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Abstract

Repeated self-administration of cocaine is associated with impairments in motivated behaviors as well as alterations in both dopamine (DA) release and neural signaling within the nucleus accumbens (NAc). These impairments are present even after several weeks of abstinence from drug taking, suggesting that the self-administration experience induces long-lasting neuroplastic alterations in the mesolimbic DA circuit. To understand these changes at the terminal level, rats were allowed to self-administer either cocaine intravenously (~1 mg/kg per infusion; Cocaine) or water to a receptacle (Control) in 2-hour sessions over 14 days, followed by 30 days of enforced abstinence. Fast-scan cyclic voltammetry was then used to record real-time DA release in either the NAc core or shell following electrical stimulations of the ventral tegmental area (VTA) in freely-moving animals. In Controls, the kinetics of DA release in the core and shell strikingly differed, with shell displaying slower release and reuptake rates than core. However, cocaine experience differentially altered these signaling patterns by NAc subregion. In the shell, Cocaine rats showed less sensitivity to the dynamic range of applied stimulations than Controls. In the core, by contrast, Cocaine rats displayed robustly reduced peak DA release given the same stimulation, while also showing slower release and reuptake kinetics. The differential effects of cocaine self-administration on terminal function between core and shell is consistent with a region-specific functional reorganization of the mesolimbic DA system following repeated, and may provide an anatomical substrate for altered cognitive function following chronic drug-taking and addiction.

71 **Significance Statement**

72 Chronic drug use alters neural signaling (particularly dopamine), even after extended periods of
73 drug abstinence. Evidence suggests that dopamine terminals may be persistently altered in
74 cocaine-experienced animals, (i.e., influencing the rates and amount of dopamine release and
75 reuptake) but it is not known whether this is a general property of the dopamine system, or if
76 instead, changes are unique within different terminal regions. Voltammetric recordings in the
77 nucleus accumbens core and shell in cocaine-experienced rats revealed region-specific
78 differences in release/reuptake kinetics relative to controls. Strikingly, while drug-naïve subjects
79 showed consistent differences in dopamine kinetics between core and shell, cocaine remodeled
80 the entire accumbens to become more "shell-like". Understanding this remodeling will be critical
81 for developing treatments to prevent drug relapse.

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Introduction

Phasic dopamine (DA) signaling in the nucleus accumbens (NAc) is implicated in learning, motivation, reward encoding and drug taking (Schultz et al., 1997, Berridge and Robinson, 1998, Berridge, 2012, Berridge and Kringelbach, 2015, Saddoris et al., 2015a). Evidence suggests DA signaling acts to modulate activity of NAc neurons by permitting plasticity for task-relevant stimuli. For example, in NAc, phasic patterns of neural activity arise only in regions where phasic DA signals are also present (Cheer et al., 2005, Cheer et al., 2007, Owesson-White et al., 2009), while blockade of the DA signal via AP-5 in the ventral tegmental area (VTA) abolishes phasic excitatory encoding in NAc neurons (Cacciapaglia et al., 2011).

Growing evidence suggests that cocaine use differentially acts on the DA system in the NAc. For example, rats willingly self-administer cocaine into the NAc shell but not core (Rodd-Henricks et al., 2002, Ikemoto, 2003). Behaviorally, while normal DA signaling encodes information about task-relevant stimuli, animals with a history of cocaine self-administration display abnormal phasic DA release patterns even following several weeks of drug abstinence that strikingly differ between core and shell (Saddoris et al., 2016b). Thus, because both acute and chronic actions of repeated cocaine experience differentially alter DA release dynamics and related associative neural encoding within neuroanatomically-distinct terminal regions (Saddoris and Carelli, 2014), it is essential to understand how drug experience may uniquely alter DA signaling in core and shell.

However, it can be difficult to determine whether altered phasic DA signaling is due to changes in either (1) the ability for DA neurons to appropriately encode task-relevant information (i.e., disruptions of limbic inputs to the VTA), (2) the ability for DA neurons to appropriately release DA (i.e., disruptions of output of VTA neuron terminals within the NAc),

or (3) some combination of the two. While we and others (Willuhn et al., 2014) have shown that cocaine alters phasic DA signaling during behavior, studies from Jones and colleagues has indicated that DA terminal function is significantly altered as well (Jones et al., 1996, Mateo et al., 2005, Yorgason et al., 2011, Calipari et al., 2014, Siciliano et al., 2015). However, in these studies, DA kinetics were often examined in *ex vivo* brain slice preparations (e.g., Ferris et al., 2013) which may differ from how these systems may operate in awake and behaving animals. Further, while few of these experiments have examined how cocaine exerts long-term effects following prolonged drug abstinence (Cameron et al., 2016, Siciliano et al., 2016), none have investigated whether the extended withdrawal from drug taking differentially affects DA signaling in core and shell.

To isolate the question of terminal function, I implanted electrical stimulation probes into the VTA of freely-moving 30-day abstinent rats with either a history of cocaine self-administration or drug-naïve controls and voltammetrically assessed the real-time kinetics of the phasic DA signal in the NAc following variations of applied stimulation frequencies and durations. Critically, voltammetry recordings were taken from both core and shell, allowing for isolation of the effects of cocaine experience on terminal function in these regions. While DA release kinetics were changed in both core and shell following cocaine self-administration experience, core kinetics were altered in a manner that resembled the shell in drug-naïve rats across several metrics. Thus, cocaine experience appears to differentially augment DA terminal function between core and shell that persists long after the cessation of drug taking.

Methods

130 *Subjects*. Male Sprague-Dawley rats (n = 31) were used and lightly food-deprived to ~90% of
131 their free-feeding weight at the time of recording (Charles River; RRID:
132 http://www.criver.com/products-services/basic-research/find-a-model/sprague-dawley-rat).
133 During all phases of the experiment, single-housed rats were allowed *ad libitum* access to water
134 in their home cages, and maintained on a 12:12 light:dark schedule. Stimulations were obtained
135 from subjects trained in appetitive conditioning experiments. Recordings during the associated
136 behavioral experiments and descriptions of those tests appear elsewhere (Sugam et al., 2012,
137 Saddoris et al., 2015a, Saddoris et al., 2015b). Experiments were performed in accordance with
138 UNC Chapel Hill Institutional Animal Care and Use Committee protocols (12-236, 11-057 and
139 09-240).
140
141 *Behavior*
142 Self-administration. Detailed descriptions of this task appear elsewhere (Saddoris and Carelli,
143 2014, Saddoris et al., 2016b). Briefly, at least one month prior to testing, a subset of rats (n=22)
144 was implanted with intrajugular catheters. Following recovery, rats were randomly assigned to
145 either the intravenous cocaine self-administration group (Cocaine; n=10) or water self-
146 administration group (Control; n=12). Cocaine was provided by the NIDA Drug Supply Program
147 (RRID: Code #9041,
148 https://www.drugabuse.gov/sites/default/files/ndspcat24thedmarch2015.pdf). All self-
149 administration sessions were performed in a standard rat chamber (Context A: 25 x 25 x 30 cm,
150 stainless steel rod floor; MED Associates, St Albans, VT). For the Cocaine subjects (Figure 1A),
151 presses on a lever below an illuminated cue light resulted in an infusion of intravenous cocaine
152 (0.33mg/infusion; ~1mg/kg) coupled with a 20s presentation of a houselight and intermittent

tone, extinguishing of the cue light and retraction of the lever. For the Controls (Figure 1A), presses on the lever under the illuminated cue light resulted in the same stimuli (houselight/tone, lever retraction and cue light extinguish), but the reinforcer was water (250 µl) delivered to a centrally-located foodcup. Controls also received yoked saline infusions based on the self-administration schedule of a Cocaine rat in an adjacent box. Both groups were allowed to press for 2hr per session for 14 sessions. Following this, all rats entered a period of enforced abstinence for 30d by remaining in their home cages in the colony room with ad libitum access to food and water.

Previous training. The group of drug-naïve rats that did not receive jugular catheters (n=9) had been previously trained to perform an instrumental discrimination; the results and descriptions of those experiments appear elsewhere (Sugam et al., 2012, Saddoris et al., 2015b). Briefly, rats in this task learned that presses on one lever resulted in one type of reward option (1 pellet), while presses on the other lever resulted in a different reward option (a larger food reward with either a delay or decreased probability of delivery). There was no effect of previous experience on any measure of DA (Water Control vs Drug Naïve Control, $F_{(1, 281)} = 0.062$, $P=0.80$), and as such both groups were collapsed into a larger Control group for all subsequent analyses (12 Controls plus 9 Drug-Naïve Controls = 21 Controls). Note that for a subset of subjects (n=8 Control; n=3 Cocaine), two recordings were taken in the same animal. Critically, the second recording was at least 300 µm ventral to the first, ensuring that the recording was taken from new tissue.

Fast Scan Cyclic Voltammetric Recordings. FSCV recordings were performed in awake and behaving rats identical to those described previously (Sugam et al., 2012). Briefly, a carbon fiber electrode was acutely lowered into the NAc core or shell using a custom manipulator, then locked in place. An Ag/AgCl-plated reference wire was inserted at the time of recording in the

contralateral hemisphere. Both the electrode and reference were connected to an amplifying headstage (UNC Chemistry, Chapel Hill). Changes in current were detected by applying ramping voltage (from -0.4V to +1.3V and back to -0.4V over 10 ms); this change was detected by software, and chemometrics were used to convert current into DA release concentrations at the recording site using HDCV Analysis (UNC, Chapel Hill). To ensure reliable comparisons between groups on measures of peak and area under the curve, the average baseline concentration prior to the event of interest (pellet delivery, stimulation) was subtracted from the concentration in each bin during the effect period. This ensured that the average baseline for each trial would be set to 0 nM, thereby isolating the absolute change in [DA] as a result of the event. Likewise, this set the cumulative DA release during the baseline to 0 nM, again effectively isolating the absolute change in cumulative [DA] release.

