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Transcriptomic Changes in Rat Cortex and Brainstem After Cortical Spreading Depression With or Without Pretreatment With Migraine Prophylactic Drugs

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Abstract: **Migraine with aura is a subtype of migraine characterized by transient neurological disturbances that usually precede headache. Cortical spreading depression (CSD) is the likely pathophysiological correlate of the aura phase of migraine, found in common and rare forms of migraine, such as familial hemiplegic migraine. CSD is a depolarization wave that propagates across the cerebral gray matter transiently suppressing neuronal activity. Prophylactic treatments for migraine, such as topiramate or valproate, reduce the number of CSD events. We evaluated changes in gene expression in rat cortex and brainstem after inducing CSD in the cortex, with and without a prophylactic treatment with topiramate or valproate. CSD induction showed similar transcriptomic profiles with and without treatment in cortex, involving genes related to hormone stimulus, apoptosis, synaptic transmission, and interleukin signaling. In brainstem, CSD with and without treatment, although to a lesser extent, also induced gene expression changes involving genes related to apoptosis. Half of the genes altered in brainstem after CSD were also differentially expressed in the same direction in cortex. No differences in gene expression were identified after CSD as a consequence of the treatments, neither in cortex nor in brainstem.**

Perspective: ***Our results suggest that early after triggering the CSD, similar consequences are seen at the genetic level with or without prophylactic treatment. Gene expression changes induced by CSD in cortex and brainstem may help to better understand the underlying mechanisms and identify targets for therapeutic approaches.***

Key words: *Cortical spreading depression, migraine, transcriptomics, topiramate, valproate.*

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Migraine is a disabling neurological disorder that manifests with recurrent and episodic headache attacks accompanied by other features such as nausea or photo- and phonophobia. Common forms of the disorder can be classified into two main types, with and without aura.[15] Migraine aura is a neurological phenomenon characterized by transient neurological disturbances that precede or accompany headache. Cortical spreading depression (CSD) is the likely electrophysiological correlate of migraine aura.[14,16,21] CSD is a neuronal phenomenon consisting of a short-lasting slow depolarization wave usually originated in the occipital cortex that moves forward, and involves changes at different levels, such as the neuronal, glial, and vascular systems. It is followed by a suppression of neuronal activity and local changes in the cerebral blood flow, and it has also been described in patients with stroke or brain injury.[7,8] It has been proposed that CSD might be able to activate the trigeminovascular system, innervating the meningeal blood vessels, and this activation is thought to be one of the mechanisms that underlie migraine pain.[13,20] It is hypothesized that CSD, when ignited, drives the migraine generator network, which involves brainstem nuclei, trigeminal nerve, and parasympathetic vasomotor efferents.[9] Conversely, the brainstem region is thought to be involved in the modulation of the migrainous cascade and a putative aura site in the migraine with brainstem aura (formerly basilar-type migraine) variant. Moreover, brainstem areas remain active during the pain phase of migraine and even after its successful treatment.[13] However, the whole development of the CSD and aura and related changes are still not well understood.

Murine animal models for CSD have been developed during the past years. CSD can be evoked by electrical or chemical stimulation.[6] Several studies have revealed gene expression changes in rat brain samples caused by CSD, mainly involving early genes and transcription factors, genes related to inflammatory response or synapse, among others.[17,26]

Prevention of CSD can be approached by migraine prophylactic drugs, some of them proven to suppress this phenomenon in animal models with a dose-dependent effect on the number and frequency of CSD events, such as the anticonvulsants topiramate (TPM) and valproate (VPA), when used as prophylactic treatments for migraine.[1,3,4,25] Moreover, the effect on gene expression of TPM and VPA has also been studied using different approaches and models, in vitro and in vivo, and these reported gene expression differences of genes related to neuronal differentiation, synaptic transmission or apoptosis.[2,5,11,19,27,28]

In this study, we aimed at performing a transcriptomic analysis in cortex and brainstem in a rat model of CSD, previously established,[6,26] to study short-term changes in gene expression induced by CSD with or without previous prophylactic treatment with TPM or VPA.

Methods

Experimental Groups

Thirty-six adult male Sprague Dawley rats (Charles River Laboratories, Barcelona, Spain) received single daily intraperitoneal injections between 9 and 10 AM for 4 weeks with saline (Fisiológico Braun .9%, B. Braun, Madrid, Spain), TPM (80 mg/kg; gift from Janssen-Cilag, Madrid, Spain), or VPA (200 mg/kg; Sanofi-Aventis, Barcelona, Spain); chosen rat strain and drug dosages were on the basis of published data.[3] The animals weighed between 215 and 360 g at the beginning of the treatment period and between 290 and 470 g on the day of recordings. Rats were randomly divided into 4 experimental groups (n = 9 per group): 1) saline + sham group: rats were injected with saline, surgery was performed but CSD was not induced; 2) saline + CSD group: rats were injected with saline, surgery was performed and CSD was induced in the right hemisphere; 3) TPM + CSD group: rats were treated with TPM, surgery was performed and CSD was induced in the right hemisphere; 4) VPA + CSD group: rats were treated with VPA, surgery was performed and CSD was induced in the right hemisphere. Animals were housed in cages of 4 in a temperature- and humidity-controlled facility with a 12-hour light cycle and with ad libitum access to food and water. The average weight of the animals was 406 ± 30 g for the saline + sham group, 396 ± 37 g for the saline + CSD group, 367 ± 41 g for the TPM + CSD group, and 387 ± 19 g for the VPA + CSD group. All experimental procedures were approved by the institutional ethics committee and guidelines for animal care were strictly followed.

