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Chronic treatment with lithium or valproate modulates the expression of *Homer1b/c* and its related genes *Shank* and *Inositol 1,4,5-trisphosphate receptor*

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Abstract

Homer proteins are associated with both dopaminergic and glutamatergic function. In addition, these proteins are implicated in many signal transduction pathways that are also putative targets of the mood stabilizers lithium and valproate (VPA). This study investigated the effect of *in vivo* chronic administration of therapeutically-relevant doses of lithium and VPA on the expression of the inducible (*Homer1a* and *ania-3*) and constitutive (*Homer1b/c*) isoforms of the *Homer1* gene in rat brain, and of two other *Homer*-related genes: *Inositol 1,4,5 trisphosphate receptor* (*IP3R*) and *Shank*. *Homer1b/c* was significantly decreased in cortex by VPA, and in striatal and accumbal subregions by both lithium and VPA. Both mood stabilizers reduced *Homer1b/c* expression in the dorsolateral caudate-putamen, while only VPA decreased gene expression in all other striatal subregions. *Shank* and *IP3R* were downregulated by both mood stabilizers in the cortex. Neither chronic lithium nor VPA affected *Homer* immediate-early genes. These results suggest that

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Contributors:

AdB: designed the study, wrote the protocol, performed the experiments, undertook the statistical analysis, and wrote the manuscript.

CT: performed the experiments, undertook the statistical analysis, and wrote the manuscript.

MC: performed the experiments, undertook the statistical analysis, and wrote the manuscript.

PXY: designed the study, wrote the protocol, and carried out drug treatment.

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lithium and VPA similarly modulate the expression of structural postsynaptic genes with topographic specificity in cortical and subcortical regions. Thus, *Homer* may represent an additional molecular substrate for mood stabilizers, and a potential link with dopaminergic function.

Keywords

bipolar disorder; gene expression; glutamate; postsynaptic density; schizophrenia; striatum

Introduction

The *Homer* family of genes encodes for scaffolding proteins that are localized at the glutamatergic postsynaptic density (PSD). These proteins have been implicated in the regulation of synaptic plasticity, as well as in animal behaviors considered relevant to the modeling of human psychiatric diseases (de Bartolomeis and Iasevoli 2003). Moreover, several lines of evidence suggest that Homer proteins may play a potential role in some neuropsychiatric disorders, such as schizophrenia (Szumlinski et al., 2006).

Multiple constitutive forms (Homer 1b/c, Homer2, and Homer 3) of Homer proteins, and two inducible forms (Homer1a and its splicing variant ania-3), which are induced in an immediate-early gene (IEG)-like fashion, have been described (Brakeman et al., 1997). Both constitutive and inducible forms have an EVH (Ena/Vasp homology) domain at the N-terminal, which allows these proteins to interact with metabotropic glutamate receptors (mGluRs) type I (mGluR1 and mGluR5) on the cell surface (Kammermeier and Worley 2007). The constitutive forms also have a coiled-coil domain at the C-terminal that is responsible for self-multimerization, whereas the inducible forms (Homer 1a and ania-3)—which lack the coiled-coil domain—cannot multimerize. Homer1a disassembles the constitutive forms, acting as a “dominant negative”, thereby modifying signal transmission initiated at mGluR1/5.

Several studies suggest that *Homer* expression can be modulated by psychotropic drugs that mainly, though not exclusively, impact dopaminergic and glutamatergic neurotransmission (Polese et al., 2002; Tomasetti et al., 2007; Zhang et al., 2007). In addition, previous studies from our laboratory demonstrated that antipsychotic drugs differentially affect *Homer1a* expression depending on the degree of dopamine D2 receptor blockade (Tomasetti et al., 2007).

Recently, we noted that the combined administration of antipsychotics and valproate (VPA) may induce differential modulation of PSD—genes particularly *Homer*-related genes—compared to the effects observed when these drugs were administered individually (Tomasetti et al., 2011). However, to the best of our knowledge, no study to date has investigated the manner in which the mood stabilizers lithium and VPA regulate *Homer* genes. This is unexpected, given that clinical (Moore et al., 2000) and preclinical (Du et al., 2004) evidence have implicated glutamate in the pathophysiology of bipolar disorder, and that dopamine neurotransmission is believed to play a role in the mechanism of action of mood stabilizers (Beaulieu et al., 2009). In addition, recent studies have directly implicated *Homer1* in the etiology of mood disorders (Rietschel et al., 2010). This dearth of information is even more surprising considering that Homer proteins modulate the phosphoinositide (PI) signaling pathway (Mao et al., 2008), one of the most extensively studied targets of lithium, and which has also recently been studied as a putative target of VPA (Tokuoka et al., 2008; Williams and Harwood 2000). Furthermore, Homer has been implicated in the modulation of the phosphatidylinositol-3-kinase – Akt (PI3K-Akt)

pathway (Ronesi and Huber 2008), which, in turn, was shown to directly regulate the function of glycogen synthase kinase 3β (GSK- 3β), a well-recognized molecular target of mood stabilizers (Gould and Manji 2005).

