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Properties of Dopamine Release and Uptake in the Songbird Basal Ganglia

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Gale, Samuel D. and David J. Perkel. Properties of dopamine release and uptake in the songbird basal ganglia. J Neurophysiol 93: 1871–1879, 2005. First published November 17, 2004; doi:10.1152/jn.01053.2004. Vocal learning in songbirds requires a basal ganglia circuit termed the anterior forebrain pathway (AFP). The AFP is not required for song production, and its role in song learning is not well understood. Like the mammalian striatum, the striatal component of the AFP, Area X, receives dense dopaminergic innervation from the midbrain. Since dopamine (DA) clearly plays a crucial role in basal ganglia–mediated motor control and learning in mammals, it seems likely that DA signaling contributes importantly to the functions of Area X as well. In this study, we used voltammetric methods to detect subsecond changes in extracellular DA concentration to gain better understanding of the properties and regulation of DA release and uptake in Area X. We electrically stimulated Ca2+- and action potential–dependent release of an electroactive substance in Area X brain slices and identified the substance as DA by the voltammetric waveform, electrode selectivity, and neurochemical and pharmacological evidence. As in the mammalian striatum, DA release in Area X is depressed by autoinhibition, and the lifetime of extracellular DA is strongly constrained by monoamine transporters. These results add to the known physiological similarities of the mammalian and songbird striatum and support further use of voltammetry in songbirds to investigate the role of basal ganglia DA in motor learning.

INTRODUCTION

The neurotransmitter dopamine (DA) plays an important role in the function of basal ganglia circuits in motor control and learning. One such circuit is the anterior forebrain pathway (AFP) in songbirds, which is required for song learning in juveniles and plasticity of adult song but not for song production (Bottjer et al. 1984; Brainard and Doupe 2000; Scharff and Nottebohm 1991; Sohrabji et al. 1990; Williams and Mehta 1999). The first component of the AFP is the basal ganglia nucleus Area X. Like the mammalian striatum, Area X receives glutamatergic projections from pallial areas (the premotor nucleus HVC, used as a proper name, and the AFP output nucleus the lateral magnocellular nucleus of the anterior nidopallium, LMAN; nomenclature following Reiner et al. 2004) and a dense dopaminergic projection from the midbrain ventral tegmental area (VTA; Fig. 1; Bottjer 1993; Lewis et al. 1981). The essential role of the AFP in song learning and adult song plasticity and the known functions of basal ganglia DA in motor control and learning in mammals lead to the hypothesis that DA may play a key part in the song learning process or other functions of Area X. Consistent with an important role for DA in information processing in Area X, DA modulates the excitability of the major cell type (the spiny neuron) in Area X and also the strength of excitatory synaptic inputs to these cells (Ding and Perkel 2002, 2004; Ding et al. 2003).

In mammals, DA neurons fire spontaneously at a slow rate and transiently burst in response to salient events such as reward or reward predicting stimuli (Hyland et al. 2002; Schultz 1998). Burst firing enhances accumulation of extracellular DA (Chergui et al. 1994) and presumably causes the phasic increases in extracellular DA concentration observed in the striatum of behaving rats (Cheer et al. 2004; Phillips et al. 2003; Robinson et al. 2002; Roitman et al. 2004). Ultimately, the dynamics of extracellular DA concentration depends critically on local factors that vary among different regions of the brain: the density of DA release sites, the rate of DA uptake through transporter proteins, and the state of release-regulating neurotransmitter receptors on DA axon terminals. These factors influence whether DA acts via phasic (subsecond) signaling, slower fluctuations and/or by tonic influence on DA receptors.

