ORIGINAL INVESTIGATION

Rate of onset of dopamine transporter inhibitors assessed with intracranial self-stimulation and in vivo dopamine photometry in rats

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Abstract

Drug self-administration and intracranial self-stimulation (ICSS) are two preclinical behavioral procedures used to predict abuse potential of drugs, and abuse-related drug effects in both procedures are thought to depend on increased mesolimbic dopamine (DA) signaling. Drug self-administration and ICSS yield concordant metrics of abuse potential across a diverse range of drug mechanisms of action. The "rate of onset," defined as the velocity with which a drug produces its effect once administered, has also been implicated as a determinant of abuse-related drug effects in self-administration procedures, but this variable has not been systematically examined in ICSS. Accordingly, this study compared ICSS effects produced in rats by three DA transporter inhibitors that have different rates of onset (fastest to slowest: cocaine, WIN-35428, RTI-31) and that produced progressively weaker metrics of abuse potential in a drug self-administration procedure in rhesus monkeys. Additionally, in vivo photometry using the fluorescent DA sensor dLight1.1 targeted to the nucleus accumbens (NAc) was used to assess the time course of extracellular DA levels as a neurochemical correlate of behavioral effects. All three compounds produced ICSS facilitation and increased DA levels assessed by dLight. In both procedures, the rank order of onset rate was cocaine > WIN-35428 > RTI-31; however, in contrast to monkey drug self-administration results, maximum effects did not differ across compounds. These results provide additional evidence that drug-induced increases in DA drive ICSS facilitation in rats and illustrate the utility of both ICSS and photometry to evaluate the time course and magnitude of abuse-related drug effects in rats.

Keywords Psychostimulants · Reuptake inhibitors · Addiction · Fluorimetry · Dopamine release · Latency

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Introduction

Many drugs of abuse with diverse pharmacological mechanisms of action share a common effectiveness to stimulate mesolimbic dopamine (DA) signaling, and this shared neurochemical effect promotes reinforcement of drug-taking behaviors in operant drug self-administration procedures (Ikemoto and Bonci 2014; Koob and Volkow 2010; Wise 2004). Rate of drug onset, defined as the velocity at which a drug produces its effect once administered, is an additional factor that can influence measures of drug reinforcement, such that faster onset is often associated with higher metrics of reinforcing efficacy (Balster and Schuster 1973; Ko et al. 2002; Panlilio et al. 1998; Wee et al. 2006; Winger et al. 2002). For example, dopamine transporter (DAT) ligands constitute one class of abused drugs (Baumann et al. 2018; Docherty and Alsufyani 2021), and Wee et al. (2006) used

a progressive-ratio drug self-administration procedure in rhesus monkeys to compare the reinforcing efficacy of DAT inhibitors that differed in their rates of distribution to the brain after systemic administration. Reinforcing efficacy was quantified as the highest break point maintained by each drug, and peak break point correlated with rate of brain distribution, such that cocaine had the fastest onset and highest break point, and slower onset DAT inhibitors had progressively lower break points.

One challenge to interpretation of these data is that drug self-administration rates can be influenced not only by reinforcing drug effects that increase response rates, but also by "reinforcement-independent rate-altering" drug effects (e.g., sedation or stereotypies) that can disrupt behavior and reduce response rates (Katz 1989; Meisch and Lemaire 1993; Negus and Banks 2011; Young and Herling 1986). Drugs with slow onsets also often have long durations of action, and if the long duration of a drug's reinforcement-independent rate-altering effects exceeds the duration of procedural parameters (e.g., the duration of intertrial intervals and/or limited holds in a progressive-ratio procedure) that might allow dissipation of those effects, then peak rates of drug self-administration may be constrained not by limits of reinforcing efficacy, but rather by sustained behavioral disruption produced by self-administered drug doses. A common manifestation of reinforcement-independent rate-altering effects is the descending limb of drug self-administration dose–effect curves. Although these descending limbs are generally less prominent under progressive-ratio schedules than under conventional fixed-ratio schedules with short post-reinforcement time outs, they can nonetheless constrain and confound breakpoint metrics of reinforcing efficacy (Roberts et al. 1999; Rowlett et al. 1996). Overall, the relative temporal parameters of a self-administration procedure can interact with the time course of self-administered drugs to yield potentially misleading metrics of reinforcement efficacy that underestimate abuse potential of drugs with slow onsets and correspondingly long durations of action.

Intracranial self-stimulation (ICSS) is another preclinical procedure that can be used to evaluate abuse-related behavioral effects and relative abuse potential of drugs (Carlezon and Chartoff 2007; Kornetsky and Bain 1992; Negus and Miller 2014; Watterson et al. 2014; Wise 1996). In ICSS, subjects are equipped with an electrode targeting a brain-reward region and trained to press a lever for pulses of electrical brain stimulation. Most drugs of abuse increase (or "facilitate") ICSS responding, and rewarding efficacy of a drug is indicated by the maximum degree of ICSS facilitation produced by any drug dose. Metrics of abuse-potential magnitude in drug self-administration and ICSS are highly correlated for drugs with a diverse range of pharmacological mechanisms of action (e.g., see Bauer et al. (2013) and Negus and Miller (2014)); however, the

impact of time course on expression of ICSS facilitation as a metric of rewarding efficacy and abuse-potential magnitude has not been systematically examined. Accordingly, one goal of the present study was to use an ICSS procedure in rats to compare the time course and magnitude of effects produced by cocaine, WIN-35428, and RTI-31, three of the four DAT inhibitors investigated in the Wee et al. (2006) drug self-administration study cited above. If slow drug onset resulted in reduced peak ICSS facilitation, this would support the broader hypothesis that slow onset is associated with reduced abuse potential. Alternatively, if peak ICSS facilitation did not vary across these drugs, this would argue against a relationship between onset rate and abuse-potential magnitude.

A second goal of the present study was to use dLight1.1 targeted to the nucleus accumbens (NAc) and in vivo photometry to evaluate the time course of changes in extracellular DA levels produced by the same three DAT inhibitors investigated in the ICSS procedure. In vivo photometry is a novel technique that permits evaluation of neurotransmitter dynamics with high temporal resolution. In this technique, modified G-protein-coupled receptors fused to fluorescent proteins can fluoresce upon ligand binding without activating intracellular signaling cascades, thus acting as genetically encoded sensors for neurotransmitter detection in vivo (Labouesse et al. 2020; Patriarchi et al. 2018; Sun et al. 2018). dLight1.1 is one such sensor that fluoresces upon DA binding (Patriarchi et al. 2018). dLight1.1 can be encoded in viral vectors and virally expressed in mesolimbic DA targets like NAc to permit photometric monitoring of drug-induced DA dynamics. Given the putative role of the mesolimbic DA pathway in mediating abuse-related effects in general and ICSS facilitation in particular, we hypothesized that these DAT inhibitors would show similar time courses to alter ICSS and the dLight1.1 photometry signal.