An intriguing connection also appears to exist between the Homer proteins, the mGluRs, intracellular calcium concentrations, and the enzyme protein kinase C (PKC) (Sala et al., 2005), whose protein levels are also affected by chronic *in vivo* administration of lithium and VPA (Manji and Chen 2002). In addition, recent observations suggest that Homers play a major role in modulating the complex signaling machinery that routes signals from postsynaptic receptors (mGluRs and N-methyl-D-aspartate receptors (NMDARs)) to the Mitogen activated protein kinase – Extracellular signal-regulate kinase1/2 (MAPK-ERK1/2) pathway (Wang et al., 2007), which has been putatively implicated in mood modulation and in the mechanism of action of mood stabilizers (Einat et al., 2003; Yuan et al., 2001).

The present study investigated the manner in which chronic administration of lithium and VPA, at therapeutic doses, modulates the inducible and constitutive transcripts of *Homer1*. We evaluated the expression of *Homer* genes by means of *in situ* hybridization histochemistry, focusing on the cortex, caudate-putamen, and nucleus accumbens; all of these areas are believed to be involved in the pathophysiology of bipolar disorder (Green et al., 2007) and in the mechanism of action of mood stabilizers (Ramaprasad et al., 2005). We chose to explore both inducible and constitutive Homer isoforms (i.e. *Homer1a* and *Homer1b/c* respectively) based on previous studies (Tomasetti et al., 2007; Iasevoli et al., 2010) showing that chronic administration of antipsychotics may affect both transcripts. A recent study from our laboratory (Tomasetti et al., 2011) analyzed *Homer1a* following the administration of VPA—alone or in association with antipsychotic drugs—in both acute and chronic paradigm of injected administration; building on that work, the present study compared the effects of both lithium and VPA on *Homer1* transcripts in a chronic paradigm of oral administration. This model was chosen in order to more closely approximate the conditions necessary to obtain pharmacological effects with mood stabilizers in clinical practice, and to assess the impact of prolonged oral drug administration on *Homer* expression.

We also evaluated the possible involvement of *Shank* and *Inositol 1,4,5 trisphosphate receptor (IP3R)*, two genes tightly related to *Homer* function, and which share a prominent role in synaptic plasticity.

Experimental Procedures

Animals, drug treatments, and tissue preparation

Adult male Wistar-Kyoto (WKY) rats (weight 175–200g) were housed in a temperature- and humidity-controlled colony room with access to water and food *ad libitum*, maintained under a 12-hour light/dark cycle, and allowed a one-week accommodation period before initiation of experiments. All experimental procedures were carried out in accordance with the guidelines published in the National Research Council (NRC) *Guide for the Care and Use of Laboratory Animals* (NIH Publication N.85–23, revised 1996), and were approved by the National Institute of Mental Health (NIMH) Animal Care and Use Committee. All efforts were made to minimize the number of animals used and their suffering.

Rats (n=5 for each experimental group) were randomly assigned to a treatment group and fed either: 1) regular rodent chow (control), 2) lithium carbonate-containing chow (2.4 g/kg), or 3) VPA-containing chow (20 g/kg) (custom produced by Bio-Serv, Frenchtown, NJ, USA) for four weeks. In addition to tap water, a bottle of saline was available to rats in order to minimize any electrochemical imbalance that might occur during the treatment. At the

end of treatment, rats were killed by decapitation between 9:00 A.M. and 12:00 P.M., trunk blood samples were collected in order to monitor drug concentrations, and the whole brain was rapidly removed, quickly frozen on powdered dry ice, and stored at -80°C . Serum drug concentrations were confirmed to be within the therapeutic range (lithium 0.5~1.0mEq/l, VPA 50~100 $\mu\text{g/ml}$).

Serial coronal sections of 12 μm were cut on a cryostat at -18°C at the level of the striatum (approx. from Bregma -1.20mm to -1.00mm), using the rat brain atlas of Paxinos and Watson (Paxinos and Watson 1997) as an anatomical reference. Sections were stored at -80°C for subsequent analysis.

Radiolabeling

Details for all the probes are listed in Table 1.

Homer 1a, *ania-3*, and *Homer1b/c* probes were oligodeoxyribonucleotides derived from identical probes used in previous hybridization studies (Polese et al., 2002; Tomasetti et al., 2007). All other probes were designed from GenBank sequences and checked with BLAST to avoid cross-hybridization. All oligodeoxyribonucleotides were purchased from MWG Biotech (Firenze, Italy).