Surgery and Electrophysiological Recordings

On the day of the experiment, rats were moved to the laboratory and were anesthetized using vaporized isoflurane (4.5% induction, 1–1.5% maintenance, in 70% N_2O/30% O_2), as previously reported,[3] and temperature was kept at 37°C. Two craniotomies were performed over the right hemisphere for KCl application and electrophysiological recordings (see Fig 1). Cortical direct current potential shifts were recorded with glass micropipettes 500 μm below the pial surface. After surgical preparation, the cortex was allowed to recover for 15 minutes under saline irrigation. CSDs were induced by placing a cotton ball soaked with KCl 1 M over the pial surface and was kept moist with 5 μL every 15 minutes. The total number of KCl-induced CSDs was counted during 1 hour of KCl application.

Preparation of Samples and RNA Isolation

After 1 hour of recordings, animals were euthanized by decapitation and brains were gently removed from the skull. Right cortices (CSD-induced), left cortices, and brainstems (including pons, medulla, and trigeminal nucleus caudalis) were dissected, flash

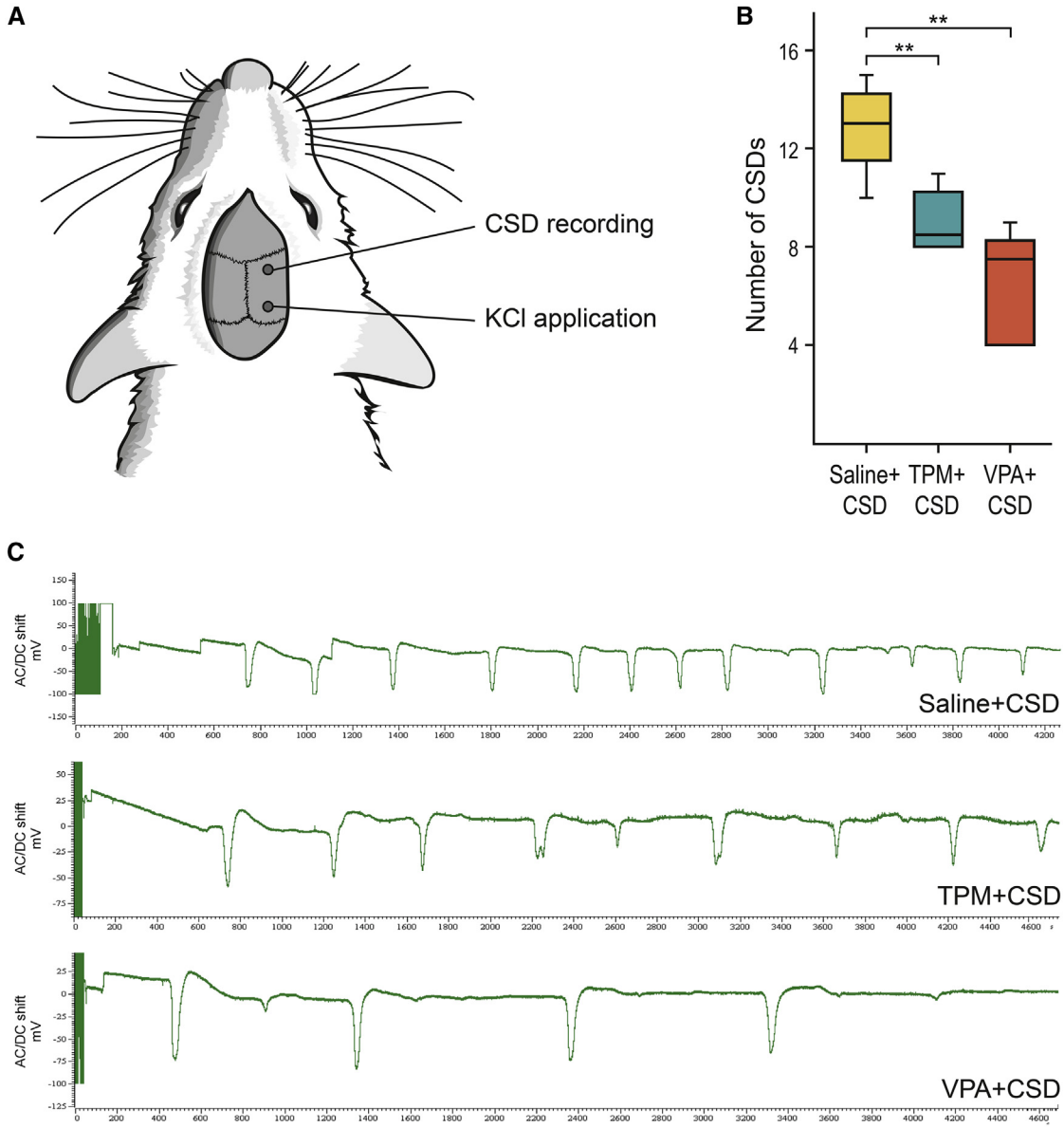

Figure 1. CSD induction and recordings. **(A)** Figure depicting surgical procedures for the CSD induction and recordings. Two burr holes were drilled over the right hemisphere at the following coordinates (mm from bregma): (1) posterior 5, lateral 2 for KCl application site; and (2) posterior .5, lateral 2 for recording site. **(B)** Boxplot indicating the number of CSDs that occurred during 1 hour of KCl induction in the saline, TPM and VPA groups. **(C)** Examples of cortical direct current potential shifts recorded during 1 hour in 1 animal of the saline (12 CSDs), TPM (9 CSDs), and VPA groups (4 CSDs). Significant differences compared with the (saline + CSD) group are indicated (**$P < .01$). Abbreviations: AC/DC, alternating current/direct current; mV, millivolts.

