Endocannabinoids mediate synaptic plasticity at glutameric synapses on spiny neurons within a basal ganglia nucleus necessary for song learning

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Thompson JA, Perkel DJ. Endocannabinoids mediate synaptic plasticity at glutameric synapses on spiny neurons within a basal ganglia nucleus necessary for song learning. J Neurophysiol 105: 1159–1169, 2011. First published December 22, 2010; doi:10.1152/jn.00676.2010.—Activation of type 1 cannabinoid receptors (CB1R) in many central nervous system structures induces both short- and long-term changes in synaptic transmission. Within mammalian striatum, endocannabinoids (eCB) are one of several mechanisms that induce synaptic plasticity at glutameric terminals onto medium spiny neurons. Striatal synaptic plasticity may contribute a critical component of adaptive motor coordination and procedural learning. Songbirds are advantageous for studying the neural mechanisms of motor learning because they possess a neural pathway necessary for song learning and adult song plasticity that includes a striato-pallidal nucleus, area X (homologous to a portion of mammalian basal ganglia). Recent findings suggest that eCBs contribute to vocal development. For example, dense CB1R expression in song control nuclei peaks around the closure of the sensori-motor integration phase of song development. Also, systemic administration of a CB1R agonist during vocal development impairs song learning. Here we test whether activation of CB1R alters excitatory synaptic input on spiny neurons in area X of adult male zebra finches. Application of the CB1R agonist WIN55212–2 decreased excitatory postsynaptic current (EPSC) amplitude; that decrease was blocked by the CB1R antagonist AM251. Guided by eCB experiments in mammalian striatum, we tested and verified that at least two mechanisms indirectly activate CB1Rs through eCBs in area X. First, activation of group I metabotropic glutamate receptors with the agonist 3,5-dihydroxyphenylglycine (DHPG) induced a CB1R-mediated reduction in EPSC amplitude. Second, we observed that a 10 s postsynaptic depolarization induced a calcium-mediated, eCB-dependent decrease in synaptic strength that resisted rescue with late CB1R blockade. Together, these results show that eCB modulation occurs at inputs to area X spiny neurons and could influence motor learning and production.

ENDOCANNABINOIDS (ECBS) MEDIATE activity-dependent changes in synaptic strength via activation of type 1 cannabinoid receptors (CB1R) throughout the central nervous system (Wilson and Nicoll 2001; Gerdenman et al. 2002; Kreitzer and Regehr 2001a,b). Activation of CB1R leads to a suppression of neurotransmitter release at both inhibitory and excitatory synapses, resulting in transient as well as long-lasting forms of synaptic plasticity (Gerdenman and Lovinger 2001, Huang et al. 2003; Chevaleyre et al. 2006; Kano et al. 2009). Although observed in several regions of the mammalian brain, including the hippocampus, neocortex, amygdala, and striatum, CB1R is most densely expressed in the outflow nuclei of the basal ganglia, substantia nigra, and globus pallidus (Herkenham et al. 1991; Herkenham 1992; Hohmann and Herkenham 2000; Rodriguez et al. 2001). With regard to medium spiny neurons in the striatum, the CB1 receptor is highly expressed on GABAergic axon terminals and expressed at low levels on glutameric axon terminals (Uchigashima et al. 2007). The striatum and the other basal ganglia regions to which it projects play critical roles in motor control and procedural learning (Graybiel et al. 1994; Hikosaka et al. 2000; Nicola 2007). Thus eCB-mediated synaptic plasticity is likely one crucial mechanism by which the basal ganglia selectively reinforce appropriate actions (Gerdeman and Lovinger 2001; Gerdeman et al. 2002; Kreitzer and Malenka 2005). Moreover, understanding its role in striatal plasticity will provide insight into the underlying mechanisms of cognitive impairment associated with cannabinoid abuse (Goonawardena et al. 2010; Puighermanal et al. 2009).

Song acquisition in songbirds represents an excellent model for studying feedback-based motor learning because adult song crystallization requires a basal ganglia circuit to process auditory and motor feedback. In the songbird telencephalon, an anterior forebrain pathway (AFP), necessary for song learning and adult song plasticity, includes a specialized striato-pallidal basal ganglia nucleus (area X) that shares many electrophysiological and morphological features with mammalian basal ganglia (for review, see Doupe et al. 2005). During song development, juvenile birds transition from variable vocal sequences to stereotyped song, a phenomenon that typifies the reinforcement-based process underlying many forms of motor learning (Troyer and Doupe 2000a,b). CB1R activation may contribute to long-term change in this circuit. Systemic activation of CB1R coincident with tutor song exposure during development induces severe vocal impairment, indicating that disruption of eCB activity interferes with the production of stereotyped song (Soderstrom and Tian 2004).

