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Adolescent Stress–Induced Epigenetic Control of Dopaminergic Neurons via Glucocorticoids

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Abstract

Environmental stressors during childhood and adolescence influence postnatal brain maturation and human behavioral patterns in adulthood. Accordingly, excess stressors result in adult-onset neuropsychiatric disorders. We describe an underlying mechanism in which glucocorticoids link adolescent stressors to epigenetic controls in neurons. In a mouse model of this phenomenon, a mild isolation stress affects the mesocortical projection of dopaminergic neurons in which DNA hypermethylation of the tyrosine hydroxylase gene is elicited, but only when combined with a relevant genetic risk for neuropsychiatric disorders. These molecular changes are associated with several neurochemical and behavioral deficits that occur in this mouse model, all of which are blocked by a glucocorticoid receptor antagonist. The biology and phenotypes of the mouse models resemble those of psychotic depression, a common and debilitating psychiatric disease.

Human behavior in adulthood is greatly influenced by various environmental conditions during childhood and adolescence (1–3). Although these environmental factors can interact with each other (4), individual responses vary, mainly because of different genetic predispositions among individuals (5). These gene-environment interactions may also underlie a variety of neuropsychiatric disorders (6). The development of means to intervene in such

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disorders, including prophylactic environmental readjustment (7), would be made easier if more were known about the underlying mechanisms and mediators of such interactions. Studies of dopamine responsiveness in conjunction with the effect of stress hormones in genetically selected rats were among the pioneering efforts in this field (8).

Maintaining mice in individual cages for 5 weeks during postnatal brain maturation, which may mimic separation from parents and family members and social isolation in humans (9), critically affects their adult behavioral patterns relative to those of mice raised in normal group housing (10). We asked whether such "suboptimal" levels of exposure (e.g., shorter duration of exposure) to the environmental stressor during adolescence might serve as a risk factor for adult behavioral deficits when combined with one or more appropriate genetic risks. We therefore exposed a newly generated transgenic mouse model with a putative dominant-negative DISC1 (disrupted in schizophrenia 1) under expression control of the prion protein promoter (DISC1-DN-Tg-PrP; see fig. S1 for characterization of this genetic model) to 3-week isolation stress. We studied four groups of mice: wild-type mice without isolation (control, CTL); wild-type mice with 3-week isolation (environmental stressor, E); DISC1-DN-Tg-PrP without isolation (genetic factor, G); and DISC1-DN-Tg-PrP with 3-week isolation (combination of genetic factor and environmental stressor, which we designate GXE) (fig. S2A).

When examining prepulse inhibition (PPI), immobility time in a forced swim test, and locomotor activity just after puberty, we did not observe any changes in behavior among CTL, E, and G mice. In contrast, GXE mice showed robust deficits in all of these behavioral paradigms (Fig. 1 and fig. S3A), which suggests that the combination of genetic factor and environmental stressor during adolescence (from 5 to 8 weeks of age) could lead to synergistic effects in the phenotypes. These behavioral deficits are unlikely to reflect major physical changes, because there were no differences in body weight among the groups (fig. S4). The trend in the behavioral changes in GXE mice was preserved in both sexes (fig. S5; see fig. S3, B and C, for data on locomotion over time).

What is the underlying mechanism of behavioral changes in GXE mice? No change in the volume of the lateral ventricles was observed between CTL and G groups (fig. S6A). At the histological level, Nissl staining showed no robust change in the frontal cortex (Fc) of GXE mice relative to the other groups (fig. S6B). There was no change in immunostaining for glial fibrillary acidic protein (GFAP), indicating no evidence of robust gliosis (fig. S6, C and D). Thus, we hypothesized that a functional alteration in neurotransmission without anatomical and histological changes might occur in this model. The total and extracellular levels of dopamine in the Fc were significantly decreased in GXE mice (Fig. 2, A and B, and fig. S7) relative to levels in the other groups. Dopaminergic change in the GXE model may have specific pathological importance, as no alterations in levels of norepinephrine and serotonin were observed (Fig. 2A). This dopaminergic change may be more specific to the projections originating from the ventral tegmental area (VTA), because there was no change in the total levels of dopamine in the caudate putamen (CPu) (Fig. 2C). In accordance with the changes in dopamine in the GXE model, a decrease in the expression levels of tyrosine hydroxylase (TH) was observed in the Fc (Fig. 2D) but not in the nucleus accumbens (NAc) (Fig. 2E). Expression of the dopamine D1 receptor (D1R) was unchanged, whereas expression of D2R was elevated in the Fc but not in the NAc (fig. S8). The GXE model displayed an augmented elevation in the levels of dopamine upon methamphetamine (METH) challenge in the NAc but not in the Fc (Fig. 2, F and G); this suggests that these neurochemical changes could consistently underlie augmented locomotor activity observed in the GXE model. Two distinct dopaminergic projections (mesocortical and mesolimbic) originating from the VTA may therefore be differentially affected in the GXE model.