frozen, and mechanically homogenized in liquid nitrogen with a mortar and pestle. Samples from right and left cortex and brainstem from a total of 36 rats were homogenized using the TissueRuptor system (Qiagen, Düsseldorf, Germany) and total RNA was isolated using the RNeasy Lipid Tissue Mini Kit (Qiagen) according to the manufacturer's protocol. RNA concentration was determined using the NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE), and integrity was evaluated using the Bioanalyzer 2100 platform (Agilent Technologies, Santa Clara, CA).

Microarray Hybridization

For the microarray experiment, 6 rats from each of the 4 groups were randomly selected. Right cortex and brainstem samples were used for the hybridizations. Samples from the remaining rats as well as left cortex samples from all animals were kept for later validation experiments. For this study we used the GeneChip Rat Genome 230 2.0 (Affymetrix, Santa Clara, CA), analyzing >30,000 transcripts and variants from 28,000 rat genes. A total of 48 chips were used in this study and hybridized with 500 ng of total RNA at the Genomics Unit of

Hospital Clínic-Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS, Barcelona, Spain). Microarray chips were scanned using a GeneChip Scanner 3000 7G System AutoLoader (Affymetrix), and Affymetrix Expression Console software v 1.2 (Affymetrix) was used to obtain raw data.

Statistical and Bioinformatic Analyses

Number of CSDs were compared between groups using the nonparametric Mann–Whitney U test, considering 1-tailed test and setting significance at $P < .05$.

Bioconductor software (www.bioconductor.org) and affy library for R environment were used to analyze the microarray data from the right cortex and brainstem samples. Quality assessment, background correction, and normalization were performed as previously described.[12] For the gene filtering we considered a signal threshold of $\log_2(100)$ and an interquartile range (IQR) >20% for variability filtration.

Class comparisons were performed for right cortex and brainstem samples separately. We used the Linear Modeling for Microarray Analysis (limma) package to compare the expression patterns among the different groups: saline + CSD versus saline + sham, TPM + CSD versus saline + sham and VPA + CSD versus saline + sham comparisons were made to assess the effect of the CSD on gene expression; TPM + CSD versus saline + CSD and VPA + CSD versus saline + CSD comparisons to assess the possible effect of CSD after previous treatment versus CSD itself; and TPM + CSD versus VPA + CSD comparison to look for potential differences between the two treatments in rats that underwent CSD. Correction for multiple testing was done by adjusting the *P* values with a false discovery rate (FDR) of 5%.

The lists of genes showing significant differential expression for every comparison in the right cortex or in the brainstem were subjected to bioinformatic analyses using different tools: DAVID Annotation Tool (david.abcc.ncifcrf.gov) for functional group overrepresentation considering Gene Ontology (GO) biological process terms; and Ingenuity Pathway Analysis (IPA) software (2013 release) to analyze enrichment of canonical pathways and gene networks (Ingenuity Systems, Redwood City, CA).

Quantitative Reverse Transcription Polymerase Chain Reaction

Validation of gene expression changes was performed in all samples previously used for the microarray hybridization and in 3 additional samples from each group, a total of 9 samples per condition. For the validation experiments we selected genes showing differential expression in the right cortex using the following criteria: 1) fold-change >1.6, 2) significant differential expression in all conditions with CSD compared with the saline + sham group, 3) present in the most enriched GO categories and/or the best scored networks. In the brainstem, because the low number of differentially expressed genes precluded a proper bioinformatic analysis, only criteria 1) and 2) were considered, and we selected genes that were also chosen for validation in the cortex. Overall, a total of 7 genes were selected for validation in cortex samples (*Gadd45g*, *Sgk1*, *Egr2*, *Egr4*, *Crem*, *Homer1*, and *Ntrk2*). Two of them were also selected for validation in brainstem samples (*Gadd45g* and *Sgk1*). For normalization we considered 16 housekeeping genes and chose 2 that did not change across conditions. The genes *Ywhaz* and *Pgk1* were assessed using quantitative reverse transcription (qRT) polymerase chain reaction (PCR) and when confirmed to remain unaltered they were used for normalization. Additionally, genes validated in the right cortex were also assessed in left cortex samples of the same animals, in which CSD was not induced.

Reverse transcription from total RNA samples was performed with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster city, CA) in all 9 samples from each group. For real-time PCR experiments we used the LightCycler 480 II system (Roche Applied Science, Penzberg, Germany) and the assays were designed with the Universal ProbeLibrary Assay Design Center software (Roche Applied Science; www.roche-applied-science.com). Statistical analyses were performed using the nonparametric Mann–Whitney U test, setting significance at $P < .05$.