The role of eCB modulation in area X is of particular interest because spiny neurons in this nucleus receive glutameric input from both the premotor nucleus HVC (proper name) and from the AFP through the lateral magnocellular nucleus of the anterior nidopallium (Bottjer et al. 1989; Farries and Perkel 2002). Spiny neurons make GABAergic inhibitory connections on area X output neurons, which are crucial for regulating AFP information flow (Farries et al. 2005; Leblois et al. 2009), and they exhibit time-locked activity during the production of syllable segments (Goldberg and Fee 2010). The capacity to integrate information from feed forward motor signals and performance feedback could allow spiny neurons to encode and exert modifications to the motor program through changes in synaptic strength. Therefore, synaptic plasticity within area
X may contribute to the trial and error learning during the sensory-motor phase of song development. Here we test whether eCB-mediated transient and long-term forms of synaptic plasticity occur in area X.

METHODS

Slice preparation. Forty-seven adult male zebra finches (Taeniopygia guttata) were obtained from our breeding colony or a commercial supplier. Birds were housed in cages of five or fewer on a 14:10-h light-dark cycle. Slicing procedures used were similar to those described by Ding et al. (2003) and were approved by the Institutional Animal Care and Use Committee at the University of Washington. Briefly, birds were anesthetized using isoflurane and decapitated. The brain was quickly removed and immersed in ice-cold artificial cerebrospinal fluid (aCSF) containing the following (in mM): 119 NaCl, 2.5 KCl, 1.3 MgSO₄, 2.5 CaCl₂, 1 NaH₂PO₄, 16.2 NaHCO₃, 11 glucose, and 10 HEpES, with an osmolality of 290–310 mosmol/kgH₂O. Slices (350– to 400-μm thick) were cut coronally with a Vibratome 1000 Plus (Vibratome, St. Louis, MO) in ice-cold aCSF and then transferred to a storage chamber containing aCSF heated to 30–35°C. The storage chamber was allowed to cool to room temperature after slicing was completed. In both the storage and recording aCSF, HEpES was replaced with equiosmolar NaHCO₃. All solutions were continuously bubbled with a gas mixture of 95% O₂:5% CO₂.

Electrophysiological recording. Slices were allowed to equilibrate for 1 h in the storage chamber before recording. For recording, a slice was submerged in a small chamber perfused (flow rate: 2–3 ml/min) with the HEpES-free aCSF containing the GABA_A receptor antagonist picrotoxin (150 μM; Sigma, St. Louis, MO), with an osmolality of 290–310 mosmol/kgH₂O. Slices were transilluminated and viewed through a dissecting stereomicroscope. When transilluminated, area X is visually identifiable as a dark region in the medial striatum, ventral to the dorsal arcopallial lamina. Neurons in area X were recorded in voltage-clamp mode using the “blind” whole cell technique (Blanton et al. 1989). Glass pipettes (5–10 MΩ) were pulled to have a tip of <2 μm in diameter (Micropipette Puller P-97; Sutter Instruments, Novato, CA) and filled with internal solution containing the following (in mM): 120 Cs-gluronate, 10 HEpES, 0.2 EGTA, 8 NaCl, 2 ATP, 0.3 GTP, 2 MgCl₂, 5 QX-314, and 10 phosphocreatine, pH 7.25–7.35. All experiments were continuously monitored (median: 38 MΩ; range: 15–58 MΩ). Experiments were excluded from further analysis if there was ≥25% change in the series resistance. Pairs of stimuli were delivered with a 50-ms interpulse interval to measure the paired pulse ratio, a test for possible involvement of presynaptic sites of action (Manabe et al. 1993).

Synaptic physiology. Excitatory postsynaptic currents (EPSCs) were evoked at either 10- or 15-s intervals by electrical stimulation with bipolar stainless-steel electrodes (Frederick Haer) placed within area X near its dorsal boundary (Fig. 1B). The recording electrode was placed near and ventral to the stimulating electrodes in most cases. AMPA glutamate receptor-mediated EPSCs were assessed at a holding potential of −80 mV. Throughout all experiments, input resistance and series resistance, key indicators of cell health and recording quality, were monitored with a 50-ms hyperpolarizing voltage pulse of 5 mV on each sweep of data collected. Series resistance was continuously monitored (median: 38 MΩ; range: 15–58 MΩ). Experiments were excluded from further analysis if there was ≥25% change in the series resistance. Pairs of stimuli were delivered with a 50-ms interpulse interval to measure the paired pulse ratio, a test for possible involvement of presynaptic sites of action (Manabe et al. 1993).