Which upstream mediator(s) may influence dopaminergic neurotransmission, and perhaps also adulthood behavioral patterns, in the GXE model? We initially addressed this question by measuring plasma corticosterone in mice after they had completed several behavioral tests, because an increase in levels of plasma corticosterone under stress conditions is frequently reported (11). There was no relative difference in the levels of plasma corticosterone among CTL, G, and E mice; only GXE mice showed significantly elevated levels of corticosterone (Fig. 3A). To determine whether this elevated corticosterone could underlie the dopaminergic changes, we administered the glucocorticoid receptor (GR) antagonist RU38486 (mifepristone, 20 mg/kg subcutaneously; unexpected side effects were not observed at this dose) (12) (fig. S2B). RU38486 successfully normalized all the dopamine-related abnormalities studied in the GXE model, including levels of basal and METH-induced extracellular dopamine, TH, and D2R (Fig. 3, B and C, and fig. S9). RU38486 also significantly ameliorated the impaired performance in PPI, forced swim test, and locomotor activity upon METH challenge in the GXE model (Fig. 3, D to F, and fig. S3D).

These results indicate that two major dopaminergic projections from the VTA are differently affected by elevated glucocorticoids in the GXE model. Do glucocorticoids primarily affect two distinct projections in a different manner? Or do they primarily affect one projection, which results in the changes in the other projection? Given that a decrease in TH expression can account for all other molecular changes (decrease in dopamine, compensatory increase in D2R) in the Fc of GXE mice, we first examined the effects of elevated glucocorticoids on the regulation of *Th* gene expression. The mouse *Th* gene contains a glucocorticoid response element (GRE)–like sequence (GGCACAGTGTGGTCT) located in the 5′ flanking DNA between positions −2435 and −2421 from the transcription start site (13). Glucocorticoids have been reported to influence DNA methylation and resultant mRNA expression in the genes containing GRE(s), such as the one that encodes FK506 binding protein 5 (FKBP5) (14). Thus, we hypothesized that glucocorticoids might affect DNA methylation of the *Th* gene differentially between two distinct dopaminergic projections, which would underlie neurochemical and behavioral changes in GXE mice. We used green and red beads that could be retrogradely transported from projected areas back to the nucleus; green beads were injected into Fc and red beads into NAc. In the VTA, most of the TH-positive neurons contained either green or red beads, representing mesocortical and mesolimbic dopaminergic projections, respectively (Fig. 4A). Therefore, we dissected the VTA, separated and purified the green and red cells by fluorescence-activated cell sorting (FACS), and examined DNA methylation of these cells by bisulfite sequencing.

When we compared the DNA methylation levels and patterns in the *Th* gene in cells projected from the VTA to the Fc (green cells) among CTL, G, E, and GXE models, GXE showed a significant increase in DNA methylation (Fig. 4, B and C). In contrast, there was no difference in DNA methylation in cells projected from the VTA to the NAc (red cells) among these four animal groups (Fig. 4D). These results are compatible with TH expression in the Fc and NAc (Fig. 2, D and E). Might excess glucocorticoid signaling in the GXE model mediate this change in DNA methylation in the cells of the mesocortical projection? The GR antagonist RU38486 normalized methylation in the GXE model (Fig. 4E). These results demonstrate glucocorticoid-induced, projection-specific epigenetic modifications in the dopamine neurons.

