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# Endocannabinoids mediate the glucocorticoid-induced inhibition of excitatory synaptic transmission to dorsal raphe serotonin neurons

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**Key points**

- The modulation of the serotonin system by glucocorticoids plays a central role in the regulation of stress responses. However, the mechanisms by which glucocorticoids regulate the excitability of dorsal raphe serotonin neurons remain unknown.
- In this study, we show that glucocorticoids rapidly inhibit glutamatergic synaptic transmission to serotonin neurons by reducing glutamate release.
- The rapid inhibition of glutamate release is not signalled by classical intracellular glucocorticoid receptors, but rather by putative membrane-located G-protein-coupled receptors.
- Activation of the membrane-located G-protein-coupled receptors increases endocannabinoid signalling, which in turn mediates the inhibition of glutamatergic transmission in the dorsal raphe.
- In the dorsal raphe, glucocorticoids increase endocannabinoid tone by inhibiting cyclooxygenase-2.

**Abstract** Glucocorticoids play a critical role in the modulation of stress responses by controlling the function of the serotonin (5-HT) system. However, the precise effects of glucocorticoids on the excitability of dorsal raphe (DR) 5-HT neurons remain unknown. In this study, we investigated the effects of glucocorticoids on excitatory synaptic transmission to putative DR 5-HT neurons. We found that corticosterone or the synthetic glucocorticoid agonist dexamethasone rapidly suppressed glutamatergic synaptic transmission to DR 5-HT neurons by inhibiting glutamate release in the DR. This inhibitory effect was mimicked by membrane-impermeable glucocorticoids, indicating the involvement of membrane-located corticosteroid receptors. The glucocorticoid-induced inhibition of glutamatergic transmission was mediated by the activation of postsynaptic G-protein-coupled receptors and signalled by retrograde endocannabinoid (eCB) messengers. Examination of the downstream mechanisms revealed that glucocorticoids enhance eCB signalling via an inhibition of cyclooxygenase-2. Together, these findings unravel a novel mechanism by which glucocorticoids control the excitability of DR 5-HT neurons and provide new insight into the rapid effects of stress hormones on the function of the 5-HT system.

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**Abbreviations** ACTH, adrenocorticotropin hormone; CORT, corticosterone; COX-2, cyclooxygenase-2; CRH, corticotropin-releasing hormone; CV, coefficient of variation; DEX, dexamethasone; DR, dorsal raphe; eCBs, endocannabinoids; HPA, hypothalamic-pituitary-adrenal; 5-HT, serotonin; PKA, protein kinase A; PLC, phospholipase C; PPR, paired pulse ratio; PVN, paraventricular nucleus of the hypothalamus; sEPSCs, spontaneous EPSCs.


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## Introduction

Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis and the serotonin (5-HT) system has long been involved in the pathophysiology of stress-related mental disorders such as affective and anxiety disorders (McEwen, 2003). Exposure to various stressors activates the HPA axis by stimulating peripheral and central inputs converging on the paraventricular nucleus of the hypothalamus (PVN). This in turn triggers the release of corticotropin-releasing hormone (CRH) into the hypophyseal portal circulation (Palkovits, 1987), leading to the secretion of adrenocorticotropin hormone (ACTH) and the release of glucocorticoids into the bloodstream. Glucocorticoids exert numerous physiological effects at the periphery and central nervous system to enable the organism to respond adequately to stress (de Kloet *et al.* 2005). The elevated level of glucocorticoids during stress also exerts a negative feedback control of the HPA axis (Palkovits, 1987) to prevent excessive and uncontrolled secretion of glucocorticoids (Dallman, 2005), which could have detrimental effects on the health of the organism (McEwen, 2003).

Previous studies have established that the activity of the HPA axis is also under the control of the 5-HT system (Lanfumey *et al.* 2008). DR 5-HT neurons project to the PVN (Sawchenko *et al.* 1983; Petrov *et al.* 1994) and establish synaptic contacts with CRH-containing neurons (Liposits *et al.* 1987). Importantly, activation of the 5-HT system enhances the activity of the HPA axis, increases the secretion of stress hormones (e.g. corticosterone, ACTH), and regulates the behavioural responses to stress (Carrasco & Van de Kar, 2003). In contrast, inhibition of the 5-HT system reduces the activity of the HPA axis and inhibits the secretion of stress hormones (Fuller & Snoddy, 1990). The 5-HT-induced activation and inhibition of the HPA axis are thought to be mediated by 5-$HT_{2C}$ and 5-$HT_{1A}$ receptors, respectively (Vielhaber *et al.* 2005; Heisler *et al.* 2007).

On the other hand, activation of the HPA axis by various stressful stimuli has been shown to modulate the function of the 5-HT system. For instance, exposure to forced swim stress increases 5-HT release in the striatum and decreases 5-HT release in the amygdala and septum (Kirby *et al.* 1995; Adell *et al.* 1997). In addition, exposure to various stress models alters the expression of 5-$HT_{1A}$ and 5-$HT_{2C}$ receptors (Mendelson & McEwen, 1991; Englander *et al.* 2005) and affects the firing rate of DR 5-HT neurons (Grahn *et al.* 1999). Despite the important role played by the HPA axis and the 5-HT system in the regulation of neuroendocrine and behaviour responses to stress, the precise mechanisms by which glucocorticoids modulate the function of DR 5-HT neurons remain unknown. In the present study, we report that glucocorticoids control the excitability of putative DR 5-HT neurons by inhibiting glutamatergic transmission. This inhibitory effect is signalled by putative G-protein-coupled receptors and involves retrograde endocannabinoid (eCB) messengers. As such, this study unravels a previously unknown mechanism by which glucocorticoids can rapidly control the function of the 5-HT system.

## Methods

### Brain slice preparation

All the experiments were conducted in juvenile (3–4 weeks old) male Sprague–Dawley rats (Harlan Laboratories Inc., Indianapolis, IN, USA) and were approved by the University at Buffalo Institutional Animal Care and Use Committee and conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. Brainstem slices containing the DR were prepared as previously described (Haj-Dahmane, 2001). Briefly, rats were anaesthetized with isoflurane and killed by decapitation. The brain was rapidly removed and placed in a cold modified artificial cerebrospinal fluid (ACSF) of the following composition (in mM): 110 choline chloride; 2.5 KCl; 0.5 $CaCl_2$; 7 $MgSO_4$; 1.25 $NaH_2PO_4$; 26.2 $NaHCO_3$; 11.6 sodium L-ascorbate; 3.1 sodium pyruvate, 25 glucose and equilibrated with 95% $O_2$–5% $CO_2$. The area of the brainstem containing the DR was isolated, and coronal slices (300–400 $\mu$m) were cut using a vibrating-blade microtome (Lancer series 1000; Leica Biosystems, St Louis, MO, USA). Slices were incubated in a holding chamber containing ACSF of the following composition (in mM): 119 NaCl; 2.5 $CaCl_2$; 1.3 $MgSO_4$; 1 $NaH_2PO_4$; 26.2 $NaHCO_3$; 11 glucose and continuously bubbled with a mixture of 95% $O_2$–5% $CO_2$ for 30–45 min at 35°C. Slices were allowed to recover at room temperature for at least 1 h before recordings were conducted. Following recovery, slices were transferred one at a time to a recording chamber (Warner Instruments, Hamden, CT, USA) mounted on an fixed upright microscope and continuously perfused at a rate of 2–3 ml $min^{-1}$ with ACSF saturated with 95% $O_2$–5% $CO_2$ and heated to 30 ± 1°C using a solution heater (Warner Instruments).

### Whole-cell recordings

Neurons of the DR were visualized using a BX 51 Olympus microscope (Olympus Company, Tokyo, Japan) equipped with a ×40 water-immersion lens, differential interference contrast and infrared optical filter. Whole-cell recordings were obtained with patch electrodes (3–5 MΩ) filled with an internal solution containing (in mM): 120 potassium gluconate; 10 KCl; 10 sodium phosphocreatine, 10 Hepes; 1 $MgCl_2$; 1 EGTA; 2 $Na_2$-ATP; 0.25 Na-GTP; pH 7.3;

osmolarity 280–290 mosmol $l^{-1}$). Membrane potentials were corrected for the liquid junction potential of this internal solution (~5 mV). Putative DR 5-HT neurons were identified using previously established electrophysiological criteria (Haj-Dahmane, 2001).