Results

CSD Induction and Effect of Treatment on CSD

Four groups of rats were obtained according to the induction of CSD and the previous drug treatment they received during 4 weeks: saline + sham, saline + CSD, TPM + CSD, and VPA + CSD. Animals did not show CSD induction under saline irrigation during 15 minutes, before KCl induction. Registers of right cortical direct current potential shifts after induction with KCl showed that treatment reduced CSD frequency, as previously described (Figs 1B and 1C).[3] When comparing the number of CSDs that occurred per hour, statistically significant differences were detected in the saline + CSD group (Median [IQR]: 13.0 [11.5–14.2] CSDs) compared with the TPM + CSD (8.5 [8.0–10.2] CSDs, $P = .00523$) and the VPA + CSD (7.5 [4.0–8.2] CSDs, $P = .00256$) groups (Fig 1B). No statistically significant differences were observed between the TPM + CSD and VPA + CSD groups. No CSDs were registered in left cortices of other experimental subjects when we induced CSDs in the right cortex (data not shown).

Transcriptional Changes Induced by CSD With or Without Treatment

To assess possible transcriptional changes caused by the induction of CSD in cortex, with or without previous prophylactic treatment (TPM or VPA), gene expression profiles of right cortex and brainstem from the four experimental groups (6 animals per group) were compared using microarray technology. First, the CSD effect was assessed comparing saline + CSD and sham + CSD groups, and then the effect of CSD with or without previous treatment was evaluated comparing saline + CSD with the TPM + CSD or VPA + CSD groups.

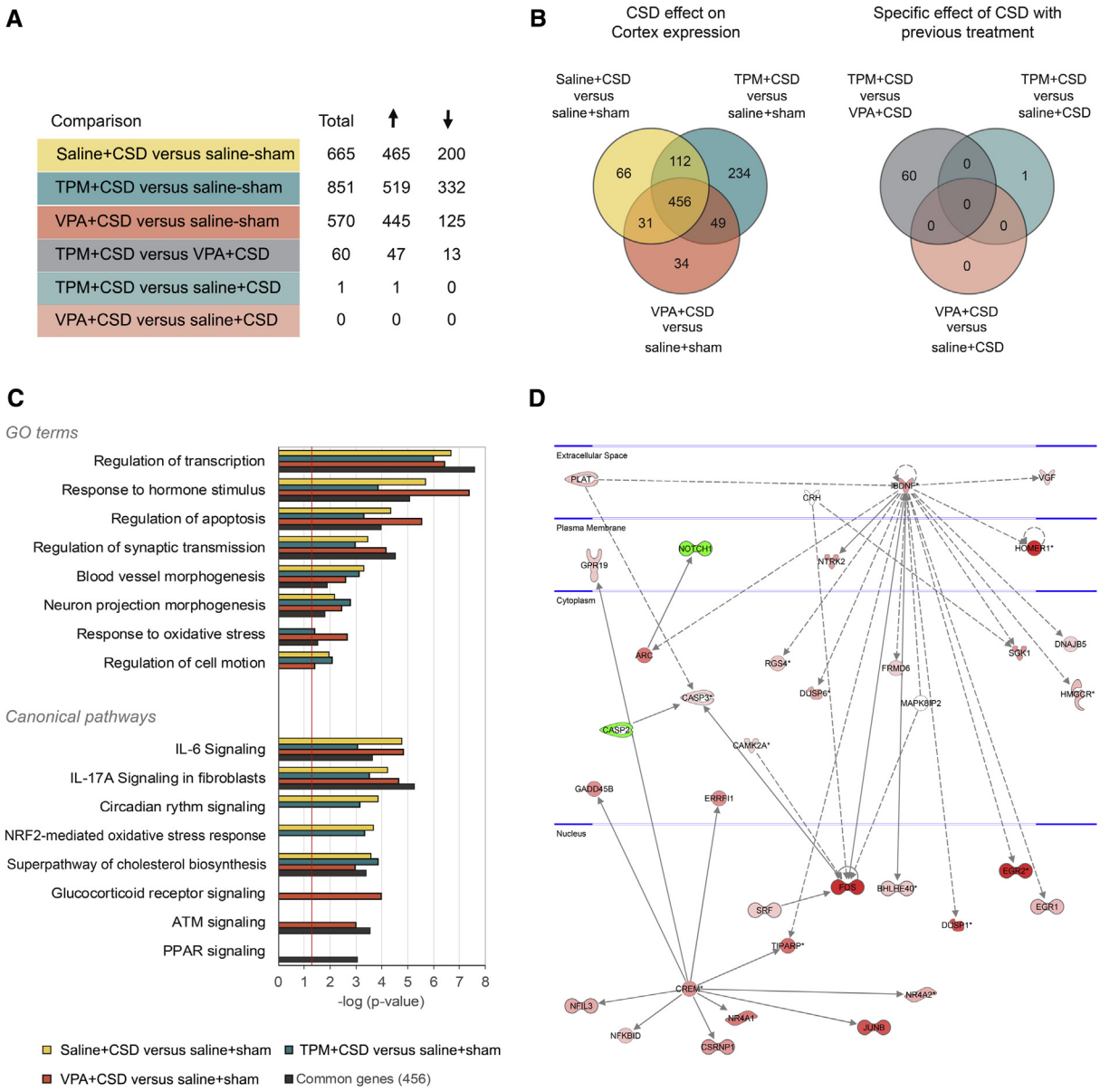

Figure 2. Gene expression changes in the right cortex induced by CSD with and without prophylactic treatment with TPM or VPA (n = 6 per group). **(A)** Total number of genes that are upregulated or downregulated identified in each comparison. **(B)** Venn diagrams of genes differentially expressed in each comparison. Intersections show genes for which expression is altered in more than 1 comparison. **(C)** Most enriched GO terms and canonical pathways identified in the genes that show differential expression after CSD. The red line indicates the significance threshold. **(D)** Best scored gene network in the comparison (saline + CSD vs saline + sham), which is involved in behavior, nervous system development, and function and hereditary disease (score 37). The network is representative of the ones identified in the other CSD groups with treatment. Abbreviations: IL, interleukin; NRF2, nuclear factor erythroid 2–related factor 2; ATM, ataxia-telangiectasia mutated; PPAR, peroxisome proliferator-activated receptors.