A key question in epigenetics is how long such chemical modifications elicited by the primary stimulus can be maintained—that is, whether this adolescent stress leads to a long-lasting change in adulthood. The genetic model was isolated once from 5 to 8 weeks (i.e., for 3 weeks, the same condition as in the GXE model) and then returned to group housing after adolescence (from 8 to 20 weeks); we designate these mice as GXE-G (fig. S2C). At

20 weeks of age, we examined DNA methylation in the *Th* gene among GXE-G, G, and CTL mice. We then compared these results at 20 weeks of age with those from the GXE model at 8 weeks (immediately after isolation). The significantly high levels of the methylation in the GXE model at 8 weeks were maintained in the GXE-G model at 20 weeks (Fig. 4F). When we compared the epigenetic status of the genetic model without adolescent isolation (G) and that with isolation (GXE-G) at 20 weeks, we also observed significant differences as a result of isolation from 5 to 8 weeks (Fig. 4F).

Our results suggest that an environmental stressor—isolation stress during adolescence—can elicit molecular, neurochemical, and behavioral deficits only when combined with an appropriate genetic risk (in this case, dominant-negative DISC1 in the GXE mice).We observed projection-specific alterations, especially those in epigenetic modifications, in VTA-originated dopaminergic neurons of the GXE model. Under this condition, molecular changes in dopaminergic projections from the VTA and associated behavioral alterations were blocked by administration of the GR antagonist RU38486. These data are compatible with a previous report that chronic administration of corticosterone alters a potassium-induced elevation of extracellular dopamine (15). In the present model, the epigenetic alterations were evident in adult animals even if they were maintained in normal group housing several weeks after the transient adolescent isolation.

A key question in neurobiology is how mesocortical and mesolimbic projections are functionally related yet regulated by different means (16). These dopaminergic projections are involved in many important brain functions, including reward-associated behavior, motivation, and cognition, which are strongly affected by environmental stressors (17). Nonetheless, the link of stressors to dopaminergic projections at the molecular level has remained elusive. Our results show that glucocorticoids influence VTA-originated dopaminergic neurons in a projection-specific manner that involves, at least in part, an epigenetic mechanism. Adult human deviant behavior possibly mediated by the dopaminergic system is long-lasting and associated with stress events in childhood and adolescence (18). These selective changes may be accounted for directly by projection-specific molecular dispositions of GRs and downstream cascades in dopamine neurons. It is also conceivable that elevated glucocorticoids may affect neurons in the hippocampus and other areas (19), which in turn regulate the epigenetic status in a projection-specific manner.

DISC1 is a risk factor for many psychiatric disorders, including schizophrenia and mood disorders (20).DISC1 is expressed in many regions in the brain, but most of the functional studies on DISC1 have been made exclusively in the cerebral cortex and hippocampus. Previously published transgenic models from multiple laboratories have made use of the α–calcium/calmodulin-dependent protein kinase II (αCaMKII) promoter that preferentially expresses DN-DISC1 in the cortex and hippocampus after birth (DISC1-DN-Tg-CaMKII) (fig. S1, D to F) (20). In contrast, the present DISC1-DN-Tg-PrP model that shows the effect of gene-environment interactions expresses DN-DISC1 widely in the brain, including the hypothalamus. This evidence may open an avenue to pursue a role for DISC1 in the hypothalamic-pituitary-adrenal (HPA) axis.

Major depression with psychotic features (psychotic depression) is a common and debilitating psychiatric illness with a 0.4% prevalence in the general population in the United States and Europe (21), affecting up to 25% of depressed patients admitted to psychiatric hospitals (22). Mifepristone (RU38486) is uniquely beneficial in psychotic depression by directly blocking GRs and indirectly modulating functional interaction of cortisol and mineralocorticoid receptors (MRs) (23). However, no preclinical model representing this serious medical condition has been available. Given the behavioral abnormalities relevant to the endophenotypes of simultaneous psychosis and depression, as