A method of selectively measuring DA release with subsecond temporal resolution seems imperative for understanding the properties and functions of DA signaling in the songbird basal ganglia. In mammals, such measurements have been achieved in brain slices and in vivo using voltammetric methods in which endogenously released DA is oxidized on the surface of a carbon fiber, and the resulting current is recorded (reviewed in Robinson et al. 2003). We applied these techniques to songbirds to determine whether we could reliably measure DA release in Area X and if the factors regulating the extracellular concentration of DA are similar in the mammalian and songbird striatum. From brain slices of Area X, we electrically stimulated Ca2+- and action potential–dependent release of an electroactive substance identified as dopamine by the shape of the voltammetric waveform, electrode selectivity, and by anatomical, neurochemical, and pharmacological evidence. The properties and regulation of DA release and uptake in Area X are similar to those reported previously in the mammalian striatum. These results extend the wealth of existing physiological and anatomical evidence for a high degree of similarity between avian and mammalian basal ganglia and verify that voltammetry will be a useful technique to measure DA release with high temporal resolution in Area X of songbirds in vivo.

METHODS

Care of birds and preparation of brain slices

All procedures were approved by the University of Washington Institutional Animal Care and Use Committee. Adult (>90 days old) male
FIG. 1. Simplified diagram of the song system. HVC (used as a proper name) projects to the robust nucleus of the archicerebellum (RA) as part of the motor pathway for song production. HVC also projects to RA indirectly via the anterior forebrain pathway, which includes Area X, the medial portion of the dorsolateral nucleus of the anterior thalamus (DLM), and the lateral magnocellular nucleus of the anterior nidopallium (LMAN). Dopaminergic inputs to Area X originate in the ventral tegmental area (VTA).

zebra finches were obtained from commercial suppliers and housed in groups of five or fewer on a 13:11-h light/dark cycle. Food and water were available at all times. Brain slices were prepared as described in detail by Stark and Perkel (1999). Birds were anesthetized with isoflurane and decapitated. The brain was removed and immersed in an ice-cold artificial cerebrospinal fluid (ACSF), which was made of the same components described above for the slicing solution except for replacement of HEPES with an additional 10 mM NaHCO₃. The ACSF was initially maintained at 35°C when the slices were transferred and allowed to cool to room temperature. All solutions were continuously bubbled with a gas mixture of 95% O₂-5% CO₂. Slices were left for at least 1 h before use.

Electrochemical recordings

To construct carbon fiber electrodes (CFEs), a single carbon fiber (15 mm long, 11 µm diam; P-25, Amoco, Tustin, CA) was inserted into the small end of a standard plastic P200 pipette tip. The plastic around the carbon fiber was melted with a heating coil in such a way that the pipette tip could be pulled by hand to a shank that sealed around the carbon fiber. The exposed carbon fiber tip was cut to a length of 30–50 µm by hand with a scalpel blade. The electrode was filled with 2 M KCl.

For recordings, slices were submerged in a small, illuminated chamber and perfused (2–3 ml/min) with ACSF warmed to 32°C. The borders of Area X were clearly visible through a dissecting microscope. The tip of the CFE was gently lowered into the slice to a depth of 50–150 µm. A bipolar, stainless steel stimulating electrode was inserted about 100 µm from the CFE. DA release was elicited by single 0.1-ms shocks (60–70 V amplitude) controlled by a stimulus isolation unit (Isoflex, AMPI, Jerusalem, Israel). Signals were amplified with a MultiClamp 700A amplifier in voltage-clamp mode and digitized with a Digidata 1322A (Axon Instruments, Foster City, CA). The electrode potential, stimulus, and data acquisition were controlled using Clampex 9.0 software (Axon Instruments). For constant potential amperometry (CPA), the CFE was held at +0.4 V; signals were low-pass filtered at 100 Hz and sampled at 10 kHz. For fast-scan cyclic voltammetry (FVC), the CFE was held at −0.4 V and a triangular waveform (−0.4 to 1 V and back at 300 mV/s, a total of 9.33 ms) was applied every 100 ms (10 Hz); signals were low-pass filtered at 2 kHz and sampled at 20 kHz. Background-subtracted cyclic voltammograms (current-voltage plots) were made by subtracting the average of the current recorded for 10 voltammetric scans (1 s) prior to stimulation from the current recorded for each voltammetric scan after stimulation. Changes in DA concentration were quantified by plotting the peak oxidation current (converted to DA concentration as described below) of the voltammograms corresponding to each 100 ms-spaced time-point after stimulation. Data were analyzed and plotted using Clampfit 9.0 (Axon Instruments) and IGOR (Wave Metrics, Lake Oswego, OR).

