ABSTRACT

The present disclosure provides methods and compositions for the treatment of neurological disorders and stress-induced conditions. Methods of increasing the levels of 5Thr3a on neuronal cells is also provided.
FIGS. 1A-1K CONTINUED

D. Evf2-3' end regulated

E. Evf2-trans regulated

F. Evf2 regulated

Graphs showing the regulation of Evf2 in different neuron subtypes.
FIGS. 1A-1K CONTINUED

G

Evf2-3' end regulated

Evf2 chr6 targets

E13.5 MGE qRT-PCR
Evf1+/3' + Evf1+/1'

Ddx6 Evl2 Ddx5 Umad1 Lsm8 Rbm28 Akp5 Cond2

- Evf1+/--
- Evf1+/+

activated repressed independent

H

Evf2-trans regulated

Evf2 chr6 targets

E13.5 MGE qRT-PCR

Ddx6 Evl2 Ddx5 Umad1 Lsm8 Rbm28 Akp5 Cond2

Evl2trans

activates independent Evf1+/--/Evl2+/+

FIGS. 2A-2J CONTINUED

F

G

H

I

J
FIGS. 3A-3N CONTINUED

SOM weight positions

$Evf2^{+/+}$

$Evf2^{TS/TS}$
FIGS. 4A-4J CONTINUED
### FIGS. 6A-6D

#### Evf IncRNA spliced products

<table>
<thead>
<tr>
<th>Evf exons 1-4</th>
<th>Evf mouse mutants</th>
<th>Ev-f-regulated (E13.5 MGE)</th>
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<tr>
<td>Exon 2: Dlx5/6ei ultraconserved enhancer sequence</td>
<td>Evf2&lt;sup&gt;+/+&lt;/sup&gt;</td>
<td>Evf2&lt;sup&gt;TST&lt;/sup&gt;</td>
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<tr>
<td>Evf2</td>
<td>5'—&lt;br&gt;1</td>
<td>2</td>
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<tr>
<td>Evf2-5'</td>
<td>5'—&lt;br&gt;1</td>
<td>2</td>
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<tr>
<td>Evf1-3</td>
<td>5'—&lt;br&gt;3</td>
<td>4</td>
</tr>
<tr>
<td>Evf2-trans</td>
<td>5'—&lt;br&gt;1</td>
<td>2</td>
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</tbody>
</table>
FIGS. 8A-8D

A. $E_{v12}^{+/+}$ and $E_{v12}^{TS/TS}$

Hits (number of nuclei in each cluster)

B. Weights from $Dix5/6UCE:Akr1b8$

C. Weights from $Dix5/6UCE:Umad1$

D. Weights from $Umad1:Akr1b8$

B. Distances between $Dix5/6UCE:Umad1$
C. Distances between $Dix5/6UCE:Akr1b8$
D. Distances between $Umad1:Akr1b8$. 

Note: The images show patterns and clusters with varying shades indicating different levels of hits or weights.
FIGS. 9A-9G

A

Evf2^{+/+}

chr6: 0-40Mb

DLX5/6UCE-4C

H3K4me3 H3K4me1 H3K27me3
FIGS. 9A-9G CONTINUED

B

$Evf2^{TS/TS}$

$chr6 : 0\text{-}40\text{Mb}$

$DLX5/6UCE$-

$H3K4me3$ $H3K4me1$ $H3K27me3$
FIGS. 9A-9G CONTINUED

C conserved

chr6 : 0-40Mb

DLX5/6UCE-4C
H3K4me3 H3K4me1 H3K27me3
FIGS. 9A-9G CONTINUED

D

Um01 (~1.6Mb from Dlx5/6UCE)

E

Lsm8 (~12Mb from Dlx5/6UCE)
Fig. 10 Microarray of E13.5 MGE: Evf2\textsuperscript{+/-TS} \div Evf2\textsuperscript{+/-}
(validated list)

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Fold change</th>
<th>p value</th>
<th>Gene Ontology/Biological Process</th>
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<tr>
<td>Cdh2</td>
<td>2.22</td>
<td>2.16e-05</td>
<td>regulation of transcription, DNA-dependent</td>
</tr>
<tr>
<td>Lin28A</td>
<td>1.49</td>
<td>0.0266</td>
<td>RNA processing, RNA metabolism</td>
</tr>
<tr>
<td>AtxT</td>
<td>1.09</td>
<td>0.8741</td>
<td>oxidation reduction</td>
</tr>
<tr>
<td>Evf2</td>
<td>1.85</td>
<td>0.55</td>
<td>transition of mitotic cell cycle</td>
</tr>
</tbody>
</table>

*validated changes by qPCR

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<tr>
<td>Evf2</td>
<td>1.85</td>
<td>transition of mitotic cell cycle</td>
<td>0.55</td>
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</tbody>
</table>

*validated changes by qPCR
Fig. 12  In vivo dosage relationships between interneuron subtype genes and *Evl2-chr6* target genes

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<tr>
<th>RSQ: qRT-PCR E13.5 MGE (*6 genotypes)</th>
<th>CALB</th>
<th>GAD1</th>
<th>NPY</th>
<th>SOM</th>
<th>5HTR3a</th>
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<td>Dlx6</td>
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<td>0.18</td>
<td>0.12</td>
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<td>Evl2</td>
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<td>0.23</td>
<td>0.09</td>
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<tr>
<td>Dlx5</td>
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<td>0.35</td>
<td>0.04</td>
<td>0.02</td>
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<td>Umad1</td>
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<tr>
<td>Lsm8</td>
<td>0.52</td>
<td>0.35</td>
<td>0.95</td>
<td>0.56</td>
<td>0.34</td>
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<td>Rbm28</td>
<td>0.22</td>
<td>0.21</td>
<td>0.07</td>
<td>0.00</td>
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<td>Akr1b8</td>
<td>0.19</td>
<td>0.63</td>
<td>0.29</td>
<td>0.14</td>
<td>0.17</td>
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</table>

RSQ >0.80

*Evl2TSm/+p, Evl2TSp/+m, Evl2TS/TS,
Dlx5/6KOm/TSp, Dlx5/6KOp/TSm, Evl1TS/TS
pooled (n=3-7 each genotype)
FIG. 13

E13.5 mouse ganglionic eminences
COMPOSITIONS AND METHODS OF TREATING NEUROLOGICAL DISORDER AND STRESS-INDUCED CONDITIONS

CROSS-REFERENCE TO RELATED APPLICATIONS


STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with government support under R01 MH090463 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

[0003] The field of the invention is the treatment of neurological diseases and stress-induced conditions, including developmental neurological disorder and mood disorders.

[0004] Early life stress and trauma is a prominent risk factor for several psychiatric illnesses, including mood and anxiety disorders (Holmes et al., 2005). Further, in utero maternal stress has been shown in clinical studies (pregnant women’s exposure to a range of traumatic, as well as chronic and common life stressors (i.e., bereavement, daily hassles, and earthquake)) to result in significant alterations in children’s neurodevelopment, including increased risk for mixed handedness, autism, affective disorders, and reduced cognitive ability. (Talge N M, Neul C, Glover V. Antenatal maternal stress and long-term effects on child neurodevelopment: how and why? J Child Psychol Psychiatry. 2007; 48(3-4):245-61). More recently, maternal antenatal anxiety and/or depression have been shown to predict increased risk for neurodevelopmental disorders in children, and to confer risk for future mental illness. (O’Connor et al., Maternal antenatal anxiety and behavioural/emotional problems in children: a test of a programming hypothesis, Child Psychol Psychiatry. 2003 October; 44(7):1025-36). While early-life stress effects and in utero effects on adult psychopathology may depend upon genetic risk, the nature of gene and environment interaction is thought to play a role in the outcome.

[0005] Mood disorders are presently treated by a number of antidepressant medications. Most of these drugs are either tricyclic antidepressants (TCAs) or selective serotonin reuptake inhibitors (SSRIs). The efficacy of these drugs differs substantially among patients. These therapies can also have significant side effects. For example, more than a third of patients taking SSRIs experience sexual dysfunction. Other problematic side effects include gastrointestinal disturbances, often manifested as nausea and occasional vomiting, agitation, insomnia, weight gain, and onset of diabetes.

[0006] Present drugs directly bind serotonin receptors to affect neuronal activity, affecting all neurons expressing serotonin receptors to increase the levels of serotonin in the central nervous system (CNS).

[0007] Therefore, there is need for additional treatment options for mood disorders by targeting novel pathways that can directly affect serotonin receptor expression in subsets of neuronal populations. The present invention is directed to meeting this and other needs.

SUMMARY OF THE INVENTION

[0008] Certain embodiments of the present disclosure substantially overcome the aforementioned drawbacks by providing a novel pathway to directly increase the level of serotonin receptor gene expression in neurons, providing a novel agent for treating neurological disorders and stress-induced conditions. Applicant has found that Evv2 long non-coding RNA modulates serotonin receptor expression by decreasing the expression of a specific enzyme, Akri88, in developing interneurons. Mice lacking Evv2 exhibit changes in behavior, including behavioral despair, learning and seizure susceptibility. This disclosure provides compositions and methods of treating neurological disorders and stress-induced conditions by treating a subject with Akri88/ B10 or an agonist thereof. Further, the disclosure provides methods and compositions for treating neurological disorders and stress-induced conditions by treating a subject with small molecule effectors or metabolites of the mevalonate pathway.