For each probe, a 50 μl labeling reaction mix was prepared on ice using DEPC-treated water, 1X tailing buffer, 7.5pmol/ μl of oligodeoxyribonucleotide, 125 Units of Terminal deoxynucleotidyl Transferase (TdT) and 100mCi ^{35}S -dATP. The mix was incubated for 20 minutes at 37°C . The unincorporated nucleotides were separated from radiolabeled DNA using ProbeQuant G-50 Micro Columns (Amersham-GE Healthcare Biosciences, Milano, Italy). To assess probe specificity, the autoradiographic signal distribution was compared and found to be consistent with previous *in situ* hybridization studies (Brakeman et al., 1997; Polese et al., 2002).

In Situ Hybridization Histochemistry

Sections were processed for radioactive *in situ* hybridization (Ambesi-Impiombato et al., 2003). All solutions were prepared with sterile double-distilled water. The sections were fixed in 4% formaldehyde in 0.12 M phosphate buffered saline (PBS, pH 7.4), quickly rinsed three times with 1X PBS, and placed in 0.25% acetic anhydride in 0.1 M triethanolamine/0.9% NaCl, pH 8.0 for 10 minutes. Next, the sections were dehydrated in 70%, 80%, 95%, and 100% ethanol, delipidated in chloroform for five minutes, rinsed again in 100% and 95% ethanol, and air-dried.

Sections were hybridized with $0.4\text{--}0.6 \times 10^6$ cpm of radiolabeled oligonucleotide in buffer containing 50% formamide, 600mM NaCl, 80mM Tris-HCl (pH 7.5), 4mM EDTA, 0.1% pyrophosphate, 0.2mg/ml heparin sulfate, and 10% dextran sulfate. Slides were covered with coverslips and incubated at 37°C in a humid chamber for 22–24 hours. After hybridization, the coverslips were removed in 1X saline sodium citrate solution (SSC) and the sections were washed 4×15 minutes in 2X SSC/50% formamide at $43\text{--}44^{\circ}\text{C}$, followed by two 30-minute washes with 1X SSC at room temperature. The slides were rapidly rinsed in distilled water and then in 70% ethanol.

Autoradiography and Image Analysis

The sections were dried and exposed to Kodak-Biomax MR Autoradiographic film (Sigma-Aldrich, Milano, Italy). A slide containing a scale of 16 known amounts of ^{14}C standards (American Radiolabeled Chemicals, St. Louis, US) was co-exposed with the samples. The autoradiographic films were exposed over 14–30 days. The optimal time of exposure was

chosen to maximize signal-to-noise ratio but to prevent optical density from approaching saturation limits. Film development protocol included a 1.5-minute dip in the developer solution and three minutes in the fixer. The quantitation of the autoradiographic signal was performed using a computerized image analysis system, including: a transparency film scanner (Microtek Europe B. V., Rotterdam, The Netherlands), an Apple iMac, and ImageJ software (v. 1.40, Rasband, W.S., <http://rsb.info.nih.gov/ij/>). Brain sections on film were captured individually. Scanned images were showed in inverted look-up table (LUT) color, although all the other original characteristics (i.e. contrast, brightness, resolution) were preserved. Each experimental group contained five animals. Each slide contained three adjacent sections of a single animal. All hybridized sections were exposed on the same sheet of X-ray film. Signal intensity analysis was carried out on digitized autoradiograms measuring mean optical density within outlined Regions of Interest (ROIs) corresponding to subregions of the cortex, caudate-putamen, and nucleus accumbens (Figure 1). These cortical and striatal subregions are structurally and functionally interconnected through projections from the cortex, which targets specific striatal sectors. Thus, ROIs were chosen based on functional mapping studies of gene expression (Willuhn et al., 2003) and other studies analyzing *Homer* expression in response to drugs (Tomasetti et al., 2011). Sections were quantitated blind to the treatment conditions. To test for inter-observer reliability an independent quantitation was performed by a second investigator. Results were considered reliable only when the statistical significance of effects obtained by the second investigator was quantitatively consistent with the results obtained by the first investigator.

Data processing

Measurements of mean optical density within ROIs were converted using a calibration curve based on the standard scale co-exposed to the sections. ^{14}C standard values were previously cross-calibrated to ^{35}S brain paste standards, in order to assign a dpm/mg tissue wet weight value to each optical density measurement through a calibration curve. For this purpose, a “best fit” 3rd degree polynomial was used. For each animal, measurements from the three adjacent sections were averaged and the final data were reported in relative disintegrations per minute (rel d.p.m.) as mean $\pm$ the standard error of the mean (S.E.M.). A one-way ANOVA was used to analyze treatment effects. Statistical significance was established at $p < 0.05$. The Tukey's *post hoc* test was used to determine the locus of effects for significant ANOVA results.

Results

The effects of chronic administration of lithium or VPA on *Homer1b/c*, *Shank*, *IP3R*, *Homer1a*, and *ania-3* gene expression in each forebrain subregion analyzed are presented in Table 2.

