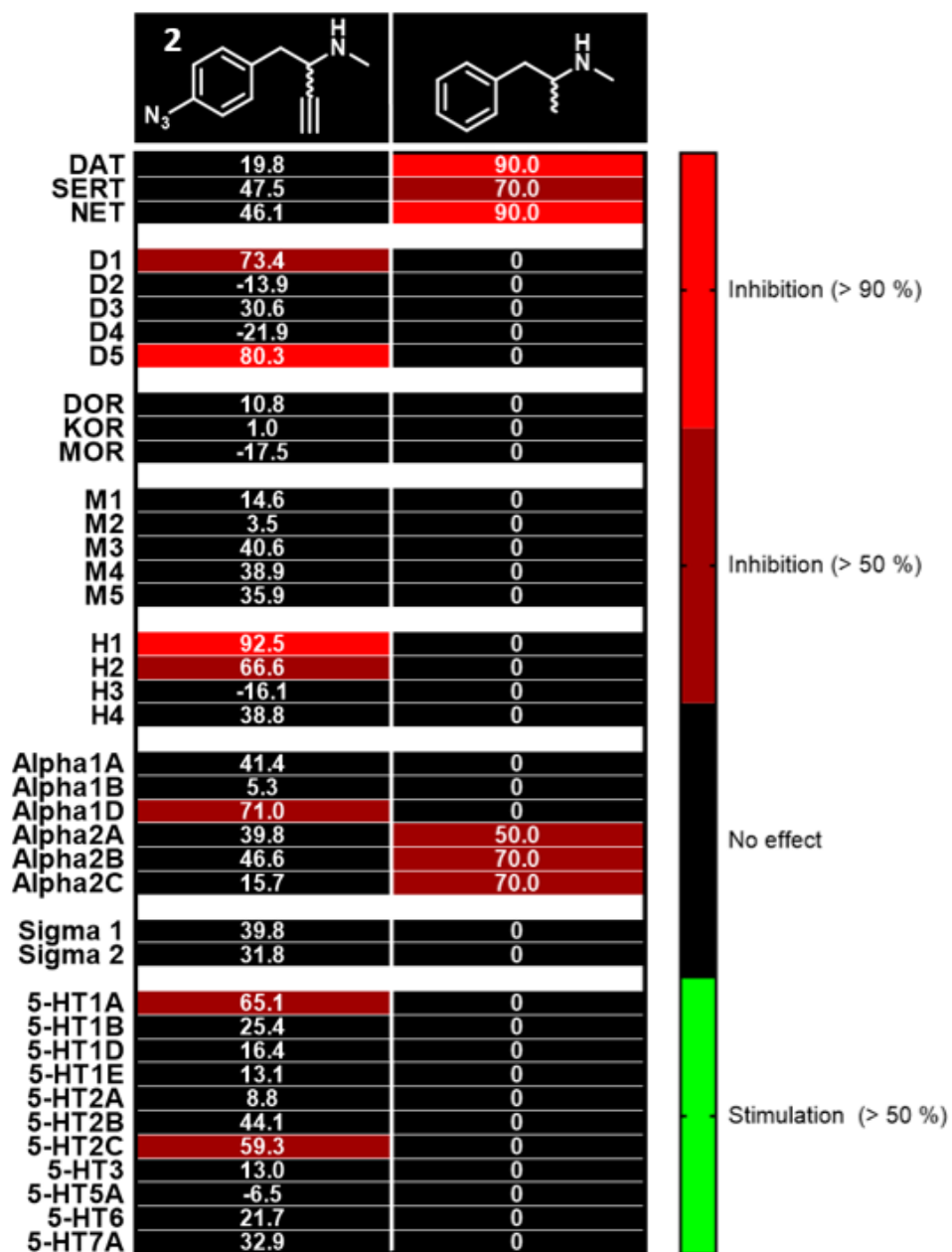
Supplemental Figure S4. Time course for specific behavioral readouts related to swimming in the novel tank test. Each data point represents preceding minute of activity for adult Casperfish treated with indicated concentrations of Meth (A), 2 (B), or co-incubation experiments. All data represented as mean \pm s.e.m. (N = 8-10 per cohort [randomly selected / sex balanced]).



Supplemental Figure S5. A) Top and side view of custom-built irradiation chamber (3.5”W x 7”L x 2.25”H, acrylic). B) The top of the chamber is lined with weather stripping to prevent damage to the UV-lamps (λ -312 nm) and the exterior covered with foil to maximize exposure to irradiation from all angles. C). Zebrafish are irradiated with UV-light for 10 min to induce covalent attachment binding partners within the brain. No changes in tank water temperatures were observed.



Supplemental Figure S6. Bioinformatic workflow.



Supplemental Figure S7. The probe (2) was screened at (10 μ M) for ability to displace binding of orthosteric radioligands for cloned human receptors. Compounds exhibiting >50% inhibition are designated as ‘active’ and flagged for follow-up K_i determination in secondary binding experiments. Results for methamphetamine are provided for comparison (approximations based on published PDSP K_i 's).

Treatment	Conc.	Subject_ID	Sex	Total	
VC	VC	1,2,3,4,5,7	Male	6	
		6,8,9,10	Female	4	
MA	5 mg/L	2,3,4,7,8	Male	5	
		1,5,6,9,10	Female	5	
	20 mg/L	1,2,3,4,10	Male	5	
		5,6,7,8,9	Female	5	
	40 mg/L	1,3,4,6,8	Male	5	
		2,5,7,9,10	Female	5	
	65 mg/L	5,10	Male	2	
		1,2,3,4,6,7,8,9	Female	8	
	2	6 mg/L	3,5,6,7,8	Male	5
			1,2,4,9,10	Female	5
25 mg/L		1,2,3,6,8	Male	5	
		4,5,7,9,10	Female	5	
50 mg/L		2,4,6,9	Male	4	
		1,3,5,7,8,10	Female	6	
MA + 2	40, 50 mg/L	1,2,4,5,6,8,9	Male	7	
		3,7,10	Female	3	

Supplemental Table 1. Catalog of sex composition of all treatments.