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ARTICLE

Early stress evokes temporally distinct consequences on the hippocampal transcriptome, anxiety and cognitive behaviour

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Abstract

The early stress of maternal separation (ES) exerts long-lasting effects on cognition and anxiety. Recent evidence indicates enhanced hippocampus-dependent spatial learning in young adult ES animals, which shifts towards a decline in long-term memory in middle-aged life. Further, we find that ES animals exhibit enhanced anxiety in young adulthood that does not persist into middle-aged life. Here, we demonstrate unique, predominantly non-overlapping, hippocampal transcriptomes in young adult and middle-aged ES animals that accompany the temporally-specific behavioural consequences. Strikingly, the extent of gene dysregulation in middle-aged ES animals was substantially higher than in young adulthood. Functional analysis revealed distinct biological processes enriched at the two ages, highlighting the temporal shift in ES-evoked gene regulation. Our results suggest that ES history interacts with aging to exacerbate age-associated transcriptional changes and cognitive decline. qPCR profiling of histone deacetylases (*Hdacs*) and histone methyltransferases (HMTs) revealed an age-dependent, opposing regulation with decreased expression noted in young adult ES animals (*Hdac 2, 7, 8, 9* and *Suv39h1*) and enhanced levels in middle-aged life (*Hdac 2, 6, 8* and *Suv39h1*). While altered expression of histone modifying enzymes did not translate into global histone acetylation or methylation changes, we noted differential enrichment of histone acetylation and methylation modifications at the promoters of multiple genes regulated in the hippocampi of young adult and middle-aged ES animals. Our results highlight the differential molecular and behavioural consequences of ES across a life-span, and suggest a possible role for epigenetic mechanisms in contributing to the temporally-specific transcriptional changes following ES.

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Key words: Epigenetic, histone deacetylase, histone methyltransferase, maternal separation, microarray.

Introduction

Adverse early experience programmes life-long changes in stress-response neurocircuitry. Early stress (ES) evokes hypothalamo-pituitary-adrenal (HPA) dysfunction (Kalinichev et al., 2002), enhanced anxiety (Lehmann et al., 1999) and perturbed cognition (Hulshof et al., 2011) in adulthood. However, the behavioural consequences of ES are not invariant across the life-span, but rather differ in nature temporally, with the observation of both adaptive and maladaptive changes. Multiple models of ES programmes enhanced anxiety and perturb the glucocorticoid-mediated, hippocampal feedback control of the HPA axis well into adulthood (Kalinichev et al., 2002; Weaver et al., 2006; Rice et al., 2008). However, it is unknown if the anxiogenic consequences are maintained across the life-span, particularly in the absence of a 'second-hit' adult stressor (Eiland and McEwen, 2012). The early stressors of maternal separation (Suri et al., 2013) or low quality maternal care (Champagne et al., 2008) are associated with improved performance on stress-associated learning tasks in young adulthood. In contrast, cognitive dysfunction ensues in middle-aged life in ES animals (Suri et al., 2013) and in the limited nesting material model of disrupted maternal care (Brunson et al., 2005; Ivy et al., 2010). This suggests that ES exposure can evoke age-dependent, differential behavioural outcomes.

Molecular changes that drive functional alterations in neurocircuits responsible for emotional and cognitive processing may contribute to the behavioural consequences of ES. Given the differential nature of behavioural outcomes across life following ES, we hypothesized that ES-evoked molecular changes in circuits such as the hippocampus, a region involved in cognition (Ferbinteanu et al., 2003) and control of stress-response pathways (Radley and Sawchenko, 2011), may differ across life. We examined the hippocampal transcriptome in young adult and middle-aged ES animals, time points at which we assessed anxiety and spatial learning. We find that ES evokes distinct, age-dependent changes in

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cognition and anxiety. The temporally-specific behavioural outcomes of ES were accompanied by unique global gene expression changes within the hippocampus. Given the key role of epigenetic machinery in contributing to persistent changes in gene expression (Kouzarides, 2007), we also examined the expression of histone deacetylases (*Hdac*) and histone methyltransferases (HMT) within the hippocampi of ES animals across life. Strikingly, ES animals exhibited an age-dependent, opposing pattern of regulation of histone modifying enzymes. Our findings indicate that ES evokes temporally-specific effects on hippocampal gene expression, spatial learning and anxiety. The differential molecular changes evoked by ES across life may establish a substrate that upon interaction with environmental context or additional stressors such as the aging process may contribute to the behavioural outcomes of ES history that emerge in a temporally distinct manner.

Method

Animals

Male Sprague–Dawley rats bred in the Tata Institute of Fundamental Research (TIFR) animal facility were maintained under group housed conditions with a 12 h light–dark cycle and *ad libitum* access to food and water. All experimental procedures were in accordance with the guidelines of the National Institute of Health Guide for Care and Usage of animals, and approved by the TIFR Institutional Animal Ethics committee.

Early stress

Litters from pregnant primiparous dams were assigned randomly to control or ES groups. ES pups were separated from dams from postnatal day 2 (PD2) to PD14 for 3 h daily and kept on euthermic pads. Following separation, the litters and their dams were returned to the home cage. Control litters were left undisturbed throughout, other than routine animal facility handling. Male ES animals were studied at two ages: 2 months in young adulthood, and 15 months in middle-aged life.

Morris water maze

The Morris water maze (MWM) test was performed in a tank (150 cm diameter) in the presence of prominent visual cues. An escape platform (10 cm diameter) was hidden below the water surface with the location fixed across MWM training, and the virtual area around the platform (58 cm diameter) was designated the target annulus. Animals were trained to locate the platform over 5 d of training, 4 trials/d (trial duration = 120 s, inter-trial interval = 60 s) and performance was determined by the latency to find the hidden platform (escape latency). Short- and long-term spatial memory was tested on probe tests (90 s) conducted 24 h and 10 d post-training, and performance was assessed by the percent time in the target annulus (n = 11/group/age). MWM behaviour was monitored and scored using an automated tracking system (Noldus Ethovision 3.1, Noldus Information Technology, Netherlands).

Open field test

To assess anxiety, 2 (n = 13–18/group) and 15 (n = 7–10/group) month old control and ES animals were tested on the open field test (OFT). Animals were introduced into a novel open field arena (100 cm × 100 cm × 100 cm) under uniform low intensity lighting, and behaviour was analysed over a period of 10 min. Anxiety behaviour was assessed by the time spent and the percent distance travelled in the central region of the arena. The behaviour of the animals in the OFT was recorded and scored using Noldus Ethovision 3.1.

Microarray

To address global transcriptional changes in ES animals, microarray analysis was performed on hippocampi from young adult (n = 4/group) and middle-aged (Controls: n = 3, ES: n = 5) control and ES animals as previously described (Benekareddy et al., 2010). In brief, hippocampal RNA was extracted using RNeasy Minikit (Qiagen, USA), Cy3 labelled and standard spike controls were used in all labelling reactions. The labelled RNA (600 ng) from each animal was fragmented and individually hybridized to a brain-specific custom rat expression array 8X15 K (024724) with 15000 features. The hybridized slides were scanned with the Agilent Microarray Scanner G Model G2505C (Agilent Technologies) and subjected to analysis using GeneSpring GX 11 software (Agilent Technologies). Cut-offs of log2>0.3 and <−0.3 were applied to the data set. Statistical analysis was performed with a t-test (significance level of <0.05) corrected for multiple comparisons using the Benjamini and Hochberg method (Benjamini and Hochberg, 1995). Array data from ES animals in young adulthood and middle-aged life compared with their respective age-matched controls described in this manuscript has been deposited in the NCBI's Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=xnydbasyscaqoxc&acc=GSE29637). Data was subjected to functional analysis using the DAVID (Database for Annotation, Visualization, and Integrated Discovery: http://david.abcc.ncifcrf.gov/) functional annotation tool.

Chromatin immunoprecipitation (ChIP)

Chromatin immunoprecipitation (ChIP) analysis was performed as previously described (Tsankova et al., 2006). Briefly, hippocampi from 2 (n = 6–9/group) and 15 (n = 7–10/group) month old control and ES animals were minced, cross-linked and sonicated to generate

400–800 bp chromatin fragments. Equal amounts of chromatin lysate (40 μg) was immunoprecipitated separately with antibodies against specific histone modifications, histone3 acetylation (H3ac), histone3-lysine 9 acetylation (H3K9ac), control nonimmune rabbit IgG (Millipore Bioscience Research Reagents), histone3-lysine 9 dimethylation (H3K9me2) and histone3-lysine 9 trimethylation (H3K9me3) (Abcam, UK). The histone modification bound antibodies were pulled down using protein A/G agarose beads, chromatin was eluted, reverse cross-linked, and proteinase K treated. Chromatin was extracted and subjected to quantitative PCR (qPCR) analysis.

