Brain dopamine and serotonin differ in regulation and its consequences

Parastoo Hashemi[a], Elyse C. Dankoski[b], Rinchen Lama[a], Kevin M. Wood[a], Pavel Takmakov[a], and R. Mark Wightman[a,b,1]

[a]Department of Chemistry and [b]Curriculum in Neurobiology, University of North Carolina, Chapel Hill, NC 27599

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Dopamine and serotonin (5-hydroxytryptamine or 5-HT) are neurotransmitters that are implicated in many psychological disorders. Although dopamine transmission in the brain has been studied extensively in vivo with fast scan cyclic voltammetry, detection of 5-HT using in vivo voltammetric methods has only recently been established. In this work we use two carbon-fiber microelectrodes to simultaneously measure dopamine release in the nucleus accumbens and 5-HT release in the substantia nigra pars reticulata, using a common stimulation in a single rat. We find that 5-HT release is profoundly restricted in comparison with dopamine release despite comparable tissue content levels. Using physiological and pharmacological analysis, we find that 5-HT transmission is mostly sensitive to uptake and metabolic degradation mechanisms. In contrast, dopamine transmission is constrained by synthesis and repackaging. Finally, we show that disruption of serotonergic regulatory mechanisms by simultaneous inhibition of uptake and metabolic degradation can have severe physiological consequences that mimic serotonin syndrome.

electrical stimulation | monoamine oxidase inhibitor | selective serotonin reuptake inhibitor

Dopamine and serotonin (5-hydroxytryptamine or 5-HT) are neurotransmitters with important, conserved roles in the vertebrate nervous system. Dopamine is important in neuronal circuitry that controls reward and in brain regions that regulate movement (1). Assigning a specific functional role to 5-HT has proven more difficult because electrophysiological recordings of 5-HT neurons reveal unchanged firing in response to most stimuli (2). Competing roles have been suggested for dopamine and 5-HT in reward circuitry, with dopamine signals predicting positive stimuli and 5-HT signals predicting negative consequences (3, 4). Biochemically, their regulation is quite similar, with similar proteins regulating synthesis, storage, release, uptake, and metabolism. To compare functional dopamine and 5-HT regulation in the brain, methods have been developed to monitor dynamic changes in their concentrations in the extracellular space.

Transient fluctuations of dopamine concentrations in the extracellular space of the nucleus accumbens core (NAc) can be evoked by electrical stimulation of the medial forebrain bundle (MFB) and have been characterized in the rat using in vivo voltammetric methods (5, 6). Dopamine is easily oxidized, and electrochemical methods such as fast-scan cyclic voltammetry can be used for detection (7). A carbon-fiber microelectrode is placed in the brain region of interest. The shape of the cyclic voltammogram identifies dopamine, and its amplitude can be used to calculate concentration. Each cyclic voltammogram can be collected in less than 10 ms, enabling fast, repetitive acquisition. Successive recordings of fluctuations in dopamine concentration lead to visualization of dopaminergic transmission events with subsecond temporal resolution. This method has shown that electrical stimulations of dopaminergic axons immediately evoke dopamine release that is rapidly uptaken by the dopamine transporter (DAT). During behavior, receipt of unexpected rewards (8) or cues that predict reward (9) result in transient changes in dopamine concentration. These transients arise from the spontaneous firing of dopaminergic neurons in the ventral tegmental area and are regulated by the same mechanisms as electrically evoked release (10).

Similar subsecond characterizations of 5-HT transmission are possible because 5-HT is also easily oxidized. However, voltammetric detection of 5-HT is complicated by oxidative products that foul the electrode surface, lowering sensitivity and temporal resolution (11). Furthermore, 5-hydroxyindole acetic acid (5-HIAA), the primary metabolite of 5-HT, can similarly degrade the electrode surface. To minimize these issues, we have modified the detection waveform to prevent formation of oxidation products that foul the electrode surface. We also electrodeposit Nafion, a cation exchange polymer, on the carbon fiber to decrease sensitivity to 5-HIAA (12, 13). Using these modifications, we have demonstrated that stimulated 5-HT release can be measured in the substantia nigra pars reticulata (SNr) by stimulating the MFB (14) at a location that also evokes dopamine release (5).

This work characterizes and compares factors that regulate extracellular concentrations of 5-HT and dopamine. In-house hardware and software developments enable application of two different waveforms at separate electrodes in different brain regions (15). Using simultaneous dopamine and 5-HT measurements evoked by a common stimulation, physiological and pharmacological manipulations of release amplitude and uptake properties were evaluated. We find that distinctly different processes govern dopamine and 5-HT dynamics. Taken together, these results show that mechanisms controlling 5-HT in the extracellular space are more stringent than those of dopamine.