Histological procedures. After an experiment was completed, the slice was fixed in paraformaldehyde (4% in 0.1 M phosphate buffer) overnight at 4°C and then transferred to 30% sucrose (in 0.1 M phosphate buffer) for cryoprotection. After at least overnight immersion in the sucrose solution at 4°C, slices were sectioned to 50-μm thickness with a freezing microtome. Resected slices were processed with Alexa-568 conjugated streptavidin (Invitrogen). Labeled neurons, which lack spontaneous activity and display a distinct current response profile to the voltage ramp (Ding and Perkel 2002; Farries and Perkel 2002).

To measure EPSC amplitude, the size of the absolute peak current following stimulation was measured. The paired-pulse ratio was calculated as the amplitude of the second EPSC divided by the amplitude of the first EPSC. To determine the effect of drug on EPSC amplitude, for each cell the peak amplitude of 10 consecutive traces just before drug application and 10 consecutive traces preceding drug washout were averaged to derive the baseline and drug amplitudes, respectively. To compare between drug manipulations the percentage change was calculated for each cell. The percentage change induced by drug application was calculated as (postdrug − predrug)/predrug * 100. Data are presented as means ± SE unless otherwise stated.

For the depolarization experiments, we delivered a single square voltage pulse from −80 to 0 mV (of either 5- or 10-s duration). To test for an immediate effect of depolarization a baseline measure (last 2 min before onset of depolarization) was compared with the first six traces (first min) following depolarization. To assess the long-term effect of depolarization on EPSC amplitude, the baseline traces were compared with the last six traces of each recording. Because depolarization experiments were initially expected to yield effects of <2 min in total duration, most cells were recorded for the duration of 5 min. Those cells from each group that were recorded for ≥10 min were assessed to determine the stability of depolarization induced long-term effects. Therefore, measurements of long-term effects of depolarization were made at both 5 and 10 min postdepolarization time points. For both 5 and 10 min postdepolarization comparisons, the percentage change was calculated for each cell as described above to compare between depolarization manipulations.

Statistics. Statistical analysis was performed with Prism 4.0 (GraphPad Software, San Diego, CA). Pre- and postcondition comparisons for both the drug and depolarization experiments were analyzed with paired t-tests. Unpaired U-tests (Mann-Whitney) were used to compare between drug group effects. A one-way ANOVA was used to test for differences in the depolarization experiment, followed by a Tukey’s multiple comparison test for interactions.
cells were inspected under a fluorescence microscope using 60 and 100 objectives for characteristic morphological features of different cell types described previously by Farries and Perkel (2002). Confocal images of several cells were acquired with an Olympus FV1000 scanning unit.

RESULTS

We recorded from a total of 74 neurons in area X from 47 adult zebra finches. All recovered cells were confirmed to be spiny neurons with post hoc histology (see METHODS).

Spiny neuron identification. On the basis of criteria described in METHODS, we were able to distinguish spiny neurons from other cell types in area X. In response to the standard voltage ramp, putative spiny neurons showed a distinct large inward current (Fig. 1C). Nonspiny neurons responded with mostly outward current and occasional inward inflections (likely unclamped sodium currents).

Exogenous activation of CB1 receptors in area X reduces synaptic strength. To determine the capacity for CB1 receptor activation to modulate spiny neuron synaptic strength, we bath applied WIN to acute brain slices while recording in whole cell mode. An example cell (Fig. 2A) shows that bath application of 1 μM WIN reduced EPSC amplitude (EPSC amplitude expressed as a percentage of baseline: 73 ± 2%). In addition, the paired pulse ratio increased following the superfusion of WIN (112 ± 6%), indicating a decrease in the probability of neurotransmitter release. Group data (Fig. 2, C–E) show that on average 1 μM WIN reduced EPSC amplitude to 67 ± 6% of baseline (n = 7; P < 0.01, paired t-test) and increased paired pulse ratio to 114 ± 5% of baseline (P < 0.05, paired t-test; Fig. 2E).