Quantitative PCR (qPCR)

Hippocampal RNA was reverse transcribed (High capacity cDNA Reverse Transcription Kit, Applied Biosystems, USA) and the synthesized cDNA was subjected to quantitative PCR (qPCR) (Applied Biosystems) with primers specific to the genes of interest. Analysis of qPCR data was performed using the ΔΔCt method as described previously (Bookout and Mangelsdorf, 2003). For analysis of microarray validation and histone modifying enzymes mRNA levels, expression data from all groups was normalized to the endogenous housekeeping gene, hypoxanthine-guanine phosphoribosyltransferase (*Hprt*) (n=9–12/group/age). An intragenic non-transcribed region, from each individual sample, was used as a normalizing control for analysis of ChIP data. Results were compared to the control group and expressed as a fold change ±S.E.M. Primers used for qPCR analysis are listed in Supplementary Table S1.

Western blot analysis

Hippocampal tissue lysates were separated by SDS polyacrylamide gel electrophoresis and proteins were transferred to polyvinyldifluoride membranes (GE Healthcare, UK). Following blocking, membranes were incubated with rabbit anti-H3 acetylation (1:1000; Millipore Research laboratories), rabbit anti-H3 K9 dimethylation (1:1000; Abcam), rabbit anti-H3 K9 trimethylation (1:1000; Abcam) or rabbit anti-β-III-tubulin (1:5000; Covance, USA). Membranes were incubated with a horseradish peroxidase conjugated donkey anti-rabbit (1:5000, GE Healthcare) secondary antibody. Protein-antibody complexes were detected with an ECL substrate (GE Healthcare). Densitometric analysis was performed using Scion image software (Scion Corporation, USA) (n=4–5/group/age).

Statistical analysis

Experiments with two groups were analysed using the unpaired Student's t-test (Instat, Graphpad Software Inc., USA). Statistical analysis of escape latencies on the MWM task was subjected to repeated measures ANOVA using the software SPSS output (IBM Corporation, USA) with paired Student's t-test for group wise *post-hoc* comparisons. Significance was determined at $p<0.05$.

Results

Early stress evokes differing age-dependent consequences on anxiety and hippocampus-dependent cognitive behaviour

We have previously shown that ES improved MWM learning in young adulthood and evoked a decline in long-term spatial memory on this task in middle-aged life (Suri et al., 2013). Here, in an independent experiment we reconfirm our previous findings that ES evokes differential behavioural outcomes on the MWM task across life. Young adult and middle-aged ES animals and their age-matched controls were trained on the MWM task (Fig. 1*a*). Consistent with our previous results, 2-month-old ES animals showed a more rapid acquisition of spatial learning as indicated by significantly lower escape latencies (Fig. 1(*b*); effect of group: $F_{1,20}$=7.56, p=0.01; Repeated measures ANOVA; n=11/group). Reduced escape latencies in ES animals as compared to controls were noted on the first training day of the MWM task (Fig. 1(*b*)), though this decrease was not statistically significant (Supplementary Figure S1) (p=0.13, Repeated measures ANOVA). Performance on the probe tests at 24 h (Fig. 1(*c*)) and 10 d (Fig. 1(*d*)) was unaltered across young adult control and ES animals. To assess anxiety, young adult ES animals were tested on the OFT. Young adult ES animals demonstrated enhanced anxiety behaviour in the OFT with significantly reduced time spent in the centre (Fig. 1(*e, f*); p=0.02, Student's t-test; n=13–18/group), and a trend towards a decrease in the percent distance travelled in the centre of the arena (p=0.06) (Supplementary Fig. S2A).

Middle-aged control and ES animals did not exhibit any significant difference in escape latencies across training (Fig. 1(*g*); effect of group: $F_{1,20}$=0.58, $p>0.05$, Repeated measures ANOVA; n=11/group) or on the probe test conducted 24 h after training (Fig. 1(*h*)). In the 10 d probe test, middle-aged ES animals showed a significant reduction in percent time spent in the target annulus (Fig. 1*i*; p=0.01, Student's t-test) indicating impaired long-term recall of spatial memory. We also tested if the enhanced anxiety on the OFT observed in young adult ES animals was maintained into middle-aged life. Middle-aged ES animals exhibited no difference in the time spent (Fig. 1(*j,k*)), or in the percent distance travelled (Supplementary Fig. S2C), in the centre of the open field arena as compared to their age-matched controls (n=7–10/group). No difference in the swim speed in the MWM (Supplementary Table S2) or the total distance travelled in the OFT (Supplementary Fig. S2B, D) was noted between control and ES animals at either of the two ages examined.

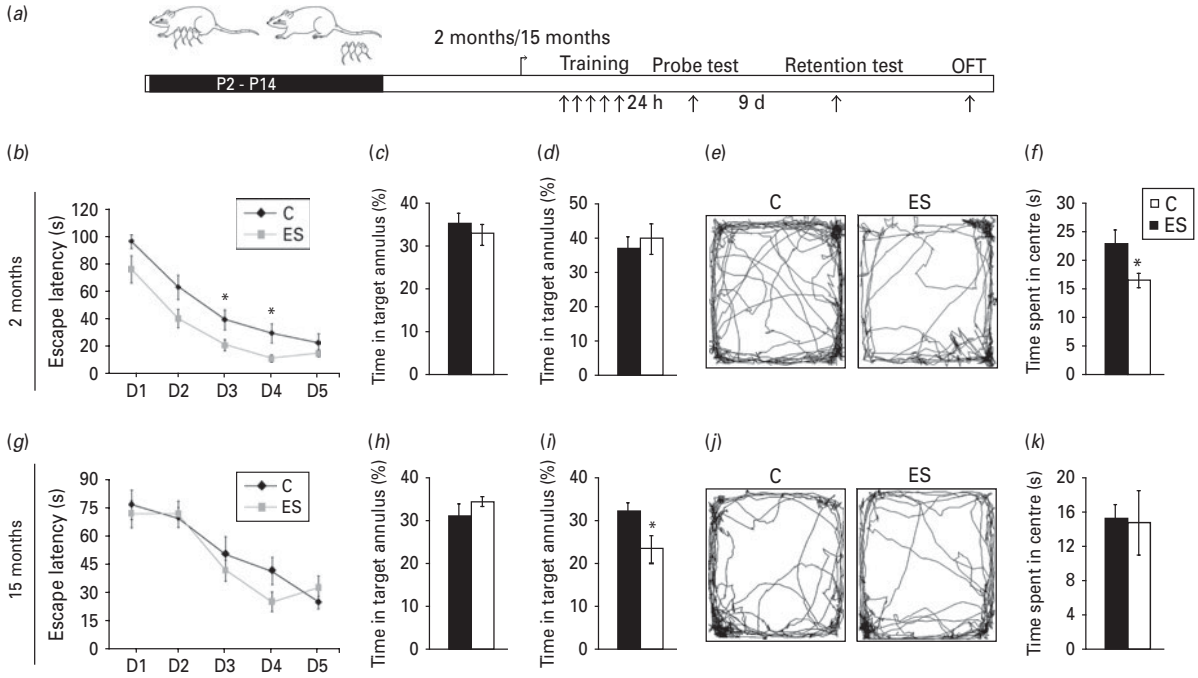


Fig. 1. Age-dependent changes evoked by early stress on hippocampal dependent cognitive function and anxiety behaviour. (*a*) Shown is a schematic representation of the experimental design. Animals with a history of the early stress of maternal separation (ES) and their age-matched controls (C) were trained in young adulthood (2 months) and in middle-aged life (15 months) (n=11/group/age) on the Morris water maze (MWM) task (5 d (D) of training, 4 trials/d). Animals were tested for short and long-term retention using probe tests 24 h and 10 d after the last day of training. Probe tests assessed the percent time spent in the target annulus. (*b*) Young adult ES animals exhibited reduced escape latency across training days as compared to age-matched controls (*p<0.05; repeated measures ANOVA). (*c, d*) Short and long-term recall of spatial memory following the end of training did not differ between the control and ES groups. (*e*) Shown is a representative track from a young adult control and ES animal in the open field arena. (*f*) Young adult ES animals exhibited enhanced anxiety behaviour as assessed by the significantly lesser time spent in the centre on the open field test (OFT) (n=13–18/group; *p<0.05; unpaired Student's t-test). Middle-aged ES animals did not differ from controls in their escape latency during MWM training (*g*) or in their performance on the probe test 24 h after the end of training (*h*). (*i*) Middle-aged ES animals exhibited a significant impairment in recollection of the hidden platform position on the probe test performed 10 d after MWM training, as assessed by a significant decline in the percent time spent in the target annulus as compared to age-matched controls (*p<0.05; unpaired Student's t-test). (*j*) Shown is a representative track from a middle-aged control and ES animal in the open field arena. (*k*) Middle-aged ES animals did not exhibit any difference in anxiety behaviour as compared to the age-matched controls when tested on the OFT (n=7–10/group). Results are expressed as the mean±S.E.M. and represent the time in seconds for escape latency during MWM training, the percent time in target annulus for the probe tests performed 24 h and 10 d after MWM training, and the time spent in seconds in the centre of the open field arena.

