Chronic Stress Triggers Social Aversion via Glucocorticoid Receptor in Dopaminoceptive Neurons

Jacques Barik *et al.*

Science **339**, 332 (2013);
DOI: 10.1126/science.1226767

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005.

do not trigger CCR7, and saw no significant effects on DC migration (Fig. 3D and fig. S8C). Together, these findings establish the functional activity of the immobilized CCL21 gradients that we identified before and show that, although DCs carry the receptor for and can respond to CXCL12, CCL21 gradients dominate. In addition, the chemokine pattern appears not to be determined by the distribution of CCL21 binding sites but most likely by the diffusion range of CCL21, which is trapped by sugar residues once it is released from the LVs (*21*).

CCL21 was shown to bind via its C-terminal domain sulfated sugars like heparin, heparan, dermatan, and chondroitin sulfates with low nanomolar affinities (*19*, *22*, *23*), potentially explaining the observed long retention times of CCL21 within the dermis and the inability of C-terminally truncated CCL21 to outcompete the endogenous gradients. To test which of the sugar moieties are involved in the immobilization of CCL21, we pretreated ear explants with sugar-degrading enzymes and found that heparitinase, which effectively removed heparan sulfates (fig. S9A), severely changed the CCL21 pattern. Quantifications revealed an almost complete flattening of the gradient and a drop to signal levels similar to control stainings, whereas tissue integrity was not affected (Fig. 3E and figs. S5G, S9B, and S10A). Consequently, DC migration in heparitinase-treated explants was severely diminished (Fig. 3F and fig. S10B). Heparan sulfate distribution patterns in untreated dermis did not match these of CCL21 (fig. S9A), corroborating the concept that not the tissue-binding sites for CCL21 but rather its distribution range determines the shape of the CCL21 gradient. These findings demonstrate that, like in the lumen of blood endothelium (*2*), interstitial CCL21 is immobilized to heparan sulfate residues, which either decorate cell surfaces or interstitial matrix components. This immobilized chemokine fraction is sufficient to guide intravasation of DCs, whereas adhesion molecules of the integrin family are dispensable for path-finding (*24*).

The term "haptotaxis" was originally introduced to describe cell migration along adhesive gradients, a phenomenon that was successfully constituted in vitro but still lacks direct in vivo support (*25*). Interstitial guidance by heparan sulfate immobilized chemokine gradients, as demonstrated here, can be viewed as a second variant of haptotaxis. The facts that (i) many chemokines bind GAGs (*26*), (ii) GAG interaction is important for the leukocyte-recruiting activity of some chemokines upon instillation into animals (*27*), and (iii) leukocytes have the ability to migrate along immobilized chemokine gradients in vitro (*28*, *29*) suggest that haptotaxis could be a widely used principle. Because immobilized gradients are insensitive to mechanical perturbations, they certainly constitute a robust and stable infrastructure for cellular guidance, whereas attraction by soluble gradients might be rather transient in nature.