[0009] In one aspect, the disclosure provides a method of treating a neurological disorder or stress-induced condition in a subject, the method comprising the steps of administering an effective amount of at least one aldo-keto reductase family 1, member b10 (Akri1b10), aldo-keto reductase family 1, member B8 (Akri1b8), an agonist of Akri1b10, or an agonist of Akri1b8 in order to alleviate, reduce or inhibit one or more symptoms of the neurological disorder or stress induced condition in the subject.

[0010] In another aspect, the present disclosure provides a method of increasing expression of 5-hydroxytryptamine receptor 3A (5Ht3a) in at least one neuron, the method comprising contacting the at least one neuron with at least one selected from the group consisting of Akri1b8, an agonist of Akri1b8, Akri1b10 and an agonist of Akri1b10, wherein the at least one neuron exhibits an increase in expression of 5Ht3a.

[0011] In another aspect, the present disclosure provides a method of increasing the serotonin level in a subject, the method comprising administering to the subject Akri1b8, an agonist of Akri1b8, Akri1b10, an agonist of Akri1b10, or a combination thereof, in an effective amount to increase the serotonin level in the subject.

[0012] In yet another aspect, the disclosure provides a method of inducing a pluripotent stem cell to differentiate into a neuron comprising culturing the pluripotent stem cell with Akri1b8, an agonist of Akri1b8, Akri1b10, an agonist of Akri1b10, or a combination thereof wherein the pluripotent stem cell differentiates into a neuron that expresses 5Ht3a.

[0013] In another aspect, the disclosure provides a method of treating a neurological disorder or stress-induced disorder, the method comprising administering in an effective amount a small molecule effector or metabolite of the mevalonate pathway, wherein administration of the small molecule effector or metabolite alleviates, reduce or inhibit at least one or more symptoms of the neurological disorder or stress-induced disorder.

[0014] The foregoing and other aspects and advantages of the invention will appear from the following description. In the description, reference is made to the accompanying drawings which form a part hereof, and in which there is shown by way of illustration at least one preferred embodi-
ment of the invention. Such embodiment does not necessarily represent the full scope of the invention, however, and reference is made therefore to the claims and herein for interpreting the scope of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawings(s) will be provided by the Office upon request and payment of the necessary fee.

[0016] FIG. 1A-1K. Evf2 enhancer lncRNA regulation of genes across 27 Mb and Dlx6 dosage regulated interneuron subtype enhancers. A. Schematic of the 27.6 Mb region of mouse chr7 encompassing Evf2 and transcriptionally regulated target genes. Evf1 and Evf2 lncRNAs are spliced, polyadenylated transcripts, transcribed over ~51 kb region. Dlx5/6 intergenic region, enhancers (Dlx5/6/UCNE, etc.), (Zrenka et al., 2000). Red arrows indicate sites of triple polyA transcription stop insertions, preventing Evf2 (Evf2TS) and Evf1 (Evf1ITS) transcription in mice. Evf2-chr7 targets, identified by microarray, and validated by qRT-PCR (FIG. 10), are asymmetrically located across 27 Mb region. The corresponding human chr7 loci are indicated. B-H. MGE qRT-PCR gene expression analysis from mouse lacking Evf2 (Evf2TS/TS, Evf2-regulated), lacking Evf1 and expressing truncated Evf2-5' (Evf1TS/TS, Evf2-3'regulated), expressing an Evf2 transgene (Evf2TS/TS-R, transregulated), or wildtype littermates (Evf1+/+, Evf2++). B. Interneuron subtype genes (Calb1, Npy, Sat, Slt3ra) in Evf2TS/TS normalized to Evf2++-, C. Evf2,5'-exon1-2, Evf1 (exon3-4), Dlx5, and Dlx6, in Evf1TS/TS (grey bars), normalized to Evf1++ (black bars). D. Interneuron subtype genes (Calb1, Npy, Sat, Sh3ta) in Evf1TS/TS normalized to Evf1++-, E. interneuron subtype genes (Calb1, Npy, Sat, Sh3ta) in Evf1TS/TS normalized to Evf1++-, F. chr7 targets: Evf2TS/TS normalized to Evf2++-, G. chr7 targets: Evf1TS/TS normalized to Evf1+++-, H. chr7 targets: Evf2TS/TS-R normalized to Evf2TS/TS (except for yellow bar, normalized to Evf2++-). A-H: n=4-7 of each genotype, values normalized to Evf2++-, Evf1++-, or Evf2TS/TS (dotted lines), A, C, I-H, red (repressed genes), grey (Dlx6, Dlx5, Rbn28, Akr1b8, Calb1), green (activated genes, Umad1, Lmna, Npy, Sat, Slt3ra), green (Evf2 independent gene, Cnd2). I-K. UCSC browser display of interneuron genes and DLX binding sites identified by ChIP-seq. Dlx6 dosage-dependent regulation of DLX binding sites is tested in luciferase reporter assays, using primary MGIE cells. Triangles represent increasing concentrations of Dlx6 plasmid; results are normalized to plasmid expressing GFP. J. Calb1 gene, Dlx6-regulated 2/3 DLX binding sites, J. Npy gene, Dlx6-regulated 2/3 DLX binding sites. K. Sat (Som) gene, Dlx5-regulated 1/3 DLX binding sites. I-K: n=12, Student's t-test values from two experiments, Student's t-test, *p<0.05, **p<0.01, ***p<0.001, error bars (S.E.M.)

[0017] FIG. 2A-J. The Evf2-Akr1b8-5l1r3a region: Akr1b8 and mevalonate pathway-regulated enhancers in the Zbb16-5l1r3a region. A, B. qRT-PCR analysis of CMGE, Evf2TS/TS normalized to Evf2++-. C. qRT-PCR analysis of CMGE, Evf2TS/TS, Akr1b8-5l1r3a expression in primary CGE transfectected with Akr1b8 (pCMV6-Akr1b8, grey bars), normalized to GFP transfectected with GFP (pCMV-GFP, black bars), n=3-6. C. UCSC Broker display of Zbb16-5l1r3a(b) (5l1r3a(b) region), and different network tools (ChIP-seq, MACS2, purple bars) in the promoter region of Zbb16 of Evf2++/vs Evf2TS/TS GE, identifies potential Akr1b8-regulated enhancers (Akr1b8-5l1r3a, Akr1b8, orange). D-F. J. Regulation of Akr1b8 in luciferase reporter assays, using primary CGE and MGIE cells. F. Akr1b8 regulation of individual enhancers Akr1b8 (1) and Akr1b8 (2), normalized to GFP expression. G-J. Dosage effects of mevalonate pathway metabolites FOH and GGOH (grey bars) on Akr1b8 luciferase reporters, normalized to buffer alone (black bars). Triangles indicate increasing concentrations (FOH: 0.1, 1, 10, or 100 μM, GGOH: 0.01, 0.1, 1, 10, or 100 μM). G-H. MGIE, n=10, and I-J. MGIE, n=12, averaged from two experiments, Student's t-test, *p<0.05, **p<0.01, ***p<0.001, error bars (S.E.M.).

[0018] FIG. 3A-N. The Evf2 RNA cloud associates with Umad1 and Akr1b8, and regulates Dlx5/6 UCE-Umad1- Akr1b8 distances in interneuron subpopulations. A. Schematic showing the distances between Dlx5/6 UCE (yellow), Umad1 (green, activated target gene), Akr1b8 (red, repressed target gene), direction of Evf2 lncRNA transcription, formation of the Evf2 RNA cloud (green dashed circle). B-C. Fluorescent in situ hybridization (FISH) of GE nuclei probed with anti-sense Evf2 RNA (green), and DNA probes, as indicated. White arrows indicate co-localization of Evf2 RNA cloud and target genes. D-G. DNA FISH of GE nuclei showing examples of Dlx5/6 UCE-gene interactions. H. Schematics summarizing the Evf2 RNA cloud localization and Dlx5/6 UCE-Umad1 Akr1b8 interactions. I-N. Comparison of distances between Dlx5/6 UCE-Umad1 Akr1b8 in Evf2++- and Evf2TS/TS GE nuclei (n=83, each genotype). I-K. Gene distances from single nuclei binned in 8 groups (<0.2 μm-2 μm), and percentages of nuclei in each bin plotted. Chi-square (χ², *p<0.05), degrees of freedom (df=7), Evf2++- (black bars) Evf2TS/TS, Evf2 density plot of gene distances shows greater density of Evf2++- nuclei (blue) outside of main cluster Evf2++- nuclei (red). M. N. Self-organizing maps (SOMs) in the Matlab neural network toolbox (NTI) and three training iterations optimally cluster gene-distance data and visualization. M. Neighbor weight distance SOMs show that ~2-fold more Evf2++- nuclei clusters Evf2++- are connected by closer distances (yellow SOMs) provide a 3-D visualization of connections between Evf2++- (orange) and Evf2TS/TS (blue) centroids.