DA release was elicited by electrical stimulation of VTA afferents via the bipolar stimulating probe. These were generated for each subject in the course of developing a training set specific for each electrode and at each recording location (Rodeberg et al., 2015). Bipolar stimulations consisted of a series of pulses (2 ms positive, 2 ms negative for a total pulsewidth of 4 ms per pulse) which varied in both frequency and number. The range of frequencies applied spanned from 12 to 60 Hz, while the number ranged from 1 to 24 pulses. To simplify this range to a single dimension, a Stimulation Index was used, which is the product of frequency X pulse number (e.g., a stimulation delivered at 20 Hz for 10 pulses would result in a Stimulation Index of 200 [i.e., 20 x 10]). Each subject received multiple stimulations that sampled throughout the Stimulation Index range (from 20 to 1440) for an average of 16 ± 6 stimulations per subject.

Determinants of DA release and reuptake kinetics

To understand the kinetics of DA release and reuptake, several metrics were adopted from those described in detail in Yorgason et al (2011). These factors are shown in Figure 1C-E. First, several points were established in the DA release curve (Figure 1C). For each trial, electrical stimulations occurred after a 5 sec baseline period, followed by 10s of a post-stimulation period. Peak DA was the greatest concentration of DA release within 3 sec following stimulation. Other points examined reuptake relative to the peak level. Half-peak was the point in the reuptake that was half of peak concentration, while T20 and T80 were periods that indicated 20% and 80% decay from peak, respectively. Finally, a 95% confidence interval around the 5 sec baseline period was established for each trial, and then computed the first point during reuptake where the [DA] returned this confidence interval following peak.

Based on these points, the latency at which the DA signal reached these points was computed (Figure 1D). Latency to peak was the time elapsed between stimulation and peak. Other factors measured relative to stimulation onset included the latency to half peak (i.e., full width at half-height; FWHH), and the latency to the return to baseline (within 95% confidence interval of baseline). Finally, the rates of change in [DA] between points were computed. These included Release Velocity (i.e., the rate of increase in [DA] between stimulation and peak), Slope (here, the average rate of uptake between T20 and T80) and V_{Max} (here, the maximum rate of uptake as estimated by the rate of change between peak and T20). Note that V_{Max} in this case is not a true measure of maximum reuptake, as this can only truly be computed with Michaelis-Menten equations when the DA transporter (DAT) is saturated. While this may be the case at the very high stimulation levels, we cannot be certain that this is the case for any of our recordings in awake and behaving rats. Further, we are interested in the maximum rate of post-peak reuptake in all of our samples, not just the very large (and physiologically unrealistic) stimulations. Thus,

our measure of V_{Max} is an estimate of this function rather than a true V_{Max}, but we feel captures an important aspect of reuptake kinetics. In contrast, our other measures presented here are not dependent on DAT saturation for accurate computation (Yorgason et al., 2011) and are presented without correction.

All factors were determined using equations based on the above criteria and were thus unbiased by group or region.

Statistical analysis

The shape of the stimulated DA traces are heavily influenced by a number of factors which tend to scale with the magnitude of the peak DA level (e.g., the latency from stimulation to a return to a post-peak baseline will positively correlate with the height of the peak [DA]). As such, in order to determine with more certainty how these factors compare, we attempted to equate the observations by two factors: peak and stimulation intensity. For peak magnitude alignments, blocks were aligned by peak responses, and were defined as low peak (<0.1 μM DA), medium-low peak (0.1-0.2 μM DA), medium-high peak (0.2-0.4 μM DA), and high peak (0.4-0.8 μM DA). Within these blocks, then, all observations were matched for peak, thus allowing for more controlled comparison of other factors (e.g., FWHH, latency to peak, etc). For the stimulation intensity, a Stimulation Index (i.e., frequency X pulse number) was used. Blocks ranged from low frequency (Stimulation Index: 40-100), medium-low frequency (Stimulation Index: 100-300), medium-high (Stimulation Index: 300-600), and high frequency (Stimulation index: >600). In general, blocks were chosen based on the relative frequency of observations between groups to ensure relatively equivalent numbers of stimulations between groups.

Each analysis used individual stimulations based on the block criteria, region (core or shell) and drug background (Cocaine or Control). Each kinetic factor was thus subject to a multi-factorial analysis of variance (ANOVA) that used either drug background or region as one factor and block as the other factor. Note that given the variability in the number of observations for any given bin within an group and/or block, we corrected for unequal N by using a weighted mean (Type III) sum of squares in our analyses. For significant main effects or interactions of either drug background or region, pairwise comparisons between the groups at each level of the block with t-tests were used as a post-hoc test. T-tests were chosen as a post-hoc test because experiment-wise post-hoc tests (e.g., Tukey HSD) use a single determinant to estimate significance based expected pairwise differences. As such, these tests will underestimate reliable differences at low stimulations and peaks, while overestimating differences at high stimulations and peaks. Therefore, t-tests at each level were independent of experiment-wise variance, and isolated the specific effects at a given level. Critically, a Bonferroni correction was used for these t-tests to control for multiple comparison error. In addition, significant main effects of block and interactions of block by region/drug orthogonal linear contrasts were used to determine whether the rates of change in the kinetic factor differed by region or drug background. Statistics for ANOVAs and pairwise comparisons were done using Statistica (vers. 12; RRID: SCR_ 024213 https://scicrunch.org/resources/Any/search?q=Statistica%20&l=Statistica) and χ^2 analysis was done using GraphPad QuickCalcs (http://graphpad.com/quickcalcs/). Graphs were generated using GraphPad Prism 6 (RRID: SCR_ 002798 http://www.graphpad.com).

Results

Data were obtained from recordings in 31 rats, which included 9 rats that were naïve to self-administration, 12 that were water self-administration controls (thus a total of 21 Controls) and 10 with a history of cocaine self-administration.

For rats with a history of self-administration, rates of self-administration pressing were similar between Cocaine subjects and controls, particularly by the end of training when pressing rates were stable (Figure 1B). Rates of self-administration of Cocaine were similar to those from previous reports that were sufficient to augment both DA release and neural signaling in the NAc (Saddoris and Carelli, 2014, Saddoris et al., 2016b). There was a significant interaction of Drug (Cocaine versus Water) X Day, $F_{(13, 143)} = 2.76$, though pairwise posthoc comparisons between groups failed to find any significant differences in press rate on any day of conditioning (Tukey: all $p>0.65$). Critically, there were no effects of Region (rats that were destined to have recordings in the core or shell) or interactions of Region with any other factor (all $P>0.65$), indicating that all subjects had equivalent training and experience with self-administration prior to recordings.

Histological placements of carbon fiber electrode tips in the NAc (Figure 2) indicated recordings from 24 locations in the core (n = 17 in Controls, n = 7 in Cocaine), and 18 locations in the shell (n = 12 in Controls, n = 6 in Cocaine). From these, I obtained 218 stimulation trials from the core of Controls and 102 stimulations of Cocaine rats, and 112 stimulation trials from the shell of Controls and 63 of Cocaine rats.

Stimulations were quantified based on the distribution of peak DA responses from each group. Peak DA stimulations were first binned in increments of 50 nM from 0 nM to 1200 nM, with a final aggregate bin comprised of all stimulations with peak DA greater than 1200 nM (Figure 3). In Controls, the distribution of peak DA in the shell following stimulations was

skewed towards lower peaks (median: 125.7 nM) compared to the core, which were more evenly spread across the distribution space (median: 248.2 nM). Indeed, the number of stimulations with a peak response of lower than 150 nM was reliably greater in shell than in core relative to the residual of the populations ($\chi^2 = 15.91$, $P < 0.0001$). In contrast, the distribution of peak DA in the core and shell following stimulation in Cocaine rats showed a different pattern. In Cocaine subjects, the distribution of peak DA was similar between core and shell (median Cocaine core: 108.5 nM; median Cocaine shell: 145.6 nM), while both groups displayed distributions that closely resembled that seen in the shell of Controls (median: 125.7 nM). Indeed, both Cocaine groups showed significantly greater numbers of peaks less than 150 nM than the Core Controls (Core Control versus Core Cocaine, $\chi^2 = 27.14$, $P < 0.0001$; Core Control versus Shell Cocaine, $\chi^2 = 7.76$, $P = 0.0053$), while neither Cocaine group differed between Shell Control in proportion of peak stimulations less than 150 nM (Shell Control versus Shell Cocaine, $\chi^2 = 0.66$, $P = 0.80$; Shell Control versus Core Cocaine, $\chi^2 = 1.76$, $P = 0.18$).