CFEs were calibrated (at the end of a day of experiments) to convert current to approximate DA concentration. The CFE tip was carefully lowered into the end of the glass tube (1.1 mm ID) from which fresh ACSF perfused the slice. A 5-s “pulse” of 1–5 µM DA or norepinephrine (NE) dissolved in oxygenated ACSF was allowed to pass through the perfusion tubing and over the CFE, and the current change was recorded with CPA or FVC. CPA calibration without attempting to mimic the extracellular ascorbic acid concentration in brain tissue typically underestimates the sensitivity of the electrode by an order of magnitude (Kawagoe and Wightman 1994; Schmitz et al. 2001). Therefore only the peak oxidation current measured with FCV was used to estimate DA concentration in this paper, and CPA measurements are reported in units of current. Our CFEs were about three times more sensitive to DA than NE.

Slices were stimulated once every 2.5 min to allow full recovery from paired-pulse depression (see RESULTS and DISCUSSION). Drugs (diluted to their final concentration in the ACSF perfusing the slice chamber) were applied after the peak amplitude of the signal was stable for at least three consecutive stimulations.

For comparisons of peak DA release and decay time constant among Area X, medial striatum (MSI), and lateral striatum (LSI), DA release was recorded at 13 locations in the striatum of each parasagittal slice. Area X was divided into four quadrants to give four of the locations. Six locations were in the MSI outside of Area X (2 anterior, 1 ventral, and 3 posterior to Area X). Three locations were dorsal but not anterior to the globus pallidus and considered to be in the LSI. The CFE and stimulating electrodes were positioned at each location (in pseudorandom order) and DA release was evoked by single shock stimulation as described above. The peak amplitude of DA release and decay time constant were determined from a single trace taken after the amplitude was stable for three consecutive stimulations (as described for drug experiments above). The values of peak DA release and decay time constant at each location within a region (Area X, MSI, and LSI) were averaged to obtain a single value for each region in the slice. These are the values plotted and used for statistical analysis. DA uptake in the mammalian striatum is modeled according to Michaelis-Menten kinetics to determine the maximum rate of uptake (Vmax). However, we do not know the value of Km (binding affinity) for the DA transporter in birds or even whether just a single transporter contributes to DA decay in the zebra finch striatum. We instead fit the latter part of the decay phase (beginning at the concentration reached at the location with the smallest amplitude of DA release) to a single exponential to determine the decay time constant (see Fig. 5A).

Measurement of monoamine tissue content

For tissue content measurements, a 400- to 500-µm-thick coronal slice within the anterior and posterior borders of Area X was prepared as described above. While the slice was still immersed in the ice-cold slicing solution, a square piece of tissue within the medial-lateral and dorsal-ventral borders of Area X was dissected with a sharp scalpel under a dissecting microscope. The dissected piece of tissue was transferred to a plastic tube and frozen on dry ice. A similar-sized piece of tissue was cut from the pallium dorsal to Area X in the same slice. Tissue samples remained frozen at −80°C or on dry ice until analyzed for monoamine content. Monoamine levels were measured by HPLC with electrochemical detection at the Neurochemistry Core Lab in Vanderbilt University’s Center for Molecular Neuroscience.
obtained from DA or NE dissolved in ACSF (Fig. 2B, inset).
The current at the peak oxidation potential measured with FCV changed with a time course similar to the current change measured with CPA (Fig. 2B). However, the time to peak and the decay of the FCV signal were slightly slower than those of the CPA signal because of adsorption of analyte to the CFE between FCV scans (Bath et al. 2000; Venton et al. 2002).