ES animals exhibit distinct hippocampal transcriptomes in young adulthood and middle-aged life

To gain a molecular understanding of the mechanisms that may underlie the temporally distinct behavioural consequences of ES, we performed microarray experiments to determine global patterns of gene expression in the hippocampi of young adult (n=4/group) and middle-aged (n=3–5/group) ES animals (Fig. 2(*a,b*)). Strikingly, we found that a history of ES evokes an age-dependent, unique hippocampal transcriptome, with a minimal overlap of genes regulated in young adult and middle-aged ES animals (Fig. 2(*c,d*), Supplementary Table S3, S5) and substantially larger number of genes altered in their expression in middle-aged ES animals (Fig. 2(*c,d*), Supplementary Table S5). The genes that demonstrated a similar regulation at the two ages were 9.5% and 3.3% of the total transcriptome regulated in young adult and middle-aged ES animals, respectively.

We also noted the enrichment of distinct functional categories of genes in young adult and middle-aged ES animals (Fig. 3(*a–c*), Supplementary Table S4, S6). Functional analysis with DAVID revealed that the young adult ES hippocampal transcriptome exhibited a significant enrichment for functional categories of genes such as plasma membrane components, intracellular signalling pathways, neurotransmitter and neuropeptide receptors, cytoskeletal components and MAP kinase signalling (Fig. 3(*b*) and Supplementary Table S4). In contrast, middle-aged ES animals showed a significant enrichment for genes involved in functional biological processes including ion binding, transcriptional regulation, neuronal dendritic projection, cellular stress response pathways, neuronal

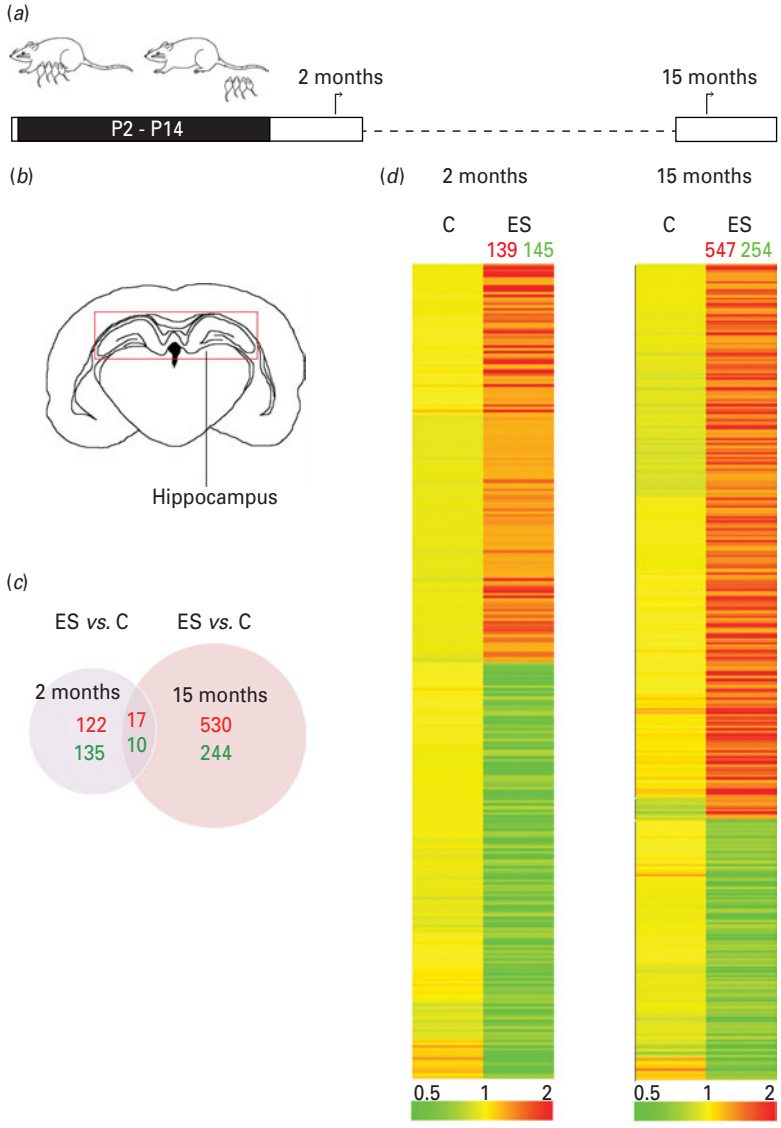
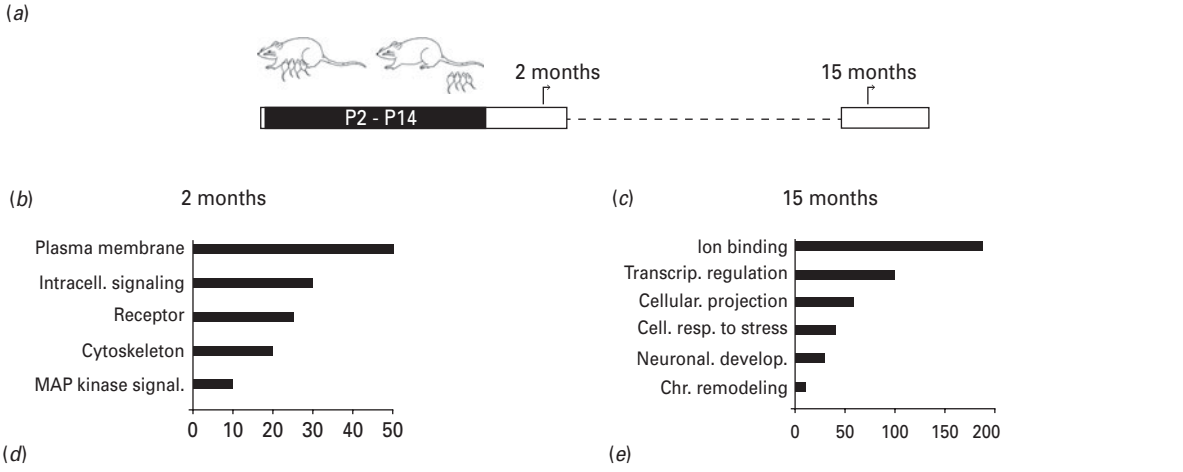


Fig. 2. Early stress is associated with differential, age-dependent changes in the hippocampal transcriptome. (*a, b*) Microarray analysis was performed to assess the hippocampal transcriptome of young adult (2 months) (n=4/group) and middle-aged (15 months) (control: n=3, ES: n=5) animals with a history of early stress (ES) as compared to their respective age-matched controls (C). (*c, d*) Microarray analysis revealed temporally distinct changes in the hippocampal transcriptome of young adult and middle-aged ES animals. (*c*) The numbers of upregulated (red) and downregulated (green) genes in 2-month-old and 15-month-old ES animals as compared to their respective age-matched controls, are represented using a Venn diagram (statistical cutoff: log2>0.3 for upregulated genes and <−0.3 for downregulated genes, p<0.05; t-test corrected for multiple comparisons). (*d*) Heat map analysis display the significantly regulated genes (p<0.05, t test corrected for multiple comparisons; upregulated genes are shown in red, downregulated genes are shown in green) in young adult and middle-aged ES animals as compared to their respective age-matched controls. Each row represents a single gene and each column represents the average fold change for the group. The data are normalized intensity values. The hippocampal transcriptome analysis reveals a larger number of genes regulated in middle-aged as compared to young adult ES animals, with minimal overlap of genes observed between these two ages.

development and chromatin remodelling (Fig. 3(*c*) and Supplementary Table S6).

qPCR validation of hippocampal gene expression changes in young adult and middle-aged ES animals

We then validated the regulation of several genes in young adult (Fig. 3(*d*); n=10–12/group) and middle-aged (Fig. 3(*e*); n=9–10/group) ES animals using qPCR analysis in tissue samples from an independent cohort of animals. Our qPCR results with young ES animals confirmed the differential regulation of several genes implicated in cognition and glutamatergic neurotransmission including protein kinase A catalytic subunit (*Prkacb*) (Nayak et al., 1998), kinase A (PKA) anchor protein 7 (*Akap7*) (Sanderson and Dell'acqua, 2011), dystrophin (*Dmd*)



