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**Cite this article:** Tinarelli F, Garcia-Garcia C, Nicassio F, Tucci V. 2014 Parent-of-origin genetic background affects the transcriptional levels of circadian and neuronal plasticity genes following sleep loss. *Phil. Trans. R. Soc. B* **369**: 20120471.

<http://dx.doi.org/10.1098/rstb.2012.0471>

One contribution of 14 to a Theme Issue 'Timing in neurobiological processes: from genes to behaviour'.

### Subject Areas:

genetics, neuroscience, behaviour

### Keywords:

parent-of-origin effects, sleep, gene, expression

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Electronic supplementary material is available at <http://dx.doi.org/10.1098/rstb.2012.0471> or via <http://rstb.royalsocietypublishing.org>.

# Parent-of-origin genetic background affects the transcriptional levels of circadian and neuronal plasticity genes following sleep loss

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Sleep homeostasis refers to a process in which the propensity to sleep increases as wakefulness progresses and decreases as sleep progresses. Sleep is tightly organized around the circadian clock and is regulated by genetic and epigenetic mechanisms. The homeostatic response of sleep, which is classically triggered by sleep deprivation, is generally measured as a rebound effect of electrophysiological measures, for example delta sleep. However, more recently, gene expression changes following sleep loss have been investigated as biomarkers of sleep homeostasis. The genetic background of an individual may affect this sleep-dependent gene expression phenotype. In this study, we investigated whether parental genetic background differentially modulates the expression of genes following sleep loss. We tested the progeny of reciprocal crosses of AKR/J and DBA/2J mouse strains and we show a parent-of-origin effect on the expression of circadian, sleep and neuronal plasticity genes following sleep deprivation. Thus, we further explored, by *in silico*, specific functions or upstream mechanisms of regulation and we observed that several upstream mechanisms involving signalling pathways (i.e. DICER1, PKA), growth factors (CSF3 and BDNF) and transcriptional regulators (EGR2 and ELK4) may be differentially modulated by parental effects. This is the first report showing that a behavioural manipulation (e.g. sleep deprivation) in adult animals triggers specific gene expression responses according to parent-of-origin genomic mechanisms. Our study suggests that the same mechanism may be extended to other behavioural domains and that the investigation of gene expression following experimental manipulations should take seriously into account parent-of-origin effects.

## 1. Introduction

Sleep is a genetically and epigenetically regulated phenomenon that is subjected to two fundamental processes: a homeostatic process and a circadian process [1]. The homeostatic process of sleep depends on previous wakefulness, representing the pressure for sleep according to the time of day. The circadian process dictates the timing of sleep; it is a self-sustained periodic mechanism that develops with approximately 24 h, cell-autonomous, oscillations. The molecular machinery that sets the circadian clock is composed of positive and negative feedback loops, which involve transcriptional and translational core elements within the cell (reviewed in [2]). Alternative translational and post-translational components participate in the fundamental modulatory mechanisms that maintain circadian timing. These activities involve several epigenetic changes, such as histone modification, acetylation and methylation [3,4], that modulate the dynamic on/off switches of physiological circadian sleep–wake processes [4]. Sleep homeostasis is biologically related to the circadian clock, and several clock gene mutations result in significant alterations to the electrophysiological measures of sleep [5–7].

Pioneering studies by Franken *et al.* [8] have shown that the genetics of mouse strains influence electrophysiological measures of sleep, for example slow oscillations in the delta frequency range (1–4 Hz), a fundamental measure of sleep intensity. Several studies in mice have shown that sleep deprivation induces changes in gene expression and it has been reported that the genetic backgrounds of mouse strains affect the transcriptional changes that follow sleep loss [9]. However, different studies have identified different classes of genes that depend on sleep [10]. Transcriptome analysis in three mice strains (AKR/J, C57BL/6J and DBA/2J) [9] described the *Homer1a* gene as an ideal sleep-dependent target that is rapidly and strongly induced by sleep deprivation in all strains.