### Stimulation and recordings

All recordings were performed from neurons located in the dorsomedial and ventromedial subdivisions of the DR. Excitatory postsynaptic currents (EPSCs) were evoked with single square-pulses (duration = 100 to 200 $\mu$s) delivered at 0.1 Hz using a patch pipette (3–5 M$\Omega$) filled with ACSF and placed (50 to 100 $\mu$M) dorsolateral to the recording sites. EPSCs were recorded from neurons voltage-clamped at −70 mV in the continuous presence of picrotoxin (100 $\mu$M) and strychnine (20 $\mu$M) to block $GABA_A$ and glycine receptor-mediated synaptic currents, respectively. The stimulus intensity was adjusted to evoke EPSCs with amplitude ranging from 100 to 250 pA, which represents 75% of the maximum EPSC amplitude. Membrane currents were amplified with an Axoclamp 2B or Multiclamp 700B amplifier (Molecular Devices, Union City, CA, USA). The membrane currents were filtered at 3 kHz, digitized at 20 kHz with Digidata 1440 and acquired using the pCLAMP 10 software (Molecular Devices). Access resistance (10–20 m$\Omega$) was monitored online using 5 mV hyperpolarizing voltage steps (200 ms duration). Recordings were discarded when the access resistance increased by more than 10%.

### Data analysis

EPSCs were analysed using the Clampfit 10.2 software (Molecular Devices). The amplitude of EPSCs was determined by measuring the average current during a 2 ms time window at the peak of each EPSC and subtracted from the baseline current determined during a 5 ms time window before the stimulus artifact. All EPSC amplitudes were normalized to the mean baseline amplitude recorded for at least 10 min before drug application. For paired pulse experiments, pairs of stimuli were given at 30 ms within pair intervals. The paired pulse ratios ($PPR = EPSC_2/EPSC_1$) were averaged for at least 60 trials in the absence and presence of glucocorticoids. For the determination of the coefficient of variation (CV), the standard deviation (SD) and the mean amplitude of EPSCs were calculated from at least 60 consecutive trials in the control condition and the presence of glucocorticoids. The CV was then calculated by the following ratio: (SD)/(EPSC mean amplitude). Glutamate-mediated spontaneous (s) EPSCs were analysed off-line using the Mini Analysis software (Synaptosoft, Decatur, GA, USA). The events were detected using amplitude detection and area threshold criteria set at 5 pA and 50 fC, respectively. The selected events were further inspected visually to prevent noise from compromising the analysis. Statistical analysis was performed using the Origin 8.0 software (Microcal Software Inc., Northampton, MA, USA). The results in the text and figures are expressed as mean ± SEM. Statistical comparisons were conducted using Student's paired $t$ test for within-group comparisons, the unpaired $t$ test for comparisons between groups, and the non-parametric Kolmogorov–Smirnov (K–S) test for comparison of sEPSCs. Statistical significance was set at $P < 0.05$.

### Chemical and drugs

Most chemicals were obtained from Fisher Scientific (Pittsburgh, PA, USA). The water-soluble dexamethasone, dexamethasone-BSA, corticosterone, spironolactone, mifiprestone, cycloheximide, nimesulide, meloxicam, aldosterone and GDP-$\beta$-S were purchased from Sigma-Aldrich (St Louis, MO, USA). Picrotoxin, strychnine, *N*-(piperidin-1-yl)-5-(4-iodophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (AM 251) and (*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone mesylate (WIN 55,212-2), PKI (6-22) and 1-[6-[[(17$\beta$)-3-methoxyestra-1,3,5(10)-trien-17-yl]amino[hexyl]-1*H*-pyrrole-2,5-dione (U 73122) were purchased from Tocris Cookson (Ellisville, MO, USA). For the experiments using CB1 receptor antagonists, type I and type II glucocorticoid receptor antagonists, phospholipase C inhibitors and protein synthesis inhibitors, slices were pre-incubated with the drugs for at least 60 min before the start of the experiments.

## Results

### Glucocorticoids inhibit AMPAR-EPSCs in putative DR 5-HT neurons

Excitatory postsynaptic currents (EPSCs) were evoked in putative DR 5-HT neurons voltage clamped at −70 mV and in the presence of $GABA_A$ and glycine receptor antagonists picrotoxin (100 $\mu$M) and strychnine (20 $\mu$M), respectively. We previously showed that under these conditions, local extracellular stimulation elicits EPSCs that were mainly mediated by the activation of AMPA receptors (Haj-Dahmane & Shen, 2005, 2009). Bath application of the stress hormone corticosterone (CORT, 300 nM) profoundly reduced the amplitude of EPSCs. A typical experiment depicting the effect of CORT on the amplitude of EPSCs is illustrated in Fig. 1*A*. On average, CORT (300 nM) reduced the amplitude of EPSCs to 60.87 ± 3.4% of the baseline ($n = 12$, $P < 0.01$ *versus*

baseline; Fig. 1*B*). Increasing the concentration of CORT to 1 $\mu$M resulted in a stronger depression of the amplitude of EPSCs (48.52 ± 7.5% of baseline, $n = 7$). In contrast to the inhibition of EPSCs, application of CORT had no effect on the holding current (control = 0.15 ± 0.64 pA, CORT = 1.05 ± 1.35 pA, $n = 12$; Fig. 1*C*) and membrane resistance (control = 378 ± 18 mΩ; CORT = 389 ± 20 mΩ, $n = 12$; Fig. 1*D*).

The CORT-induced inhibition of EPSCs was mimicked by the synthetic glucocorticoid agonist dexamethasone (DEX). Indeed, bath application of DEX (300 nM) reduced the amplitude of EPSCs to 62.5 ± 2.5% of baseline ($n = 8$, $P < 0.01$ *versus* baseline; Fig. 2*A*). The depression of EPSC amplitude induced by DEX was also concentration dependent, with the threshold effect obtained at ∼100 nM and maximal effect induced by 10 $\mu$M (Fig. 2*D*, $n = 8$). In contrast to the effect of CORT and DEX, administration of the endogenous mineralocorticoid aldosterone (ALDO, 1 $\mu$M) had no effect on the amplitude of EPSCs (94.85 ± 4.5% of baseline, $n = 7$; Fig. 2*C*). Similar results were obtained with the corticoid precursor cholesterol (10 $\mu$M) ($n = 4$ cells, data not shown), indicating that the effects of glucocorticoids are steroid specific.

To determine the mechanism by which glucocorticoids inhibit the amplitude of EPSCs, we examined the effect of DEX on the PPR and CV, two parameters that reflect changes in the probability of neurotransmitter release. We found that the DEX-induced inhibition of EPSCs was associated with a significant increase in both the PPR (control = 1.093 ± 0.02; DEX = 1.413 ± 0.04, $n = 6$, $P < 0.05$; Fig. 3*B*) and CV (control = 0.23 ± 0.03; DEX = 0.473 ± 0.06, $n = 5$; $P < 0.05$; Fig. 3*C*), suggesting that glucocorticoids reduced glutamate release. Consistent with this notion, administration of DEX (300 nM) significantly reduced the frequency (K–S test; $P < 0.01$; Fig. 3*F*), but not the amplitude of sEPSCs (Fig. 3*G*). The averaged frequency of sEPSCs was reduced from 4.58 ± 0.69 Hz in the control condition to 2.16 ± 0.45 Hz in the presence of DEX (300 nM) (*t* test, $P < 0.05$, $n = 7$). The averaged amplitudes of all selected sEPSCs recorded in the absence and presence of DEX (300 nM)