Materials and methods

Methods

Subjects Adult male and female Sprague–Dawley rats ($N = 24$; Envigo, Indianapolis, IN, USA) were individually housed with free access to food and water in the home cage and maintained on a 24-h light/dark cycle with lights on from 6:00 am to 6:00 pm. All experiments were performed during the light phase of the cycle. Animal-use protocols were approved by the Institutional Animal Care and Use Committee and complied with the National Research Council Guide for the Care and Use of Laboratory Animals.

Drugs Cocaine hydrochloride and WIN-35428 tartrate were provided by the National Institute on Drug Abuse Drug Supply Program (Rockville, MD, USA). RTI-31 tartrate was supplied by RTI International (Durham, NC, USA). All drug solutions were prepared in sterile bacteriostatic saline (Hospira Inc., Lake Forest, IL, USA) and administered by intraperitoneal (IP) injection. Drug doses are expressed as their salt form.

Intracranial self-stimulation procedures

Overview ICSS studies were conducted using equipment, surgical procedures, and training procedures described previously (Baird et al. 2021; Bonano et al. 2014; Davies et al. 2020; Johnson et al. 2018) (see Supplementary Material for additional details). Briefly, rats were implanted with electrodes targeting the medial forebrain bundle and trained to press a lever under a fixed-ratio 1 schedule for pulses of electrical brain stimulation. Daily sessions were composed of sequential, 10-min components. Each component consisted of ten 1-min frequency trials, and the brain-stimulation frequency decreased across trials from 158 to 56 Hz in 0.05 log increments.

The study compared the time course of effects produced by cocaine (1–10 mg/kg), WIN-35428 (0.1–0.32 mg/kg), and RTI-31 (0.032–0.1 mg/kg). Each drug and its saline control were evaluated in a separate cohort of rats ($n = 6–7$; 3–4 males and 3 females), and dose order within each group was randomized using a Latin-square design. Test sessions consisted of three daily baseline components followed first by IP injection of saline or a test-drug dose and then by 12 successive 10-min test components for the first 120 min. Additional test components beginning 300 and 1440 min post-injection were included for WIN-35428 and RTI-31.

Data analysis The primary dependent measure was the total number of stimulations delivered across all 10 frequencies during each 10-min component. To normalize these raw data, results for each test component in each rat were expressed as a percentage of the mean total stimulations per component earned during the baseline components for that rat on that day (%BL). During the initial 120 min of each session, when test components were presented continuously, we observed a modest but significant decline in responding over time after saline administration. To adjust for this declining baseline and isolate drug-induced changes in ICSS performance, data were further transformed into a "Difference Score," calculated as the $\%BL_{drug} - \%BL_{saline}$ at the corresponding time point.

Difference scores were averaged across rats and analyzed by repeated-measures ANOVA. A significant ANOVA was followed by Dunnett's post hoc test. The criterion for significance was $p < 0.05$. Data relating time on the X axis to "Difference Score" on the Y axis were also used to calculate four additional values for each drug: (1) maximum effect (E_{max}), (2) time of maximum effect (T_{max}), (3) time to 50% of maximum effect (T_{50}), and (4) potency to produce a Difference Score of 75 (ED_{75}). Values were considered to differ if 95% confidence limits did not overlap.

To provide a more granular analysis of ICSS data at selected times, we also evaluated the total number of stimulations during each 1-min frequency trial. To normalize these raw data, reinforcement rates from each trial in each rat were converted to percent maximum control rate (% MCR), with MCR defined as the mean maximal rate observed during the "baseline" components for that rat on that day. Normalized ICSS rates at each frequency were used to generate and compare "frequency-rate" curves at times of approximate peak effect for each drug.

In vivo photometry procedures

Surgery General details for stereotaxic surgery are provided in Supplementary Material. A Hamilton Neuros™ syringe and an electronic syringe pump (World Precision Instruments, Sarasota, FL) mounted into the stereotaxic frame was used to inject adeno-associated virus encoding dLight1.1 (pAAV-CAG-dLight1.1, Addgene cat # 111,067-AAV5 at a titer of 1.7×10^{13} genomic copies per ml) (Patriarchi et al. 2018). One microliter of virus was injected at 4 nl/s rate targeting the left NAc (1.5 mm posterior to bregma, 1.8 mm lateral to midline suture, and 7.0 mm ventral to skull surface) followed immediately by the implantation of a fiber-optic cannula (core diameter 400 μm, numerical aperture (NA) 0.66; Doric Lenses Inc., Quebec, Canada) at the same coordinates but at 6.3 mm ventral to skull surface.

Apparatus Excitation light from two light-emitting photodiodes (LEDs): M405L4 and M470L4 (Thorlabs Inc., Newton, NJ) bandpass filtered at 405/30 nm and 466/40 nm, respectively, was focused using a 0.54 NA multimode collimator (Thorlabs Inc., Newton, NJ) to a 0.57 NA Pigtailed 1×1 Fiber-optic Rotary Joint with an optical swivel (Doric Lenses Inc., Quebec, Canada). The fiber-optic cable was long enough to allow free exploratory behavior by the rat in the recording chamber. dLight1.1 emission was bandpass filtered at 525/39 nm and measured using a photomultiplier (PMT) (Photon Technology International Inc., Lawrenceville, NJ). The 405 nm LED and the 470 nm LED were amplitude modulated at 756 and 1197 Hz, respectively. The emitted signals were recovered using two dual-analog lock-in amplifiers (one per channel) (Lerner et al. 2015). A digitally controlled electric shutter was placed in the excitation path. Shutter control and analog signal digitalization were

performed using a Digidata 1322A controlled by Clampex 8 software (Axon Instruments, Inc., Foster City, CA). Figure S1 shows a schematic diagram of the photometry system. The dLight DA sensor was excited using light at two wavelengths, 405 nm and 470 nm, and light emission was detected at a common 525/39 nm single band. The 405 nm excitation corresponds to the isosbestic point of the sensor, insensitive to changes in DA concentration (Lerner et al. 2015; Patriarchi et al. 2018). In contrast, light arising from 470 nm excitation is proportional to the DA level (Patriarchi et al. 2018).

Testing Photometry testing was conducted in five males given that the ICSS studies indicated similar temporal parameters of drug effects in males and females. There were two inclusion criteria: (1) the presence of a spontaneous DA signal observed in the 470 nm channel and (2) a positive increase in DA fluorometric signal after 10 mg/kg cocaine IP injection. Before each test session, the autofluorescence of the photometer's fiber-optic array was photobleached for 4 h at full power. The light power was 0.40 and 0.35 mW/mm^2 for the 405 and 470 nm LEDs, respectively, at the end of the fiber-optic cable for the experiments. The fiber-optic cable was connected to the optic cannula, the rat was placed into an acrylic chamber ($30.5 \times 30.5 \times 37$ cm), the indicated drug (or saline) solution was administered IP, and the fluorometric response from the animal was recorded for 90 min measuring both channels (405 nm and 470 nm), simultaneously. Data acquisition was automated using episodic recording with Clampex 8.0 software. All settings were identical between trials.