In our study, we have tested for the first time whether the effect of genetic background on sleep-dependent gene expression is determined in a parent-of-origin manner. Parent-of-origin effects (i.e. genomic imprinting) have been suggested to modulate fundamental aspects of sleep. Clinical observations of neurodevelopmental sleep disorders suggest that genomic imprinting plays a pivotal role in the architecture of both rapid eye movement (REM) and non-REM (NREM) sleep [11–13]. Interestingly, diverse sleep deficits occur in diseases, such as Prader–Willi syndrome (PWS) and Angelman syndrome (AS), which are classically characterized by opposing imprinting profiles. PWS is caused by maternal duplications/paternal deletions of alleles on chromosome 15q11–13, whereas AS is associated with paternal duplications/maternal deletions on the same region, 15q11–13. The former is characterized by REM sleep abnormalities, excessive sleepiness and core temperature abnormalities [14–17], while the latter is characterized by reductions in sleep. Sleep abnormalities associated with the PWS/AS imprinting region may be linked to the *UBE3A* gene. Indeed, the lack of the maternal allele of *Ube3a* in mice results in reduced NREM sleep, deterioration in REM sleep and an increased frequency of waking during the dark-to-light transition [18]. Moreover, serotonin (5-HT) 2A receptors, mediating aminergic inhibition of REM-on cells [19], are primarily expressed by maternal alleles [20]. The importance of studying the link between parental genomic background and sleep has been emphasized by our recent study in mice [21]. We have shown that loss of imprinting of the maternally imprinted gene *Gnas* dramatically affects REM and NREM physiology in mice [21]. To test the hypothesis that parent-of-origin genetic background affects the expression of specific genes, determining the presence or the absence of a homeostatic response to sleep loss, we studied reciprocal crosses of two mouse strains that differ in their homeostatic responses to sleep deprivation: AKR/J and DBA/2J. These two strains have distinct delta-power profiles [8] and different gene expression responses [9,22] after sleep deprivation. While AKR/J mice exhibit dramatic increases in delta power after 6 h of sleep deprivation, DBA/2J mice present a milder response following the same deprivation protocol [8]. Furthermore, AKR/J mice show a greater increase in mRNA levels of core circadian clock genes, such as *Bmal1*, *Clock*, *Cry1*, *Cry2*, *Per1* and *Per2*, after 6 h of sleep deprivation than do DBA/2J mice [22].

The rationale for our study involves the phenotypic expression patterns of reciprocal heterozygous  $F_1$  mice. A parent-of-origin effect would lead to a differential phenotype (i.e. different gene expression) between two reciprocal  $F_{1S}$  (hereafter referred to as  $F_1$  and  $F_{1r}$ ). For the purpose of

this study, we screened a large list of genes that are involved in circadian, sleep, genomic imprinting and neuronal plasticity regulation in the prefrontal cortex (PFC), as this brain area has been closely linked to sleep function in mammals [23,24]. Remarkably, we detected a sleep-dependent modulation of certain genes that depends on an individual's parental background. This proves, for the first time, that parent-of-origin effects regulate specific sleep-dependent genetic mechanisms.

## 2. Material and methods

### (a) Animals and procedures

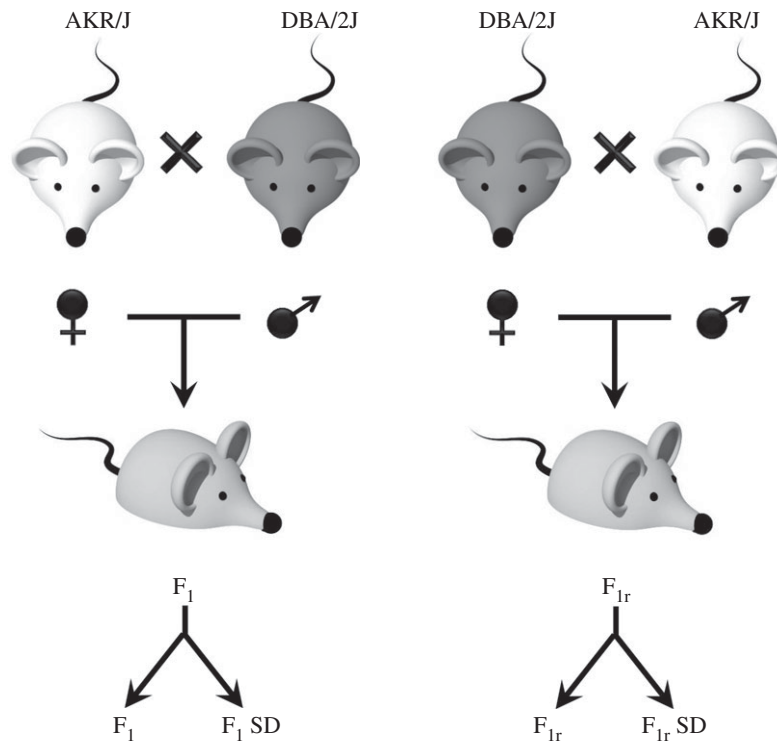
The initial AKR/J mouse strain was obtained from Jackson Laboratories (Bar Harbour, USA) and the DBA/2J strain was obtained from Charles River (Wilmington, USA). The mice were kept in an IIT (Istituto Italiano di Tecnologia) animal facility and bred in reciprocal crosses to obtain two different experimental cohorts, AKR/J $\times$ DBA/2J  $F_1$  mice (the maternal strain is reported first) and DBA/2J $\times$ AKR/J  $F_{1r}$  mice. Each cohort included a total of six males (13 weeks old) that were equally subdivided into a sleep-deprived (SD) group and a control group (figure 1). All mice were group-housed a week before the experiment, with food and water ad libitum, under a 12 L:12 D cycle (lights on from 7.00 to 19.00). On the day of the experiment, SD mice underwent 6 h of sleep deprivation starting at 7.00. At 13.00, SD was interrupted, and the mice were left undisturbed for 1 h before they were sacrificed and their PFC tissue was collected. Tissue from the control group was collected at the same time, but control group mice were not subjected to sleep deprivation. All sleep experiments were conducted in the home-cage environment and all procedures were performed under the guidance of the Italian Policy (licence number 039, expires on 15 June 2015).

