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Nicotinic receptors regulate the dynamic range of dopamine release in vivo

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Koranda JL, Cone JJ, McGehee DS, Roitman MF, Beeler JA, Zhuang X. Nicotinic receptors regulate the dynamic range of dopamine release in vivo. *J Neurophysiol* 111: 103–111, 2014. First published October 2, 2013; doi:10.1152/jn.00269.2013.—Nicotinic acetylcholine receptors (nAChRs) are expressed presynaptically on dopamine axon terminals, and their activation by endogenous acetylcholine from striatal cholinergic interneurons enhances dopamine release both independently of and in concert with dopamine neuron activity. Acute nAChR inactivation is believed to enhance the contrast between low- and high-frequency dopamine cell activity. Although these studies reveal a key role for acute activation and inactivation of nAChRs in striatal microcircuitry, it remains unknown if chronic inactivation/desensitization of nAChRs can alter dopamine release dynamics. Using in vivo cyclic voltammetry in anaesthetized mice, we examined whether chronic inactivation of nAChRs modulates dopamine release across a parametric range of stimulation, varying both frequency and pulse number. Deletion of β2*nAChRs and chronic nicotine exposure greatly diminished dopamine release across the entire range of stimulation parameters. In addition, we observed a facilitation of dopamine release at low frequency and pulse number in wild-type mice that is absent in the β2* knockout and chronic nicotine mice. These data suggest that deletion or chronic desensitization of nAChRs reduces the dynamic range of dopamine release in response to dopamine cell activity, decreasing rather than increasing contrast between high and low dopamine activity.

chronic nicotine; β2 nicotinic subunit; dopamine release; in vivo cyclic voltammetry; dorsolateral striatum

DOPAMINE release plays a critical role in reinforcement learning and motivated behaviors (Balleine et al. 2007; Beeler 2011; Beeler et al. 2010; Berridge 2004; Berridge et al. 2009; Everitt and Robbins 2005; Humphries and Prescott 2010; Kheirbek et al. 2009; Nicola 2007; Redgrave et al. 2011; Salamone et al. 2007; Schultz 2002). β2*-Containing nicotinic acetylcholine receptors (nAChRs) on dopamine terminals potently regulate dopamine release. Activation of presynaptic nAChRs on dopamine terminals enhances dopamine release both independently of (Cachope et al. 2012; Threlfell et al. 2012) and in concert with dopamine neuron activity (Rice and Cragg 2004; Zhang and Sulzer 2004; Zhou et al. 2001). Acute blockade or desensitization of β2*nAChRs lowers the probability of dopamine release from striatal terminals in response to single-pulse stimulation (Exley and Cragg 2008; Rice and Cragg 2004; Zhang and Sulzer 2004; Zhang et al. 2009a, 2009b). In these studies, increasing stimulus frequency diminishes or overcomes the inhibitory effect of acute blockade or desensitization of nAChRs, although the extent of that recovery is controversial. At higher frequency stimulation, acute nAChR blockade has been observed to enhance (Exley et al. 2008; Rice and Cragg 2004), diminish (Zhang et al. 2009a), or have no effect on (Zhang and Sulzer 2004; Zhang et al. 2009b) dopamine release. Despite these different observations, it has been proposed that acute nAChR inactivation enhances the contrast between high and low dopamine cell activity, presumably improving signal-to-noise ratio (Exley and Cragg 2008; Rice and Cragg 2004; Zhang and Sulzer 2004; Zhang et al. 2009a; 2009b).

Although these studies reveal the effects of acute nAChR activation or inactivation on dopamine release in isolated striatal microcircuitry in a slice preparation, they do not address the question more relevant to nicotine addiction: how does chronic nicotine exposure affect the dynamics of dopamine release in an intact animal? To explore this question, we evoked dopamine release and parametrically varied both stimulation frequency and number of current pulses in vivo using an anesthetized mouse preparation. Changes in evoked dopamine release were measured in the dorsolateral striatum using fast-scan cyclic voltammetry. We compared evoked dopamine release in wild-type (WT) mice with that in mice lacking the β2* nAChR subunit or mice exposed to chronic, intermittent nicotine.

MATERIALS AND METHODS

Animals

Mice were housed in standard conditions on a 12:12-h light-dark cycle in a temperature- and humidity-controlled facility and allowed ad libitum access to standard chow and water. β2*-Subunit knockout (β2*KO) mice (Picciotto et al. 1997, 1998) were backcrossed with C57Bl6/J mice from Jackson Laboratory. Heterozygote offspring were then bred to generate β2*KO and age-matched WT littermate controls. For long-term nicotine studies (see below), C57Bl6/J mice were obtained from Jackson Laboratory. Males and females aged 10–14 wk at recording were used. All procedures were in accordance with the guidelines of and approved by the Institutional Animal Care and Use Committee at the University of Chicago.