Each rat was tested with drug doses that produced peak ICSS facilitation. Each rat was tested twice with each drug dose; however, technical issues in some trials required omission of a test. Additionally, separate saline control trials were conducted in association with each test drug dose in each rat, such that a drug trial was conducted at the beginning of the week, and a saline control trial was conducted 3 days later for each rat. The order for testing the three different drugs was randomized across rats.

Data analysis The 405 nm and 470 nm signals were expressed as a ratio (470 nm/405 nm). To assess the trajectory of drug effects across the entire 90-min recording period, data were averaged across each 30-s recording period to yield a mean point for every minute. An edge-artifact (when shutter opens and closes) was not included in the averaging. Data throughout each session in each rat were then normalized to the initial signal by dividing the mean ratio trace for each point by the first data point in that session ($F_{470/480}/F_0$). We observed a modest but significant decline in the signal after saline vehicle administration. To adjust for

this declining baseline, data were further transformed into a "Difference Signal," calculated as the mean drug dose signal minus the mean saline signal at the corresponding time point in that rat. The onset phase of the Difference Signal time course was fitted to a logistic and polynomial equations using Origin 2021 software. The logistic fittings were used to determine the maximal amplitude (E_{max}), and the time to reach 50% the maximal signal (T_{50}) of the trace. The polynomial fitting was used to determine time to reach E_{max} (T_{max}). Data were averaged across rats, and mean E_{max}, T_{50}, and T_{max} values were considered different if 95% confidence limits did not overlap.

To provide a more granular analysis of photometry data, the discrete DA transients (or events) observed within 30-s recording sweeps in the 470/405 trace were studied using Clampfit 10.2 software. The amplitude, width, and frequency of the events were measured on six sweeps (180 s total recording time) at the peak response time for each compound and at the corresponding time after saline injections. For each event, "amplitude" was defined as the magnitude of the transient measured from the starting value (immediately before the event began) to the peak, and "width" was defined as the time from the start to the end of the event (Figure S2). "Frequency" was defined as the number of events counted in 180 s expressed in Hz. Since event amplitude and width constitute multiple measurements per animal, comparison between test and control groups was performed using hierarchical statistics (nested t-test) (Eisner 2021; Sikkel et al. 2017). Frequency of events was a single measure per animal, and comparison between groups was performed using a paired t-test.

Immunostaining Brains were fixed using 4% paraformaldehyde in PBS by intracardiac perfusion. Subsequently, brain sections were incubated for 48 h with an anti-green fluorescent protein antibody conjugated to Alexa Fluor 546 (sc-9996 AF546, Santa Cruz Biotechnology Inc., Dallas, TX) at 4 °C. DAPI was used as counterstaining. After staining, the slices were mounted on slides using anti-fading media (ProLong Diamond Antifade, ThermoFisher Scientific). Images were acquired using an Olympus IX70 epifluorescence microscope ($4 \times$ objective) equipped with a digital EMCCD camera (Andor Technology).

Results

Intracranial self-stimulation

Baseline and saline-control ICSS data are shown in Figure S3. Figure 1 shows the time course of drug effects on ICSS expressed as Difference Scores for cocaine
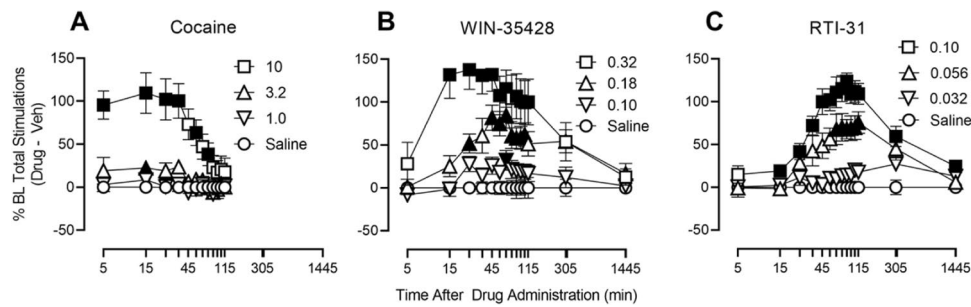
Fig. 1 Time course of ICSS difference scores for cocaine, WIN-35428, and RTI-31. Abscissae: Time in min (log scale) after administration of IP saline or test drug, with numerals indicating the midpoint of each 10-min component (e.g., the number "5" indicates the midpoint of the component conducted from 0 to 10 min after injection). Ordinates: Number of stimulations per component expressed as Difference Scores (%BL Drug − %BL Vehicle). Each data point represents the mean ± SEM of 6–7 rats, and filled points indicate a significant difference from saline vehicle at that time point using a repeated-measures two-way ANOVA (cocaine, RTI-31) or mixed-effects analysis (WIN-35428) followed by a Dunnett post hoc test ($p < 0.05$). Statistics for significant dose × time interactions were as follows: cocaine ($F(3.155, 15.78) = 7.244$; $p = 0.0026$), WIN-35428 ($F(3.062, 15.15) = 5.600$; $p = 0.0084$), RTI-31 ($F(4.123, 24.74) = 9.908$; $p < 0.0001$). A mixed-effects analysis was used for WIN-34528 because there was missing data for one rat at the 1445-min time point for doses of 0.10 and 0.18 mg/kg

(1–10 mg/kg), WIN-35428 (0.1–0.32 mg/kg), and RTI-31 (0.032–0.1 mg/kg). Table 1 shows E_{max}, T_{50}, and T_{max} values for the highest dose of each drug. All three drugs produced dose- and time-dependent ICSS facilitation with statistically similar E_{max} values; however, time courses differed. In general, cocaine had the fastest onset and shortest duration, whereas RTI-31 had the slowest onset and longest duration. T_{max} values for both cocaine and WIN-35428 were significantly faster than for RTI-31. T_{50} values had the same rank order as T_{max}, and T_{50} for WIN-35428 was significantly faster than for RTI-31; cocaine onset occurred in less than 5 min and was too rapid to determine a precise T_{50}. Higher doses were tested in a subset of rats for each drug (18 mg/kg cocaine, 0.56 mg/kg WIN-35428, and 0.18 mg/kg RTI-31), but these higher doses produced severe behavioral disruption (e.g., stereotypies) and maintained lower peak levels of ICSS facilitation. As a result, these higher doses were not tested further.

Figure 2 shows dose–effect data for all three test drugs at the approximate T_{max} time of peak effect. Regression analysis showed a significant linear dose–effect relationship for each drug, and ED_{75} values are shown in Table 1. WIN-35428 and RTI-31 were approximately 38 and 110 times more potent than cocaine, respectively, to facilitate ICSS. Each drug produced qualitatively similar dose-dependent leftward/upward shifts in ICSS frequency-rate curves.