well as the pharmacological responses, the GXE mouse may be a promising model for psychotic depression. The availability of a preclinical model would allow us to study underlying pathological mechanisms, including those in the premorbid and prodromal stages, and explore novel therapeutic strategies. Such a model could provide a good template not only for screening compounds with better efficacy and fewer side effects but also for prophylactic environmental readjustment, which is crucially important in clinical psychiatry.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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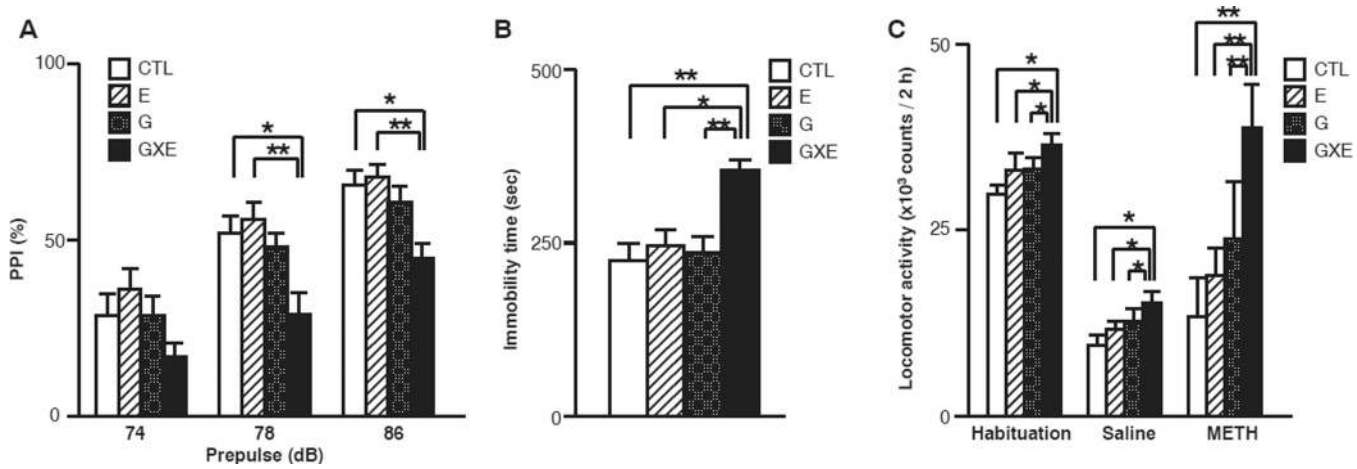

Fig. 1.

The GXE mouse model: Behavioral abnormalities in DISC1-DN-Tg-PrP after exposure to adolescent isolation stress for 3 weeks. (**A**) Deficits in PPI. (**B**) Impaired performance in the forced swim test. (**C**) Aberrant locomotor activity. CTL, wild type without isolation; E, wild type with isolation; G, DISC1-DN-Tg-PrP without isolation; GXE, DISC1-DN-Tg-PrP with isolation. Values are means ± SE; $^{**}P < 0.01$, $^{*}P < 0.05$. Numbers of animals and statistical information are described in tables S1 and S2.