Chronic Nicotine Administration

C57Bl6/J mice (Jackson Laboratory) received either 100 μg/ml (free base) nicotine via the drinking water daily for 2–4 wk or regular water. This dose did not alter daily water intake (nicotine: 4.896 ±

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0.1902 ml; control: 5.014 ± 0.2915 ml; $t = 0.3376$, $P = 0.7444$), similar to previous studies (Matta et al. 2007; Meliska et al. 1995; Robinson et al. 1996; Rowell et al. 1983). Additionally, this schedule of nicotine administration mimics nicotine dosing in human smokers allowing for prolonged, intermittent exposure to nicotine (Matta et al. 2007). Mice were maintained on this schedule of nicotine dosing until they were removed from their home cage, anesthetized, and prepped for voltammetry recordings. Forty-five to 60 min elapsed between removal from home cage and first voltammetric recording. Because nicotine has a half-life of 6–8 min in mice (Matta et al. 2007), potential acute nicotine effects were minimized or absent. There were no observed differences in dopamine release between the β2*WT littermate controls and control C57Bl6/J mice obtained from Jackson Laboratory that did not receive nicotine in their drinking water [$F_{(1,6)} = 0.23$, $P = 0.647$]. Thus data from these two groups were pooled together and collectively referred to as WT.

Fast-Scan Cyclic Voltammetry

Carbon fiber electrode construction. Carbon fiber microelectrodes were fabricated in house. Individual carbon fibers (7-μm diameter; Goodfellow Cambridge, Huntingdon, UK) were aspirated into glass pipettes (0.6-mm O.D., 0.4-mm I.D.; A-M Systems, Carlsborg, WA) and then pulled on a vertical electrode puller (Narishige, East Meadow, NY). The seal of each electrode was evaluated under a light microscope, and the exposed portion of the carbon fiber was cut to ~75 μm. A silver print coated wire was inserted into the lumen of the pipette to establish contact with the carbon fiber. Generally, the same electrode was used each day, and groups were interleaved with the order switching every day so that potential error from slight variations in electrodes would be distributed equally between groups. At the conclusion of each experiment, carbon fiber electrodes were calibrated in a flow cell using 1 μM dopamine. Results are reported as dopamine concentration determined for each recording based on individual electrode calibrations. The average calibration factor equaled 68.9 ± 5.90 nM/nA.

Surgery and recording. Mice were removed from their home cages, immediately anesthetized with urethane (2.5 g/kg ip), and mounted in a stereotaxic frame (KOPF, Tujunga, CA). A bipolar, twisted, tungsten stimulating electrode (tip separation ~1.0 mm; Plastics One, Roanoke, VA) was lowered into the substantia nigra [SN: anteroposterior (AP), −3.2 mm; lateral (Lat), 0.5–0.8 mm medial boundary relative to bregma; dorsoventral (DV), 4.5 mm from the brain surface] while a carbon fiber microelectrode was lowered into the ipsilateral dorsolateral striatum (DLS: AP, +1.1 mm; Lat, 2.0 mm relative to bregma; DV, 2.5–3.0 mm from the brain surface). Additionally, a chloride-coated silver wire (Ag-AgCl) reference electrode was implanted in the contralateral forebrain. Fast-scan cyclic voltammetry (FSCV) was performed as described previously (Day et al. 2007; Roitman et al. 2004, 2010) using Tarheel CV software for data acquisition and analysis. Briefly, once the carbon fiber microelectrode was positioned, the electrode was periodically scanned from a holding potential of −0.4 V (relative to Ag-AgCl) to +1.3 V and back (400 V/s). Each voltage scan produces a large charging current that becomes highly stable. Voltage scans were first applied at 60 Hz for 30 min to allow the charging current to stabilize. After 30 min, the frequency of applied voltage scans was lowered to 10 Hz, the frequency at which dopamine measurements were made. After a stable background was achieved, each 15-s data collection file was background subtracted by averaging the current obtained from 10 voltage scans before SN stimulation (see below) from the remainder of the scans (background subtraction). Both the carbon fiber and stimulating electrodes were lowered in 100-μm increments to optimize evoked dopamine release. At the initial and each subsequent location, the SN was stimulated by administering 24 monophasic current pulses (4 ms/pulse) at a rate of 60 Hz (150 μA) while voltammetric recordings were made in the DLS. Once the peak dopamine signal was optimized, pulse number (1–24 pulses) was altered across a range of frequencies (5–60 Hz) in descending order. SN stimulation was delivered every 2 min, and peak oxidation current was measured. Preliminary studies showed evoked dopamine release was independent of stimulation history.

Dopamine identification. For each mouse, current vs. electrode potential (cyclic voltammogram, CV) during stimulation of the SNc with the highest stimulation parameters (24 pulses, 60 Hz) was plotted and dopamine was identified the basis of the unique chemical signature of the analyte, consisting of a current occurring at approximately +0.6 V on the positive-going voltage sweep and approximately −0.2 V on the negative-going voltage sweep (oxidation and reduction peaks, respectively; Phillips et al. 2003). This CV served as the "template" for dopamine. CVs from individual SN stimulations using other parameters were subjected to linear regression analysis the template. If a value of $R^2 \geq 0.7500$ was obtained (Phillips et al. 2003; Roitman et al. 2004), then the peak oxidation current was recorded, whereas CVs that did not meet this criterion were assigned a value of 0.

Electrode Placement

At the end of each experiment, 2 μl of trypan blue dye (Sigma, St. Louis, MO) were injected at the carbon fiber recording depth, and animals were then euthanized and perfused. Light microscopy was used to confirm to confirm carbon fiber electrode placement within the DLS. Figure 1*F* shows placement of working electrodes.