Although this study was not designed a priori to investigate sex differences in drug effects, it did include both male and female rats, and Table S1 shows T_{50}, T_{max}, E_{max}, and ED_{75} values for each drug segregated by sex. For all three drugs, E_{max}, T_{50}, and T_{max} were similar in males and females, and 95% confidence limits overlapped. ED_{75} values were also statistically similar for cocaine and WIN-35428; however, for RTI-31, 95% confidence limits did not overlap, and the ED_{75} was lower in females.

In vivo photometry

Four to 5 weeks after surgery, rats showed clear spontaneous activity in the 470 nm DA-sensitive channel (Fig. 3A), whereas the 405 nm channel displayed less activity (Fig. 3B). The 405 nm signal accounts mostly for small

Table 1 ICSS difference score and photometry (dLight) difference signal data, mean (95% CI)

| ICSS	E_{max} (%BL Drug − %BL Veh)	T_{50} (min)	T_{max} (min)	ED_{75} (mg/kg)
Cocaine	122.6 (79.2–166.0)	< 5	13.0 (7.1–23.8)	5.4 (3.5–7.9)
WIN-35428	160.2 (113.5–207.0)	8.2 (4.3–15.7)	24.7 (16.6–36.8)	0.14 (0.11–0.17)
RTI-31	132.4 (107.0–157.8)	32.0 (28.4–36.1)	83.0 (75.0–91.7)	0.049 (0.039–0.058)
dLight	E_{max} (Drug − Veh) $\Delta[F_{(470/405)}/F_0]$	T_{50} (min)	T_{max} (min)	
Cocaine	0.068 (0.022–0.113)	1.5 (0.3–2.8)	11.9 (5.4–18.4)	
WIN-35428	0.072 (0.044–0.101)	8.5 (5.0–12.0)	39.8 (29.1–50.6)	
RTI-31	0.047 (0.022–0.071)	21.6 (18.1–25.1)	66.6 (50.4–82.7)	
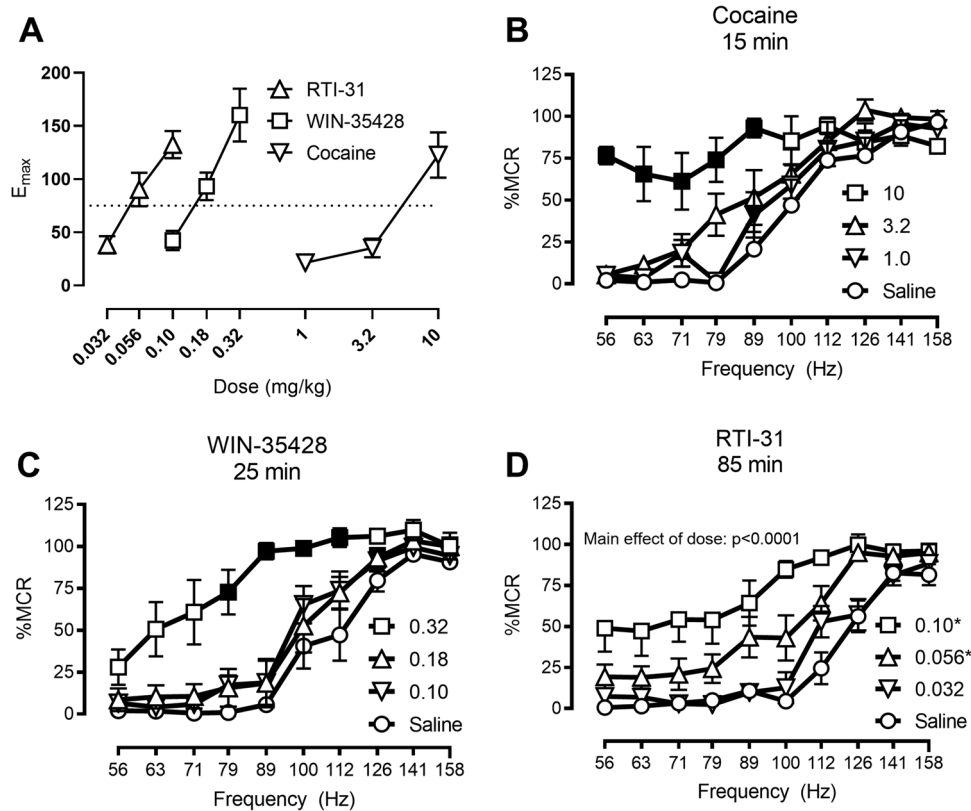
Fig. 2 Potency comparisons for effects produced by cocaine, WIN-35428, and RTI-31. Panel (**A**) shows dose–effect curves determined at the approximate T_{max} for each drug. Abscissa: Drug dose in mg/kg (log scale). Ordinate: E_{max} for each drug dose. Dotted line indicates the difference score used for relative potency comparisons (ED_{75}). Each point represents the mean $\pm$ SEM of 6–7 rats. Statistics for linear regressions are as follows: cocaine ($p = 0.0035$; $r^2 = 0.5901$), WIN-35428 ($p = 0.0001$; $r^2 = 0.6118$), RTI-31 ($p < 0.0001$; $r.^2 = 0.6012$). Panels (**B–D**) show frequency-rate curves for cocaine, WIN-35428, and RTI-31 during the component closest to their calculated T_{max}. Abscissae: Frequency of electrical brain stimulation in Hz (log scale). Ordinates: Number of stimulations per trial expressed as a percentage of daily maximum control rate (MCR). Each data point represents the mean $\pm$ SEM from 6 to 7 rats, and filled points represent frequencies at which ICSS rates were different compared to saline as determined by a repeated-measures two-way ANOVA followed by a Dunnett post hoc test ($p < 0.05$). Two-way ANOVA for frequency $\times$ dose interactions were as follows: cocaine ($F(4.028, 20.14) = 5.873$; $p = 0.0026$), WIN-35428 ($F(3.355, 16.77) = 3.922$; $p = 0.0242$), RTI-31 ($F(3.888, 23.33) = 2.651$; $p = 0.0600$). Although the frequency $\times$ dose interaction was not significant for RTI-31, there was a significant main effect of dose ($F(1.553, 9.316) = 40.88$; $p < 0.0001$). RTI-31 only: Asterisks indicate doses at which ICSS rates collapsed across all frequencies were different compared to saline as determined by a repeated-measures one-way ANOVA followed by a Dunnett post hoc test ($p < 0.05$). One-way ANOVA for dose $F(1.328, 7.968) = 51.98$; $p < 0.0001$

transitions resulting from motion of the optical swivel as the animal explored the chamber during the recording. To correct for both the movement artifact and the initial fast component of photobleaching once light is turned on (see Fig. 3A and B), a ratio was computed for signals between the 470 and 405 nm channels (Fig. 3C). This ratio signal shows a stable mean over the 30-s recording epoch while retaining the transitions related to spontaneous DA release. The traces show spontaneous transients or "events" of ~ 1 s duration (see zoom insert in Fig. 3C and Figure S2).

