Supporting Information

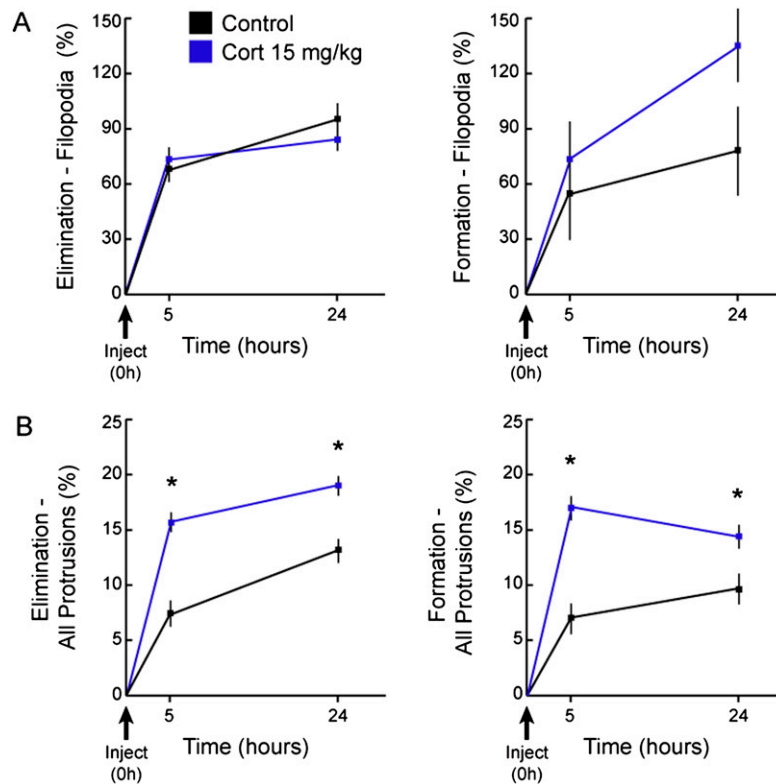## Liston and Gan 10.1073/pnas.1110444108

Fig. S1. Corticosterone effects on filopodium turnover. (*A*) Corticosterone (15 mg/kg) had no significant effect on filopodium elimination [$F(1,15) = 0.25$, $P = 0.62$] or formation [$F(1,15) = 2.58$, $P = 0.129$] over intervals of 5 and 24 h in the barrel cortex of P30 mice. (*B*) To confirm that corticosterone effects on spine turnover occurred independently of changes in filopodia, dendritic spines and filopodia were pooled and analyzed together using two-factor ANOVA (corticosterone treatment and time) while controlling for number of filopodia observed as a covariate. Corticosterone effects on turnover were comparable with those effects depicted in Fig. 1 *B* and *C*, with enhanced elimination [$F(1,15) = 44.4$, $P < 0.001$] and formation [$F(1,15) = 31.6$, $P < 0.001$] evident at both time points. Error bars = SEM. *Significantly different from corresponding control ($P < 0.05$).
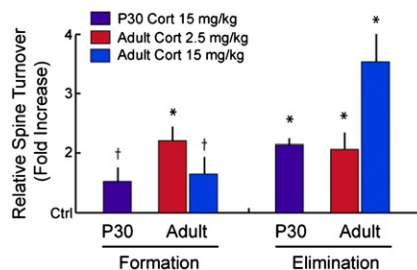
Fig. S2. Glucocorticoids enhanced relative spine turnover rates after controlling for baseline spine turnover in age-matched controls. The relative increase in spine turnover was slightly greater in adults than adolescents after normalizing for differing baseline turnover rates in controls. Relative spine turnover rates (formation on the left and elimination on the right) are depicted in terms of a fold increase in turnover relative to the corresponding control. Error bars = SEM. *Significantly different relative to corresponding control ($P < 0.05$). †Difference approached significance at $P < 0.10$ relative to corresponding control. Tables S1–S3 show statistics and additional details.