[0019] FIG. 4A-J. Evf2 regulates Dlx5/6 UCE interactions across chr6. A-C. Integrated Circos plots indicating Dlx5/6 UCE interaction sites across chr6 (inner panels showing interactions identified by 4C-seq of GE), and corresponding H3Kme profiles (identified by native ChIP-seq of GE, H3K4me3 [green], H3K4me1 [purple], H3K27me3 [red] peaks). A. enriched in Evf2++- (positively regulated), B. enriched in Evf2TS/TS (-, negatively regulated), C. conserved (detected in both Evf2++- and Evf2TS/TS, Evf2-independent). D-E. Upper panels indicate the distribution of Evf2-regulated Dlx5/6 UCE interacting sites (Evf2++- (+, orange empty circles, Evf2TS/TS (-, empty blue circles. Lower panels indicate the density of Evf2-regulated Dlx5/6 UCE interacting sites. D. Across entire chr6 (~150 Mb), E. Across 40 Mb of chr6 (including 27 Mb region containing the...
Dlx5/6UCE bait and transcriptional target genes, Umad1, Lsm8, Rbm28, and Akr1b8. F-J. Normalized read counts of histone modifications H3K4me3, H3K4me1, H3K27me3, and H3K27ac with respect to distance from Dlx5/6UCE-chromatin interacting sites. Histone modification plots showing p-value calculations at ±0.2 kb, ±0.6 kb, and ±1.0 kb, indicated by grey bars; pink line (p<0.05) on the right y-axis indicates the cut-off for significant differences. E. Evf2-2* comparison of histone modification profiles at Evf2-Dlx5/6UCE-chr6 sites (+, orange solid line) and (−, orange dashed line), (unpaired t-test). G. Evf2-2* comparison of histone modification profiles at Evf2-Dlx5/6UCE-chr6 sites (+, blue solid line) and (−, blue dashed line), (unpaired t-test). H. Comparison of histone modifications profiles at Evf2-Dlx5/6UCE-chr6 sites (+) in Evf2-2* (orange line), and Evf2-2* (blue line), (paired t-test). I. Comparison of histone modification profiles at Evf2-Dlx5/6UCE-chr6 sites (+) in Evf2-2* (orange line), and Evf2-2* (blue line), (paired t-test). J. Comparison of histone modification profiles at conserved Dlx5/6UCE-chromatin interaction sites (Evf2-independent, I) in Evf2-2* (orange line), and Evf2-2* (blue line), (paired t-test).

[0020] FIG. 5A-E. Biological significance of Evf2-Dlx5/6UCE-chromatin interactions. A. Schematic showing EVF2 regulation of genes located across a 27 Mb region of mouse chr6. Evf2 is transcribed from the Dlx5/6UCE (yellow +), and also transcribed antisense to Dlx6. Evf2 represses Dlx6, Rbm28 and Akr1b8 (red boxes) through Evf2-2* Dlx5/6UCE-containing region. Dlx6 dosage regulates enhancers in interneuron subtype genes (Calb1, Npy, Sat), contributing to interneuron diversity. Akr1b8, an aldoketoreductase and mevalonate pathway metabolites (FOH and GOGH) regulate enhancers at the promoter of Zbtb16, downstream of the interneuron subtype gene (5Htr3a). Evf2 activates Umad1 and Lsm8 (green boxes), activating Zbtb16 through transcription mechanisms (green arrow). Umad1 and Lsm8 dosage are linked genetically to subtype gene dosage (Umad15Htr3a, Umad1Sst, Lsm8Npy), through unknown mechanisms. Evf2 regulation of interneuron subtype genes depends on embryonic brain region (MGE vs. CGE). Evf2-chromatin target gene organization is conserved with human chr7, except Umad1 is located 88 Mb Y of Dlx5/6UCE (7p21). Despite this inversion, Dlx5/6UCE-Umad1 and Dlx5/6UCE-Akr1b810 interactions are conserved in mouse E13, 5GE and developing human brain (orange arrows). B. The Evf2 RNA cloud (dashed green circle) localizes to both activated and repressed target genes in the instructive 27 Mb region (orange box). Along chr6, Dlx5/6UCE interaction sites are divided into Evf2 positively (+, green arrow), negatively (−, grey arrow), and independent (I, grey arrow), indicating that Evf2 regulates both the number and position of (+) and (−) sites. Histone modifications distinguishing between (+) and (−), where active marks H3K4me3/1 and H3K27ac are enriched at (+) compared to (−) sites. C. Venn diagram showing the relationship between genes near Evf2-regulated (+ green circle, − red circle) and independent (I, grey circle) Dlx5/6-chromatin sites. D. Gene ontology (GO) analysis of Dlx5/6UCE-chromatin interactions in mouse E13, 5GE, indicating specific biological processes at genes near (I) and (−) sites, but not (+) sites. E. Venn diagram showing Dlx5/6UCE-genome interactions that are conserved between human chr7 (developing brain) and mouse chr6 (E13, 5GE) (black numbers, human, Hu, white numbers, mouse, Mo). ~44% of Dlx5/6UCE-chr7 gene interactions are conserved with mice (orange circle overlap with deep yellow), while ~51% are Evf2-regulated (green and red overlap with deep yellow).

[0021] FIG. 6A-D. Evf2-chromatin-targets and interneuron subtype gene expression: dose-dependent and differential roles of the Evf2-2* region, 3′ end and trans effects. A. Table summarizing necessary and sufficient regulatory roles of different Evf IncRNA spliced forms in E13.5 MGE. Evf exons are labeled (1-4), repressed genes (red), and activated genes (green). Pink Star: correlation between repressed targets and interneuron subtype gene regulation. B. Taqman E13.5 MGE qRT-PCR analysis from 6 different mouse mutants, where maternal (m) and paternal (p) alleles are indicated: Evf2T2Sm*+p, Evf2T2Sp*+m, Evf2T2TS/TS, Dlx5/6OMn/TSp, Dlx5/6OKp/TSm, Dlx5/6OKp/TSm. Values are normalized between Evf2Evf2-chromat target genes (Evf2-2*, Dlx5, Dlx6, Umad1, Lsm8, Rbm28, Akr1b8), and Gad1 interneuron subtype genes (Gad1, Calb1, Npy, Som, 5Htr3a), reveal dose dependent relationships for 4/35 gene pairs. X-axis: Evf2-chromatin target genes, Y-axis: interneuron subtype genes: Dlx6: Calb1 (r2=0.81), Lsm8:Npy (r2=0.95), Umad1:5Htr3a (r2=0.93), Umad1:Som/Sst1 (r2=0.86), n=4-7 of each genotype (individuals analyzed for Evf2TS/TS and EvtTTS/TS, pooled values for Evf2T2Sm*+p, Evf2T2Sp*+m, Dlx5/6OMn/TSp, Dlx5/6OKp/TSm. Values are normalized to +/− litters. Schematics for each genotype are shown on the right. C. Taqman E13.5 MGE qRT-PCR analysis from Dlx5/6OK/T S showing genetic rescue of Dlx5/6 rescues effects on interneuron subtype genes, normalized to Dlx5/6+/+Evf2+ litters, n=4 each genotype, Student’s t-test, **p<0.001. D. Evf2-regulated histone lysine methylation (H3K4me3) changes in promoter regions of interneuron subtype genes (Calb1, Npy, Som/Sst1). UCSC browser profiles of anti-H3K4me3, native ChIPseq results compare profiles in Evf2+ and Evf2TS/TS E13.5 GE chromatin. Despite subtle changes in (~2-fold), IDR-MACS2 peaks are indicated by black tracks, where darker bars indicate higher peak densities (black-grey). MACS2 identities differential peaks (pink tracks), expressed in -log10(p-value). Computationally predicted enhancer sites are indicated at the top (Enhancers mm9; FANTOM, UCSC).

[0022] FIG. 7A-E. Genetic and epigenetic analysis of the Evf2-Akr1b8-5Htr3a axis. A. Schematic of E13.5 MGE mouse embryonic brain indicating sub-divisions of embryonic ganglionic eminences (LGE, MGE, CGE), with the red dotted line showing the region dissected to separate MGE from CGE. Taqman qRT-PCR analysis of Nkx2.1 (a marker for MGE), confirming accuracy of dissections between MGE (Nkx2.1 detected), and CGE (Nkx2.1 not detected), n=2 pools each region, *p<0.02. B. Taqman qRT-PCR analysis of E13.5 CGE, Evf2T2TS/TS normalized to Evf2+/+, Akr1b8, Dlx6, retinoid receptor alpha (RxRα), interneuron subtype genes in E13.5 CGE, Akr1b8−/− normalized to Akr1b8+/+. Loss of Akr1b8 does not affect interneuron subtype gene expression in CGE. C. Taqman qRT-PCR analysis of RxRα in LGE, MGE, and CGE of Evf2+/+ and Evf2T2TS indicates no effects of Evf2 loss in any region of the GE; p<0.05, n=4. D. Taqman qRT-PCR analysis of E13.5 MGE Evf2T2TS/TS, Akr1b8−/− normalized to Evf2+/+, Akr1b8+/+. Akr1b8 loss does not rescue Evf2 effects on 5Htr3a in MGE (Akr1b8−, 5Htr3a), n=6-15 of each genotype; Student’s t-test, **p<0.01, error bars (S.E.M). E. Evf2-regulated histone lysine methylation (H3K4me3, H3K4me1, H3K27me3) changes in the Zbtb16-5Htr3a region. UCSC browser pro-
files of native differential ChIPseq results compare profiles in Evf2+/+ and Evf2TS/TS E13.5 GE chromatin. IDR-MACS2 peaks are indicated by black tracks, where darker bars indicate higher peak densities (black g grey). MACS2 identifies differential peaks (pink tracks), expressed in -log10 (P-value). Computationally predicted enhancer sites are indicated at the top (Enhancers mm9, FANTOM, UCSC).