To quantify changes at a larger time scale suitable for tracking onset of drug effects, photometry determinations were performed once per minute for 90 min. At this time scale, a time-dependent decline in the ratio signal was evident after saline or drug administration (Fig. 3D). To control for the declining saline baseline and to isolate drug-induced changes in the ratio signal, drug data were normalized to vehicle data using the Difference Signal (Fig. 3E). Figure 3F shows the time course of change in Difference Signals produced by cocaine (10 mg/kg), WIN-35428 (0.32 mg/kg), and RTI-31 (0.1 mg/kg) doses that produced maximal ICSS facilitation. Table 1 shows E_{max}, T_{50}, and T_{max} data for each treatment. As in the ICSS studies, all three drugs produced time-dependent increases in the Difference Signal with similar E_{max} values but different rates of onset. Cocaine had the fastest onset (i.e., the lowest T_{50}, and T_{max}), whereas RTI-31 had the slowest onset (i.e., the highest T_{50}, and T_{max}).

Figure 4 shows the effect of cocaine (10 mg/kg), WIN-35428 (0.32 mg/kg), and RTI-31 (0.1 mg/kg) on the 470/405 ratio signal immediately after the IP injection (T_0) and at

the time of peak response (T_{max}). All three DAT inhibitors increased the signal amplitude at sweep T_{max} compared to sweep T_0, consistent with its increased mean value described in Fig. 3F. Conversely, control saline injections produce no change in signal amplitude at matching times (right panels in Fig. 4). Event analysis (see Figure S2) showed no significant change in the relative amplitude of the individual DA events, but events lasted longer in the presence of all three DAT inhibitors (Table 2 and Figure S4). Additionally, cocaine and WIN-35428 significantly decreased event frequency, and the effect of RTI-31 on event frequency approached significance (Table 2 and Figure S5). The decrease in event frequency reflected the presence of longer and/or fused DA events.

Comparison of ICSS and photometry data

Comparison of T_{50} and T_{max} values across procedures in Table 1 indicates that, in general, each drug had a similar time course for both ICSS facilitation and stimulation of the dLight photometry signal. The only exception was the T_{50} value for RTI-31, which was significantly faster in photometry than in ICSS. Figure 5 graphically compares the time courses of ICSS and photometry effects produced by 10 mg/kg cocaine, 0.32 mg/kg WIN-35428, and 0.1 mg/kg RTI-31. For each drug, results shown in Fig. 1 (ICSS) and in Fig. 3F (dLight) were normalized to % E_{max}. The photometry procedure here described was devised to yield a 10 × faster data-acquisition rate than the ICSS procedure. At this higher acquisition rate, photometry better resolved the onset rate for cocaine effects (Fig. 5A). However, despite the differences in sampling rates, the onset and duration of cocaine effects were closely aligned across procedures. The onset of WIN-35428 was slower than the onset of cocaine in both procedures (Fig. 5B), but again, the time courses of ICSS and photometry effects for WIN-35428 were closely aligned. The slowest onset in both procedures was produced by RTI-31 (Fig. 5C), and this drug also showed the greatest divergence in rates of onset across ICSS and photometry procedures, consistent with the faster T_{50} in photometry than in ICSS.

Discussion

This study compared effects of three DAT inhibitors with different time courses in assays of ICSS to evaluate abuse-related behavioral effects and in vivo photometry to evaluate extracellular DA dynamics. There were three main findings. First, ICSS distinguished onset rates of these drugs, but peak ICSS facilitation did not differ between drugs. Second, in vivo photometry with the dLight1.1 sensor targeting the NAc also distinguished onset rates of increased DA levels produced by these drugs, but again, peak photometry signals did not differ significantly between drugs. Lastly, there was

a close alignment of onset rates for ICSS and photometry effects of the fastest onset drug cocaine, but time courses appeared to diverge as onset rate became slower. These results show that ICSS and in vivo photometry can assess the onset rate and magnitude of abuse-related drug effects on behavior and mesolimbic DA signaling. Moreover, because slower onsets were not associated with reduced peak values in either procedure, these results do not support the hypothesis that slow onset reduces abuse-potential magnitude. Additionally, these results are consistent with a key role for DA in the NAc area as a mediator of drug-induced ICSS facilitation in rats.

Impact of drug time course on ICSS metrics of abuse potential Preclinical testing plays a key role in predicting abuse potential and guiding regulatory control of new psychoactive substances (FDA 2017). Drug self-administration is the most widely used family of procedures for preclinical abuse-potential testing, and positive reinforcing effects indicative of abuse potential depend on factors that include both the pharmacodynamic mechanism of drug action and the time course of drug effect (Ator and Griffiths 2003; Carter and Griffiths 2009; O'Connor et al. 2011). ICSS is an alternative approach that has similar predictive validity relative to drug self-administration across a wide range of pharmacodynamic mechanisms of action (Negus and Miller 2014). More specifically, results for intraperitoneally administered drugs from the present ICSS procedure in rats correlated with results from studies of intravenous drug self-administration under a progressive-ratio schedule in rhesus monkeys for a range of monoamine transporter substrates that varied in pharmacodynamic selectivity for DA and serotonin transporters (Bauer et al. 2013; Wang and Woolverton 2007; Wee et al. 2005). However, the role of time course has not been systematically examined as a determinant of abuse-related ICSS facilitation indicative of abuse potential. Accordingly, a major goal of this study was to compare drug effects in our rat ICSS procedure using a set of DAT inhibitors that have different time courses and that maintained different progressive-ratio breakpoints in rhesus monkeys (Wee et al. 2006).