Observations were then binned into larger blocks by peak DA (0-0.1μM [Low], 0.1-0.2 μM [Medium-Low], 0.2-0.4 μM [Medium-High], 0.4-0.8 μM [High], and >0.8 μM [Very High]) to assess whether there were differences at the higher peaks that were not immediately discernable with 50 nM bins (data not shown). Here, we replicated the previous observation that there were significantly more low peak stimulations in the shell than core in Controls (Low block, $\chi^2 = 10.18$, $P = 0.0014$), but also now demonstrate that core stimulations produced a greater number of higher peaks than shell in the Medium-High block, $\chi^2 = 5.33$, $P = 0.021$, and a nearly-significant trend in the High Peak block, $\chi^2 = 3.73$, $P = 0.053$. However, cocaine experience significantly shifted this distribution downward in the core. As a result, there were more stimulations that elicited low peaks in the core of Cocaine animals than Controls (Low

Peak block, $\chi^2 = 19.22$, $P < 0.0001$), and fewer higher-magnitude peaks (High Peak block, $\chi^2 = 8.01$, $P = 0.002$; Very High block, $\chi^2 = 13.98$, $P = 0.0001$). In contrast, the distribution of peak responses in the shell was less affected by cocaine. There were no differences between control and cocaine groups in any bin less than 0.8 μM (all $P > 0.13$), though cocaine appeared to have eliminated the Very High peaks seen in Controls ($\chi^2 = 4.47$, $P = 0.03$). Interestingly, there were no differences in distributions between Shell Controls and Core Cocaine in any block (all $P>0.11$). Indeed, the only difference between the Shell Control and combined Cocaine groups (Cocaine Core plus Cocaine Shell) was at the Very High block ($\chi^2 = 6.75$, $P = 0.01$; all others, $P>0.25$); whereas there were robust differences between Core Control and the combined Cocaine groups (Low, $\chi^2 = 11.65$, $P = 0.0006$; High, $\chi^2 = 4.98$, $P = 0.03$; Very High $\chi^2 = 8.65$, $P = 0.003$). Thus, the distribution of peak responses in cocaine-experienced animals was much more consistently similar to that normally found in the shell, but distinctly unlike that typically found in the core.

Differential Core and shell release kinetics in Controls

It was next important to understand whether release and reuptake kinetics differed by region and/or cocaine experience. However, because many factors in these measures can be intrinsically correlated (e.g., larger peaks will also typically show a slower return to baseline), it was important to control for at least one factor when making comparisons between observations. Thus, data were compared using two organizing principles. First, data were grouped based on peak DA (as above) regardless of stimulation intensity. However, because the extremely few observations in the Very High block, analysis was performed within and across 4 blocks (Low,

Medium-Low, Medium-High, and High Peak), and between region (core, shell) and drug history (Control, Cocaine). Then, these same data were grouped based on stimulation intensity (regardless of peak) based on the Stimulation Index (i.e., frequency of stimulation X number of pulses), also grouped by a 4-block design (Low, Medium-Low, Medium-High, and High Stimulation). Representative color plots from the core and shell in Control and Cocaine groups are shown in Figure 4A-D.

Peak-aligned stimulated DA events revealed multiple factors that differed between core and shell. Despite similar peaks, multiple measures of response kinetics in the shell in Controls were reliably slower than in the core. However, following cocaine experience, both core and shell kinetics more obviously resembled normal shell responses (Figure 4E). This was formalized by running a 3-way ANOVA that used Group (Core Control, Core Cocaine, Shell Control, Shell Cocaine) and Blocks of peak DA height (Low, Medium-Low, Medium-High, and High) as factors across a variety of kinetics measures. In general, on the majority of these measures, peak-aligned DA responses supported the hypothesis that cocaine experience shifted core DA release kinetics into a more shell-like pattern. For pairwise t-test comparisons between groups, please see Tables 1 and 2 for Bonferroni-corrected p-values.

First, it was important to show that aligning by peak resulted in similar groups of data within blocks across treatment groups (Figure 5A). This was largely true, though there was a modest interaction between Group X Block, $F(9, 425) = 2.25$, $P = 0.02$. However, no posthoc pairwise comparisons reached significance at any block between groups or drug background, indicating that peak DA was consistent across all groups and blocks, and therefore allowing for direct comparisons of kinetics of stimulations with matched peaks.

Several kinetic factors were then explored. First, applied stimulation frequency (Figure 5B) indicated a modest interaction of Group X Block, $F(9, 425) = 1.94$, $P = 0.045$, which was due largely to Core Controls showing lower applied frequencies in the Low Peak block than both Shell Controls ($P=0.007$) and both Cocaine groups ($P=0.001$). In contrast, the Shell Controls were not different from both Cocaine groups in this block ($P=0.66$). Further, planned linear contrasts indicated that Core Controls showed a linear increase in peak as a function of frequency, $F(1,425) = 26.8$, $P < 0.0001$ while no other group showed any such linear response (all $P > 0.17$). Indeed, the orthogonal linear contrast between Core Control versus all other groups was significant, $F(1, 425) = 12.4$, $P = 0.0005$, while the contrast between Shell Control and both Cocaine groups was not, $F(1, 425) = 0.03$, $P = 0.85$. Thus, while Core Controls showed linear increases in peak with increases in applied stimulation frequency, all other groups were less dynamically related to this parameter.

Next, the total DA release between stimulation and the return to baseline was measured (area under the curve [AUC]; Figure 5C). Despite similar peaks, there was a significant main effect of Group, $F(3, 425) = 15.71$, $P < 0.00001$, which indicated a significant pairwise difference between Core Controls and Core Cocaine ($P = 0.00001$), but no difference between Shell Controls and Shell Cocaine ($P > 0.10$). There was a further Group X Block interaction, $F(9, 425) = 3.70$, $P = 0.0002$. Specifically, while all groups showed significant linear increases in AUC across blocks (all $P < 0.00001$), Core Controls increased at a slower rate across blocks than Core Cocaine, $F(1, 425) = 8.50$, $P = 0.004$ and Shell Controls, $F(1, 425) = 18.29$, $P = 0.00002$, while there was no difference in the linear change across blocks between the Shell Controls and Core Cocaine, $F(1, 425) = 0.04$, $P = 0.84$. Consistent with previous findings, Core Controls showed consistently smaller AUC compared to Shell Controls, particularly in the Large Peak

block (P=0.008), which Shell Controls did not show a difference in AUC compared to either Cocaine group (P=0.23 Shell Cocaine; P=0.90, Core Cocaine) in this block.

Next, kinetics related to DA release rates were examined using Release Velocity (the rate of DA release per second between stimulation and peak; Figure 5D) and the latency to reach peak [DA] (Figure 5E). Release Velocity showed clear differences between Core Controls and other groups as indicated by both a main effect of Group, $F(3, 425) = 67.01$, $P < 0.00001$, and a Group X Block interaction, $F(9, 425) = 3.13$, $P = 0.001$. Core Controls showed significantly faster Release Velocity than each of the other groups at all blocks (all $P < 0.00001$), but no other groups differed from each other (all $P > 0.10$). While all groups exhibited significant linear contrasts across blocks (all $P < 0.0001$), Core Controls showed more rapid increases in Release Velocity across blocks than Core Cocaine, $F(1, 425) = 6.29$, $P = 0.01$, Shell Controls, $F(1, 425) = 9.16$, $P = 0.003$, and Shell Cocaine, $F(1, 425) = 17.09$, $P = 0.0004$. However, linear contrasts between Shell Controls and either Cocaine group were not different (both $P > 0.20$).

Cocaine experience also reliably affected latency to reach peak [DA] (Figure 5E). There was a main effect of Group, $F(3, 425) = 147.8$, $P < 0.0001$ and a Group X Block interaction, $F(9, 425) = 12.66$, $P < 0.0001$. Unlike the previous metrics, Latency to Peak showed the most profound changes in the shell rather than core following cocaine experience. Shell Cocaine was significantly slower to reach peak than all other groups (all $P < 0.00001$), which was due to slowed rates in the Low Peak block compared to all other groups in that block (all $P < 0.00001$). However, the average response of the cocaine-experienced groups was remarkably similar to the shell; a contrast comparing Shell Controls to the averaged Cocaine groups was not significant, $F(1, 425) = 1.31$, $P = 0.26$, while a contrast comparing Core Controls to the Cocaine groups was highly significant, $F(1, 425) = 242.9$, $P < 0.00001$. Thus, for both releaser metrics, both Cocaine

groups were much closer to the Shell Controls in both rate and rates of change across blocks than Core Controls.

Finally we examined how peak-grouped signals differed in reuptake dimensions including V_{Max} (i.e., the maximum rate of reuptake between peak and 20% decay from peak [T20]), Full Width and Half Height (FWHH; time between stimulation and 50% peak [DA] following peak), Slope (change in DA between 20% decay from peak [T20] and 80% decay from peak [T80]), and the latency to return to post-peak baseline (as determined by a 95% confidence interval around the pre-stimulation baseline).

V_{Max} rates of reuptake mirrored those obtained from Release Velocity (Figure 5F). A strong main effects of Group, $F(3, 425) = 43.11$, $P < 0.00001$, and a Group X Block interaction, $F(9, 425) = 2.64$, $P = 0.006$, was due almost exclusively to differences between Core Controls and all other groups (group-wise comparisons versus Core Control, all $P < 0.00001$). In contrast, there were no group-wise differences between Shell Controls and either of the Cocaine groups (both $P > 0.75$). Likewise, the change in reuptake across blocks increased faster in Core Controls relative to each of the other groups (all linear contrast comparisons, $P < 0.004$), whereas these rates did not differ between Shell Controls and either of the Cocaine groups (both $P > 0.40$).

In contrast, FWHH appeared to more closely resemble latency to peak measures (Figure 5G). Again, a main effect of Group, $F(3, 425) = 116.1$, $P < 0.00001$, and a Group X Block interaction, $F(9, 425) = 7.52$, $P < 0.00001$, which was largely due to differences in slowed rates in the Shell Cocaine group compared to all other groups (all $P < 0.00001$). As with Latency to Peak, FWHH showed an interesting property in which the average Cocaine response was reliably

different from Core Controls using a linear contrast, $F(1, 425) = 149.76$, $P < 0.00001$, while the Cocaine groups were not different from Shell Controls, $F(1, 425) = 0.87$, $P = 0.39$.

Reuptake during Slope showed a significant main effect of Group, $F(3, 425) = 4.89$, $P = 0.003$, but no interaction of Group X Block ($P = 0.29$; Figure 5H). This modest effect appeared to be due to a significantly faster clearance rate in Core Controls than all the other groups (all pairwise comparisons versus Core Control, $P < 0.02$), while there were no differences between either of the Cocaine groups relative to the Shell Controls ($P > 0.80$).