### (b) Quantitative real-time PCR

Total RNA was extracted from approximately 1 g of snap-frozen PFC using the Rneasy Microarray tissue mini kit (Qiagen, Hilden, Germany). RNA samples were quantified with an ND1000 Nanodrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Reverse transcription of 1  $\mu$ g of RNA was performed using the RT<sup>2</sup> First Strand Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. RT-qPCR was conducted using a custom RT<sup>2</sup> Profiler PCR array for 234 imprinted, circadian and epigenetic-related genes, based on a 384-well plate format developed by Sabioscience Qiagen technical service (Carlsbad, USA; electronic supplementary material, tables S1 and S2). Reconfirmation experiments for the genes of interest were performed using a different set of primers (table 1). RT-qPCR was performed on a ViiA 7 Real-time System machine (Applied Biosystem, Foster City, CA, USA) using the following conditions: 10 min at 95°C, 40 cycles of denaturation at 95°C for 15 s and an annealing and extension step at 60°C for 1 min. Each sample was run to obtain average Ct values according to the manufacturer's specifications. All samples were normalized against a panel of four different housekeeping genes: *Gapdh*, *Gusb*,  *$\beta$ -actin* and *B2M*. Expression levels relative to these housekeeping genes were determined by the calculation of  $\Delta$ Ct, and the data are expressed as  $2^{-\Delta\Delta Ct}$ , where  $\Delta\Delta Ct$  is the difference between the SD and not-SD cohorts.

### (c) Statistical analysis

Statistically significant gene expression differences between SD and not-SD mice were visualized by pooling together the two normal sleep cohorts as a control group ( $F_1$  and  $F_{1r}$ ) (figure 2c).



**Figure 1.** The creation of reciprocal cohorts permits the investigation of the role of parental epigenetic controls after SD. A schematic of experimental design and reciprocal crossing mating is presented. The  $F_1$  generation was obtained from an AKR/J $\times$ DBA/2J crossing, and the  $F_{1r}$  generation was obtained from a DBA/2J $\times$ AKR/J breeding. Males (13 weeks old) from both  $F_1$  and  $F_{1r}$  were used as follows: three were maintained under sleep deprivation (SD), and three were subjected to a standard sleep pattern protocol as controls.

**Table 1.** Primers used for the confirmation of the presence of the genes of interest.

primer	sequence
<i>Rian</i> forward	AGGATTGATTGTGCTGTAGAGT
<i>Rian</i> reverse	CCTCACTGTCTCCATTCCAA
<i>Dlk1</i> forward	ACAATGGAACCTGCGTGGA
<i>Dlk1</i> reverse	CTTGTGCTGGCAGTCCTT
<i>Mtrnr2</i> forward	AAAGGAGGGTTCAACTGTCT
<i>Mtrnr2</i> reverse	CCAAGGGTCTTCTCGTCTT
<i>Prok2</i> forward	TGCTGTGCTGCAGTATCT
<i>Prok2</i> reverse	TCTTCTTCTGCCTTCCA
<i>Per2</i> forward	AGTACACCACCCCTTACAAGCT
<i>Per2</i> reverse	GACACGGCAGAAAAAGATTCTC
<i>Egr1</i> forward	CCTATGAGCACCTGACCACAGAGT
<i>Egr1</i> reverse	CTCGTCTCCACCATCGCCTTCT
<i>Fos</i> forward	ACAGCCTTCTACTACCAT
<i>Fos</i> reverse	GCACTAGAGACGGACAGA
<i>Gapdh</i> forward	GAACATCATCCCTGCATCCA
<i>Gapdh</i> reverse	CCAGTGAGCTTCCCGTTCA
$\beta$ -Actin forward	AAGTGTTACAGGAAGTCC
$\beta$ -Actin reverse	ATAATTTACAGAAAGCAATGC

Statistically significant parent-of-origin-regulated genes were identified by splitting the controls of these sleep-modulated genes and comparing the fold change of  $F_1$  SD/ $F_1$  versus  $F_{1r}$  SD/ $F_{1r}$  (figure 2d). In both cases, a two-way ANOVA

with Bonferroni's multiple comparison test analysis was performed ( $*p < 0.05$ ;  $**p < 0.01$ ;  $***p < 0.001$ ). All average data are presented as the mean  $\pm$  s.e.m.