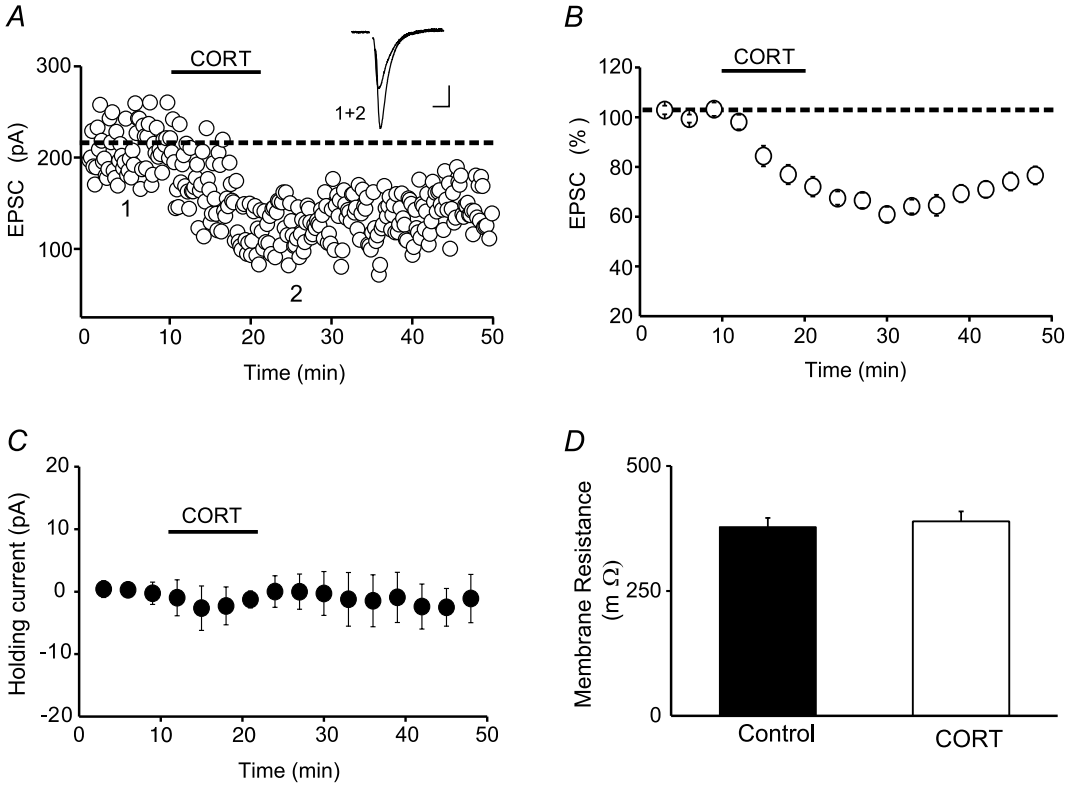


**Figure 1. The stress hormone corticosterone inhibits the amplitude of AMPAR-EPSCs in putative DR 5-HT neurons**
*A*, a representative experiment depicting the impact of corticosterone (CORT, 300 nM) on the amplitude of evoked AMPAR-EPSCs. Inset is superimposed EPSC traces taken at time points indicated by the numbers in panel *A*. *B*, summary graph of time course and magnitude of the CORT-induced inhibition of AMPAR-EPSCs ($n = 12$) in putative DR 5-HT neurons. *C* and *D* depict summary graphs of the effects of CORT on the holding current and membrane resistance, respectively. Note that CORT (300 nM) had no effect on the holding current ($n = 12$) and membrane resistance ($n = 12$). Calibration bars: 50 pA, 10 ms.

were $25.89 \pm 3.5$ pA and $27.67 \pm 2.8$ pA, respectively ($n = 7$). Taken together, these results clearly indicate that glucocorticoids inhibit the strength of glutamate synapses of putative DR 5-HT neurons by reducing glutamate release.

### The inhibition of AMPAR-EPSCs is not mediated by intracellular corticosteroid receptors

The relatively rapid (4–5 min) onset of the glucocorticoid-induced inhibition of AMPAR-EPSCs suggests that this effect is unlikely to be mediated by classical intracellular type I/II corticosteroid receptors. To test this notion, we examined the effect of the membrane-impermeable glucocorticoid dexamethasone conjugated to bovine serum albumin (DEX-BSA) on the amplitude of EPSCs. We found that bath application of DEX-BSA (10 $\mu$M) significantly reduced the amplitude of EPSCs to $74.9 \pm 6.5\%$ of the baseline ($n = 6$, $P < 0.05$ *versus* baseline; Fig. 4*A*). When compared to the effect of DEX and CORT, the depression of EPSCs induced by DEX-BSA developed slowly with an onset of 10–15 min. The relatively slow time course of the effect of DEX-BSA most likely reflects a poor diffusion of the DEX-BSA in the brain slices.

To further assess the involvement of intracellular corticosteroid receptors, we examined the effect of intracellular infusion of CORT (1 $\mu$M) through the recording electrodes on both the amplitude and PPR of EPSCs. We reasoned that if the glucocorticoid-induced inhibition of EPSCs were to be mediated by intracellular corticosteroid receptors, intracellular administration of glucocorticoids, which presumably activate these receptors, should reduce the amplitude of EPSCs and increase the PPR. We found that dialysis of DR 5-HT neurons with an internal solution containing corticosterone (1 $\mu$M) did not significantly affect the amplitude of EPSCs ($EPSC_{3\ min} = 102 \pm 1.3\%$ of baseline; $EPSC_{20\ min} = 99.4 \pm 5.2\%$ of baseline, $n = 6$, Fig. 4*B*) nor the PPR ($PPR_{3\ min} = 100.3 \pm 2.99\%$ of baseline; $PPR_{20\ min} = 99.15 \pm 2.86\%$ of baseline, $n = 6$; Fig. 4*B*), suggesting that activation of intracellular corticosteroid receptors is not required for the glucocorticoid-induced inhibition of EPSCs.

Type I/II intracellular corticosteroid receptors mediate their physiological effects by regulating gene expression and protein synthesis (Datson *et al.* 2001). Therefore, to further examine their role in the inhibition of EPSCs, we tested the effect of cycloheximide, a protein translation inhibitor, on the glucocorticoid-induced inhibition of EPSCs. We found that treatment of slices with cycloheximide (10 $\mu$M) had no effect on the glucocorticoid-induced inhibition of EPSCs (control $= 44.45 \pm 7.4\%$ of baseline; cycloheximide $= 41.15 \pm 4.52\%$ of baseline, $n = 6$; Fig. 4*C*), suggesting that the glucocorticoid-induced inhibition of EPSCs is not signalled by a genomic pathway. Taken together, these results strongly indicate that the glucocorticoid-induced inhibition of AMPAR-EPSCs is unlikely to be signalled by classical intracellular corticosteroid receptors, but rather by membrane-located corticosteroid receptors.

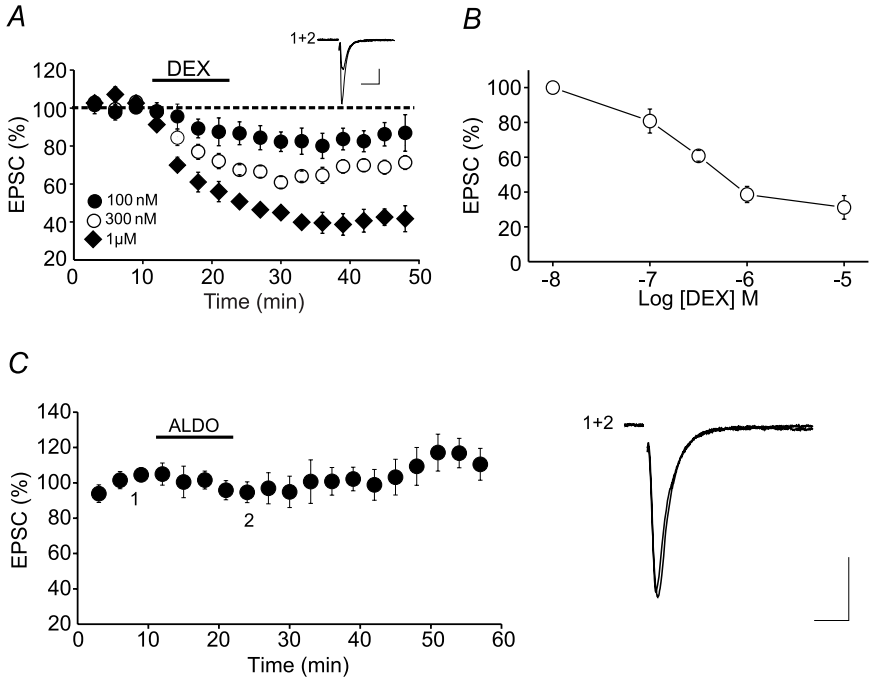


**Figure 2. The synthetic glucocorticoid agonist dexamethasone (DEX) mimics the effect of CORT**
*A*, summary graph illustrates the time course and magnitude of the inhibition of AMPAR-EPSCs induced by DEX at 100 nM (●, $n = 6$), 300 nM (○, $n = 10$) and 1 $\mu$M (◆, $n = 8$). *B*, plot of the dose–response curve of DEX-induced inhibition of EPSC amplitude. *C*, the mineralocorticoid agonist aldosterone (ALDO) had no effect on the amplitude of EPSCs. Left panel is a summary plot of the effect of aldosterone (ALDO, 1 $\mu$M) on EPSCs. Right panel illustrates averaged EPSC traces collected at the time points indicated by the numbers in the left panel. Calibration bars: 50 pA, 10 ms.