Return to Baseline latency was largely determined by region rather than drug experience (Figure 5I). There was a main effect of Group, $F(3, 425) = 19.02$, $P < 0.0001$, but no interaction of Group X Block ($P = 0.06$). This group effect was not due to drug condition within a region (Core Control vs Core Cocaine, $P = 0.08$; Shell Control vs Shell Cocaine, $P = 0.10$), but rather to slower baseline return in the shell than the core in both drug conditions (Core Control vs Shell Control, $P = 0.003$; Core Cocaine vs Shell Cocaine, $P = 0.0001$).

For the final set of analyses, data were aligned by the intensity of the applied stimulation (i.e., Stimulation Index). In general, cocaine experience had distinctly different effects on how stimulations affected DA release across regions (for pairwise t-test comparisons between groups, please see Tables 3 and 4 for Bonferroni-corrected p-values). In the core (Figure 6A), DA release was significantly decreased relative to controls with the same stimulation parameters, while in the shell (Figure 6B), cocaine experience produced more subtle effects that impact the dynamic range of the DA response. Grouping data into blocks by Stimulation Index according to a scale that roughly doubled in intensity between blocks, there was an overall significant difference in distribution between groups, $\chi^2 = 48.25$, $P < 0.00001$ (Figure 6C). Follow-up tests indicated that

Core Controls had more low-intensity stimulations than both Core Cocaine (Stim Index 0-50: χ^2 = 10.58, P = 0.001) and Shell Controls (Stim Index 0-50: χ^2 = 3.85, $P < 0.05$). In contrast, the Core Cocaine group showed similar numbers of observations at in the low-intensity range as Shell Controls (Stim Index 0-50: χ^2 = 3.02, *n.s.*) and Shell Cocaine subjects (Stim Index 0-50: χ^2 = 0, *n.s.*). Likewise, there were no differences between Shell Control and Shell Cocaine subjects in this bin (Stim Index 0-50: χ^2 = 2.17, *n.s.*). At the high end of the stimulation intensity range, there were fewer stimulations in the Core Controls than the mean of the Cocaine groups (Stim Index >600: χ^2 = 4.14, P = 0.04), while Shell Controls showed similar numbers as the Cocaine groups (Stim Index >600: χ^2 = 0.01, *n.s.*).

As above, several metrics were quantified to assess features of kinetics, though as the peaks were unequal, only a subset of measures was analyzed: Peak [DA], Release Velocity and V_{max}. Consistent with peak-aligned measures above, cocaine experience shifted core DA release dynamics towards a more shell-like pattern across multiple metrics. For example, Peak [DA] exhibited a main effect of group, F(3, 391) = 7.01, P = 0.0001 (Figure 6D), which was due to significantly higher peaks overall in the Core Control group than both Core Cocaine (P = 0.001) and Shell Cocaine subjects (P = 0.02); Shell Controls did not differ from either Cocaine group (Core Cocaine, P = 0.09; Shell Cocaine, P = 0.41). Planned contrasts indicated that while both Control groups exhibited significant linear increases in DA as a function of increasing Stimulation Index (Core: F(1, 391) = 23.89, P < 0.00001; Shell: F(1, 391) = 10.03, P = 0.002), Core Cocaine subjects showed a nearly-significant trend in this direction, F(1, 391) = 3.74, P = 0.053, while Shell Cocaine subjects showed no relationship between Stimulation and DA, F(1, 391) = 0.40, P = 0.53.

Similar patterns were found for Release Velocity (Figure 6E) and V_{max} (Figure 6F). Both showed significant main effects of group (Release Velocity: $F(1, 391) = 33.87$, $P < 0.00001$; V_{max}: $(1, 391) = 31.75$, $P < 0.00001$), and both posthoc examinations revealed that Core Controls exhibited faster release and reuptake than each of the other groups (all $P < 0.00001$), while Shell Controls did not differ from either Cocaine group (all $P > 0.59$). Indeed, planned contrasts indicated that only Core Controls displayed a linear correlation between applied stimulation and release (Release Velocity: $F(1, 391) = 14.91$, $P = 0.0001$) and reuptake (V_{max}: $F(1, 391) = 14.43$, $P = 0.0002$), while none of the other groups showed this correspondence (all $P > 0.08$).

Discussion

Here, voltammetrically-recorded rapid DA release was measured in the NAc core and shell following electrical stimulation of VTA afferents in freely-moving rats. While the present data replicate well-established differences between core and shell in normal animals (Jones et al., 1996, Mateo et al., 2005, Addy et al., 2010), abstinence from cocaine self-administration significantly alters this relationship. In general, cocaine-experienced subjects displayed DA release kinetics that became significantly more similar to normal shell kinetics regardless of region. Specifically, while Core Cocaine subjects displayed generally lower peak [DA], peak-matched stimulations produced slowed kinetic responses of both release and reuptake for Cocaine rats relative to Controls. In contrast, both Shell Cocaine and Core Cocaine subjects were often similar to Shell Controls on a wide variety of metrics regardless of whether the observations were aligned by peak or by applied stimulation intensity. Collectively, these observations suggest that prior cocaine experience differentially alters DA terminal function in a

region-specific manner, which likely has important ramifications for understanding altered neuroplasticity in cocaine-experienced populations, even long after the cessation of drug taking behaviors.

To understand the function of normal phasic DA signaling in the brain, it is critical to consider a variety of factors including temporal dynamics of the signal, the neuroanatomical terminal region for DA afferents, and the behaviorally-relevant task being encoded. There are well-known intrinsic differences in signaling kinetics between core and shell due to neuroanatomical features of these regions. For example, NAc shell expresses a decreased density in the DA transporter (DAT) compared to the core, and as such, displays reliably slower synaptic reuptake of released DA (Jones et al., 1996). The present study replicates this previous work by demonstrating slower reuptake in the shell than core in Controls by multiple metrics including V_{Max}, FWHH, Slope, and the Latency to Return to Baseline. These effects were largely true whether stimulations were aligned by stimulation parameters or by peak DA response.

In addition to these reuptake measures, there were reliable differences in release kinetics between core and shell in Controls, including faster Release Velocity and Latency to Peak. For example, frequency-aligned DA kinetics (e.g., Release Velocity, V_{max}, Peak [DA]) in the core linearly scaled with applied stimulations, while these same factors in the shell remained relatively flat regardless of stimulation intensity. This sensitivity of peak DA release arising from the intensity of impulse activity may support a functional role in normal behavioral task signaling. For example, in the NAc core peak DA during predictive cues in a value-based decision-making task reliably scales with the animal's preferred option when weighing cost-benefit choices, while DA release in the NAc shell showed similar DA peaks in the same conditions (Day et al., 2010, Sugam et al., 2012). Thus, a coupling between excitability and the

511 magnitude of the DA response may indicate an intrinsic aspect of core DA signaling that encodes
512 value by the relative peak for various stimuli (Saddoris et al., 2015b).

513 In contrast to these normal differences between core and shell, abstinence from cocaine
514 self-administration induced a more homogenous DA release pattern between subregions that
515 were similar in several aspects to drug-naïve shell kinetics, and were consistent across multiple
516 metrics and alignment properties. For the present study, both Core Cocaine and Shell Cocaine
517 showed a peak-aligned distribution of responses that was statistically similar to Shell Controls,
518 and which reliably differed from Core Controls. For example, while stimulations in Core
519 Controls resulted in peak [DA] in the core that ranged between 40-1200 nM, stimulations in the
520 Shell Controls and both Cocaine groups produced peak DA release in the core that were
521 primarily below 200 nM. Thus, cocaine experience appeared to shift the DA response in the core
522 away from a widely dynamic response into a much narrower and smaller peak response typical
523 of the shell, similar recent findings obtained in a slice preparation (Siciliano et al., 2016).

524 While largely having more dramatic effects on core DA terminals, cocaine experience
525 nonetheless induced some consistent changes in stimulated DA release in the shell as well. Here,
526 DA release and reuptake kinetics (specifically, Release Velocity, Latency to Peak, and FWHH)
527 were slower in Cocaine rats than Controls, but only at Low levels of DA release (<200 nM).
528 However, these lower peak DA responses are typical of the normal physiological range of peak
529 [DA] observations (i.e., 40-150 nM) typically seen in freely-moving rats in the NAc shell using
530 an acute FSCV electrode (Aragona et al., 2008, Beyene et al., 2010, Wheeler et al., 2011,
531 Cacciapaglia et al., 2012, Saddoris et al., 2015a). Thus, these somewhat limited effects may have
532 significant ramifications for normal DA signaling during behavioral tasks. Further, Stimulation-
533 aligned data suggests that cocaine flattens the dynamic range of the DA response, with a

generalized response at all applied stimulation intensities rather than a linear scaling of DA with stimulation changes.

Remarkably, the pattern of augmented DA release kinetics does not clearly mirror findings of dysfunctional DA signals during motivated learning behaviors (Spoelder et al., 2015, Saddoris et al., 2016b). In a recent finding, we showed that phasic DA release elicited by rewarding stimuli during associative learning was significantly impaired in both core and shell, though these deficits were distinct within subregion. In the core, peak DA in cocaine-experienced rats failed to differentially encode information about reward-predictive and irrelevant stimuli, instead displaying differences between cues several seconds after cue onset. Further, we found exaggerated DA release in the core during reward receipt in cocaine-experienced rats. In contrast, cocaine experience proved devastating to shell, where neither cues nor rewards elicited DA that was above baseline (Saddoris et al., 2016b).

