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Resilience to chronic stress is mediated by noradrenergic regulation of dopamine neurons

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Dopamine (DA) neurons in the ventral tegmental area (VTA) help mediate stress susceptibility and resilience. However, upstream mechanisms controlling these neurons remain unknown. Noradrenergic (NE) neurons in the locus coeruleus, implicated in the pathophysiology of depression, have direct connections within the VTA. Here we demonstrate that NE neurons regulate vulnerability to social defeat through inhibitory control of VTA DA neurons.

Given that dopaminergic mesocorticolimbic pathways are implicated in regulating vulnerability to emotional stress[1–4], we investigated whether modifications of NE transmission could be observed in these pathways following chronic social defeat. Locus coeruleus (LC) NE neurons, through direct anatomical[5] and functional[6] connections within the VTA, could be an intermediate between external stressors and regulation of the stress response by VTA DA neurons. Animals exposed to a 10-d repeated social defeat stress procedure (**Fig. 1a**) can be separated into susceptible and resilient on the basis of a social avoidance measure. Susceptible defeated mice showed robust social aversion in the presence of a CD1 mouse, whereas resilient defeated mice (37% of the mice tested) displayed an interaction score similar to that of undefeated control mice, as expected[1,7] (**Fig. 1b** and **Supplementary Fig. 1a**). NE tissue levels in the VTA, measured by HPLC on a small cohort of mice, was lower in susceptible mice than in resilient mice (**Fig. 1c**), whereas no change was observed in other limbic areas, including the nucleus accumbens (NAc) and the prefrontal cortex (PFC) (**Fig. 1d**). Furthermore, a strong correlation, which was not driven by outliers (Grubbs test: control $t = 1.51$, $P = 1$; susceptible $t = 1.93$, $P = 0.21$; resilient: $t = 2.03$, $P = 0.19$), was found between NE levels in the VTA and social interaction times (**Fig. 1c**). This suggests that NE transmission in the VTA might be a critical factor in determining vulnerability to emotional stress, although a definitive answer should be provided by direct assessment of extracellular NE release by *in vivo* microdialysis. We confirmed that, as previously demonstrated[1–3], susceptible mice exhibited higher excitability of VTA DA neurons compared with control and resilient mice (**Supplementary Fig. 1b–g**). Susceptible mice also exhibited higher DA levels in the NAc but not in the PFC, suggesting higher DA release from VTA DA neurons projecting into the NAc in susceptible mice (**Supplementary Fig. 1h**).

Next we investigated the activity state of LC NE neurons. We found a global decrease in the number of cells immunoreactive for the immediate early gene product c-fos in the LC of susceptible mice compared to controls, an effect not observed in resilient mice (**Supplementary Fig. 2a**). Using fluorescent retrograde tracer beads injected into the VTA, we specifically measured the neuronal activation of LC NE neurons projecting to the VTA (**Supplementary Fig. 2b–d**). Lower c-fos expression observed in susceptible mice was fully accounted for by NE (tyrosine hydroxylase (TH)-positive) neurons projecting to the VTA (positive for beads) (**Fig. 1e**). However, the numbers of both non-NE-activated cells (TH-negative) projecting to the VTA (positive for beads) and NE-activated cells (TH-positive) not projecting to the VTA (negative for beads) were unchanged (**Fig. 1f,g**). Moreover, we found a strong correlation between the number of activated VTA-projecting NE neurons and social interaction time (**Fig. 1e**), suggesting that both the activation state of VTA-projecting LC NE neurons and the amount of NE released into the VTA are critical factors in determining vulnerability to emotional stress.

Changes in noradrenergic control of VTA neurons in response to emotional stress would thus modulate the neuronal activation of the VTA DA pathways to set the behavioral outcome. Since resilient animals do not show differential c-fos expression in LC NE neurons projecting to the VTA, additional presynaptic mechanisms may be accounting for the greater NE release observed in these mice. Dysregulations in NE synthesis, release and reuptake have been previously demonstrated in response to chronic stress[8–10], and such modifications are candidates for further understanding increased NE levels in the context of resilience.

To investigate whether NE neurotransmission is necessary to induce resilience to social defeat, we engineered mice with selective brain-specific NE depletion[11]. In conditional $VMAT2^{DBHcre}$ knockout (KO) mice, the *loxP*-flanked (floxed) vesicular monoamine transporter-2 (*VMAT2*, or *Slc18a2*) gene was specifically spliced in NE neurons by Cre recombinase expressed under the dopamine β-hydroxylase (*Dbh*) promoter. We assessed the specificity of the DBHcre strain using a tdTomato reporter mouse, and the efficiency of *VMAT2* splicing was confirmed by the specific disappearance of *VMAT2* mRNA hybridization in the LC as well as by the marked decrease in NE (**Supplementary Fig. 3a–c**). No alteration in the survival rate, growth and motor ability of the $VMAT2^{DBHcre}$ KO mice was found (**Supplementary Fig. 3d–f**), ruling out any confounding effect on more complex behavior.

Chronic social defeat (10 d) triggered social avoidance in both wild-type (WT) and KO genotypes (**Fig. 2a** and **Supplementary Fig. 4a–c**). However, only 5.3% of the KO defeated mice displayed a resilient