Table S1.   Glucocorticoids rapidly and potently enhance dendritic spine turnover in vivo (accompanies Fig. 1 *B* and *C*)

	Control (%)	N	Corticosterone (2.5 mg/kg)	n	t	Corticosterone (15 mg/kg)	n	t
Formation								
Main effect of corticosterone	$F(2,41) = 61.3$							
Main effect of time	$F(3,41) = 9.19$							
Interaction (h)	$F(4,41) = 9.67$							
5	1.40 ± 0.20	3	6.47 ± 0.69	3	**7.02**	12.00 ± 0.64	6	**11.1**
12	4.63 ± 0.61	6				7.92 ± 0.90	5	**3.11**
24	4.61 ± 0.64	5	8.43 ± 0.55	3	**4.04**	8.37 ± 0.47	6	**4.82**
72	7.44 ± 0.38	11				11.40 ± 1.76	3	**3.58**
Elimination								
Main effect of corticosterone	$F(2,41) = 133.0$							
Main effect of time	$F(3,41) = 41.7$							
Interaction (h)	$F(4,41) = 1.16$							
5	1.67 ± 0.47	3	4.40 ± 0.55	3	**3.79**	10.78 ± 0.88	6	**9.19**
12	4.42 ± 0.76	6				12.62 ± 0.78	5	**7.49**
24	5.97 ± 1.09	5	11.17 ± 1.11	3	**3.13**	14.92 ± 0.52	6	**7.85**
72	9.13 ± 0.52	11				19.63 ± 0.69	3	**9.74**

Images of barrel cortex were obtained at postnatal day (P) 30 followed by an i.p. injection of corticosterone (2.5 or 15 mg/kg) or vehicle (DMSO). The spine formation and elimination rates below were quantified by acquiring repeated images of the same dendritic segments over 24 h or after 3 d of daily corticosterone injections. In all tables, elimination and formation rates are expressed as a percent of the total number of spines quantified in the initial image for the specified interval. *n*, number of subjects. *F* statistics derive from univariate ANOVA with corticosterone status and time as fixed factors. Posthoc *t* statistics describe contrast with corresponding control. Data are means ± SEM. Statistics in bold are significant at $P < 0.05$.

Table S2.   Spine turnover rates (72 h) were quantified in adults after 3 d of daily corticosterone (15 mg/kg) (accompanies Fig. 1*D*), and 72-h data for P30 adolescents are repeated here for comparison with adults

	Control (%)	n	Corticosterone (2.5 mg/kg)	n	t	Corticosterone (15 mg/kg)	n	t
Formation								
Main effect of corticosterone	$F(2,11) = 11.3$							
P30	7.44 ± 0.38	11				11.40 ± 1.76	3	**3.58**
~P120	3.06 ± 0.34	8	6.77 ± 0.78	3	**5.19**	5.07 ± 0.97	3	**2.56**
Elimination								
Main effect of corticosterone	$F(2,11) = 33.4$							
P30	9.13 ± 0.52	11				19.63 ± 0.69	3	**9.74**
~P120	2.94 ± 0.30	8	6.10 ± 0.78	3	**4.78**	10.43 ± 1.42	3	**8.05**

Table S3.   Relative changes in 72-h spine turnover rates were quantified in adults after 3 d of daily corticosterone (15 mg/kg) based on the same data as in Tables S1 and S2 (accompanies Fig. 1*E*)

Age	Corticosterone (2.5 mg/kg)	n	t	Corticosterone (15 mg/kg)	n	t
Relative formation (fold increase vs. control)						
P30				1.53 ± 0.24	3	*2.24*
~P120	2.21 ± 0.26	3	**4.76**	1.66 ± 0.32	3	*2.08*
Relative elimination (fold increase vs. control)						
P30				2.15 ± 0.08	3	**15.3**
~P120	2.07 ± 0.26	3	**4.03**	3.55 ± 0.48	3	**5.32**

For each subject in the corticosterone-treated groups, the relative change in spine formation was calculated by dividing the observed formation by the group mean for control subjects. The same calculation was performed for spine elimination rates. The data below represent group means (±SEM) of these individual relative changes expressed as *x*-fold increase relative to controls. *t* statistics denote results of one-sample *t* test of relative increase vs. zero. Statistics in bold are significant at $P < 0.05$; statistics in italics approach significance at $P < 0.10$.