References and Notes

1. J. Middleton *et al.*, *Cell* **91**, 385 (1997).
2. X. Bao *et al.*, *Immunity* **33**, 817 (2010).
3. R. Alon, *Immunity* **33**, 654 (2010).
4. E. C. Butcher, L. J. Picker, *Science* **272**, 60 (1996).
5. S. H. Wei, I. Parker, M. J. Miller, M. D. Cahalan, *Immunol. Rev.* **195**, 136 (2003).
6. F. Castellino *et al.*, *Nature* **440**, 890 (2006).
7. L. I. R. Ehrlich, D. Y. Oh, I. L. Weissman, R. S. Lewis, *Immunity* **31**, 986 (2009).
8. B. Boldajipour *et al.*, *Cell* **132**, 463 (2008).
9. T. Okada *et al.*, *PLoS Biol.* **3**, e150 (2005).
10. B. McDonald *et al.*, *Science* **330**, 362 (2010).
11. D. Alvarez, E. H. Vollmann, U. H. von Andrian, *Immunity* **29**, 325 (2008).
12. H. Pflicke, M. Sixt, *J. Exp. Med.* **206**, 2925 (2009).
13. R. Förster *et al.*, *Cell* **99**, 23 (1999).
14. A. Link *et al.*, *Nat. Immunol.* **8**, 1255 (2007).
15. M. R. Britschgi, S. Favre, S. A. Luther, *Eur. J. Immunol.* **40**, 1266 (2010).
16. P. Baluk *et al.*, *J. Exp. Med.* **204**, 2349 (2007).
17. A. Levchenko, P. A. Iglesias, *Biophys. J.* **82**, 50 (2002).
18. K. Schumann *et al.*, *Immunity* **32**, 703 (2010).
19. J. Hirose *et al.*, *Biochim. Biophys. Acta* **1571**, 219 (2002).
20. J. L. de Paz *et al.*, *ACS Chem. Biol.* **2**, 735 (2007).
21. P. Müller, A. F. Schier, *Dev. Cell* **21**, 145 (2011).
22. M. Bax, S. J. van Vliet, M. Litjens, J. J. García-Vallejo, Y. van Kooyk, *PLoS One* **4**, e6987 (2009).
23. K. Uchimura *et al.*, *BMC Biochem.* **7**, 2 (2006).
24. T. Lämmermann *et al.*, *Nature* **453**, 51 (2008).
25. R. J. Petrie, A. D. Doyle, K. M. Yamada, *Nat. Rev. Mol. Cell Biol.* **10**, 538 (2009).
26. A. Rot, U. H. von Andrian, *Annu. Rev. Immunol.* **22**, 891 (2004).
27. C. L. Salanga, T. M. Handel, *Exp. Cell Res.* **317**, 590 (2011).
28. A. Rot, *Eur. J. Immunol.* **23**, 303 (1993).
29. U. Haessler, M. Pisano, M. Wu, M. A. Swartz, *Proc. Natl. Acad. Sci. U.S.A.* **108**, 5614 (2011).

Acknowledgments: We thank M. Frank for technical assistance and S. Cremer, P. Schmalhorst, and E. Kiermaier for critical reading of the manuscript. This work was supported by a Humboldt Foundation postdoctoral fellowship (to M.W.), the German Research Foundation (Si1323-1,2 to M.S.), the Human Frontier Science Program (HFSP-RGP0058/2011 to M.S.), the European Research Council (ERC StG 281556 to M.S.), and the Swiss National Science Foundation (31003A_127474 to D.F.L., 130488 to S.A.L.). The authors declare no conflicts of interest. The data reported in the manuscript are tabulated in the main paper and in the supplementary materials.

Supplementary Materials

www.sciencemag.org/cgi/content/full/339/6117/328/DC1
Materials and Methods
Figs. S1 to S10
References (*30*)
Movies S1 to S4

6 August 2012; accepted 20 November 2012
10.1126/science.1228456

Chronic Stress Triggers Social Aversion via Glucocorticoid Receptor in Dopaminoceptive Neurons

Jacques Barik,[1,2,3,4]* Fabio Marti,[3,4,5] Carole Morel,[3,4,5] Sebastian P. Fernandez,[3,4,6] Christophe Lanteri,[2,3,7] Gérard Godeheu,[2,3,7] Jean-Pol Tassin,[2,3,7] Cédric Mombereau,[3,4,8] Philippe Faure,[3,4,5] François Tronche[1,2,3,4]*

Repeated traumatic events induce long-lasting behavioral changes that are key to organism adaptation and that affect cognitive, emotional, and social behaviors. Rodents subjected to repeated instances of aggression develop enduring social aversion and increased anxiety. Such repeated aggressions trigger a stress response, resulting in glucocorticoid release and activation of the ascending dopamine (DA) system. We bred mice with selective inactivation of the gene encoding the glucocorticoid receptor (GR) along the DA pathway, and exposed them to repeated aggressions. GR in dopaminoceptive but not DA-releasing neurons specifically promoted social aversion as well as dopaminergic neurochemical and electrophysiological neuroadaptations. Anxiety and fear memories remained unaffected. Acute inhibition of the activity of DA-releasing neurons fully restored social interaction in socially defeated wild-type mice. Our data suggest a GR-dependent neuronal dichotomy for the regulation of emotional and social behaviors, and clearly implicate GR as a link between stress resiliency and dopaminergic tone.