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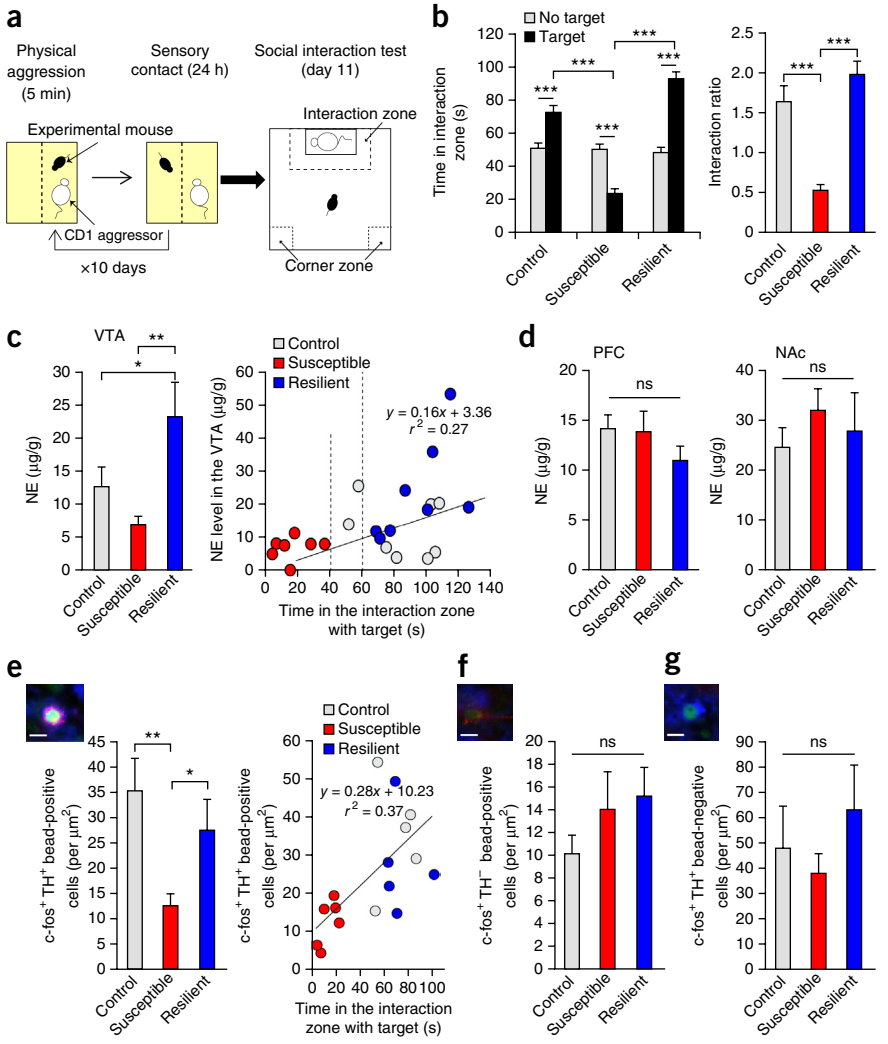

Figure 1 Susceptibility to social defeat decreases both NE in the VTA and neuronal activation of LC NE neurons projecting to the VTA. (**a**) Schematic of the 10-d social defeat stress procedure and the social interaction test. (**b**) Time in the interaction zone ($F_{(2,73)} = 58.1$, $P < 0.001$; left) in control mice ($n = 30$) and socially defeated resilient mice ($n = 17$) was significantly increased during the second phase in the presence of CD1 mice (target) compared with the first phase with no target, whereas it was significantly decreased in susceptible defeated mice ($n = 29$) (*post hoc* Fisher LSD $^{***}P < 0.001$). The time spent in the social interaction zone with target and the interaction ratio [(interaction time, target present)/(interaction time, target absent)] ($F_{(2,73)} = 22.6$, $P < 0.001$; right) were significantly decreased in susceptible mice compared with both control and resilient mice (*post hoc* $^{***}P < 0.001$). (**c**) The concentration of NE (µg/g) in the VTA, as measured by HPLC (left), was significantly decreased in susceptible mice ($n = 7$) compared with resilient mice ($n = 8$) (control mice $n = 8$; $F_{(2,20)} = 4.8$, $P = 0.02$; *post hoc* $^{*}P = 0.05$; $^{**}P = 0.007$), and there was a significant correlation (right) between the NE concentration (µg/g) and the time spent in the interaction zone in the presence of the target ($t_{22} = 2.76$, $r^2 = 0.27$, $P = 0.011$). (**d**) No change in NE concentration (µg/g) was found in the PFC ($F_{(2,20)} = 0.38$, $P = 0.68$, not significant (ns)) or the NAc ($F_{(2,20)} = 1.17$, $P = 0.33$, ns) in response to chronic social defeat stress. (**e**) The number of activated NE neurons in the LC projecting to the VTA (c-fos$^+$ TH$^+$ bead-positive cells per µm^2; $F_{(2,13)} = 5.8$, $P = 0.015$) was significantly lower in susceptible mice ($n = 6$) compared with control mice ($n = 5$) and resilient mice ($n = 5$) (*post hoc* $^{*}P = 0.043$, $^{**}P = 0.005$; scale bar, 10 µm), and there was a significant correlation (right) between the number of activated NE neurons in the LC projecting to the VTA (c-fos$^+$ TH$^-$ bead-positive cells per µm^2) and the time spent in the interaction zone in the presence of the target ($t_{16} = 2.86$, $r^2 = 0.37$, $P = 0.013$). (**f**) The number of activated non-NE neurons in the LC projecting to the VTA (c-fos$^+$ TH$^-$ bead-positive cells per µm^2) was not altered by the chronic social defeat procedure ($F_{(2,13)} = 0.77$, $P = 0.48$, ns; scale bar, 10 µm). (**g**) The number of activated NE neurons in the LC that do not project to the VTA (c-fos$^+$ TH$^+$ bead-negative cells per µm^2) was unchanged in response to the social defeat procedure ($F_{(2,13)} = 0.82$; $P = 0.46$, ns; scale bar, 10 µm). Error bars, s.e.m.

phenotype, as compared with 30.4% in WT defeated mice (**Fig. 2b**). *VMAT2*^{DBHcre} KO mice already showed a higher propensity to susceptibility (**Fig. 2c**) after only 1 d of stress, demonstrating the large effect of NE transmission on eliciting resilience to social defeat.

Given the role of VTA DA neuronal excitability in vulnerability to social defeat[1–4,12,13], we performed *in vivo* extracellular single-unit recordings on VTA DA neurons in *VMAT2*^{DBHcre} mice. We found an increase in both the spontaneous firing rate and the bursting activity of VTA DA neurons in KO mice compared with WT (**Fig. 2d**), with a decrease in low-firing-rate neurons and an increase in highly bursting neurons (**Fig. 2e–f**). These results indicate that NE neurotransmission likely promotes resilience to social defeat by inhibiting VTA DA neuronal activity.