Table S4. Glucocorticoids enhance spine turnover in multiple cortical regions (accompanies Fig. 2)

	Control (%)	n	Corticosterone (15 mg/kg)	n	t
24-h Spine formation at P30					
Main effect of corticosterone		$F(1,21) = 58.3$			
Main effect of region		$F(2,21) = 8.94$			
Interaction		$F(2,21) = 0.62$			
Region					
Barrel	4.61 ± 0.64	5	8.37 ± 0.47	6	**4.82**
M1	6.11 ± 0.64	5	9.33 ± 0.69	3	**3.27**
M2	6.63 ± 0.45	3	11.30 ± 0.59	5	**5.46**
24-h Spine elimination at P30					
Main effect of corticosterone		$F(1,21) = 172.5$			
Main effect of region		$F(2,21) = 2.70$			
Interaction		$F(2,21) = 0.40$			
Region					
Barrel	5.97 ± 1.09	5	14.92 ± 0.52	6	**7.85**
M1	6.93 ± 0.63	5	14.03 ± 0.56	3	**7.59**
M2	7.52 ± 0.81	3	16.62 ± 0.64	5	**8.81**

Images of barrel, primary motor (M1) cortex, or secondary motor cortex (M2) were obtained at P30, which was followed immediately by i.p. injection of vehicle (DMSO) or corticosterone (15 mg/kg). A second image was obtained 24 h later. F statistics below derive from univariate ANOVA, with corticosterone status and brain region as fixed factors. Posthoc t statistics describe contrast with corresponding control. Data are means $\pm$ SEM. Statistics in bold are significant at $P < 0.05$. Corticosterone enhanced both elimination and formation in all three areas of cortex. Spine formation rates were slightly higher in primary and secondary motor cortex than in barrel cortex [$F(2,21) = 8.94$, $P = 0.002$], but there was no significant interaction between corticosterone and brain region for either measure, indicating a comparable enhancing effect in all three areas of cortex.

Table S5. Glucocorticoid deprivation blocks dendritic spine remodeling in the developing barrel cortex at P30 (accompanies Fig. 3B)

Treatment	N	Formation (%)	SEM	t	$F(3,18)$
3-d Spine formation at P30					
Control	11	7.44	0.38		**35.9**
Dexamethasone (0.1 mg/kg)	4	1.38	0.30	**9.02**	
Dex+cort (5 mg/kg)	4	5.33	0.51	**2.97**	
Dex+cort (10 mg/kg)	3	9.00	0.64	1.93	
3-d Spine elimination at P30					
Control	11	9.13	0.52		**42.7**
Dexamethasone (0.1 mg/kg)	4	2.40	0.42	**7.34**	
Dex+cort (5 mg/kg)	4	10.43	0.48	1.40	
Dex+cort (10 mg/kg)	3	15.03	1.01	**5.23**	

Images of barrel cortex were acquired at P30, and second images were acquired 3 d later. During the interim, the dexamethasone treatment group received two time daily injections (i.p.) of low-dose dexamethasone at 0.1 mg/kg, which suppresses endogenous corticosterone release. The dex+cort groups were also treated with supplemental corticosterone at the specified dose one time daily in addition to dexamethasone. Controls received two time daily injections of vehicle (DMSO). F statistics derive from univariate ANOVA with treatment group as a fixed factor. Posthoc t statistics describe contrast with corresponding control. Statistics in bold are significant at $P < 0.05$.

Table S6. Glucocorticoid deprivation blocks dendritic spine remodeling in the developing barrel cortex at P21 (accompanies Fig. 3C)

Treatment	n	Formation (%)	SEM	T	F(1,8)
1-d Spine formation at P21					
Control	6	14.70	1.70		**39.2**
Dexamethasone 0.1 mg/kg	4	1.25	0.39	**7.71**	
1-d Spine elimination at P21					
Control	6	15.37	0.60		**339.8**
Dexamethasone 0.1 mg/kg	4	1.25	0.21	**22.3**	

Here, the initial images were acquired at P21, and second images were acquired 1 d later. Treatments and statistics are as described above.