Homer1b/c (Figure 2)

We analyzed the autoradiographic signal of *Homer1b/c* mRNA in the cortex, caudate-putamen, nucleus accumbens core, and nucleus accumbens shell. In the cortex, the *Homer1b/c* signal was significantly reduced in cingulate (Cg; $p=0.0137$; $F_{(dl)}=5.491_{(2,18)}$), medial agranular (M2; $p=0.016$; $F_{(dl)}=5.249_{(2,18)}$), motor (M1; $p=0.0337$; $F_{(dl)}=4.115_{(2,18)}$), and insular (I; $p=0.0158$; $F_{(dl)}=5.265_{(2,18)}$) areas by VPA treatment compared to control. The lithium-treated group displayed a similar pattern of *Homer1b/c* signal reduction in cortical subregions as the VPA-treated group, but these results did not reach statistical significance.

In the striatum, the ANOVA analysis revealed that both lithium and VPA significantly decreased the *Homer1b/c* signal compared to control in the dorsolateral caudate-putamen (CPDL; $p=0.0001$; $F_{(dl)}=15.552_{(2,18)}$). In all the other subregions of the caudate-putamen,

Homer1b/c expression was significantly reduced only by VPA compared to control (dorsomedial (CPDM; $p=0.004$; $F_{(dl)}=7.398_{(2,18)}$), ventrolateral (CPVL; $p=0.002$; $F_{(dl)}=8.536_{(2,18)}$), and ventromedial (CPVM; $p=0.009$; $F_{(dl)}=6.108_{(2,18)}$).

In the core of the nucleus accumbens, the densitometric analysis of autoradiographic images showed a statistically significant decrease in *Homer1b/c* expression after chronic treatment with VPA compared to control ($p=0.015$; $F_{(dl)}=5.432_{(2,18)}$). No significant results were found in the nucleus accumbens shell.

Shank (Figure 3, panels a and c)

Shank mRNA was detected in the frontal and parietal cortices, and a lower signal was detected in the caudate-putamen and nucleus accumbens. The densitometric analysis of autoradiographic images showed a significant decrease of *Shank* signal in M2 with VPA treatment compared to control and lithium ($p=0.0107$; $F_{(dl)}=5.993_{(2,17)}$), and in M1 compared to control ($p=0.034$; $F_{(dl)}=4.153_{(2,17)}$). In Cg ($p=0.0047$; $F_{(dl)}=7.466_{(2,18)}$), SS (somatosensory cortex; $p=0.0157$; $F_{(dl)}=5.359_{(2,17)}$) and I ($p=0.0178$; $F_{(dl)}=5.155_{(2,17)}$), VPA significantly reduced *Shank* expression compared to lithium. No significant differences were noted in striatal subregions.

IP3R (Figure 3, panel b and d)

The *IP3R* mRNA autoradiographic signal was distributed in both cortical and striatal regions. Significant differences between groups were detected in all subregions of both frontal and parietal cortices. Post-hoc tests showed that lithium significantly reduced the *IP3R* signal in Cg ($p=0.0456$; $F_{(dl)}=4.038_{(2,12)}$), M1 ($p=0.048$; $F_{(dl)}=3.952_{(2,12)}$), SS ($p=0.0181$; $F_{(dl)}=5.712_{(2,12)}$), and I ($p=0.0346$; $F_{(dl)}=4.510_{(2,12)}$) compared to control. In the striatum, chronic treatment with VPA significantly decreased the *IP3R* signal in CPDL compared to control ($p=0.0265$; $F_{(dl)}=4.863_{(2,13)}$).

Homer1a and ania-3

Homer1a and *ania-3* gene expression was detected in several cortical and subcortical brain regions of the control group, with a signal distribution similar to that obtained in previous studies (Polese et al., 2002; Tomasetti et al., 2007); however, no significant differences between treatment groups were noted for either gene in all analyzed areas.

Discussion

Lithium and VPA affect Homer1b/c expression in brain areas involved in mood modulation

Several lines of evidence suggest that Homer proteins may be involved in multiple signal transduction pathways—including PI3K-Akt, IP3R, and MAPK-ERK1/2—all of which have also been implicated in the putative mechanism of action of mood stabilizers and in the pathophysiology of mood disorders. This study is the first to demonstrate that chronic *in vivo* treatment with therapeutically relevant doses of lithium or VPA decreases the transcription of *Homer1b/c*, the constitutive isoform of the *Homer1* gene; notably, this gene has been implicated in the pathophysiology of psychiatric disorders (de Bartolomeis and Iasevoli 2003; Szumlinski et al., 2006). We found that *Homer1b/c* expression was significantly reduced in the cingulate, medial agranular, motor, and insular subregions of the cortex by chronic treatment with VPA. In the striatum, both lithium and VPA downregulated *Homer1b/c* in the dorsolateral caudate-putamen, but only VPA administration reduced expression of the gene in other striatal subregions (dorsomedial, ventrolateral, and ventromedial) and in the nucleus accumbens core.