To assess whether NE neurotransmission is sufficient to counteract vulnerability to social defeat, we pharmacologically increased NE release. We treated control and susceptible mice with the α2 noradrenergic receptor antagonist idazoxan (2 mg/kg, intraperitoneally) starting immediately after the 10 d of defeat. Idazoxan increases both neuronal firing in the LC[14] and NE release[15] by blocking presynaptic α2 noradrenergic receptors (**Fig. 3a**). Chronic treatment (1 week of daily injection), but not acute (injection 30 min before the social interaction test) treatment, led to decreased social avoidance, thereby reversing the susceptible phenotype of defeated mice to a resilient phenotype (**Fig. 3b** and **Supplementary Fig. 5a,b**). In control animals, idazoxan treatment had no effect on social interaction (**Supplementary Fig. 5c**). This effect was also seen after 1 week of treatment with reboxetine, a selective NE reuptake inhibitor that increases extrasynaptic NE, triggering resilience in susceptible animals (**Supplementary Fig. 6a**). Interestingly, acute idazoxan treatment did not change the basal firing rate of VTA DA neurons between 5 and 35 min after injection, but it increased the bursting activity 25 min after injection (**Supplementary Fig. 6b–d**). By contrast, after chronic daily treatment with idazoxan, we observed decreased bursting activity of VTA DA neurons compared with NaCl-treated mice, whereas we found no effect on the firing rate (**Fig. 3c**). Specifically, we observed a decreased proportion of highly bursting VTA DA neurons in mice treated chronically with idazoxan compared with NaCl-treated mice (**Fig. 3d**). This demonstrates that NE

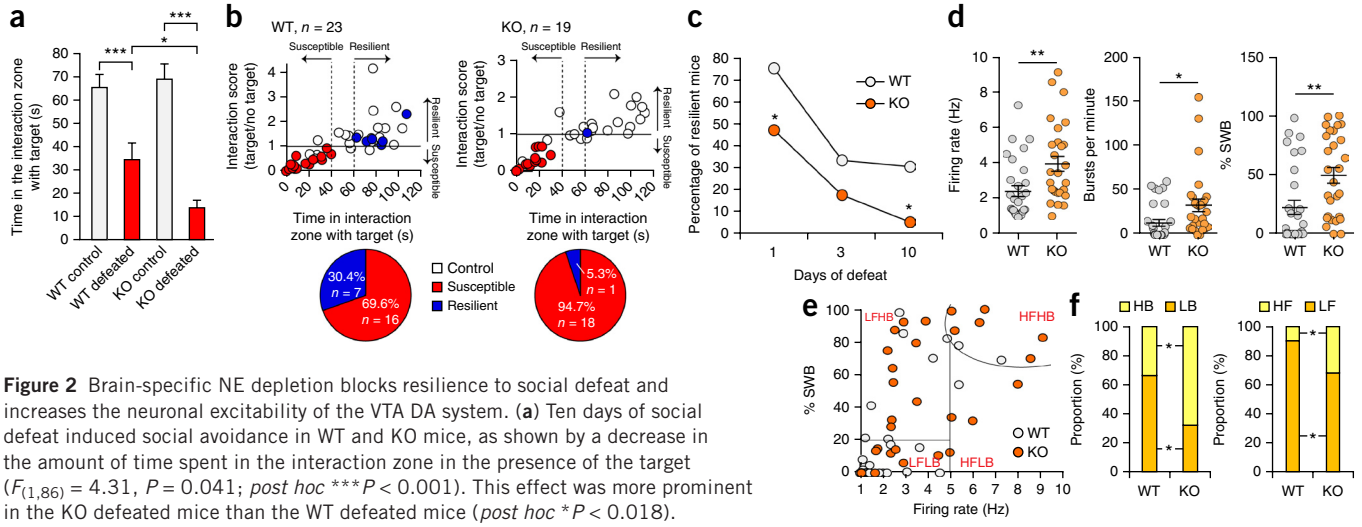

Figure 2 Brain-specific NE depletion blocks resilience to social defeat and increases the neuronal excitability of the VTA DA system. (**a**) Ten days of social defeat induced social avoidance in WT and KO mice, as shown by a decrease in the amount of time spent in the interaction zone in the presence of the target ($F_{(1,86)} = 4.31$, $P = 0.041$; *post hoc* $^{***}P < 0.001$). This effect was more prominent in the KO defeated mice than the WT defeated mice (*post hoc* $^{*}P < 0.018$). (**b**) The correlation between the interaction score (target versus no target) and the time spent in the interaction zone with the target (top) showed that 30.4% of the WT defeated mice had a resilient phenotype, as compared with 5.3% in the KO defeated mice (bottom). (**c**) The decrease in proportion (%) of resilient KO mice was observed not only after 10 d of social defeat ($^{*}P = 0.0069$) but also after only 1 d of social defeat ($^{*}P = 0.05$). (**d**) The firing rate ($^{**}P = 0.0038$; left), the number of bursts per minute ($^{**}P = 0.014$; middle) and the percentage of spikes within bursts (% SWB; $^{**}P = 0.0033$; right) were significantly increased in KO mice ($n = 28$) compared with their WT littermates ($n = 30$). (**e**) The repartition of the different categories of DA neurons (LFLB: low frequency, low burst; LFHB: low frequency, high burst; HFLB: high frequency, low burst; HFHB: high frequency, high burst) was modified in KO mice compared with WT. (**f**) KO mice showed a significantly increased proportion of higher bursting neurons ($^{*}P = 0.0028$; left; LB, low burst; HB, high burst) and a decreased proportion of lower frequency neurons ($^{*}P = 0.021$; right; LF, low frequency; HF, high frequency) compared with WT. Error bars, s.e.m.

neurotransmission is not only necessary but sufficient to promote resilience to emotional stress and that the NE regulation of VTA DA neuron bursting activity may be the critical factor for its effect on resilience.

To demonstrate the circuit specificity of NE neurotransmission in the VTA in promoting resilience to chronic social defeat, we used optogenetics to stimulate LC NE fibers in the VTA of susceptible animals. We injected a Cre-dependent adeno-associated virus expressing channelrhodopsin-eYFP (AAV1-ChR2-eYFP) into the LC of DBHcre-tdTomato mice (**Fig. 3e**). We validated the specificity and efficiency of virus expression in the soma and in the VTA fibers of LC NE neurons (**Supplementary Fig. 7a**,**b**) and validated its function *in vitro* and *in vivo* to ensure spatial and temporal activation of the stimulated LC NE neurons (**Supplementary Fig. 7c**–**h**).

Chronic optogenetic stimulation of LC NE fibers projecting to the VTA (20 min daily during 1 week) reversed the susceptible phenotype of defeated mice into a resilient phenotype (**Fig. 3f** and **Supplementary Fig. 8a,b**). This effect was not observed after acute stimulation (5 min during the social interaction test). In control animals, optogenetic stimulation of the LC NE projections to the VTA had no effect on social interaction behavior (**Supplementary Fig. 8c**). This demonstrated the ability of LC NE neuron stimulation to promote resilience to emotional stress, through NE neurotransmission specifically to the VTA.

