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SUPPORTING INFORMATION

The coaction of tonic and phasic dopamine dynamics

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Materials and Methods

Chemicals. Dopamine hydrochloride, DOPAC, trizma hydrochloride, calcium chloride, magnesium chloride, α-methyl-DL-tyrosine ester hydrochloride (AMPT), and pargyline hydrochloride were purchased from Sigma Aldrich (St. Louis, MO). GBR-12909 dihydrochloride was purchased from Tocris Bioscience (Bristol, UK). Sodium chloride and sodium bicarbonate were purchased from EMD (Gibbstown, NJ). Sodium phosphate and ascorbic acid were purchased from Mallinckrodt (Phillipsburg, NJ). Liquion™ (LQ-1105, 5% by weight Nafion®) was purchased from Ion Power Solutions (New Castle, DE). All neurotransmitters were weighed and dissolved in 0.1 N $HClO_4$ to make 1.0 mM stock solutions. Immediately prior to experiments, the stock solutions were diluted to the desired concentration in pH = 7.4 artificial cerebral-spinal fluid (15 mM Tris, 126 mM NaCl, 2.5 mM KCl, 25 mM $NaHCO_3$, 2.0 mM NaH_2PO_4, 1.2 mM Na_2SO_4, 1.2 mM $CaCl_2$, and 2.0 mM $MgCl_2$).

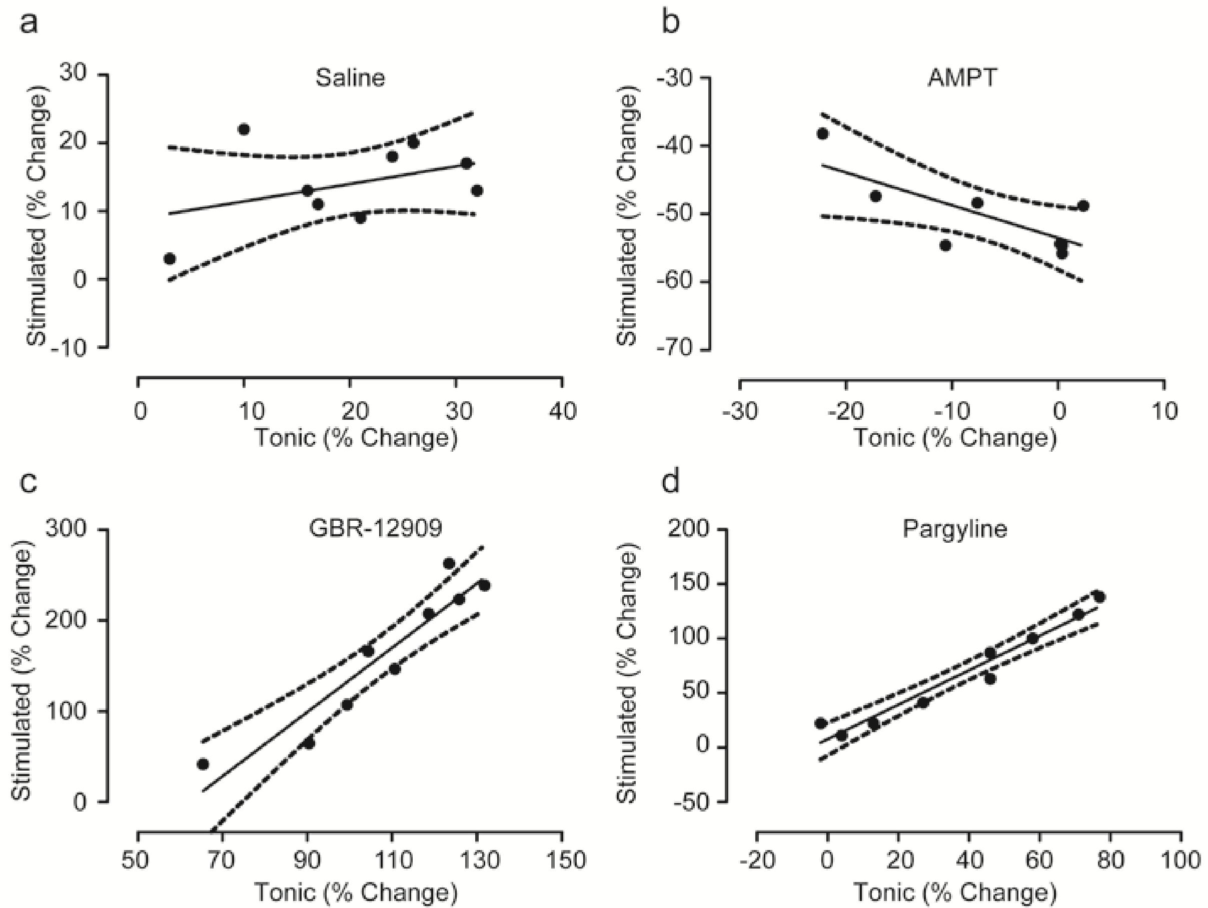
Electrode fabrication. Cylindrical carbon-fiber microelectrodes were prepared according to Cahill and colleagues.[1] In summary, a T-650 carbon fiber (7 µm diameter, Cytec Thornel, Woodland Park, NJ) was aspirated into a 0.68 mm internal diameter glass capillary (A-M Systems, Inc., Sequim, WA). Capillaries were subsequently heated and pulled to a fine seal using a type PE-2 pipette puller (Narishige, Japan). Electrodes were then cut to 50 µm in length and coated with Nafion by electrodeposition at +1.0 V for 30 seconds as previously described.[2] A Ag/AgCl reference electrode was prepared by soaking a silver wire (0.25 mm, Alfa Aesar) in chlorine bleach overnight (Food City, Mesa, AZ).