The shape of the voltammogram strongly suggested we were measuring release of DA and/or NE (DA and NE produce very similar voltammograms). In mammals, it is commonly assumed that CFEs solely measure DA, and not NE, release in the striatum because CFEs are about three times more sensitive to DA than NE (see METHODS) and the density of DA innervation and DA tissue content are much greater than those of NE in the striatum (DA:NE content is 100:1 in the caudate and 10:1 in the nucleus accumbens of rats; Garris et al. 1993; Kuhr et al. 1986). Similarly, in zebra finches, the tyrosine hydroxylase (TH; the rate-limiting enzyme in the synthesis of both DA and NE) positive fibers that project heavily to Area X originate from cell bodies in VTA that are TH positive but not positive for dopamine-β-hydroxylase (DβH; an enzyme involved in conversion of DA to NE and thus a marker of noradrenergic neurons), and lesions of VTA completely abolish catecholamine histofluorescence in Area X (Bottjer 1993; Lewis et al. 1981; Mello et al. 1998). DβH-positive fibers are sparse in Area X and surrounding striatal areas (Mello et al. 1998). We directly measured monoamine content in tissue homogenate from Area X and from pallium located dorsal to Area X (a region with much lighter TH immunostaining) using liquid chromatography. Levels of NE, serotonin (5-hydroxytryptamine, or 5-HT), and the 5-HT metabolite 5-hydroxyindole-3-acetic acid (5-HIAA) were not significantly different in Area X and pallium (P = 0.4, paired t-test), whereas, as expected, levels of DA and its metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were significantly greater in Area X than in pallium (P < 0.05; Fig. 3). DA was on average 40 times more abundant than NE in Area X (P < 0.0001, paired t-test; Fig. 3). This result is qualitatively the same as that of Harding et al. (1998), who found DA was about five times more abundant than NE in Area X in 90-day old zebra finches (the difference in absolute amounts of DA and NE measured here and by Harding et al. might be due to differences in release and metabolism of catecholamines while handling the bird or during tissue collection). Thus the zebra finch striatum seems to receive, like the striatum of mammals and other
birds (Reiner et al. 1994), a very dense DA innervation and a relatively sparse noradrenergic innervation. Although tissue content is only a measure of what may potentially be released by local stimulation in brain slices (for instance, it does not distinguish between neurotransmitter in the releasable pool of vesicles versus storage pools; Garris and Wightman 1994), the higher sensitivity of CFEs for DA than NE, anatomical data, and relative amounts of DA and NE contained in Area X suggest that the CPA and FCV signals resulting from electrical stimulation in Area X are a measure of DA release and that NE is unlikely to make more than a small contribution to these signals.

To ask whether the DA release we evoked is dependent on action potentials and Ca\(^{2+}\) entry, we used CPA to measure the peak amplitude of DA release after blocking action potentials with TTX (2 \(\mu\)M), preventing Ca\(^{2+}\) entry by removing Ca\(^{2+}\) from the ACSF bathing the slice, or blocking voltage-gated Ca\(^{2+}\) channels with Cd\(^{2+}\) ions (100 \(\mu\)M CdCl\(_2\)). TTX \((n = 6)\), removal of extracellular Ca\(^{2+}\) \((n = 6)\), or Cd\(^{2+}\) \((n = 3)\) all significantly reduced the peak amplitude of evoked DA release by 80–100\% \((P < 0.001, t\)-test\), and the effects of Ca\(^{2+}\) removal and Cd\(^{2+}\) were at least partially reversible (Fig. 4). Thus, in Area X as in mammalian striatal slices, local electrical stimulation elicits Ca\(^{2+}\)- and action potential–dependent release of DA.