Transcriptional Changes Induced by CSD in Cortex

To assess the effect of CSD on gene expression in the right cortex we first performed a comparison between the nontreated group with CSD saline + CSD and the group in which CSD was not induced (saline + sham). This comparison showed a total of 665 genes with significant differential expression, most of them upregulated (Figs 2A and 2B; Supplementary Table 1).

Additionally, we evaluated changes in gene expression caused by CSD in rats that had been pretreated with either TPM or VPA, by comparing the TPM + CSD and VPA + CSD groups with the saline + CSD group, and no significant differences were observed except for a single gene (*Stk32c*) that was upregulated in the TPM + CSD group (Figs 2A and 2B). No other differences in gene expression could be detected when we used a less restrictive FDR threshold of 20% instead of 5%. Consistently, the comparison of any of the treated groups, TPM + CSD or VPA + CSD with the saline + sham group displayed changes in gene expression similar to the comparison between saline + CSD and saline + sham (Fig 2C; Supplementary Tables 2 and 3). Most of the genes, a total of 456, were present in the 3 lists of differentially expressed genes in the same direction in the 3 CSD groups compared with saline + sham (Fig 2B; Supplementary Table 4).

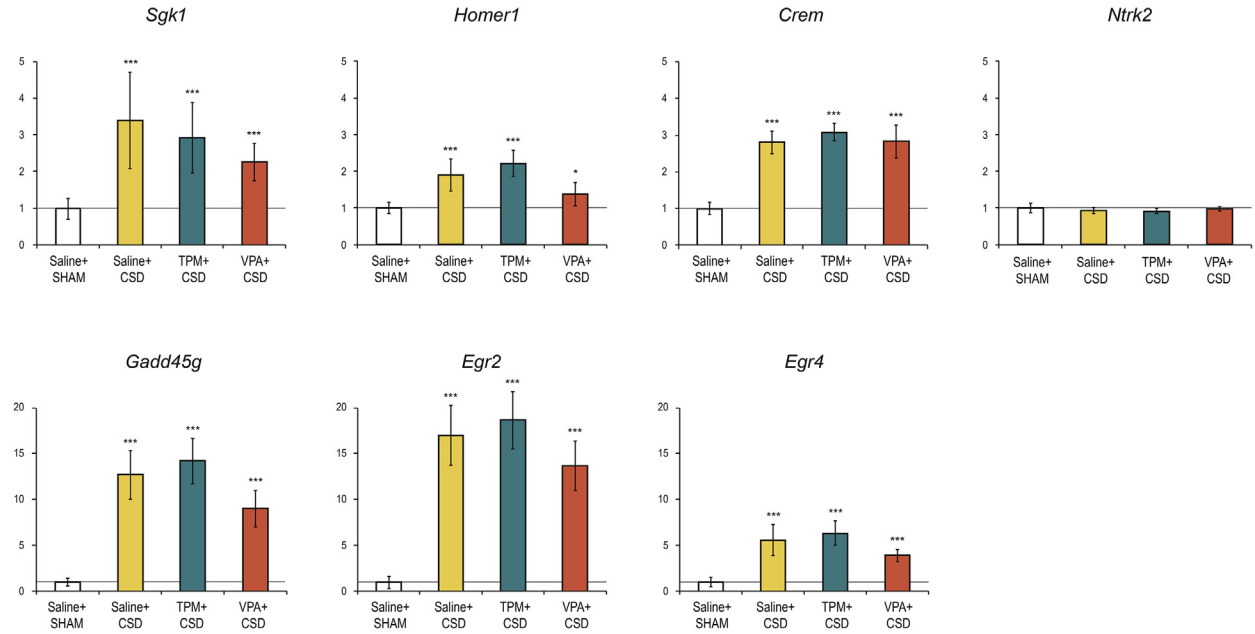

Figure 3. qRT-PCR validation of gene expression changes identified in the right cortex in the microarray analysis. Transcription levels of 7 representative genes were determined using qRT-PCR in 36 rats (n = 9 per group). Significant differences compared with the saline + sham group normalized to *Ywhaz* are indicated (*P < .05; **P < .01; ***P < .001). Error bars indicate SD. Similar results were obtained when normalized to *Pgk1*.