Results

Simultaneous Dopamine and 5-HT Release Evoked by a Common Stimulation. A carbon-fiber microelectrode was placed in the NAc of each rat, and the dopamine waveform was applied. A second microelectrode was placed in the ipsilateral SNr and used the 5-HT waveform. Both waveforms are illustrated in Fig. 1, *Left*. Release was evoked in both brain regions by electrically stimulating the ipsilateral MFB (60 Hz, 2-s duration, biphasic pulses, 2 ms each phase, 350 μA), shown in the color plots in Fig. 1. Each color plot encodes 300 cyclic voltammograms recorded over 30 s around the stimulation (initiated at 5 s, vertical black line). The shape of the cyclic voltammograms (upper current–voltage curve, Fig. 1, *Left*) recorded in the NAc and SNr identify dopamine and 5-HT, respectively. Strikingly, the amplitude of stimulated dopamine is ∼300 times greater than that of 5-HT. The uptake rates of the neurotransmitters are more comparable. Dopamine and 5-HT transporters follow Michaelis-Menten kinetics. At low concentrations the rate constant for dopamine

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[1]To whom correspondence should be addressed. E-mail: rmw@unc.edu.

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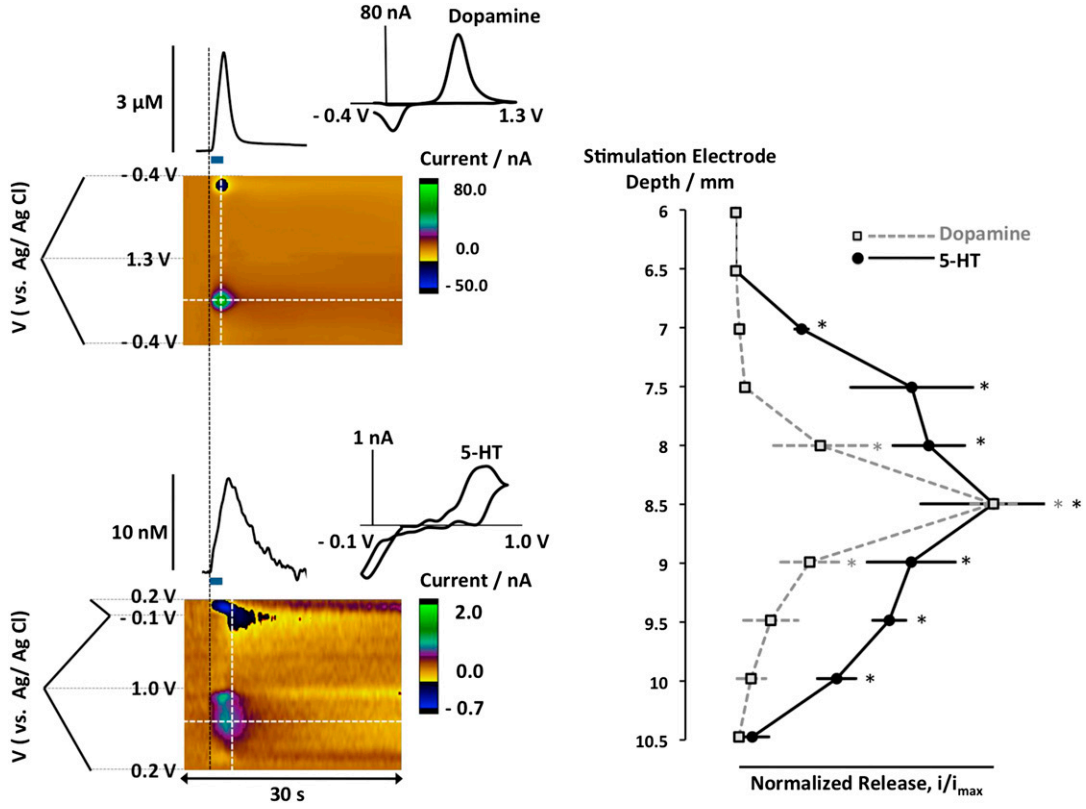

Fig. 1. *Left*: Representative color plots with potential on the *y* axis, time on the *x* axis, and the current in false color. Horizontal white dashed line was used to construct the current vs. time traces plotted above the color plots. Stimulation onset (dashed black vertical line) and duration is represented by the blue bar under the current vs. time traces. *Insets*: Cyclic voltammograms taken at the vertical white dashed lines. *Right*: Averaged, normalized responses recorded in the NAc (dopamine) and SNr (5-HT) as the stimulating electrode was lowered down a vertical tract to the MFB ($n = 6$). Asterisks indicate normalized 5-HT maximal release values that are significantly different from the corresponding dopamine normalized release values ($P < 0.05$).

uptake in the NAc ($k = V_{max}/K_m$) is ~14 s^{-1} (16), and in the SNr, it is ~4 s^{-1} for 5-HT uptake (14).