### The inhibition of AMPAR-EPSCs is mediated by putative G-protein-coupled receptors

Previous studies in other brain areas have shown that glucocorticoids can induce a rapid modulation of synaptic transmission by either the activation of membrane-located type I/II corticosteroid receptors (Karst *et al.* 2005; Olijslagers *et al.* 2008) or putative G-protein-coupled receptors (Di *et al.* 2003). To test these possibilities, we first examined the effect of spirinolactone, a type I corticosteroid receptor antagonist on the glucocorticoid-induced inhibition of EPSCs. Blockade of these receptors with spirinolactone (10 $\mu$M) did not affect the DEX-induced inhibition of EPSC amplitude (control = 59.97 ± 5.68% of baseline, spirinolactone = 56.47 ± 6.25% of baseline, $n = 7$; Fig. 5*A*). Similarly, treatment of slices with the type II corticosteroid receptor antagonist mifiprestone (10 $\mu$M) failed to block the DEX-induced inhibition of EPSCs (control = 59.97 ± 5.68% of baseline; mifiprestone = 64.07 ± 4.47% of baseline, $n = 7$; Fig. 5*A*). These results suggest that the inhibition of AMPAR-EPSCs by glucocorticoids in DR 5-HT neurons is unlikely to be signalled by membrane-located type I/II corticosteroid receptors.

We next examined whether the glucocorticoid-induced inhibition of AMPAR-EPSCs is signalled by putative G-protein-coupled receptors. We tested the effect of postsynaptic intracellular application of the membrane-impermeable G-protein inhibitor GDP-$\beta$-S (500 $\mu$M: for at least 10 min), a manipulation that blocks

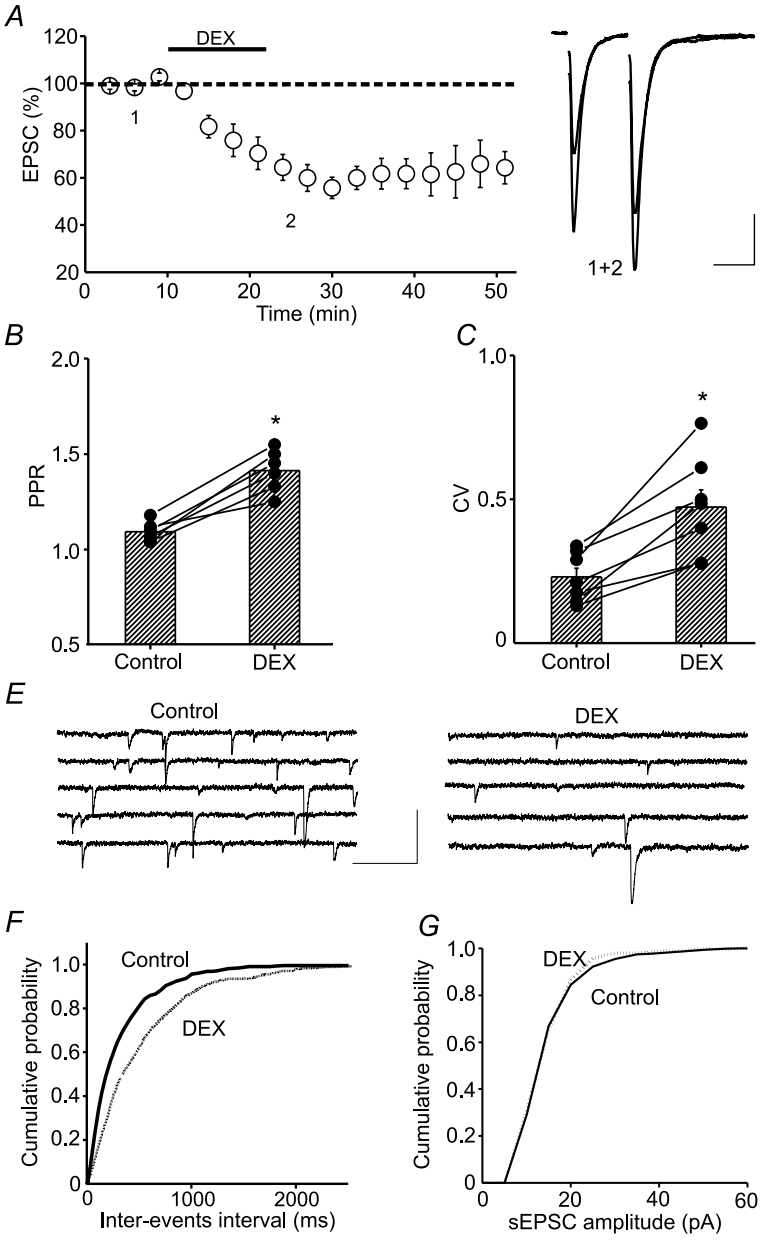


**Figure 3. Glucocorticoids inhibit AMPAR-EPSCs in putative DR 5-HT neurons by reducing glutamate release**
*A*, summary graph of dexamethasone (DEX)-induced inhibition of AMPAR-EPSCs elicited by pairs of stimuli with an inter-stimulus interval of 50 ms. Right panel is superimposed averaged pairs of EPSC traces taken at the time points indicated by the numbers in the left panel. Calibration bars: 50 pA, 20 ms. *B*, summary histogram of the average paired pulse ratio (PPR) of EPSCs obtained in the control condition ($n = 6$) and the presence of DEX ($n = 6$, $^*P < 0.05$). Note that the inhibition of EPSCs is accompanied by a significant increase in the PPR. *C*, summary histogram of the CV determined in the absence and presence of DEX (300 nM). Note that dexamethasone significantly increases the CV ($n = 6$; $^*P < 0.05$). *E*, sample graph illustrates sEPSCs recorded in the control condition (left panel) and in the presence of DEX (300 nM, right panel). Calibration bars: 50 pA, 300 ms. *F*, plots of the cumulative probability curves of the inter-event interval obtained in the control condition (continuous line) and in the presence of DEX (300 nM, dashed line) for the neuron illustrated in *E*. Note that DEX (300 nM) induces a significant ($P < 0.05$; K–S test) rightward shift of the cumulative probability curve of the inter-event interval, indicating an inhibition of sEPSC frequency. *G*, plots of the cumulative probability curves of sEPSC amplitude obtained in the control condition (continuous line) and in the presence of DEX (300 nM; dashed line). Note that DEX has no effect on the amplitude distribution of sEPSCs.

postsynaptic G-protein-coupled receptors (Haj-Dahmane & Shen, 2005) on the glucocorticoid-induced inhibition of EPSCs. As illustrated in Fig. 5*B*, replacing GTP with the GDP-$\beta$-S in the pipette solution completely prevented the inhibition of EPSCs induced by DEX (1 $\mu$M) (GTP = 51.72 ± 3.5% of baseline, $n = 7$; GDP-$\beta$-S = 97.36 ± 3.12% of baseline, $n = 7$, $P > 0.05$ *versus* baseline, $P < 0.05$ *versus* GTP; Fig. 5*B*), indicating that the effect of glucocorticoids requires the activation of G-proteins. More importantly, the finding that inhibition of postsynaptic G-proteins abolished the DEX-induced inhibition of AMPAR-EPSCs suggests that the effect of glucocorticoids is mediated by postsynaptic G-protein-coupled receptors.

The finding that the glucocorticoid-induced inhibition of EPSCs is initiated by the activation of postsynaptic G-protein-coupled receptors, yet mediated by a decrease in glutamate release, implies that this response is signalled by retrograde messengers. We have previously shown that eCBs synthesized and released from putative DR 5-HT neurons act as retrograde messengers and inhibit glutamate release via the stimulation of presynaptic cannabinoid CB1 receptors (Haj-Dahmane & Shen, 2005, 2009). Therefore, it is possible that eCBs are the retrograde messengers that mediate the rapid effect of glucocorticoids on EPSCs. To test this possibility, we examined the impact of CB1 receptor antagonist AM 251 on the glucocorticoid-induced inhibition of EPSCs. Pretreatment of slices with AM 251 (3 $\mu$M) almost abolished the DEX-induced inhibition of EPSCs (control = 46.89 ± 2.59% of baseline, AM 251 = 93.59 ± 4.28% of baseline, $P < 0.01$ *versus* control, $n = 8$; Fig. 6*A*). Conversely, activation of CB1 receptors with Win 55,212-2 not only mimicked, but also occluded the glucocorticoid-induced inhibition of EPSCs. Indeed, bath application of Win 55,212-2 (10 $\mu$M) strongly reduced the amplitude of EPSCs (53.5 ± 3.8% of baseline, $P < 0.01$ *versus* baseline, $n = 8$; Fig. 6*B*). In the presence of Win 55,212-2, administration of DEX (1 $\mu$M) failed to induce further inhibition of EPSCs (51.89 ± 2.8% of