Although distinct differences exist between VPA and lithium, the direction of the effects of both these mood stabilizers on *Homer* expression in cortical and subcortical regions is consistent with these compounds having a potential common transductional pathway; this feature, in turn, has been considered pivotal in elucidating the mechanism of action of these drugs, which both act as mood stabilizers despite their structural differences. Nevertheless, it is noteworthy that both of these mood stabilizers affected *Homer1b/c* expression in brain regions that have specifically been implicated in the pathophysiology of bipolar disorder (for a review, see Green et al., 2007). Indeed, several studies have demonstrated that functionally distinct cortical subregions project to specific striatal areas, and that analyzing gene expression changes induced by psychotropic drugs may elucidate the functional domains affected by these drugs (Willuhn et al., 2003). In the present study, VPA modified the expression of *Homer1b/c* in cortical regions critical to motor control (medial agranular and motor cortices) and to cognitive and behavioral aspects of emotion (cingulate and insular cortices). The cortical premotor and motor areas project specifically to the dorsolateral regions of the striatum, which specifically control somatomotor inputs; cingulate and insular cortices project to the ventral striatum, which is mostly implicated in behavioral control. Albeit preliminary, these data describing the different response pattern of constitutive *Homer* genes to different mood stabilizers may provide an additional avenue of investigation for clarifying their mechanism of action in specific brain areas involved in mood modulation.

Functional implications of Homer1 modulation by mood stabilizers

Given the multifunctional role of Homers as scaffolding and adaptor proteins, the effects of lithium and VPA on *Homer1b/c* expression suggest a direct impact on postsynaptic remodeling. The reduced transcription of *Homer1b/c* in cortical and subcortical regions might involve the putative modulation of signal transduction pathways starting at mGluR1/5. For instance, decreased Homer1b/c function may impair the coupling of mGluRs1/5 to IP3Rs mediated by Homer clusters (Sala et al., 2005), thereby lowering calcium-dependent signal transduction. This might thus constitute an additional mechanism of action for lithium and VPA; both agents have already been shown to reduce IP3 and diacylglycerol signaling, which comprises a crucial pathway for synaptic plasticity (Tokuoka et al., 2008; Williams and Harwood 2000).

Moreover, Homer proteins are known to organize the distribution of mGluRs1/5 in specific neuronal sectors. In particular, *Homer1b/c* expression appears to be essential for arranging mGluRs1/5 in membrane clusters on dendritic spines in order to facilitate excitatory neuronal responses (Kammermeier 2006). Therefore, it is possible that *Homer1b/c* downregulation, induced by both lithium and VPA, might lead to lowered sensitivity of the postsynaptic neuron to excitatory stimuli via the rearranging of mGluR1/5 localization.

Finally, Homer1b/c has been reported to play a major role in linking mGluRs1/5 with the postsynaptic effectors MAPK-ERK1/2 in a calcium-independent manner, generating a signaling pathway that acts in parallel to and coordinates with the conventional IP3R-calcium pathway (Mao et al., 2005). Thus, a decrease in Homer1b/c function by chronic treatment with lithium or VPA might affect ERK1/2 activation and, consequently, its impact on gene transcription.

Furthermore, these effects of lithium and VPA on Homer1b/c-mediated mGluR signaling may contribute to the overall ability of these two mood stabilizers to treat the elevated glutamatergic activity found in specific brain areas of patients with bipolar disorder (Hashimoto et al., 2007). This is consistent with the evidence that both lithium and VPA may also reduce brain NMDA-mediated calcium signaling in distinct regions of the forebrain (Basselin et al., 2006; Basselin et al., 2008).

Is Homer1 a possible link between dopamine and mood stabilizers?

Several studies implicated *Homer* genes in transductional pathways specifically correlated to dopaminergic function, and downstream of dopaminergic receptors (Iasevoli et al., 2009). Our previous work (Polese et al., 2002; Tomasetti et al., 2007) described a peculiar pattern of *Homer* gene induction in the rat forebrain following the acute or chronic administration of typical and atypical antipsychotics. In particular, the expression of the inducible isoforms of *Homer1* seems to be correlated with the degree of dopamine D2 receptor blockade induced by various antipsychotics. Recent evidence suggests that both lithium and VPA may affect dopaminergic function in multiple ways; for instance, both mood stabilizers can reverse the abnormal behaviors induced by indirect agonists (e.g., apomorphine or amphetamine) (Kim et al., 2008; Umeda et al., 2006). Other studies noted that VPA may potentiate dopamine efflux by antipsychotics in cortical regions (Ichikawa et al., 2005). Recent work from our laboratory demonstrated that VPA, when co-administered with a typical or atypical antipsychotic, may modify the impact on *Homer*-related postsynaptic gene expression exerted by individual administration of these drugs (Tomasetti et al., 2011); interestingly, this evidence is highly consistent with recent observations that both mood stabilizers might directly act on dopaminergic transductional pathways, such as the Akt-GSK3 β cascade (Beaulieu et al., 2009). Given the putative role of Homer proteins in the signaling cascade of dopamine receptors in cortical and striatal regions, our finding that lithium and VPA modulate *Homer1b/c* may provide additional information regarding the possible impact of these two agents on dopaminergic signaling.

