Supplementary Methods (Hardaway and Wang *et al.*)

C. elegans **Strains**

The *dat-1(ok157)III* strain was obtained from J. Duerr and J. Rand (Oklahoma Medical Research Foundation, Oklahoma City), and is a complete loss of function mutation that eliminates the majority of the DAT-1 coding sequence. Rescue of this deletion via 6-hydroxydopamine toxicity and for swimming behavior was performed in McDonald *et al.* and Hardaway and Hardie *et al* respectively[1,2]. A strain producing a loss of function disruption of DOP-3 *(dop-3(vs106)X)* was obtained from the Caenorhabditis Genetics Center, which is funded by the NIH Office of Research Infrastructure Programs (P40 OD010440). The specific impact of this deletion on *dop-3* signaling was demonstrated by rescue of exogenous DA-induced paralysis by introduction of a wild type *dop-3* fragment in Chase *et al*[3]. We obtained *cat-2(tm2261)* from Shohei Mitani at the National Bioresource Project at Tokyo Women's Medical University and backcrossed it three times to the N2 strain before use. The *cat-2(tm2261)* deletion exhibits altered 2-nonanone avoidance, which was rescued by introduction of a wild type *cat-2* transgene[4], and exhibits significantly reduced tissue DA levels relative to the N2 strain[2].

Assessment of Swip Behavior

In both batch and automated analyses, we generated synchronous populations of these strains by hypochlorite treatment and harvesting arrested L1 animals. L1s were plated at a moderate density on fresh NGM/OP50 plates and incubated at temperatures ranging from 12 to 20 degrees. We observe no differences in Swip for N2 and *dat-1* animals reared between these temperatures. On test days, middle stage L4 animals were identified by characteristic morphological features and used for behavior as N2 animals show some stochastic Swip and quiescence bouts prior to and during the last larval molt. At this stage, the crescent structure should be fully formed and visible and the worms should be actively crawling. Young adults should not be used for Swip as we observe a reduction in penetrance following the L4 stage. We placed worm test plates at room temperature for 15 min to acclimate to room temperature (22°C) before testing. A stock of IMI (Sigma – Cat No. I7379) was dissolved in water to 100 mM on test days and serially diluted in water to the desired concentrations. For batch analyses, 100 µL of water or drug were dispensed in multiple wells of a Pyrex Spot Plate (Fisher catalog number 13-748B) and 10-15 L4 animals were gently transferred using an eyelash pick. After 10 min., we recorded the number of paralyzed worms/total worms. Two independent observers performed assays over several weeks using multiple worm preparations, blind to genotype or drug manipulation. For automated video analysis, single L4 hermaphrodites were placed in 20 µL of water or drug in a single well of a Pyrex Spot Plate, and 10 min. movies (uncompressed AVI format) of their swimming behavior were captured as described in the

supplementary materials. Every worm line was recorded sequentially and in parallel over several days to ensure that strains were identical in age.

Tracker2.0 Validation
Following the analysis of individual tracker files generated by Tracker2.0, we compared the individual worm frequency plots against the raw video to validate the fidelity of tracking. Short bouts of paralysis or slow movement provided convenient frequency markers for this process. In cases of bad tracking, the text files were omitted from further analysis or the raw video was retracked using Pause/Reseed to ensure proper placement of the spine.

SwimR Tools
We used validated tracker files (see above) to generate a frequency matrix using SwimR. This matrix was analyzed using the SwimR command (see SwimR manual) according to the default parameters except for outlier analysis. We used a high MAD score (15.4667) for each group that we analyzed, which prevented SwimR from excluding any outliers from subsequent analysis. We reasoned that drug application would produce bimodal effects and that exclusion based on the median of a sample group would exclude genuine drug responsive animals. Therefore all genotypes, including no drug, were analyzed in this way.

Statistical Analysis
SwimR generates several output images and text files of the raw data used to generate them (see Supplementary Materials). Raw data from SwimR was input into Prism 6.0a (Graphpad, La Jolla,CA) and subsequent analyses performed as described in the text or figure legends. IMI dose response curves in Figure 1B were generated by nonlinear regression using a fixed slope and normalized response condition. Values for *dat-1* are connected by a simple line and do not represent a non-linear regression.

References

1. Mcdonald, P. W. *et al.* Vigorous motor activity in Caenorhabditis elegans requires efficient clearance of dopamine mediated by synaptic localization of the dopamine transporter DAT-1. *Journal of Neuroscience* **27,** 14216–14227 (2007).
2. Hardaway, J. A. *et al.* Forward Genetic Analysis to Identify Determinants of Dopamine Signaling in Caenorhabditis elegans Using Swimming-Induced Paralysis. *G3: Genes| Genomes| …* (2012).
3. Chase, D. L., Pepper, J. S. & Koelle, M. R. Mechanism of extrasynaptic dopamine signaling in Caenorhabditis elegans. *Nat Neurosci* **7,** 1096–1103 (2004).
4. Kimura, K. D., Fujita, K. & Katsura, I. Enhancement of Odor Avoidance Regulated by Dopamine Signaling in Caenorhabditis elegans. *Journal of*

Neuroscience **30,** 16365–16375 (2010).