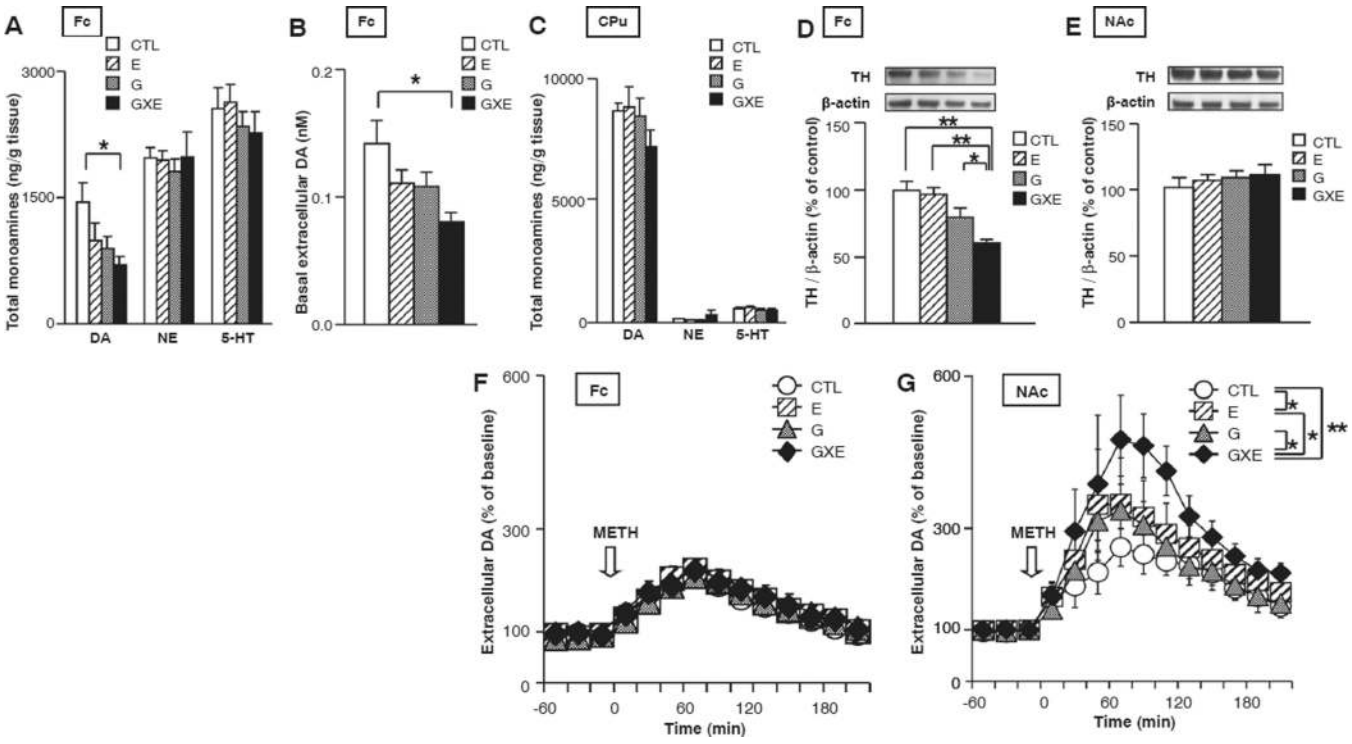

Fig. 2.

Dopaminergic disturbances in the GXE model. (**A**) Levels of total content of monoamines [dopamine (DA), norepinephrine (NE), and serotonin (5-HT)]. (**B**) Levels of extracellular basal DA in the Fc. (**C**) Levels of total content of monoamines in the CPu. (**D**) Levels of TH in the Fc. (**E**) Levels of TH in the NAc. (**F**) Extracellular DA levels upon METH challenge in the Fc. (**G**) Extracellular DA levels upon METH challenge in the NAc. Total and extracellular levels of neurotransmitters were measured by high-performance liquid chromatography and in vivo microdialysis, respectively. Values are means ± SE; $^{**}P < 0.01$, $^{*}P < 0.05$.