Traumatic experiences and social stress, such as aggression, may contribute to the onset of psychiatric disorders, including post-traumatic stress disorder and major depression (*1*, *2*). These situations trigger stress responses including the activation of the hypothalamic-pituitary-adrenal (HPA) axis and the release of its final output, the glucocorticoid hormones (GCs). This mechanism is central to the orchestration of the physiological and behavioral responses of vertebrates as they adapt to environmental stimuli (*3*). However, disproportionate or excessively long-lived stress responses expose the organism to deleterious outcomes and can precipitate the development of psychiatric disorders such as pathological anxiety, depression, and inability to socially perform (*4*–*6*). The molecular and cellular mechanisms underlying the etiology of stress-related psychiatric conditions remain unclear. Hence, understanding how individuals control emotions and cope with stressful events is a major clinical concern in the treatment of psychiatric illnesses.

GCs activate two related nuclear receptors: the glucocorticoid receptor (GR) expressed ubiquitously, and the mineralocorticoid receptor (MR), present in discrete brain regions. Both act as transcription

factors, controlling gene expression in the nucleus and participating in the rapid modulation of neuronal excitability at the membrane. During stress response, MR is involved in the appraisal of novel situations, whereas GR facilitates the consolidation of stress-related information (*7*). Molecular genetic approaches showed that GR modulates stress-related behaviors, including emotional behaviors, cognitive functions, and addictive states (*8*–*10*).

Male mice are territorial animals attacking any conspecific intruder; this behavior is modulated by HPA axis activity and dopamine (DA) (*11*–*13*). High GC levels lower the threshold for attack latency, possibly involving MR, whereas low levels trigger inappropriate attack targeting (*11*, *14*). We addressed here the role of GR in persistent behavioral changes after repeated social defeat. Social defeat repeated daily for 10 days leads to increased anxiety and social aversion (*15*). A single aggression in naïve mice led, 45 min later, to a marked increase (factor of >60) in circulating GC levels (basal, 6.2 ± 1.3 ng/ml; acute aggression, 392.6 ± 51 ng/ml; $n = 6$, $P < 0.01$, unpaired t test). Stressors that ultimately trigger a rise in glucocorticoid levels can alter the excitability of midbrain dopamine-releasing neurons (DA neurons) and consequently affect DA release (*16*). These neurons from the ventral tegmental area (VTA) project to limbic and cortical structures and participate in the modulation of motor control, reward, and emotions (*17*–*19*). They are activated in response to psychosocial stimuli (*17*) and sustain social aversion in mice exposed to repeated aggression by increasing brain-derived neurotrophic factor (BDNF) levels (*20*). Furthermore, they participate in the modulation of anxiety-like behaviors and cognitive functions (*21*).

Could GR activation in DA neurons be involved in social aversion? Mice selectively deprived of GR in DA neurons (*10*) ($Nr3c1^{loxP/loxP}$;TgBACDATiCre mice, hereafter termed GR^{DATCre}) and control littermates were subjected to daily social defeats for 10 consecutive days (see supplementary text) and compared to undefeated littermates. We measured their reactivity and habituation to a new environment by recording daily locomotor activity prior to each aggression. Undefeated GR^{DATCre} and control littermates exhibited similar expected novelty-reaction and habituation of exploratory behaviors that became apparent throughout the course of the experiment (fig. S1A). In contrast, both groups of defeated mice rapidly displayed neophobic responses marked by a pronounced hypolocomotion already seen after the first days of aggression. To assess social behavior under basal conditions or after aggression, we recorded the time spent establishing social contacts with an unfamiliar mouse, as previously described (*20*), in a low-luminosity (20 lux) environment to minimize the impact of emotional behaviors. Undefeated control and GR^{DATCre} mice spent a comparable amount of time performing social behavior. This was measured by the increase of time spent in the vicinity of a male mouse contained in a polycarbonate box placed in an open field. Repeated defeat triggered social avoidance in both genotypes, indicated by a substantial reduction in the time spent performing social contacts. This rules out the involvement of GR in DA neurons as a mediator of the outcome of chronic social defeat stress (Fig. 1A). This paradigm also engendered, regardless of genotype, strong anxiety-like behaviors as assessed by the elevated zero-maze and the dark-light box, two tests based on approach-avoidance conflicts (fig. S1, B and C), and no change in despair behavior as measured by the forced-swim test (fig. S1D). These results show that although stress provoked changes in behavior after repeated social defeat, this did not involve the activation of GR in DA cells.