Statistical Analysis

The data were tested for significance using ANOVA (R statistical software, version 2.12.1 2010-12-16; The R Foundation for Statistical Computing, http://www.r-project.org). Frequency and pulse number were treated as categorical factors rather than continuous variables, because we cannot assume that either is a linear function. The Kaplan-Meier cumulative survival plot using the LogRank test (Mantel-Cox test) was used to quantify failure to evoke measurable dopamine release.

RESULTS

Loss of β2 nAChR Subunits and Chronic Nicotine Inhibits Stimulated Dopamine Release*

We first analyzed dopamine release measured in the DLS of intact mice following electrical stimulation of the SN with a single pulse of stimulation. Stimulated dopamine release was significantly lower (Fig. 1, *A* and *B*) in both mice with a genetic deletion of the β2* nAChR subunit (β2*KO: 1.586 ± 1.023 nM; $t = 4.852$, $P < 0.001$; $n = 5$) and WT mice exposed to chronic nicotine (cNIC: 6.268 ± 1.819 nM; $t = 3.717$, $P < 0.01$; $n = 6$) compared with WT mice (29.59 ± 4.118 nM; $n = 9$). We next applied a train of 5 pulses administered at 20 Hz, a stimulation pattern within the reported physiological range of phasic dopamine neuron firing (Grace and Bunney 1984; Hyland et al. 2002; Schultz 1986). Similar to results with single-pulse stimulation, dopamine release was significantly lower (Fig. 1, *A* and *B*, 20 Hz) in both β2*KO (10.95 ± 5.473 nM; $t = 2.516$, $P = 0.0143$; $n = 5$) and cNIC mice (23.20 ± 7.232 nM; $t = 1.982$, $P = 0.037$; $n = 6$) compared with WT mice (71.27 ± 18.35 nM; $n = 9$). These differences were not the result of changes in uptake kinetics, because the time for peak dopamine current to decay by 50% (T_{50}) was not different between groups [data not shown; $F_{(2,56)} = 0.82$, $P > 0.40$].