**Regional comparison of DA release and uptake in the avian striatum**

The peak DA concentration measured at the CFE depends on the density of DA release sites, the amount of DA released at each site, and the distance DA diffuses from each release site (largely controlled by the rate of DA uptake through transporters). Peak DA release and the rate of DA uptake thus reflect important properties of DA signaling and vary across different regions of the mammalian brain. For instance, in rats, peak DA release is greater and DA uptake rate faster by an order of magnitude in the striatum than in the prefrontal cortex and amygdala (Garris and Wightman 1994). Also, in both the rodent and primate striatum, peak DA release tends to be greater and uptake faster in “motor” compared with “limbic” striatal subregions (Cragg 2003; Cragg et al. 2000, 2002; Garris and Wightman 1994; Jones et al. 1995, 1996). To determine whether peak DA release and the kinetics of DA uptake are different among Area X and other parts of zebra finch striatum, we recorded DA release in response to a single shock in three different regions of the striatum (Area X, MSt outside of Area X, and LSt) in parasagittal slices \((n = 8);\) see METHODS). FCV was used so that we could compare peak release amplitude in terms of estimated concentration (see METHODS). To compare rate of DA uptake, we fit the latter part of the decay phase to a single exponential and measured the time constant (see METHODS and Fig. 5A). Peak DA concentration after a single shock varied from about 0.5 to 2.5 \(\mu\)M (similar to concentrations observed in mammalian striatal slices) and was significantly greater in Area X than MSt and LSt \((P < 0.05,\) repeated measures ANOVA and Tukey’s multiple comparisons test; Fig. 5B). This is consistent with the greater intensity of TH-expressing fibers (and thus probably

**FIG. 4.** A: example of an experiment showing effect of removing extracellular Ca\(^{2+}\) \((0 \text{ Ca}\(^{2+}\)) or applying TTX on DA release. B: CPA traces from the time-points indicated in A. Stimulation occurred at 1 s. C: inhibition of DA release in all experiments with TTX \((n = 6)\), 0 Ca\(^{2+}\) \((n = 6)\), and Cd\(^{2+}\) \((n = 3)\).
and LSt (a constant was not significantly different among Area X, MSt, and LSt) and thus faster uptake of DA in Area X, but the decay time might also result in a greater density of monoamine transporters and release sites in Area X than surrounding striatum in adult zebra finches (Soha et al. 1996). Greater fiber density might also result in a greater density of monoamine transporters and thus faster uptake of DA in Area X, but the decay time constant was not significantly different among Area X, MSt, and LSt ($P = 0.38$, repeated measures ANOVA; Fig. 5C).

**Regulation of DA uptake through monoamine transporters**

In mammals, DA is rapidly diluted as it diffuses from the release site, and thereafter, the time and distance DA travels and interacts with receptors is heavily influenced by the rate of uptake via the DA transporter (DAT) (Cragg and Rice 2004). To assay the influence of monoamine transporters on DA transmission in Area X, we measured (with CPA) the half-life (time to 50% decay) of DA released by a single shock in the presence of monoamine uptake transporter inhibitors. The DAT inhibitors GBR-12935 (GBR; $5 \, \mu M$, $n = 6$) and nomifensine ($5 \, \mu M$, $n = 5$) increased the half-life of DA released in Area X by over 350%, indicating that DA transmission is tightly controlled by uptake through the DAT in Area X (both effects $P < 0.05$, t-test; Fig. 6). Surprisingly, the NE transporter (NET) inhibitors desipramine ($1 \, \mu M$, $n = 6$) and maprotiline ($10 \, \mu M$, $n = 5$) at concentrations that are specific to NET in mammals also substantially increased the half-life of DA released in Area X (by over 800 and 300%, respectively; both effects $P < 0.01$, t-test; Fig. 6).

Expression of DAT or NET is specific to the cells that synthesize and release the corresponding neurotransmitter in mammals; however, both DAT and NET can transport either catecholamine (reviewed in Torres et al. 2003). The NET (located on NE-releasing axons) has a significant role in the uptake of DA in the rat prefrontal cortex, where DA innervation is sparse compared with the striatum and DAT expression is low (Moron et al. 2002; Mundorf et al. 2001; Sesack et al. 1998). The NET does not contribute to DA uptake in the mammalian striatum (Cragg 2003; Cragg et al. 2000, 2002; Jones et al. 1995, 1996). Even in the nucleus accumbens shell, where there is moderate NE innervation and expression of NET and where DAT expression is less dense compared with the dorsal striatum and nucleus accumbens core, NET inhibitors do not affect DA uptake in normal or DAT knock-out mice (Berridge et al. 1997; Budygin et al. 2002; Delfs et al. 1998; Jones et al. 1997; Schroeter et al. 2000). Hence, the possibility that NET located on NE axons contributes strongly to DA uptake in Area X seems unlikely given the very high-density of DA fibers compared with NE fibers. Another possibility is that DA axons in Area X express two transporters—one pharmacologically DAT-like and the other NET-like—that both contribute significantly to DA uptake. If either of the possibilities stated above were true, the combination of a DAT inhibitor and NET inhibitor might have greater effect on DA half-life in Area X than either of the drugs alone. Co-application of GBR and desipramine ($n = 5$) or GBR and maprotiline ($n = 5$) had no further effect on DA half-life than desipramine or maprotiline alone ($P = 0.28$ and $P = 0.93$, respectively, unpaired t-test; Fig. 6), suggesting instead that both types of drugs (DAT and NET inhibitors) act on the zebrafish DAT. These results show that the spatial and temporal influence of DA in Area X, as in the mammalian striatum, are strongly restricted by the action of DA transporters.