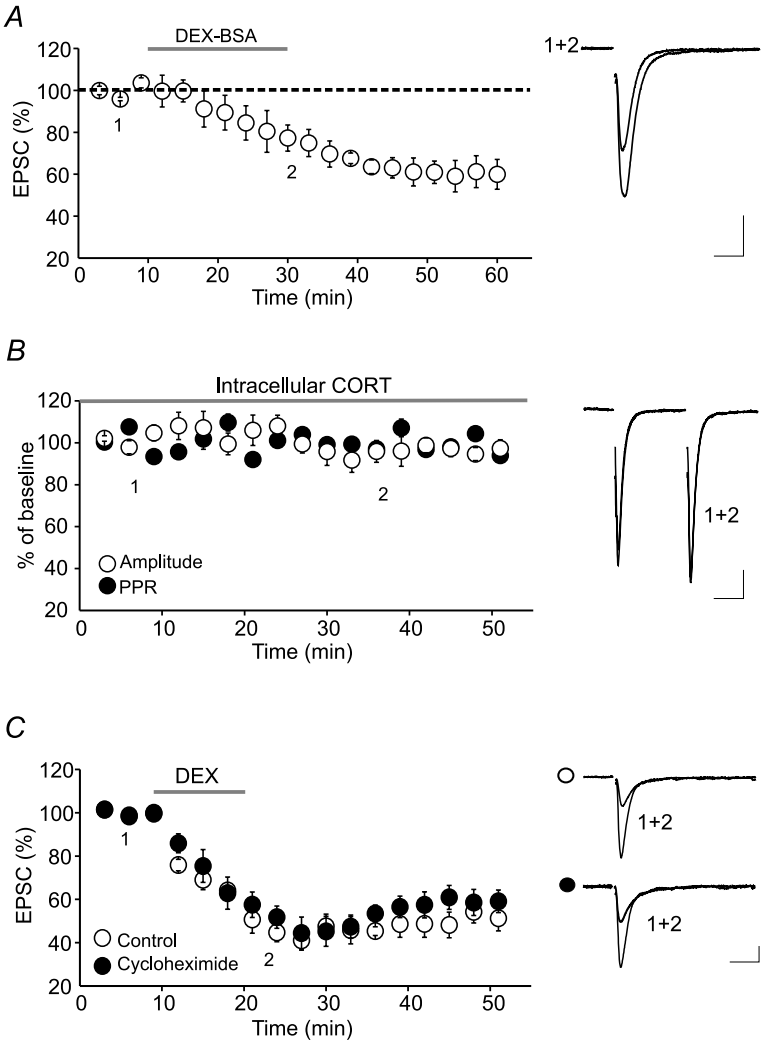


**Figure 4. The glucocorticoid-induced inhibition of AMPAR-EPSCs is not mediated by the activation of intracellular corticosteroid receptors**
*A*, the membrane-impermeable glucocorticoid DEX-BSA inhibits the amplitude of AMPAR-EPSCs. Left panel is a summary graph of the effect of bath applied DEX-BSA (10 $\mu$M) on the amplitude of EPSCs. Right panel illustrates superimposed EPSC traces taken at time points indicated by numbers in the left panel. *B*, postsynaptic intracellular application of corticosterone (CORT; 1 $\mu$M) has no effects on the amplitude and PPR of AMPAR-EPSCs. Left panel illustrates a summary graph of the effects of intracellular application of corticosterone (CORT) on the amplitude (○, $n = 7$) and PPR (●, $n = 7$). Right panel is superimposed EPSC traces taken at the time point indicated by numbers in the left panel. *C*, inhibition of protein synthesis has no effect on the DEX-induced inhibition of EPSCs. Left panel is a summary plot of DEX effects on EPSC amplitude obtained in control slices (○, $n = 7$) and in slices treated with the protein synthesis inhibitor cycloheximide (10 $\mu$M, ●, $n = 7$). Right panel depicts superimposed EPSC traces collected at ime points indicated by the numbers in the left panel. Calibration bars: 50 pA, 10 ms.

baseline, $P > 0.05$ *versus* Win, $n = 7$; Fig. 6*B*). To ensure that the lack of glucocorticoid effect in the presence of Win 55,212-2 reflected a genuine occlusion, we also examined the effect of glucocorticoids on EPSCs in slices pretreated with Win 55,212-2 (10 $\mu$M). As expected for an occlusion effect, pretreatment with Win 55,212-2 prevented the inhibition of EPSCs induced by DEX (1 $\mu$M) (96.58 $\pm$ 6.7% of baseline, $n = 7$; Fig. 6*C*). Collectively, these results strongly indicate that eCBs are the retrograde messengers that mediate the glucocorticoid-induced inhibition of glutamatergic synaptic transmission to putative DR 5-HT neurons.

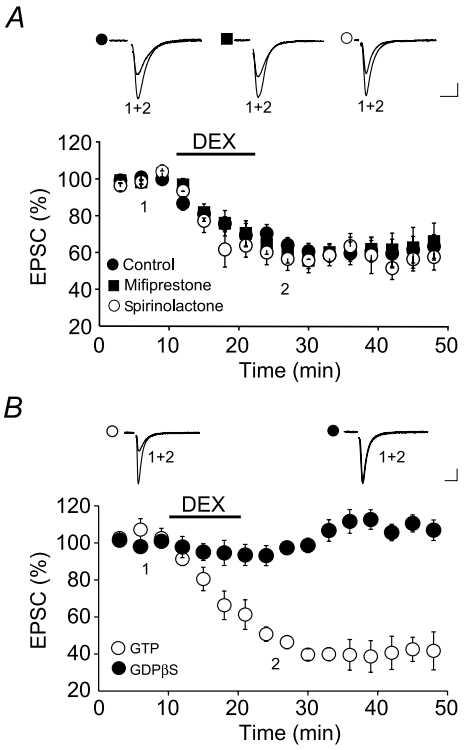


**Figure 5. Putative G-protein-coupled receptors mediate the glucocorticoid-induced inhibition of AMPAR-EPSCs**
*A*, type I and type II corticosteroid receptor antagonists spirinolactone and mifiprestone pretreatment have no significant effects on the dexamethasone (DEX)-induced inhibition of AMPAR-EPSCs. Lower panel is a summary graph of the effect of DEX (1 $\mu$M) on the amplitude of EPSCs obtained in the control condition (●, $n = 8$), the presence of spirinolactone (10 $\mu$M, ○, $n = 8$) and mifiprestone (10 $\mu$M, ■, $n = 8$). Upper panel depicts averaged EPSC traces collected at the time points indicated by the numbers in the lower panel. *B*, blocking postsynaptic G-protein signalling with GDP-$\beta$-S abolished the DEX-induced inhibition of EPSCs. Lower panel is a summary graph of the effect of DEX (1 $\mu$M) on the amplitude of EPSCs recorded with an internal solution containing GTP (250 $\mu$M, ○, $n = 7$) and GDP-$\beta$-S (250 $\mu$M, ●, $n = 7$). Upper panel represents superimposed EPSC traces taken before (1) and during bath application of DEX (2). Calibration bars: 50 pA, 10 ms.

## Glucocorticoids enhance eCB signalling by inhibiting COX-2

Results from a previous study in the PVN have suggested that membrane glucocorticoid receptors can enhance