(d)

	qPCR		Microarray	
Gene	Fold change	*P* value	Fold change	*P* value
Upregulated	ES *vs.* C		ES *vs.* C	
Akap7	1.16±0.03	0.039	1.28±0.08	0.035
Art5	1.60±0.18	0.007	1.46±0.24	0.040
Dmd	1.25±0.09	0.043	3.03±0.61	0.010
Htr2a	1.66±0.14	0.001	1.27±0.08	0.022
Kitlg	2.20±0.13	0.001	1.67±0.15	0.002
Prkacb	1.16±0.06	0.026	1.24±0.06	0.006
Map3k1	1.76±0.15	0.001	1.23±0.07	0.015
Downregulated				
Capn5	0.86±0.04	0.010	0.29±0.03	0.001
Htr5a	0.73±0.05	0.047	0.74±0.05	0.017
Kcna3	0.80±0.03	0.001	0.61±0.04	0.005
Ppp3ca	0.72±0.10	0.064	0.69±0.06	0.044
Slc6a9	0.65±0.03	0.003	0.64±0.07	0.019
Unvalidated genes				
Atgr2	1.56±0.35	0.088	1.41±0.19	0.034
Cald1	0.97±0.08	0.830	1.38±0.13	0.012
Grik2	1.06±0.03	0.181	1.26±0.03	0.019

(e)

	qPCR		Microarray	
Gene	Fold change	*P* value	Fold change	*P* value
Upregulated	ES *vs.* C		ES *vs.* C	
Cav1	1.37±0.15	0.041	1.65±0.14	0.025
Capn7	1.24±0.05	0.029	1.71±0.14	0.019
H2afx	1.39±0.13	0.034	1.71±0.13	0.005
Hif1a	1.26±0.05	0.029	1.45±0.09	0.025
Ppp1r11	1.47±0.13	0.003	1.37±0.04	0.004
Ppp3cb	1.51±0.11	0.001	1.38±0.06	0.007
Siah1	1.12±0.03	0.009	1.81±0.04	0.025
Downregulated				
Akap9	0.69±0.06	0.005	0.77±0.07	0.057
Btg2	0.83±0.03	0.046	0.62±0.06	0.017
Rcan3	0.74±0.02	0.002	0.33±0.03	0.001
Ryr3	0.55±0.10	0.037	0.40±0.10	0.014
Stat3	0.74±0.08	0.043	0.64±0.67	0.021
Unvalidated genes				
Epo	0.45±0.13	0.131	0.39±0.06	0.012
Fut4	0.84±0.12	0.293	0.53±0.03	0.034
Tcfap4	0.82±0.10	0.159	0.65±0.08	0.050

Fig. 3. Distinct functional categories of genes regulated in young adult and middle-aged early stress (ES) animals. (*a*) The genes regulated in young adult and middle-aged ES animals were analysed using the software DAVID to determine the functional categories regulated. (*b*) Functional analysis of ES-regulated genes at 2 months of age, using the software DAVID, revealed a significant enrichment of functional categories including plasma membrane components, intracellular signalling pathways, neurotransmitter and neuropeptide receptors, cytoskeletal proteins and MAP kinase signalling pathway components. (*c*) In contrast, middle-aged ES animals revealed a significant regulation of genes involved in ion binding, transcriptional regulation, cellular projection, cellular stress response pathways, neuronal development and chromatin remodeling. (*d, e*) Quantitative PCR experiments performed for select genes using an independent cohort of 2-month-old (*d*) and 15-month-old (*e*) control (C) and ES (n=9–12/group/age; unpaired Student's t-test) animals validated the regulation observed in the microarray. Values are expressed as mean±S.E.M. fold change of control.

(Daoud et al., 2009), MAP kinase kinase kinase 1 (*Map3k1*) (Schafe et al., 1999), glycine transporter (*Slc6a9*) (Singer et al., 2009), stem cell factor (*Kitlg*) (Motro et al., 1996), subunits of calcineurin (*Ppp3ca*) (Malleret et al., 2001) and the calcium-dependent protease calpain 5 (*Capn5*) (Touyarot et al., 2002) (Fig. 3(*d*)), *N*-methyl-D-aspartate receptor NMDAR1 (*Grin1*) (Fold change: Control=1.00±0.010, ES=1.29±0.02, p=0.0007) (Nakazawa et al., 2004) and transient receptor potential cation channel, subfamily C, member 5 (*Trpc5*) (Fold change: Control=1.00±0.10, ES=1.59±0.12, p=0.003) (Fortin et al., 2012)

Middle-aged ES animals exhibited regulation of genes involved in calcium homeostasis, namely the protease calpain 7 (*Capn7*) (Vosler et al., 2008), specific calcineurin subunits (*Ppp3cb*), the regulator of calcineurin (*Rcan 3*) (Foster et al., 2001) and the ryanodine receptor 3 (*Ryr3*)

(*a*)

	2 months	15 months
Hdac 1	0.97±0.06	0.92±0.04
Hdac 2	0.83±0.04*	**1.17±0.03***
Hdac 3	1.46±0.05*	1.04±0.13
Hdac 4	1.01±0.06	1.00±0.05
Hdac 5	1.06±0.05	0.86±0.18
Hdac 6	1.01±0.04	1.54±0.12*
Hdac 7	0.78±0.07#	1.07±0.09
Hdac 8	0.52±0.08*	1.37±0.11*
Hdac 9	0.77±0.03*	1.12±0.10
Hdac 10	0.78±0.10	0.76±0.31
Hdac 11	0.93±0.04	1.12±0.11
G9a/Ehmt2	0.99±0.04	0.93±0.05
Suv39h1	0.70±0.07*	1.68±0.12*

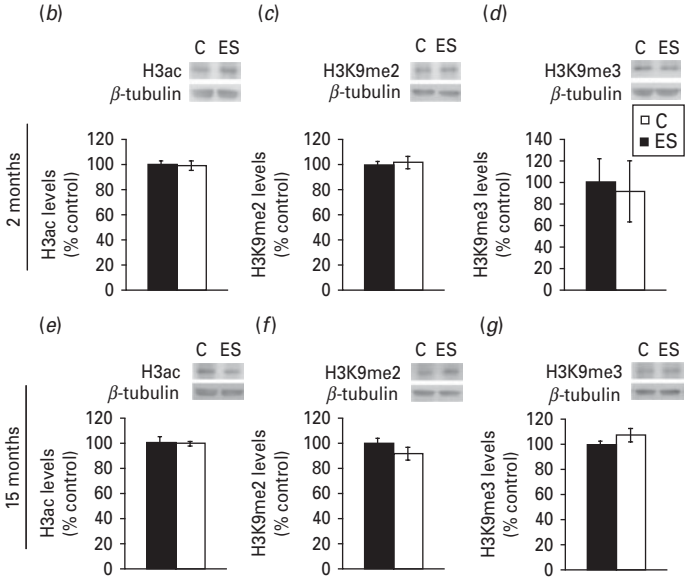


Fig. 4. Early stress evokes temporally-distinct changes in the hippocampal expression of histone modifying enzymes. Quantitative PCR analysis of mRNA levels of histone deacetylases (*Hdac1* to *Hdac11*) and histone methyltransferases- *G9a/Ehmt2* and suppressor of variegation 39H1 (*Suv39h1*) was performed in young adult (2 months) and middle-aged (15 months) animals with a history of early stress (ES) and their respective age-matched controls (C) (n=9–12/group/age). (*a*) Expression analysis revealed a significant decline in *Hdac2, Hdac8 and Hdac9* and a trend towards a decline in *Hdac7* ($^{\#}p$=0.07) expression in young adult ES animals. Young adult ES animals also exhibited an increased expression of *Hdac3*. (*a*) In contrast, an enhancement in expression of *Hdac2, Hdac6* and *Hdac8* was observed in ES animals in middle-aged life. (*a*) An opposing age-dependent regulation of the histone methyltransferase *Suv39h1* was also observed, with a decline in expression noted in young adult ES animals and an increase in expression in middle-aged ES animals. The global hippocampal histone3 acetylation (H3ac) (*b, e*), histone3 lysine-9 dimethylation (H3K9me2) (*c, f*) or histone3 lysine-9 trimethylation (H3K9me3) (*d, g*) levels as assessed using western blot analysis were not altered in young adult or middle-aged ES animals (n=4–5/group/age). Values are expressed as mean±S.E.M. fold change of control for qPCR analysis and mean±S.E.M. percentage of control for western blot analysis ($^{\#}p$=0.07, $^{*}p$<0.05; unpaired Student's t-test).