Dopaminoceptive neurons of the nucleus accumbens (NAc) exert positive feedback on DA neuron activity through projection onto the VTA, relayed locally by interneurons (*22*, *23*). GC-mediated stress effects on the dopaminergic pathway could, alternatively, involve postsynaptic neurons bearing DA receptors. We therefore studied animals deprived of GR in dopaminoceptive neurons (*10*) ($Nr3c1^{loxP/loxP}$;TgYAC-D1aCre mice, hereafter termed GR^{D1Cre} mice). In this model, GR is absent from 87 to 88% of striatal and accumbal neurons as well as from neurons located in deep cortical layers (fig. S2, A and B). Remarkably, social avoidance was completely abolished in GR^{D1Cre} mice. Undefeated control and GR^{D1Cre} mice behaved normally during the social interaction test when presented with an unfamiliar mouse (Fig. 1B). However, defeated control mice displayed a strong social avoidance, whereas defeated GR^{D1Cre} mice socially interacted like undefeated mice (Fig. 1B). The number and duration of contacts established by undefeated controls and defeated GR^{D1Cre} mice with the box containing the target mouse were comparable (Fig. 1, C

[1]Molecular Genetics, Neurophysiology and Behavior Group, Centre National de la Recherche Scientifique (CNRS) Unité Mixte de Recherche (UMR) 7224, 75005 Paris, France. [2]Institut National de la Santé et de la Recherche Médicale (INSERM) U952S, 75005 Paris, France. [3]Université Pierre et Marie Curie, 75005 Paris, France. [4]Laboratory of Excellence (Labex) Biological Psychiatry Laboratory, 75005 Paris, France. [5]Neurophysiology and Behavior Group, CNRS UMR7102, 75005 Paris, France. [6]Neurotransmission and Development Group, INSERM U839, 75005 Paris, France. [7]Physiopathology of Addiction and Relapse Group, CNRS UMR7224, 75005 Paris, France. [8]Molecular and Cellular Mechanisms of Cortical Development Group, INSERM U839, 75005 Paris, France.

*To whom correspondence should be addressed. E-mail: francois.tronche@upmc.fr (F.T.); jacques.barik@snv.jussieu.fr (J.B.)