We first examined dopamine and 5-HT release as a function of the stimulating electrode's dorsoventral position. Fig. 1, *Right* shows the normalized amplitude of evoked 5-HT in the SNr and dopamine in the NAc obtained at varying stimulating electrode locations as it was lowered in 0.5-mm increments from −6 to −10.5 mm through the MFB. Stars indicate that significant release was evoked in that location ($P < 0.05$). Both 5-HT and dopamine release were maximal with the stimulating electrode at −8.5 mm. The 5-HT response profile was broader, with measurable release at stimulation electrode depths between 7.0 and 10.0 mm below dura, whereas dopamine could only be recorded between 8.0 and 9.0 mm below dura.

Effect of Stimulation Parameters on Release. We varied stimulus parameters in the MFB to examine their effects on release relative to that obtained with our maximal conditions (depth, 8.5 mm from dura, 60 Hz: 2-s duration, biphasic pulses, 2 ms each phase, 350 µA). Dopamine and 5-HT release are both sensitive to stimulus pulse width (5), reaching a maximum at 2 ms (Fig. 2*A*). Dopamine release increases with stimulus intensity up to 350 µA with 2-ms-wide pulses (17), and 5-HT responds similarly (Fig. 2*B*). Stimulated dopamine release diminishes when stimulations are repeated rapidly (18). Using maximal stimulation trains repeated every minute, we found that dopamine release decreased with consecutive stimulations, dropping to 38% ± 7% of the maximum normalized value ($n = 6$, $P < 0.05$) after the 20th stimulation (Fig. 2*C*). In contrast, maximal 5-HT release did not show signs of depletion.

Pharmacology of Synthesis, Packaging, Release, Uptake, and Metabolism. Various aspects of dopamine transmission, including synthesis (5), packaging (19), autoreceptor regulation of release (6), uptake (16, 20), and metabolism (21), were characterized with pharmacology and in vivo voltammetry. In this study we compared the dopaminergic and serotonergic responses to these manipulations. The averaged predrug and drug responses of stimulated 5-HT release are shown in Figs. 3 and 4 (*Top*), and the responses for dopamine to equivalent treatments are shown immediately below (*Upper Middle*). Histograms with amplitude and $t_{1/2}$ (both expressed relative to predrug value) are displayed in Figs. 3 and 4 (*Lower Middle* and *Bottom*), respectively. Where the response to pharmacological treatment is significantly different from predrug, statistical significance ($P < 0.05$) is noted with an asterisk. Stimulation duration is indicated by horizontal bars below each concentration vs. time plot.

We investigated the role of synthesis on dopamine and 5-HT release by administering NSD 1015 (100 mg kg^{-1}), an aromatic amino acid decarboxylase inhibitor (Fig. 3, *Left*). Inhibition of decarboxylase results in accumulation of L-DOPA and 5-hydroxytryptophan (22). Sixty minutes after NSD 1015 administration, dopamine release was reduced to 18.0% ± 4.5% of its original amplitude ($n = 6$, $P < 0.05$). In contrast, 5-HT release was reduced to 48.1% ± 2.8% of its pretreatment amplitude at the same time point ($n = 6$, $P < 0.01$). There were no significant effects of NSD 1015 on the $t_{1/2}$ of dopamine or 5-HT.

Dopamine and 5-HT are both released via exocytosis from vesicular stores. We investigated the significance of rapid vesicular packaging in both systems by comparing the effects of a vesicular monoamine transporter 2 (VMAT2) inhibitor, tetrabenazine (10 mg kg^{-1}) (23, 24). Tetrabenazine significantly reduced dopamine