(Marks, 1997) (Fig. 3(*e*)). Genes implicated in cellular stress response pathways, including the transcription factors B-cell translocation gene-2 (*Btg2*) (Tirone, 2001), hypoxia inducible factor (*Hif1a*) (Aminova et al., 2005), seven in absentia homolog 1 (*Siah1*) (Shang et al., 2012) and the histone variant *H2afx* (Sokolov et al., 2007) were found to be regulated (Fig. 3(*e*)). Though we observed few genes that exhibited an age-dependent opposing regulation, members of specific gene families were regulated in a contrasting manner in young adult and middle-aged ES animals, with a decline in *Capn5* and *Ppp3ca* in young adulthood and an induction of *Capn7* and *Ppp3cb* in middle-aged ES animals. These qPCR validation experiments using separate cohorts of young adult and middle-aged ES animals confirmed the regulation of 15/20 (70%) and 13/20 (65%), respectively, of the genes identified as significantly regulated in our arrays.

Early stress evokes age-dependent changes in the expression of specific histone modifying enzymes

ES animals exhibit global gene expression changes that emerge at different epochs of life, with alterations observed long after the initial stressor experience. We hypothesized that histone-modifying enzymes may contribute to the age-dependent gene expression changes through altering promoter-associated histone modifications. We profiled the expression of several *Hdacs* (*Hdac1* to *Hdac11*) and the HMTs, *G9a* and suppressor of variegation 39H1 (*Suv39h1*) within the hippocampi of young adult and middle-aged ES animals. We focused on these particular histone modifying enzymes as they are reported to influence both cognitive function and modulate stress-associated behavioural adaptations (Renthal et al., 2007; Guan et al., 2009; Schaefer et al., 2009; Kilgore et al., 2010).

qPCR analysis revealed an opposing pattern of *Hdac* expression in young adult and middle-aged ES animals. We observed a significant decline in *Hdac2* (p=0.006) and *Hdac8* (p<0.001) levels in the hippocampi of young adult ES animals (Fig. 4(*a*); Student's t-test; n=10–12/group). In contrast, *Hdac2* (p=0.03) and *Hdac8* (p=0.006) expression were upregulated in middle-aged ES animals (Fig. 4(*a*); Student's t-test; n=9–10/group). While other *Hdacs* examined did not show such biphasic regulation, several *Hdacs* were decreased in expression in young

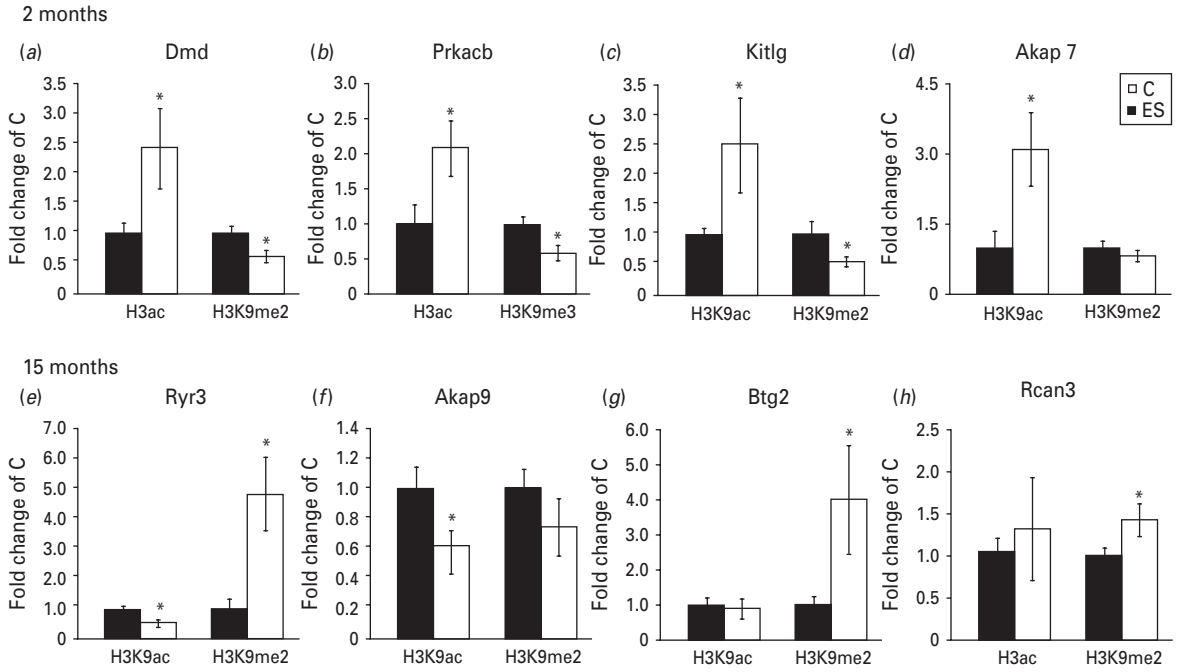


Fig. 5. Early stress (ES) evokes altered epigenetic histone modifications at the promoters of regulated genes. ES evoked activating and repressive histone modifications associated with specific genes found regulated in young adult and middle-aged animals (n=6–10/group/age). Genes that showed enhanced hippocampal expression in young adult (2 months) ES animals namely, dystrophin (*Dmd*) (*a*) and protein kinase A subunit b (*Prkacb*) (*b*), and kit ligand (*Kitlg*) (*c*) and kinase A anchoring protein 7 (*Akap7*) (*d*) exhibited a significant enhancement in levels of H3 acetylation (H3ac) and H3K9 acetylation (H3K9ac) respectively, when compared to age-matched controls. (*a, b, c*) The enhanced activating modifications were accompanied by a decline in the repressive H3K9 dimethylation (H3K9me2) and H3K9 trimethylation (H3K9me3) modifications at the promoters of *Dmd* (*a*) and *Kitlg* (*c*), and *Prkacb* (*b*), respectively. In contrast, 15-month-old ES animals exhibited a significant decline in H3K9ac levels at the promoters of the downregulated genes ryanodine receptor 3 (*Ryr3*) (*e*) and protein kinase A anchoring protein 9 (*Akap9*) (*f*) when compared to age-matched controls. An increase in the repressive H3K9me2 histone modification was also found associated with the promoters of the downregulated genes *Ryr3* (*e*), B-cell translocation gene2 (*Btg2*) (*g*) and repressor of calcineurin (*Rcan3*) (*h*) in middle-aged ES animals. All values are expressed as mean±S.E.M. fold change of control (*p<0.05; unpaired Student's t-test).

adult ES animals including *Hdac7* (p=0.07) and *Hdac9* (p=0.005) (Fig. 4(*a*)). In contrast, concomitant with the increased expression of *Hdac2* and *Hdac8*, we observed a significant increase in *Hdac6* mRNA levels (p=0.004) in the hippocampi of middle-aged ES animals. In variance with the general pattern of decreased *Hdac* expression in the hippocampi of young adult ES animals, *Hdac3* showed enhanced expression at this age (p=0.001) with no change observed in expression in middle-aged life. The expression of the other *Hdacs* examined (*Hdac1, 4, 5, 10* and *11*) was unaltered in ES animals at both ages (Fig. 4(*a*)).

We also examined the expression of *G9a* and *Suv39h1*, HMTs responsible for the repressive H3K9me2 and H3K9me3 histone modifications (Kouzarides, 2007) and implicated in cognition (Gupta et al., 2010). qPCR analysis revealed a significant decline in hippocampal *Suv39h1* expression in young adult ES animals (p=0.05) and increased *Suv39h1* expression in middle-aged life (p=0.009) (Fig. 4(*a*)), with no significant change in *G9a/Ehmt2* (Fig. 4(*a*)) at the two ages. While our qPCR studies clearly indicate altered expression of several *Hdacs* and *Suv39h1* at both 2 and 15 months of age in ES animals (n=9–12/group/age), other than the regulation of *Suv39h1* in 15-month-old ES animals we did not observe these changes in our array data (n=3–5/group/age), possibly as a consequence of difference in sensitivity of qPCR *vs.* array (Chuaqui et al., 2002; Morey et al., 2006), the statistical cut-offs applied to array data and the differences in sample size. The altered expression of specific *Hdacs* and HMTs in young adult and middle-aged ES animals, did not however translate into global changes in H3ac (Fig. 4(*b,e*)), H3K9me2 (Fig. 4(*c,f*)) and H3K9me3 (Fig. 4(*d,g*)) in the hippocampi of ES animals at these ages (n=4–5/group/age).