The 5-HT transporter (SERT) inhibitor fluvoxamine ($5 \, \mu M$, $n = 5$) and the monoamine oxidase (MAO-B) inhibitor pargyline ($20 \, \mu M$, $n = 3$) had no effect on DA half-life in Area X ($P = 0.86$ and 0.53, respectively, t-test; Fig. 6). This is consistent with our other evidence (most importantly, the shape of the voltammogram) that 5-HT and DOPAC do not contribute to our CPA and FCV measurements and shows that SERT and MAO-B are not involved in the rapid decay of extracellular DA in Area X.

**FIG. 5.** A: example FCV recordings from Area X (circles), MSt outside of Area X (triangles), and LSt (squares). Stimulation occurred at 1 s. Latter parts of the decay phase are fit to a single exponential (shown with solid lines) beginning at the same concentration (see METHODS). B: peak DA release in the Area X, medial striatum (MSt), and lateral striatum (LSt) are plotted as open circles and are connected by lines for measurements made in the same slice ($n = 8$). Mean value for each area is indicated by an open bar. Error bars represent SE. C: decay time constants in Area X, MSt, and LSt plotted as in B.
Control of DA release by presynaptic neurotransmitter receptors

In mammals, extracellular DA inhibits its own release by binding to D2 receptors on DA axon terminals. We tested the effect of the D2 agonist quinpirole (10 μM) and D2 antagonist sulpiride (10 μM) on the peak amplitude of DA release in Area X recorded with CPA. Since DA can activate α2 noradrenergic receptors (Cornil et al. 2002; Zhang et al. 1999) and α2 receptor agonists reduce DA release in the mammalian striatum (Trendelenburg et al. 1994; Yavich et al. 1997), we also tested the possibility that α2 receptors can act as release-regulating autoreceptors on DA terminals in Area X using the α2 receptor agonist clonidine (10 μM) and antagonist yohimbine (10 μM). Quinpirole decreased DA release by ~50% (n = 5, P < 0.0001, t-test; Fig. 7). The effect of quinpirole was significantly reduced by sulpiride (n = 5; P < 0.001, 1-way ANOVA with Tukey’s multiple comparisons test) and was not affected by yohimbine (n = 3, P > 0.05, same test); a small (<10%) but significant effect of quinpirole on DA release persisted in the presence of sulpiride (n = 5, P < 0.001, t-test). Sulpiride itself increased DA release by ~35% (n = 7, P < 0.01, t-test), suggesting that D2 receptors are tonically active and inhibiting DA release in our slice preparation. Tonic D2 activation in our slice preparation might be the result of constitutive DA release that is independent of the activity of DA cell bodies, which were not present in our slices. Clonidine decreased DA release by ~25% (n = 6, P < 0.001, t-test). The effect of clonidine was blocked by yohimbine (n = 3, P < 0.01, 1-way ANOVA with Tukey’s multiple comparisons test) but not by sulpiride (n = 3, P > 0.05). The block of clonidine by yohimbine was complete (n = 3, P = 0.38, t-test). Yohimbine alone had no effect on DA release in Area X (n = 6, P = 0.56, t-test).

