

Adult hippocampal neurogenesis buffers stress responses and depressive behaviour

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Glucocorticoids are released in response to stressful experiences and serve many beneficial homeostatic functions. However, dysregulation of glucocorticoids is associated with cognitive impairments and depressive illness^{1,2}. In the hippocampus, a brain region densely populated with receptors for stress hormones, stress and glucocorticoids strongly inhibit adult neurogenesis³. Decreased neurogenesis has been implicated in the pathogenesis of anxiety and depression, but direct evidence for this role is lacking^{4,5}. Here we show that adult-born hippocampal neurons are required for normal expression of the endocrine and behavioural components of the stress response. Using either transgenic or radiation methods to inhibit adult neurogenesis specifically, we find that glucocorticoid levels are slower to recover after moderate stress and are less suppressed by dexamethasone in neurogenesis-deficient mice than intact mice, consistent with a role for the hippocampus in regulation of the hypothalamic–pituitary–adrenal (HPA) axis^{6,7}. Relative to controls, neurogenesis-deficient mice also showed increased food avoidance in a novel environment after acute stress, increased behavioural despair in the forced swim test, and decreased sucrose preference, a measure of anhedonia. These findings identify a small subset of neurons within the dentate gyrus that are critical for hippocampal negative control of the HPA axis and support a direct role for adult neurogenesis in depressive illness.

Functional granule neurons are generated in the hippocampus throughout life by a multistep process that begins with glial fibrillary acidic protein (GFAP)-expressing radial cell precursors^{8,9}. To investigate the role of adult neurogenesis in hippocampal function, we created mice that express herpes simplex virus thymidine kinase (TK) under control of the GFAP promoter (TK mice; Fig. 1a). Thymidine kinase renders mitotic cells sensitive to the antiviral drug valganciclovir but spares post-mitotic cells¹⁰. Stellate astrocytes and radial neuronal precursor cells both express GFAP, and both cell types express thymidine kinase in the transgenic mice. However, the number of astrocytes is unaltered by treatment with valganciclovir in TK mice (Fig. 1b, c), consistent with a lack of cell proliferation in astrocytes in the adult brain¹¹. In contrast, immature neurons expressing doublecortin (DCX) were virtually eliminated in the dentate gyrus of valganciclovir-treated TK (v-TK) mice (Fig. 1d and Supplementary Fig. 1). One-day-old neuronal progenitors, identified with DCX and the cell-cycle marker bromodeoxyuridine (BrdU), were reduced in v-TK mice by 99% relative to control conditions (Fig. 1e). Transgene expression alone and valganciclovir treatment of wild-type (WT) mice had no effect (Fig. 1e). v-TK mice showed weight gain comparable to v-WT mice (Supplementary Fig. 2), and histopathological examination found no abnormalities in the small intestine or submucosal or myenteric plexuses (not shown), indicating that the gastrointestinal effects described in another strain expressing thymidine kinase under the

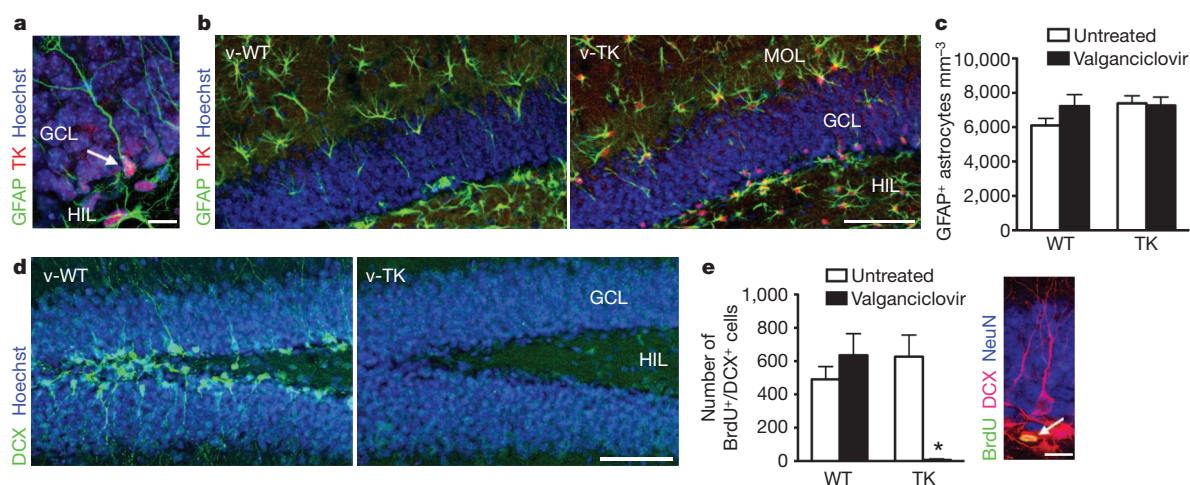


Figure 1 | GFAP-TK mice show specific inhibition of adult neurogenesis. **a**, Confocal image of endogenous GFAP and transgenic thymidine kinase expression in a radial precursor cell in the dentate gyrus (arrow). **b**, Confocal photographs of valganciclovir-treated mice show GFAP⁺ astrocytes in the hilus and molecular layer in both genotypes, despite strong thymidine kinase expression in all GFAP-expressing cells in TK mice. **c**, Valganciclovir treatment did not affect numbers of GFAP⁺ astrocytes (genotype effect $F_{1,20} = 1.7$, $P = 0.2$; valganciclovir effect $F_{1,20} = 1.0$, $P = 0.3$; interaction $F_{1,20} = 1.5$, $P = 0.2$; $n = 6$ per group), confirming the expectation that valganciclovir does not kill astrocytes, which are post-mitotic in the adult. **d**, Confocal photographs

of dentate gyrus doublecortin (DCX) immunostaining in mice treated with valganciclovir (v-WT and v-TK) for 4 weeks. DCX⁺ young neurons are abundant in v-WT mice but absent in v-TK mice. **e**, The number of BrdU⁺/DCX⁺ young neurons was unaltered in v-WT mice but reduced by 99% in v-TK mice (genotype effect $F_{1,20} = 20$, $P = 0.0002$; valganciclovir effect $F_{1,20} = 19$, $P = 0.0003$; interaction $F_{1,20} = 40$, $P < 0.0001$; * $P < 0.001$ versus untreated TK and v-WT; $n = 6$ per group). Inset shows example of 1-day-old BrdU⁺/DCX⁺ neuron (arrow). Error bars, s.e.m.; scale bars, 10 μm in **a**, **e**, 100 μm in **b**, **d**. MOL, molecular layer; GCL, granule cell layer; HIL, hilus.