Altered histone modifications at the promoters of specific regulated genes in young adult and middle-aged ES animals

We next examined whether the altered regulation of select genes in the hippocampi of young adult and middle-aged ES animals was associated with altered histone modifications within their promoter regions. In particular we focused on H3ac, H3K9ac, H3K9me2 and H3K9me3 histone modifications, which involve a role for the HDACs

and HMTs (Kouzarides, 2007) found regulated in ES animals at the two ages examined. Enhanced expression of *Dmd, Prkacb, Kitlg* and *Akap7* in young adult ES animals was accompanied by a significant enrichment of the activating histone modifications H3ac at the *Dmd* ($p=0.05$), *Prkacb* ($p=0.04$) and *Akap7* ($p=0.03$) (Fig. 5(*a,b*), Supplementary Table S7; Student's *t*-test; $n=6$–9/group) promoters, and H3K9ac at the *Kitlg* ($p=0.03$) and *Akap7* ($p=0.02$) promoters (Fig. 5(*c,d*), Supplementary Table S7). Concomitant with the enhanced activating histone modifications, we also noted a decline in repressive histone modifications at the promoters of *Dmd* ($p=0.02$) and *Kitlg* ($p=0.03$) (H3K9me2), and *Prkacb* (H3K9me2, H3K9me3) (H3K9me2: $p=0.08$; H3K9me3: $p=0.04$) (Fig. 5(*a–c*), Supplementary Table S7) in the hippocampi of young adult ES animals. A trend towards an increase in the H3K9me3 ($p=0.09$) histone modification was observed at the *Kitlg* promoter at this age (Supplementary Table S7). H3K9ac and H3K9me3 at the *Dmd* promoter, H3K9ac at the *Prkacb* promoter, H3ac at the *Kitlg* promoter, and H3K9me2 and H3K9me3 at *Akap7* promoter were not altered in young adult ES animals as compared to their age-matched controls (Supplementary Table S7).

We next examined epigenetic histone modifications within the promoters regions of specific genes selectively regulated in middle-aged life. Given the increase in the hippocampal expression of epigenetic enzymes linked to repressed promoter activity (*Hdac2, 6, 8* and *Suv39h1*) in middle-aged ES animals, we focused in particular on histone modifications within the promoters of the downregulated genes *Ryr3, Akap9, Btg2* and *Rcan3*. We observed a significant increase in the repressive H3K9me2 modification at the *Ryr3* ($p=0.005$), *Btg2* ($p=0.02$) and *Rcan3* ($p=0.05$) promoters in the hippocampi of middle-aged ES animals (Fig. 5(*e,g,h*); Supplementary Table S7, Student's *t*-test; $n=7$–10/group). We also noted decreased levels of H3K9ac at the *Ryr3* ($p=0.02$) and *Akap9* ($p=0.04$) promoters (Fig. 5(*e,f*), Supplementary Table S7). H3ac and H3K9me3 at the *Ryr3* promoter, H3ac, H3K9me2 and H3K9me3 at the *Akap9* promoter, H3ac, H3K9ac and H3K9me3 at the *Btg2* and *Rcan3* promoters were unaltered in middle-aged ES animals (Supplementary Table S7). Collectively, our results demonstrate that long after ES experience, epigenetic histone modifications may contribute to the hippocampal gene regulation pattern that emerges in an age-dependent fashion.

Discussion

We find that ES effects on gene expression and behaviour emerge in an age-dependent fashion and vary significantly across life. ES animals in young adulthood exhibited improved spatial learning and enhanced anxiety. However, the behavioural changes were not invariant across life, with the heightened anxiety noted in young adulthood reverting to baseline as ES animals aged and an impairment of long-term spatial recall noted to emerge in middle-aged ES animals. The novel finding of our study is that the distinct behavioural changes that ensue in young adult and middle-aged ES animals are accompanied by hippocampal gene expression changes that vary starkly at these time-points. The ES-evoked hippocampal transcriptome showed minimal overlap at the two ages examined, with an overall increase in the number of regulated genes in middle-aged ES animals. Further, we noted that the biological processes enriched in the hippocampal transcriptome of young adult *vs.* middle-aged ES animals differed substantially. Profiling analysis of histone modifying enzymes revealed an opposing regulation, with several repressive histone modifying enzymes reduced in young adult ES animals and enhanced expression of these classes of epigenetic enzymes in middle-aged ES animals. Distinct age-dependent effects on epigenetic machinery that emerge in ES animals may contribute to the age-dependent gene regulation. We find altered histone acetylation and methylation marks at the promoters of specific genes regulated in young adult and middle-aged ES animals. Collectively, our findings demonstrate that a history of ES programmes transcriptional and behavioural consequences that differentially emerge at distinct epochs of life.

We have previously shown that young adult ES animals exhibit improved acquisition on the stress-associated MWM learning task (Suri et al., 2013), accompanied by enhanced anxiety. These findings are in agreement with observations of improved cognitive performance in models of postnatal novelty (Tang et al., 2006; Reeb-Sutherland and Tang, 2011) and juvenile adverse experience (Avital and Richter-Levin, 2005) and reports of increased anxiety that ensues following ES experience (Lehmann et al., 1999; Rice et al., 2008). However, our results of improved spatial learning differ from prior studies that showed either no change or impaired cognitive performance in models of ES, with possible explanations for such discrepancies arising from use of different rodent strains and variations of the early stressors (Huot et al., 2002; Grace et al., 2009; Hulshof et al., 2011). Enhanced anxiety could contribute to enhanced arousal, differential behavioural reactivity and increased attention to escape cues in the stressful MWM task, thus facilitating acquisition of learning. It has been previously suggested that enhanced anxiety following ES may contribute to adaptive mechanisms that facilitate survival under adverse environmental contexts (Beery and Francis, 2011).

Interestingly, ES-mediated improvements in learning and enhanced emotionality were not maintained across life, with a shift towards impaired long-term spatial memory and no change in anxiety noted in middle-aged ES animals. Our results suggest the possibility that the physiological stressor of aging may serve as a precipitating

factor for the early emergence of age-associated memory impairments in ES animals. These findings are in agreement with a prior report from a model of maternal neglect where animals exhibited cognitive impairments in middle-aged life (Brunson et al., 2005; Ivy et al., 2010). While the aging process in interaction with ES history appears to hasten cognitive decline, we did not observe an age-associated exacerbation of anxiety in middle-aged ES animals. Rather, middle-aged ES animals did not differ in their behaviour from age-matched controls on the OFT. Further experiments are required to address whether the maintenance of anxiety is dependent on the nature of environmental context in adulthood, with the possibility that in the absence of strong 'second-hit' stressors, anxiety evoked by ES may wane across life.

In keeping with the age-dependent behavioural consequences of ES, we find unique gene expression changes in the hippocampi of young adult and middle-aged ES animals further highlighted by the enrichment of distinct functional categories. The hippocampal transcriptome in young adulthood revealed altered expression of multiple pathways that could contribute to the ES-evoked improvement of cognitive performance. We noted regulation of several genes associated with cellular cytoskeleton networks that play a role in altering synapse dynamics, neurite outgrowth, or neuronal plasticity, processes implicated in learning and memory (Harvey and Svoboda, 2007; Caroni et al., 2012). Further, altered expression of the MAP kinase signalling pathway in young adult ES animals may also contribute to improved cognitive function noted at this age (Valjent et al., 2001; Xia and Storm, 2012). Concomitant with the enhanced anxiety behaviour observed at this age we also find the regulation of several genes implicated in pathogenesis of psychiatric disorders, such as *Prkacb, Htr5a, Htr2a* and *Slc6a9* (Jokela et al., 2007; Shelton, 2007; Javitt, 2009; Xu et al., 2012). In this regard the *Htr2a* receptor upregulation is particularly interesting, given we have previously reported enhanced cortical 5-HT_2 receptor function in ES animals (Benekareddy et al., 2010) and shown that postnatal pharmacological blockade of the 5-HT_2 receptor prevents the emergence of anxiety in ES animals (Benekareddy et al., 2011).

Strikingly, the pattern of gene dysregulation in middle-aged ES animals was unique to this timepoint and showed minimal overlap with the changes observed in young adult ES animals. Paralleling the impairments of hippocampus-dependent cognitive function in middle-aged ES animals we noted the regulation of functional categories of genes implicated in ion binding and calcium homeostasis (calpain, calcineurin, *Rcan3, Ryr3*) that may modulate synaptic plasticity and hippocampal-dependent cognition (Malleret et al., 2001; Touyarot et al., 2002; Hoeffer et al., 2007; Adasme et al., 2011) . Normal calcium homeostasis plays a critical role in neuronal survival (Celsi et al., 2009), and its disruption may contribute to the exacerbation of aging-related neuronal damage and cognitive decline in middle-aged ES animals (Hajieva et al., 2009; Oliveira and Bading, 2011). Middle-aged ES animals also demonstrated the functional enrichment of cellular stress response pathways. It is noteworthy that these enriched functional categories namely cellular response to stress, cell development and ion binding altered in middle-aged ES animals, are also reported to be differentially regulated in the brains of aged animals (Blalock et al., 2003; Burger et al., 2007, 2008). This suggests that middle-aged ES animals may exhibit accelerated age-associated transcriptional regulation of a subset of the genome.