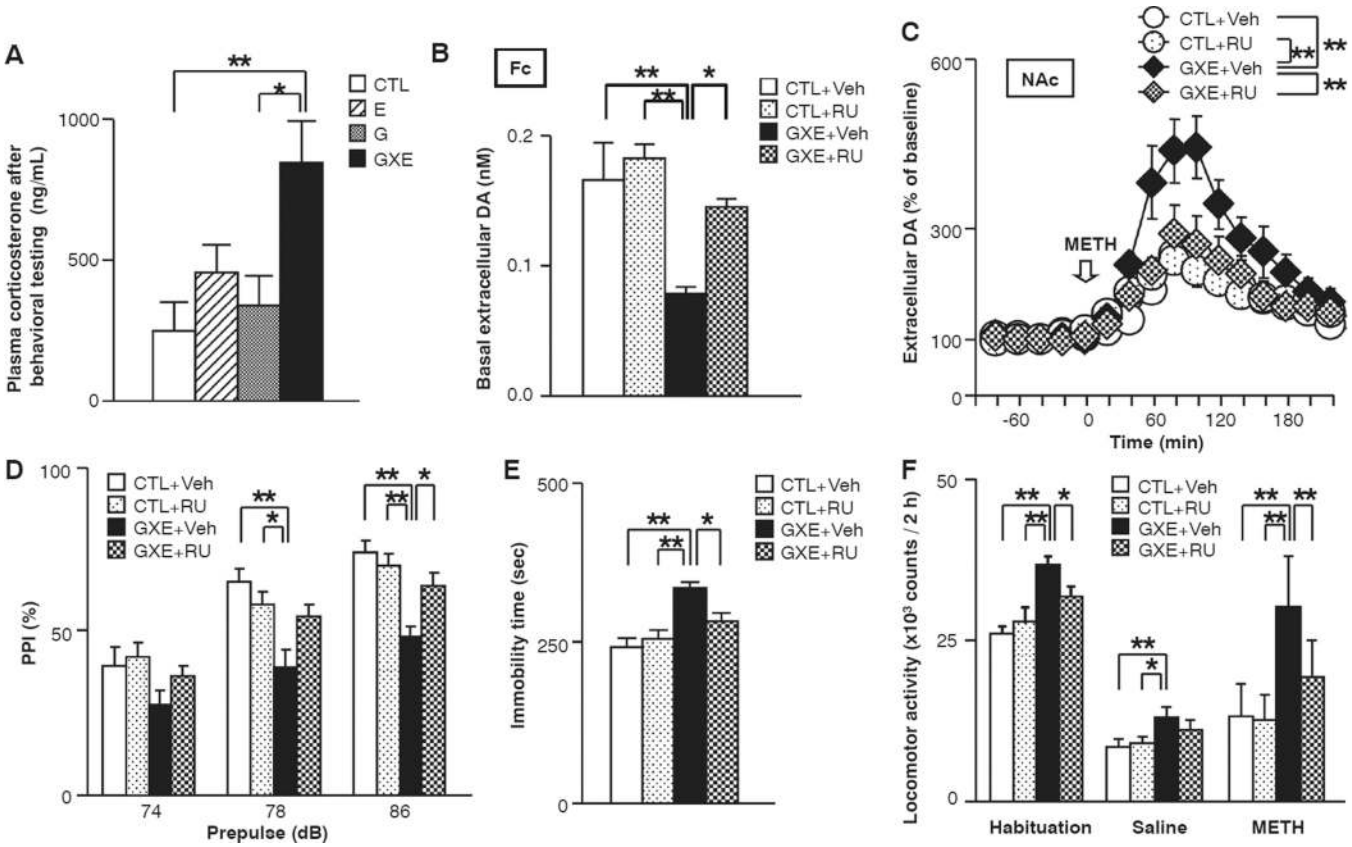

Fig. 3.

Influence of glucocorticoids on neurochemical and behavioral abnormalities in the GXE model. (**A**) Levels of plasma corticosterone after behavioral tests. (**B**) Effects of the GR antagonist RU38486 on levels of extracellular basal DA in the Fc. (**C**) Effects of RU38486 on levels of extracellular DA upon METH challenge in the NAc. (**D** to **F**) Effects of RU38486 on performance of PPI (D), forced swim test (E), and locomotor activity (F). Veh, treated with vehicle; RU, treated with RU38486. Values are means ± SE; $^{**}P < 0.01$, $^{*}P < 0.05$.