Lithium and VPA modulate Shank and IP3R expression: possible differential regional impact on calcium-dependent synaptic plasticity

Shank and *IP3R*—the two *Homer*-related genes analyzed—were both found to be affected in cortical regions after chronic administration of either lithium or VPA, although some differences in their modulation exist. Indeed, *Shank* expression was mildly impacted by chronic lithium in the cortex, while VPA significantly downregulated gene expression in motor and insular cortices. It is important to note that although both mood stabilizers promoted *IP3R* cortical downregulation, only lithium treatment significantly decreased *IP3R* expression in almost all cortical subregions. In contrast, in the dorsolateral caudate-putamen, *IP3R* was downregulated by VPA, while lithium slightly—and non-significantly—decreased gene expression.

Shank proteins are known to dually interact with NMDARs through a PSD95-GKAP (postsynaptic density 95/guanylate kinase-associated protein) complex, and with mGluRs1/5 by linking Homer1b/c, which interacts with IP3Rs to connect this machinery to the intracellular calcium stores (Sheng and Kim 2000). Notably, recent studies demonstrated that Shank and Homer1b/c play a major role in regulating calcium intracellular oscillations by interacting directly with each other and with IP3Rs localized on calcium cisterns (Sala et al., 2005). Therefore, the impact on *Shank* and *IP3R* transcripts following chronic administration of either lithium or VPA in both cortex and striatum may reflect the overall regulation of calcium-dependent glutamatergic activity exerted by these two mood stabilizers in cortical and subcortical regions. However, the differences in the expression patterns of *Shank* and *IP3R* by the two distinct mood stabilizers may suggest a differential regulation of calcium intracellular networks with a possible neuroanatomical specificity. Indeed, although lithium and VPA share common mechanisms of action with respect to the intracellular pathways activated (e.g. Akt/GSK3 β signaling; phosphoinositide pathway) (Gould and Manji, 2005; O'Donnell et al., 2000), several reports have demonstrated that the effects of the two mood stabilizers may diverge depending upon neural cell type (Jin et al., 2005; Jonathan Ryves et al., 2005). Moreover, lithium and VPA have also been reported to impact gene expression with differential regional specificity (Chen et al., 1999). The present

findings contribute additional data regarding this issue, thereby stimulating further investigation to elucidate the mechanisms by which lithium and VPA may differentially trigger calcium-dependent pathways in a region-specific manner.

In addition, the fact that both *Shank* and *IP3R*—like *Homer1b/c*—were modulated in brain regions specifically involved in cognitive-behavioral and motor aspects of emotion may further confirm the essential role that these PSD proteins play in the transductional pathways implicated in the mechanism of action of mood stabilizers.

Lithium and VPA did not impact Homer1 inducible transcripts: new ways to synaptic rearrangements by mood stabilizers?

In contrast to the constitutive isoform of the *Homer1* gene, neither the inducible form *Homer1a* nor its splice variant *ania-3* were affected by chronic administration of lithium or VPA. These results are consistent with other reports showing a negligible induction of IEGs (i.e. *c-fos*) following the chronic administration of lithium or VPA (Chen et al., 1999). Moreover, the present findings support our previous work in this area (Tomasetti et al., 2011), which found that neither acute nor chronic VPA administration modulated *Homer1a* expression, even though the co-administration of VPA with an antipsychotic induced changes in gene expression that differed from those elicited by the individually-administered compounds. These findings suggest that chronic treatment with mood stabilizers—although they impact *Homer*-dependent signalling—may not affect the *Homer* constitutive/inducible forms ratio, as seen in our previous study of antipsychotics (Iasevoli et al., 2010). Thus, it is more likely that they have a direct impact on structural constituents of the postsynaptic network, such as *Homer* long forms, *Shank*, and *IP3Rs*.

Taken together, these results provide the first evidence that chronic administration of the mood stabilizers lithium and VPA modulate *Homer1b/c* and its related postsynaptic genes *Shank* and *IP3R*. Notably, all of these genes encode multifunctional proteins that interact with well-known targets of mood stabilizers, and all have been implicated in long-term synaptic changes. Therefore, *Homer* genes may represent a new molecular substrate for the mechanism of action of mood stabilizers, a finding that clearly warrants further investigation.