In contrast to their different break points in a progressive-ratio drug self-administration procedure in monkeys (Wee et al. 2006), cocaine, WIN35428, and RTI-31 produced similar magnitudes of peak facilitation in the rat ICSS procedure. Thus, for these compounds with a shared mechanism of action but different time courses, peak magnitudes of ICSS facilitation did not correspond to peak break points in the drug self-administration procedure. Because slower onset was not associated with reduced ICSS facilitation as a metric of abuse-potential magnitude, the present results do not support the hypothesis that slower onset reduces abuse potential. Rather, these findings are consistent with other
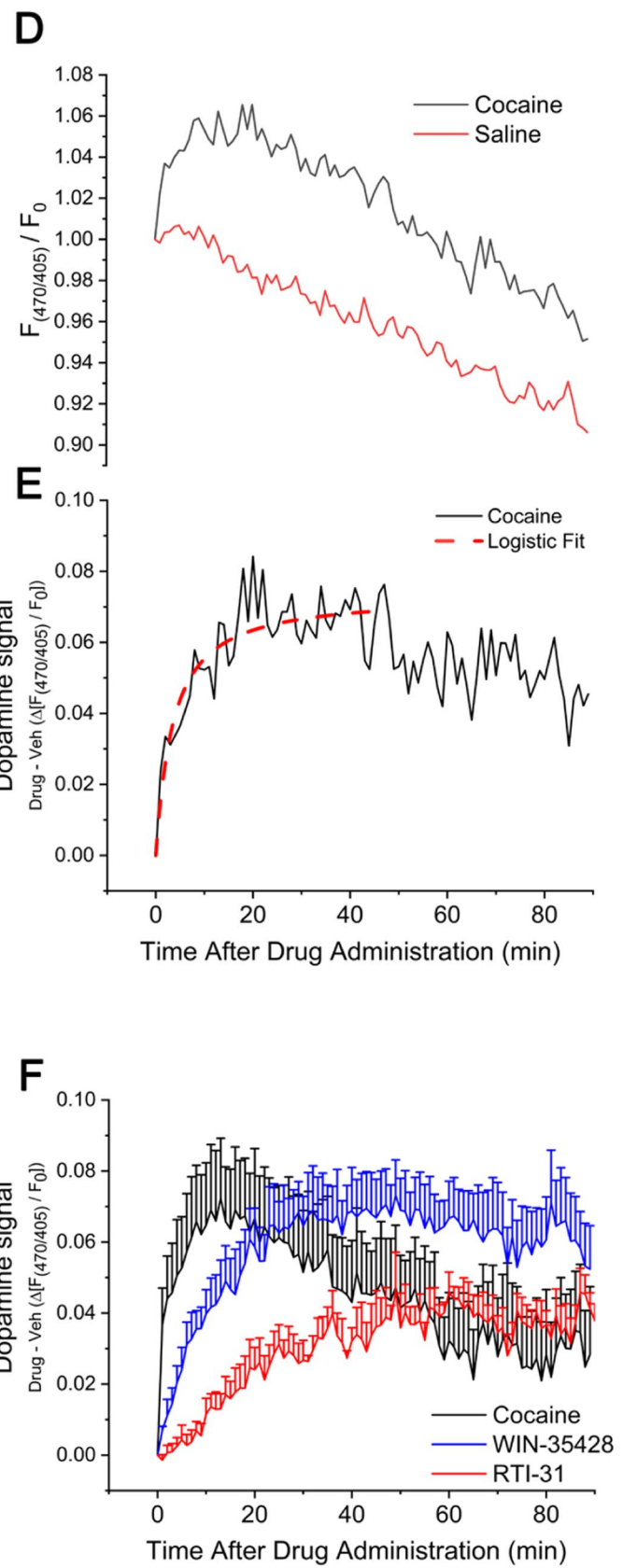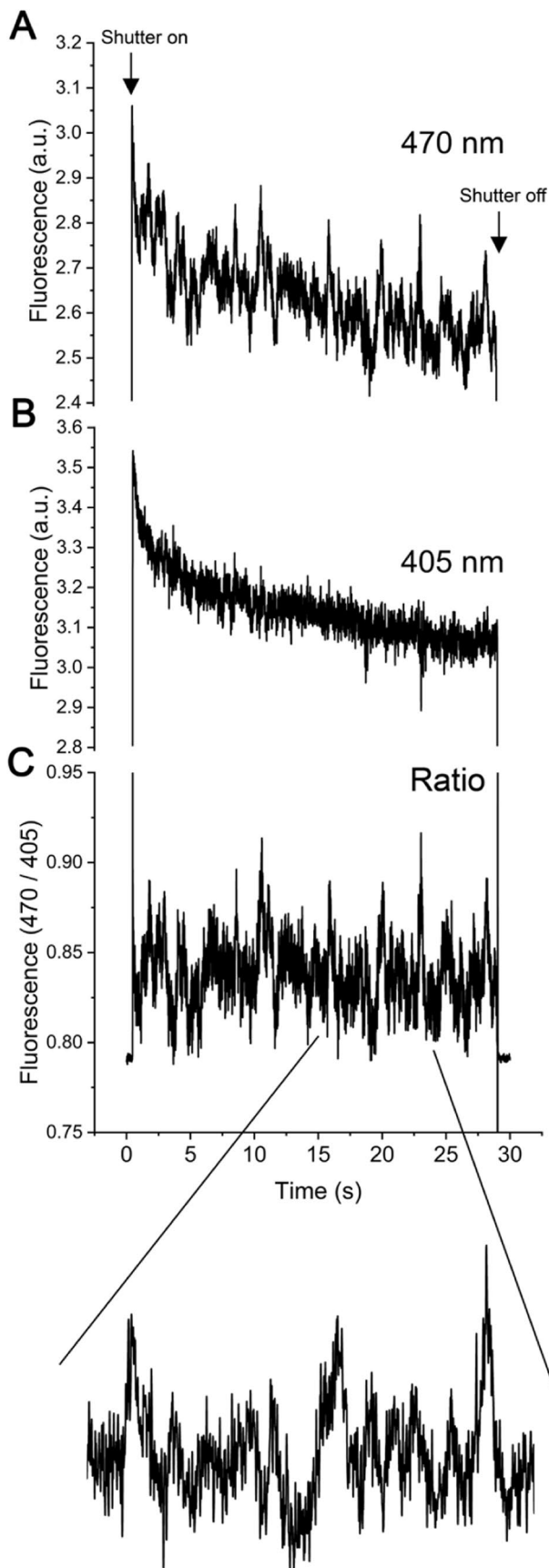
◄**Fig. 3** Measurement of dopamine signals using ratiometric photometry targeting the NAc in vivo. Four to 6 weeks after dLight1.1-AAV injection and optic canula implantation, DA signals were measured in awake and behaving rats. (**A**) Representative 30-s recording of the demodulated signal arising from the 470 nm excitation. (**B**) Simultaneous signal demodulated from the isosbestic 405 nm excitation. (**C**) The ratio between channels ($F_{470/405}$) serves as a "corrected" signal, revealing transients of ~ 1 s duration characteristic of spontaneous DA release (see insert). (**D**) dLight1.1 signals were recorded beginning immediately after cocaine (or saline) injection 90 min. Each 30 s sweep ($F_{470/405}$) was averaged to get a single intensity point per minute. The resulting trace was divided by the first point ($F_{470/405}/F_0$). (**E**) The "saline" trace was subtracted from the "cocaine" trace (cocaine–vehicle) yielding a new trace showing the mean DA changes along the full experimental time. A logistic fit (red dashed trace) was used to estimate T_{50} and E_{max} (see Table 1). (**F**) The experiments were performed for cocaine (black trace), WIN-35428 (blue trace), and RTI-31 (red trace) in 5 rats. The traces represent the mean + SEM. The maximal response (E_{max}), half time to maximal response (T_{50}), and time for maximal response (T_{max}) were calculated and are shown in Table 1

data to suggest that metrics of abuse potential in drug self-administration procedures are vulnerable to reinforcement-independent rate-altering effects (Katz 1989; Meisch and Lemaire 1993; Negus and Banks 2011; Young and Herling 1986) and may underestimate abuse potential of slow-onset long-acting drugs that produced sustained behavioral disruption (Roberts et al. 1999).