Antagonism of the CB1 receptor blocks exogenous eCB-mediated reduction of synaptic strength. To confirm that WIN-mediated suppression of EPSC amplitude was the result of activation of CB1 receptors, we next applied a CB1 receptor antagonist before and during the superfusion of WIN. An example cell (Fig. 2B) shows that 4 μM AM251 delivered along with 1 μM WIN blocked the reduction in synaptic strength induced by exogenous CB1 receptor activation (93 ± 2% of baseline) with no change in paired pulse ratio (100 ± 3% of baseline).
average, AM251 blocked the WIN-induced depression of EPSC amplitude (98 ± 5% of baseline; n = 6; P = 0.65, paired t-test; Fig. 2, C–E). In Fig. 2D, comparison between the two drug conditions using an unpaired U-test (Mann-Whitney) shows that the decrease in EPSC amplitude following application of WIN was blocked by AM251 (73 and 95%, WIN and AM251, respectively; medians of %baseline; P < 0.01). In addition, the paired pulse ratio remained unaltered in the presence of AM251 (100 ± 4% of baseline; P = 0.98, paired t-test). Together these data establish that activation of CB1 receptors reduces glutamate release from HVC and lateral magnocellular nucleus of the anterior nidopallium terminals onto spiny neurons (Fig. 2E).

Activation of group I metabotropic glutamate receptors induced eCB-mediated suppression of EPSC amplitude on spiny neurons. To test for endogenous eCB-mediated retrograde suppression, we manipulated an upstream target in the eCB cascade: the group I metabotropic glutamate receptor (mGluR). Activation with the group I mGluR (subtypes 1 and 5) agonist DHPG induces suppression of synaptic strength in the hippocampus, cerebellum, auditory brainstem, and ventral striatum (Rouach and Nicoll 2003; Maejima et al. 2001; Ohno-Shosaku et al. 2002a; Kreitzer and Malenka 2005).

We found that in area X bath application of DHPG (100 μM) readily induced a depression of synaptic transmission (48 ± 7% of baseline; n = 7; P < 0.001, paired t-test; Fig. 3A), which was blocked by the CB1 antagonist AM251 (98 ± 7% of baseline; n = 8; P = 0.72, paired t-test). A comparison between the two drug conditions using an unpaired U-test (Mann-Whitney) shows that the decrease in EPSC amplitude following application of WIN was blocked by AM251 (45 and 99% for DHPG and AM251, respectively; medians of %baseline; P < 0.01; Fig. 3B). Consistent with a presynaptic locus of modulation the significant increase in paired pulse ratio during the application of DHPG (123 ± 10% of baseline; P < 0.05, paired t-test) was blocked by AM251 (94 ± 6%; P = 0.95, paired t-test; Fig. 3C).

Fig. 2. Exogenous activation of type 1 cannabinoid receptors (CB,R) with WIN55212–2 (WIN). A: an example cell showing that exogenous activation of CB,R with the synthetic CB,R agonist WIN (1 μM) reduced EPSC amplitude (73 ± 2% of baseline; means ± SE); time course of experiment in which each point represents the average amplitude of 4 EPSCs. Insets: sample excitatory postsynaptic current traces from the baseline period (a) and during WIN application (b); traces are averages of 10 consecutive responses. B: an example cell showing that blockade of CB,R activation with application of the CB,R antagonist AM251 (4 μM) prevents the decrease in EPSC amplitude induced by WIN application (91 ± 2%; means ± SE); time course of experiment in which each point represents the average amplitude of 4 EPSCs. Insets: sample excitatory postsynaptic current traces from the baseline period (a) and during WIN application (b); traces are averages of 10 consecutive responses. C: time-course representation of summary data shows that application of AM251 blocked the reduction in EPSC amplitude induced by CB,R activation with WIN alone. D: comparison between the 2 drug conditions using an unpaired U-test (Mann-Whitney) shows that the decrease in EPSC amplitude following application of WIN was blocked by AM251 (median values 73 and 95% for WIN and AM251, respectively; *P < 0.01). E: scatterplot of paired-pulse ratio (PPR) plotted for each cell (baseline PPR on x-axis against postdrug PPR on y-axis). Consistent with a presynaptic locus of the suppression of EPSC amplitude, we observed an increase in the paired pulse ratio (140%) following application of WIN (114 ± 5% of baseline; P = 0.05, paired t-test; means ± SE), indicating a decrease in transmitter release probability. Application of AM251 blocked the increase in PPR induced by CB,R activation (100 ± 4% of baseline; P = 0.98, paired t-test; means ± SE).
Ten second postsynaptic depolarization alone induced an immediate and prolonged depression of EPSC amplitude that was blocked by AM251. We examined the capacity of postsynaptic depolarization alone to suppress excitatory activity and whether the subsequent suppression was mediated by eCBs. We observed that a 10-s depolarization effectively decreased EPSC amplitude measured in the first minute following depolarization, whereas a shorter depolarizing voltage pulse of 5 s was insufficient to induce a decrease in EPSC amplitude [EPSC amplitude expressed as a percentage of baseline: 84 ± 3% (P < 0.001) and 99 ± 5% (P = 0.75) for 10- and 5-s depolarization, respectively; Fig. 4, A and B]. In addition, we observed that the EPSC amplitude following a 10-s depolarizing voltage pulse continued to decline for ~10 min after depolarization; this prolonged depression following depolarization is unusual compared with the transient depression typically observed in other systems (Wilson and Nicoll 2001; Ohno-Shosaku et al. 2001; Wilson et al. 2001; Yoshida et al. 2002; Diana et al. 2002). To assess long-term changes induced by 10 s postsynaptic depolarization, we measured changes in EPSC amplitude at two additional time points: 5 and 10 min postdepolarization (see METHODS). At 5 min postdepolarization, 5-s depolarization had no effect on EPSC amplitude (100 ± 4%; P = 0.85; Fig. 4A). However, the decrease in EPSC amplitude following a 10-s depolarization was sustained (73 ± 5%; P < 0.001). Similar findings were observed at 10 min postdepolarization [10 s: 54 ± 11% (P < 0.05) and 5 s: 101 ± 11% (P = 0.94)].