Thus, while stimulated DA in the shell in the present study was less obviously affected by cocaine than in the core, phasic DA release in the shell during motivated behavior was profoundly impaired. This dissociation suggests that DA terminals in the shell remain functional, yet are unable to normally signal the significance of behavioral events. This inability to track behavioral stimuli despite relatively normal DA terminal function suggests a profound change in the mesolimbic circuitry induced by repeated cocaine experience, though whether this functional disconnection is due to changes in VTA inputs and/or local modulation of DA afferents has yet to be explored. In the core, however, there were some features during the learning task (Saddoris et al., 2016b) that complement the present finding. For example, DA signals in cocaine-experienced rats for the CS+ presentations was relatively sustained throughout the cue rather than briefly at cue onset, a dynamic more linked to the shell than core (Cacciapaglia et al., 2012,

557 Saddoris et al., 2015a). Further, while DA signaling for predicted rewards by typically
558 disappears in the core with training, consistent with reward prediction error hypotheses (Schultz
559 et al., 1997, Pan et al., 2005), fully-anticipated rewards persistently elicit large DA release events
560 in cocaine-experienced rats (Saddoris et al., 2016b), a pattern of activity more typically found in
561 the shell (Cacciapaglia et al., 2012, Saddoris et al., 2015a). Further, we have recently reported
562 that shell (but not core) DA release in drug-naïve rats tracks differences in reward magnitude, but
563 in cocaine-experienced rats, this differential DA release pattern for reward magnitude is found in
564 the core instead of the shell (Saddoris et al., 2016a). Thus, cocaine experience induces striking
565 changes in the functional properties of the NAc core and shell which are differentially manifest
566 in behavioral and synaptic properties in a region-specific fashion.

567 Collectively, these findings suggest that the core becomes more shell-like in its response
568 dynamic to phasic DA signals following experience with cocaine self-administration. This
569 hypothesis is consistent with previous reports which have shown that motivationally-relevant
570 encoding of relevant stimuli shifts dorsolaterally in the striatum in drug-experienced animals
571 (Takahashi et al., 2007, Willuhn et al., 2012). These shifts are predicted by the anatomical
572 organization of the mesolimbic system wherein complex "loops" of connections involving the
573 striatum, limbic cortex and midbrain result in learned information synapsing at increasingly
574 dorsal and lateral targets within the circuitry over repeated experience (Haber et al., 2000, Haber
575 et al., 2006, Haber, 2014). Indeed, disruption of earlier portions of these circuits can prevent
576 these shifts in normal animals (Belin and Everitt, 2008, Belin et al., 2009, Willuhn et al., 2012),
577 suggesting dorsolateral shifts in encoding may reflect transitions to more habitual kinds of
578 information (Robbins and Everitt, 2002).

Likewise, in cocaine-experienced rats, this dorsolateral shift appears to involve not just the neural output of the striatum, but also the DAergic input. This appearance of a functional dorsolateral shift in DA signaling properties may thus explain aspects of addiction as a chronically-relapsing disorder; with functional changes in signaling along a dorsolateral axis within the striatum, representations of drugs and drug-associated stimuli may be encoded in a more habit-like manner and therefore more resilient against treatment. Indeed, we and others have shown that repeated drug intake biases animals towards a strong sign-tracking phenotype wherein outcome-associated stimuli take on abnormally-high salience (McClory and Spear, 2014, Robinson et al., 2015, Spoelder et al., 2015, Saddoris et al., 2016b), and that sign-tracking responses are insensitive to changes in value of the associated outcome (i.e., more habit-like) (Nasser et al., 2015). In conclusion, the present findings provide evidence for a functional alteration in DA terminals for the core and shell in cocaine-experienced animals, patterns of which either reflect (core) or are distinct from (shell) behaviorally-elicited DA signals. Future studies will investigate the causes for these neuroplastic changes, and may provide insight into potential therapeutics to reverse these alterations.

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732

733 ***Figure Legends***

734 **Figure 1**. ***a.*** Schematic of experimental design. ***b***. Reinforced presses across the 14d of self-
735 administration training for Controls and Cocaine rats. ***c-e***. Schematic of different metrics of DA
736 release kinetics. ***a***. Points in the release kinetics in relation to the peak DA release (i.e., point of
737 greatest [DA] following stimulation). Half Peak is the point at exactly half of peak concentration,
738 Return to BL is the point at which the [DA] was within a 95% confidence interval of the
739 baseline, and T20 and T80 reflect 20% decrease and 80% decrease in [DA] from peak,
740 respectively. Area Under the Curve (AUC) was estimated by summing the [DA] in each 100-ms
741 bin between stimulation and Return to BL. ***b***. Latency measures derived from the points of
742 release and reuptake from (***a***). Latency to peak, Full Width at Half Height (i.e., latency from
743 stimulation to Half Peak), and Return to BL Latency are relative to stimulation, while T20 and
744 T80 Latencies are relative to Peak. ***c***. Rates of change relative to points during release. Release
745 velocity is the rate of increase in [DA] from stimulation to peak, V_{Max} is the rate of uptake
746 between the peak and T20, Slope is the rate of uptake between T20 and T80.

747 **Figure 2**. Placement of electrodes during recording in either Controls (top row) or Cocaine rats
748 (bottom row). Black circles show placements for core, gray circles indicate shell.

749 **Figure 3**. Distribution of peak [DA] amplitude from stimulation trials in the NAc core (Control:
750 black, Cocaine: blue) and NAc shell (Control: gray, Cocaine: red). Peak [DA] responses for each
751 stimulation were binned by 50 ms epochs from 0-1200 nM, while all stimulations that were
752 greater than 1200 nM represented the final bin. Proportion reflected the number of stimulations
753 in that bin as a proportion of all stimulations from that group.**Control Core vs Control Shell;
754 §Control Core vs Cocaine Core; $^{@}$Control Shell vs Cocaine Shell, P<0.001 for relevant χ^2.

755 **Figure 4**. Representative color plots of stimulated DA release in NAc core (***a-b***) and NAc shell
756 (***c-d***). ***e***. Overlapped traces of DA elicited by electrical stimulation in the core and shell of
757 Controls and Cocaine-experienced subjects from the representative color plots in ***a-d***.

758 **Figure 5**. Kinetic factors of DA release aligned by peak [DA] in Control Core (black squares),
759 Cocaine Core (blue squares), Control Shell (gray circles) and Cocaine Shell (red circles)
760 recordings. **Control Core vs Control Shell; $^{\Delta}$Control Core vs Both Cocaines; §Control Core vs
761 Cocaine Core; $^{@}$Control Shell vs Cocaine Shell; ‡Control Shell vs Both Cocaines, P<0.01
762 (Bonferroni-corrected α for multiple comparisons).

763 **Figure 6**. Average phasic DA release in the NAc core (***a***) and shell (***b***) of Controls (black/gray)
764 Cocaine self-administering rats (blue/red) in Stimulation Index-aligned bins. ***c***. For each drug
765 group and region, the proportion of cells (out of all observations) in each Stimulation Index bin.
766 Note $\log_2$ scale used to show the loss specifically of the low stimulation index observations in the
767 Cocaine groups. Peak [DA] (***d***), Rise Velocity (***e***) and VMax (***f***) for treatment groups across
768 Stimulation Intensity bins. **Control Core vs Control Shell; $^{\Delta}$Control Core vs Both Cocaines;
769 §Control Core vs Cocaine Core; $^{@}$Control Shell vs Cocaine Shell; ‡Control Shell vs Both
770 Cocaines, P<0.01 (Bonferroni-corrected α for multiple comparisons).

771

Table Legends

Table 1. Significance (p-value) of pairwise t-tests at each peak bin (Low [<0.1 μM DA], Medium-Low [0.1-0.2 μM DA], Medium-High [0.2-0.4 μM DA] and High [0.4-0.8 μM DA]) between Core Control and Shell Control (left), Core Control and Core Cocaine (middle) and Shell Control and Shell Cocaine (right). ***Bold Italics****, P<0.01 (significant after Bonferroni correction); *Italics only*, P<0.05 (not significant after Bonferroni correction).

Table 2. Significance (p-value) of pairwise t-tests at each peak bin (Low [<0.1 μM DA], Medium-Low [0.1-0.2 μM DA], Medium-High [0.2-0.4 μM DA] and High [0.4-0.8 μM DA]) between Core Control and Shell Control (left; repeated from Table 1), Core Control and average of Both Cocaine groups (core and shell; middle) and Shell Control average of Both Cocaine groups (core and shell; right). ***Bold Italics****, P<0.01 (significant after Bonferroni correction); *Italics only*, P<0.05 (not significant after Bonferroni correction).

Table 3. Significance (p-value) of pairwise t-tests at each Stimulation Index bin (Low [100-300], Medium-Low [300-600], Medium-High [600-1200] and High [>1200]) between Core Control and Shell Control (left), Core Control and Core Cocaine (middle) and Shell Control and Shell Cocaine (right). ***Bold Italics****, P<0.01 (significant after Bonferroni correction); *Italics only*, P<0.05 (not significant after Bonferroni correction).

Table 4. Significance (p-value) of pairwise t-tests at each Stimulation Index bin (Low [100-300], Medium-Low [300-600], Medium-High [600-1200] and High [>1200]) between Core Control and Shell Control (left, repeated from Table 3), Core Control and average of Both Cocaine groups (core and shell; middle) and Shell Control average of Both Cocaine groups (core and shell; right). ***Bold Italics****, P<0.01 (significant after Bonferroni correction); *Italics only*, P<0.05 (not significant after Bonferroni correction).