Bioinformatic analyses with DAVID identified similar enriched GO categories in the three sets of differentially expressed genes in the animals in which CSD was induced (with or without pretreatment). Thus, the same categories were evident when we considered the genes present in each of these three lists as well as the list of the 456 shared genes: response to hormone stimulus, regulation of transcription, regulation of synaptic transmission, neuron projection morphogenesis, and vascular development were some of the most representative (Fig 2C). Using IPA we identified several common enriched canonical pathways: interleukin 6 and 7 signaling, glucocorticoid receptor signaling, or cholesterol biosynthesis. The best scored IPA network identified in the comparison of saline + CSD versus saline + sham was related to nervous system development and function, and the *Crem* gene was one of the main nodes of the network (Fig 2D). When considering the list of shared genes for the three CSD groups (456 genes) this was also the best scored network, and similar results were obtained when using the gene lists from TPM + CSD and VPA + CSD versus saline + sham.

Finally, the comparison between the two groups of rats in which CSD was induced after treatment (TPM + CSD vs VPA + CSD) resulted in a list of 60 genes also after applying a 5% FDR (Figs 2A and 2B; Supplementary Table 5), 43 of them present in the comparisons mentioned previously and 17 new. Most of them were only nominally significant for the TPM + CSD versus saline + CSD (Supplementary Table 6). All 17 transcripts were only differentially expressed in the TPM + CSD group, because the VPA + CSD group showed no differences in gene expression in these genes compared with the saline + CSD group (Supplementary Table 6), and two of them showed a fold-change < −1.6 (*Col1a2* and *Ogn*) in the TPM + CSD group compared with the VPA + CSD and saline + CSD groups. The two best scored networks for the differentially expressed genes between the TPM + CSD and VPA + CSD groups were related to inflammatory response and cellular assembly. No significantly enriched GO categories were identified.

A total of 7 genes were selected to validate gene expression changes in the right cortex: *Sgk1, Homer1, Crem, Ntrk2, Gadd45g, Egr2, and Egr4*. Overexpression was confirmed for 6 of them in all CSD groups compared with the saline + sham group, with the only exception of *Ntrk2* (Fig 3, Supplementary Table 7).

Although CSD propagates only in the ipsilateral cortex, some expression changes have also been described in the contralateral region.[17] To assess this event, genes validated in the right cortex were evaluated in left cortex samples, where CSD was not induced. For the 6 validated genes (*Sgk1, Homer1, Crem, Gadd45g, Egr2, and Egr4*) only *Sgk1* showed overexpression, whereas the other 5 remained unaltered (Supplementary Fig 1).

Transcriptional Changes Induced by CSD in the Brainstem

Transcriptomic changes were also studied in brainstem samples, when performing the same comparisons mentioned previously. Changes in gene expression were identified for all CSD groups (saline/TPM/VPA) compared with saline + sham (Figs 4A and 4B). Compared with cortex, a lower number of genes were differentially expressed: 98 genes identified in the saline + CSD versus saline + sham groups, and 62 and 68 for the TPM + CSD and VPA + CSD versus the saline + sham group,

A

Comparison	Total	↑	↓
[Saline+CSD] vs [Saline-SHAM]	98	53	45
[TPM+CSD] vs [Saline-SHAM]	62	26	36
[VPA+CSD] vs [Saline-SHAM]	68	41	26
[TPM+CSD] vs [VPA+CSD]	0	0	0
[TPM+CSD] vs [Saline+CSD]	0	0	0
[VPA+CSD] vs [Saline+CSD]	0	0	0