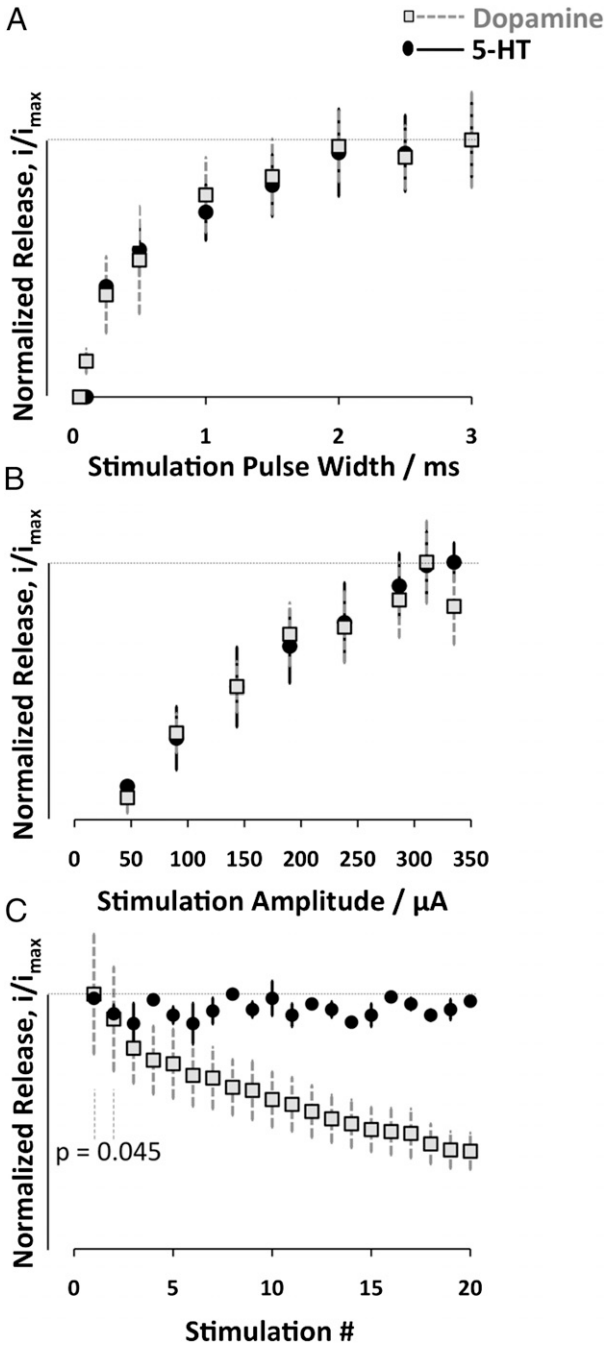

Fig. 2. Averaged, normalized dopamine and 5-HT maximal release with (*A*) variation of stimulation pulse width and (*B*) stimulation current amplitude ($n = 6$ for each transmitter). (*C*) Averaged, normalized dopamine and 5-HT responses to repeated stimulation trains ($n = 6$). Stimulations were 120 biphasic pulses at 60 Hz, 350 μA, and 2 ms delivered once every 60 s for 20 min.

release to 6.3% ± 3.0% of its predrug value 60 min after its administration ($n = 5$, $P < 0.05$; Fig. 3, *Center*). Effects on 5-HT release were not significant (71.6% ± 8.8% of predrug value; $n = 5$). However, the time delay between stimulation onset and maximum signal increased from 2.21 ± 0.15 s to 3.52 ± 0.34 s ($n = 5$, $P < 0.05$) after tetrabenazine administration. Tetrabenazine reduced dopamine release close to the limit of detection; therefore, $t_{1/2}$ analyses for statistical significance were not possible. The $t_{1/2}$ of 5-HT was significantly increased, from 1.9 + 0.25 s to 2.59 ± 0.43 s (135% ± 21% of original; $n = 5$, $P < 0.05$).

To examine autoreceptor control of release at 5-HT terminals in the SNr, we administered methiothepin (20 mg kg^{-1}), a nonselective 5-HT 1a and 1b receptor antagonist (25). Stimulated 5-HT release increased to 161% ± 10% of its original (Fig. 3, *Right*) ($n = 6$, $P < 0.05$). We administered raclopride, a D2 receptor antagonist, to examine the effects of autoreceptors in the dopaminergic system (26). Raclopride (2 mg kg^{-1}) increased stimulated dopamine release to 184% ± 34% ($n = 6$, $P < 0.05$) of the predrug value. Although raclopride had no significant effects on $t_{1/2}$ for dopamine, methiothepin significantly increased $t_{1/2}$ for 5-HT, from 1.36 ± 0.17 s to 1.99 ± 0.24 s ($n = 6$, $P < 0.05$).