To conclude, we have demonstrated that NE neurotransmission from the LC to the VTA is both necessary and sufficient to promote resilience to social defeat. Moreover, our data suggested that modifying NE tone selectively affects VTA DA neurons projecting to the NAc. NE-depleted *VMAT2*^{DBHcre} KO mice showed a lack of resilience to social defeat, mirroring the induction of susceptibility obtained by optogenetic stimulation of the VTA–NAc but not the VTA–PFC pathway[4]. In contrast, chronic treatment with idazoxan led to decreased neuronal excitability of the VTA DA system and counteracted susceptibility to social defeat to induce resilience, as observed after optogenetic inhibition of the VTA–NAc dopaminergic pathway[4]. While the activity of VTA DA neurons has been previously validated as a biological marker of stress susceptibility in anesthetized animals[1,3,4], confirming these results in awake animals would be of great interest. It will also be of interest to investigate whether the role of NE in stress vulnerability can be extended to other experimental models and stress modalities. For instance, phasic photoactivation of VTA DA neurons was shown to rescue depression-like behavior induced by unpredictable chronic mild stress, rather than promoting susceptibility to social defeat[16]. The precise postsynaptic mechanisms by which NE may control VTA neurons excitability—namely, α1NR located on DA neurons[17] or on GABA afferents[18] or excitatory hyperpolarization-activated current and inhibitory K^+ currents[19]—remain to be elucidated. Understanding such mechanisms would help to puzzle out the differential acute versus chronic effect of NE on behavior. Chronic, but not acute, increase of NE release may, for instance, induce postsynaptic NE receptor desensitization[20], which would decrease the neuronal excitability of the VTA DA system, in contrast to the increased bursting activity observed after acute idazoxan treatment. In conclusion, we have characterized a new neural circuit underlying resilience to chronic emotional stress, provided a rationale for the use of NE releaser and suggested future directions for developing depression treatments targeting NE transmission.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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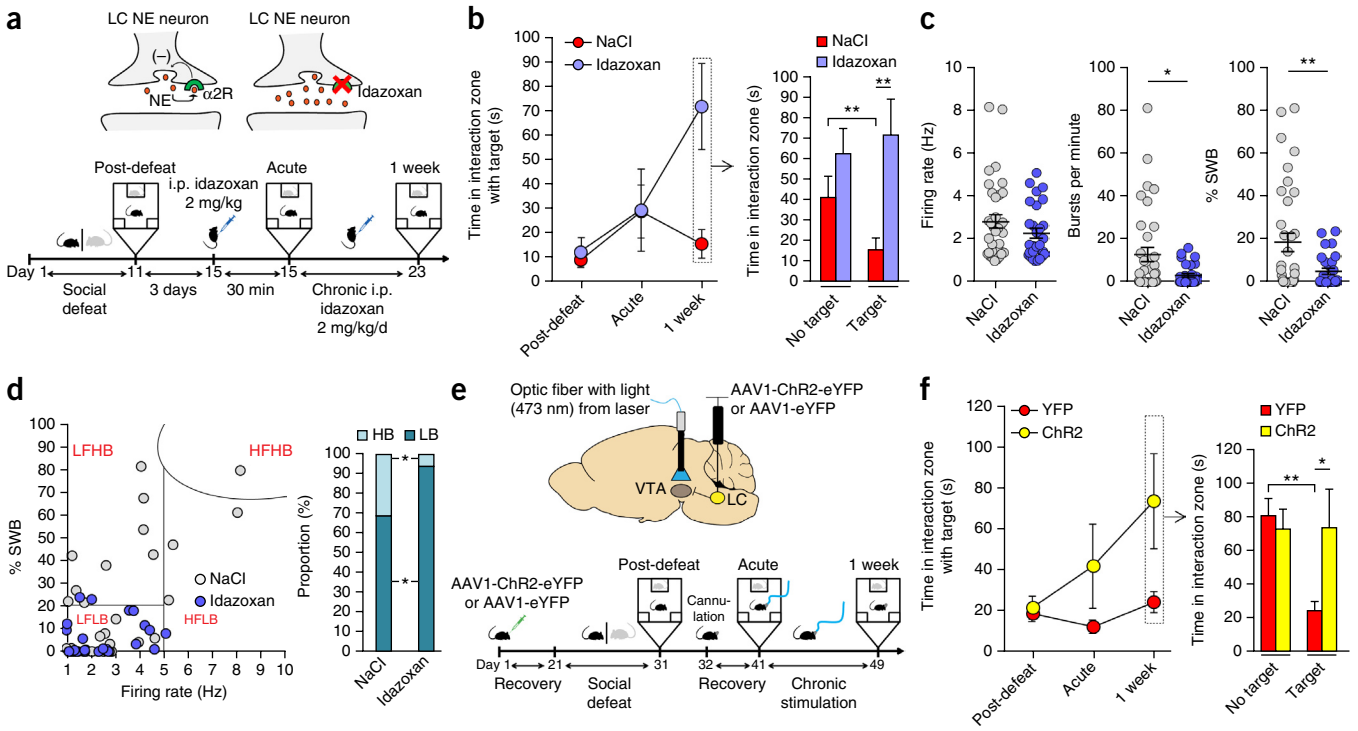

Figure 3 Chronic treatment with idazoxan or optogenetic stimulation of noradrenergic fibers in the VTA counteracts susceptibility to social defeat. (**a**) Idazoxan action mechanisms and experimental timeline. α2R, α2 adrenoceptor. (**b**) After 10 d of social defeat, 1 week of idazoxan treatment (2 mg/kg, intraperitoneally) in susceptible mice counteracted this phenotype to induce a resilient-like phenotype whereas acute treatment 30 min before the social interaction test had no effect ($F_{(2,32)} = 7.1$, $P = 0.003$, left). After 1 week of treatment ($F_{(1,16)} = 7.87$, $P = 0.013$, right), the time spent in the interaction zone with a target by the idazoxan-treated susceptible mice ($n = 8$) was significantly increased compared with that in NaCl-treated susceptible mice ($n = 10$; *post hoc* $^{**}P = 0.0014$), while the interaction time with no target was similar between groups (*post hoc* $P = 0.58$, ns). Moreover, the time spent in the interaction zone with a target was significantly lower compared to the time spent without a target in the NaCl-treated mice (*post hoc* $^{**}P = 0.0015$) whereas there was no differences for the idazoxan-treated mice (*post hoc* $P = 0.75$, ns). (**c**) The number of bursts per minute ($t = 2.54$, $^{*}P = 0.014$; middle) and the percentage of spikes within a burst (% SWB; $t = 2.78$, $^{**}P = 0.0072$; right) were significantly decreased in idazoxan-treated mice ($n = 29$) as compared with NaCl-treated mice ($n = 35$) whereas the firing rate (Hz; $t = 1.35$, $P = 0.18$; left) was unchanged. (**d**) The repartition of the different categories of DA neurons (LFLB: low frequency, low burst; LFHB: low frequency, high burst; HFLB: high frequency, low burst; HFHB: high frequency, high burst) was modified in idazoxan-treated mice as compared with NaCl-treated mice (left). Idazoxan-treated mice showed a significantly decreased proportion of higher bursting neurons ($^{*}P = 0.002$; left; LB, low burst; HB, high burst) as compared with NaCl-treated mice (right). (**e**) Optogenetic stimulation protocol and experimental timeline. (**f**) After 10 d of social defeat, 1 week of optogenetic stimulation of LC NE fibers in the VTA of susceptible mice counteracted this phenotype to induce a resilient-like phenotype whereas acute stimulation during the social interaction test had no effect ($F_{(2,20)} = 3.14$, $P = 0.065$; left). After 1 week of stimulation ($F_{(1,10)} = 9.25$, $P = 0.012$; right), the time spent in the interaction zone with the target of the ChR2-stimulated susceptible mice ($n = 6$) was significantly increased compared with YFP-stimulated susceptible mice ($n = 6$) (*post hoc* $^{*}P = 0.027$) whereas the interaction zone time with no target was similar between groups (*post hoc* $P = 0.70$, ns). Moreover, the time spent in the interaction zone with a target was significantly lower compared to the time spent without a target in the YFP-stimulated mice (*post hoc* $^{**}P = 0.0017$) whereas there was no difference for the ChR2-stimulated mice (*post hoc* $P = 0.96$, ns). Error bars, s.e.m.