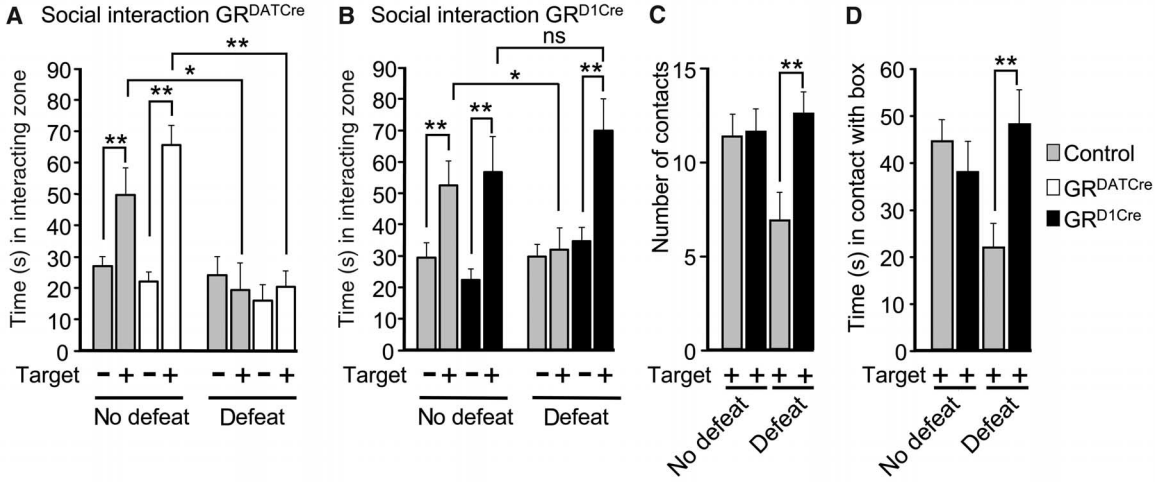

Fig. 1. GR in dopaminoceptive neurons, but not in DA neurons, selectively drives social aversion triggered by chronic social stress. (**A**) Undefeated control (gray) and GR^{DATCre} mice (white) spent more time in the virtual interacting zone when a mouse was contained in a box present in the open field (Target+) than when no mouse was introduced (Target–). In contrast, defeated control and GR^{DATCre} mice displayed social aversion (interaction defeat × target, $F_{1,44} = 19.5$, $P < 0.0001$) but no genotype effect (no interaction defeat × target × genotype, $F_{1,44} = 0.3$, $P > 0.05$). (**B**) Undefeated control (gray) and GR^{D1Cre} (black) mice displayed expected social interactions. Defeated control animals exhibited social aversion, whereas defeated GR^{D1Cre} mice exhibited social interaction (interaction defeat × target × genotype, $F_{1,44} = 9.7$, $P < 0.01$). (**C** and **D**) The numbers of contacts (C) and time spent in physical contact with the box (D) by defeated GR^{D1Cre} mice were higher than in defeated controls but similar to undefeated control mice [interaction genotype × defeat, $F_{1,44} = 4.3$, $P < 0.05$ (C); $F_{1,44} = 7.3$, $P < 0.01$ (D)]. $n = 12$ per group; $^*P < 0.05$, $^{**}P < 0.01$ (post hoc Bonferroni test).

and D). This difference in social aversion cannot be attributed to a defect in social memory, as both control and mutant mice equally performed in a social discrimination task (fig. S3A). This effect was not due to differences in circulating corticosterone levels or in HPA axis reactivity after social defeat, which were similar in GR^{D1Cre} mice and control littermates (Fig. 2A).

GRs in dopaminoceptive neurons are essential for adapting to a chronic social stress and translating this experience-dependent social behavior into a learned prediction of a recurrent danger. The absence of GR from dopaminoceptive neurons specifically affected social avoidance without modifying other behavioral changes induced by repeated defeats, such as induced anxiety-like behaviors (Fig. 2B and fig. S3B) and induced hyporeactivity to a novel environment (fig. S3C). Nor were despair behaviors affected either (fig. S3D). The lack of social aversion of defeated GR^{D1Cre} mice could result from early GR gene inactivation due to early developmental Cre expression (*24*). This is probably not the case, because chronic GR blockade (using RU486) before each daily aggression prevented social aversion, hence mimicking the dopaminoceptive-targeted GR gene inactivation (fig. S3E). Furthermore, stress triggered physiological adaptations, such as impoverishment of fur state, decreased body and thymus weights, and adrenal hypertrophy in both genotypes (fig. S4, A to D).

The change in social avoidance observed in GR^{D1Cre} mice could result from a general deficit in fear memory trace formation. To evaluate changes in learned fear, we subjected animals to classical fear conditioning training with three consecutive inescapable footshocks. The day after training, animals were reexposed to the same context and the conditional freezing response was quantified. GR^{D1Cre} mice and control littermates exhibited similar levels of emotional reactivity to learned aversive stimuli, ruling out the possibility that the lack of social avoidance could reflect an altered fear response (Fig. 2C).

To gain insight into the putative circuitry underpinning this failure of social avoidance, we measured DA release in the NAc after aggression by in vivo microdialysis in freely moving mice. Social defeat triggered a robust release of DA in control animals, whereas this effect was markedly diminished in GR^{D1Cre} littermates (Fig. 3A). The defective stress-elicited DA release in GR^{D1Cre} mice suggests that DA neuro-