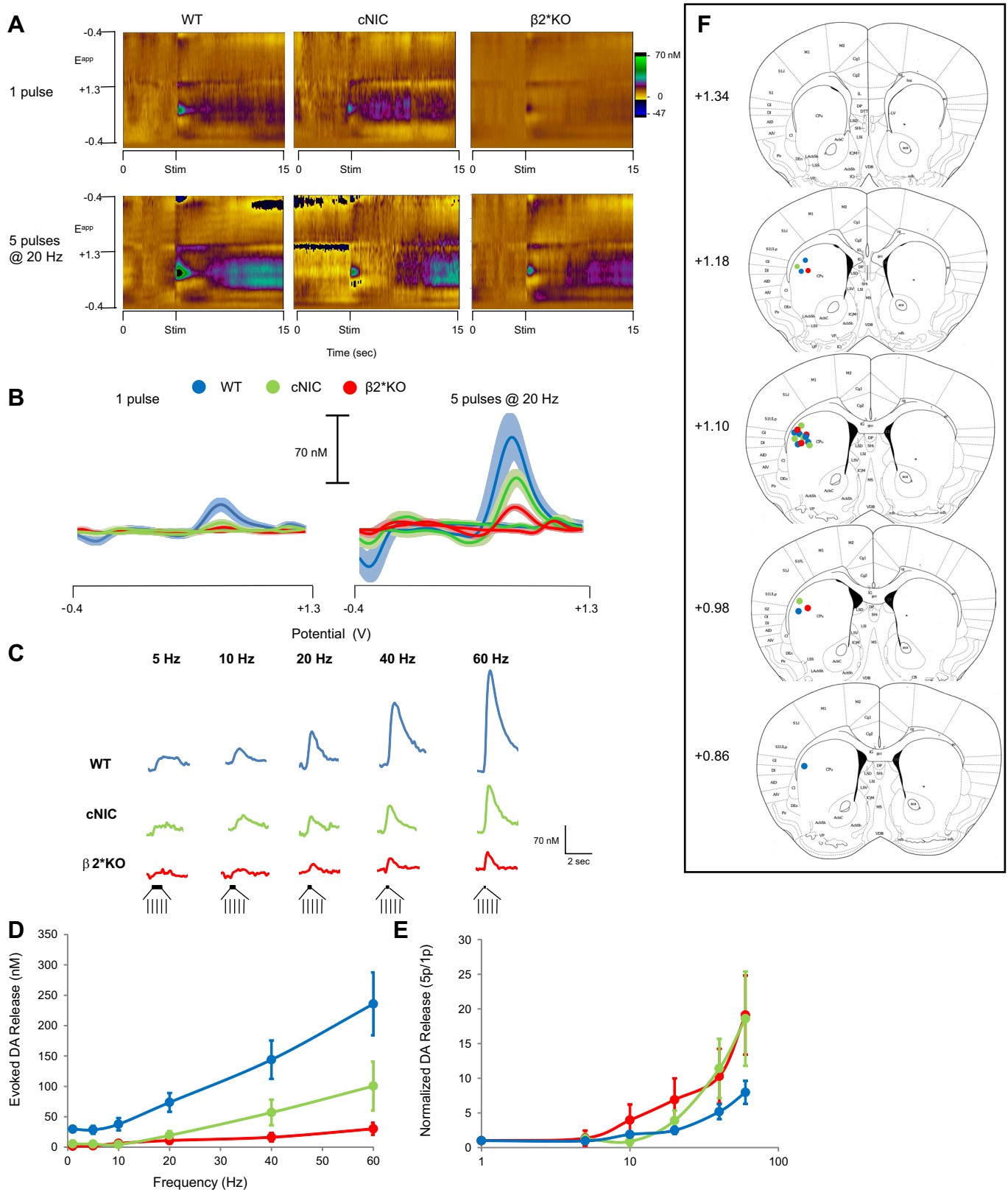

Fig. 1. Frequency-dependent dopamine (DA) release in the intact mouse. DA release was stimulated by applying a train of 5 pulses to the substantia nigra (SN). *A*: example color plots of DA release in the dorsolateral striatum (DLS) from an individual wild-type (WT), chronic nicotine-treated (cNIC), and $\beta 2$*-subunit knockout ($\beta 2$*KO) mice showing current plotted in pseudocolor following a single pulse of stimulation (*top*) or 5 pulses administered at 20 Hz (*bottom*). *B*: averaged cyclic voltammograms showing characteristic electrochemical fingerprint of DA with the oxidation current occurring at about +0.6 V and the reduction current occurring at −0.2 V following a single pulse of stimulation (*left*) or 5 pulses administered at 20 Hz (*right*). *C*: averaged current-time traces at the indicated stimulation frequencies. *D*: average absolute DA release within each group across frequencies. *E*: DA release following 5 pulses of stimulation across frequencies normalized to single-pulse stimulation within each group. WT, $n = 9$; cNIC, $n = 6$; $\beta 2$*KO, $n = 5$. Error bars indicate SE. *F*: placement of cyclic voltammetry working electrodes.

*Loss of β2*nAChRs and Chronic Nicotine Increases the Frequency Dependence of Dopamine Release*

In vitro studies have observed decreased dopamine release under acute nicotinic blockade/desensitization that is pronounced at low frequencies. As stimulation frequency increases, this inhibitory effect is diminished (Exley et al. 2008; Rice and Cragg 2004; Zhang and Sulzer 2004; Zhang et al. 2009a, 2009b). To test whether deletion of the β2*-subunit or chronic nicotine exposure altered the frequency dependence of dopamine release, we applied five pulses at increasing frequencies. Although all groups showed a frequency-dependent increase in dopamine release [Fig. 1, *C* and *D*; frequency, $F_{(4,64)} = 23.0$, $P < 0.001$], β2*KO and cNIC groups exhibited consistently lower dopamine release at all frequencies compared with WT [group, $F_{(2,15)} = 5.6$, $P < 0.05$]. In fact, at the highest frequency tested, 60 Hz, dopamine release was reduced to 38% and 18% of WT release in cNIC and β2*KO mice, respectively. This suggests that in vivo, increasing frequency does not overcome reduced dopamine release associated with β2* deletion and chronic nicotine treatment. After normalization of five-pulse release to that observed with a single pulse, the main effect of group is no longer significant [Fig. 1*E*; group, $F_{(2,15)} = 1.25$, $P = 0.312$]. Both the β2*KO and cNIC groups show greater increase in release with increasing frequency compared with WT [group × frequency: β2*KO, $F_{(4,46)} = 4.13$, $P < 0.01$; cNIC, $F_{(4,48)} = 2.88$, $P < 0.05$]. However, in the context of overall reduction in absolute release, this apparent increased responsiveness to frequency represents greater frequency dependence. Normalization obscures the dramatically reduced range of dopamine release in the β2*KO and cNIC mice. This suggests that deletion of β2* nicotinic subunits and chronic nicotine induce a loss of function that decreases contrast between high- and low-frequency activity.

Dopamine Release is Preferentially Facilitated at Low Frequencies in WT Mice

To systematically examine the effects of different activity patterns on dopamine release in vivo, we varied the number of stimulation pulses across a range of frequencies. We evaluated differences between groups in absolute dopamine release (Fig. 2, *left*) and release normalized to peak dopamine release at 24 pulses for each frequency tested (Fig. 2, *right*). In WT mice, dopamine release at low frequencies appeared to be facilitated such that it increased rapidly with increasing pulse number and reached asymptote at 3 or 10 pulses at 5 and 10 Hz, respectively, with additional pulses having little effect on release (Fig. 2, *A* and *B*; 5, 10 Hz). In contrast, at higher frequencies, dopamine release increased linearly with additional pulses (Fig. 2, *C–E*). At the highest frequency (60 Hz) and pulse number tested (24 pulses), the amount of dopamine released did not asymptote (Fig. 2*E*). This contrasts with slice studies, where dopamine release in the dorsal striatum does not scale with pulse number at high frequencies but asymptotes after 2–4 pulses (Exley et al. 2008; Zhang et al. 2009a, 2009b). In vivo, we observe that release asymptotes in response to pulse number at lower but not higher frequencies, suggesting that in vivo dopamine release can reflect both the frequency and duration of high-frequency burst activity. In contrast, facilitation and asymptote of release at low frequencies rapidly establishes a stable dopamine signal within a brief window. The differential modulation of release at low and high frequencies facilitates a wide dynamic range in dopamine signaling.