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AUTHOR CONTRIBUTIONS

The studies were conceived and designed by B.G. and E.I. Experiments were performed by E.I. and L.P. with contributions from Q.R. for *in vivo* optogenetic experiments; Q.R. and F.M. for *in vivo* electrophysiological recording; E.G., G.M. and J.R. for surgeries; A.G. and L.M. for neurochemical analysis; A.T. and N.M. for the histology; and B.A. and S.W. for patch-clamp recording. The paper was written by B.G., E.I. and L.P., and was edited by the other authors.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Animals. Animal housing, breeding and care were performed in accordance with the Canadian Council on Animal Care guidelines (http://ccac.ca/en_/standards/guidelines/) and the Animal Care Committee from the Douglas Institute Research Center. The mice were kept under standard conditions at 22 ± 1 °C, a 60% relative humidity, and a 12-h light-dark cycle with food and water available *ad libitum*.

The floxed *VMAT2* mouse strain was obtained from the Mouse Clinical Institute (Institut Clinique de la Souris, MCI/ICS, Illkirch, France). The DBHcre mice (B6.FVB(Cg)-*Tg(Dbh-cre)KH212Gsat*/Mmucd; stock number 031028-UCD) were supplied by the Mutant Mouse Regional Resource Centers (MMRRC). Reporter tdTomato mice (B6;129S6-*Gt(ROSA)26Sor*$^{tm9(CAG\text{-}tdTomato)Hze}$/J) were supplied by the Jackson Laboratory (Maine, USA).

To assess specific expression of the Cre recombinase under the DBH promoter (**Supplementary Fig. 3a**), DBHcre mice were crossed with the tdTomato floxed reporter mice. The brain-specific NE-depleted mice (*VMAT2*^{DBHcre}) were obtained by crossing heterozygous *VMAT2* floxed mice with DBHcre mice to obtain heterozygous offspring, which when crossed produced wild-type (WT; 7/16), knockout (KO; 3/16) and heterozygote (6/16) mice at the expected ratio (**Supplementary Fig. 3d**). After weaning and sexing, mice were housed in groups of four or five animals per cage.

Male C57BL/6 mice (Charles River), *VMAT2*^{DBHcre} WT and KO mice and DBHcre-tdTomato mice 2–4 months old were used as experimental subjects in the social defeat study, during which they were housed individually. CD1 male retired breeders (Charles River), used as resident mice, were housed individually. All behavioral experiments were performed during the light cycle.

Drugs. Idazoxan hydrochloride (Sigma-Aldrich) diluted in NaCl 0.9% was administered daily intraperitoneally for a period of 1 week at a dose of 2 mg/kg. Reboxetine hydrochloride (Tocris) diluted in NaCl 0.9% was administered daily intraperitoneally for a period of 1 week at a dose of 20 mg/kg. Idazoxan and reboxetine were first administered 30 min before the social interaction test and the last injection was performed 24 h before the social interaction test.

Chloral hydrate 8% (400 mg/kg, Sigma-Aldrich) diluted in NaCl 0.9% was administered intraperitoneally to anesthetize the mice before conducting surgery for *in vivo* electrophysiological recordings. Quinpirole hydrochloride (Sigma-Aldrich) and eticlopride hydrochloride (Sigma-Aldrich) were administered intraperitoneally at 1 mg/kg prepared in NaCl 0.9%.

Chronic social defeat stress. The social defeat protocol was conducted according to the model described by Golden *et al.* (2011)[7]. CD1 aggressor mice were screened over 3 consecutive days and selected according to the following criteria: (i) a latency of attack under 1 min, (ii) consistent bouts over 3 min and (iii) attacking for 2 consecutive days. The defeated mice were subjected to a daily 5-min physical interaction with an aggressive CD1 mouse for 10 consecutive days (**Fig. 1a**). Following the daily defeat, the animal remained in the aggressor's home cage on the other side of a perforated translucent Plexiglas divider to allow visual, auditory and olfactory interaction with the aggressor for 24 h until the next defeat. The control mice (or undefeated mice) were housed in pairs separated by a Plexiglas divider and were never exposed to the CD1 aggressors. Each day, the defeated and the control mice were placed with a different cagemate. To homogenize groups, mice were assigned to control and defeated groups according to their age, weight and cagemate.

Immediately after the last defeat, the experimental mice were singly housed and were tested 24 h later in the social interaction test (**Fig. 1a**). This test was composed of two phases of 2.5 min each in which the mouse was placed in an open field (45 cm × 45 cm × 45 cm) with a Plexiglas wire mesh enclosure (10 cm wide × 6.5 cm deep × 42 cm high). The time spent in the interaction zone surrounding the wire mesh enclosure was measured over 2.5 min during a first phase in which the wire mesh was empty (no target) and a second phase in which an unfamiliar CD1 mouse was placed inside the wire mesh (target). Between the two phases, the experimental mouse was returned to his home cage for 30 s.