Fig. 4, *Left* shows the effects of inhibition of the 5-HT uptake transporter (SERT) and DAT. SERT was inhibited with the selective serotonin reuptake inhibitor citalopram (10 mg kg^{-1}) (27), whereas GBR 12909 (15 mg kg^{-1}) was used to inhibit DAT (28). Citalopram significantly increased stimulated 5-HT amplitude, to 476% ± 134% of its original value ($n = 6$, $P < 0.05$) and significantly increased $t_{1/2}$, from 2.27 ± 0.07 s to 7.35 ± 1.46 s ($n = 6$, $P < 0.05$). GBR 12909 significantly increased stimulated dopamine amplitude, to 279% ± 70% of its predrug value ($n = 6$, $P < 0.05$) and significantly increased $t_{1/2}$, from 0.86 ± 0.14 s to 2.12 ± 0.2 s ($n = 6$, $P < 0.05$).

Fig. 4, *Right* compares the effects of monoamine oxidase (MAO) inhibition with pargyline (75 mg kg^{-1}) on dopamine and 5-HT–stimulated release (21). 5-HT release amplitude increased to 349% ± 60% of its original value ($n = 6$, $P < 0.05$), whereas dopamine release did not increase significantly (175.1% ± 27.3%; $n = 6$, $P > 0.05$). There were no significant effects of pargyline on dopamine $t_{1/2}$; however, the 5-HT $t_{1/2}$ significantly increased, from 1.58 ± 0.07 s to 2.97 ± 0.49 s ($n = 6$, $P < 0.05$).

SSRI and MAOI Administration. Fig. 5 shows spontaneous efflux of dopamine (Fig. 5*A*) and 5-HT (Fig. 5*B*) occurring after respiratory arrest caused by administration of citalopram (10 mg kg^{-1}) followed by pargyline (150 mg kg^{-1}). The concentration of

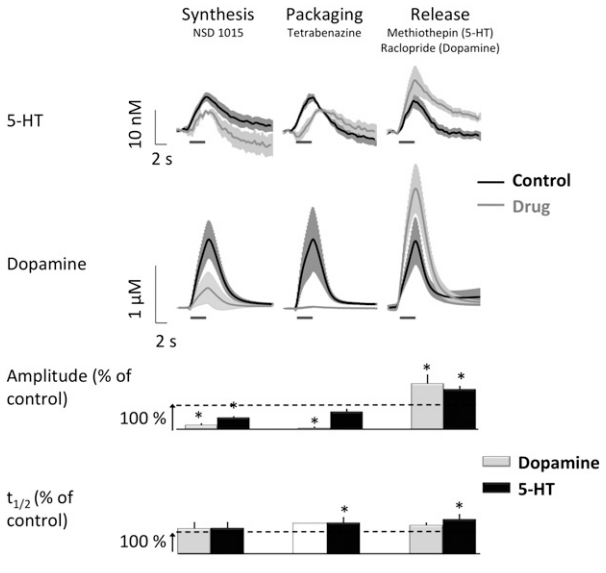

Fig. 3. Comparison of averaged responses of 5-HT in the SNr (*Top*; $n = 5$) and dopamine in the NAc (*Upper Middle*; $n = 6$) to pharmacological inhibition of synthesis, packaging, and release (left to right). Synthesis was inhibited with NSD 1015, and vesicular packaging was inhibited with tetrabenazine. Release was increased by autoreceptor antagonists (methiothepin for 5-HT and raclopride for dopamine). *Lower Middle* and *Bottom*: Histograms of the percent change in amplitude and $t_{1/2}$ induced by the drug 60 min after its administration. Asterisks on the histogram indicate a significant change from predrug values ($P < 0.05$).