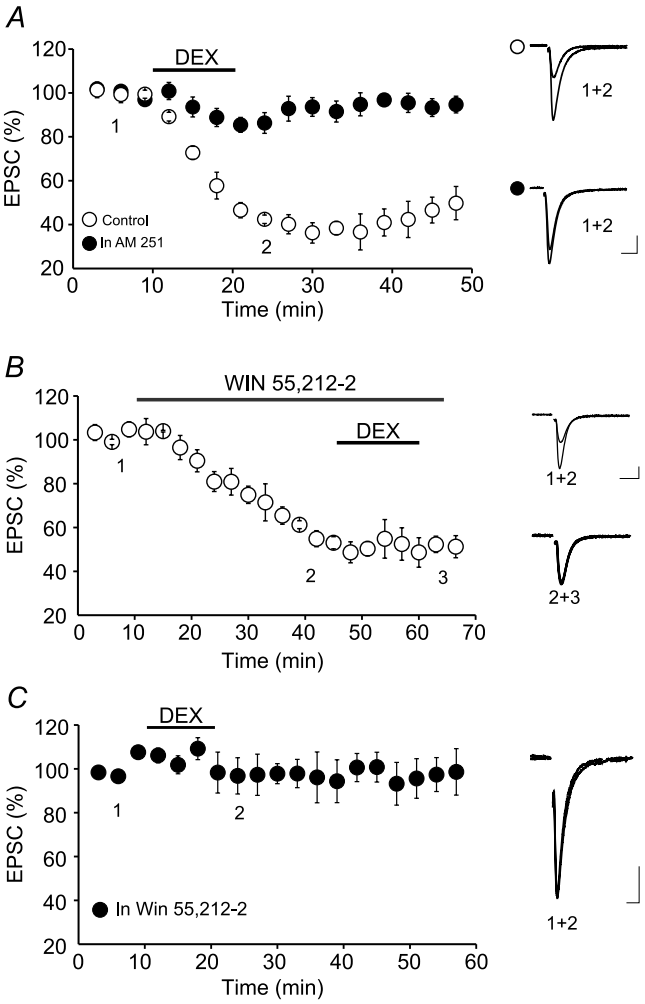


**Figure 6. Retrograde eCB signalling mediates the glucocorticoid-induced inhibition of AMPAR-EPSCs**
*A*, blockade of CB1 receptors with AM 251 antagonizes the dexamethasone (DEX)-induced inhibition of EPSCs. Left panel is a summary graph of the effect of DEX (1 $\mu$M) on the amplitude of EPSCs obtained in control condition (○, $n = 8$) and in slices pretreated with AM 251 (3 $\mu$M, ●, $n = 7$). Right panel illustrates averaged EPSC traces collected at the time points indicated by the numbers in the left panel. *B*, activation of CB1 receptors mimics and occludes the effects of DEX on EPSC amplitudes. Left panel is a summary graph of the effect of CB1 agonist Win 55,212-2 (10 $\mu$M) and Win 55,212-2 + DEX (1 $\mu$M) on the amplitude of EPSCs. Right graph depicts averaged EPSC traces taken at the time points indicated by numbers in the left panel. *C*, left panel illustrates the effect of DEX (1 $\mu$M) on the amplitude of AMPAR-EPSCs obtained in slices pretreated with the CB1 receptor agonist Win 55,212-2 (10 $\mu$M). Right graph depicts averaged AMPA-EPSC traces taken at the time points indicated by numbers in the left graph. Calibration bars: 50 pA, 10 ms.

eCB release and suppress synaptic transmission via the activation of the cAMP/protein kinase A (PKA) pathway (Di *et al.* 2003). To test whether this pathway also mediates the effect of glucocorticoids in the DR, we examined the effect of inhibiting postsynaptic PKA using PKI (6–22), a membrane-impermeable inhibitor of PKA, on the DEX-induced inhibition of EPSCs. Surprisingly, we found that postsynaptic intracellular infusion of PKI (6–22) (1 $\mu$M; via the path electrodes), a manipulation that has previously been shown to inhibit the cAMP/PKA pathway (Chevaleyre *et al.* 2007; Haj-Dahmane & Shen, 2010), had no effects on the inhibition of EPSCs induced by DEX (300 nM) (control = 66.54 ± 4.6% of baseline; $n = 7$; PKI = 68.38 ± 3.28% of baseline, $n = 7$; Fig. 7*A*). Such a result suggests that activation of the postsynaptic cAMP/PKA pathway is not required for the glucocorticoid-induced increase in eCB release and the subsequent inhibition of AMPAR-EPSCs in putative DR 5-HT neurons.

Glucocorticoids are also known to activate the phosphatidylinositol-specific phospholipase C (PLC) pathway (Graber & Losa, 1995; Marchetti *et al.* 2003), a major pathway for the synthesis of the eCB 2-arachidonoyl glycerol;. Therefore, we assessed whether activation of this pathway could contribute to the glucocorticoid-induced increase in eCB tone. We examined the effect of PLC inhibitor on the glucocorticoid-induced inhibition of EPSCs. Unexpectedly, treatment of slices with the PLC inhibitor U73122 (10 $\mu$M), a manipulation that has been shown to inhibit PLC-dependent eCB synthesis in DR 5-HT neurons (Haj-Dahmane & Shen, 2005), did not significantly alter the effect of DEX on EPSCs. On average, DEX (300 nM) reduced the amplitude of EPSCs to 61.58 ± 4.85% and 63.95 ± 4.35% of baseline in control conditions and the presence of U73122, respectively ($n = 6$; Fig. 7*B*). The lack of effect of the PLC inhibitor U73122 on DEX-induced inhibition of EPSCs strongly suggests that the glucocorticoid-induced increase in eCB tone is not mediated by the PLC pathway.

Previous results have suggested that glucocorticoids can induce a post-transcriptional inhibition of neuronal cyclooxygenase type 2 (COX-2) (Zhang *et al.* 1999), an enzyme that plays a key role in the degradation of eCBs into eCB-derived prostanoids (Kozak *et al.* 2000). In addition, inhibition of COX-2 has been shown to increase eCB tone and facilitate eCB-dependent inhibition of synaptic transmission in the other brain areas (Kim & Alger, 2004; Slanina & Schweitzer, 2005). Thus, it is possible that glucocorticoids could increase eCB tone by inhibiting eCB degradation. To test this possibility, we first examined whether non-steroid inhibitors of COX-2 could mimic the inhibitory effect of glucocorticoids on EPSCs. Bath administration of nimesulide (10 $\mu$M) reduced the amplitude of EPSCs recorded from putative DR 5-HT neurons (47.49 ± 5.06% of baseline, $P < 0.05$ *versus* baseline, $n = 11$; Fig. 8*A*). Similarly, bath application of meloxicam (10 $\mu$M), a structurally different COX-2 inhibitor (Vane *et al.* 1998) reduced the amplitude of EPSCs (58.67 ± 5.11% of baseline; $n = 7$, data not shown). The inhibition of EPSCs induced by COX-2 inhibitors was associated with a significant increase in PPR (PPR control = 1.04 ± 0.05; PPR nimesulide = 1.3 ± 0.028;

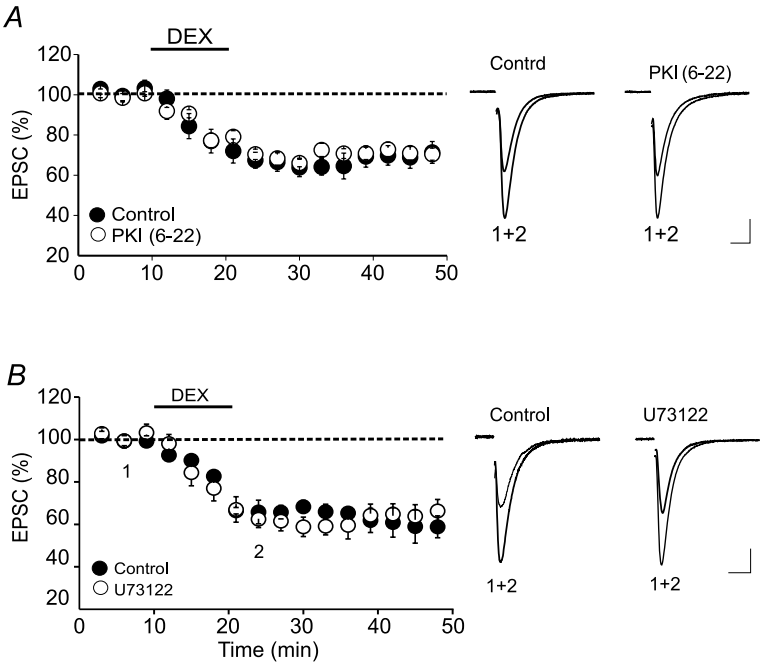


**Figure 7. The glucocorticoid-induced increase in eCB tone is not mediated by the cAMP/PKA or PLC pathways**
*A*, activation of the postsynaptic cAMP/PKA pathway is not required for glucocorticoid-induced inhibition of AMPAR-EPSCs. Left graph illustrates the effect of DEX (300 nM) on the amplitude of EPSCs recorded using a control internal solution (●, $n = 8$) and an internal solution containing PKI (6–22) (1 $\mu$M), a membrane-impermeable PKA inhibitor (○, $n = 7$). Right graph is superimposed averaged EPSCs taken at the time points indicated by the numbers in the left panel. Note that postsynaptic intracellular infusion of the PKA inhibitor had no effects on the DEX-induced inhibition of EPSCs. *B*, the glucocorticoid-induced inhibition of EPSCs is not signalled by the PLC pathway. Left panel is a summary graph of the DEX-induced inhibition of EPSCs obtained in control slices (●, $n = 7$) and in slices treated with the PLC inhibitor U73122 (10 $\mu$M, $n = 7$). Note that inhibition of PLC had no effect on the inhibition of EPSCs induced by DEX (300 nM). Right panel illustrates superimposed EPSC traces taken at the time points indicated by numbers in the left panel. Calibration bars: 50 pA, 10 ms.

$n = 11$; $P < 0.05$) and CV (control $= 0.19 \pm 0.03$; CV nimesulide $= 0.32 \pm 0.053$; $n = 11$; $P < 0.05$), indicating that the inhibition of EPSCs was mediated by a decrease in glutamate release. Pretreatment of slices with the CB1 receptor antagonist AM 251 (3 $\mu$M) completely blocked the depression of EPSCs induced by COX-2 inhibitors (Fig. 8*A*; control $= 47.49 \pm 5.06\%$ of baseline; in AM 251 $= 96.3 \pm 5.6\%$; $n = 7$; $P < 0.05$, unpaired $t$ test). Taken together, these results indicate that COX-2 inhibitors suppress glutamate release in the DR via an increase in eCB tone.