*Facilitation of Dopamine Release at Low Frequencies is Abolished Following Deletion of β2*nAChR*

In the β2*KO mice, dopamine release was drastically reduced across all pulses and frequencies tested compared with that in WT mice [Fig. 2; main effect of group, $F_{(1,10)} = 10.7$, $P < 0.01$; frequency, $F_{(3,10)} = 9.7$, $P < 0.01$; pulse number, $F_{(7,70)} = 36.2$, $P < 0.001$]. At low frequencies, evoked dopamine release in β2*KO mice did not rapidly increase with pulse number and asymptote at low pulse numbers as observed in WT mice [Fig. 2, *A* and *B*, 5 Hz: group, $F_{(1,10)} = 8.3$, $P < 0.05$, group × pulse, $F_{(7,76)} = 2.09$, $P = 0.054$; 10 Hz: group, $F_{(1,10)} = 17.6$, $P < 0.01$, group × pulse, not significant]. For example, in WT mice, 5 pulses at 5 Hz elicited ~75% of maximal dopamine release at that frequency (Fig. 2*A*, *right*). In contrast, the same stimulation (5 pulses at 5 Hz) only elicited ~9% of maximal release in β2*KO mice (Fig. 2*A*, *right*). To assess the relative failure rate of dopamine release as a function of pulse number and frequency, we constructed survival plots for each frequency (i.e., "survival" of release as pulse number decreases), where failures were defined as currents that were too small to allow clear determination that dopamine was the oxidized species (see MATERIALS AND METHODS). In the β2*KO mice, release probability is greatly reduced across all pulse numbers at 5 Hz, with much higher failure rates (Fig. 3*A*; $\chi^2 = 8.227$; df = 1; $P < 0.005$). Together, these data suggest β2* deletion degrades the low activity facilitation observed in WT.

In contrast, at higher frequencies (40 and 60 Hz), the shape of the β2*KO curves is similar to that of WT, where dopamine release increases linearly with pulse number (Fig. 2, *D* and *E*), with comparable failure rates (Fig. 3*B*; $\chi^2 = 3.328$, df = 1; $P > 0.0681$). When dopamine release is normalized to maximal release at each frequency, increased release with increasing pulse numbers is preserved in β2*KO mice at 40 and 60 Hz [Fig. 2, *E* and *F*, *right*; 40 Hz, $F_{(1,12)} = 0.03$, $P = 0.85$; 60 Hz, $F_{(1,12)} = 0.55$, $P = 0.47$], although this obscures the overall reduction in release (Fig. 2, *E* and *F*, *left*). Although both groups show monotonically increasing release with increased stimulation, absolute dopamine levels are drastically lower across all conditions in the β2*KO relative to WT mice, and the absolute difference between dopamine release at high and low stimulation is also greatly reduced in β2*KO mice.

Long-Term Nicotine Exposure Reduces Absolute dopamine Release and Degrades Facilitation of Low-Frequency Activity

A group of WT mice were administered chronic nicotine (100 μg/ml) in their drinking water for a minimum of 2 wk, providing intermittent access to nicotine similar to that seen in human smokers (Grabus et al. 2005; Matta et al. 2007). Similar to results in β2*KO mice, chronic nicotine reduced absolute dopamine release across all pulses and frequencies tested [Fig. 2; main effect of group, $F_{(1,11)} = 5.8$, $P < 0.05$; frequency, $F_{(3,11)=} = 10.7$, $P < 0.01$; pulse number, $F_{(7,77)} = 38.7$, $P < 0.001$]. Chronic nicotine, however, does not completely abolish but severely diminishes nAChR facilitation of dopamine release at low frequencies. Consistent with this partial retention of facilitation, we observe a trend toward increased failure rate of dopamine release between cNIC and WT mice at 5 Hz (Fig.