FSCAV data collection. FSCAV was performed as previously described.[3] Briefly, data was collected using WCCV 2.0, which is custom hardware and software developed by Knowmad Technologies, LLC. (Tucson Az) The voltammetric waveform was generated and the data was acquired using a PCIe-6341 DAC/ADC Card (National Instruments, Austin TX). A CMOS precision analog switch, ADG419 (Analog Devices) was used to control the application of the

computer-generated waveform to the electrode. The logic was controlled using the PCIe-6341 National Instruments interface card and in-house software. This switch was used to apply either the triangle waveform (-0.4 V to 1.3 V, scan rate = 1200 V/s) or a constant potential (-0.4 V) to the electrode. Statistical analysis was performed using Prism 5 (Graph Pad, La Jolla, CA). Data collected from FSCAV measurements was fit using a previously developed model (Comsol Multiphysics 4.0, Los Angeles CA).[3,5]

***In vivo* measurements.** Mouse surgery and handling were in compliance with Wayne State University's Guide for the Care and Use of Laboratory Animals, approved by the Institutional Animal Care and Use Committees (IACUC). Male mice (C57BL/6J, 20 – 25 g; Charles River Laboratories, Wilmington, MA) were anesthetized with 25% w/w urethane in saline solution (0.7 mL per 100 g mouse weight) and mounted in a stereotaxic frame (Kopf Instruments, Tujunga, CA). Holes were drilled in the skull to allow access to the nucleus accumbens for microelectrode placement (stereotaxic coordinates from bregma: 1.1 mm anterior; 1.3 mm lateral; 4.2 - 4.7 mm ventral) and to allow access to the medial forebrain bundle (MFB) for stimulator placement (stereotaxic coordinates from bregma: 1.06 mm posterior; 1.25 mm lateral; 5.0 mm ventral). Stereotaxic coordinates were referenced from Paxinos and Frankli.[6] Body temperature was maintained at 37 °C with a constant temperature heating pad (Braintree, Braintree, MA). A bipolar stimulating electrode (Plastics One, Wallingford, CT) provided constant-current, stimulation pulses to the MFB. To avoid electrical crosstalk, the stimulation did not occur during individual cyclic voltammograms, but during the rest period between them. The stimulus was optically isolated from the signal generation apparatus (NeuroLog System, Hertfordshire, England). A 40 pulse, 60 Hz biphasic (±350 μA, 2 ms per phase) stimulation was used for all experiments. Pargyline (75 mg kg^{-1} *i.p.*),[7] GBR 12909 (10 mg kg^{-1} *i.p.*),[8] and AMPT (250 mg kg^{-1} *i.p.*)[9] were dissolved in 0.9% sodium chloride Hospira (Lake Forest, IL) and injected into the peritoneal cavity at a volume of 0.1 mL 20 g^{-1} body weight.

Correlation of phasic and tonic dopamine concentration changes



S1: Correlation of phasic and tonic response. **a**) Saline, **b**) AMPT, **c**) GBR-12909, and **d**) Pargyline. The percent change in phasic response is plotted on the ordinate, and the percent change in tonic concentrations is plotted on the abscissa. Saline (R^2 = 0.17, p = 0.27), AMPT (R^2 = 0.56, p = 0.032), GBR-1290 (R^2 = 0.88, p =0.0002), and pargyline (R^2 = 0.95, p <0.0001). Dashed lines indicated 95 % confidence interval.

Table 1: Sensitivity for dopamine, DOPAC, and ascorbic acid.

Charge (pC)			Sensitivity (pC/μM)		
200 nM DA	20 μM DOPAC	225 μM AA	DA	DOPAC	AA
5 ± 1	1.6 ± 0.4	0.4 ± 0.4	23 ± 5	0.08 ± 0.02	0.002 ± 0.002

The limits of integration were determined for dopamine, and then voltammograms from DOPAC and ascorbic acid were analyzed with the same limits. The resultant charge was normalized for concentration giving a sensitivity in pC/μM ± SEM, n = 3 electrodes.

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