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GFAP promoter⁸ are absent in this line of mice. Taken together, these data indicate that v-TK mice have a specific loss of adult neurogenesis without detectable effects on astrocytes or general health.

The hippocampus provides negative control of the HPA axis^{6,7}, but the circuitry involved is not well understood. Because adult hippocampal neurogenesis is highly sensitive to stress and glucocorticoids³, we hypothesized that adult neurogenesis may be important for hippocampal regulation of the HPA axis. We therefore examined serum levels of corticosterone, the predominant rodent glucocorticoid, in several conditions that activate the HPA axis. Neurogenesis-deficient v-TK and control v-WT mice had equivalent levels of corticosterone at the onset of both the light and the dark phase (Supplementary Fig. 3), indicating that adult-born neurons are not required for normal circadian fluctuation of glucocorticoids. v-WT and v-TK mice also had similar corticosterone levels after exposure to a novel environment (Supplementary Fig. 3), a mild stressor, consistent with previous findings¹².

To test the response to, and recovery from, a moderate psychological stressor, we subjected mice to 30 min of restraint and measured corticosterone 0, 30 or 60 min later (Fig. 2a). v-TK mice and v-WT mice had similar levels of corticosterone immediately after termination of the stressor. However, v-TK mice had elevated corticosterone relative to v-WT mice 30 min after stress, suggesting impaired negative feedback control of glucocorticoid release similar to that observed in mice with complete loss of glucocorticoid receptors in the forebrain¹³. To investigate whether the hypersecretion of glucocorticoids habituates, mice were subjected to daily restraint for 16 additional days, and corticosterone was measured on the final day. As on the first day of restraint, v-TK mice had elevated corticosterone relative to v-WT mice (Fig. 2b). Control TK mice, which were never treated with valganciclovir and therefore had normal levels of neurogenesis, had levels of corticosterone identical to untreated WT mice 30 min after restraint (Fig. 2c). Therefore, corticosterone

hypersecretion in neurogenesis-deficient mice is not caused by non-specific or insertion site effects of the thymidine kinase transgene.

The experiments above indicate that adult neurogenesis regulates the endocrine stress response, but they do not pinpoint the brain region involved. Hippocampal damage primarily alters the response to psychological stressors such as restraint, which produce fear without causing a direct threat to well-being, but does not typically affect responses to physical stressors, such as hypoxia, haemorrhage, inflammation or anaesthesia⁷. Consistent with HPA regulation at the level of the hippocampus, v-WT and v-TK mice showed comparable elevations in corticosterone after exposure to isoflurane anaesthesia (Supplementary Fig. 4). Additionally, in agreement with the lack of global HPA dysregulation, we found no evidence for reduced cell birth in the hypothalamic paraventricular nucleus of v-TK mice (Supplementary Fig. 5).

To test the contribution of adult neurogenesis in other regions directly, we exploited the spatial specificity of X-irradiation to reduce hippocampal neurogenesis while sparing neurogenesis in the subventricular zone^{12,14}, a source of GFAP⁺ neuronal precursors added to the olfactory bulb throughout adulthood^{8,15}. Irradiated mice had elevated corticosterone during recovery from restraint stress (Fig. 3), replicating the initial finding with an independent ablation method. In addition, there was no relationship between the extent of subventricular zone neurogenesis inhibition and the corticosterone response in irradiated mice (Fig. 3). Therefore, the most parsimonious interpretation of our results is that inhibition of adult neurogenesis in the dentate gyrus leads to hypersecretion of glucocorticoids in response to stress.

A functional link between new neurons and anxiety/depression has been suggested by the demonstration that some antidepressant effects on behaviour are blocked by irradiation^{12,16–18}. However, normal anxiety- and depressive-like behaviour in animals lacking neurogenesis has led

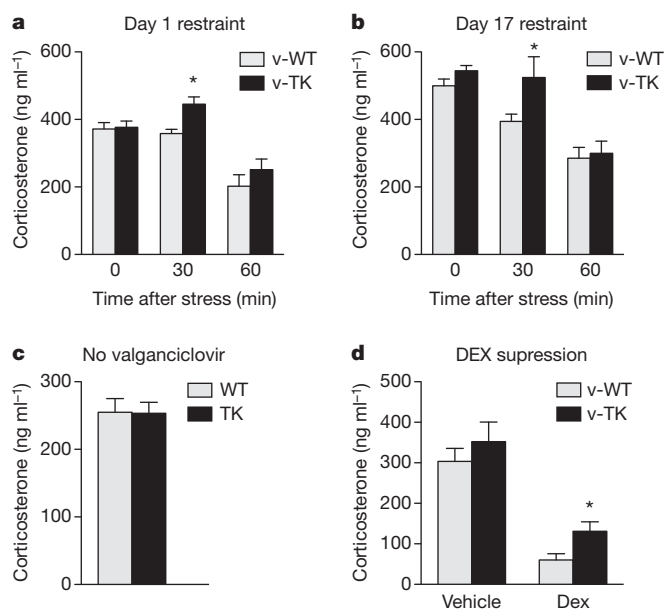


Figure 2 | The glucocorticoid response to stress is increased in neurogenesis-deficient mice. **a**, Restraint, a moderate psychogenic stressor, resulted in higher corticosterone in neurogenesis-deficient v-TK mice than in v-WT mice 30 min after the end of stress. **b**, The effect of restraint was still observed after repeated exposure to stress (for both days: genotype effect $F_{1,65} > 5$, $P < 0.05$; time effect $F_{2,65} > 24$, $P < 0.001$; $*P < 0.05$ post hoc versus v-WT; $n = 6–17$ per group per time point). **c**, In untreated control mice, corticosterone levels 30 min after restraint stress were not different between WT and TK mice, indicating that altered glucocorticoid response to stress is not a non-specific effect of transgene expression ($t_{30} = 0.1$, $P = 0.95$; $n = 16$ per group). **d**, Valganciclovir-treated v-TK mice show impaired dexamethasone suppression of corticosterone in response to restraint ($*t_{13} = 2.5$, $P = 0.03$; $n = 7–8$ per group). Error bars, s.e.m.