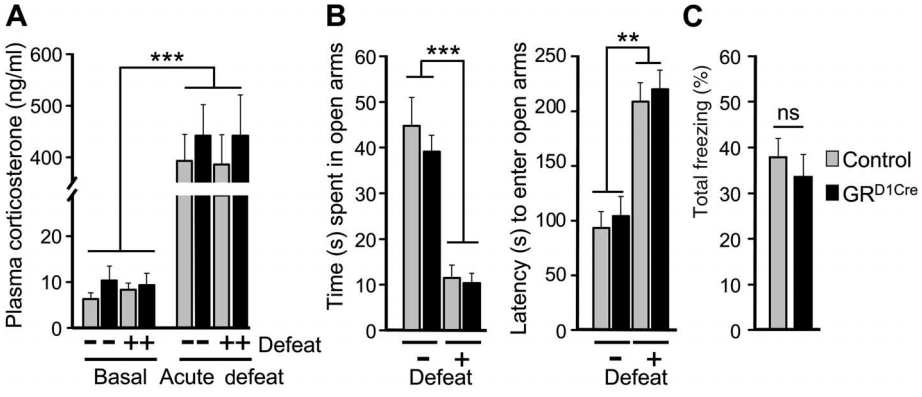

Fig. 2. GR in dopaminoceptive neurons is dispensable for emotional behavior and HPA axis reactivity subsequent to chronic social stress. (**A**) Levels of plasma corticosterone differed neither in naïve nor in chronically defeated control and GR^{D1Cre} mice under basal conditions or after an additional acute social defeat episode. Effect of acute stress ($F_{1,36}$ = 314.9, P < 0.0001) with no genotype ($F_{1,36}$ = 1.3, P > 0.05) or defeat ($F_{1,36}$ = 0.14, P > 0.05) effect. Circulating GC levels in control and defeated GR^{D1Cre} mice (black) and control littermates (gray) are shown under basal conditions or after an acute defeat. (**B**) Anxiety was measured within an elevated zero-maze in undefeated and defeated animals. After chronic defeat stress in control and GR^{D1Cre} mice, the latency to enter (right panel) and the time spent (left panel) in the open arms was equally increased and decreased, respectively. Effect of defeat (time in open arms $F_{1,44}$ = 63.3, P < 0.001; latency $F_{1,44}$ = 43.3, P < 0.001), no effect of genotype (time in open arms $F_{1,44}$ = 0.8, P > 0.05; latency $F_{1,44}$ = 0.5, P > 0.05). (**C**) Quantification of freezing behavior in control and GR^{D1Cre} mice reexposed to the conditioning context 24 hours after conditioning with footshocks (unpaired t test, P > 0.05). n = 5 to 7 mice per group (A), n = 12 mice per group [(B) and (C)]; **P < 0.01, ***P < 0.001 (post hoc Bonferroni test).