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Abbreviations

AcCo	nucleus accumbens core
AcSh	nucleus accumbens shell
ANOVA	analysis of variance
Bcl-2	B-cell lymphoma 2 protein

BAD	Bcl-2-associated death promoter
BDNF	brain-derived neurotrophic factor
Cg	cingulate cortex
CPDM	dorsomedial caudate-putamen
CPDL	dorsolateral caudate-putamen
CPVL	ventrolateral caudate-putamen
CPVM	ventromedial caudate-putamen
CREB	c-AMP response element-binding protein
ERK	extracellular-signal regulated kinase
GSK-3β	glycogen synthase kinase 3 β
I	insular cortex
IEGs	immediate-early genes
IP3	inositol 1,4,5-trisphosphate
IP3Rs	IP3 receptors
M1	motor cortex
M2	medial agranular cortex
MAPK	mitogen-activated protein kinase
mGluRs	metabotropic glutamate receptors
NMDARs	N-methyl-D-aspartate glutamate receptors
PBS	phosphate buffered saline
PI	phosphoinositide
PI3K-Akt	phosphatidylinositol-3-kinase
PKC	protein kinase C
PSD	postsynaptic density
ROI	region of interest
SS	somatosensory cortex
SSC	saline sodium citrate solution
VPA	valproate

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Figure 1.

Panel a Diagram of regions of interest (ROIs) quantitated on autoradiographic film images in rat forebrain (section coordinates are approximate from Bregma 1.20 mm to 1.00 mm). 1 = cingulate cortex (Cg); 2 = medial agranular cortex (M2); 3 = motor cortex (M1); 4 = somatosensory cortex (SS); 5 = insular cortex (I); 6 = dorsomedial caudate-putamen (CPDM); 7 = dorsolateral caudate-putamen (CPDL); 8 = ventrolateral caudate-putamen (CPVL); 9 = ventromedial caudate-putamen (CPVM); 10 = core of nucleus accumbens (AcCo); 11 = shell of nucleus accumbens (AcSh). Modified from Paxinos and Watson (1997).

Panel b. Illustration of cortico-striatal projections among the ROIs in which gene expression induced by mood stabilizers was measured. PL/IL = prelimbic/infralimbic cortices (not shown).

Figure 2.

Panel a autoradiographic film images of *Homer 1b/c* mRNA detected by means of *in situ* hybridization histochemistry in coronal brain sections after chronic treatment with control (CTR), lithium (Li), or valproate (VPA).

Panel b: *Homer 1b/c* mRNA levels in subregions of the cortex, caudate-putamen, and nucleus accumbens. Data are reported in relative d.p.m. as mean $\pm$ S.E.M. Tukey's *post hoc* test: * = vs. CTR ($p < 0.05$)

Figure 3.

Panels a and b: Autoradiographic film images of *IP3* and *Shank* mRNA detected by means of *in situ* hybridization histochemistry in coronal brain sections after acute treatment with control (CTR), lithium (Li), or valproate (VPA).

Panels b and c: *IP3* and *Shank* mRNA levels after chronic treatment in the subregions of the cortex, caudate-putamen, and nucleus accumbens. Data are reported in relative d.p.m. as mean $\pm$ S.E.M. Tukey's *post hoc* test: * = vs. CTR ($p < 0.05$).

Table 1Probes for *In Situ* Hybridization Histochemistry

Probe	cDNA length (bp)	cDNA position	mRNA	GenBank #
<i>Homer1a</i>	48	2527–2574	<i>Homer1a</i>	U92079
<i>Ania-3</i>	48	1847–1894	<i>ania-3</i>	AF030088
<i>Homer1b/c</i>	48	1306–1353	<i>Homer1b/c</i>	AF093268
<i>Shank</i>	48	2757–2804	<i>Shank1</i>	AF131951
<i>IP3R</i>	48	7938–7985	<i>IP3R</i>	J05510

Table 2

mRNA levels of *Homer1b/c*, *Shank*, *IP3*, *Homer1a* and *ania-3* after chronic administration of lithium or valproate