In several regions of the mammalian brain, transient suppression of presynaptic transmitter release through postsynaptic depolarization is regulated by the retrograde activity of eCBs. As one test of the source of the suppression following a 10-s depolarization, we observed that the paired pulse ratio was significantly increased from baseline at 5 and 10 min postdepolarization (change in paired pulse ratio expressed as %baseline; 118 ± 6 and 145 ± 24% at 5 and 10 min, respectively; Fig. 4C). Therefore, to assess whether putative retrograde eCB-activity mediated the suppression observed following the 10-s depolarization, we superfused AM251 before and after postsynaptic depolarization. We found that blockade of CB1R activation prevented the synaptic depression induced by 10 s postsynaptic depolarization both at the first minute following depolarization (EPSC amplitude expressed as %baseline: 107 ± 4%; P = 0.34) and 5 min postdepolarization (100 ± 5%; P = 0.87).
Fig. 4. A: time-course representation of summary data shows that a 10-s but not a 5-s voltage pulse is sufficient to induce eCB-mediated suppression of EPSC amplitude. B: EPSC amplitude following the 10-s voltage pulse was significantly decreased compared with both 5 s (*P < 0.01) and 10 s with AM251 (***P < 0.001); however, there was no difference between the change in EPSC amplitude for 5 and 10 s with AM251. Also, at 5 min after depolarization (Dep.), EPSC amplitude following the 10-s voltage pulse was significantly smaller than after either 5- or 10-s depolarization with AM251 (for both P < 0.01). There was, however, no difference between the change in EPSC amplitude for 5-s depolarization and 10-s depolarization with AM251. Finally, at 10 min postdepolarization, an unpaired U-test (Mann-Whitney) showed that following a 10-s voltage pulse, EPSC amplitude was significantly decreased compared with a 5-s voltage pulse. C: analysis of EPSC amplitudes in the 10-s voltage pulse group shows that the PPR does not significantly differ from baseline until 5 min postdepolarization (118 ± 6%; *P < 0.05, paired t-test; means ± SE), but that the elevated PPR is sustained 10 min postdepolarization. This coincides with the sustained decrease in EPSC amplitude (145 ± 24%; P < 0.05, paired t-test; first 3 scatterplots: •, individual cells; ○, the average). *P < 0.05, **P < 0.01, ***P < 0.001.
Two cells in the 10-s depolarization with AM251 group were recorded for 10 min and EPSC amplitude measured at the 10-min time point in these cells fell within the range of the 5-s cells at 10 min (97 ± 3%). Statistical analysis (one-way ANOVA) revealed an effect of voltage pulse duration on EPSC amplitude at 1 min postdepolarization [F(2,29) = 11.63; P < 0.001]. Analysis of post hoc comparisons (Tukey’s multiple comparison) showed that EPSC amplitude following the 10-s voltage pulse was significantly decreased compared with both 5 (P < 0.01) and 10 s with AM251 (P < 0.001); there was, however, no difference between the change in EPSC amplitude for 5 and 10 s with AM251. Similarly, at 5 min postdepolarization, a one-way ANOVA revealed an effect of voltage pulse duration on EPSC amplitude [F(2,29) = 10.86; P < 0.001]. Post hoc comparisons (Tukey’s) at 5-min depolarization showed that EPSC amplitude following the 10-s voltage pulse was significantly decreased compared with both 5 and 10 s with AM251 (for both P < 0.01). There was no difference between the change in EPSC amplitude for 5 and 10 s in the presence of AM251. Finally, at 10 min postdepolarization, an unpaired U-test (Mann-Whitney) showed that following a 10-s voltage pulse EPSC amplitude was significantly decreased compared with the change in EPSC amplitude following a 5-s voltage pulse (P < 0.05; Fig. 4B). The two cells recorded for 10 min postdepolarization following the 10-s voltage pulse with application of AM251 showed no effect on EPSC amplitude similar to the 5-s depolarization group at 10 min postdepolarization.