Although peak ICSS facilitation did not vary across the drugs tested here, it is relevant to note that ICSS readily permitted assessment of their distinct time courses. For example, the procedure used here determined ICSS frequency-rate curves every 10 min for the first 120 min, and included additional probes after 5 h and 24 h if necessary. This approach provided sufficient temporal resolution to differentiate the rates of onset and durations of action of the three drugs tested. Moreover, time courses for these drugs determined in the ICSS procedure correspond well with time courses assessed in rats and mice using other dependent measures such as locomotor activity (de Saint Hilaire et al. 1995; Hemby et al. 1995; Tolliver and Carney 1995), DAT binding (Wee et al. 2006), and DA release (see photometry
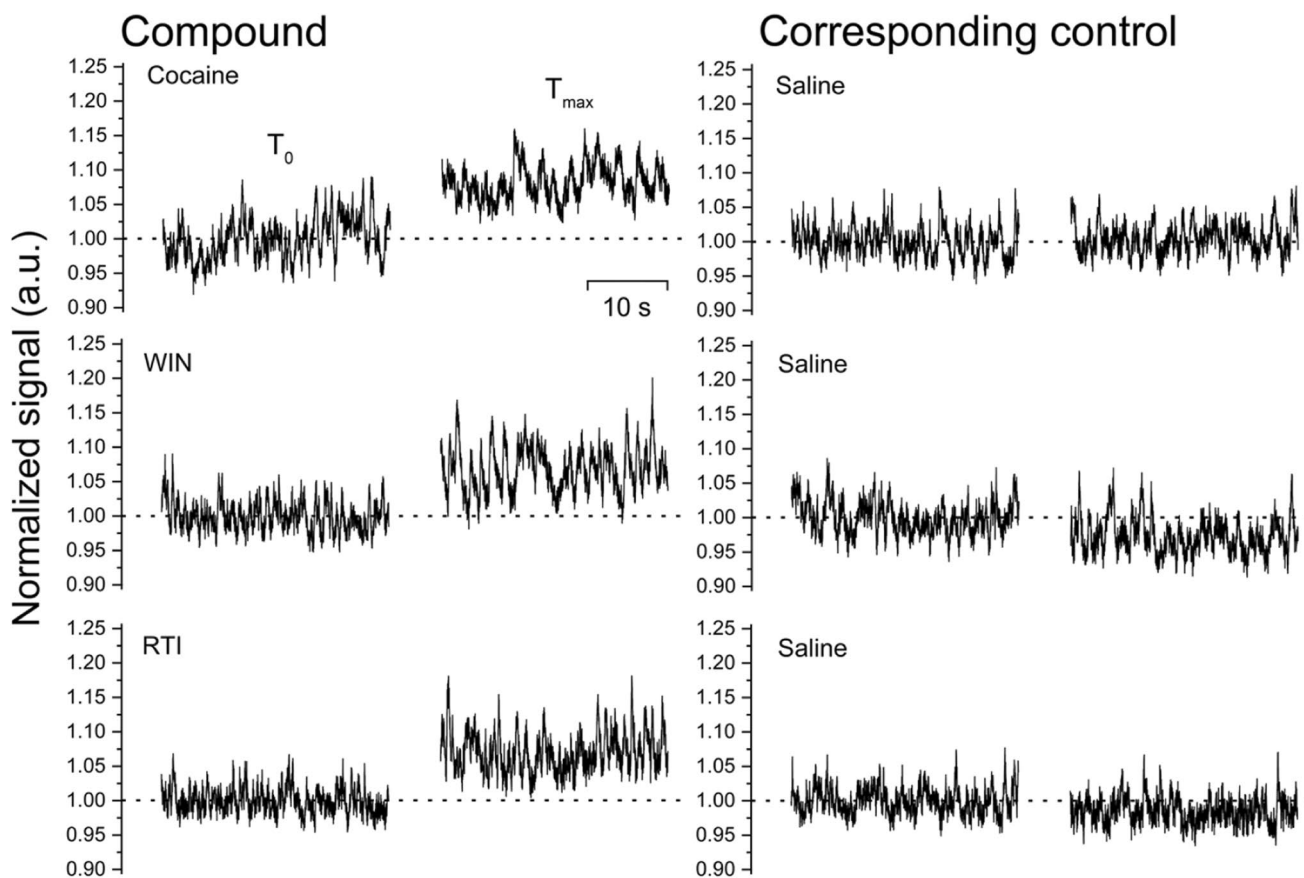

Fig. 4 Thirty second traces of the DA signal immediately after injection (T_0) and at the time of peak response (T_{max}). The 470/405 signals were normalized to the mean value of the T_0 sweep for comparison. The T_0 sweep and one T_{max} sweep are shown. Event analysis for the 8–15 min time band for cocaine, 30–35 min for WIN-35428 (WIN), and 60–65 min for RTI-31 (RTI) are shown in Table 2. The response for the same animal at a matched time after saline injection is shown in the right panels (corresponding control)

Table 2 Analysis of events detected using photometry (mean ± SD)

Time after injection (compound)	Amplitude (normalized)	Width (s)	Frequency (Hz)
8–13 min (cocaine)	0.993 ± 0.337 (N = 5; n = 422)	1.258 ± 0.758* (N = 5; n = 422)	0.469 ± 0.081[&&] (N = 5)
30–35 min (WIN)	1.125 ± 0.476 (N = 5; n = 581)	0.945 ± 0.584*** (N = 5; n = 581)	0.646 ± 0.060[&] (N = 5)
60–65 min (RTI)	1.131 ± 0.521 (N = 5; n = 561)	0.935 ± 0.622** (N = 5; n = 561)	0.623 ± 0.149[†] (N = 5)
Time after injection (matched control)	Amplitude	Width (s)	Frequency (Hz)
8–13 min (saline)	1.000 ± 0.406 (N = 5; n = 586)	0.899 ± 0.555 (N = 5; n = 586)	0.651 ± 0.058 (N = 5)
30–35 min (saline)	1.000 ± 0.411 (N = 5; n = 704)	0.677 ± 0.410 (N = 5: n = 704)	0.782 ± 0.115 (N = 5)
60–65 min (saline)	1.000 ± 0.387 (N = 5; n = 672)	0.658 ± 0.379 (N = 5; n = 672)	0.747 ± 0.151 (N = 5)

*$p = 0.0231$ vs control, nested t-test; **$p = 0.0027$ vs control, nested t-test; and ***$p = 0.0003$ vs control, nested t-test. [&]$p = 0.0204$ vs control, paired t-test; [&&]$p = 0.0093$ vs control, paired t-test; and [†]$p = 0.0579$ vs control, paired t-test. N = number of animals and n = number of events

data below). Thus, ICSS procedures permit assessment of both the time course and magnitude of abuse-related ICSS facilitation (Negus and Miller 2014).

ICSS studies included both male and female rats in accordance with National Institutes of Health Guidelines (Miller et al. 2017); however, these studies were not intended or powered to determine sex differences in drug effects, so any conclusions regarding the role of sex as a determinant of drug effects are only tentative. In general, the DAT inhibitors studied here tended to have higher potencies and E_{max} values in females than in males, but this difference reached our criterion for significance only for the potency of RTI-31. An earlier study that conducted a careful and fully powered assessment of cocaine effects on ICSS in male and female rats also found no sex differences (Stratmann and Craft 1997).