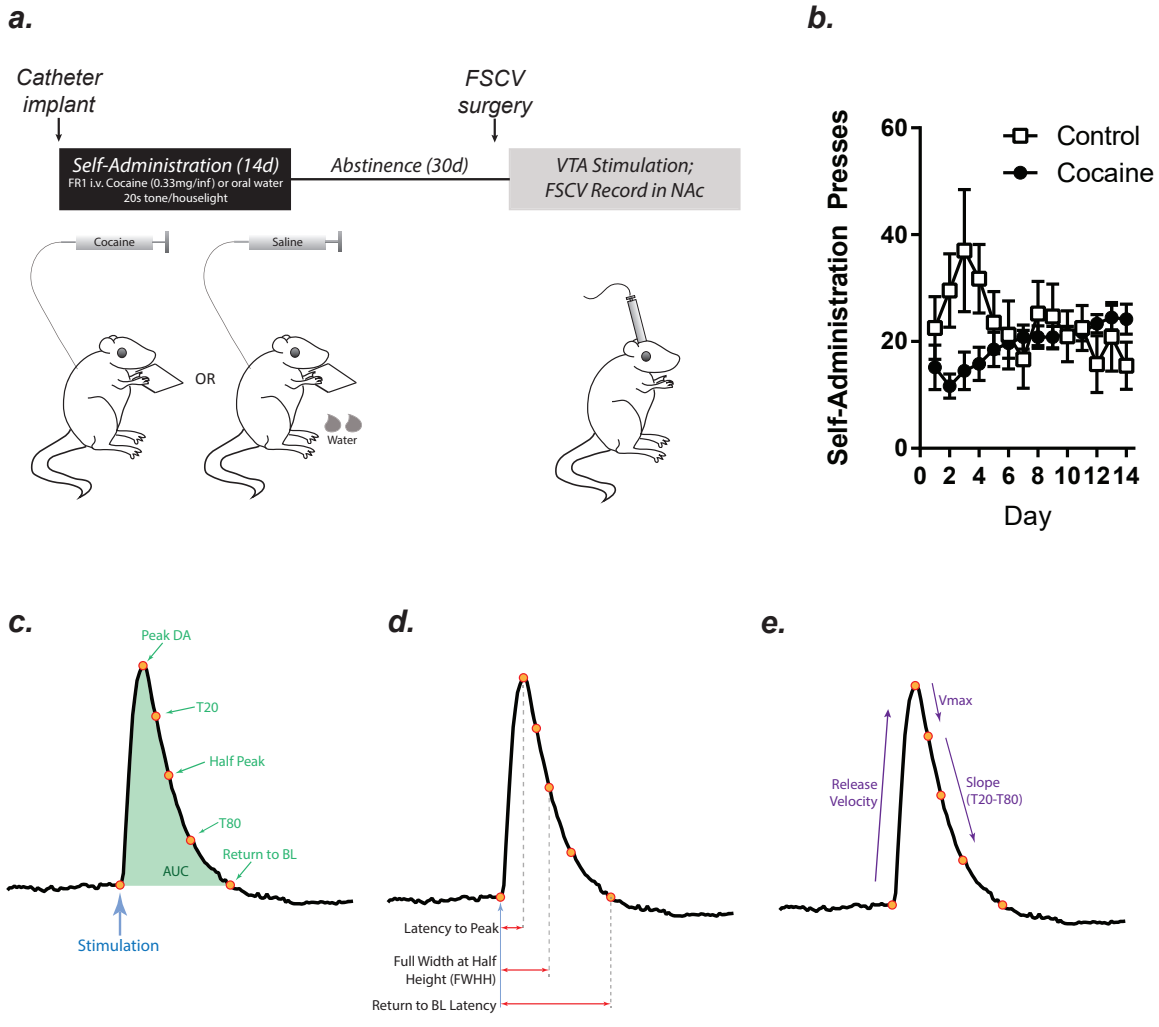


Figure 1

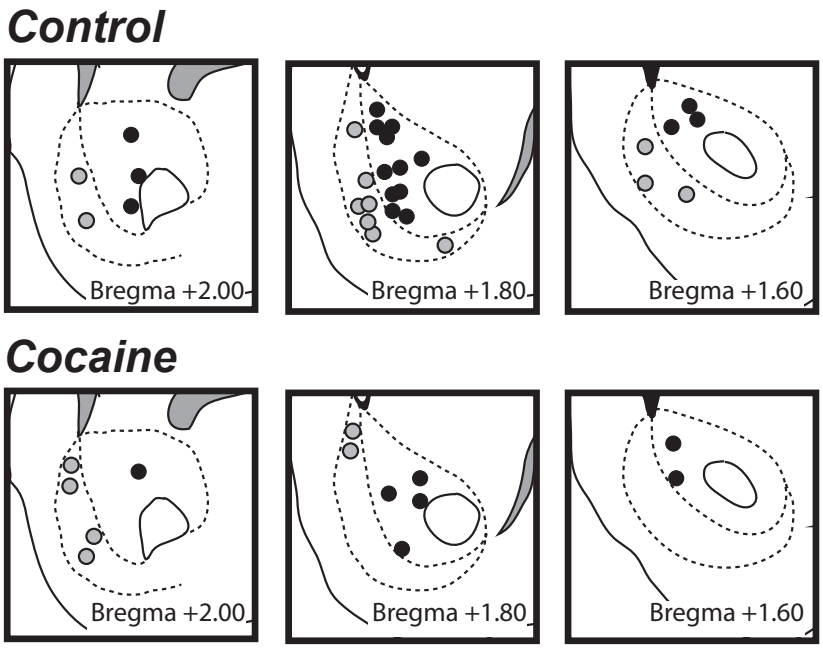


Figure 2

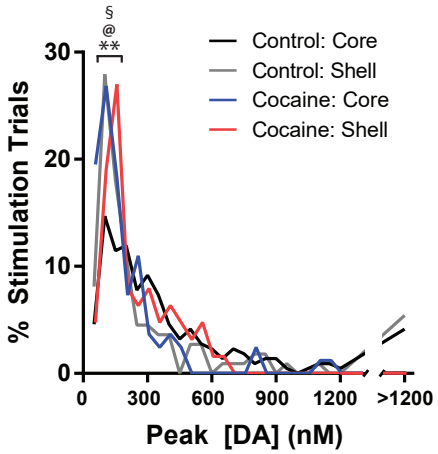
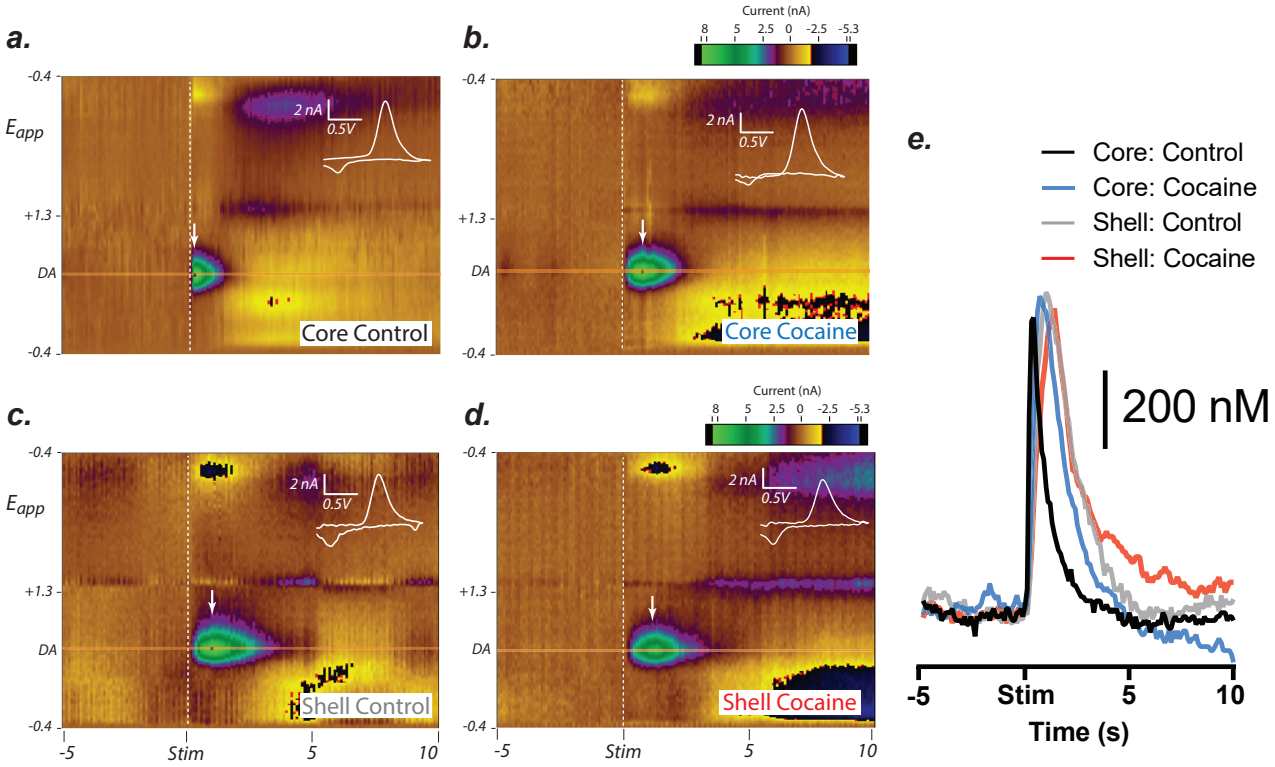
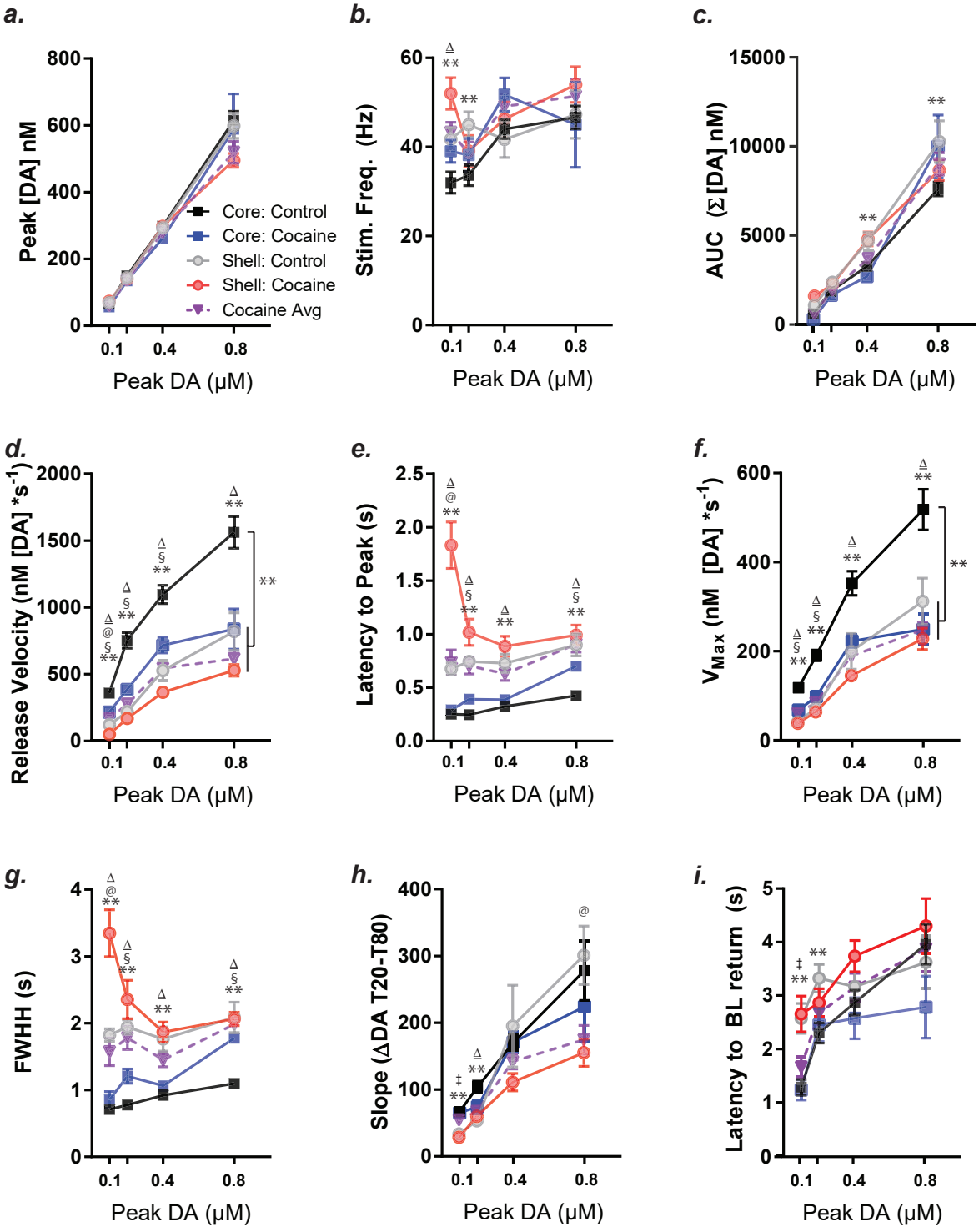
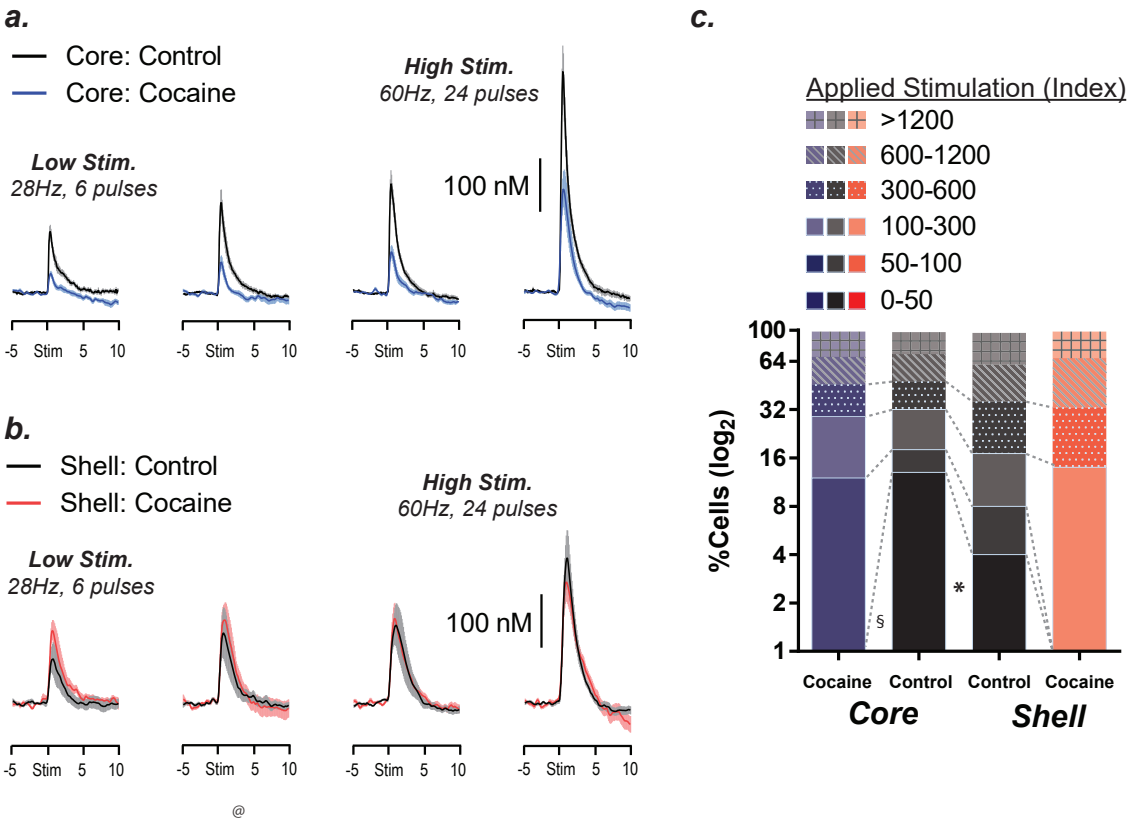


Figure 3







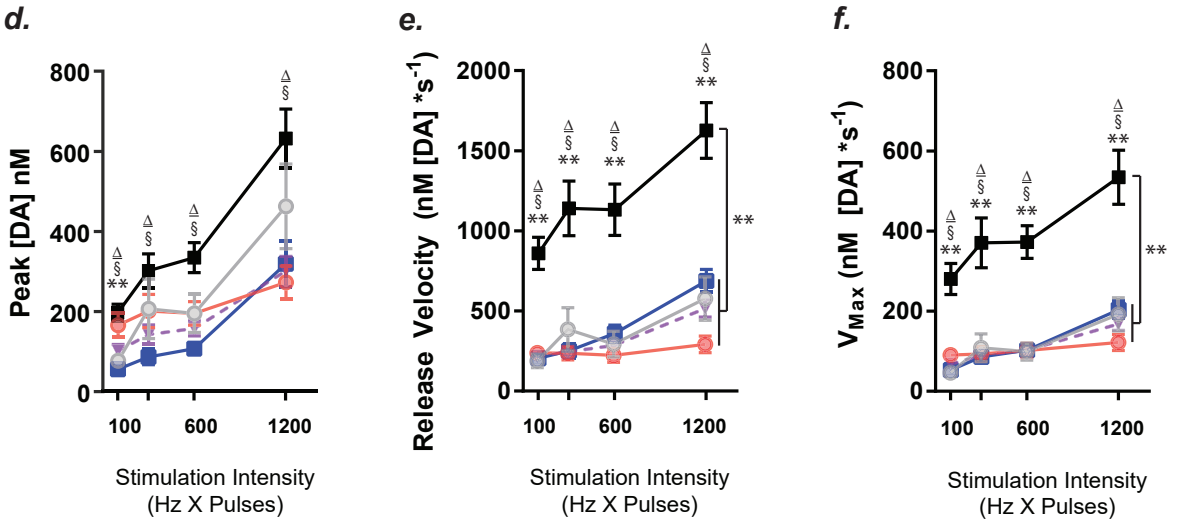


Table 1. Peak-Aligned pairwise comparisons (individual drug groups)

p-values (t-test)	Core (Control) vs. Shell (Control)				Core (Control) vs. Core (COCAINE)				Shell (Control) vs Shell (COCAINE)			
Peak [DA]	0. 1 µM	0.2 µM	0.4 µM	0.8 µM	0.1 µM	0.2 µM	0.4 µM	0.8 µM	0.1 µM	0.2 µM	0.4 µM	0.8 µM
Peak	0.87	*0.02*	0.97	0.32	*0.03*	***0.001****	0.07	0.66	0.81	0.42	0.94	*0.03*
Freq.	***0.007****	***0.001****	0.54	0.91	*0.04*	0.28	0.10	0.85	*0.03*	0.20	0.43	0.34
AUC	*0.02*	*0.05*	***0.001****	***0.008****	0.06	0.12	0.11	0.09	0.10	0.52	0.88	0.24
Rise Velocity	***<0.0001****	***<0.0001****	***<0.0001****	***0.004****	***0.002****	***<0.0001****	***0.008****	0.07	***0.0004****	0.06	0.06	0.07
Lat. Peak	***<0.0001****	***<0.0001****	***<0.0001****	***<0.0001****	0.27	***<0.0001****	0.12	***0.003****	***<0.0001****	*0.02*	0.22	0.53