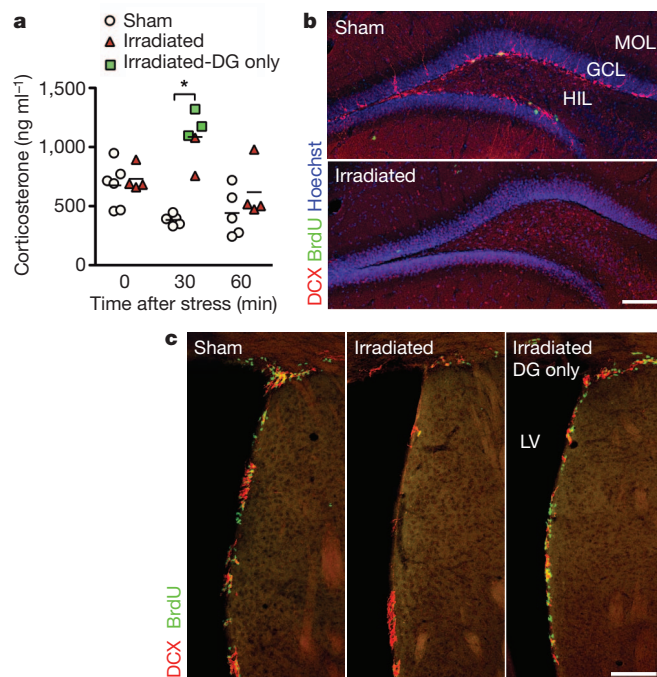


Figure 3 | Increased stress response is not due to reduced neurogenesis in the subventricular zone. **a**, Increased corticosterone response 30 min after restraint was confirmed in mice in which neurogenesis was reduced by irradiation (irradiation effect $F_{1,50} = 2.0$, $P = 0.16$; time effect $F_{2,50} = 5.1$, $P = 0.01$; irradiation \times time interaction $F_{2,50} = 5.7$, $P = 0.006$; $*P < 0.01$ post hoc; $n = 4–6$ per group per time point). Green squares at the 30 min time point indicate corticosterone levels in irradiated mice that showed unaffected neurogenesis in the subventricular zone. **b**, Confocal images of BrdU⁺ and DCX⁺ cells in the dentate gyrus; neurogenesis was reduced in all irradiated mice. **c**, Confocal images of BrdU⁺ and DCX⁺ cells illustrating sparing of neurogenesis in the subventricular zone. Scale bars, 100 μ m. LV, lateral ventricle.

to speculation that impaired neurogenesis does not directly contribute to the aetiology of depression in adulthood^{4,5}. Our findings above suggest that stress may be a key unexplored factor linking adult-born neurons to anxiety- and depressive-like behaviours.

To probe a potential interaction between stress, neurogenesis and depression, we first used the dexamethasone suppression test, which is commonly used to test HPA axis feedback in depressed patients. A subgroup of depressed patients, and mice that display depressive behaviours, show impaired inhibition of endogenous glucocorticoids by the synthetic glucocorticoid dexamethasone^{19,20}. We found that dexamethasone effectively suppressed the restraint-induced rise in corticosterone to near basal levels in v-WT mice (Fig. 2d). However, the level of corticosterone in dexamethasone-injected v-TK mice was significantly higher than that in v-WT mice, consistent with a depressive-like phenotype.

We next examined whether adult neurogenesis regulates the behavioural response to stress in the novelty-suppressed feeding (NSF) test, which shows robust effects of antidepressants that are blocked by irradiation^{12,18}. In this test, food-deprived mice are introduced to a novel open field containing a food pellet at its centre and the latency to begin feeding is recorded¹². When assessed in the NSF test under normal conditions, v-WT and v-TK mice showed similar feeding latencies (Fig. 4a), indicating similar levels of approach-avoidance behaviour. Restraint stress just before testing, however, significantly increased the feeding latency in v-TK mice while having no effect on v-WT mice. Moreover, by the end of the 10 min test only 53% of stressed v-TK mice had fed compared with 92% of stressed v-WT mice (Fig. 4b). Mice from all groups consumed food upon returning to their home cage, indicating that decreased motivation to eat was not responsible for change in behaviour (Supplementary Fig. 6). Thus, adult neurogenesis does not alter behaviour under baseline conditions in this test, consistent with previous observations^{12,18}, but buffers the effects of stress on feeding behaviour.

Because the NSF test is associated with both anxiety- and depressive-like behaviour, we investigated the interaction of neurogenesis and stress in additional behavioural tests. In the elevated plus maze, a test of anxiety-like behaviour, there was an anxiogenic effect of stress but no significant difference between v-WT and v-TK mice (Supplementary Fig. 7). We next tested depressive-like behaviour, using the forced swim test, in which rodents are placed in an inescapable cylinder of water and immobility is used as a measure of behavioural despair²¹. Under control conditions neurogenesis-deficient v-TK mice became immobile more rapidly and for a greater duration than v-WT mice (Fig. 4c, d), consistent with a depressive phenotype. In v-WT mice, restraint stress reduced the latency to become immobile and increased total immobility to the level of v-TK mice. Thus, neurogenesis-deficient mice displayed a depressive phenotype at baseline, which could be induced in intact mice by acute stress. Consistent with previous reports^{16,18,22}, v-WT and v-TK mice showed similar levels of immobility during later stages of the test, when high (potentially ceiling) levels of behavioural despair are observed²¹ (Supplementary Fig. 8).

Anhedonia is a hallmark symptom of depression and, in rodents, presents as a decrease in preference for a sucrose solution compared with water²³. To assess whether adult neurogenesis is required for hedonic behaviours, we habituated v-WT and v-TK mice to freely available water and 1% sucrose for 3 days. Both groups similarly preferred sucrose (Supplementary Fig. 9a). Then, after water and sucrose deprivation, the bottles were reintroduced and preference was measured during a 10 min test. To introduce an aspect of reward-based decision making²³, bottle locations were switched for the test. Although v-WT mice showed a preference for sucrose as before, neurogenesis-deficient v-TK mice showed no sucrose preference during the test (Fig. 4e). The decreased sucrose preference in v-TK mice was observed not only in the 10-min test but also during the next night (Fig. 4f). Overall consumption levels were not different, indicating that preference differences did not result from altered thirst or motivation to drink (Supplementary Fig. 9a–d). No differences were