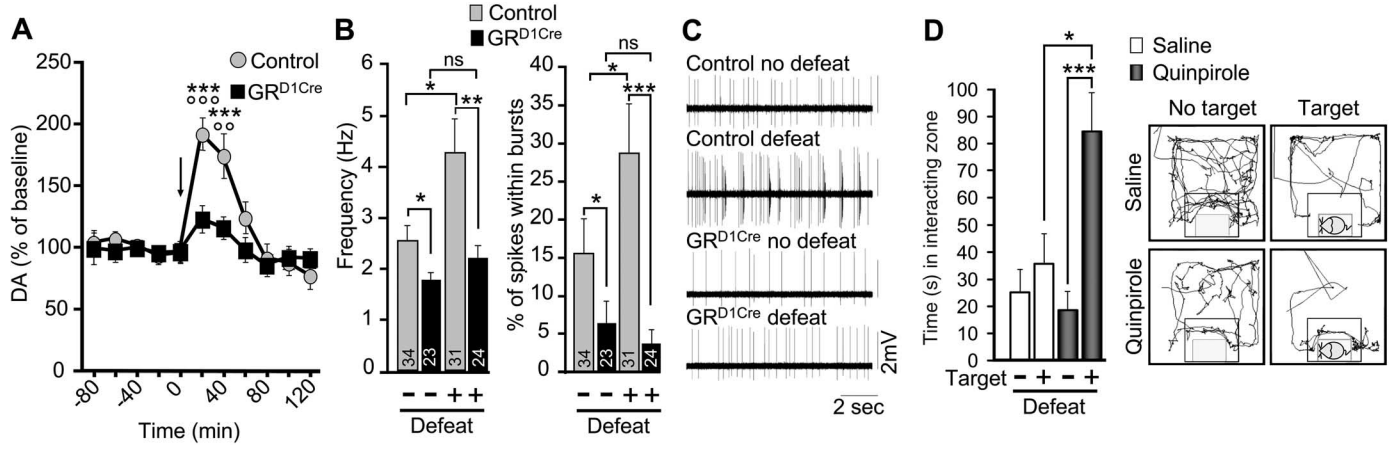

Fig. 3. Increase of in vivo firing of VTA neurons is required for the expression of social aversion. (**A**) An acute social defeat stress (arrow) fails to induce significant DA overflow, as measured by microdialysis, in the NAc of GR^{D1Cre} mice relative to control littermates (interaction defeat × genotype $F_{10,130}$ = 4.7, P < 0.0001). Stress versus respective baseline: ***P < 0.001. Control versus mutant: °°P < 0.01, °°°P < 0.0001. n = 7 to 8 mice per group. (**B**) Firing rate (left panel) and percentage of spikes within bursts (right panel) of VTA DA cells in undefeated or defeated control and GR^{D1Cre} mice in vivo. Numbers indicate the number of DA cells in corresponding groups; n = 24 to 34 neurons from 6 to 8 mice per group. (**C**) Representative recordings obtained from control and GR^{D1Cre} mice after repeated social defeat. (**D**) An acute quinpirole injection to defeated control mice prior to the social interaction test reverses social aversion, whereas saline fails to do so. Left panel: Quantification of the time spent in the interacting zone by defeated mice injected with saline (white bars) or quinpirole (0.03mg/kg, black shaded bars) in the presence of an empty box (Target–) or containing a mouse (Target+). Right panel: Representative trackings of defeated mice after either saline or quinpirole administration (interaction target × treatment $F_{1,38}$ = 7.4, P < 0.01). n = 10 to 11 mice per group. *P < 0.05, **P < 0.01, ***P < 0.001 (post hoc Bonferroni test).

transmission is a primary component of social stress–induced neuroadaptations. To further support this observation, we examined the electrophysiological properties of VTA DA neurons in vivo. These cells exhibit two discernible patterns of activity—slow, single-spike firing and fast-bursting activity—with the possibility of switching among these modes (*25*, *26*). This mode switching allows flexibility within the circuitry. It has been associated with reward-related behaviors (*27*) and has been suggested to promote alertness in cases of threatening situations (*28*). The frequency and the number of bursting events of DA neurons from defeated control mice was significantly increased relative to those of undefeated controls (Fig. 3, B and C, and fig. S5, A to D) (*29*). In contrast, DA neurons of defeated GR^{D1Cre} mice, although still expressing the GR gene, failed to demonstrate any significant electrophysiological adaptations, hence mirroring the lack of social avoidance (Fig. 3, B and C).

We further investigated this non–cell-autonomous effect of GR gene inactivation on dopamine-releasing neurons. Although VTA DA neurons of GR^{D1Cre} mice already exhibit a diminished basal activity, as we previously observed (*10*), their functioning is not completely abolished. Indeed, eticlopride [a DA D2 receptor (D2-R) antagonist] comparably increased VTA DA neuron electrophysiological activity in both control and mutant mice (fig. S5E). Moreover, these DA neurons are capable of sustaining a prolonged DA release in awake GR^{D1Cre} mice acutely challenged with morphine (*30*), indicating that these cells can be physiologically active under certain circumstances. The lack of electrophysiological adaptations observed in GR^{D1Cre} mice further supports the existence of a positive feedback loop that regulates VTA firing, and that is essential for adapting to threatening situations. We next asked whether this increase in DA neurons firing is a prerequisite for social avoidance or a subsequent neuroadaptation to stress. Just before the social interaction test, defeated and undefeated control mice received an acute injection of saline or quinpirole, a DA D2-R agonist that, when administered at low doses, primarily stimulates somatodendritic D2 autoreceptors and consequently suppresses DA neuron activity (*31*, *32*). Social interaction was similar in undefeated mice acutely challenged with saline or quinpirole (fig. S5F). However, whereas saline-injected defeated mice exhibited the expected social avoidance, quinpirole efficiently restored social interaction (Fig. 3D).

Our results show that in dopaminoceptive neurons, GR, a transcription factor induced by stress response, promotes social aversion but not other anxiety-like behaviors also induced after repeated aggression. This effect is likely to occur via a positive feedback loop from the NAc to the VTA. Our findings also suggest the existence of a discrete specification of neuronal circuits underlying behavioral outcomes of social stress. Our demonstration that a pharmacological intervention can restore social interaction suggests the possibility of normalizing specific negative outcomes of traumatic events.