The mechanisms that underlie the age-dependent emergence of gene regulation and behavioural outcomes in animals with a history of ES are at present unknown. However it can be hypothesized that overlap of ES with critical periods of postnatal development may set up alterations in key neuroendocrine (Kalinichev et al., 2002), neurotransmitter and neurotransmitter receptor (Goodfellow et al., 2009; Benekareddy et al., 2010) systems that on interaction with environmental contexts and the aging process could precipitate altered gene expression and behavioural outcomes. Alternatively, possible mechanisms could involve the influence of ES on epigenetic machinery, which through their manifold effects on gene expression may serve to programme differential molecular and behavioural changes that emerge across the life-span in ES animals. Our results indicate that the expression of several HDACs and HMTs is perturbed following ES, with an opposing pattern of expression at the two ages. While we noted a decline in HDACs and HMTs in young adult ES animals (*Hdac2, Hdac7, Hdac8, Hdac9* and *Suv39h1*), in contrast an upregulation was observed in middle-aged life (*Hdac2, Hdac6, Hdac8 and Suv39h1).* Such differential consequences on *Hdac* and HMT expression are in keeping with the distinct transcriptional and behavioural changes noted at these two ages. However, we did not observe global changes in histone acetylation or methylation signatures within the hippocampi of ES animals in young adulthood or middle-aged life. Rather, we noted differential enrichment of histone modifications at the promoters of specific regulated genes in ES animals at the two ages, suggesting a role for epigenetic mechanisms in mediating the distinct transcriptional changes observed in ES animals at different epochs of life.

In summary, our results demonstrate ES-evoked effects on cognition and anxiety behaviour that vary in a temporal manner, accompanied by non-overlapping hippocampal transcriptional alterations, including an opposing regulation of *Hdacs* and *HMTs* in young adult and middle-aged ES animals. These findings highlight the importance of examining the influence of ES history across temporal windows, as the molecular and behavioural outcomes likely vary across epochs of life. Our results suggest that a history of ES interacts with the physiological process of aging to evoke an exacerbation

of age-related cognitive decline and to compound the regulation of hippocampal gene expression.

Supplementary material

For supplementary material accompanying this paper, visit http://dx.doi.org/10.1017/S1461145713001004.

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Statement of Interest

None.

References

Adasme T, Haeger P, Paula-Lima AC, Espinoza I, Casas-Alarcón MM, Carrasco MA, Hidalgo C (2011) Involvement of ryanodine receptors in neurotrophin-induced hippocampal synaptic plasticity and spatial memory formation. Proc Natl Acad Sci USA 108:3029–3034.

Aminova LR, Chavez JC, Lee J, Ryu H, Kung A, Lamanna JC, Ratan RR (2005) Prosurvival and prodeath effects of hypoxia-inducible factor-1alpha stabilization in a murine hippocampal cell line. J Biol Chem 280:3996–4003.

Avital A, Richter-Levin G (2005) Exposure to juvenile stress exacerbates the behavioural consequences of exposure to stress in the adult rat. Int J Neuropsychopharmacol 8:163–173.

Beery AK, Francis DD (2011) Adaptive significance of natural variations in maternal care in rats: a translational perspective. Neurosci Biobehav Rev 35:1552–1561.

Benekareddy M, Goodfellow NM, Lambe EK, Vaidya VA (2010) Enhanced function of prefrontal serotonin 5-HT(2) receptors in a rat model of psychiatric vulnerability. J Neurosci 30:12138–12150.

Benekareddy M, Vadodaria KC, Nair AR, Vaidya VA (2011) Postnatal serotonin type 2 receptor blockade prevents the emergence of anxiety behaviour, dysregulated stress-induced immediate early gene responses, and specific transcriptional changes that arise following early life stress. Biol Psychiatry 70:1024–1032.

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Statist Soc Ser B 57:289–300.

Blalock EM, Chen KC, Sharrow K, Herman JP, Porter NM, Foster TC, Landfield PW (2003) Gene microarrays in hippocampal aging: statistical profiling identifies novel processes correlated with cognitive impairment. J Neurosci 23:3807–3819.

Bookout AL, Mangelsdorf DJ (2003) Quantitative real-time PCR protocol for analysis of nuclear receptor signalling pathways. Nucl Recept Signal 1:e012.

Brunson KL, Kramar E, Lin B, Chen Y, Colgin LL, Yanagihara TK, Lynch G, Baram TZ (2005) Mechanisms of late-onset cognitive decline after early-life stress. J Neurosci 25:9328–9338.

Burger C, López MC, Feller JA, Baker HV, Muzyczka N, Mandel RJ (2007) Changes in transcription within the CA1 field of the hippocampus are associated with age-related spatial learning impairments. Neurobiol Learn Mem 87:21–41.

Burger C, Lopez MC, Baker HV, Mandel RJ, Muzyczka N (2008) Genome-wide analysis of aging and learning-related genes in the hippocampal dentate gyrus. Neurobiol Learn Mem 89:379–396.

Caroni P, Donato F, Muller D (2012) Structural plasticity upon learning: regulation and functions. Nat Rev Neurosci 13:478–490.

Champagne DL, Bagot RC, van Hasselt F, Ramakers G, Meaney MJ, de Kloet ER, Joëls M, Krugers H (2008) Maternal care and hippocampal plasticity: evidence for experience-dependent structural plasticity, altered synaptic functioning, and differential responsiveness to glucocorticoids and stress. J Neurosci 28:6037–6045.

Chuaqui RF, Bonner RF, Best CJM, Gillespie JW, Flaig MJ, Hewitt SM, Phillips JL, Krizman DB, Tangrea MA, Ahram M, Linehan WM, Knezevic V, Emmert-Buck MR (2002) Post-analysis follow-up and validation of microarray experiments. Nat Gen Supplement 32:509–514.

Celsi F, Pizzo P, Brini M, Leo S, Fotino C, Pinton P, Rizzuto R (2009) Mitochondria, calcium and cell death: a deadly triad in neurodegeneration. Biochim Biophys Acta 1787:335–344.

Daoud F, Candelario-Martinez A, Billard JM, Avital A, Khelfaoui M, Rozenvald Y, Guegan M, Mornet D, Jaillard D, Nudel U, Chelly J, Martinez-Rojas D, Laroche S, Yaffe D, Vaillend C (2009) Role of mental retardation-associated dystrophin-gene product Dp71 in excitatory synapse organization, synaptic plasticity and behavioural functions. PLoS ONE 4:e6574. doi: 10.1371/journal.pone.0006574.

Eiland L, McEwen BS (2012) Early life stress followed by subsequent adult chronic stress potentiates anxiety and blunts hippocampal structural remodeling. Hippocampus 22:82–91.

Ferbinteanu J, Ray C, McDonald RJ (2003) Both dorsal and ventral hippocampus contribute to spatial learning in Long-Evans rats. Neurosci Lett 345:131–135.

Fortin DA, Srivastava T, Dwarakanath D, Pierre P, Nygaard S, Derkach VA, Soderling TR (2012) Brain-derived neurotrophic factor activation of CaM-kinase kinase via transient receptor potential canonical channels induces the translation and synaptic incorporation of GluA1-containing calcium-permeable AMPA receptors. J Neurosci. 32:8127–8137.

Foster TC, Sharrow KM, Masse JR, Norris CM, Kumar A (2001) Calcineurin links Ca2+ dysregulation with brain aging. J Neurosci 21:4066–4073.

Goodfellow NM, Benekareddy M, Vaidya VA, Lambe EK (2009) Layer II/III of the prefrontal cortex: inhibition by the serotonin 5-HT1A receptor in development and stress. J Neurosci 29:10094–10103.

Grace L, Hescham S, Kellaway LA, Bugarith K, Russell VA (2009) Effect of exercise on learning and memory in a rat model of developmental stress. Metab Brain Dis 24:643–657.

Guan JS, Haggarty SJ, Giacometti E, Dannenberg JH, Joseph N, Gao J, Nieland TJ, Zhou Y, Wang X, Mazitschek R, Bradner JE, DePinho RA, Jaenisch R, Tsai LH (2009) HDAC2 negatively regulates memory formation and synaptic plasticity. Nature 459:55–60.

Gupta S, Kim SY, Artis S, Molfese DL, Schumacher A, Sweatt JD, Paylor RE, Lubin FD (2010) Histone methylation regulates memory formation. J Neurosci 30:3589–3599.

Hajieva P, Kuhlmann C, Luhmann HJ, Behl C (2009) Impaired calcium homeostasis in aged hippocampal neurons. Neurosci Lett 451:119–123.