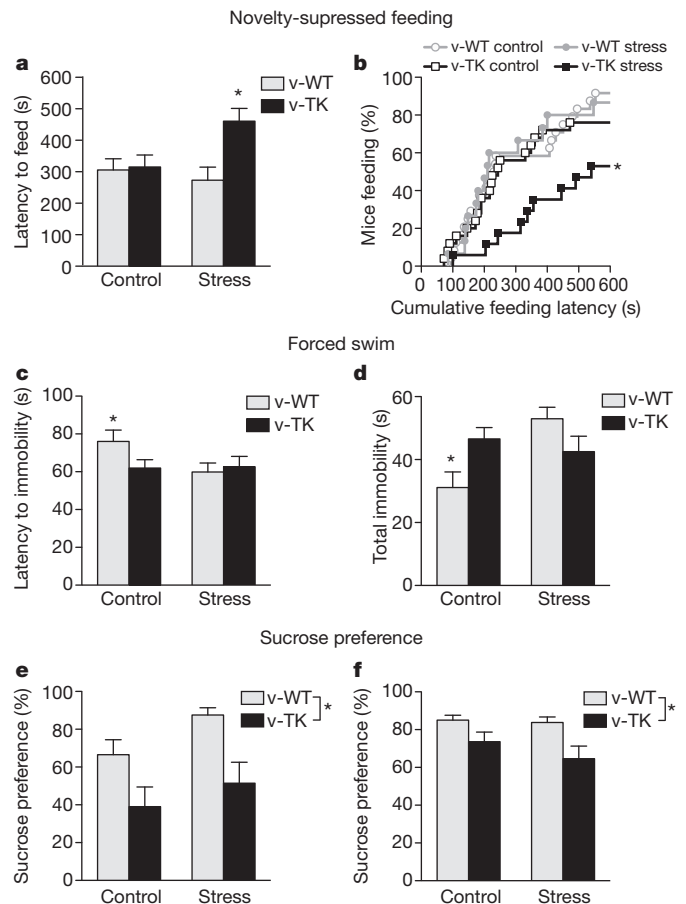


Figure 4 | Mice lacking neurogenesis show increased anxiety/depression-like behaviours. **a**, In the NSF test, v-TK mice showed increased latency to feed in a novel environment after restraint stress but not under control conditions (genotype effect $F_{1,75} = 5.9$, $P = 0.02$; stress effect $F_{1,75} = 1.9$, $P = 0.17$; genotype \times stress interaction $F_{1,75} = 4.8$, $P = 0.03$; * $P < 0.05$ versus v-TK control and * $P < 0.01$ versus v-WT stressed; $n = 13$ –25 per group). **b**, Cumulative distribution of feeding latencies for the NSF test (log-rank test; * $P < 0.05$ versus all other groups). **c**, Neurogenesis-deficient v-TK mice became immobile faster in the forced swim test. Restraint stress reduced the latency to immobility in v-WT mice but did not affect v-TK mice (genotype effect $F_{1,88} = 1.1$, $P = 0.3$; stress effect $F_{1,88} = 2.2$, $P = 0.14$; genotype \times stress interaction $F_{1,88} = 2.6$, $P = 0.11$; * $T_{46} = 2.1$, $P < 0.05$ versus v-WT stressed; v-TK control versus v-TK stressed $T_{42} = 0.1$, $P = 0.9$; $n = 22$ –26 per group). **d**, Under control conditions, the total time spent immobile was greater in v-TK mice than in v-WT mice. Restraint stress significantly increased total immobility in v-WT mice but had no effect on v-TK mice (genotype effect $F_{1,88} = 0.3$, $P = 0.6$; stress effect $F_{1,88} = 4.2$, $P = 0.04$; genotype \times stress interaction $F_{1,88} = 9.1$, $P = 0.003$; * $P < 0.05$ versus control v-TK, * $P < 0.001$ versus stressed v-WT, stressed v-WT versus stressed v-TK $P > 0.05$; $n = 22$ –26 per group). **e**, Neurogenesis-deficient v-TK mice showed reduced preference for sucrose in an acute test, compared with v-WT mice, under both control and restraint conditions (genotype effect $F_{1,20} = 11.2$, $P < 0.01$; stress effect $F_{1,20} = 3.1$, $P = 0.09$; genotype \times stress interaction $F_{1,20} = 0.2$, $P = 0.7$; $n = 4$ –8 per group). **f**, Sucrose preference remained lower in v-TK mice than v-WT mice during the subsequent dark cycle (genotype effect $F_{1,25} = 6.8$, $P = 0.01$; stress effect $F_{1,25} = 0.8$, $P = 0.4$; genotype \times stress interaction $F_{1,25} = 0.5$, $P = 0.5$; $n = 4$ –10 per group). Error bars, s.e.m.

observed in WT and control TK mice that were not treated with valganciclovir, indicating that anhedonic behaviour does not result from thymidine kinase expression alone (Supplementary Fig. 9e–g). The loss of sucrose preference in v-TK mice was observed whether or not the mice were restrained before testing, perhaps reflecting a basic difference between reward-related behaviours tested in this paradigm and the stress response behaviours tested in despair and avoidance situations. Taken together, our behavioural results suggest that adult

neurogenesis buffers the effect of previous stress in the NSF test, buffers the effect of inescapable stress in the forced swim test and enhances reward-seeking behaviour independent of stress in the sucrose preference test.

Elucidating the strong but poorly understood association between stress and depression is critical for development of more effective treatments^{1,2}. The hippocampus has long been known to regulate the HPA axis^{6,7}, and the importance of the hippocampus for emotional behaviour is emerging²⁴. Our data show that adult-born hippocampal granule neurons dynamically regulate stress reactivity at both the endocrine and behavioural levels. A direct role for adult neurogenesis in depression-like behaviour was observed in three behavioural situations that are commonly used to assess antidepressant efficacy and characterize the development of a depressive phenotype in response to chronic stress^{12,18,25}. In addition to its effects on emotional behaviour, stress is an important modulator of learning and memory²⁶. Our results therefore also suggest that buffering of stress responses by new neurons may play a role in learning and memory under novel or aversive conditions, in addition to any more direct function of young neurons in encoding of information. Because the production of new granule neurons is itself strongly regulated by stress and glucocorticoids³, this system forms a loop through which stress, by inhibiting adult neurogenesis, could lead to enhanced responsiveness to future stress. This type of programming could be adaptive, predisposing animals to behave in ways best suited to the severity of their particular environments²⁷. However, maladaptive progression of such a feed-forward loop could potentially lead to increased stress responsiveness and depressive behaviours that persist even in the absence of stressful events.

METHODS SUMMARY

All procedures followed the Institute of Laboratory Animal Research guidelines and were approved by the Animal Care and Use Committee of the National Institute of Mental Health. Transgenic mice expressing HSV-TK under the GFAP promoter were generated from a previously generated plasmid²⁸ using standard techniques and bred on a mixed C57Bl/6:CD-1 background. Male v-WT and v-TK mice were treated with valganciclovir for 8 weeks (dexamethasone experiment), 10–19 weeks (endocrine), 12 weeks (behaviour) or 4 weeks (histology; histology after 12 weeks in Supplementary Fig. 1), beginning at 8 weeks of age. Male C57Bl/6 mice were irradiated under pentobarbital anaesthesia, as described previously²⁹, and tested 9 weeks later. For immunohistochemical analyses, mice were given BrdU 6 weeks (for analysis of the paraventricular nucleus) or 24 h before being killed, brain sections were immunostained as previously described²⁹ and labelled cells were counted stereologically.