These results indicate that DA release in Area X is inhibited by activation of D2 and α2 receptors. To determine whether endogenously released DA can activate these receptors and inhibit further DA release (autoinhibition), we measured (with CPA) the ratio of the peak amplitude of DA release caused by single shocks separated by short time intervals (the paired-pulse ratio, or PPR) in normal conditions and in the presence of sulpiride or yohimbine at the same recording position. For intervals close enough that the second release event occurred during the decay of DA released from the first shock, the amplitude of the second release event was determined by subtracting the record of DA release caused by a single pulse from the two-pulse record (Cragg 2003; Phillips et al. 2002). In control conditions, there was a time-dependent paired-pulse depression (PPD) of DA release in Area X (maximum ~80% depression at 1 s; Fig. 8, A and B). Sulpiride (n = 5) or yohimbine (n = 5) partially decreased the magnitude of PPD (sulpiride 3 times more so than yohimbine), suggesting that DA release in Area X inhibits its own release by activating D2 and more modestly, α2 receptors. The effect of sulpiride on the PPR was significant (P < 0.05, paired t-test) for the intervals from 0.1 to 3 s, and that of yohimbine for the intervals from 0.05 to 1 s. To determine the time course and magnitude of autoinhibition mediated by D2 and α2 receptors, we subtracted the PPR measured for each interpulse interval from the two-pulse record (Cragg 2003; Phillips et al. 2002). D2 receptor–mediated autoinhibition was activated within 50 ms of the first stimulation pulse, reached a maximum at 500 ms.
accounting for 30% of the PPD), and terminated by about 5 s after the initial pulse. The time course and magnitude of D2-mediated autoinhibition measured in Area X slices are similar to measurements in mammalian striatal slices (Phillips et al. 2002). Autoinhibition mediated by α2 receptors in Area X followed a similar time course but was weaker (accounting for no more than 10% of the PPD) and shorter lasting (<2 s).

The difference in the magnitudes of the D2 and α2 receptor–mediated effects could be due to receptor numbers, efficiency of activation by DA, and intracellular signaling pathways used. The relative magnitude of the effect of evoked DA release on D2 and α2 receptors is also influenced by the fact that D2 receptors are already partially activated in the slice, whereas α2 receptors are not (Fig. 7).

What is the origin of the substantial D2/α2-independent component of the PPD? Release of other neurotransmitters besides DA by the first shock might contribute to time-dependent depression of subsequent DA release. Glutamate, GABA, and acetylcholine (ACh) can all modulate DA release in the mammalian striatum (Avshalumov et al. 2003; Rice and Cragg 2004; Schmitz et al. 2002; Zhang and Sulzer 2003, 2004; Zhou

FIG. 7. A: average time course of the effects of quinpirole (D2 agonist, open circles, n = 5), sulpiride (D2 antagonist, open squares, n = 7), clonidine (α2 agonist, filled circles, n = 6), and yohimbine (α2 antagonist, filled squares, n = 6). Error bars indicate SE. B: change in peak amplitude of DA release after application of sulpiride (Slp, n = 7), quinpirole (Qnp, n = 5), quinpirole in the presence of sulpiride (n = 5), quinpirole in the presence of yohimbine (Yhm, n = 3), yohimbine (n = 6), clonidine (Cln, n = 6), clonidine in the presence of yohimbine (n = 3), and clonidine in the presence of sulpiride (n = 3). Individual experiments are plotted with circles. Mean for each group is shown with an open bar.