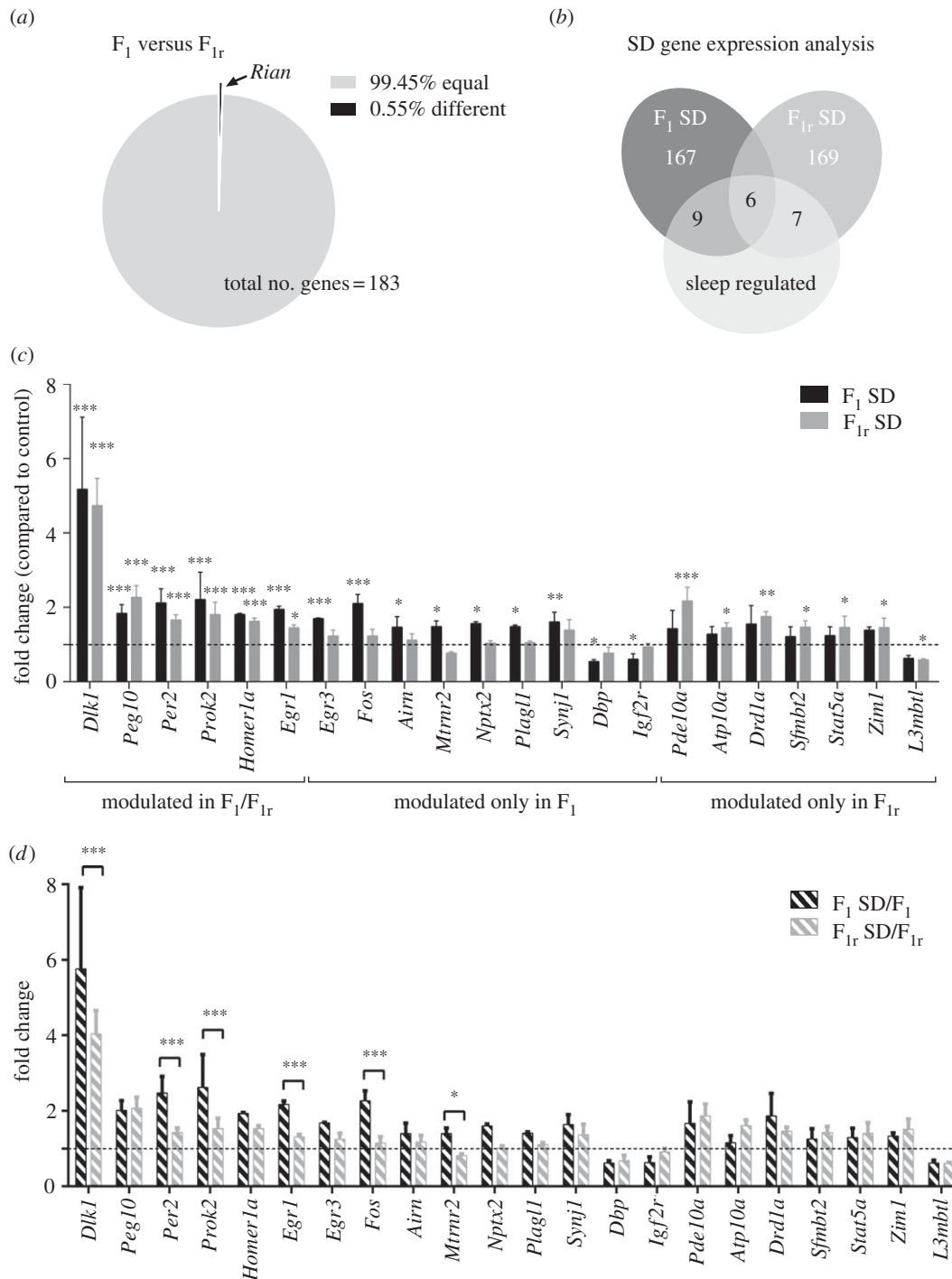
#### (d) Ingenuity pathway analysis

Significantly enriched functional classes and upstream regulators (figure 4 and electronic supplementary material, figure S1) were identified through Ingenuity Pathway Analysis (Ingenuity Systems, www.ingenuity.com) running a core-analysis using as input the 22 regulated genes detected in PFC samples of  $F_1$  SD/ $F_{1r}$  SD reciprocal crosses and the initial set of 230 genes as background. A complete list of all the identified significant classes is reported in the electronic supplementary material, table S3.