Serum corticosterone was measured by radioimmunoassay (MP Biomedicals) from submandibular blood samples obtained directly from the home cage condition or after exploration of a novel box, restraint or isoflurane exposure. For the dexamethasone suppression test, dexamethasone (Sigma; 50 µg kg⁻¹ in propylene glycol) or vehicle were injected 90 min before restraint, and blood was sampled immediately after 10 min restraint.

Behavioural tests were performed after 30 min of restraint or directly from the home cage. Different cohorts of mice were tested in the NSF test, elevated plus maze, forced swim test and sucrose preference test as previously described^{12,18,21,30}. Statistical analyses were performed by *t*-test, log-rank test or ANOVA with Fisher's least significant difference test for post-hoc comparisons. Significance was set at *P* < 0.05.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 7 April; accepted 13 June 2011.

Published online 3 August 2011.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank A. Sowers and J. Mitchell for help with irradiation, M. Brenner for providing the plasmid construct, GlaxoSmithKline for providing the antibody against HSV-TK, and K. Sanzone, F. Kamhi, L. Glover, S. Ferrante and L. Grigereit for assisting with mouse breeding and treatment. This research was supported by the Intramural Program of the National Institute of Mental Health, National Institutes of Health, USA, ZIA MH002784 (to H.A.C.).

Author Contributions J.S.S. performed histological, endocrine and behavioural experiments, analysed data and wrote the paper; A.S. performed behavioural experiments and analysed behavioural data; H.A.C. performed endocrine experiments, analysed data and wrote the paper; J.P. and M.B. generated the transgenic mice.

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METHODS

All procedures followed the Institute of Laboratory Animal Research guidelines and were approved by the Animal Care and Use Committee of the National Institute of Mental Health.

Transgenic animals. Transgenic mice expressing HSV-TK under the GFAP promoter (GFAP-TK) were generated on a C57Bl/6 background using the pGfa2-TK1 plasmid²⁸ applying standard techniques and were outcrossed onto a mixed C57Bl/6:CD-1 background. For all GFAP-thymidine kinase experiments, male mice were weaned at 3 weeks of age, genotyped by PCR, and housed four per cage with mixed genotype siblings. Mice were housed on a 12 h light/dark cycle with lights on at 6.00 a.m. All experiments were performed on different, naive cohorts of mice except the home cage, exploration and 17 day restraint corticosterone experiments, which were performed on the same cohort. Beginning at 8 weeks of age, valganciclovir was mixed into powdered chow (0.3%, approximately $35 \text{ mg kg}^{-1} \text{ day}^{-1}$) for 4 days, alternating with standard pelleted chow for 3 days. Mice were treated for 8 weeks before dexamethasone testing, 10–19 weeks before other endocrine testing and 12 weeks before behavioural testing. Histology was performed after 4 weeks, to assess effectiveness when animals were younger and neurogenesis was higher but was also routinely confirmed after longer treatments in tested mice (Supplementary Fig. 1). All mice within each experiment were tested at the same time point after the start of treatment.

Irradiation. Male C57Bl/6 mice were irradiated under pentobarbital anaesthesia, as described previously²⁹, and tested 9 weeks later.

Immunohistochemical analyses. Immunohistochemistry was performed on 40- μm sections as previously described²⁹, using the following primary antibodies: goat anti-GFAP (Santa Cruz Biotechnology), rabbit anti-HSV-thymidine kinase (gift from GlaxoSmithKline), goat anti-doublecortin (Santa Cruz Biotechnology), rat anti-BrdU (Accurate) and mouse anti-BrdU (BD Biosciences). Alexa-conjugated secondary antibodies made in donkey (Invitrogen) and the nuclear counterstain Hoechst 33258 (Sigma) were used for all fluorescent labelling. The ABC method (Vector Labs) and DAB were used for enzymatic staining with cresyl violet nuclear counterstaining.

For dentate gyrus analyses, valganciclovir-treated and untreated WT and TK mice were injected once with BrdU (200 mg kg^{-1}), after 4 weeks of treatment, and perfused 24 h later. Stereological counts of BrdU⁺ cells were performed on a 1 in 12 series of sections in the dentate gyrus. Counts of BrdU⁺/DCX⁺ neurons were obtained by multiplying total BrdU⁺ cell counts by the proportion that expressed DCX. To quantify GFAP⁺ astrocytes, the most medial 500 μm of the molecular layer of the suprapyramidal blade was imaged with a confocal microscope (Olympus FV300) and a $\times 60$ oil-immersion lens. The total number of GFAP⁺ cells was divided by the volume examined for each animal to obtain a measure of astrocyte density.

For analyses of the paraventricular nucleus, mice were given BrdU water (1 mg ml^{-1}) for 1 week, beginning in the fifth week of valganciclovir treatment, and perfused 6 weeks later. Stereological counts were performed in a one in two series of sections through the paraventricular nucleus.

For irradiation experiments, mice received a single BrdU injection and were perfused 24 h later. Immunostaining for BrdU and DCX was used to confirm a reduction in dentate gyrus neurogenesis, and BrdU and DCX labelling was examined in the subventricular zone of mice from the 30 min post-restraint group to assess the spatial specificity of irradiation-induced reduction of adult neurogenesis.

Stress and corticosterone measurement. To measure circadian fluctuations in baseline corticosterone, submandibular blood samples were obtained directly from the home cage condition, using animal lancets (Medipoint) at 7.00–8.00 a.m. or 7.00–8.00 p.m. Blood samples were centrifuged, and serum was collected and stored at -80°C until use. Serum corticosterone was measured by radioimmunoassay (MP Biomedicals).

For exploration stress, mice were placed in an open field (white plastic box, $50 \text{ cm} \times 50 \text{ cm} \times 50 \text{ cm}$) for 5 min and allowed to explore. Blood samples were

obtained 30 min later. During the interval between exploration and blood sampling, mice were placed individually in clean, empty cages to prevent social interactions that could influence HPA activity. Morning and evening exploration occurred between 8.00 and 11.00 a.m. and 6.00 and 8.00 p.m., respectively. Locomotor behaviour was analysed using Ethovision software (Noldus).

For all restraint stress experiments, mice were restrained for 30 min in decapicones (Baintree Scientific). For isoflurane experiments, mice were exposed to 4% isoflurane in oxygen for 30 min. Blood samples were obtained 0, 30 or 60 min after stress on different subsets of mice; all mice were stressed at the same time and each mouse was used for only one of the three time points. For the 30- and 60-min groups, mice were individually placed in empty cages between stress and blood sampling. Restraint and isoflurane experiments were performed between 12.00 and 4.00 p.m.