Homer1b/c				
	Lithium	Valproate	P-Value	F _(df) -Value
<i>Cg</i>	93.96±2.9	87.52±2.1*	0.0137	5.491 _(2, 18)
<i>M2</i>	90.96±3.2	87.41±2.1*	0.016	5.249 _(2, 18)
<i>MI</i>	90.54±3.4	88.57±2.2*	0.0337	4.115 _(2, 18)
<i>SS</i>	92.57±3.7	89.4±3.0	0.117	2.414 _(2, 18)
<i>I</i>	85.59±2.5*	86.68±2.8*	0.0158	5.265 _(2, 18)
<hr/>				
<i>CPDM</i>	92.6±2.3	83.9±2.5*	0.004	7.398 _(2, 18)
<i>CPDL</i>	88.3±2.1*	80.7±1.7*	0.0001	15.552 _(2, 18)
<i>CPVL</i>	91.8±2.8	83.3±1.9*	0.002	8.536 _(2, 18)
<i>CPVM</i>	92.2±3	89.6±2.8*	0.009	6.108 _(2, 18)
<hr/>				
<i>AcCo</i>	90.4±3.6	85.7±2*	0.015	5.432 _(2, 17)
<i>AcSh</i>	96.8±3.2	89.5±3	0.154	2.091 _(2, 17)
<hr/>				
Shank				
	Lithium	Valproate	P-Value	F _(df) -Value
<i>Cg</i>	103.48±2.1	96.35±0.6	0.0047	7.466 _(2, 17)
<i>M2</i>	101.26±2.1	94.6±0.7*	0.0107	5.993 _(2, 17)
<i>MI</i>	99.53±1.8	94.85±1*	0.034	4.153 _(2, 17)
<i>SS</i>	101.06±1.5	96.53±0.9	0.0157	5.359 _(2, 17)
<i>I</i>	102.61±2.6	94.74±1.6	0.0178	5.155 _(2, 17)
<hr/>				
<i>CPDM</i>	100.9±3.2	94.9±2.5	0.29	1.326 _(2, 18)
<i>CPDL</i>	101.3±3.5	97.9±2.9	0.679	0.395 _(2, 18)
<i>CPVL</i>	102.6±3.3	96.7±2.3	0.333	1.167 _(2, 18)
<i>CPVM</i>	104.6±3.4	99.2±2.2	0.3	1.288 _(2, 18)
<hr/>				
<i>AcCo</i>	101.5±2.2	96.9±1.3	0.352	1.107 _(2, 18)
<hr/>				
IP3R				
	Lithium	Valproate	P-Value	F _(df) -Value
<i>Cg</i>	90.25±1.5*	93.64±2.2	0.0456	4.038 _(2, 12)
<i>M2</i>	86.79±1.2	92.55±3	0.0823	3.097 _(2, 12)
<i>MI</i>	83.35±1.4*	86.73±2	0.048	3.952 _(2, 12)
<i>SS</i>	86.06±0.9*	86.85±1.8	0.0181	5.712 _(2, 12)
<i>I</i>	89.82±1.2*	90.17±1.4	0.0346	4.510 _(2, 12)
<hr/>				
<i>CPDM</i>	89.63±1.9	89.12±2.4	0.096	2.821 _(2, 13)
<i>CPDL</i>	83.65±1.6	81.3±2.8*	0.0265	4.863 _(2, 13)
<i>CPVL</i>	87.53±2.2	83.51±2.4	0.138	2.311 _(2, 13)
<i>CPVM</i>	86.87±1.6	85.66±1.9	0.0788	3.128 _(2, 13)
<hr/>				
<i>AcCo</i>	95.17±2.1	92.22±0.9	0.296	1.336 _(2, 13)

IP3R						
Shank						
	Lithium	Valproate	P-Value	F _(ab) -Value	Lithium	Valproate
<i>AcSh</i>	102.4±2.2	97.2±1.4	0.248	1.505 _(2, 18)	89.39±2	90.13±2.9
						0.0423
						4.074 _(2, 13)
Ania-3						
Homer1a						
	Lithium	Valproate	P-Value	F _(ab) -Value	Lithium	Valproate
<i>Cg</i>	85.63±7.4	88.4±11.2	0.671	0.410 _(2, 13)	90.13±5.1	89.67±6
						0.448
<i>M2</i>	87.03±5.6	90.31±10.3	0.556	0.614 _(2, 13)	90.91±3.9	89.93±5.9
						0.332
<i>MI</i>	88.85±3.8	103.32±9.9	0.369	1.075 _(2, 13)	93.2±2.9	93.43±4.7
						0.398
<i>SS</i>	90.19±8	100.15±8	0.553	0.620 _(2, 13)	93.54±3.7	101.19±3.7
						0.264
<i>I</i>	80.74±5.1	83.74±7	0.0594	2.822 _(2, 13)	88.67±3.8	95.55±3.8
						0.107
						2.569 _(2, 16)
CPDM						
	98.3±11.3	87.2±8.6	0.258	1.483 _(2, 15)	93.8±4.4	97.3±3.3
						0.466
						0.805 _(2, 14)
CPDL						
	95.7±4.5	88.4±5.4	0.364	1.082 _(2, 15)	99.2±4.8	98.2±2.8
						0.921
						0.082 _(2, 14)
CPVL						
	97.6±6	91.7±4.4	0.486	0.756 _(2, 15)	95.9±2.9	100±2.4
						0.481
						0.770 _(2, 14)
CPVM						
	93.8±4.6	85.1±6.3	0.191	1.849 _(2, 15)	92.4±2.2	96.9±3.9
						0.29
						1.351 _(2, 14)
AcCo						
	94.9±5.4	88.6±5.7	0.463	0.810 _(2, 15)	94.7±2.9	96±4.4
						0.544
						0.635 _(2, 14)
AcSh						
	104.2±6.7	92.0±7	0.593	0.541 _(2, 15)	97.5±2.7	95.7±5.3
						0.788
						0.242 _(2, 14)