Ten-second depolarization-induced suppression requires calcium and is not rescued by late AM251 application. Transient change in synaptic strength following brief postsynaptic depolarization [depolarization induced suppression of inhibition or excitation (DSI/DSE)] has been described in several disparate brain regions, each with distinct kinetics (for review, see Alger 2002). However, in most forms of DSI/DSE so far observed, depolarization-induced changes in synaptic activity depend on the influx of Ca2+ through voltage-gated calcium channels because eCBs are produced in response to Ca2+ elevation (Alger 2002; Kano et al. 2009). DSE in particular is abolished by intracellular application of the calcium chelator BAPTA (Kreitzer and Regehr 2001b). Therefore, to assess whether induction of eCB-mediated DSE in area X depends on an influx of Ca2+, recordings were made with patch pipettes containing 10 mM BAPTA.

Figure 5A shows that induction of eCB-mediated DSE in area X depends on postsynaptic elevation of calcium. A Kruskal-Wallis test to assess the change in EPSC amplitude following DSE with BAPTA application revealed no differences between EPSC amplitude at baseline, at 1 min post, and at the final minute following DSE [F(2,24) = 0.594; P = 0.743]. Comparison of 10-s DSE cells with BAPTA application and control cells (10-s depolarization alone) using an unpaired U-test (Mann-Whitney) showed that DSE was blocked by intracellular BAPTA application (106 and 45% for BAPTA and control, respectively; medians of %baseline; P < 0.001; Fig. 5A2). In addition to comparing mechanisms of DSE induction between mammalian DSE and avian basal ganglia DSE, we also examined the similarity of mechanisms contributing to maintenance of suppression following depolarization. Evidence from Ronesi et al. (2004) suggests that CB1 activation is necessary for induction but not maintenance of striatal eCB-long-term depression (LTD); antagonism of CB1 R 1 min after high frequency stimulation blocks eCB-LTD, but CB1R blockade 3 min after induction produces variable blockade and CB1R blockade 10 min after induction fails to block eCB-LTD. To test whether maintenance of the prolonged suppression following 10 s postsynaptic depolarization in area X requires sustained activation of CB1R, we applied a CB1R antagonist (AM251) at 2 or 5 min after inducing DSE.

Figure 5B1 shows that application of AM251 2 min after depolarization blocked prolonged suppression of synaptic activity. On the other hand, blockade of CB1R 5 min after depolarization failed to reverse the synaptic suppression. Comparison of 2-min AM251 group with the equivalent time point postdepolarization in the 5-min AM251 group using an unpaired U-test (Mann-Whitney) showed that the DSE was reversed when AM251 was applied 2 min after depolarization but not when it was applied 5 min after depolarization (103 and 59% at 2 and 5 min, respectively; medians of %baseline; P < 0.05; Fig. 5B2). These data indicate that, similar to mammalian striatal eCB-LTD, sustained activation of CB1R is not necessary to maintain the reduction in synaptic strength; rather, transient eCB activity interacts with another putative presynaptic signal (Ronesi et al. 2004).

DISCUSSION

We report here that activation of CB1 receptors at glutamatergic synapses onto spiny neurons in the avian basal ganglia nucleus area X reduces synaptic strength through reduced presynaptic release probability. In addition, activation of group I mGluRs with the agonist DHPG induced a similar presynaptic depression. This effect was blocked by the CB1R antagonist AM251, consistent with mGluR activation leading to production of eCBs, indirectly activating CB1R (Maejima et al. 2001; Kreitzer and Malenka 2005; Narushima et al. 2006a, 2007; Fig. 3). We also observed a form of DSE that occurs following 10 s postsynaptic depolarization and that lasts minutes rather than seconds. This form of DSE is triggered by elevated calcium and blocked in the presence of a CB1R antagonist. Consistent with presynaptic high-frequency stimulation-induced eCB-LTD in mammalian striatum, we found that CB1R activation is necessary for induction but not maintenance of prolonged suppression of synaptic strength (Ronesi et al. 2004). Overall, these findings suggest that eCB-mediated striatal plasticity may be one mechanism through which eCBs are involved in vocal learning.