References and Notes

1. K. L. Huhman, *Horm. Behav.* **50**, 640 (2006).
2. I. Kelleher *et al.*, *Br. J. Psychiatry* **193**, 378 (2008).
3. E. R. de Kloet, I. E. de Jong, M. S. Oitzl, *Eur. J. Pharmacol.* **585**, 473 (2008).
4. E. R. de Kloet, M. Joëls, F. Holsboer, *Nat. Rev. Neurosci.* **6**, 463 (2005).
5. B. S. McEwen, *Ann. N.Y. Acad. Sci.* **1032**, 1 (2004).
6. R. M. Sapolsky, *Science* **308**, 648 (2005).
7. F. L. Groeneweg, H. Karst, E. R. de Kloet, M. Joëls, *J. Endocrinol.* **209**, 153 (2011).
8. F. Tronche *et al.*, *Nat. Genet.* **23**, 99 (1999).
9. Q. Wei *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 11851 (2004).
10. F. Ambroggi *et al.*, *Nat. Neurosci.* **12**, 247 (2009).
11. J. Haller, M. R. Kruk, *Neurosci. Biobehav. Rev.* **30**, 292 (2006).
12. J. Haller, S. Millar, J. van de Schraaf, R. E. de Kloet, M. R. Kruk, *J. Neuroendocrinol.* **12**, 431 (2000).
13. D. I. Beiderbeck *et al.*, *Psychoneuroendocrinology* **37**, 1969 (2012).
14. A. H. Veenema, O. C. Meijer, E. R. de Kloet, J. M. Koolhaas, B. G. Bohus, *Horm. Behav.* **43**, 197 (2003).
15. V. Krishnan, E. J. Nestler, *Nature* **455**, 894 (2008).
16. M. Marinelli, in *Stress and Addiction*, M. Al'Absi, Ed. (Academic Press/Elsevier, Burlington, MA, 2007), pp. 41–83.
17. M. Marinelli, C. N. Rudick, X. T. Hu, F. J. White, *CNS Neurol. Disord. Drug Targets* **5**, 79 (2006).
18. O. Valenti, D. J. Lodge, A. A. Grace, *J. Neurosci.* **31**, 4280 (2011).
19. J. W. Dalley, B. J. Everitt, *Semin. Cell Dev. Biol.* **20**, 403 (2009).
20. O. Berton *et al.*, *Science* **311**, 864 (2006).
21. L. S. Zweifel *et al.*, *Nat. Neurosci.* **14**, 620 (2011).
22. Y. Xia *et al.*, *J. Neurosci.* **31**, 7811 (2011).
23. N. Omelchenko, S. R. Sesack, *Synapse* **63**, 895 (2009).
24. T. Lemberger *et al.*, *BMC Neurosci.* **8**, 4 (2007).
25. A. A. Grace, B. S. Bunney, *J. Neurosci.* **4**, 2877 (1984).
26. A. A. Grace, B. S. Bunney, *J. Neurosci.* **4**, 2866 (1984).
27. J. Mirenowicz, W. Schultz, *Nature* **379**, 449 (1996).
28. V. Krishnan *et al.*, *Cell* **131**, 391 (2007).
29. J. L. Cao *et al.*, *J. Neurosci.* **30**, 16453 (2010).
30. J. Barik *et al.*, *Biol. Psychiatry* **68**, 231 (2010).
31. A. Usiello *et al.*, *Nature* **408**, 199 (2000).
32. A. Anzalone *et al.*, *J. Neurosci.* **32**, 9023 (2012).

Acknowledgments: We thank S. Wonnacott for critical reading of the manuscript, and staff from the IFR83 animal facility for assistance. This work was funded by CNRS, INSERM, the Agence Nationale de la Recherche (TIMMS grants), the UPMC (Emergence program), the Région Île-de-France (NeRF), the European Union (FP7 STREPs PheComp and NovelTune), the Bettencourt Schueller Foundation, and the Mission Interministérielle de Lutte contre la Dépendance et la Toxicomanie. J.B. has been supported by the Fondation pour la Recherche Médicale. The authors declare no competing financial interests.

Supplementary Materials

www.sciencemag.org/cgi/content/full/339/6117/332/DC1
Materials and Methods
Figs. S1 to S5
References (*33*–*39*)

2 July 2012; accepted 21 November 2012
10.1126/science.1226767

Adolescent Stress–Induced Epigenetic Control of Dopaminergic Neurons via Glucocorticoids

Minae Niwa,[1,2,3] Hanna Jaaro-Peled,[2] Stephanie Tankou,[2] Saurav Seshadri,[2] Takatoshi Hikida,[2,4] Yurie Matsumoto,[1,3] Nicola G. Cascella,[2] Shin-ichi Kano,[2] Norio Ozaki,[3] Toshitaka Nabeshima,[1,5,6]* Akira Sawa[2]*

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 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