For the dexamethasone suppression test, mice were injected with dexamethasone (Sigma; $50 \mu\text{g kg}^{-1}$ in propylene glycol) or vehicle 90 min before restraint. Blood samples were taken immediately after 10 min restraint.

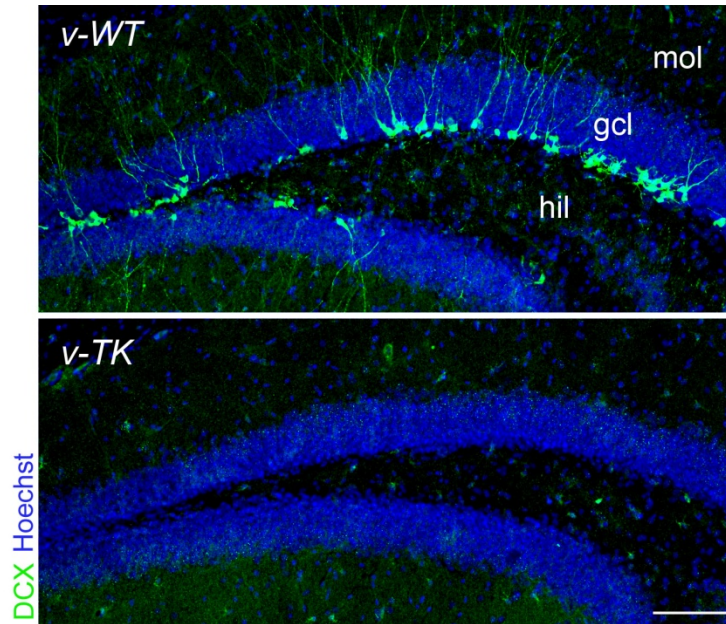
Novelty-suppressed feeding. Mice were handled for 2 min day^{-1} for 3 days before testing to familiarize them with experimenter handling. Food was removed from the cage 24 h before testing. Mice were weighed just before food deprivation and again before testing to assess body weight loss. For testing, mice were placed for 10 min in a brightly lit open field ($50 \text{ cm} \times 50 \text{ cm} \times 50 \text{ cm}$ white plastic boxes containing bedding) with a food pellet at the centre on a slightly (1 cm) elevated platform. Mice were either placed in the arena directly from their home cage or after 30 min of restraint stress and 2 min back in their home cage. Behaviour was videotaped, and the latency for each mouse to begin feeding was scored, offline, by an experimenter blind to the genotype and condition for each mouse. Upon returning to their home cage, the total amount of food consumed during a 5-min period was analysed to test whether feeding differences in the novel environment were due to differences in hunger/motivation.

Elevated plus maze. Mice were handled for 3 days and were placed in an elevated plus maze for 5 min either directly from their home cage or after 30 min restraint and 2 min back in their home cage. Behaviour was tracked using Ethovision software and the amount of time spent in the open arms was measured.

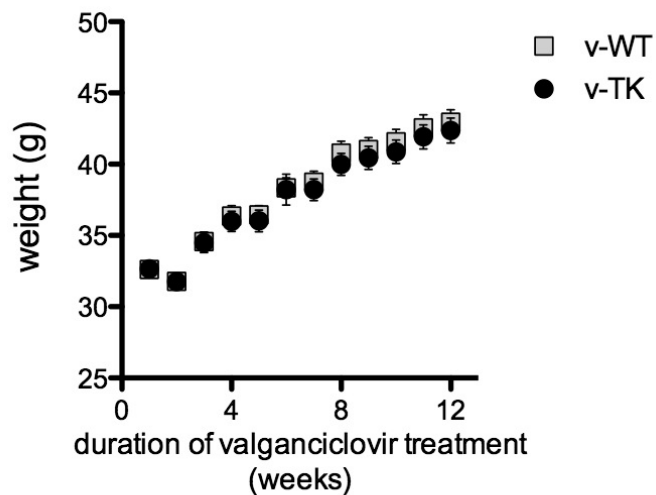
Forced swim test. Mice were individually placed in a Plexiglas cylinder (19 cm diameter, 30 cm height) containing 19 cm water ($23 \pm 1^\circ\text{C}$) and were videotaped for 6 min. Active (swimming, climbing, struggling) or passive (immobility) behaviours were scored using a time sampling technique to rate the predominant behaviours in each 5-s interval. In contrast to protocols designed to detect reductions in immobility²¹ (for example, scoring minutes 2–6 of testing, when immobility is very high in controls, to detect antidepressant effects), the first 2 min of the test were scored separately to better detect potential increases in immobility. The latency to become immobile for the first time was also measured. After the swim session, mice were dried and placed in a cage surrounded by a heating pad. The water was changed between each animal.

Sucrose preference. Mice were not handled but were individually housed and given a water bottle containing water and a second with 1% sucrose with the left/right location balanced across animals. After 3 days of habituation, both bottles were removed at 12.00 p.m.³⁰. Beginning at 6.30 p.m., mice were subjected to either 30-min restraint or brief experimenter handling, returned to their home cage, and given access to water and sucrose with bottles in the reversed left/right location. Bottles were weighed before testing and again after 10 min, and sucrose preference was expressed as $(\Delta\text{weight}_{\text{sucrose}})/(\Delta\text{weight}_{\text{sucrose}} + \Delta\text{weight}_{\text{water}}) \times 100$. The bottles were weighed again at 1 h and on the subsequent morning, and sucrose preference over this interval was used as a long-term, overnight measure.

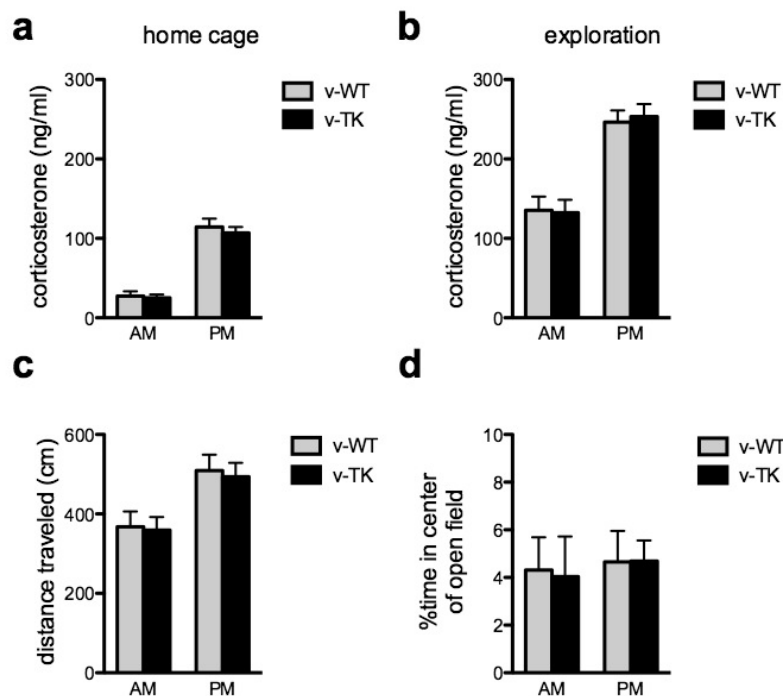
Statistical analyses. Statistical analyses were performed by *t*-test, log-rank test or ANOVA with Fisher's LSD test for post-hoc comparisons. Significance was set at $P < 0.05$.