Ecb-mediated striatal plasticity: comparison between mammalian striatum and songbird area X. In mammalian striatum, postsynaptic release of eCBs induces long-term depression of excitatory synaptic activity through activation of the CB1 receptor on presynaptic boutons (Gerdeeman et al. 2001; Gerdeeman and Lovinger 2001; Gerdeeman et al. 2002). Release of eCBs, primarily dependent on elevation of intracellular calcium levels, can be triggered by several different mechanisms: activation of D2 dopamine receptors (Giufrida et al. 1999) or group I mGluRs (Maejima et al. 2001; Varma et al. 2001), and presynaptic high frequency conditioning (Auclair et al. 2000; Huang et al. 2001; Gerdeeman and Lovinger 2001; Gerdeeman et al. 2002; for review of eCB-mediated synaptic plasticity, see Chevaleyre et al. 2006). Here, we found that
within area X, exogenous activation of the CB₁ receptor is sufficient to depress synaptic transmission. Moreover, similar to mammalian medium spiny neurons, we demonstrated that activation of the group I mGluRs induces eCB-mediated synaptic plasticity in area X spiny neurons. Future experiments will test whether spiny neurons in area X exhibit forms of eCB-mediated synaptic plasticity requiring presynaptic stimulation paired with postsynaptic depolarization.

Interest in eCB regulation of synaptic transmission increased with the discovery that eCBs are the retrograde signaling molecules responsible for reducing inhibitory activity in hippocampal cells (Ohno-Shosaku et al. 2001; Wilson and Nicoll 2001) and both inhibitory and excitatory activity in the cerebellum (Kreitzer and Rehger 2001a,b; Yoshida et al. 2002). Although synthetic cannabinoids suppress excitatory transmission in the striatum, recent studies suggest that DSE cannot be triggered at medium spiny neurons with depolarization alone (Kreitzer and Malenka 2005; Narushima et al. 2006a). These reports used a 5-s duration voltage pulse; our findings with this stimulus are consistent with those reports. Contrary to DSI in the striatum, for which depolarization alone is sufficient (Narushima et al. 2006a), to induce DSE reliably in the striatum requires coupling depolarization with activation of group I mGluRs (Narushima et al. 2006a; Uchigashima et al. 2007). However, the fact that activation of group I mGluRs alone can induce a rapid suppression of EPSC amplitude confounds interpretation of the contribution that each mechanism makes to triggering eCB-mediated suppression of synaptic activity. Our finding that a 10-s voltage pulse alone is sufficient to induce suppression of EPSCs suggests that the combination of group I mGluR activation with a 5-s depolarization may additively increase intracellular calcium, thereby inducing synaptic suppression, whereas a 5-s depolarization alone may be ineffective due to inadequate eCB synthesis. At minimum, we confirm through intracellular application of a Ca²⁺ chelator that the striatal eCB-mediated DSE reported here requires an elevation of Ca²⁺, consistent with previous observations of postsynaptic depolarization induced short-term plasticity (Alger 2002).

Work from the hippocampus provides one explanation for the difference in the efficacy of postsynaptic depolarization to induce DSI or DSE. Ohno-Shosaku et al. (2002b) found that excitatory transmission is less sensitive to the suppressive effects of postsynaptic depolarization than inhibitory transmis-
sion. Thus a decrement in sensitivity to postsynaptic depolarization for excitatory transmission may explain the absence of DSE following a 5-s depolarization in the striatum (Ohno-Shosaku et al. 2002b; Narushima et al. 2006a). This explanation would support the possibility that 10-s depolarization was sufficient to induce suppression in area X because the decreased sensitivity of excitatory transmission was compensated for by greater eCB synthesis.

Most notably, the DSE we report here in spiny neurons of area X differs from previously reported observations of DSE in that it persists for minutes rather than decaying over tens of seconds. In the mammalian brain, agonist-activated CB₁R at cortical terminals onto spiny neurons induces an atypical time course of synaptic depression; unlike eCB-mediated synaptic change in other areas of the mammalian brain, in the striatum agonist-activated CB₁R-mediated depression cannot be fully reversed with washout of the agonist and application of a CB₁ receptor antagonist (Robbe et al. 2001; Kreitzer and Malenka 2005). It is possible that release of eCBs through depolarization alone was sufficient to induce and maintain the sustained synaptic depression. Because the effect of a 10-s depolarization was blocked by intracellular calcium chelation, this form of DSE likely involves similar induction mechanisms as previously reported in rodents. Although a 10-s depolarization is highly nonphysiological, it was sufficient to reveal endogenous cannabinoid activity. In future experiments, it will be of interest to test whether more physiological depolarization protocols (i.e., bursts of action potentials) can induce DSE in songbird basal ganglia.