Criteria for separating susceptible from resilient mice were defined according to their respective social interaction behavior as previously published[3,5,6,15,21]. Resilience was defined by a ratio greater than 1 of the time spent in the interaction zone during phase 2 (target) over phase 1 (no target). In addition, resilient mice needed to spend more than 60 s interacting with the CD1 mouse. Susceptibility, conversely, was defined as a ratio under 1 and an interaction time less than 40 s with the CD1 during phase 2. In agreement with these criteria, the *K*-means cluster analysis of our own data provided an interaction time with CD1 of 27.99 ± 15.7 s (n = 29) and 89.35 ± 19.3 s (n = 17) and a ratio of 0.63 ± 0.38 and 1.87 ± 0.69 for vulnerable and resilient mice, respectively.

Locomotor activity. Spontaneous locomotion was measured in an Omnitech Digiscan activity monitor. Plexiglas open-field chambers (40 cm × 40 cm) with photocells placed on bottom and lateral surfaces allowed measurement of horizontal activity at 5-min intervals for 3 h.

Rotarod. Motor coordination was assessed by an accelerating rotarod (Roto-Rod, Series 8, IITC Life Sciences). On the first day, mice were habituated during three trials (inter-trial interval 30 min) at rotating speed ranges of 6–12, 6–24 and 6–48 accelerating rpm for a maximum period of 2 min. The next day, the latency to fall was recorded over three trials at a rotating speed range from 6 to 48 accelerating rpm for a maximum of 5 min.

Stereotaxic surgery. *Retrobead injections.* Mice were anesthetized with isoflurane gas combined with oxygen and placed on the stereotaxic apparatus. Once the skull was exposed and holes drilled bilaterally, 200 nl of red retrobeads (Lumafluor, Naples, USA) were injected at a 0° angle into the VTA (AP −3.15 mm; LM ±0.5 mm; DV −4.5 mm). Mice were allowed to recover for 1 week before being exposed to the chronic social defeat stress procedure.

Virus injections. Recombinant adeno-associated virus AAV1 carrying EF1a.DIO.hChR2(H134R)-eYFP.WPRE.hGH (Penn Vector Core, AV-1-20298P) or control EF1a.DIO.eYFP.WPRE.hGH (Penn Vector Core, AV-1-27056) was stereotaxically infused bilaterally adjacent to the LC (AP −5.45 mm; LM +/− 1.28 mm; DV −3.65 mm) of DBHcre-tdTomato mice anesthetized with isoflurane gas combined with oxygen. DBHcre-tdTomato mice were randomly assigned to viral injection. Virus injections (0.5 µl per side at a rate of 0.05 µl/min) were done through an internal cannula connected via tubing to a 10-µl Hamilton microsyringe mounted on a micro-drive pump (WPI, Sarasota, FL, USA). Mice were allowed to recover for 1 week before starting the chronic social defeat stress procedure. Animals with no bilateral viral expression were discarded from the study.

Implantation of optical fibers. The AAV1-injected DBHcre-tdTomato mice were implanted with custom-made optical fibers (200 µm core) 24 h after the social interaction test performed at the end of the 10-d social defeat protocol. Mice were anesthetized with isoflurane and placed on a stereotaxic frame. A midline incision was made, holes were drilled in the skull, and optical fibers (5 mm in length) were implanted bilaterally at a 10° angle into the VTA (AP −3.2 mm; LM ± 1.15 mm; DV −3.4 mm) and fixed using dental cement. Mice were allowed to recover for 3 d before starting optical stimulation and behavioral testing. Animals with optical fiber implants outside the target area were discarded from the study.

Tissue processing and immunohistochemistry. *Histology in retrobead-injected mice.* Two hours after the social interaction test, mice were perfused with 0.9% NaCl followed by 4% paraformaldehyde (PFA). Brains were collected and post-fixed in 4% PFA for 2 h, and kept in a 15% sucrose solution at 4 °C before being sliced into coronal sections (40 µm thick) using a cryostat (Leica CM3050S).

Free-floating sections of C57BL/6 mice brains were washed in PBS 0.1 M, then treated in 3% H_2O_2 for 20 min. After washes, slices were incubated overnight in a goat anti–c-fos primary antibody (1:500; Santa Cruz, sc-52) diluted in PBS 0.1 M, 2% normal horse serum (NHS) and 0.3% Triton. After washes, slices were incubated for 2 h in a biotinylated secondary horse anti-goat antibody (1:500; Vector Laboratories, BA-9500) diluted in PBS 0.1 M with 2% NHS and 0.3% Triton. The staining was amplified with an avidin-biotin complex (Elite ABC kit, Vector Laboratories) and visualized with DAB (Sigma-Aldrich, St. Louis, MO, USA). After washes, sections were mounted on gelatin-coated slides, dried, dehydrated and coverslipped.

Free-floating slices of C57BL/6 mice brains injected with red retrobeads into the VTA were rinsed in PBS 0.1 M and incubated overnight with rabbit anti-TH (1:4,000; Santa Cruz, sc-14007) and goat anti–c-fos (1:500; Santa Cruz, sc-52) primary antibodies diluted in PBS 0.1 M, 2% normal donkey serum (NDS) and 0.3% Triton. After washes, slices were incubated for 2 h in secondary antibodies, a donkey anti-rabbit Alexa 647 (1:500; Life Technologies, A-31573) and a

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donkey anti-goat Alexa 488 (1:500; Life Technologies, A-11055) diluted in PBS 0.1 M with 2% NDS and 0.3% Triton. Sections were then rinsed and mounted on gelatin-coated slides under Vectashield mounting medium.

The number of c-fos, TH and/or bead-positive cells in the LC was counted bilaterally in one section out of every three, from bregma 4.96 to 5.52 mm. For each animal, the number of c-fos–positive cells was normalized by the surface area (μm^2) of the region on each section. Cells were counted using a ×20 objective lens with a conventional light microscope. All counting analyses were performed blinded to the experimental group. All antibodies were used as instructed by the manufacturer.

Histology after optical stimulation. After completion of experiments, AAV1 virus–injected DBHcre-tdTomato mice were perfused transcardially with 0.9% NaCl followed by 4% paraformaldehyde (PFA). Brains were extracted and post-fixed in 4% PFA for 2 h, and kept in a 15% sucrose solution at 4 °C before being sliced into coronal sections (40 µm thick) using a cryostat (Leica CM3050S).

For soma colocalization in the LC, free-floating sections from DBHcre-tdTomato ChR2-eYFP transduced mice were washed in PBS 0.1 M and incubated overnight with a rabbit anti-GFP primary antibody (1:5,000, Abcam, ab290) diluted in PBS 0.1 M, 2% NDS and 0.3% Triton. After washes, slices were incubated for 2 h in secondary antibody, a donkey anti-rabbit Alexa 488 (1:500; Life Technologies, A-21206) diluted in PBS 0.1 M with 2% NDS and 0.3% Triton. Sections were then rinsed and mounted on gelatin-coated slides under Vectashield mounting medium.