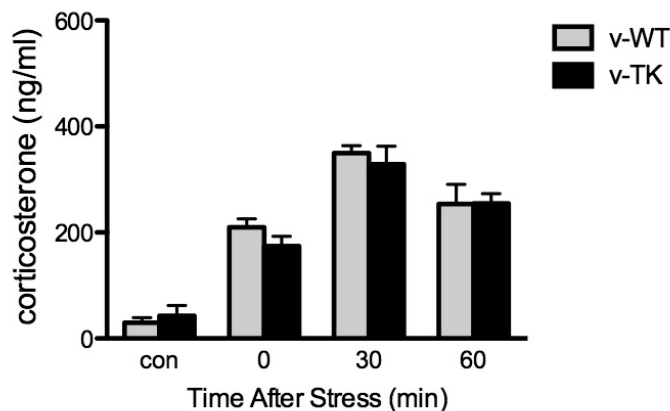
Supplementary Figure 1: Confocal photograph of doublecortin immunostaining following 12 weeks of valganciclovir treatment. Extending the 4 week treatment results in Fig. 1, neurogenesis remained suppressed in v-TK mice after longer valganciclovir treatments, as employed in the endocrine and behavior experiments. Scale bar 100 μ m. Mol, molecular layer; gcl, granule cell layer; hil, hilus.



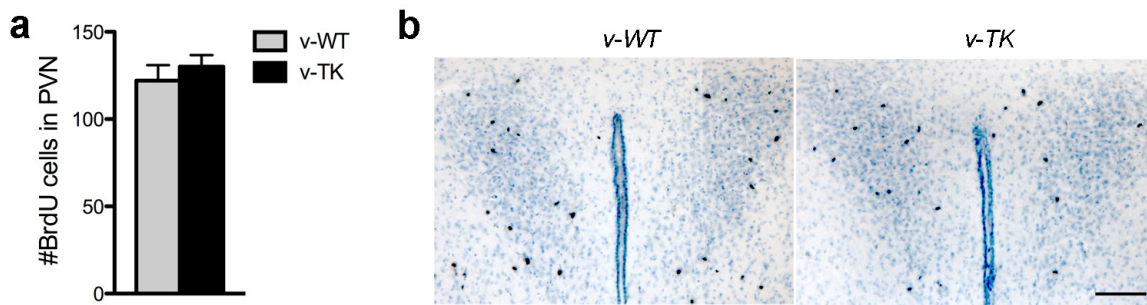
Supplementary Figure 2: Body weights were not significantly different in v-WT and v-TK mice (2-way repeated measures ANOVA, genotype effect $F_{1,759}=0.2$, $P=0.69$, duration effect $F_{11,759}=239$, $P<0.0001$, genotype x duration interaction $F_{11,759}=0.4$, $P=0.97$).



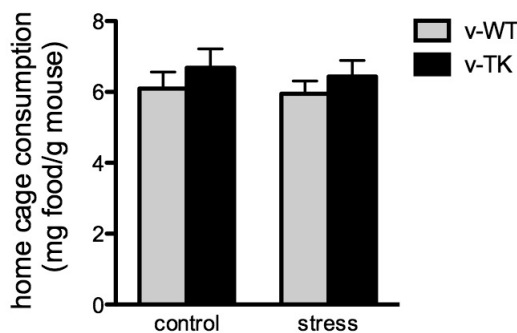
Supplementary Figure 3: Circadian fluctuations in corticosterone and baseline exploratory behavior. a) Circadian fluctuation in serum corticosterone in the home cage condition is normal in treated v-TK mice (genotype effect $F_{1,64}=0.4$, $P=0.52$; time of day effect $F_{1,64}=123$, $P<0.001$). b) Corticosterone levels did not differ between v-WT and v-TK mice following exposure to a novel open field, a mild stressor (genotype effect $F_{1,66}=0.02$, $P=0.90$; time of day effect $F_{1,66}=53$, $P<0.001$). c) Total locomotor distance did not differ between v-WT and v-TK mice during exploration of the novel environment (genotype and interaction effects $F_{1,66}<1$, $P>0.7$, time of day effect $F_{1,66}=14$, $P<0.001$). d) Time spent in the center of the open field (10 cm x 10 cm) did not differ between v-WT and v-TK mice (genotype and interaction effects both $F_{1,66}<1$, $P>0.7$; time of day effect $F_{1,66}=4$, $P<0.001$).



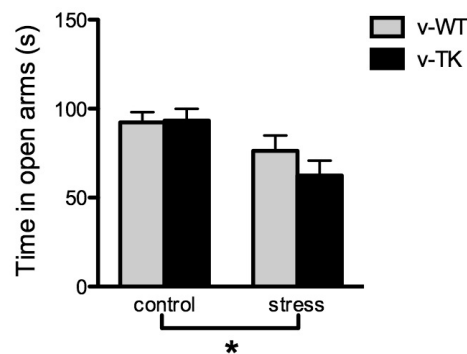
Supplementary Figure 4: Corticosterone response to isoflurane anesthesia, a moderate physical stressor, was unchanged in v-TK mice (genotype effect $F_{1,31}=0.4$, $P=0.55$; time effect $F_{3,31}=49$, $P<0.001$; interaction $F_{3,31}=0.3$, $P=0.8$).



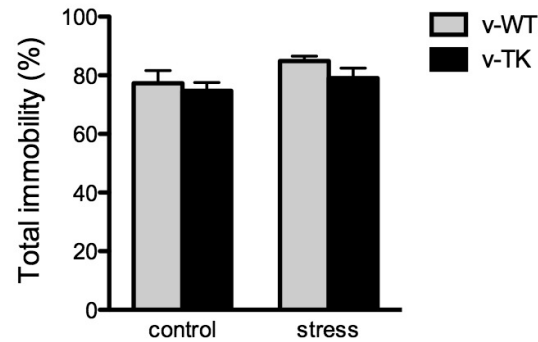
Supplementary Figure 5: Cell addition in the hypothalamic paraventricular nucleus (PVN), which drives the HPA response to stress, is not reduced in v-TK mice ($T_{11} = 0.7$, $P=0.5$), suggesting that any neurogenesis that might occur in the adult PVN is not altered in our transgenic model. b) BrdU immunostaining (black) in the PVN, as seen with Nissl counterstain (blue). Scale bar 100 μm .