Harvey CD, Svoboda K (2007) Locally dynamic synaptic learning rules in pyramidal neuron dendrites. Nature 450:1195–1200.

Hoeffer CA, Dey A, Sachan N, Wong H, Patterson RJ, Shelton JM, Richardson JA, Klann E, Rothermel BA (2007) The down syndrome critical region protein RCAN1 regulates long-term potentiation and memory via inhibition of phosphatase signalling. J Neurosci 27:13161–13172.

Huot RL, Plotsky PM, Lenox RH, McNamara RK (2002) Neonatal maternal separation reduces hippocampal mossy fiber density in adult Long Evans rats. Brain Res 950:52–63.

Hulshof HJ, Novati A, Sgoifo A, Luiten PG, den Boer JA, Meerlo P (2011) Maternal separation decreases adult hippocampal cell proliferation and impairs cognitive performance but has little effect on stress sensitivity and anxiety in adult Wistar rats. Behav Brain Res 216:552–560.

Ivy AS, Rex CS, Chen Y, Dube C, Maras PM, Grigoriadis DE, Gall CM, Lynch G, Baram TZ (2010) Hippocampal dysfunction and cognitive impairments provoked by chronic early-life stress involve excessive activation of CRH receptors. J Neurosci 30:13005–13015.

Javitt DC (2009) Glycine transport inhibitors for the treatment of schizophrenia: symptom and disease modification. Curr Opin Drug Discov Devel 12:468–478.

Jokela M, Keltikangas-Jarvinen L, Kivimaki M, Puttonen S, Elovainio M, Rontu R, Lehtimaki T (2007) Serotonin receptor 2A gene and the influence of childhood maternal nurturance on adulthood depressive symptoms. Arch Gen Psychiatry 64:356–360.

Kalinichev M, Easterling KW, Plotsky PM, Holtzman SG (2002) Long-lasting changes in stress- induced corticosterone response and anxiety-like behaviours as a consequence of neonatal maternal separation in Long-Evans rats. Pharmacol Biochem Behav 73:131–140.

Kilgore M, Miller CA, Fass DM, Hennig KM, Haggarty SJ, Sweatt JD, Rumbaugh G (2010) Inhibitors of class 1 histone deacetylases reverse contextual memory deficits in a mouse model of Alzheimer's disease. Neuropsychopharmacology 35:870–880.

Kouzarides T (2007) Chromatin modifications and their function. Cell 128:693–705.

Lehmann J, Pryce CR, Bettschen D, Feldon J (1999) The maternal separation paradigm and adult emotionality and cognition in male and female Wistar rats. Pharmacol Biochem Behav 64:705–715.

Malleret G, Haditsch U, Genoux D, Jones MW, Bliss TV, Vanhoose AM, Weitlauf C, Kandel ER, Winder DG, Mansuy IM (2001) Inducible and reversible enhancement of learning, memory, and long-term potentiation by genetic inhibition of calcineurin. Cell 104:675–686.

Marks AR (1997) Intracellular calcium-release channels: regulators of cell life and death. Am J Physiol 272:H597–H605.

Morey JS, Ryan JC, Van Dolah FM (2006) Microarray validation: factors influencing correlation between oligonucleotide microarrays and real-time PCR. Biol Proced Online 8:175–193.

Motro B, Wojtowicz JM, Bernstein A, van der Kooy D (1996) Steel mutant mice are deficient in hippocampal learning but not long-term potentiation. Proc Natl Acad Sci USA 93:1808–1813.

Nayak A, Zastrow DJ, Lickteig R, Zahniser NR, Browning MD (1998) Maintenance of late-phase LTP is accompanied by PKA-dependent increase in AMPA receptor synthesis. Nature 394:680–683.

Nakazawa K, McHugh TJ, Wilson MA, Tonegawa S (2004) NMDA receptors, place cells and hippocampal spatial memory. Nat Rev Neurosci 5:361–372.

Oliveira AM, Bading H (2011) Calcium signalling in cognition and aging-dependent cognitive decline. Biofactors 37:168–174.

Radley JJ, Sawchenko PE (2011) A common substrate for prefrontal and hippocampal inhibition of the neuroendocrine stress response. J Neurosci 31:9683–9695.

Reeb-Sutherland BC, Tang AC (2011) Dissociation between neonatal novelty-induced preferential maternal care and enhancement in cognitive, social, and emotional functions. Behav Brain Res 224:318–325.

Renthal W, Maze I, Krishnan V, Covington HE III, Xiao G, Kumar A, Russo SJ, Graham A, Tsankova N, Kippin TE, Kerstetter KA, Neve RL, Haggarty SJ, McKinsey TA, Bassel-Duby R, Olson EN, Nestler EJ (2007) Histone deacetylase 5 epigenetically controls behavioural adaptations to chronic emotional stimuli. Neuron 56:517–529.

Rice CJ, Sandman CA, Lenjavi MR, Baram TZ (2008) A novel mouse model for acute and long-lasting consequences of early life stress. Endocrinology 149:4892–4900.

Sanderson JL, Dell'acqua ML (2011) AKAP signalling complexes in regulation of excitatory synaptic plasticity. Neuroscientist 17:321–336.

Schafe GE, Nadel NV, Sullivan GM, Harris A, LeDoux JE (1999) Memory consolidation for contextual and auditory fear conditioning is dependent on protein synthesis, PKA, and MAP kinase. Learn Mem 6:97–110.

Schaefer A, Sampath SC, Intrator A, Min A, Gertler TS, Surmeier DJ, Tarakhovsky A, Greengard P (2009) Control of cognition and adaptive behaviour by the GLP/G9a epigenetic suppressor complex. Neuron 64:678–691.

Singer P, Yee BK, Feldon J, Iwasato T, Itohara S, Grampp T, Prenosil G, Benke D, Mohler H, Boison D (2009) Altered mnemonic functions and resistance to *N*-Methyl-D-Aspartate receptor antagonism by forebrain conditional knockout of glycine transporter 1. Neuroscience 161:635–654.

Shang J, Liu N, Tanaka N, Abe K (2012) Expressions of hypoxic stress sensor proteins after transient cerebral ischemia in mice. J Neurosci Res 90:648–655.

Shelton RC (2007) The molecular neurobiology of depression. Psychiatr Clin North Am 30:1–11.

Sokolov MV, Dickey JS, Bonner WM, Sedelnikova OA (2007) gamma-H2AX in bystander cells: not just a radiation-triggered event, a cellular response to stress mediated by intercellular communication. Cell Cycle 6:2210–2212.

Suri D, Veenit V, Sarkar A, Thiagarajan D, Kumar A, Nestler EJ, Galande S, Vaidya VA (2013) Early stress evokes age-dependent biphasic changes in hippocampal neurogenesis, Bdnf expression, and cognition. Biol Psychiatry 73:658–666.

Tang AC, Akers KG, Reeb BC, Romeo RD, McEwen BS (2006) Programming social, cognitive, and neuroendocrine

development by early exposure to novelty. Proc Natl Acad Sci USA 103:15716–15721.

Tirone F (2001) The gene PC3(TIS21/BTG2), prototype member of the PC3/BTG/TOB family: regulator in control of cell growth, differentiation, and DNA repair? J Cell Physiol 187:155–165.

Touyarot K, Poussard S, Cortes-Torrea C, Cottin P, Micheau J (2002) Effect of chronic inhibition of calpains in the hippocampus on spatial discrimination learning and protein kinase C. Behav Brain Res 136:439–448.

Tsankova NM, Berton O, Renthal W, Kumar A, Neve RL, Nestler EJ (2006) Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. Nat Neurosci 9:519–525.

Valjent E, Caboche J, Vanhoutte P (2001) Mitogen-activated protein kinase/extracellular signal-regulated kinase induced gene regulation in brain: a molecular substrate for learning and memory? Mol Neurobiol 23:83–99.

Vosler PS, Brennan CS, Chen J (2008) Calpain-mediated signalling mechanisms in neuronal injury and neurodegeneration. Mol Neurobiol 38:78–100.

Weaver IC, Meaney MJ, Szyf M (2006) Maternal care effects on the hippocampal transcriptome and anxiety-mediated behaviours in the offspring that are reversible in adulthood. Proc Natl Acad Sci USA 103:3480–3485.

Xia Z, Storm DR (2012) Role of signal transduction crosstalk between adenylyl cyclase and MAP kinase in hippocampus-dependent memory. Learn Mem 19:369–374.

Xu Z, Zhang Z, Shi Y, Pu M, Yuan Y, Zhang X, Li L, Reynolds GP (2012) Influence and interaction of genetic polymorphisms in the serotonin system and life stress on antidepressant drug response. J Psychopharmacol 26:349–359.