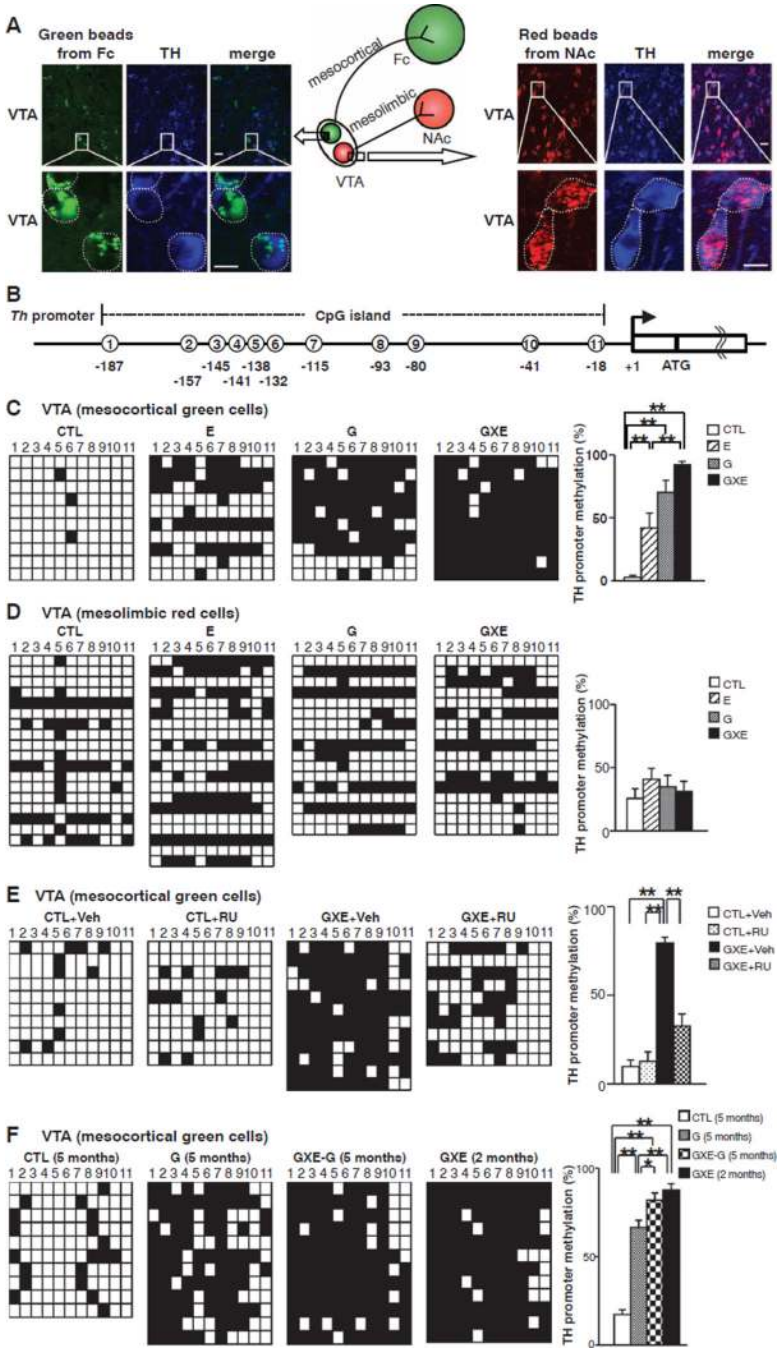

Fig. 4.

Distinct influence of glucocorticoids on epigenetic modification of the *Th* genes between mesocortical and mesolimbic dopaminergic projections in the GXE model. (**A**) Confocal images of TH-immunopositive cells in the VTA (blue), which are also labeled with retrogradely transported beads in mesocortical neurons (green) and those in mesolimbic neurons (red), respectively. Scale bars, 20 and 10 μm for upper and lower panels, respectively. (**B**) Graphic representation of the bisulfite-sequenced region in the promoter of the *Th* gene. The 11 CpG sites inside the island are indicated by open circles. The transcription start site (arrow), first exon (white box), and translation start site (ATG) are

shown. (**C**) DNA methylation pattern of the *Th* promoter in the VTA neurons projected to the Fc. (**D**) DNA methylation pattern of the *Th* promoter in the VTA neurons projected to the NAc. (**E**) Effects of RU38486 on increased DNA methylation of the *Th* promoter in the VTA neurons projected to the Fc. (**F**) Long-lasting change in the DNA methylation of the *Th* promoter in the VTA neurons projected to the Fc. GXE-G, GXE returned to group housing from 8 to 20 weeks. The columns and rows represent the 11 CpG sites and the sequenced clones, respectively. Black and white squares indicate methylated and unmethylated CpG sites, respectively. Results were obtained from at least three independent experiments. In right panels of (C) to (F), values are means ± SE; $^{**}P < 0.01$, $^{*}P < 0.05$.