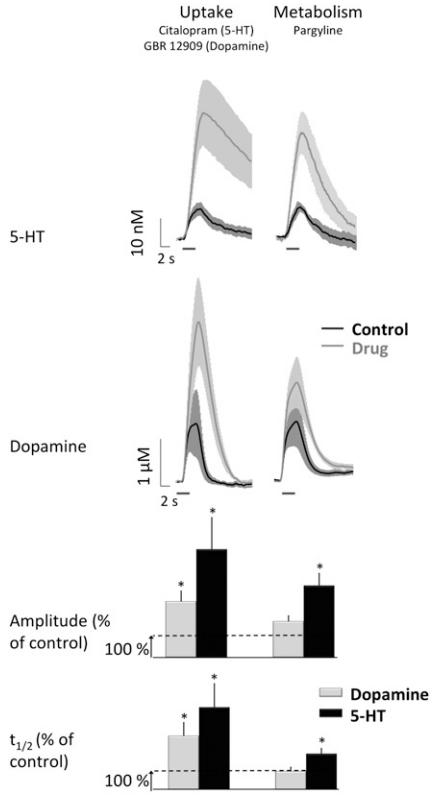

Fig. 4. Comparison of averaged responses of 5-HT in the SNr (*Top*; $n = 5$) and dopamine in the NAc (*Upper Middle*; $n = 6$) to pharmacological inhibition of uptake and metabolism by MAO. Uptake was inhibited by GBR 12909 for dopamine and citalopram for 5-HT. Pargyline inhibited MAO and is effective for both neurotransmitters. *Lower Middle* and *Bottom*: Histograms displaying the percent change in amplitude and $t_{1/2}$. Asterisks on the histogram indicate a significant change from predrug values ($P < 0.05$).

dopamine efflux at its peak averaged 37.7 ± 3.4 μM ($n = 4$), whereas 5-HT efflux was significantly lower at 0.296 ± 0.080 μM ($n = 4$, $P < 0.001$) (Fig. 5*C*). The time elapsed between onset and peak of efflux also differed significantly, averaging 77 ± 4 s for dopamine and 266 ± 50 s for 5-HT ($n = 4$ for each, $P < 0.01$) (Fig. 5*F*). Fig. 5 *D* and *E* compare responses of body temperature and heart rate between anesthetized rats that received electrical stimulation and two injections of saline 35 min apart ($n = 4$, white) or electrical stimulation followed by citalopram (T1) and pargyline (T2) ($n = 6$, black). Pargyline administration coincides with a significant decrease in body temperature (Fig. 5*D*) and heart rate (Fig. 5*E*) that persists throughout the remainder of the experiment ($P < 0.05$).

Discussion

5-HT Release Regulation Is More Stringent than for Dopamine. In this work electrical stimulation of the MFB simultaneously evoked dopamine release in the NAc and 5-HT release in the SNr. Stimulation at a common site that releases different neurotransmitters provides a way to compare dynamic alterations in their regulatory mechanisms (29). A particularly noteworthy finding was that dopamine release was ~300 times greater than 5-HT release, despite similar tissue content in the two regions examined [90 ng/mg protein for dopamine in the NAc (16) and 21 ng mg^{-1} protein for 5-HT in the SNr (30)]. Thus, despite comparable stores, the releasable pool of 5-HT in the SNr is miniscule compared with the releasable pool of dopamine in the NAc. Distinct storage and releasable pools for both 5-HT and dopamine have been described (31, 32), but the large difference in their relative size had not previously been appreciated. Moreover, we previously noted that evoked release of 5-HT in the SNr is much lower in vivo compared with its electrically evoked release from SNr slice preparations (14). This suggests that in vivo 5-HT release is subject to tighter control mechanisms than both 5-HT in slices and dopamine in vivo.

To identify possible mechanisms for these differences, we explored the effects of the electrical stimulation on the dopamine and 5-HT axons that course through the MFB. Dopaminergic and serotonergic fiber bundles responded similarly to the stimulation parameters tested, giving greater release with wide electrical pulses and large current amplitudes (Fig. 2). These properties are consistent with our predictions, given that both fibers are unmyelinated (33, 34). Varying the dorsoventral location of the stimulating electrode revealed that 5-HT release can be evoked over greater region than dopamine, suggesting that serotonergic fibers have a broader topographic distribution. However, this is not sufficient to explain the 300-fold greater release of dopamine because even direct stimulation of serotonergic cell bodies in the dorsal raphe evokes comparably low 5-HT release (14).

The disparity between 5-HT and dopamine release amplitudes is attributed to differences in the readily releasable pool. Dopamine release was sensitive to repetitive application of stimulation trains, exhibiting a fatigue in release that did not occur for 5-HT in the SNr. The diminished release of dopamine after repeated stimulations has been attributed to depletion of the releasable pool (35). In contrast, some 5-HT may be stored in dense core vesicles (36) or other compartments that do not exocytose. This would produce effects consistent with a small quantity of 5-HT available for release.