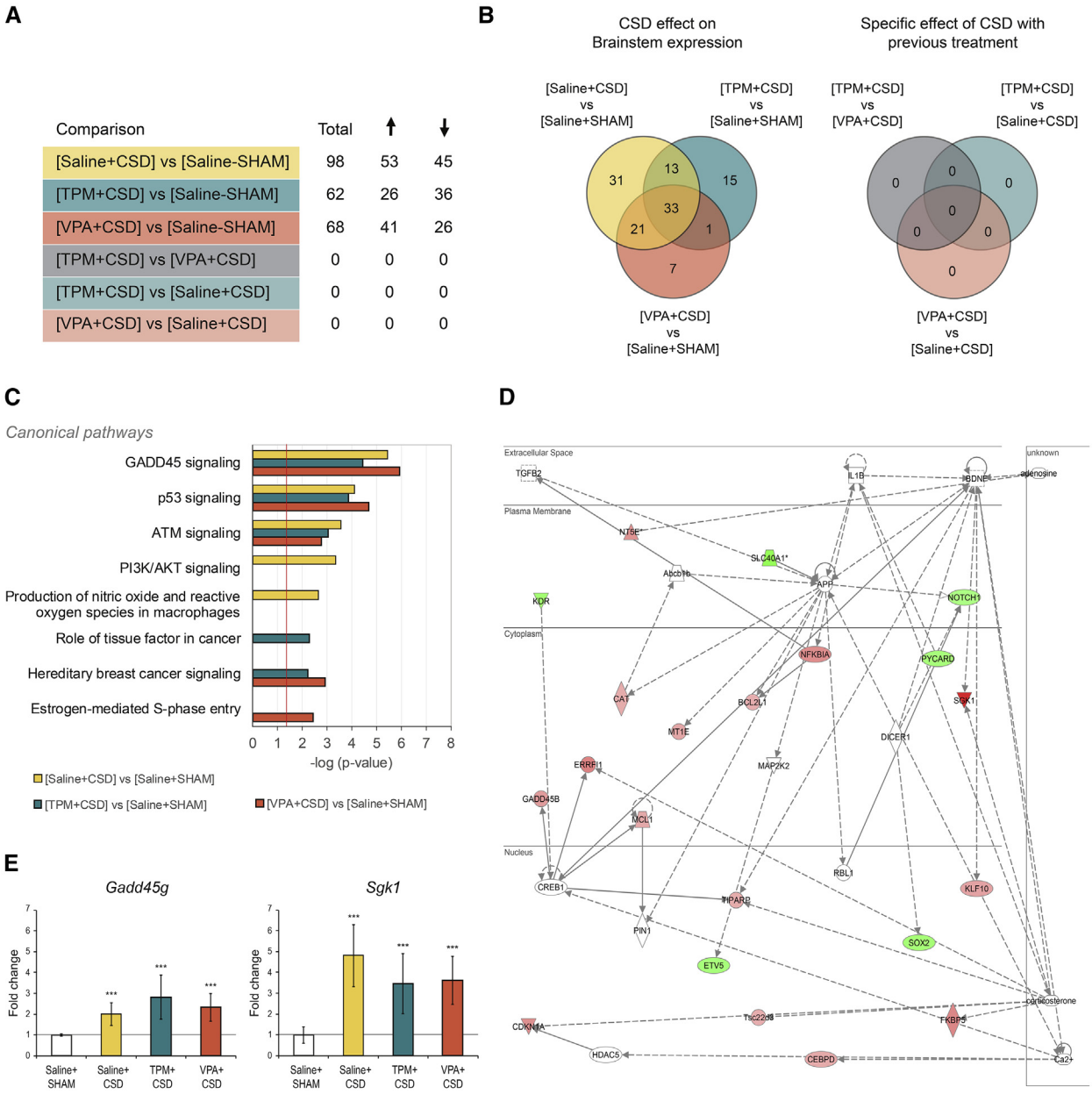

Figure 4. Gene expression changes in brainstem induced by CSD with and without prophylactic treatment with TPM or VPA (n = 6 per group). **(A)** Total number of genes that are upregulated or downregulated identified in each comparison. **(B)** Venn diagrams of genes differentially expressed in each comparison. Intersections show genes for which expression is altered in more than 1 comparison. **(C)** Most enriched canonical pathways identified in the genes that show differential expression after CSD. The red line indicates the significance threshold. **(D)** Best scored gene network in the comparison of saline + CSD versus saline + sham, which is involved in cell death and survival, nervous system development and function, and tissue morphology (score 36). The network is representative of the ones identified in the other CSD groups with treatment. **(E)** qRT-PCR validation of 2 genes showing differences in gene expression in the brainstem (n = 9 per group). Significant differences compared with saline + sham group normalized to *Ywhaz* are indicated (*$P < .05$; **$P < .01$; ***$P < .001$). Error bars indicate SD. Similar results were obtained when normalized to *Pgk1*. Abbreviations: GADD45, growth arrest and DNA-damage-inducible 45; ATM, ataxia-telangiectasia mutated; PI3K/ATK, phosphatidylinositol-4,5-bisphosphate 3-kinase/Protein kinase B.

respectively (Figs 4A and 4B; Supplementary Tables 8–10). A total of 33 genes were shared between the 3 comparisons (Fig 4B; Supplementary Table 11). No other comparison showed differences in gene expression (Fig 4B). Bioinformatic analyses with IPA highlighted apoptosis and cell death mechanisms in brainstem induced by CSD. The best scored networks identified in the genes differentially expressed in all 3 CSD groups (treated and nontreated vs saline + sham) were related to apoptosis, and the two most representative canonical pathways were Gadd45 signaling and p53 signaling for all 3 CSD groups compared with saline + sham (Figs 4C and 4D). No significantly enriched GO categories were identified after applying multiple testing corrections. Two genes were selected for validation using qRT-PCR in brainstem samples: *Gadd45g* and *Sgk1*. Upregulation of both was confirmed in all 3 CSD groups compared with saline + sham (Fig 4E).

Discussion

In this study we present a transcriptomic analysis in a rat model of CSD in two brain areas: the right cortex, where CSD was induced, and the brainstem. The latter is a region closely related to CSD activation mechanisms in which transcriptomic changes induced by CSD have not been explored. To our knowledge, this is the first study to evaluate CSD short-term effects in gene expression with and without a previous prophylactic treatment (TPM or VPA). Also to our knowledge, this is the first study that has assessed gene expression changes in the brainstem after CSD induction in the cortex.

The results of this study show that CSD has a noticeable short-term effect on gene expression in the cortex from the hemisphere where CSD was induced, either with or without pretreatment with TPM or VPA, two prophylactic drugs for migraine. Consistently, several groups of genes whose expression is altered by CSD in our study have previously been reported as involved in this process.[26] The most representative groups include genes involved in synaptic transmission (*Bdnf*, *Arc*, *Camk2a*, or *Ntrk2*), regulation of transcription (*Btg2*, *Fos*, or *Jun*) and response to organic substances (*Egr1*, *Egr2* or *Ccl2*), in agreement with previous reports.[26] We validated changes in gene expression of several genes involved in processes that are potentially relevant for CSD. These include *Gadd45g*, implicated in cell injury responses such as DNA repair and apoptosis,[22] and previously related to ischemic cortex and spreading depression[23,26]; *Sgk1*, related to brain injury and playing a protective role in the regulation of neuron apoptosis[30]; the early growth response genes *Egr2* and *Egr4* involved in cellular growth and differentiation, as well as in neuronal function,[18] and previously shown to be differentially expressed after CSD induction.[26] Another validated gene that has long been related to play a role in neuronal functions is *Crem*, shown to be upregulated after brain injury and relevant to the neuronal apoptosis process.[29] *Homer1* is involved in synaptic plasticity and architecture, and related to synaptic rearrangements in response to dopaminergic and glutamatergic signaling, which strongly connects it to the pathophysiology of neuropsychiatric disorders.[10]