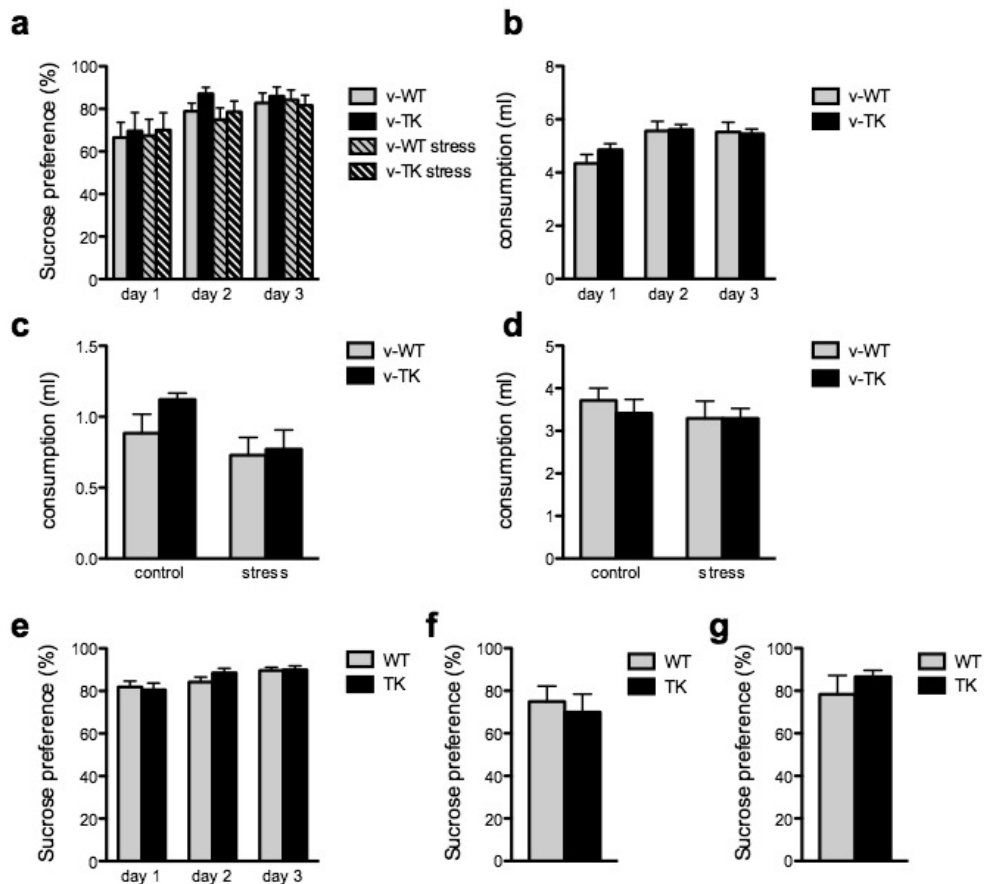
Supplementary Figure 6: Home cage food consumption following the NSF test. v-WT and v-TK mice did not consume different amounts of food upon returning to their home cage (genotype effect $F_{1,82}=1.3$, $P=0.2$; stress effect $F_{1,82}=0.2$, $P=0.7$; interaction $F_{1,82}=0.01$, $P=0.9$).



Supplementary Figure 7: Anxiety-related behavior in the elevated plus maze. Stress significantly reduced time spent in the open arms of the maze in both genotypes. Stressed v-TK mice spent 18% less time in the open arms than stressed v-WT mice, which was not significantly different (genotype effect $F_{1,46}=0.7$, $P=0.4$; stress effect $F_{1,46}=9$, $P<0.01$; interaction $F_{1,46}=0.9$, $P=0.3$).



Supplementary Figure 8: v-WT and v-TK mice did not show differences in total immobility during minutes 2-6 of forced swim testing (genotype effect $F_{1,88}=1.9$, $P=0.2$; stress effect $F_{1,88}=3.9$, $P=0.05$; genotype x stress interaction $F_{1,88}=0.3$, $P=0.6$).



Supplementary Figure 9: Sucrose preference habituation and consumption (a-d) and sucrose preference in mice not treated with valganciclovir (e-g). a) Pre-test habituation. Sucrose preference during habituation was not different between v-WT and v-TK mice; both groups showed increased sucrose preference across days of habituation (2 way repeated measures ANOVA, control and to-be-stressed mice pooled; genotype effect $F_{1,42}<0.1$, $P=0.9$; day effect $F_{2,42}=12$, $P<0.0001$; interaction $F_{2,42}=0.4$, $P=0.7$; day 3 vs. day 1 post hoc $P<0.05$ for both v-WT and v-TK). b) Total consumption (sucrose + water) was not different in v-WT and v-TK mice (2 way repeated measures ANOVA, control and to-be-stressed mice pooled; genotype effect $F_{1,42}=0.2$, $P=0.6$; day effect $F_{2,42}=16$, $P<0.0001$; interaction $F_{2,42}=1.2$, $P=0.3$). c)

Total consumption during the 10 min test was not significantly different between groups but there was a trend for stress to reduce overall consumption (genotype effect $F_{1,20}=1.1$, $P=0.3$; stress effect $F_{1,20}=3.5$, $P=0.08$; interaction $F_{1,20}=0.5$, $P=0.5$). d) There were no neurogenesis- or stress-related differences in total consumption on the night following the 10 min test (genotype effect $F_{1,20}=0.2$, $P=0.7$; stress effect $F_{1,20}=0.6$, $P=0.4$; interaction $F_{1,20}=0.2$, $P=0.7$). e) Pre-test habituation in WT and TK mice (not treated with valganciclovir) revealed no genotype differences in sucrose preference (genotype effect $F_{1,20}=0.2$, $P=0.3$; effect of day $F_{2,20}=11.5$, $P=0.0005$; interaction $F_{2,20}=1.3$, $P=0.3$). f) There was no difference in sucrose preference in untreated WT and TK mice during the 10 min test ($T_{10}=0.4$, $P=0.7$). g) There was no overnight difference in sucrose preference in untreated WT and TK mice during the dark cycle immediately following 10 min test ($T_{10}=0.9$, $P=0.4$).