We distinguished the genes that were regulated in  $F_1$  SD from those regulated in  $F_{1r}$  SD to calculate the enrichment values in the Upstream analysis. Some representative classes are shown in figure 4b.

### 3. Results and discussion

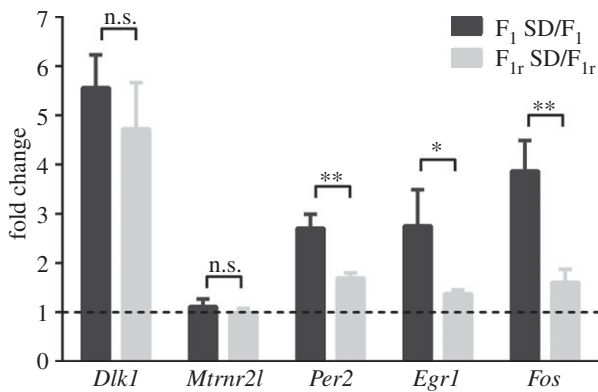
Of the 230 genes selected across genomic imprinting, circadian clock and neural plasticity domains (see electronic supplementary material, tables S1 and S2), 20% (47 genes) presented very low expression levels ( $Ct \geq 30$ ) in our PFC samples. We compared the gene expression values of the other 80% (183 genes) between  $F_1$  and  $F_{1r}$  control mice and no differences, with the exception of the expression of the *Rian* gene, were observed between the two control groups (figure 2a). The *Rian* gene was retested in a subsequent RT-PCR experiment with the second set of primers (table 1), and non-statistically significant differences were found between the two  $F_1$  progenies (data not shown). These results suggest that, under a normal sleep regimen, we observed no parental effects on gene expression.



**Figure 2.** Gene expression of 230 genes in F<sub>1</sub> and F<sub>1r</sub> mice cohorts after sleep deprivation. (a) Representation of the statistical analysis of the gene expression of 183 detectable targets (Ct ≤ 30) among the F<sub>1</sub> and F<sub>1r</sub> cohorts. (b) Visual classification of the gene expression profiles of F<sub>1</sub> and F<sub>1r</sub> animals after SD. Sleep-regulated genes compose 8.2% and 7.1% of the total genes of the F<sub>1</sub> and F<sub>1r</sub> groups, respectively. (c) Gene expression levels were quantified using Qiagen RT-PCR custom plates. Bars represent the mean + s.e.m. of three different samples for F<sub>1</sub> SD and F<sub>1r</sub> SD mice. \**p* < 0.05; \*\**p* < 0.01; \*\*\**p* < 0.001 by two-way ANOVA plus Bonferroni's post-test. (d) Parent-of-origin regulation of sleep-dependent genes among F<sub>1</sub> SD/F<sub>1</sub> and F<sub>1r</sub> SD/F<sub>1r</sub>. Bars represent the mean + s.e.m. of three different samples for F<sub>1</sub> SD and F<sub>1r</sub> SD mice related to their control groups (F<sub>1</sub> or F<sub>1r</sub>, respectively). \**p* < 0.05; \*\**p* < 0.01; \*\*\**p* < 0.001 by two-way ANOVA plus Bonferroni's post-test.

In order to maximize the effect of sleep deprivation, we pooled together the two normal sleep groups into one group (referred to as a unique 'control' group). Two-way ANOVA statistical analysis with Bonferroni's multiple comparisons of the 182 detectable genes revealed minimal gene expression changes in both SD progenies with respect to control. Specifically, 15 (8.2%) and 13 (7.1%) genes were sleep modulated in F<sub>1</sub> and F<sub>1r</sub>, respectively, with six genes in common between the two cohorts (figure 2*b,c*). Among the transcripts that were overexpressed after sleep deprivation

in both F<sub>1</sub> and F<sub>1r</sub> progenies, we found *Homer1a* and *Per2*, which confirms the findings of previous studies that these genes have fundamental roles in sleep homeostatic mechanisms [9,25,26]. *Homer1a* upregulation was lessened in DBA/2J mice when compared with AKR/J mice [27] immediately after sleep deprivation. *Per2* has also been described as differentially upregulated between the two strains [26] in the same conditions. One hour after sleep deprivation, we observed differences in the fold changes of both *Homer1a* and *Per2* gene expression levels in the reciprocal crosses of the two