Another possibility suggested through work by Ronesi et al. (2004) is that CB₁R activation is necessary only for induction, but not maintenance, of eCB-mediated suppression. We tested for this possibility by delaying the application of a CB₁R antagonist after postsynaptic depolarization. Consistent with a differential contribution of eCBs to the induction and maintenance of LTD, we find that early (2 min after depolarization), but not late (5 min after depolarization), CB₁R blockade rescues the decrement in current amplitude following 10-s postsynaptic depolarization. Thus it is possible that the 10-s depolarization used here causes the release of eCBs sufficient to induce suppression but that some other mechanism maintains the suppression. Mechanisms for synapse-selective activation of CB₁ receptors have been observed at rodent spiny neurons. Singla et al. (2007) have shown evidence for synapse-specific induction of eCB-LTD for spiny neurons in mammalian striatum through the concurrence of low frequency presynaptic activity and CB₁R activation.

Relation to other forms of synaptic modulation in area X. Other forms of synaptic modulation of glutamatergic inputs to spiny neurons have been previously reported in area X. For example, glutamatergic synaptic transmission is suppressed by D₁ dopamine receptor activation, which is mimicked by a forskolin-triggered increase in adenylyl cyclase activity (Ding and Perkel 2003). Moreover, forskolin-mediated suppression of EPSC amplitude does not depend on CB₁R activation but rather on adenosine A₁ receptors. In mammals, modulation of eCB release through dopamine modulation occurs primarily through activation of D₂ receptors (Giuffrida et al. 1999; Kreitzer and Malenka 2005).

In addition to pharmacologically mediated synaptic change, glutamatergic inputs to spiny neurons also exhibit Hebbian long-term potentiation (Ding and Perkel 2004), which requires high-frequency presynaptic stimulation paired with postsynaptic depolarization. It is not known whether eCB modulation contributes to this form of synaptic plasticity. This form of long-term potentiation requires both pre- and postsynaptic activation. When only the postsynaptic cell was depolarized with four pulses of a 1-s duration, a small depression was observed, reminiscent of that observed here.

E CB-mediated striatal plasticity and vocal learning. Avian and mammalian CB₁Rs are highly conserved, exhibiting 92% predicted amino acid identity between zebra finch and human (Soderstrom and Johnson 2000, 2001). Immunocytochemistry reveals diffuse punctate CB₁R expression throughout the zebra finch telencephalon and thalamus, with a noticeably higher intensity of expression localized to song control nuclei (Soderstrom and Tian 2006). Expression of CB₁R within song control nuclei increases over the course of song development, peaking at ~80 dph, which corresponds with the closure of the sensorimotor phase of song learning (Soderstrom and Tian 2006). Systemic exposure to cannabinoid agonists during late postnatal development markedly alters song learning by increasing the incidence of nonlearned syllables and decreasing production of tutor-matched syllables, essentially disrupting syllable stereotypy (Soderstrom and Johnson 2003; Soderstrom and Tian 2004). Moreover, exposure to cannabinoids during adulthood reduces locomotor activity and overall daily song bout production but has no effect on song structure, which is consistent with the idea that eCB modulation contributes to vocal learning.

Our findings build on the existing knowledge of CB₁R activity in the song control circuit of the zebra finch in two important ways. First, this is the first report on the electrophysiological effects of CB₁R activation in the zebra finch central nervous system. We find effects of CB₁R activation on palliostriatal excitatory synapses that are very similar to those observed at mammalian corticostriatal synapses. Second, our finding that eCBs mediate the synaptic depression caused by mGluR activation or by postsynaptic depolarization indicates a physiological role for eCBs. Area X is essential for vocal learning in juveniles and for social context-dependent song plasticity in adults (Bottjer et al. 1984; Olveczky et al. 2005; Kao et al. 2005, 2008; Leblois et al. 2010) and also because CB₁R activation alters song learning (Soderstrom and Tian 2004). Thus the forms of synaptic plasticity that we describe here are well placed to contribute to these forms of behavioral plasticity. It is also possible that the dopaminergic system, which receives indirect inputs from area X (Gale et al. 2008) and makes a strong projection back to area X (Lewis et al. 1981; Bottjer et al. 1989; Bottjer 1993; Soha et al. 1996; Gale et al. 2008; Person et al. 2008), is implicated in reinforcing template-matched vocal sequences, interacts with the eCB mechanisms in area X, and contributes to the refinement of song during vocal learning.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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