When we assessed differences in gene expression in animals with CSD compared with animals where CSD was induced after treatment with either TPM or VPA, no significant changes were found. This indicates that, in our experimental conditions, the prophylactic treatment has little effect on gene expression after CSD. Also, when we compared the groups TPM + CSD versus VPA + CSD, only a few genes showed differential expression. In agreement with the results presented in this report, previous studies support the fact that treatments can modify the frequency of CSD.[1,3,4,25] Also, gene expression changes have previously been reported for both treatments, always without subsequent CSD induction. VPA treatment for 30 days induced changes in gene expression in the rat brain, involved in different functions such as synaptic transmission, transcription factors, ion channels, or apoptosis.[5] In a murine model of diabetes treated during 7 days with TPM, 7 genes were differentially expressed in hypothalamus. Interestingly, in our study two of them (*Nr4a2* and *Pld1*) were found differentially expressed in the cortex in the TPM + CSD group compared with the saline + sham group, but showing opposite directions compared with those observed in hypothalamus without CSD[19] (Supplementary Table 2). In the case of *Nr4a2*, as well as other members of the family (*Nr4a1* and *Nr4a3*), all three genes showed, in our study, similar increased expression in all three CSD groups (saline/TPM/VPA) compared with the saline + sham group (Supplementary Table 4). Therefore, our results indicate that when CSD is triggered, prophylactic treatments may not significantly have an effect on those gene expression changes induced by the subsequent spreading depression, or their effect may be covered up by this process, at least at a short-term level.

We also focused on the left cortex, where the CSD was not induced. Thus, we evaluated in the left hemisphere of the brain the expression of those genes that had been found differentially expressed in the right cortex as a consequence of the CSD. In previous publications it has been reported that CSD propagates unilaterally within the ipsilateral hemisphere,[24] and also that only some particular genes undergo expression changes in the contralateral cortex.[17] Consistently, our results show that most of the gene expression changes induced by CSD in the right cortex that we validated were not seen in the left cortex (5 of 6 genes remained unaltered). However, to get a global view on gene expression alterations in the noninduced cortex versus the induced one, transcriptomic studies should be performed also in the contralateral cortex.

Gene expression changes after inducing CSD in the right cortex were evaluated in the brainstem, a brain area thought to be involved in the modulation of migraine. Compared with cortex, a smaller number of altered genes were found, half of them common between both areas and showing the same direction. The best scored canonical pathways in brainstem were related to apoptosis, pointing at Gadd45g signaling as the most significant one and to the *Sgk1* gene, which is part of the best ranked network. Expression changes after CSD in these two genes, involved in apoptosis but also in response to brain injury, were validated in this brain area. Therefore, our results suggest that CSD effects can reach other nervous system regions at least at gene expression level, and that some common molecular mechanisms may be involved.

Our results should be interpreted in the context of several methodological considerations. Some limitations are: 1) the time point after CSD induction, 1 hour, defines our work as a short-term study of CSD effects, with no focus on long-term consequences; 2) the cortex samples included the whole cortex in which CSD was induced, and were not as narrow as the ones dissected in previous studies (eg, the hind limb cortex[26]); 3) brainstem samples included the whole brainstem and not specific regions related to migraine (eg, A11, locus coeruleus); 4) we did not assess the effect of the treatments before CSD, thus effects of the treatment itself could not be assessed;

5) although treated animals showed a smaller number of CSDs, in all studied groups the number of CSDs induced was quite high and may not be comparable with spontaneous CSD in humans; 6) the effect of serial CSDs above a certain threshold may have the same effect on gene expression, and the effect of a single CSD with and without treatment may differ considerably from the results obtained in this study; 7) effects on gene expression have been evaluated at a transcriptional level, but other processes can be involved in which regulation takes place at post-transcriptional, translational, or post-translational levels. However, several strengths of our study reinforce the validity of our results: 1) the high number of replicates per experimental group, 6 in the microarray and 9 for qRT-PCR validations; 2) our results are consistent with previous reports assessing CSD; and 3) different brain areas were assessed separately, providing a more comprehensive vision of the changes that take place when CSD is triggered.

Conclusions

Our study shows that CSD induces short-term transcriptomic changes in cortex, consistent with previous literature, and that after CSD no differences in gene expression can be identified between animals treated with prophylactic drugs (TPM or VPA) and those not treated. CSD can affect the ipsilateral cortex as well as other brain areas in which the CSD is not induced, like the brainstem. We observed common gene expression changes between the brainstem and the cortex, suggesting similar molecular mechanisms. Further studies assessing different time points may help to better understand the course of transcriptomic changes that take place when CSD is triggered after a previous drug treatment. We think that assessing the changes induced by CSD and by prophylactic treatments in an animal model may help to elucidate the main altered pathways in the migraine aura and provide insights on the underlying physiological mechanisms also in humans, although our results have limited clinical relevance and the translation of the findings requires further investigation.

Supplementary Data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.jpain.2016.11.007.

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