[0023] FIG. 8A-D. Self-organizing map analysis of Dlx5/6UCE-Umada1-Akr1b8 gene distances in Evf2+/+ vs Evf2TS/TS E13.5 GE nuclei. Dlx5/6UCE gene distances, and gene- gene distances were calculated for 83 nuclei from Evf2+/+ and Evf2TS/TS by DNA FISH: Dlx5/6/Umada1, Umada1, Akr1b8, and Dlx5/6/Akr1b8. Self-organizing maps (SOMs) were generated in the Matlab neural net-work toolbox (NNT) using three training iterations to optimally cluster gene-distance data and visualization (www.mathworks.com/help/nnet/ys/chs-ter-data-with-a-self-organizing-map. html). The NNT provides algorithms and applications to create and visualize neural networks, including methods for clustering data www.mathworks.com/help/nnet/index.html. A. Blue hexagons represent clusters, with the number of nuclei indicated in each cluster. B-D. Weights from each distance are indicated by color, with lowest (yellow) highest (black). B. Distsances between Dlx5/6UCE-Umada1. C. Distsances between Dlx5/6UCE-Akr1b8. D. Distances between Umada1-Akr1b8.

[0024] FIG. 9A-G. Evf2-dependent and independent Dlx5/6UCE interactions and histone lyme methylation effects across chr6 (0-40 Mb) and at Evf2-chr6 target genes. A-C. Integrated Circos plots indicating Dlx5/6UCE interaction sites across chr6 (0-40 Mb), with the Dlx5/6UCE bait and long-range transcriptionally regulated target genes (Umada1, Lsm8, Rbm28, Akr1b8) labeled. Inner panels show Dlx5/6UCE interactions identified by 4C-seq; surrounding panels show histone lysine methylation profiles (MACS2 enriched (+/−) or conserved (1) identified by native ChIPseq of E13.5 GE, H5K4me3 [green], H5K4me1 [purple], H5K27me3 [red]. A. enriched in Evf2+/+, positively regulated), B. enriched in Evf2TS/TS (−, negatively regulated), C. conserved (detected in both Evf2+/+ and Evf2TS/TS, 1, Evf2-independent). D-G. Zoomed in regions of Dlx5/6UCE interacting sites (+, red region) and (−, yellow region) aligned with differential histone lysine methylation effects at Evf2-chr6 target genes. D. Umada1, E. Lsm8, F. Rbm28, G. Akr1b8. Only differential ChIPseq peaks are indicated. Taken from Wust1 genome browser site. Evf2-dependent changes in histone lyme methylation at (+) and (−) do not follow general correlations with respect to transcriptional regulation.


[0026] FIG. 11. Table of microarray analysis of gene expression in E13.5 MGE Evf2+/+,Evf2TS/TS, complete list

[0027] FIG. 12. RSQ Table of in vivo dosage relationships between interneuron subtype genes and Evf2-chr6 target genes

[0028] FIG. 13 is a schematic of a model summarizing spatial dependence of the Evf2-Akr1b8-5HT3a pathway in E13.5 mouse brain ganglionic eminence (MGE and CGE), beginning with Sh activation of Dlx1/2 and Evf1/2 (Kohut et al. 1998; Feng et al. 2006), and ending with differential regulation of 5HT3a.

[0029] FIG. 14. A diagram depicting the role of AKR1B10 in the Mevalonate Pathway. Figure from Rizner 2012 (“Enzymes of the AKR1B and AKR1C subfamilies and uterine diseases”, Frontiers in Pharmacology, vol. 3, Article 34, March 2012). AKR1B10, AKR1C3, and AKR1C1 catalyze the reduction of all-trans-retinal and 9-cis-retinal to their corresponding retinols, respectively. The reverse reaction is catalyzed by alcohol dehydrogenases (ADHs). Retinal is further oxidized by aldehyde dehydrogenases (ALDHs) to form retinoic acid, which by binding to the retinoic acid receptor (RAR) and the retinoid X receptor (RXR) stimulates cell differentiation. Retinoic acid is further metabolized by CYP26A to form 4-hydroxy-retinoic acid. (Adapted from Endo et al., 2011.)

[0030] FIG. 15. A diagram depicting the implications of AKR1B and AKR1C enzymes in retinoid signaling. Figure from Rizner 2012. Prenylation involves transfer of farnesyl pyrophosphate (FPP) or geranylgeranyl pyrophosphate (GGPP) by farnesyl transferase (FTI) and geranylgeranyl transferase 1 and 2 (GGT1 and GGT2), respectively, to various proteins including small GTPases (RAS and RHO). The reverse reaction that releases farnesol (FA) and geranyl geranial (GGAL) is catalyzed by prenyl cestyle (PCLY). Farnesyl pyrophosphate and geranylgeranly pyrophosphate thus serve as substrates of FTI and GGT1/GGT2 but can also be dephosphorylated to form farnesol (FOH) and geranylgeranyl (GGOH). FOH and GGOH are oxidized to FA and GGAL by alcohol dehydrogenases (ADHs) and by yet unidentified enzymes to farnesic acid (FA), geranylgeranylic acid (GGA) and other metabolites. The reduction of FA and GGAL to FOH and GGOH is catalyzed by AKR1B and AKR1C enzymes. This reaction indirectly recovers substrates for further formation of active prenyl pyrophosphates. Additionally, reduction of GGAL to GGOH prevents FA and GGA and the metabolites with potential apoptotic effects. (Adapted from Endo et al., 2011.)

DETAILED DESCRIPTION OF THE INVENTION

[0031] The present disclosure provides compositions and methods for the treatment of neurological disorders or stress-induced conditions. Applicant surprisingly found that Evf2 long non-coding RNA modules serotonin receptor expression by increasing the expression and activation of alko-keto reductase 1B8 (Akr1b8) (mouse) in developing interneurons. By increasing the levels of Akr1b8/10, neuronal cells increase expression and activation of 5-hydroxytryptamine receptor 3A (5HT3a) and 5HT3a. The expression of 5HT3a receptor leads to the increase in serotonin in a subset of neuronal cells. This increase in serotonin can alleviate, reduce, attenuate or inhibit one or more symptoms of a neurological disorder or stress-induced condition.

[0032] This disclosure provides compositions and methods of treating neurological disorders and stress-induced conditions by treating a subject with an effective amount of Akr1b8/B10, or an agonist thereof. Further, the disclosure provides methods and compositions for treating neurological disorders and stress-induced conditions by treating a subject with small molecule effectors or metabolites of the mevalonate pathway. The present disclosure demonstrates that the activation of alko-keto reductase1b8 (Akr1b8) or the
human homolog Akr1b10 regulates and activates S1Hr3α, and in turn increases the level of serotonin in the brain.

[0033] The present disclosure provides improved compositions and methods of treating neurological disorders, including mood disorders, over the standard serotonin specific reuptake inhibitors (SSRIs) by directly regulating and increasing the amount of S1Hr3α (serotonin receptor) on a subset of neuronal cells, which in turn regulates serotonin levels in the brain of a subject.

[0034] In some embodiments, the present disclosure provides a method of treating a neurological disorder or stress induced condition in a subject. The method comprises administering at least one of aldo-keto reductase family 1, member b10 (Akr1b10), aldo-keto reductase family 1, member b8 (Akr1b8), an agonist of Akr1b10, or an agonist of Akr1b8 in an effective amount to alleviate, reduce or inhibit one or more symptoms of the neurological disorder or stress induced condition in the subject.

[0035] Aldo-keto reductase family 1, member b10 (Akr1b10) (SEQ ID NO: 1) is the human homolog of aldo-keto reductase family 1, member b8 (Akr1b8) (SEQ ID NO: 3) in mice. Akr1b10 belongs to the AKR superfamily composed of more than 100 proteins that are structurally and/or functionally conserved in hierarchy of organisms from bacteria to humans. Akr1b10 is a monomeric enzyme with NADPH as a co-enzyme, and its enzyme activity is regulated by S-thiolation at the protein level. It is contemplated that either Akr1b10 or Akr1b8 can be used in the methods of the present disclosure. In some embodiments, a polypeptide comprising or consisting of SEQ ID NO:1 or SEQ ID NO:3 are used.

[0036] The Akr1b10 or Akr1b8 protein used in methods of the present disclosure may be a recombinant form of the protein or a protein directly or indirectly linked to an exogenous tag or agent. Suitable tags are known in the art and include, but are not limited to, affinity or epitope tags (nonlimiting examples include, e.g., cMyc, HIS, FLAG, V5-tag, HA-tag, NE-tag). Suitable agents include agents that help with the bioavailability or targeting of the protein, for example, but not limited to, agents that specifically target the blood brain barrier to allow for translocation of the proteins into the brain of a subject. In some embodiments, the Akr1b10 or Akr1b8 protein or agonists thereof may be directly or indirectly linked to an antibody or molecule with blood-brain barrier or blood-CSF barrier penetrant properties. For example, antibodies having binding specificity for the blood brain barrier are known in the art and include, but are not limited to, an antibody specific for a blood-brain barrier (BBB) receptor (BBB) which allows for BBB transcytosis properties, a polypeptide or liposome that allows for BBB transport. In some embodiments, the blood brain barrier receptor is selected from the group consisting of transferrin receptor (TfR), insulin receptor, insulin-like growth factor receptor (IGF receptor), low density lipoprotein receptor-related protein 8 (LRP8), low density lipoprotein receptor-related protein 1 (LRP1), and heparin-binding epidermal growth factor-like growth factor (HB-EGF). Suitable BBBRs are discussed for example in WO2012/075037, WO/2014/033074 and WO2015101586, the contents of which are incorporated by reference in their entirety.