Table S7. Corticosteroid receptor antagonists disrupt spine dynamics (accompanies Fig. 4)

Treatment	n	Formation (%)	SEM	T	F(5,18)
1-d Spine formation at P30					
Control	5	4.61	0.64		**34.3**
MR antagonist (20 mg/kg)	3	1.03	0.09	**5.52**	
GR antagonist (20 mg/kg)	3	1.30	0.21	**3.81**	
Corticosterone (15 mg/kg)	6	8.37	0.47		
Cort+MR antagonist	4	2.65	0.34	**8.85**	
Cort+GR antagonist	3	1.37	0.78	**8.17**	
1-d Spine elimination at P30					
Control	5	5.97	1.09		**57.7**
MR antagonist (20 mg/kg)	3	1.43	0.48	**3.04**	
GR antagonist (20 mg/kg)	3	6.03	0.55	0.04	
Corticosterone (15 mg/kg)	6	14.9	0.52		
Cort+MR antagonist	4	1.68	0.36	**18.6**	
Cort+GR antagonist	3	12.9	0.98	1.98	

Images of barrel cortex were acquired at P30, and second images were acquired 1 d later. Subjects received a single i.p. injection of the labeled treatment immediately after the initial imaging session. MR (mineralocorticoid receptor) antagonist, spironolactone; GR (glucocorticoid receptor) antagonist, mifepristone. Controls received a single injection of vehicle (DMSO). *F* statistics derive from univariate ANOVA with treatment group as a fixed factor. Posthoc *t* statistics describe contrast of corticosteroid receptor antagonist vs. control (MR and GR antagonist rows) or corticosterone (Cort+MR antagonist and Cort+GR antagonist rows). Statistics in bold are significant at $P < 0.05$.

Table S8. Brief glucocorticoid exposure increases spine turnover but preserves most spines established early in development (accompanies Fig. 5 *B–D*)

Treatment	n	Elimination (%)	t	F(1,7)	Elimination (old)	t	Elimination (new)	t
1-d Spine formation (P30–P31)								
Control	4	4.45 ± 0.67		**9.78**				
Corticosterone (15 mg/kg) × 1	5	8.36 ± 0.97	**3.13**					
1-d Spine elimination (P30–P31)								
Control	4	6.96 ± 0.67		**39.0**	3.53 ± 0.63		30.26 ± 2.57	
Corticosterone (15 mg/kg) × 1	5	12.40 ± 0.56	**6.24**		4.92 ± 0.45	1.85	59.78 ± 4.42	**5.37**

In this experiment, subjects were imaged at P23 and P30, and then, they were reimaged on P31, 24 h after a single injection of corticosterone (15 mg/kg) or vehicle (DMSO). Formation and elimination rates (percentages) are presented as means ± SEM. Elimination rates for spines that were formed early in development before P23 (elimination old) were distinguished from those rates that were formed after P23 (elimination new). *F* statistics derive from univariate ANOVA with treatment group as a fixed factor. Posthoc *t* statistics describe contrast with corresponding control. Statistics in bold are significant at $P < 0.05$.

Table S9. Chronic glucocorticoid excess leads to loss of spines formed early in development

Treatment	n	Elimination (%)	t	$F_{(1,7)}$	Elimination (old)	t	Elimination (new)	t
10-d Spine formation (P30–P40)								
Control	4	11.36 ± 0.64		0.34				
Corticosterone (15 mg/kg) × 10	5	10.60 ± 1.25	0.58					
10-d Spine elimination (P30–P40)								
Control	4	12.06 ± 0.58		**41.9**	5.14 ± 0.54		52.51 ± 2.48	
Corticosterone (15 mg/kg) × 10	5	22.70 ± 1.72	**5.87**		16.46 ± 0.90	**11.8**	78.61 ± 3.61	**6.45**

In a second experiment, subjects were again imaged at P23 and P30, and then, they were reimaged at P40 after 10 d of daily injections of corticosterone (15 mg/kg) or vehicle. Data presentation and statistics are as described above. Statistics in bold are significant at $P < 0.05$.