Temporal resolution of photometry measures of DA in the NAc area ICSS facilitation is mechanistically driven by the augmentation of DA levels in the NAc (Negus and Miller 2014). Thus, DA elevation in this brain area upon psychostimulant administration should precede or match the kinetics of ICSS facilitation onset. Studies in animals using microdialysis techniques showed that administration of DAT inhibitors like cocaine produce a robust increase in NAc DA (Carboni et al. 1989; Johnson et al. 2018; Nicolaysen et al. 1988). However, microdialysis has poor temporal resolution limited by the slow diffusion of solutes through the dialysis membrane and a slow sampling rate. Indeed, DA detection using microdialysis after cocaine administration often appears slower than ICSS facilitation (Johnson et al. 2018). Fast-scan cyclic voltammetry offers better temporal
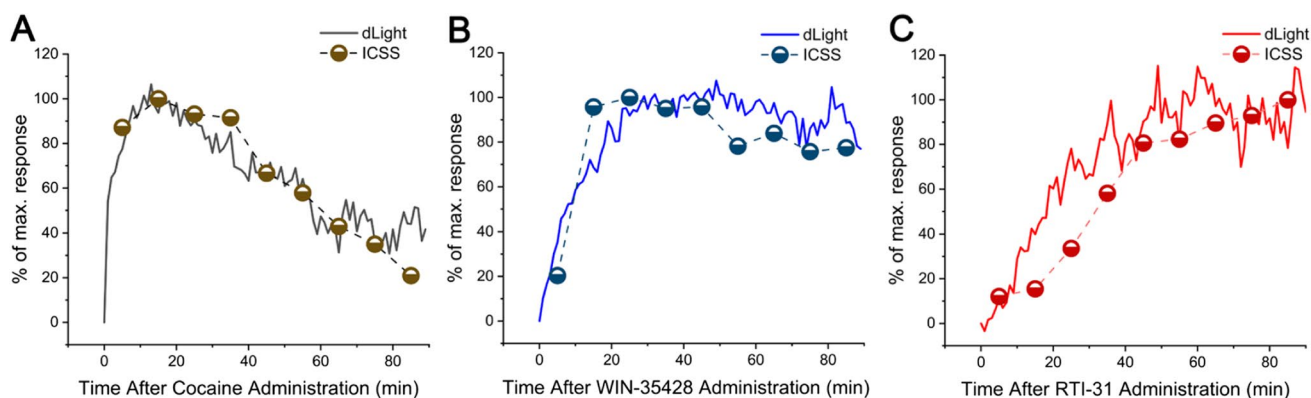

Fig. 5 The expression of abuse-related behavior (ICSS facilitation) matches the time course of increased DA levels in the NAc (dLight) in awake rats. Overlay of ICSS and dLight results shown in Figs. 1 and 3F, respectively. The mean values obtained for (**A**) cocaine (IP, 10 mg/kg), (**B**) WIN-35428 (IP, 0.32 mg/kg), and (**C**) RTI-31 (IP, 0.1 mg/kg) were expressed as a percentage of the maximal response for each trace, and ICSS and dLight responses were plotted together as function of time. ICSS and dLight experiments were performed in different groups of rats (ICSS N = 6–7 rats and dLight N = 5 rats)

resolution for detection of DA dynamics in brain. However, this procedure is used primarily to study DA dynamics in slice preparations (Brodnik et al. 2017; George et al. 2021; Jones et al. 1996) and in anesthetized animals (Gomez et al. 2021; Oleson et al. 2009; Yuen et al. 2021). The use of this procedure in awake, behaving animals is technically challenging, but several groups have used it for in vivo determinations (Ebner et al. 2010; Fortin et al. 2015; Heien et al. 2005; Oleson et al. 2009; Stuber et al. 2005). Using fast-scan cyclic voltammetry, Heien et al. (2005) described a fast tonic increase of NAc DA with a $T_{50} \sim 0.5$ min after intravenous cocaine administration in awake rats (Heien et al. 2005), consistent with our results obtained using dLight photometry and IP cocaine administration. Thus, dLight photometry affords high temporal resolution better than microdialysis and similar to that provided by fast-scan cycle voltammetry.

All three test drugs increased the spontaneous event duration (or "width"), which is consistent with an inhibition of DA reuptake by these drugs. The tonic increase of extracellular [DA], experimentally measured as an elevation of the 470/405 signal amplitude (Fig. 4), can directly result from the DA reuptake inhibition. Other indirect effects of these drugs such as longer (or more frequent) busting behavior of DA neurons may also contribute to longer DA releasing events; future experiments measuring extracellular DA and intracellular $[Ca^{2+}]$ in DA neurons, simultaneously, may help to determine the role DA neuron excitability in tonic and phasic effects produced by these reuptake inhibitors in vivo.

To better estimate tonic changes in DA after drug administration, we devised a protocol averaging data of 30-s recordings each minute for 90 min after injection. Cocaine was the fastest, followed by WIN-35428, and RTI-31 was the slowest at increasing the DA signal, showing the same rank order as values observed for ex vivo DAT binding reported by Wee et al. (2006) in rats. Cocaine showed $\sim$ ninefold faster T_{50} using dLight vs. ex vivo binding, but the slower onset drug RTI-31 had a T_{50} only twofold faster in dLight. This suggests that euthanasia and ex vivo tissue processing required to assess DAT binding kinetics may delay metrics of drug-effect onset, and this delay may be especially prominent for rapid-onset drugs (Heien et al. 2005).

Alignment of ICSS and photometry measures The temporal parameters for drug effects on ICSS facilitation and DA using dLight targeted to the NAc were closely aligned. All compounds elevated DA at the same time or earlier than they facilitated ICSS, consistent with the hypothesis that increased tonic DA is necessary for ICSS facilitation (Negus and Miller 2014). The two fastest agents (cocaine

and WIN-35428) showed overlapping kinetics across both procedures. For the slower acting drug (RTI-31), the T_{50} value for ICSS facilitation was delayed by $\sim$ 10 min compared to the dLight response. The basis for this delay requires further study; however, one possibility is that more gradual increases in DA may engage mechanisms of acute tolerance (Bradberry 2002; Maisonneuve and Kreek 1994) that limit effect magnitude before drug effects have peaked. Such mechanisms may have contributed to the non-significant trend for a lower dLight E_{max} by RTI-31 and a delayed expression of ICSS facilitation relative to increased NAc DA.

Summary Overall, these results provide additional evidence that tonic drug–induced increases in DA drive ICSS facilitation in rats. In addition, in vivo studies using both (a) ICSS facilitation as a behavioral measure and (b) DA dynamics assessed with genetically encoded fluorescent sensors as a neurochemical measure offer temporal resolution sufficient to characterize the rate of onset for abuse-related drug effects. This onset rate can contribute to preclinical predictions of reinforcing effects and abuse potential of drugs.

Declarations

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