Further, suitable blood-brain barrier targeting antibodies are discussed in, for example, US2008/0019984, US20150196663, U.S. Pat. No. 5,004,697, WO 2016094566, PCT/US2007/070587, US20170174778, which are incorporated by reference in their entirety.

Suitable blood brain barrier polypeptides are known in the art and include, but are not limited to, polypeptides discussed in WO2014076655 A1, WO2003089815 A2, WO2016079315, U.S. Pat. No. 7,902,156, WO2016079315, among others.

[0037] Suitable Akr1b10 agonists are known in the art and include, but are not limited to, for example, tolrestat, EBPC (Ethyl-1-benzylyl-3-hydroxy-2(5H)-oxoppyrrole-4-carboxylate), zoprelstat, sorbinil, epalrestat, fidarestat, statil (3-(4-Bromo-2Hfluorobenzyl)-4-oxo-3H-phthalizin-1-y]l]acetate acid), isothiocyanic acid, androst-4-ene-3,6-dione, androst-4-ene-3,6-diol, PGA1, apigenin, luteolin, 7-hydroxyflavone, magnolol, honokiol, resveratrol, BDMC (desmethoxycurcumin), butein, oleanolic acid, and y-mangostin, CAPE (acecic acid phenethyl ester), 3-(4-hydroxy-2-methoxyphenyl) acrylic acid 3-(3-hydroxyphenyl)propyl ester, MTF (9-methyl-2,3,7-trihydroxy-6-fluorone), (Z)-2-(4-methoxyphenylimino)-7-hydroxy-N-(pyridine-2-yl)-2H-chromene-3-carboxamide, 7-hydroxy-2-(4-methoxyphenylimino)-2H-chromene-3-carboxylic acid benzylamid, UV2008, androstane-3β,5α,6β,9-tetrol, JF0604, JF0049, VA (glycyrrhetinic acid), dihydroartemisin, thymoquinone, and others, some of which can be found in Huang et al. “Aldo-Keto Reductase Family 1 Member B10 Inhibitors: Potential Drugs for Cancer Treatment, Recent Patents on Anti-Cancer Drug Discovery 2016, 11, 184-196, the contents of which are incorporated by reference in their entirety.

[0038] In further embodiments, the Akr1b10 and Akr1b8 agonist may include, but are not limited to Akr1b10 antisense RNA, Evf2 antisense RNA, Dlx6 antisense RNA, Akr1b10 siRNA, Akr1b8 siRNA, Evf2 siRNA, Dlx6 siRNA, or combinations thereof. Suitable antisense RNA can be derived from one skilled in the art, for example using SEQ ID NO:1, A single-stranded RNA (antisense RNA (asRNA)) is complementary to a messenger RNA (mRNA) strand transcribed within a cell, the asRNA and are from about 15 to 30 bp long. siRNA consists of two RNA strands, an antisense (or guide) strand and a sense (or passenger) strand, which form a duplex from about 19-25 bp in length, usually with a 3' nucleotide overhang. siRNA against Akr1b10 can also be found commercially sold by a number of companies, for example, Ambion Inc (Austin, Tex., e.g., Sense (AGAGGAUGUGAUGUGUCAUTTESEQ ID NO:5) and anti-sense (AUGAACAUCACAUCCUCUGGSEQ ID NO:6) oligonucleotides available for purchase) and from Novus Biologics (Littleton Colo.). Suitable siRNA or asRNA can be derived by one skilled in the art using the sequences of Dlx6 and Evf2 known in the art, for example, from Dlx6 using SEQ ID NO: 52 or 53, and Evf2 using SEQ ID NO:53 and 54.

[0039] In some embodiments, the Akr1b10, Akr1b8, Akr1b10 or Akr1b8 agonists of the present disclosure may be delivered to neurons by use of a suitable expression vector for delivery into the subject. A recombinant expression cassette comprising a polynucleotide encoding the protein or agonist of the present invention is also contemplated. The polynucleotide may be under the control of a transcriptional promoter allowing the regulation of the transcription of the polynucleotide in a host cell.

[0040] The present disclosure also provides a recombinant expression cassette comprising a polynucleotide according to embodiments of the present disclosure under the control of a transcriptional promoter allowing the regulation of the
transcription of the polynucleotide in a host cell, e.g., a neuronal cell. The polynucleotide can also be linked to appropriate control sequences allowing the regulation of its translation in a host cell.

[0041] The present disclosure also provides a recombinant vector (e.g., a recombinant expression vector) comprising a polynucleotide according to the present invention. Advantageously, the recombinant vector is a recombinant expression vector comprising an expression cassette according to the present disclosure.

[0042] The term “vector,” as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as “expression vectors.”

[0043] In some embodiments, the expression vector is a viral vector. Suitable viral vectors are known in the art and include, but are not limited to, for example, an adenovirus vector; an adeno-associated virus vector; a pox virus vector, such as a fowlpox virus vector; an alpha virus vector; a baculoviral vector; a herpes virus vector; a retrovirus vector, such as a lentivirus vector; a Modified Vaccinia virus Ankara vector; a Ross River virus vector a Sindbis virus vector; a Semliki Forest virus vector; and a Venezuelan Equine Encephalitis virus vector.

[0044] In some embodiments, a viral vector comprising at least one DNA regulatory sequence, e.g., enhancer, is provided. The DNA regulatory sequence is a nucleic acid sequence which is able to increase transcription of the target gene (for example, by leading to an increase in the number of transcripts produced over a given period of time, in comparison to the number of transcripts produced in the same period of time in the absence of the enhancer). The DNA regulatory sequence may be located anywhere in the viral vector, for example upstream or downstream of the promoter and gene. In some embodiments, the DNA regulatory sequence is an enhancer and able to be present in either orientation.

[0045] Suitable DNA regulatory sequences include, but are not limited to, for example, the DNA regulatory sequences comprising or consisting of AkRfE1, AkRfE2, DLX binding site in (Zbb16-5lf3a), CALB1-DLX6 regulated enhancer 1, CALB1-DLX6 regulated enhancer 3, NPY-DLX6 regulated enhancer 1, NPY-DLX6 regulated enhancer 2, SST-DLX6 regulated enhancer 3, homologous sequences thereof, or fragments thereof. Suitably, in some embodiments, the DNA regulatory elements are selected from the group consisting of SEQ ID NO:26, SEQ ID NO:29, SEQ ID NO:32, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:41, SEQ ID NO:44, SEQ ID NO:47, SEQ ID NO:50 and a combination thereof.

[0046] In the context of the DNA regulatory sequence or fragment thereof comprises or consists of a nucleic acid sequence having at least 70% (such as at least 75, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 99 or 100%) sequence identity to the nucleic acid sequence of SEQ ID NO:26, SEQ ID NO:29, SEQ ID NO:32, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:41, SEQ ID NO:44, SEQ ID NO:47, or SEQ ID NO:50, more preferably at least 75%, alternatively at least 80%. Proposed enhancers in humans can be found in SEQ ID NOs:55-60.

[0047] Suitably, in one embodiment the enhancers described herein of the 5lf3a gene are regulated by AkRf8. In some embodiments, these DNA regulatory sequences are used in viral vectors to specifically target and express genes in neurons expressing 5lf3a.

[0048] In some embodiments, the expression vector further contains at least one DNA regulatory sequence, e.g., an enhancer, that enhances neuronal cells resulting in an increased expression of 5lf3a receptors. In some embodiments, the expression vector further contains at least one DNA regulatory sequence and the nucleotide sequence for AkRfE10, AkRfE8, AkRfE10 agonist or AkRfE8 agonist as described herein to target expression in neuronal cells resulting in an increased expression of 5lf3a receptors. Suitable DNA enhancers include, but are not limited to, for example, AkRfE1, AkRfE2, DLX binding site in (Zbb16-5lf3a), CALB1-DLX6 regulated enhancer 1, CALB1-DLX6 regulated enhancer 3, NPY-DLX6 regulated enhancer 1, NPY-DLX6 regulated enhancer 2, SST-DLX6 regulated enhancer 3, homologous sequences thereof, or fragments thereof. In a preferably embodiment, the DNA regulatory elements are selected from the group consisting of SEQ ID NO:26, SEQ ID NO:29, SEQ ID NO:32, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:41, SEQ ID NO:44, SEQ ID NO:47, SEQ ID NO:50, a homologous human sequence thereof.

[0049] In a further embodiment, the viral vectors may target other cells that endogenously express AkRfE10, for example liver cells.

[0050] As used herein, the term “gene” encompasses both protein-coding and non protein-coding genes. Thus, in one embodiment, the viral vector comprises at least one protein-coding gene. In another embodiment, the viral vector comprises at least one non protein-coding gene. The non protein-coding gene may encode an mRNA. Thus, in one embodiment, the non protein-coding gene encodes a small interfering RNA (siRNA), a lncRNA, or an antisense RNA. Genes suitable for use in the present invention include, but are not limited to, those coding for the following: AkRfE8, AkRfE10, an agonist of AkRfE8, and an agonist of AkRfE10, DLX6 and Etv2.

[0051] The present disclosure also provides a host cell containing a recombinant expression cassette or a recombinant expression vector according to an embodiment of the present disclosure. The host cell is either a prokaryotic or eukaryotic host cell. The host cell is capable of expressing the proteins or agonists of the present disclosure. Suitable host cells include, but are not limited to, mammalian cells and yeast cells. In some embodiments, the host cell is used to produce large quantities of the protein or agonist for use in the methods of the present disclosure.