**Figure 3.** Parent-of-origin regulation of sleep-dependent gene expression. The genes shown were quantified by RT-PCR with the second set of primers (table 1). Bars represent the mean + s.e.m. of three different samples for F<sub>1</sub> SD and F<sub>1r</sub> SD mice related to their control groups (F<sub>1</sub> or F<sub>1r</sub>, respectively). \**p* < 0.05; \*\**p* < 0.01; \*\*\**p* < 0.001 by Bonferroni's test. n.s., Non-significant.

strains (figure 2c). The other four genes modulated by sleep depletion in both progenies were *Dlk1*, *Peg10*, *Prok2* and *Egr1* (figure 2c). We also found nine genes that were differentially regulated in AKR/J×DBA/2J F<sub>1</sub> SD (*Egr3*, *Fos*, *Airn*, *Mtrnr2*, *Nptx2*, *Plagl1*, *Synj1*, *Dbp* and *Igf2r*) and seven genes that were differentially regulated in DBA/2J×AKR/J F<sub>1r</sub> SD (*Pde10a*, *Atp10a*, *Drd1a*, *Sfmbt2*, *Stat5a*, *Zim1* and *L3mbtl*; figure 2b,c).

By splitting the control group in F<sub>1</sub> and F<sub>1r</sub>, we studied whether these sleep-deprivation-regulated genes are controlled in a parent-of-origin manner. Comparing the fold change of F<sub>1</sub> SD/F<sub>1</sub> versus F<sub>1r</sub> SD/F<sub>1r</sub>, we found that *Dlk1*, *Per2*, *Prok2*, *Egr1*, *Fos* and *Mtrnr2* regulation 1 h after sleep deprivation was significantly different among reciprocal crosses (figure 2d); therefore, these genes are subjected to a parent-of-origin regulation after sleep deprivation.

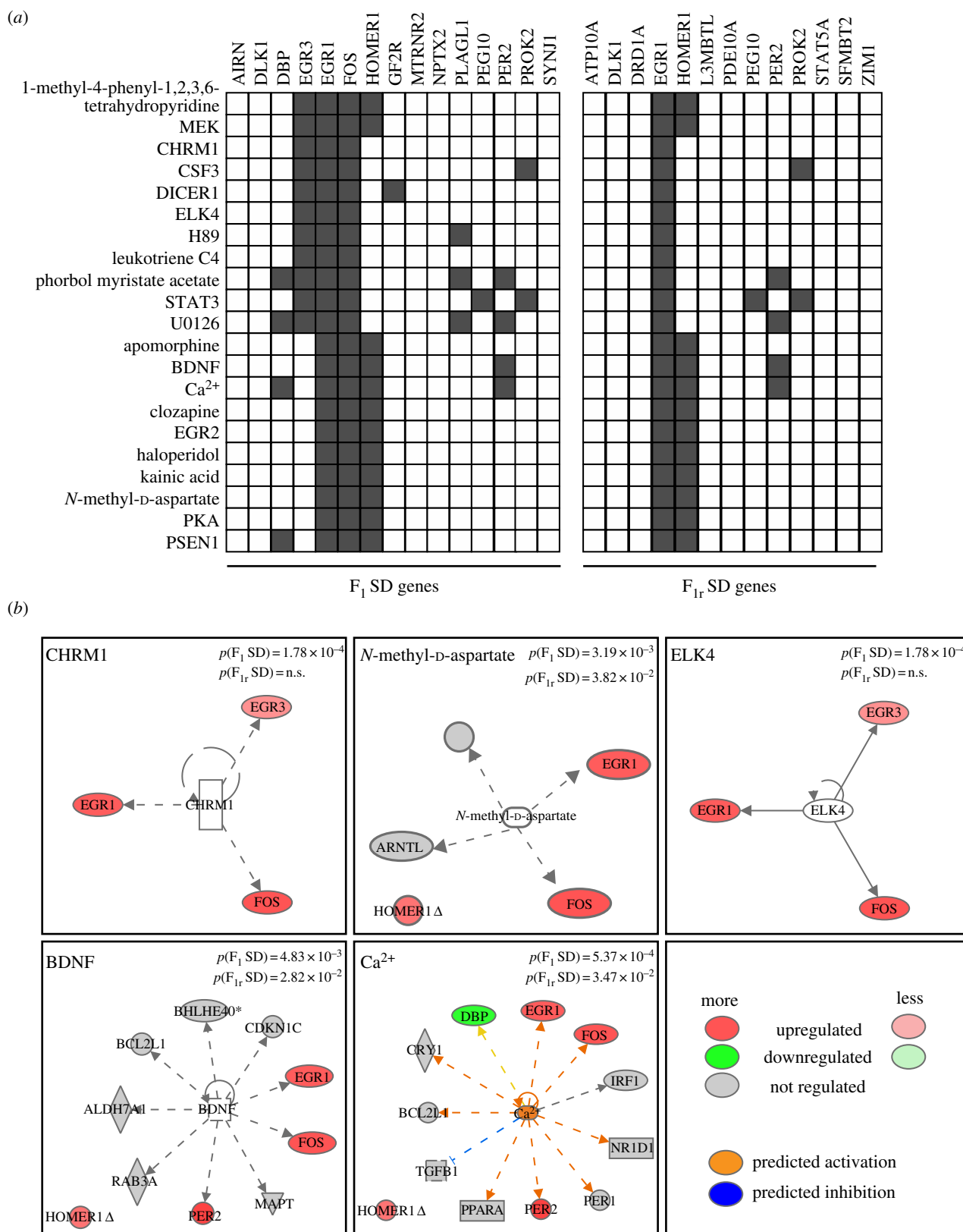
Furthermore, we tried to confirm the differential regulation of these six genes between the reciprocal crosses by repeating the RT-PCR with a different set of primers (figure 3 and table 1). In this second analysis, non-statistical differences were found between offspring after sleep deprivation in *Dlk1* and *Mtrnr2* expression (figure 3), whereas *Prok2* was not detected (data not shown). On the other hand, the rest of genes tested were consistent with the previous observation. Figure 3 shows that *Per2*, *Egr1* and *Fos* are upregulated after 1 h of sleep rebound following sleep deprivation in the F<sub>1</sub> progeny (F<sub>1</sub> SD/F<sub>1</sub>) but not in the reciprocal cohort (F<sub>1r</sub> SD/F<sub>1r</sub>). This result demonstrates that *Per2*, *Egr1* and *Fos* are genes subjected to a parent-of-origin regulation following sleep loss.