For fiber colocalization in the VTA, free-floating sections from DBHcre-tdTomato ChR2-eYFP transduced mice were washed in PBS 0.1 M and incubated overnight with rabbit anti-GFP (1:5,000, Abcam, ab290) and mouse anti-TH (1:4,000, Millipore, MAB318) primary antibodies diluted in PBS 0.1 M, 2% NDS and 0.3% Triton. After washes, slices were incubated for 2 h in secondary antibodies, a donkey anti-rabbit Alexa 488 (1:500; Life Technologies, A-21206) and a donkey anti-mouse Alexa 546 diluted in PBS 0.1 M with 2% NDS and 0.3% Triton. Sections were then rinsed and mounted on gelatin-coated slides under Vectashield mounting medium.

For c-fos immunostaining in the LC of DBHcre-tdTomato mice, ChR2-eYFP transduced mice implanted with optical fibers in the LC were perfused with 0.9% NaCl followed by 4% paraformaldehyde (PFA). Brains were collected and post-fixed in 4% PFA for 2 h, and kept in a 15% sucrose solution at 4 °C before being sliced into coronal sections (40 µm thick) using a cryostat (Leica CM3050S). Free-floating slices from the LC were rinsed in PBS 0.1 M and incubated overnight with a goat anti–c-fos primary antibody (1:500; Santa Cruz, sc-52) diluted in PBS 0.1 M, 2% normal donkey serum (NDS) and 0.3% Triton. After washes, slices were incubated for 2 h in secondary antibody, a donkey anti-goat Alexa 647 (1/500; Life Technologies, A-21447) diluted in PBS 0.1 M with 2% NDS and 0.3% Triton. Sections were then rinsed and mounted on gelatin-coated slides under Vectashield mounting medium.

Autoradiographic *VMAT2 in situ* hybridization. Brains of 21-day-old mice were collected after decapitation and frozen in isopentane at −30 °C. Brains were sliced into coronal sections (10 µm thick) using a cryostat (Leica CM3050S) and rinsed in 0.1 M PBS, SSC 10× and treated with 0.25% ethanol. [^{35}S]dATP oligonucleotides (5′-GAG GAA CAC GAT GAA CAG GAT CAG CTT GCG CGA GT-3′; 5′-CTA CGA CGG TGA GCA GCA TGT TGT CTA GCA GCA G-3′) were synthesized with terminal transferase (Amersham Biosciences) to obtain a specific activity of 5×10^{-8} dpm/µg. Sections were covered with 70 µl of hybridization mix and 5×10^{-5} dpm of each labeled oligonucleotide, and incubated overnight at 42 °C in a humid chamber. Following washes and dehydration, slides were air-dried and exposed to a BAS-SR Fujifilm Imaging Plate for 5 d. The plates were scanned with a Fujifilm BioImaging Analyzer BAS-5000. Identification of regions was based on the Franklin and Paxinos Mouse Atlas[22].

High-performance liquid chromatography. HPLC was performed (i) on micropunches of 0.5 mm diameter for the ventral tegmental area (VTA), the nucleus accumbens (NAc) and the prefrontal cortex (PFC) of C57BL/6 mice 24 h after the social interaction test, and (ii) on the whole brains of naive 4-month-old *VMAT2*^{DBHcre} WT and KO mice.

After decapitation, brains were collected, frozen in isopentane at −30 °C and stored at −80 °C. Whole brains or micropunches of specific structures were homogenized in a solution containing 225 µl (whole brain) or 45 µl (punches) of 0.25 M perchlorate and 75 µl or 15 µl, respectively, of DHBA (100 mg/ml), which served as an internal standard. Following centrifugation at 9,300*g* for 15 min at 4 °C, the supernatant was isolated to detect DA, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), NE, serotonin (5-HT) and 5-hydroxyindolacetic acid (HIAA) using high pressure liquid chromatography with electrochemical detection (HPLC-EC). Samples were run through a Luna C18 (2) 75 × 4.6 mm 3 µm analytical column at a flow rate of 1.5 ml/min and the electrochemical detector (ESA Coularray, model #5600A) was set at a potential of −250 mV and +300 mV. The mobile phase consisted of 0.6% methanol, 0.341 mM 1-octanesulfoic acid sodium salt, 168.2 mM sodium acetate, 66.6 mM citric acid monohydrate, 0.025 mM disodium ethylenediamine tetraacetate (EDTA) and 0.71 mM triethylamine adjusted to pH 4.0–4.1 with acetic acid. Using ESA's CoulArray software, the position of the peaks for each metabolite was compared to an external standard solution containing 25 ng/ml DHBA, DA, NE, 5-HT, DOPAC, HVA and 50 mM acetic acid. In parallel, pellets were reconstituted in 50 µl of 0.1 N NaOH and kept for protein quantification using a BCA Protein Assay Kit (Fisher Scientific, Ontario, Canada). Each analyzed sample was measured in µg per gram of protein.

***In vivo* electrophysiological recordings.** Mice were anesthetized with chloral hydrate (400 mg/kg, i.p.) and additional injections were administered to maintain the anesthesia throughout the experiment. Although caution must be taken when interpreting data from anesthetized animals, we used chloral hydrate because it is known to preserve the spontaneous pattern of discharge of DA neurons observed in awake animals[23]. The mice skull was opened, a hole was drilled above the VTA and the dura mater was retracted. Recording electrodes were pulled with a Narishige electrode puller from borosilicate glass capillaries with outer and inner diameters of 1.50 and 1.17 mm, respectively (Harvard Apparatus), and filled with a 0.5 M sodium acetate solution. The electrode was placed above the VTA from a range of 0.48 to 0.55 mm lateral to the midline and from 3.08 to 3.3 mm caudal to the bregma, and lowered down into the VTA from 3.9 mm to 5 mm ventral to the cortical surface using a microdrive (model 650; David Kopf Instruments, Tujunga, CA). A reference electrode was placed adjacent to the subcutaneous tissue. After being amplified and filtered at 30 kHz with a low-pass Bessel filter by a high-impedance amplifier (Axoclamp 900A System, Molecular Devices, Sunnyvale, CA) and digitalized at 20 KHz by a CED1401 interface system (Cambridge Electronics Design), the electrical signals were monitored through an oscilloscope and an audio monitor. Action potential waveform durations were measured during the acquisition without any additional filter or signal treatment. Data acquisition and analysis was performed with SPIKE 2 software. The DC offset is numerically removed from the signals in SPIKE 2 after acquisition. When a single unit was recorded, the series of action potentials was observed on the oscilloscope.