Dopamine Release Is Synthesis and Packaging Sensitive, Whereas 5-HT Is an Uptake/Metabolism-Controlled System. Several of the pharmacological agents investigated in this work (Figs. 3 and 4) inhibit the same processes in dopaminergic and serotonergic neurons. For example, inhibition of aromatic amino acid decarboxylase with NSD 1015 inhibits synthesis of both 5-HT and dopamine (22). Because the preceding experiments suggest that the releasable pool of dopamine is more sensitive to depletion, we predicted that releasable 5-HT would also be less sensitive to synthesis inhibition, and this was found to be the case. 5-HT and dopamine are both packaged into vesicles via the action of VMAT2 (37), and inhibition of this transporter with tetrabenazine resulted in a greater decrease in dopamine release than 5-HT. We propose that a smaller releasable pool contributes to lower release amplitudes, which in turn reduces the requirement for packaging in serotonergic terminals. This is supported by the comparatively moderate response of 5-HT to manipulations that affect synthesis and packaging. There was, however, a significant increase in the $t_{1/2}$ for 5-HT clearance. An increase in cytoplasmic 5-HT caused by VMAT2 inhibition could decrease uptake rates by altering the concentration-dependent driving force for SERT, a common feature of amine transporters (38). There was a delay in the onset of 5-HT release that may be an indication of tetrabenazine-insensitive vesicular pools, possibly dense core vesicles, compensating for the demand on release.

After release, one fate for monoamines is metabolic degradation by MAO (39). Prior work has shown that there is a small increase in dopamine release after MAO inhibition, presumably because repackaging into vesicles becomes more likely during a reduction in metabolic degradation (21). However, the increase in stimulated 5-HT release after MAO inhibition is greater than threefold, indicating that serotonergic neurons have greater regulation by MAO. Moreover, whereas MAO inhibition had no effect on the rate of dopamine clearance, a significant increase in the $t_{1/2}$ of the 5-HT signal indicates that this treatment caused a reduction in 5-HT reuptake rate. Similar to inhibition of

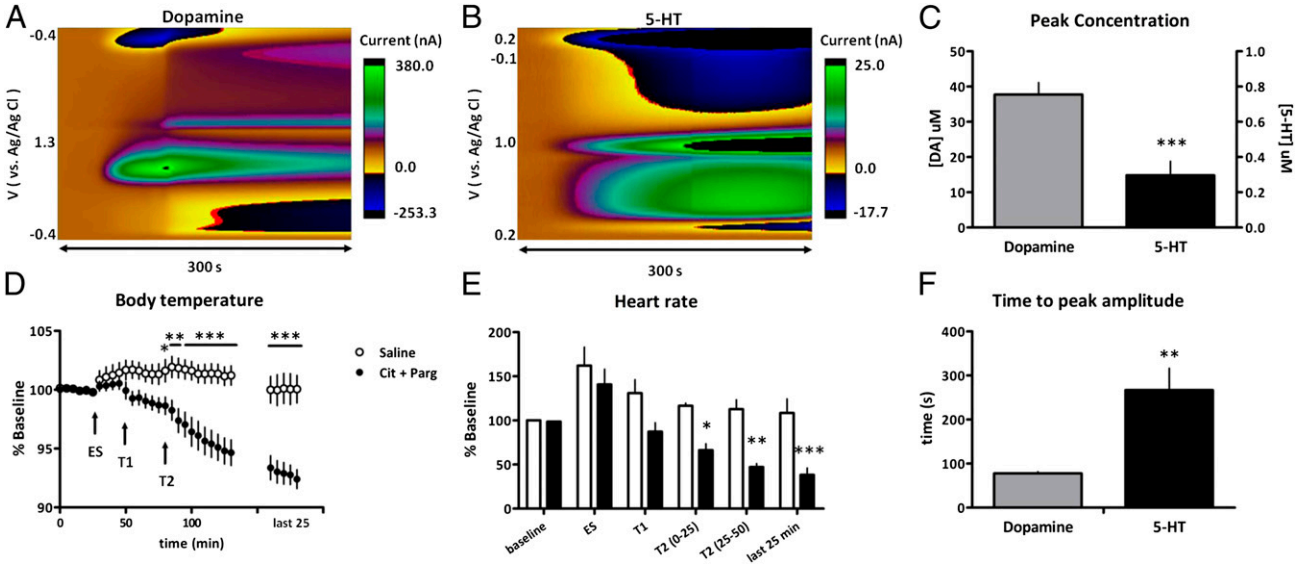

Fig. 5. Effects of citalopram and pargyline. (*A* and *B*) Representative results from individual animals for dopamine (NAc) and 5-HT (SNr) efflux that both occur spontaneously immediately after respiratory arrest. (*C*) Maximal dopamine and 5-HT concentrations ($n = 4$ for each). (*D*) Response of body temperature relative to baseline (0–25 min) during electrical stimulations (six 2-s stimulations, 5 min apart, ES), after citalopram (T1), and after pargyline (T2). Drug-treated animals shown in filled circles ($n = 6$) and saline controls shown in open circles ($n = 4$). (*E*) Response of heart rate to electrical stimulations (ES), citalopram (T1), and pargyline (T2). Drug-treated animals shown in filled bars ($n = 4$), saline controls shown in open bars ($n = 4$). (*F*) Mean time between onset of efflux and peak of efflux for dopamine and 5-HT ($n = 4$). Asterisks indicate significant differences between groups (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

VMAT2, MAO inhibition could alter the driving force of SERT, resulting in decreased rate of uptake.