*Per2* is a core regulator of the circadian clock machinery and has been described as differentially regulated between the two parental strains used in this study. Specifically, *Per2* upregulation was lessened in AKR/J mice when compared with DBA/2J mice [26]. This effect was detected both after sleep deprivation and 2 h from the end of deprivation. In our study, we showed that *Per2* is modulated by sleep deprivation in both reciprocal crosses of these strains; however, this regulation is genotype dependent. This confirms the existence of a parent-of-origin regulation of this gene under sleep deprivation. Moreover, *Fos* and *Egr1* are immediate early genes (IEGs) that were previously reported to respond to sleep deprivation according to an individual's specific genetic background [9]. Following sleep deprivation, expression levels of *Fos*, *Egr1*

and *Egr3* were reported to significantly increase in AKR/J mice but not in DBA/2J mice [9]. *Egr1* and *Egr3* exhibit circadian oscillations with differential regulation between light and dark periods in the suprachiasmatic nucleus of the hypothalamus, the master clock of the body [28,29]. Both *Egr1* and *Fos* are reported to respond to the photic phase shift of the circadian clock [30]. The light intensity required to induce *Egr1* expression can be 10 times less than the amount necessary to produce a circadian phase shift [30]. *Egr1* and *Egr3* are characterized by peculiarly timed regulatory mechanisms. *Egr1* and *Egr3* mRNA levels peak 30–60 min after seizure activity in hippocampus granule cells [31], while their protein levels peak at different time scales: EGR1 protein levels peak 1 h after treatment and return to background levels in 3–4 h, while EGR3 protein levels peak after 4–6 h and basal levels are restored after 24 h [31]. The different temporal patterns of the molecular circuits of *Egr1* and *Egr3* could represent a common genetic mechanism that, at a cellular level, acts at different timescales.