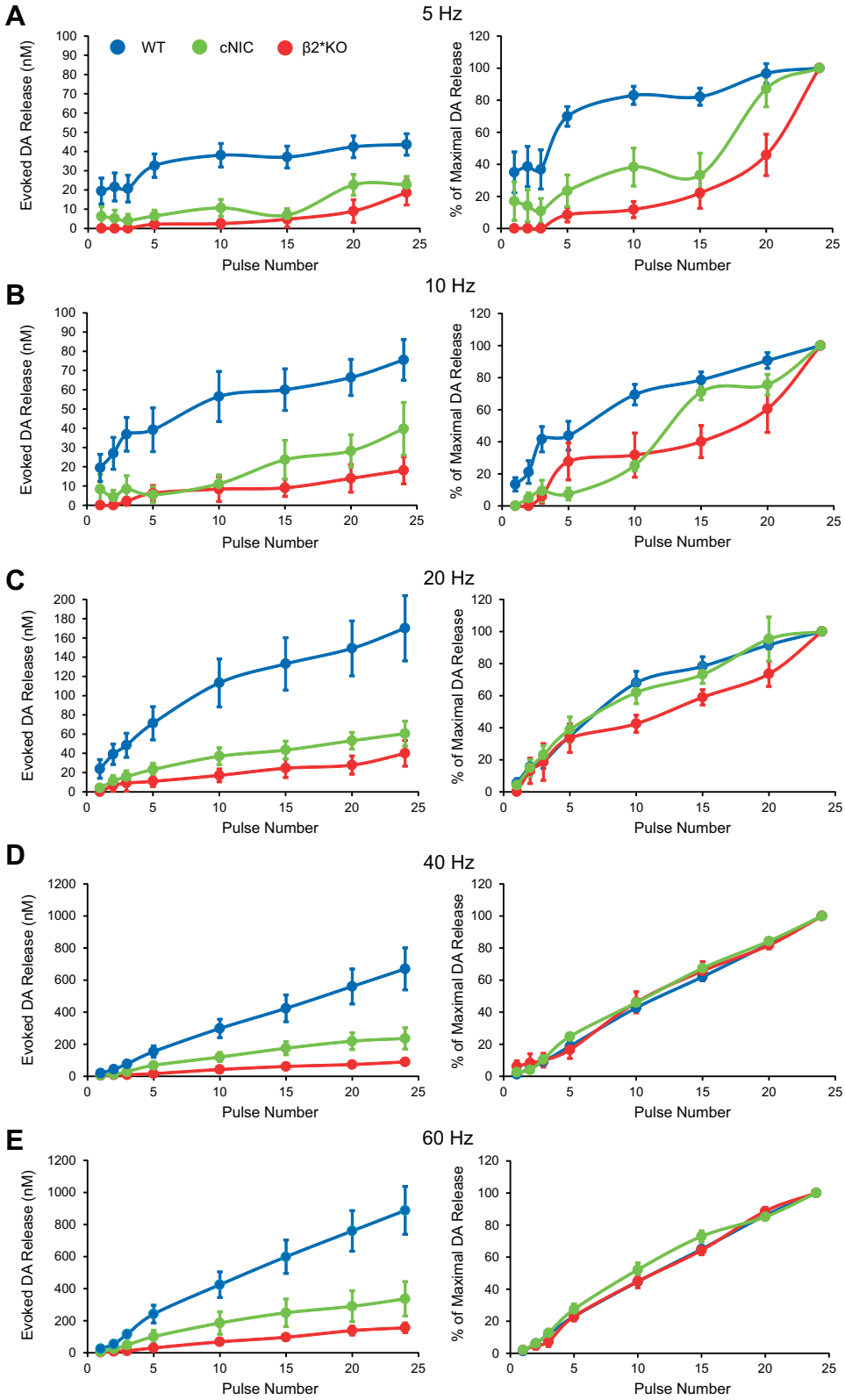

Fig. 2. β2*-Containing nicotinic acetylcholine receptor (β2*nAChR), β2*KO, and chronic nicotine treatment alters stimulated DA release in vivo. Absolute (*left*) and normalized (*right*) DA release in vivo is shown following stimulation at 5 (*A*), 10 (*B*), 20 (*C*), 40 (*D*), or 60 Hz (*E*). DA release was normalized to peak DA release (i.e., 24 pulses) at each respective frequency. WT, $n = 9$; cNIC, $n = 6$; β2*KO, $n = 5$. Error bars indicate SE. Statistics are reported in RESULTS.

3*A*; 5 Hz, $\chi^2 = 3.358$, df = 1; $P = 0.067$). At higher frequencies (40 and 60 Hz), cNIC mice show the same monotonic linear relationship between pulse and dopamine release seen in both WT control and β2*KO mice (Fig. 2, *C–E*; no statistically significant differences between groups), with comparable failure rates (Fig. 3*B*; $\chi^2 = 0.4773$, df = 1; $P = 0.48$). As with the β2*KO mice, when the data are calculated as percent of maximal dopamine release (Fig. 2, *right*), the difference in absolute dopamine release at higher frequencies is masked. However, dopamine release is still significantly lower across

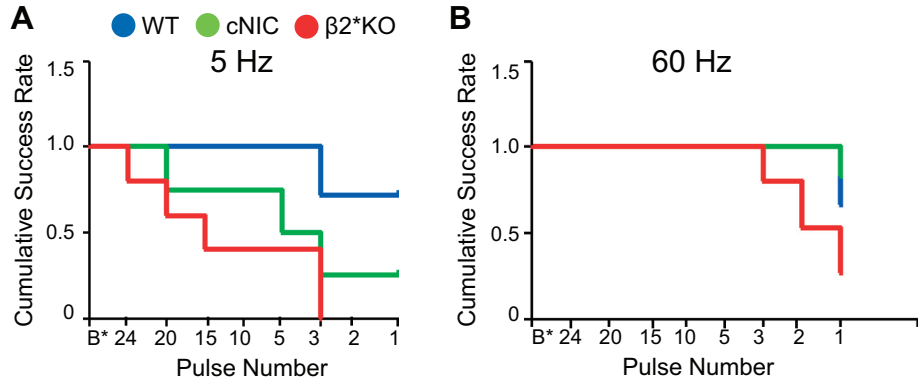

Fig. 3. Increased failure of evoked DA release in the absence of β2* nAChR subunits. *A*: Kaplan-Maier cumulative survival plot of successful evoked DA release as a function of pulse number (in reverse, from high to low pulse number) following stimulation at 5 (*A*) and 60 Hz (*B*). Starting populations: WT, $n = 9$; cNIC, $n = 6$; β2*KO, $n = 5$.

all pulses and frequencies tested, and the contrast between absolute release at high and low stimulation, as in the β2*KO mice, is greatly reduced.