If the depression of EPSCs induced by glucocorticoids is indeed mediated by inhibition of COX-2, non-steroid COX-2 inhibitors (i.e. nimesulide, meloxicam) should occlude the effect of glucocorticoids. To test this idea, we examined the ability of DEX to inhibit EPSCs in control slices and in slices pretreated with nimesulide (10 $\mu$M). As expected for an occlusion effect, treatment of slices with nimesulide (10 $\mu$M) completely prevented the DEX-induced inhibition of EPSCs (control $= 55.74 \pm 4.56\%$ of baseline; nimesulide $= 103.47 \pm 8.21$ of baseline, $n = 7$, $P < 0.05$ *versus* control; Fig. 8*B*). The findings that COX-2 inhibitors mimic and occlude the glucocorticoid-induced inhibition of EPSCs indicate that inhibition of COX-2 is the signalling mechanism by which membrane glucocorticoid receptors increase in eCB tone and inhibit AMPAR-EPSCs in putative DR 5-HT neurons.

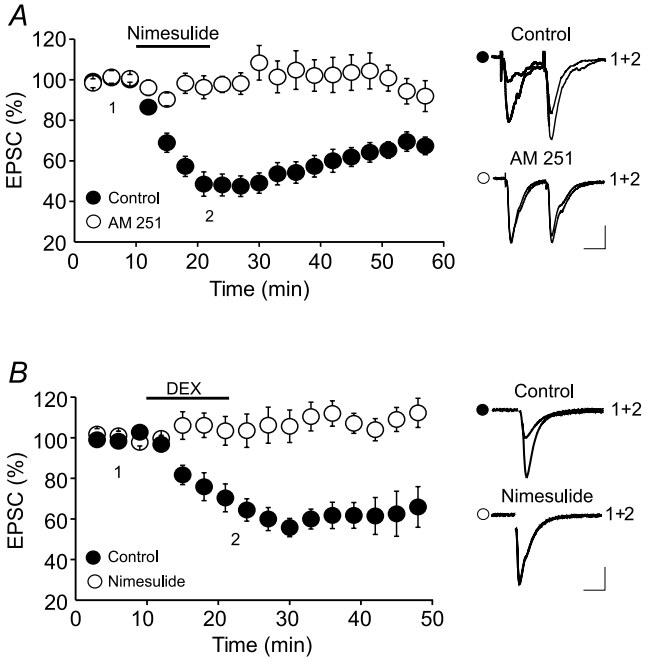


**Figure 8. The glucocorticoid-induced increase in eCB signalling is mediated by inhibition of COX-2**
*A*, the COX-2 inhibitor nimesulide mimics the effect of glucocorticoids on the amplitude of AMPAR-EPSCs via eCB signalling. Left graph is a summary of the effect of nimesulide (10 $\mu$M) on the amplitude of EPSCs obtained in control conditions (●, $n = 11$) and in slices treated with the CB1 receptor antagonist AM 251 (○, 3 $\mu$M, $n = 7$). Blocking CB1 receptors totally abolished the nimesulide-induced inhibition of EPSCs. Right panel illustrates superimposed pairs of EPSCs sampled at time points indicated by the numbers in the left graph. Note that the inhibition of EPSCs and the increase in PPR induced by nimesulide were blocked in the presence of AM 251. *B*, inhibition of COX-2 occludes the effect of DEX-induced inhibition of EPSCs. Left graph summarizes the effect of DEX (1 $\mu$M) on EPSCs in the control condition (●, $n = 9$) and in slices pretreated with nimesulide (10 $\mu$M, ○, $n = 7$). Right panel illustrates averaged EPSC traces taken at time points indicated by the numbers in the left panel. Calibration bars: 50 pA, 10 ms.

## Discussion

The functional interaction between the HPA axis and the 5-HT system plays a central role in the regulation of physiological responses to stress and the pathophysiology of stress-related affective disorders (McEwen, 2003). As such, determining the mechanisms by which stress hormone glucocorticoids regulate DR 5-HT neurons may have important functional and clinical implications. In the present study, we report that glucocorticoids reduce glutamatergic synaptic transmission to putative DR 5-HT neurons by inhibiting glutamate release. This inhibitory effect is not signalled by classical intracellular glucocorticoid receptors, but rather by putative G-protein-coupled receptors and involves retrograde eCB messengers. A novel finding of the present study is that the glucocorticoid-induced increase in eCB signalling is mainly mediated by an inhibition of COX-2. Such findings unravel previously unsuspected mechanisms by which glucocorticoids can rapidly modulate the excitability of DR 5-HT neurons.

### Mechanisms of the glucocorticoid-induced inhibition of glutamatergic transmission in the DR

Accumulating evidence has established that glucocorticoids can rapidly modulate the strength of synaptic transmission in various brain areas, such as the hippocampus (Karst *et al.* 2005; Olijslagers *et al.* 2008), amygdala (Karst *et al.* 2010) and hypothalamus (Di *et al.* 2003). Consistent with this notion, the present study shows that glucocorticoids rapidly inhibit glutamatergic synaptic transmission to putative DR 5-HT neurons. This inhibitory effect is mainly mediated by a decrease in glutamate release as indicated by the increase in the PPR and CV of EPSCs and by the decrease in the frequency, but not the amplitude of sEPSCs. This rapid inhibition of glutamate release is mimicked by a membrane-impermeable glucocorticoid DEX-BSA and is not blocked by protein translation inhibitors. Based on these findings, we propose that the glucocorticoid-induced inhibition of glutamate

release is mediated by membrane-located corticosteroid receptors. Interestingly, such a conclusion is consistent with the presence of glucocorticoid and mineralocorticoid binding sites on pre- and postsynaptic membranes in the mammalian brain (Johnson *et al.* 2005; Prager *et al.* 2010). In addition, activation of membrane-located corticosteroid receptors has also been shown to mediate the glucocorticoid-induced rapid regulation of synaptic transmission (Di *et al.* 2003; Karst *et al.* 2005, 2010; Olijslagers *et al.* 2008) and ion channel function in other brain areas (ffrench-Mullen, 1995; Olijslagers *et al.* 2008). As such, the present study further supports the notion that membrane located-corticosteroid receptors play a prominent role in mediating the rapid effects of glucocorticoids on synaptic transmission and neuronal excitability in the mammalian brain.

Based on the findings that the glucocorticoid-induced inhibition of EPSCs is not blocked by type I/II corticosteroid receptor antagonists, we propose that the rapid effect of glucocorticoid in the DR is unlikely to be mediated by membrane-located type I/II corticosteroid receptors. This conclusion is in contrast with previous studies showing that membrane-located type I corticosteroid receptors mediate the glucocorticoid-induced rapid modulation of glutamatergic transmission in the hippocampus (Karst *et al.* 2005; Olijslagers *et al.* 2008) and basolateral amygdala (Karst *et al.* 2010). It is worth noting that in these limbic areas, glucocorticoids increase glutamatergic transmission by enhancing glutamate release and/or AMPAR function (Olijslagers *et al.* 2008; Karst *et al.* 2010). This effect is mediated by membrane receptors that exhibit 10-fold higher affinity for glucocorticoids (Karst *et al.* 2010) than those reported in the present study. Therefore, it is conceivable that different types of membrane-located corticosteroid receptors may mediate the opposing effects of glucocorticoids on glutamatergic synaptic transmission. Consistent with this idea, we show that the effect of glucocorticoids in DR 5-HT neurons is signalled by putative G-protein-coupled receptors. The involvement of G-protein-coupled receptors is consistent with earlier reports showing that corticosteroids bind to membrane-located G-protein-coupled receptors (Orchinik *et al.* 1992; Maier *et al.* 2005). Furthermore, the activation of G-protein-coupled receptors has also been shown to mediate the glucocorticoid-induced inhibition of glutamate release in the paraventricular nucleus of the hypothalamus (PVN) (Di *et al.* 2003) and the regulation of ion channels (i.e. calcium channels) in others brain areas (ffrench-Mullen, 1995). Together these observations strengthen the notion that signalling through G-protein-coupled receptors represents a common mechanism by which glucocorticoids can induce a rapid modulation of neuronal excitability in the mammalian brain. However, future molecular studies are required to characterize these receptors and determine their precise signalling mechanisms.