[0052] Suitable agonists of either AkRfE10 or AkRfE8 can also be used in the methods of the present disclosure. Agonists of AkRfE10 or AkRfE8 include any chemical, protein or molecule that is able to elicit similar downstream activation of AkRfE10 or AkRfE8. In the present disclosure, an agonist of AkRfE10 or AkRfE8 would be able to elicit the increased expression of 5lf3a receptor on one or more neuronal cells.

[0053] The “treating” or “treatment” of a neurological disorder, stress-induced condition or mood disorder includes, but is not limited to, reducing, inhibiting, allevi-
ating or attenuating at least one or more symptoms of the neurological condition, stress-induced condition, or mood disorder.

[0054] The terms “effective amount” or “therapeutically effective amount” refer to an amount sufficient to effect beneficial or desirable biological and/or clinical results.

[0055] The terms “subject” and “patient” are used interchangeably and refer to any animal (e.g., a mammal), including, but not limited to, humans, non-human primates, rodents, and the like, which is to be the recipient of a particular treatment. Typically, the terms “subject” and “patient” are used interchangeably herein in reference to a human subject.

[0056] Neurological disorders or stress-induced conditions that can be treated by the methods provided herein include, but are not limited to, developmental neurological disorders, mood disorders, drug addiction, and the like. These disorders include, but are not limited to, for example, depression, anxiety disorder, panic disorder, obsessive-compulsive disorder (OCD), eating disorder, chronic pain, post-traumatic stress disorder (PTSD), epilepsy, drug addiction, and the like.

[0057] Many types of depression that may be treated by the methods of the disclosure include, but are not limited to, the three main types of clinical depression: major depressive disorder; dysthymic disorder; and bipolar depression, the depressed phase of bipolar disorder and any illness in which depression or depressive tendencies are a factor such as, inter alia, ADD (attention deficit disorder), ADHD (attention deficit hyperactivity disorder), Autism, anxiety, panic, bipolar disorder, depression, GAD (generalized anxiety disorder), OCD (obsessive compulsive disorder), PTSD (post-traumatic stress disorder), Phobias, Schizophrenia, Convulsions, Anxiety, Depression, Mania, Manic-depressive psychosis, and other mood disorders. Within these types are variations in the number of associated mental symptoms, and their severity and persistence.

[0058] A subject experiencing major depressive disorder may suffer from, among other symptoms, a depressed mood or loss of interest in normal activities that lasts most of the day, nearly every day, for at least two weeks. Such episodes may occur only once, but more commonly occur several times in a lifetime. Dysthymic disorder, a chronic but less severe type of depression, unlike major depressive disorder, does not strike in episodes, but is instead characterized by milder, persistent symptoms that may last for years. Although it usually doesn’t interfere with everyday tasks, people with this milder form of depression rarely feel like they are functioning at their full capacities. Bipolar disorder cycles between episodes of major depression, similar to those seen in major depressive disorder, and highs known as mania.

[0059] In some embodiments, the neurological disorder is a developmental neurological disorder. Developmental neurological disorders are impairments of the growth and development of the brain or central nervous system, including disorders of brain function that affect emotion, learning ability, self-control, and memory during growth and development. Suitable neurological developmental disorders include, but are not limited to, autism and autism spectrum disorders, Asperger’s syndrome, mental retardation, tic disorder, Tourette’s syndrome, attention deficit hyperactivity disorder, learning disabilities, schizophrenia, schizotypal disorder, addiction, and the like.

[0060] In some embodiments, the neurological disorder is a mood disorder. The mood disorder may include, but is not limited to, for example, major depressive disorder, unipolar major depressive episode, dysthymic disorder, treatment-resistant depression, bipolar depression, adjustment disorder with depressed mood, cyclothymic disorder, atypical depression, seasonal affective disorder, melancholic depression, psychotic depression, post-schizophrenic depression, depression due to a general medical condition, post-viral fatigue syndrome, chronic fatigue syndrome, and the like.

[0061] In another embodiment, the stress-related condition is selected from, but not limited to, posttraumatic stress disorder, acute stress disorder, adjustment disorder, bereavement related disorder, panic disorder, obsessive-compulsive disorder (OCD), eating disorder, chronic pain, posttraumatic stress disorder (PTSD), general anxiety disorder, social anxiety disorder, and anxiety disorder, due to a medical condition.

[0062] In some embodiments, the neurological disorder is epilepsy. In other embodiments the stress-induced condition is drug addiction.

[0063] In other embodiments, the present disclosure provides methods of increasing serotonin levels in a subject. The method comprises administering Akr1b10, Akr1b8 or an agonist of Akr1b10/Akr1b8 in an amount effective to increase the level of serotonin in the subject. In some embodiments, the increased serotonin levels are present in a subject suffering from a neurological disorder or stress-induced condition.

[0064] An increased level of serotonin in a subject may include, but is not limited to, an increase in serotonin levels of at least 5%, suitably at least 10%, alternatively at least 15%, alternatively at least 20% in the subject.

[0065] In further embodiments, the present disclosure provides a method of increasing expression of 5-hydroxytryptamine receptor 3A (5Htr3a) in at least one neuron, the method comprising contacting the at least one neuron with at least one of Akr1b8, an agonist of Akr1b8, Akr1b10, or an agonist of Akr1b10, wherein the at least one neuron has an increased expression of 5Htr3a on its surface. The Akr1b8, an agonist of Akr1b8, Akr1b10, or an agonist of Akr1b10 is provided in an effective amount to increase the expression of 5Htr3a on the surface of the neuron.

[0066] Not to be bound by any theories, it is believed that the increased expression of 5Htr3a on neurons leads to an increased level of serotonin signaling by the neuron. In turn, this increased signaling leads to a reduction or inhibition of one or more symptoms associated with the neurological disorder or stress-induced condition.

[0067] The term neuron includes interneurons. The interneurons may be found within the cortex of the brain of the subject. In some embodiments, the neuron is a human neuron.

[0068] The present disclosure further provides methods of inducing a pluripotent stem cell to differentiate into a neuron comprising culturing the pluripotent stem cell in the presence of at least one of Akr1b8, an agonist of Akr1b8, Akr1b10, or an agonist of Akr1b10, wherein the pluripotent stem cell differentiates into a neuron that expresses 5Htr3a on its surface. Suitable pluripotent stem cells include, but are not limited to, embryonic stem cells (ES cells) and induced pluripotent stem (iPS) cells. In some embodiments, the ES cell or iPS cell is a human cell. The method includes culture steps, conditions and medium to drive the ES or iPS cell
toward neural differentiation which are known by one skilled in the art. Suitable culture steps can be found in, for example, Takahashi, K. and S. Yamanaka, Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell, 2006. 126(4): p. 663-76, the contents of which are incorporated by reference in its entirety. Akr1b10 can be used in the recent trans-differentiation methods based on Yamanaka et al, Cell 2006, for example, methods as described in Ebert, A. D., et al., EZ spheres: a stable and expandable culture system for the generation of pro-rosette multipotent stem cells from human ESCs and iPSCs. Stem Cell Res, 2013. 10(3): p. 417-27; Hunsberger et al., Induced Pluripotent Stem Cell Models to Enable In Vitro Models for Screening in the Central Nervous System, Stem Cells Dev 2015 Aug; 24(16):1852-64, Abranches E, Silva M, Pradier L, Schulz H, Hummel O, Henrique D, et al. (2009) Neural Differentiation of Embryonic Stem Cells In Vitro: A Road Map to Neurogenesis in the Embryo. PLoS ONE 4(7): e6286. doi:10.1371/journal.pone.0006286, the contents of which are incorporated by reference in their entirety.

[0069] In some embodiments, the compositions may be used to alleviate or reduce one or more symptom or sign associated with the mood disorder, including, but not limited to, depression and any illness in which depression or depressive tendencies are a factor such as, inter alia, ADD (attention deficit disorder), ADHD (attention deficit hyperactivity disorder), Autism, anxiety, panic, bipolar disorder, depression, GAD (generalized anxiety disorder), OCD (obsessive compulsive disorder), PTSD (post-traumatic stress disorder), Phobias, Schizophrenia, Convulsions, Anxiety, Depression, Mania, Manic-depression, Psychosis, and other mood disorders.

[0070] Certain aspects of the present disclosure provide compositions which reduce or even substantially or completely diminish depression. In additional aspects, certain embodiments of the present disclosure provide methods leading to functional improvement after mood disorders or depressive events.

[0071] Further, the present disclosure provides methods and compositions for increasing the activation and regulation of 5HT3a receptors by targeting the malonate pathway with small molecule effectors or metabolites of the malonate pathway.

[0072] A further embodiment provides a method of treating a neurological disorder or stress-induced disorder, the method comprising: administering a small molecule effector or metabolite of the malonate pathway, wherein administration of the small molecule effector or metabolite alleviates, reduces or inhibits at least one symptom of the neurological disorder or stress-induced disorder. The malonate pathway is shown in FIGS. 14 and 15 and is described in Rizner et al., 2012, the contents of which are incorporated by reference in its entirety. The ability to modulate the malonate pathway with AKR1B10/1B8 allows for the treatment of a neurological disorder, stress induced disorder, and other suitable disorders, including additional small molecule effectors or metabolites that directly alter the malonate pathway. For example, in some embodiments, the small molecule effectors of the malonate pathway include, but are not limited to, e.g. FOH, GGOH, antisense RNA regulators of Akr1b10 and Ev12.