Vmax	***<0.0001****	***<0.0001****	***<0.0001****	0.01	***0.0008****	***<0.0001****	*0.02*	0.08	0.63	0.52	0.53	0.36
FWHH	***<0.0001****	***<0.0001****	***<0.0001****	***<0.0001****	0.28	***<0.0001****	0.15	***0.003****	***<0.0001****	0.14	0.67	0.93
Slope (T20-T80)	***<0.0001****	***<0.0001****	0.52	0.80	0.84	0.05	0.98	0.98	0.41	0.37	0.21	***0.009****
BL Return	***0.0002****	***0.002****	0.60	0.63	0.84	0.83	0.56	0.34	0.87	0.23	0.34	0.35
T20 Latency	***<0.0001****	***<0.0001****	***<0.0001****	***<0.0001****	0.16	***<0.0001****	0.12	***<0.0001****	***<0.0001****	0.07	0.69	0.89
T80 Latency	***<0.0001****	***<0.0001****	*0.02*	0.85	0.91	0.10	0.80	0.40	***0.0006****	0.87	0.41	0.08

Significance (p-value) of pairwise t-tests at each peak bin (Low [<0.1 µM DA], Medium-Low [0.1-0.2 µM DA], Medium-High [0.2-0.4 µM DA] and High [0.4-0.8 µM DA]) between Core Control and Shell Control (left), Core Control and Core Cocaine (middle) and Shell Control and Shell Cocaine (right). ***Bold Italics****, P<0.01 (significant after Bonferroni correction); *Italics only*, P<0.05 (not significant after Bonferroni correction).