The recorded neurons were defined as DA neurons according to the following set of features (**Supplementary Fig. 1b–d**): (i) a typical triphasic action potential with a marked negative deflection, (ii) >2.0 ms triphasic action potential with a width >1.1 ms, (ii) a slow firing rate of 1–10 Hz. Two criteria were used to confirm burst firing: (i) onset was defined by two consecutive spikes within an interval <80 ms and (ii) termination was defined by an inter-spike interval >160 ms. These electrophysiological properties distinguish DA from non-DA neurons[24,25]. Pharmacological characterization was performed on the last recorded neurons of the experiment (**Supplementary Fig. 1e**) by acute injection of a DA D2 receptor agonist (quinpirole, 1 mg/kg, i.p.) followed by an acute injection of the DA D2 receptor antagonist (eticlopride, 1 mg/kg, i.p.). DA cell's firing was recorded for 10 min and analyzed with the following parameters: (i) the average firing rate (Hz), (ii) the number of bursts per minute and (iii) the percentage of spike within a burst (% SWB).

The effect of idazoxan injected i.p. at 2 mg/kg on the VTA DA neuron activity was analyzed by (i) recording a VTA DA neuron for 10 min to establish a baseline and (ii) recording the VTA DA neuron for 35 min following an i.p. injection. The injection and recording of one VTA DA neuronal cell firing was performed on individual mice.

Patch-clamp recordings. Coronal slices (250 µm thickness) were prepared from DBHcre-tdTomato mice injected with AAV1-ChR2-eYFP virus in the LC (2 to 4 months old, 3 to 5 weeks after viral injection). An *N*-methyl-D-glucamine (NMDG) protective recovery solution, adapted to patch-clamp recordings in

older mice, was used for slicing and prepared according to the protocols described at http://brainslicemethods.com/. Briefly, mice were deeply anesthetized using a ketamine/xylazine/acepromazine mix and intracardially perfused with 4 °C slicing solution containing (in mM) 93 NMDG, 93 HCl, 30 $NaHCO_3$, 25 glucose, 20 HEPES, 10 $MgSO_4$, 5 sodium ascorbate, 2.5 KCl, 3 sodium pyruvate, 2 thiourea, 1.2 NaH_2PO_4 and 0.5 $CaCl_2$ (pH 7.3–7.4, continuously oxygenated with 95% O_2/5% CO_2). Sections were cut on a vibratome (Leica), transferred to 32 °C slicing solution for 10 min and then transferred to room temperature ACSF for 1 h before recording (ACSF (in mM): 124 NaCl, 24 $NaHCO_3$, 12.5 glucose, 5 HEPES, 2.5 KCl, 2 $MgSO_4$, 2 $CaCl_2$ and 1.2 NaH_2PO_4, pH 7.3–7.4, continuously oxygenated with 95% O_2/5% CO_2). Recordings were performed in ACSF perfused at 5 mL/min at 25 °C. Patch glass micropipettes (Warner Instruments) had a resistance 2.5–5 MΩ and the intra-pipette solution contained (in mM) 144 potassium gluconate, 10 HEPES, 3 $MgCl_2$, 2 Na_2-ATP, 0.3 GTP and 0.2 EGTA, adjusted to pH 7.2 with KOH. Junction potential was not corrected for (−9 mV), and all cells analyzed had an access resistance below 30 MΩ. Recordings were performed with an Axon Multiclamp 700B amplifier (Molecular Devices), filtered online (0.5–10,000 Hz) and sampled at 20 kHz.

Whole-cell patch-clamp recordings were performed on eYFP- and tdTomato-positive cells to quantify direct responses to blue light as previously described[26]. Briefly, ChR2-eYFP-positive neurons were first characterized in current-clamp mode by applying hyperpolarizing and depolarizing current steps (10-pA increments) from −60 mV. Spontaneous baseline activity was recorded for 1–5 min. Neurons were then maintained at −55 mV and pulses of blue light (5 ms) were delivered at frequencies of 1–50 Hz to quantify action potential fidelity. For each frequency, three sweeps of 20 light pulses were recorded and the percentage of AP fired was averaged from the three recordings. Neurons were also recorded in voltage-clamp mode (holding = −70 mV) to measure photocurrent size in response to a 500-ms pulse of blue light. Light was delivered through a custom-made LED system consisting of a blue LED (473 nm, Luxeon) coupled to a 1-mm-diameter polymer light-guide (Edmund Optics) with light intensity set at 35 mW.

***In vivo* optical stimulation.** Optical stimulations were generated by a waveform generator (Master 9, AMPI) triggering two blue-diode lasers (473 nm, LaserGlow). Each laser was connected to the optical fibers via a patch cord composed of a 200-µm optical fiber and a black furcation tubing. AAV1 virus–injected DBHcre-tdTomato mice were given phasic (10 Hz, 10 ms) blue light pulses over 500 ms every 20 s. For acute stimulation, mice received 5 min stimulation during the two phases of the social interaction test. For chronic stimulation, mice received 20 min daily stimulation in their home cage for 1 week.

Statistical analysis. No statistical methods were used to predetermine sample sizes, but our sample sizes were similar to those generally employed in literature for the same protocols[3,4,27]. Statistical analyses were performed using Statistica software. The Shapiro-Wilk test for normality of sample distribution and Levene's test for homogeneity of variances were used. The results are expressed as mean ± s.e.m. According to the experimental design, multiple groups comparison were performed using a one-way factorial ANOVA, a one-way repeated-measures ANOVA or a two-way factorial ANOVA. The between-subjects factors were group (control versus susceptible versus resilient), genotype (WT versus KO), stress (control versus defeated), treatment (NaCl versus idazoxan) and virus (YFP versus ChR2). The within-subject factors were target (no target versus target) and time (post-defeat versus acute versus 1 week). These multiple group comparisons were followed by a Fisher LSD *post hoc* test for two per two comparisons when appropriate. For the comparison of two independent groups ($VMAT2^{DBHcre}$ WT versus KO; idazoxan-versus NaCl-treated mice), we used a two-sided *t*-test by groups. For the comparison of proportion (LF versus HF; LB versus HB), we used a chi-squared test. For the correlative analysis, a Pearson correlation was performed. Correlation data are presented as scatter plots. $P < 0.05$ was selected to reflect statistical significant differences between groups. No randomization method was used to assign subjects in the experimental groups or to collect and process data.

A **Supplementary Methods Checklist** is available.

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