[0073] Aspects of the disclosure described with respect to the former method can be applicable to the latter method, and vice versa, unless the context clearly dictates otherwise.

[0074] The methods disclosed herein can include a conventional treatment regimen, which can be altered to include the steps of the methods described herein. The methods disclosed herein can include monitoring the patient to determine efficacy of treatment and further modifying the treatment in response to the monitoring. The methods disclosed herein can include administering a therapeutically effective amount of Akr1b10, Akr1b8, an agonist of Akr1b10, or an agonist of Akr1b8.

[0075] In some embodiments, compositions for use in carrying out the method claims are provided. Suitable compositions comprise an effective amount of Akr1b10, Akr1b8, an agonist of Akr1b10, or an agonist of Akr1b8, and a pharmaceutically acceptable carrier.

[0076] The term “pharmaceutically acceptable carrier” refers any carrier, diluent or excipient which is compatible with the other ingredients of the formulation and not deleterious to the recipient.

[0077] The active agent is preferably administered with a pharmaceutically acceptable carrier selected on the basis of the desired route of administration and standard pharmaceutical practice. The active agent may be formulated into dosage forms according to standard practices in the field of pharmaceutical preparations. See Alphonso Gennaro, ed., Remington’s Pharmaceutical Sciences, 18th Ed., (1990) Mack Publishing Co., Easton, Pa. Suitable dosage forms may comprise, but are not limited to, for example, tablets, capsules, solutions, parenteral solutions, troches, suppositories, or suspensions.

[0078] For oral administration, the active agent may be combined with one or more solid inactive ingredients for the preparation of tablets, capsules, pills, powders, granules or other suitable oral dosage forms. By way of example only, the active agent may be combined with at least one excipient, including, but not limited to, fillers, binders, humectants, disintegrating agents, solution retarders, absorption accelerators, wetting agents absorbents or lubricating agents.

[0079] For parenteral administration, the active agent may be mixed with a suitable carrier or diluent, including, but not limited to, water, an oil (e.g., a vegetable oil), ethanol, saline solution (e.g., phosphate buffered saline or saline), aqueous dextrose (glucose), and related sugar solutions, glycerol, or a glycol such as propylene glycol or polyethylene glycol. Stabilizing agents, antioxidant agents and preservatives may also be added. Suitable antioxidant agents include, but are not limited to, sulfite, ascorbic acid, citric acid and its salts, and sodium EDTA. Suitable preservatives include, but are not limited to, benzalkonium chloride, methyl- or propyl-paraben, and chlorobutanol. The composition for parenteral administration may take the form of an aqueous or nonaqueous solution, dispersion, suspension, or emulsion.

[0080] The composition is preferably in unit dosage form. In such form the preparation may be divided into unit doses containing appropriate quantities of the active component. The unit dosage form may be a packaged preparation, the package containing discrete quantities of preparation, such as, but not limited to, packeted tablets, capsules, and powders in vials or ampoules. Also, the unit dosage form may be a capsule, tablet, cachet, or lozenge itself, or it can be the appropriate number of any of these in packaged form.
[0081] The present invention has been described in terms of one or more preferred embodiments, and it should be appreciated that many equivalents, alternatives, variations, and modifications, aside from those expressly stated, are possible and within the scope of the invention. (Feng et al., 2006).

[0082] The invention will be more fully understood upon consideration of the following non-limiting examples.

Example 1: Evf2 Enhances IncRNA Functionally and Spatially Organizes Megabase Distant Genes in Developing Forebrain

[0083] Gene regulation requires selective targeting of DNA regulatory enhancers over megabase (Mb) distances. Here, Applicant shows that Evf2, a Dlx5/6 ultraconserved enhancer (Dlx5/6UCE) IncRNA, regulates genes that are asymmetrically-positioned across 27 Mb. Evf2 localizes to both activated (Umd1, ~1.6 Mb distant and repression (Akr1b8, ~27 Mb distant) targets in mouse developing forebrain, controlling distances between Dlx5/6UCE and transcriptional targets in interneuron subpopulations. Through both short-range (Dlx6 anti-sense) and long-range (Akr1b8) repression, the Evf2-5UCE region regulates multiple interneuron subtype genes, linking the melanotone pathway and interneuron development. Surprisingly, Evf2 regulates the number and position of hundreds of Dlx5/6UCE-chr6 interaction sites across chr6 (~150 Mb), without affecting transcription. Active histone lysine modifications distinguish Evf2 positively- and negatively-regulated Dlx5/6UCE-chr6 sites, supporting that many sites are marked before Evf2 regulation. These studies reveal that an autosomal cloud-forming enhancer IncRNA regulates genes through antisense and chromosome topological mechanisms, and also controls the 3D architecture of an entire chromosome.

[0084] Enhancers are defined as DNA sequences capable of regulating genes at a distance, independent of orientation. Early studies show regulatory interactions between the sonic hedgehog (Shh) limb enhancer (ZRS) and the Shh gene, despite a 1 Mb distance (Anderson et al., 2014; Lettice et al., 2003). Technological advances in understanding chromosome topology (Dekker, 2016) reveal that the majority of promoter interactions (~93%) are distal, rather than proximal (de Laat and Duboule, 2013; Sanyal et al., 2012). In addition, validated enhancer regulatory landscapes in vertebrates span ~1 Mb, and facilitate tissue-specific and/or developmentally programmed gene expression. Colinarity is an elegant example of enhancer regulatory landscapes that contain functionally and spatially organized HoxA and HoxD genes involved in body patterning (Kmita and Duboule, 2003). HoxD genes located at the borders of topological domains are subject to a developmentally-dependent switch in domain regulation, providing a mechanism for HoxD gene colinearity in vertebrate limbs (Andrey et al., 2013). However, many enhancer regulatory landscapes organize megabase distant genes, and therefore, enhancer-dependent, selective regulation of genes over long distances remains a fundamental question in biology.

[0085] Applicant’s work on Evf2, a splice and polyadenylated enhancer lncRNA (Feng et al., 2006) indicated that Evf2 is transcribed from Dlx5/6UCE (Zerucha et al., 2000), and regulates Dlx5/6UCE activity in trans (Feng et al., 2006). Evf2 is expressed at sites of interneuron birth in mouse embryonic forebrain (E13.5 medial and caudal ganglionic eminences [MGE, CGE]), recruits transcription factors to the Dlx5/6UCE (Bond et al., 2009), forms a large DLX1 homeodomain containing ribonucleoprotein complex (Evf2-RNP), and directly inhibits BRG1(SMARCA4) ATPase and chromatin remodeling activities (Cafligas et al., 2015). In adult mice, Evf2 loss causes GABAergic circuitry defects, supporting enhancer lncRNA biological significance (Bond et al., 2009).

[0086] Evf2 forms one-two RNA clouds per nucleus in developing interneurons (Feng et al., 2006), similar to clouds described for imprinting and dosage compensation IncRNAs (Brockdorff, 2011; Redrup et al., 2009). While the dosage compensation IncRNA Xist controls chromosome topology across the inactivated X-chromosome (Giorgi et al., 2015; Nora et al., 2012), evidence supporting chromosome-wide effects of autosomal IncRNAs is lacking. This Example demonstrates that Evf2 targets Dlx5/6UCE interactions to sites across chr6 (~150 Mb), but affecting gene expression only across a ~27 Mb region. These interactions extend beyond the limit of ~1 Mb for the majority of enhancer regulatory landscapes. Thus, in addition to antisense regulation, these data support biologically significant, instructive and permissive roles of an enhancer IncRNA through control of chromosome topology.

[0087] Results

[0088] The Evf2-5’ Enhancer-Containing Region Regulates Interneuron Subtype Genes

[0089] GABAergic interneurons in the adult brain display the greatest diversity of any cell type, partly due to expression of interneuron subtype specific genes (DeFelipe et al., 2013). In mice, Dlx homeodomain transcription factors, originally identified by homology to fly dIPl play critical roles throughout interneuron development, from their birth and migration in the GE’s (Anderson et al., 1997; Price et al., 1991), to adult activity-dependent maturation (De Marco Garcia et al., 2011). In mouse GE’s, Shh induces GABAergic interneuron specification, activating Dlx, Evfs, and an embryonic form of glutamate decarboxylase 1 (Gad1), the rate-limiting enzyme in GABA production (Feng et al., 2006; Kohzt et al., 1998). While embryonic Shh and Dlx genes contribute to interneuron diversity (Cobos et al., 2005; Long et al., 2007; Xu et al., 2010), the role of Evf2 has not been shown.

[0090] Using mice lacking Evf2 (Evf2-5’; FIG. 1A) and Evf2+/- (Bond et al., 2009), transcription stop insertion (TS) in Evf exon 1, Evf5’; FIG. 1A), the effects of Evf2 loss on interneuron subtype gene expression in MGE were determined. Evf2 activates and represses interneuron subtype genes in MGE, with greater than two-fold changes in serotonin receptor 3a (5Htr3a), and subtle changes in calbindin 1 (Calb1), neuropeptide Y (Npy), and somatostatin (Sst, Som) (FIG. 1B). While Sst and 5Htr3a constitue two of the three major interneuron subclasses (Rudy et al., 2011), parvalbumin, which marks the third major interneuron class, and other interneuron subtype genes (vasoactive intestinal peptide (VIP) and calretinin) are not expressed this early in development (not shown).