DISCUSSION

In the present in vivo study, we find both genetic deletion of β2*-subunits (β2*KO) and chronic nicotine (cNIC) dramatically reduces dopamine release across all frequencies and pulse numbers tested. Although we see increased frequency dependence of dopamine release in the absence of β2*nAChRs and following chronic nicotine exposure, increasing frequency does not overcome reduced dopamine release; the magnitude of reduction remains substantial even at high frequencies. These data suggest that chronically inactivated or desensitized β2*nAChRs greatly attenuate the dynamic range of dopamine release in response to dopamine cell activity.

In WT mice, we observe a facilitation of release in response to low stimulation protocols. At low frequencies (5 and 10 Hz), dopamine release is relatively insensitive to pulse number and quickly asymptotes, facilitating rapid, stable readout of low-frequency stimulation. In contrast, at high frequencies (40–60 Hz), dopamine release increases linearly with pulse number, faithfully reporting the length of the stimulus train and scaling with frequency, essentially encoding the number of pulses per unit time. This differs from prior in vitro studies that found dopamine release in the dorsal striatum remains relatively insensitive to increasing pulse number at high-frequency stimulation (Exley et al. 2008, 2012; Threlfell and Cragg 2011; Zhang et al. 2009a, 2009b). This may be explained by the fact that we stimulate the dopamine cell bodies in the midbrain, whereas in vitro studies stimulate dopamine terminals locally within the striatum, and local stimulation likely depolarizes many more dopamine terminals than stimulation of the cell bodies. We chose not to stimulate the striatum to avoid activation of cholinergic interneurons and the subsequent acetylcholine release that can directly induce dopamine release, independent of dopamine cell activity (Cachope et al. 2012; Threlfell et al. 2012). Thus the observed dopamine release in the current study arises from activation of dopamine cell bodies.

In β2*KO and cNIC mice, dopamine release is drastically reduced across all stimulation parameters. Because we stimulated the midbrain, these data suggest a nAChR contribution to in vivo dopamine release, independent of direct cholinergic interneuron stimulation. In addition, the low-frequency facilitation observed in WT is severely reduced, whereas scaling of dopamine release relative to pulse number is maintained at higher frequencies. If release at higher frequencies is normalized to release at one pulse, the contrast between high and low frequencies is higher in β2*KO and cNIC mice. This increased contrast, however, has to be understood in the context of an overall decrease in dopamine release. In terms of absolute magnitude of release, the difference between release at low and high frequencies in the β2*KO and cNIC mice is actually reduced: less contrast. The apparent increased contrast observed through normalization arises from an increased frequency dependence that reflects a nicotinic mediated loss rather than gain of function. Thus we propose that nAChR activation enhances the dynamic range of striatal dopamine release in response to dopamine cell activity, providing a more reliable and robust signal with greater discrimination between frequencies. On the other hand, chronic inactivation or desensitization of β2*nAChRs compromises dopamine release at all frequencies. As a consequence, β2*KO and cNIC mice may operate within a degraded range of dopamine release, where low-frequency signals become difficult to distinguish from noise and high-frequency signals must be discriminated within a much narrower, compressed range of dopamine release.

In the current study, we administered chronic nicotine to mice via the drinking water. This method is analogous to human smoking and allows for intermittent access to nicotine over a prolonged period without additional stressors such as a chronic implant or multiple injections (Matta et al. 2007). Because mice accessed nicotine through their drinking water, we could not control the precise timing of nicotine exposure. However, mice rapidly metabolize nicotine (half-life: 6–8 min), and data collection did not begin until 45–60 min after the mice were removed from their home cage. Therefore, the observed decrease in dopamine release is unlikely due to the nicotine's direct actions at nAChRs. Rather, the observed decrease in dopamine release following chronic nicotine exposure likely reflects long-lasting neuroadaptations that arise in response to repeated desensitization of β2*-containing nAChRs. For example, chronic nicotine has been associated with a functional upregulation of α4β2*-containing nAChRs in the striatum (Buisson and Bertrand 2001; Govind et al. 2009, 2012; Mugnaini et al. 2002; Nguyen et al. 2003; Perez et al. 2009; Vallejo et al. 2005; Xiao et al. 2009). However, recent studies have shown that chronic nicotine either downregulates or does not alter α6β2*nAChR expression (Even et al. 2008; McCallum et al. 2006a, 2006b; Mugnaini et al. 2006; Nguyen et al. 2003; Perez et al. 2008; Perry et al. 2007; Walsh et al.

2008), leaving open the question as to the potential contribution of functional up- or downregulation of β2-containing receptors to the diminished dopamine release observed here. α6β2* receptors in particular are found exclusively on dopamine neurons (Champitaux et al. 2003; Gotti et al. 2010; Marks et al. 2011; Perry et al. 2007; Salminen et al. 2004; Yang et al. 2011) and have been shown to potently regulate striatal dopamine release (Exley et al. 2008; Grady et al. 2007; Perez et al. 2008, 2009). Thus reduced dopamine release observed in mice chronically treated with nicotine may arise as a consequence of downregulation of α6β2*nAChRs on dopamine cell terminals.