Table 2. Peak-Aligned pairwise comparisons (collapsed drug groups)

p-values (t-test)	Core (Control) vs. Shell (Control)				Core (Control) vs. BOTH COCAINES				Shell (Control) vs BOTH COCAINES			
Peak [DA]	0. 1 µM	0.2 µM	0.4 µM	0.8 µM	0.1 µM	0.2 µM	0.4 µM	0.8 µM	0.1 µM	0.2 µM	0.4 µM	0.8 µM
Peak [DA]	0.87	0.23	0.97	0.32	0.11	***<0.0001****	0.28	0.26	0.86	0.24	0.49	0.13
Freq.	***0.007****	***0.002****	0.54	0.91	***0.001****	0.12	0.18	0.34	0.66	0.10	0.13	0.53
AUC	*0.02*	*0.03*	***0.001****	***0.008****	0.98	0.72	0.20	0.07	0.05	*0.04*	0.07	0.33
Rise Velocity	***<0.0001****	***<0.0001****	***<0.0001****	***0.004****	***<0.0001****	***<0.0001****	***<0.0001****	***<0.0001****	0.07	0.08	0.82	0.17
Lat. Peak	***<0.0001****	***<0.0001****	***<0.0001****	***<0.0001****	***0.0004****	***<0.0001****	***<0.0001****	***<0.0001****	0.67	*0.70*	0.37	0.96
Vmax	***<0.0001****	***<0.0001****	***<0.0001****	0.01	***<0.0001****	***<0.0001****	***<0.0001****	***0.0008****	*0.05*	0.20	0.48	0.35
FWHH	***<0.0001****	***<0.0001****	***<0.0001****	***<0.0001****	***0.0004****	***<0.0001****	***<0.0001****	***<0.0001****	0.32	0.47	0.12	0.64
Slope (T20-T80)	***<0.0001****	***<0.0001****	0.52	0.80	0.16	***0.001****	0.10	0.19	***0.003****	0.08	0.27	*0.01*
BL Return	***0.0002****	***0.002****	0.60	0.63	0.16	0.30	0.49	0.87	***0.007****	0.05	0.99	0.70
T20 Latency	***<0.0001****	***<0.0001****	***<0.0001****	***<0.0001****	***0.0002****	***<0.0001****	***<0.0001****	***<0.0001****	0.62	0.50	0.07	0.71
T80 Latency	***<0.0001****	***0.0006****	*0.02*	0.85	***0.0008****	***0.0001****	***0.007****	***0.001****	0.06	0.25	0.27	0.16

Significance (p-value) of pairwise t-tests at each peak bin (Low [<0.1 µM DA], Medium-Low [0.1-0.2 µM DA], Medium-High [0.2-0.4 µM DA] and High [0.4-0.8 µM DA]) between Core Control and Shell Control (left; repeated from Table 1), Core Control and average of Both Cocaine groups (core and shell; middle) and Shell Control average of Both Cocaine groups (core and shell; right). ***Bold Italics****, P<0.01 (significant after Bonferroni correction); *Italics only*, P<0.05 (not significant after Bonferroni correction).

Table 3. Stimulation Index-Aligned pairwise comparisons (individual drug groups)

p-values (t-test)	Core (Control) vs. Shell (Control)				Core (Control) vs. Core (COCAINE)				Shell (Control) vs Shell (COCAINE)			
Stim. Index	100	300	600	1200	100	300	600	1200	100	300	600	1200
Peak [DA]	***0.002****	0.96	*0.02*	0.48	***<0.0001****	***0.003****	***0.0006****	***0.009****	*0.02*	0.96	0.99	0.14
Freq.	0.97	***0.002****	0.46	1.00	0.24	0.73	0.87	0.14	0.12	0.37	0.76	1.00
AUC	*0.01*	0.98	0.21	0.45	***0.0006****	***0.003****	***0.002****	*0.02*	0.06	0.70	0.83	0.14
Rise Velocity	***0.0006****	***0.006****	***0.0003****	***<0.0001****	***0.0002****	***0.0002****	***0.006****	***0.0006****	0.31	0.42	0.49	0.14
Lat. Peak	***0.002****	***<0.0001****	***<0.0001****	***<0.0001****	0.45	0.36	0.61	0.11	0.07	**0.002***	***0.005****	*0.01*
Vmax	***0.002****	***0.003****	***<0.0001****	***0.0003****	***0.001****	***0.001****	***0.0002****	***0.004****	***0.01****	0.78	0.78	0.16
FWHH	***<0.0001****	***<0.0001****	***<0.0001****	***<0.0001****	0.15	0.52	0.59	0.09	0.89	0.05	***0.003****	0.13
Slope (T20-T80)	***0.001****	0.81	***0.004****	0.26	***0.009****	*0.03*	***0.002****	0.09	***0.004****	0.77	0.68	0.10
BL Return	0.26	0.74	0.96	0.91	0.07	*0.01*	0.11	*0.03*	0.56	0.28	0.10	0.88
T20 Latency	***<0.0001****	***<0.0001****	***<0.0001****	***<0.0001****	*0.02*	*0.32*	0.09	0.07	0.30	0.10	0.28	0.31
T80 Latency	***0.006****	0.25	*0.02*	***0.0004****	0.53	0.06	0.56	0.09	0.21	0.72	0.66	0.18

Significance (p-value) of pairwise t-tests at each Stimulation Index bin (Low [100-300], Medium-Low [300-600], Medium-High [600-1200] and High [>1200]) between Core Control and Shell Control (left), Core Control and Core Cocaine (middle) and Shell Control and Shell Cocaine (right). ***Bold Italics****, P<0.01 (significant after Bonferroni correction); *Italics only*, P<0.05 (not significant after Bonferroni correction).

Table 4. Stimulation Index-Aligned pairwise comparisons (collapsed drug groups)

p-values (t-test)	Core (Control) vs. Shell (Control)				Core (Control) vs. BOTH COCAINES				Shell (Control) vs BOTH COCAINES			
Stim. Index	100	300	600	1200	100	300	600	1200	100	300	600	1200
Peak [DA]	***0.002****	0.96	*0.02*	0.48	***0.0008****	***0.003****	***0.0001****	***0.0003****	0.36	0.37	0.41	0.07
Freq.	0.97	***0.002****	0.46	1.00	0.06	0.36	0.90	0.26	0.21	0.54	0.55	0.36
AUC	*0.01*	0.98	0.21	0.45	*0.02*	*0.03*	***0.007****	***0.003****	0.51	*0.44*	0.31	*0.02*
Rise Velocity	***0.0006****	***0.006****	***0.0003****	***<0.0001****	***<0.0001****	***<0.0001****	***<0.0001****	***<0.0001****	0.43	0.27	0.92	0.69
Lat. Peak	***0.002****	***<0.0001****	***<0.0001****	***<0.0001****	***0.005****	***0.002****	***0.0005****	***<0.0001****	0.92	0.52	0.33	0.10
Vmax	***0.002****	***0.003****	***<0.0001****	***0.0003****	***0.0002****	***<0.0001****	***<0.0001****	***<0.0001****	0.07	0.55	0.79	0.30
FWHH	***<0.0001****	***<0.0001****	***<0.0001****	***<0.0001****	0.02	***0.001****	***<0.0001****	***<0.0001****	0.42	0.74	0.79	*0.02*
Slope (T20-T80)	***0.001****	0.81	***0.004****	0.26	***0.002****	***0.006****	***<0.0001****	***0.001****	***0.007****	0.59	0.70	0.21
BL Return	0.26	0.74	0.96	0.91	0.99	0.30	0.60	0.39	0.25	0.28	0.74	*0.03*
T20 Latency	***<0.0001****	***<0.0001****	***<0.0001****	***<0.0001****	***0.0004****	***0.0007****	***<0.0001****	***<0.0001****	0.09	0.70	0.60	***0.0003****
T80 Latency	***0.006****	0.25	*0.02*	***0.0004****	0.83	0.51	*0.05*	0.23	*0.02*	0.10	0.75	0.07

Significance (p-value) of pairwise t-tests at each Stimulation Index bin (Low [100-300], Medium-Low [300-600], Medium-High [600-1200] and High [>1200]) between Core Control and Shell Control (left, repeated from Table 3), Core Control and average of Both Cocaine groups (core and shell; middle) and Shell Control average of Both Cocaine groups (core and shell; right). ***Bold Italics****, P<0.01 (significant after Bonferroni correction); *Italics only*, P<0.05 (not significant after Bonferroni correction).