Our study demonstrates for the first time that the involvement of certain genes in sleep homeostatic mechanisms is parent-of-origin dependent. Thus, we further explored whether the list of parent-of-origin-regulated genes observed in our study might be implicated in specific functions or upstream mechanisms of regulation. We performed a functional analysis searching for classes that are significantly enriched for either F<sub>1</sub> SD- or F<sub>1r</sub> SD-regulated genes (see electronic supplementary material, table S3). We observed that several differentially enriched upstream mechanisms (see the electronic supplementary material, figure S1 for the complete list) involving these IEGs in the F<sub>1</sub> SD include chemicals (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, H89, leukotriene C<sub>4</sub>, phorbol myristate acetate, U0126, apomorphine, clozapine, haloperidol, kainic acid, *N*-methyl-D-aspartate (NMDA) and Ca<sup>2+</sup>), signalling pathways (CHRM1, DICER1, PKA and PSEN1), growth factors (CSF3 and brain-derived neurotrophic factor (BDNF)) and transcriptional regulators (EGR2 and ELK4) (figure 4a). Relevant gene networks for the identified upstream regulators are depicted in figure 4b. Most of these gene networks are related to synaptic transduction, which suggests that specific parent-of-origin mechanisms can modulate sleep-dependent synaptic plasticity mechanisms in the brain. For example, NMDA is the agonist for the ionotropic glutamate receptor NMDAR, which is a pivotal ion channel implicated in the regulation of synaptic functions in the central nervous system [32]. The G protein-coupled receptor CHRM1 (cholinergic receptor muscarinic 1) can also modulate neuronal excitability and synaptic transmission [33] by interacting with glutamatergic neurotransmitter systems, for example NMDARs [34,35], and by potentiating or inhibiting NMDARs in a cell-dependent manner [36].

Ca<sup>2+</sup> influx through NMDARs is essential for long-lasting changes in synaptic efficacy, such as long-term potentiation (LTP) and long-term depression [37]. Calcium is widely known to be a major player in neuronal intracellular communication and signalling processes capable of activating and promoting gene transcription in the nucleus. Indeed, Ca<sup>2+</sup> influx in the postsynaptic terminal via NMDARs and L-type voltage activates calcium channels and stimulates the production of the second messenger 3'-5'-cyclic adenosine monophosphate (cAMP) by adenylyl cyclase. cAMP activates PKA (protein kinase A), among other important targets in memory processing. Once activated, PKA and other plasticity-associated kinases can phosphorylate and activate the cAMP response element-binding protein (CREB), which



regulates the transcription of genes involved in synaptic plasticity, memory and cell survival [38–41]. BDNF is largely expressed in the nervous system [42]. BDNF regulates several aspects of neurodevelopment and synaptic plasticity (see [43] for review), and BDNF-mediated signalling induces synaptic potentiation and plasticity in cortical networks during

wakefulness, playing a crucial role in the synaptic homeostasis regulation of sleep [44]. *Egr1* and *Fos* are tightly linked to neuronal plasticity and memory [45,46]. *Egr1* is implicated in the maintenance of synaptic plasticity and is necessary for the persistence of LTP [47] and the consolidation of different forms of long-term memory as well as during the transition

from short- to long-term memories [48,49]. Interestingly, sleep is significantly involved in the regulation of brain plasticity and cognition (see [50] for review). A series of studies have shown that PKA and CREB signalling pathways promote wakefulness [51]. The same results were obtained in mice lacking two of the three isoforms of CREB: the loss of alpha and delta causes a reduction in CREB activity and a reduction in wakefulness during the light-off period, with respect to controls [52–54].

Although our study focused only on few genes, our functional analysis identified specific domains such as behaviour, neurological diseases and cell death and survival as enriched processes involving these IEGs (see electronic supplementary material, table S3). Our study suggests that the role of sleep in neuroprotection can be determined by parental epigenetic mechanisms. Indeed, *Homer1a* is implicated in intracellular calcium homeostasis and sleep restorative mechanisms [9], and *Egr1* and *Fos* are involved in molecular neuroprotective responses, for example those triggered by ischaemia [55]. Altogether, these data indicate that sleep restriction activates molecular pathways associated with the preservation of neuronal integrity [56].

In our study, we concentrated on the PFC, one of the main targets of the restorative effects of sleep on cognition [57]. The PFC is pivotal in coordinating high-level cognitive processes, such as response inhibition, higher order attention processes, working memory and episodic learning memory [58–62]. Different phases of sleep were reported to be associated with specific activation and deactivation modes of PFC regions [63,64]. Moreover, the disruption or the alteration of normal sleep–wake cycles or circadian rhythms delayed the time required by the PFC to achieve the attention levels of other brain cortical regions [65]. In addition, sleep deprivation alters the neuronal functionality and gene expression profile in the PFC [66–68]. The evidence that neuronal plasticity genes (and possibly many neuronal plasticity pathways) are differently regulated in the PFC according to parent-of-origin mechanisms casts a new light on the epigenetic regulation of these genes in sleep and sleep-related functions.

**Acknowledgements.** We thank Raquel Garcia Garcia for graphical support. We also thank Glenda Lassi for reading and discussion of the manuscript, Riccardo Navone and Daniela Cantatore for assistance with the management of the mouse colonies.

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