### Role of eCB signalling and the COX-2 pathway

We have shown that glucocorticoids inhibit glutamate release by the activation of postsynaptic G-protein-coupled receptors. A straightforward interpretation of this finding is that the action of glucocorticoids is mediated by retrograde messengers. Consistent with this interpretation, our data show that the effect of glucocorticoids is blocked by CB1 receptor antagonists and mimicked by CB1 receptor agonists. These findings enable us to conclude that eCBs are the retrograde messengers that mediate the glucocorticoid-induced inhibition of glutamate release in putative DR 5-HT neurons. Such a conclusion is in agreement with previous studies showing that eCBs act as retrograde messengers at glutamate synapses of putative DR 5-HT neurons (Haj-Dahmane & Shen, 2005, 2009). More importantly, the finding that eCBs mediate the effects of glucocorticoids in the DR is consistent with previous *in vitro* and *in vivo* studies showing that glucocorticoids increase eCB levels in other brain areas (Di *et al.* 2003; Hill *et al.* 2010), and with the growing consensus that the behavioural and neurophysiological effects of glucocorticoids and stress are mediated, at least in part, by the recruitment of eCB system (de Oliveira Alvares *et al.* 2010; Hill & McEwen, 2010; Hill *et al.* 2011).

The finding that inhibition of the cAMP/PKA pathway in postsynaptic neurons has no effects on the glucocorticoid-induced increase in eCB tone and subsequent inhibition of EPSCs appears in conflict with previous studies showing that glucocorticoid receptors enhance eCB release in the PVN via activation of the cAMP/PKA pathway (Di *et al.* 2003; Malcher-Lopes *et al.* 2006). However, it should be noted that in these studies (Di *et al.* 2003; Malcher-Lopes *et al.* 2006), the role of the cAMP/PKA pathway was examined using bath application of membrane-permeable PKA inhibitors (i.e. staurosporine and Rp-cAMP), which inhibit both pre- and postsynaptic cAMP/PKA pathways. Because presynaptic CB1 receptors inhibit neurotransmitter release via modulation of the cAMP/PKA pathway (Chevaleyre *et al.* 2007; Haj-Dahmane & Shen, 2010), it is possible that the effect reported in these studies (Di *et al.* 2003; Malcher-Lopes *et al.* 2006) could be attributed, at least in part, to an alteration of the function of presynaptic CB1 receptors. In the present study, we specifically inhibit the postsynaptic cAMP/PKA pathway by using the intracellular application of membrane-impermeable PKA inhibitors. Under these conditions, we observe no effect of cAMPA/PKA pathway inhibitors on the glucocorticoid-induced inhibition of EPSCs. Based on

theses observations, we propose that in DR 5-HT neurons, the glucocorticoid-induced increase in eCB signalling is mediated by postsynaptic G-protein-coupled receptors, but does not require the activation of the postsynaptic cAMP/PKA pathway.

The present study reveals that PLC-mediated eCB synthesis does not mediate the glucocorticoid-induced increase in eCB tone. In contrast, blockade of COX-2-dependent eCB degradation not only induces an eCB-dependent depression of EPSCs, but also occludes the effect of glucocorticoids. These findings lead to the conclusion that the glucocorticoid-induced increase in retrograde eCB signalling in DR 5-HT neurons is most likely mediated by an inhibition of COX-2. The conclusion that glucocorticoids enhance eCB tone by inhibiting COX-2 is consistent with a high level of expression and postsynaptic localization of the COX-2 enzyme in DR 5-HT neurons (Breder *et al.* 1995). In addition, the involvement of COX-2 is in agreement with previous findings that glucocorticoids inhibit the activity of COX-2 (Zhang *et al.* 1999), and that the inhibition of this enzyme induces an eCB-dependent inhibition of synaptic transmission (Kim & Alger, 2004; Slanina & Schweitzer, 2005). As such, inhibition of COX-2 may represent a signalling mechanism by which glucocorticoids shift arachidonic acid metabolism toward the accumulation of eCBs, which in turn mediate the rapid effects of stress and glucocorticoids in the mammalian brain.

The above observations lead us to propose a mechanistic model (Fig. 9) describing the cellular mechanism by which glucocorticoids exert a rapid modulation of the excitability of DR 5-HT neurons. The increase of glucocorticoids in response to stress activates membrane-located G-protein-coupled receptors, which increase eCB tone via the inhibition of COX-2. The release of eCBs, in turn, mediates the retrograde inhibition of glutamatergic synaptic transmission to putative DR 5-HT neurons.

## Functional implications

Most of the previous studies examining the effect of glucocorticoids on the 5-HT system have focused on the long-term effects of these hormones on 5-HT neurotransmission. Generally, these studies report that exposure to glucocorticoids or various stressors enhances the expression of tryptophan hydroxylase (Abumaria *et al.* 2008), reduces the expression of 5-$HT_{2C}$ receptors (Holmes *et al.* 1995) and downregulates the function of the 5-$HT_{1A}$ receptors (Joels *et al.* 1991; Laaris *et al.* 1995). These effects are mediated by classical intracellular glucocorticoid receptors and involve the regulation of gene expression. In the present study, we report that, via a non-genomic pathway, glucocorticoids can rapidly modulate the excitability of putative DR 5-HT neurons by inhibiting glutamatergic synaptic transmission. This inhibitory effect may represent a cellular mechanism by which stress reduces the overall activity of DR 5-HT neurons and inhibits 5-HT release in their projection areas (Kirby *et al.* 1995; Adell *et al.* 1997). More importantly, because the activation of the 5-HT system stimulates the HPA axis and increases the secretion of glucocorticoids

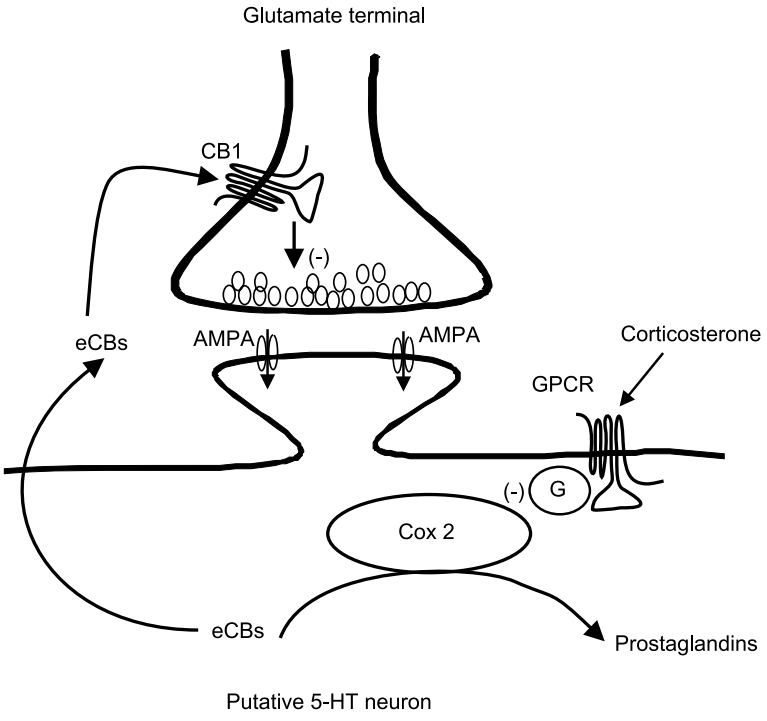


**Figure 9. A model of the rapid signalling mechanism by which glucocorticoids inhibit glutamate synapses on putative DR 5-HT neurons**
This model describes how glucocorticoids inhibit glutamate release via the activation of membrane-located G-protein-coupled receptors. This effect increases eCB signalling through the inhibition of COX-2, the degradation enzyme of eCBs. The eCBs released from putative DR 5-HT neurons activate presynaptic CB1 receptors and suppress glutamatergic synaptic transmission in the DR.

(Heisler *et al.* 2007); the rapid inhibition of DR 5-HT neurons may function as an additional feedback control of glucocorticoid secretion. This long feedback control from the DR may contribute, at least in part, to a timely termination of the HPA axis activation following exposure to stress.

It has long been recognized that the enhancement of eCB signalling and the stimulation of CB1 receptors reduces stress responses and exerts anxiolytic-like effects (Arevalo *et al.* 2001; Haller *et al.* 2004). These effects are mediated, at least in part, via the modulation of the 5-HT system (Greibel *et al.* 2005). The finding that glucocorticoids modulate the excitability of putative DR 5-HT neurons via an increase in eCB tone provides a potential cellular mechanism for the way in which the eCB system is recruited in the regulation of stress-related behaviours. However, future studies are required to further determine the precise role of eCB signalling in DR 5-HT neurons in the long-term regulation of the stress response and how various stressors may impact eCB-induced modulation of DR 5-HT neurons.

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## Author contributions

S.H.-D. designed the research and wrote the manuscript. J.W. and S.H.-D. performed the experiments and analysed the data. R.-Y.S. contributed to data analysis and manuscript preparation. All authors approved the final version of the manuscript.

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