FIG. 8. A: paired-pulse ratio (PPR) of peak amplitude of DA release for various interpulse intervals in control conditions (filled circles) and (at the same recording position) in the presence of the D2 receptor antagonist sulpiride (open circles; n = 5). Error bars represent SE. Inset: example traces of DA release in response to paired stimulation (1-s interpulse interval) in the presence and absence of sulpiride. B: same as A except showing the PPR in control conditions (filled circles; different experiments from those in A) and in the presence of the α2 receptor antagonist yohimbine (open circles; n = 5). C: time course of D2 (filled circles) and α2 (open circles) receptor-mediated autoinhibition of DA release. Each point represents the PPR in control conditions subtracted from the PPR in the presence of drug. Error bars represent SE.
et al. 2001). Nicotine (10 μM, n = 3), the nicotinic receptor antagonist mecamylamine (10 μM, n = 7), the muscarinic receptor agonist pilocarpine (30 μM, n = 3) and antagonist atropine (20 μM, n = 4), and the nonspecific cholinergic agonist carbachol (20–100 μM, n = 6) all had no effect on the amplitude of DA released by a single shock in Area X (data not shown). Although spontaneously active cholinergic interneurons are present in brain slices of Area X (Carrillo and Doupe 2004; Farries and Perkel 2002), these results suggest that, unlike in mammals (Zhou et al. 2001), DA release is not regulated by tonic ACh release in Area X. However, these experiments do not definitively rule out the possibility that release-regulating nicotinic ACh receptors are present on DA axons in Area X. The group 1 metabotropic glutamate receptor (mGluR) agonist DHPG (100 μM, n = 5), the groups 1 and 2 mGluR agonist ACPD (100 μM, n = 6), and the GABA<sub>B</sub> receptor agonist baclofen (30 μM, n = 7) significantly reduced the amplitude of DA released by a single shock in Area X by 40 ± 9% (SD), 28 ± 11%, and 22 ± 11%, respectively (P < 0.01, t-test; data not shown). Activation of mGluR, GABA<sub>B</sub>, or other receptors by endogenous neurotransmitter might contribute to the observed PPD. A portion of the autoinhibition-independent PPD might also be due to depletion of docked vesicles at DA synapses after local stimulation in brain slices. The slow, autoinhibition-independent PPD is less pronounced in vivo in mammals (Benoit Marand et al. 2001; Montague et al. 2004; Schmitz et al. 2003).

**Summary and conclusions**

We have shown that electrical stimulation in Area X from adult zebra finches results in Ca<sup>2+<sup>- and action potential–dependent release of a substance identifiable as DA by the following electrochemical, anatomical, and pharmacological evidence.

1) The shape of the voltammogram obtained with FCV after electrical stimulation in Area X is identical to that of exogenous DA and known to be unique to DA and NE.
2) The CFEs used are three times more sensitive to DA than NE.
3) Area X receives a rich projection from neurons in VTA that synthesize DA but not NE.
4) The tissue content of DA is about 40 times more abundant than NE in Area X.
5) The half-life of the released substance is dramatically increased by drugs known to block the uptake of DA through proteins that transport DA.
6) The released substance inhibits its own release by activating D2 DA receptors with a time course similar to that of D2 receptor–mediated autoinhibition of DA release in mammalian striatal slices.

The factors shown to regulate release and uptake of DA in Area X are very similar to those reported for the mammalian striatum, suggesting a common functional design for DA neurotransmission in mammalian and songbird basal ganglia.

Mammalian DA neurons spontaneously fire action potentials at a slow rate in vivo; uptake and tonic depression of release by D2 autoreceptors help set the steady-state extracellular concentration of DA in the striatum. During burst firing, DA released at short interspike intervals accumulates faster than uptake can remove it, resulting in a phasic increase in extracellular DA that reaches farther from the release site, has an increased probability of activating low-affinity receptors, and transiently reduces (via autoinhibition) subsequent DA release by the slow, tonic discharge of DA neurons. This amplification of extracellular DA in the striatum might be important for effectively transmitting the phasic signal represented by burst firing of DA neurons.

The temporal dynamics and function of DA signaling in behaving songbirds are not known. The results presented here lend confidence to the possibility of using voltammetry to measure changes in extracellular DA concentration with sub-second temporal resolution in Area X in vivo, although even more caution will be required in interpreting the identity of catecholamines contributing to voltammetric signals in vivo. Since DA plays such an important role in mammalian basal ganglia function, knowing the conditions that cause DA release and the cellular effects of DA in Area X will likely contribute to understanding how the AFP functions in songbirds, which may in turn prove useful as a model of basal ganglia function in general.

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**References**