In addition to being expressed on dopamine terminals, β2*nAChRs are expressed in the midbrain and are known to regulate dopamine cell activity. For example, activation of β2*nAChRs is thought to be necessary for dopamine neurons to switch from tonic to phasic firing (Changeaux 2010; Quik and Wonnacott 2011), and in an anesthetized, in vivo preparation, β2*KO mice exhibit reduced spontaneous dopamine activity with virtually no spontaneous phasic activity (Changeaux 2010; Mameli-Engvall et al. 2006). Moreover, prior studies have shown that chronic nicotine functionally upregulates α4β2* on GABAergic neurons (but not dopamine cell bodies) in the SN (Nashmi et al. 2007), increasing inhibitory tone on dopamine neuron activity (Nashmi et al. 2007; Tapper et al. 2004, 2007). Thus it is possible that changes in dopamine cell responsiveness to stimulation resulting from either upregulation of α4β2* on GABAergic neurons following chronic nicotine or genetic deletion of β2* on dopamine neurons could contribute to the reduced dopamine release observed in the current study. However, Stuber and colleagues (van Zessen et al. 2012) recently showed that optogenetic stimulation of midbrain GABAergic neurons applied simultaneously with electrical stimulation of midbrain dopamine cells significantly reduced tonic, but not phasic, dopamine release in the nucleus accumbens. Thus, although an overall decrease in dopamine neuron activation might account for reduced dopamine release at low frequencies, it cannot account for reduced release at higher frequencies. The mechanism underlying the reduction in high-frequency dopamine release is unknown; however, chronically decreased dopamine activity may induce a reduction in the size of the readily releasable pool (RRP) of dopamine (Hartman et al. 2006; Maffei et al. 2006; Turrigiano 2011). Alternatively, reduced dopamine terminal β2*nAChR expression may diminish the efficacy of dopamine neurons to replenish the RRP following high-frequency stimulation (Kile et al. 2010; Venton et al. 2006). Such changes in the RRP may explain why increasing pulse number or frequency is not sufficient to overcome the reductions in dopamine release observed in cNIC and β2*KO mice.

Overall, our data suggest that chronic nicotine, acting via β2*nAChRs, alters dopamine release dynamics, reducing release sensitivity and constricting the range of dopamine release in response to dopamine cell activity. It is difficult to speculate how these alterations in dopamine signaling may contribute to nicotine addiction, but we suggest that a chronically restricted range of activity-dependent dopamine release may make low-frequency signals difficult to discern from noise and diminish differences in release between higher frequencies. Thus a chronically restricted range of dopamine release may alter the striatal decoding of reward signaling. Indeed, several studies have shown that chronic nicotine (Johnson et al. 2008; Kenny and Markou 2005; Kenny et al. 2006) and exposure to other drugs of abuse can alter the sensitivity of brain reward systems as measured by intracranial self-stimulation (Hollander et al. 2012; Kenny et al. 2003, 2006). Moreover, because dopamine intimately influences corticostriatal plasticity (Calabresi et al. 2007; Lerner and Kreitzer 2011; Reynolds and Wickens 2002; Shen et al. 2008), a chronically restricted range of activity-induced dopamine release may alter corticostriatal synaptic plasticity, changing reinforcement learning in response to reward signals. The net result might be to make reinforcement learning processes dependent on circulating nicotine levels.

Finally, it is of interest to note that epidemiological studies have consistently demonstrated that smoking inversely correlates with incidence of Parkinson's disease (PD; Chen et al. 2010; Gorrell et al. 1999; Morens et al. 1995; Quik 2004). PD risk decreases with greater number of years and packs of cigarettes smoked, and following smoking cessation, risk gradually normalizes. It is intriguing to ask whether the reduction in dopamine release we observe following chronic nicotine exposure may underlie this apparent protective effect of chronic nicotine. It seems paradoxical that chronic nicotine induces the very problem it is putatively protecting against, reduced dopamine. One possibility is that chronic nicotine exposure reduces dopamine release, which, in turn, induces neuroadaptations that "inoculate" against the deleterious effects of dopamine denervation during early stages of PD, possibly protecting against aberrant corticostriatal plasticity associated with dopamine blockade or denervation (Beeler 2011; Beeler et al. 2010, 2012; Zhuang et al. 2013).

Overall, our results suggest nicotinic receptor activation provides a gain mechanism for activity-dependent dopamine release, facilitating release in response to low-frequency activity and increasing the dynamic range of dopamine release across frequencies. Loss of β2*-containing nAChRs and chronic nicotine exposure degrades the range of dopamine release. A chronically restricted range of activity-induced dopamine release, in turn, may alter the striatal decoding of reward signaling and alter corticostriatal plasticity and learning in response to those signals. Moreover, just as chronic alterations in nicotinic signaling induce long-term neuroadaptations, chronically reduced dopamine release may induce further neuroadaptations comprising part of a cascade of neural changes in response to chronic nAChR inactivation or desensitization.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.L.K., D.S.M., M.F.R., J.A.B., and X.Z. conception and design of research; J.L.K. and J.J.C. performed experiments; J.L.K. and M.F.R. analyzed data; J.L.K., J.J.C., D.S.M., M.F.R., J.A.B., and X.Z. interpreted results of experiments; J.L.K. and J.A.B. prepared figures; J.L.K. drafted manuscript; J.L.K.,

J.J.C., D.S.M., M.F.R., J.A.B., and X.Z. edited and revised manuscript; J.L.K., J.J.C., D.S.M., M.F.R., J.A.B., and X.Z. approved final version of manuscript.

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