Another means for extracellular regulation is uptake via transporters. However, because different receptors and transporters regulate dopamine and 5-HT release, it was necessary to administer different agents to evaluate these control points. We found that both neurotransmitter systems were sensitive to selective inhibition of their transporters (Fig. 4*D*), results consistent with previous work (27, 40). Serotonin autoreceptors are known to suppress 5-HT release in a manner similar to the regulation of dopamine release by the dopamine autoreceptor in the NAc (41–43). We used methiothepin, a nonselective 5-HT autoreceptor antagonist to target the multiple 5-HT autoreceptors (44). After raclopride and methiothepin, release of dopamine and 5-HT, respectively, was moderately increased. This suggests that regulation by 5-HT autoreceptors is not responsible for the differences between 5-HT and dopamine release. The increase in the $t_{1/2}$ of the 5-HT signal after methiothepin administration is consistent with the suggestion by Daws et al. (45, 46) that 5-HT_{1B} autoreceptors modulate 5-HT clearance.

Disrupting 5-HT Control Mechanisms Results in Serotonin Syndrome. Within 2.5 h of SERT and MAO inhibition respiratory arrest was followed by spontaneous efflux of dopamine and 5-HT (Fig. 5 *A* and *B*). Synchronized dopamine and 5-HT efflux likely reflects reversal of their transporters, which use secondary active transport coupled to the respiration-sensitive Na^+/K^+ ATPase (38). The spontaneous efflux preceding death resembles evoked release in that the maximum 5-HT amplitude is much lower than for dopamine (Fig. 5*C*). Furthermore, 5-HT efflux is prolonged (Fig. 5*D*), adding support to the concept that 5-HT is constrained more tightly with neurons. After citalopram, pargyline administration led to decreases in heart rate and body temperature, leading up to eventual death (Fig. 5 *D* and *E*). These findings concur with previous reports that combined administration of MAO and SERT, but not DAT, inhibitors cause fatalities in rats (47). In humans, this pharmacological combination is known to induce serotonin syndrome, which arises from excess serotonergic activity in the central nervous system (48–50). Our findings reveal the consequences of serotonin syndrome in anesthetized animals.

Conclusions

In prior work we used a similar comparative approach to compare release of norepinephrine and dopamine evoked by a single stimulation and found that their release and uptake characteristics were quite similar (29). In this work, high time resolution recordings revealed much greater differences in regulation of dopamine and 5-HT. We found that 5-HT release is highly regulated and is dominated primarily by reuptake/inactivation systems that could not be unraveled with slower monitoring techniques, such as microdialysis. Moreover, use of electrochemical monitoring has revealed neurochemical processes that occur as a result of serotonin syndrome fatality.

Methods

Full experimental procedures are provided in *SI Methods*.

Surgical Procedures. Stereotaxic surgeries for voltammetric measurements were performed as previously described (12). Briefly, Nafion-modified carbon-fiber microelectrodes were implanted in the SNr and in NAc. A bipolar stainless steel stimulating electrode was implanted into the MFB. As noted, heart rate, breathing, and body temperature were monitored in some animals (*SI Methods*).

Voltammetric Procedures. Quad Universal Electrochemical Instrument (UEI) potentiostats described previously (15) were modified by enabling independent control of the potential on two pairs of operational amplifiers in the head stage. A data acquisition system capable of generating two independent waveforms and collecting from two sets of channels was previously described (15). All potentials reported are against Ag/AgCl.

Drugs and Reagents. Pharmaceutical-grade pargyline hydrochloride, NSD 1015 (3-hydroxybenzylhydrazine dihydrochloride), GBR 12909 dihydrochloride, raclopride, methiothepin, citalopram, and tetrabenazine were administered i.p.

Data Analysis. Two-tailed Student's *t* tests were performed on paired data sets. $P < 0.05$ was taken as significant. Error bars are given as ±SEM. Two-way ANOVA was used for the analysis of body temperature and heart rate.

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