[0091] In order to distinguish between the roles of Evf2-5’ (UCE-containing) from Evf2-3’ (UCE-lacking) regions, Evf2-5’ mice were generated (inserting TS into Evf exon 5, Evf15; FIG. 1A). Evf15’ insertion truncates Evf2, generating an enhancer-lacking form (Evf2-5’), and preventing transcription of Evf1 (FIG. 1C). Evf2-5’ retains enhancer transcription and Dlx6 anti-sense transcription, the latter consistent with the finding that Dlx6 expression does not
change in EvI1T527S (FIG. 1C). However, similar to EvI2T527S, Dlx5 is increased in EvI1T527S (FIG. 1C). Therefore, EvI2T527S is both necessary and sufficient for Dlx6 repression, while EvI2T527S is required for Dlx5 repression. In EvI1T527S, interneuron subtype gene expression is not affected (FIG. 1D). Given that EvI1 continues to be expressed in EvI2T527S (Bond et al., 2009), EvI1 is not sufficient to regulate interneuron subtype genes in EvI2T527S/MGE. Therefore, EvI2 truncation, rather than EvI1 loss, is responsible for interneuron subtype gene regulatory differences between EvI1T527S and EvI2T527S (FIG. 6, pink star). Analysis of EvI2T527S/MGE indicates that EvI2 expressed from a transgene at ~38% wildtype levels (Berghoff et al., 2013) does not rescue interneuron subtype genes (FIG. 1E), supporting that the EvI2-5 enhancer-containing region controls interneuron subtype gene expression through cis-mechanisms. The combined genetic data indicates that the EvI2-5 enhancer-containing region is both necessary and sufficient for regulating Dlx6 and interneuron subtype genes.

**[0092]** EvI2 Activates and Represses Asymmetrically Positioned Genes Across 27 Mb

**[0093]** In order to identify genes involved in EvI2-dependent regulation of interneuron subtype genes, Applicant compared gene expression between EvI2+/− and EvI2T527S MGE using microarray analysis (FIG. 10, validated targets, FIG. 11, complete list). Microarray analysis indicates that the majority of validated targets are located on mouse chr6 (EvI2-chr6 targets) (FIG. 10). With the exception of overlapping Dlx6 (anti-sense), EvI2-chr6 target genes are organized asymmetrically, 5' of the EvI2 transcription start site, across 27 Mb (FIG. 1A). Evolutionarily conserved organization of 5/6 of the EvI2-chr6 target genes in human chr7 supports a potentially significant biological role (FIG. 1A). Asymmetric positioning of EvI2-chr6 targets and synteny with human chr7 led to further focus on the significance and mechanism of EvI2-chr6 target gene regulation.

**[0094]** EvI2-5UCE Represses Dlx6, Rbm28, and Akrb18

**[0095]** In addition to repressing Dlx5 and Dlx6, as shown previously (Bond et al., 2009)), EvI2 represses long-range targets Rbm28 and Akrb18, and activates long-range targets Umda1 and Lsm8 (FIG. 1A, 1F, FIG. 10). Comparisons of EvI2-chr6 targets in EvI2T527S and EvI2T527S MGE show that EvI2T527S is both necessary and sufficient for Rbm28 and Akrb18 repression, while EvI2-3 is required for Umda1 and Lsm8 activation (FIG. 1F, G). Therefore, EvI2 repression of Dlx6 and long-range targets requires the EvI2-5UCE region, while activation requires the EvI2-3 region. In EvI2T527S, partial rescue of Lsm8 supports EvI2-dependent trans-activation of at least one EvI2-chr6 long-range target gene.

**[0096]** Gene expression analysis correlates EvI2-5 repression of EvI2-chr6 targets (Dlx6, Rbm28, Akrb18) with regulation of interneuron subtype gene expression (FIG. 1, compare B, D, compare F, G). FIG. 6A summarizes the relative roles of the EvI2-5 UCE region, 3' end, and trans effects on EvI2-chr6 targets and interneuron subtype genes, highlighting the correlation between Dlx6, Rbm28, Akrb18 repression and interneuron subtype gene regulation (pink star).

**[0097]** The EvI2-Antisense Target Dlx6, Regulates Multiple Interneuron Subtype Genes

**[0098]** Using a genetic approach, Applicant next analyzed gene expression from 6 mouse mutants with different combinations of EvI2T527S, EvI1T527S, and Dlx5/6KO (Merlo et al., 2002) alleles (FIG. 6B, 6C). In Dlx5/6KO, EvI2T527S mice, rescue of Dlx6 and Dlx5, also rescues interneuron subtype gene effects (FIG. 6C). Furthermore, 4/35 possible dose-dependent relationships between five interneuron subtype and seven EvI2-chr6 target genes are detected at an r2 > 0.8, including Dlx6 and Callb1 (r2 = 0.81) (FIG. 12). In order to test whether Dlx6 dosage directly regulates Callb1 expression, Applicant used anti-DLX ChIPseq to identify potential enhancers in E13.5GE, and found three DLX binding sites within ~50 kb of the Callb1 gene (FIG. 11). Transfection into primary cultures of MGE shows that Dlx6 dosage regulates Callb1-enhancers (sites 1 and 3) in luciferase reporter assays (FIG. 11). Anti-DLX ChIPseq identifies DLX binding sites near Npy and Sst genes, also regulated by Dlx6 in a dose-dependent manner (FIG. 1J, K). Thus, Dlx6 activates and represses multiple interneuron subtype enhancers (5/6 tested), supporting that Dlx6 dosage contributes to interneuron diversity as early as E13.5. These data support that EvI2 repression of Callb1 and activation of Npy and Sst occur through Dlx6 antisense regulation.

**[0099]** Regional Control of the EvI2-Akr1b8-5Htr3a Axis Involves the Mevalonate Pathway and Akr1b8 Regulated Enhancers

**[1000]** Morphologically and molecularly distinct lateral, medial and caudal ganglionic eminences (LGE, MGE and CGE, FIG. 7A) are sites of interneuron birth in the embryonic brain, and contribute to interneuron diversity (Gelman and Marin, 2010; Nery et al., 2002; Wachtet al., 2010; Wichterle et al., 2001). Given that the CGE is a major source of 5Htr3a-expressing interneurons (Rudy et al., 2011), Applicant next analyzed EvI2- gene regulation in CGE. Analysis of interneuron subtype gene expression profiles in EvI2T527S CGE shows that both Sst and 5Htr3a increase, with no effects on Calb or Npy levels (FIG. 2A). However, EvI2-chr6 targets show a similar profile of activation and repression compared to MGE (compare FIG. 1F and FIG. 2B), with two exceptions: (1) subtle Dlx5 repression is not observed in CGE, (2) Akr1b8 repression is greater in CGE (~5-fold) compared to MGE (~7-fold).

**[1001]** Therefore, although EvI2 regulation of EvI2-chr6 targets is similar in MGE and CGE, interneuron subtype gene expression differs for all four interneuron subtype genes as follows: (1) EvI2 represses Callb in MGE, but not CGE, (2) EvI2 activates Npy in MGE, but not CGE, (3) EvI2 activates Sst and 5Htr3a in MGE, but represses Sst and 5Htr3a in CGE (compare FIGS. 1B and 2D). Together, these data show that EvI2 control of interneuron subtype gene expression in embryonic brain is regionally regulated, depending on MGE or CGE origin.

**[1002]** Applicant next determined whether correlations between Akr1b8 and 5Htr3a, the most highly regulated EvI2-chr6 target and interneuron subtype gene, respectively, reflect direct regulation. Although the loss of Akr1b8 (Akr1b8−/−) does not affect interneuron subtype gene expression in CGE (FIG. 7B), loss of Akr1b8 from EvI2T527S partially rescues 5Htr3a levels in EvI2T527S, Akr1b8 double homoyzogte CGE (FIG. 2C). Thus, EvI2 represses 5Htr3a, in part, through Akr1b8 repression in CGE. Furthermore, Akr1b8 transfection into CGE primary cultures increases endogenous Akr1b8 and 5Htr3a levels (FIG. 2D). Differential analysis of ChIPseq peaks identifies EvI2-dependent changes in H3K4me3 (active promoters), H3K27me3 (silent chromatin), and H3K4me1 (enhancers) in two regions located at the Zbtb16 5' end (~63 kb downstream of the
Ev2 regulates Dhs5/6UCE-Umad1-Akr1b8 chromosome topology in interneuron subpopulations.

Ev2 regulates chromosome topology in interneuron subpopulations by coordinating the positioning and regulation of Akr1b8 and Umad1 genes. Ev2 RNA clouds interact with Dhs5/6UCE and long-distance targets Akr1b8 and Umad1. These interactions are conserved in single nuclei, indicating a divergence between these two nuclei. The interaction between Ev2 RNA clouds and Dhs5/6UCE-Umad1-Akr1b8 is not associated with repressed genes (Fig. 3C). Therefore, Ev2 regulates distance profiles (Fig. 3K). A 3D density map of nucleic acid coordinates demonstrates the distribution of Ev2**-/- (red) and Ev2**+/+ (blue) nuclei, and indicates increased clustering by Ev2**-/- nuclei (Fig. 3I). Self-organizing maps (SOMs) optimally cluster gene-distance data and reveal two clusters of Ev2**-/- (orange) and Ev2**+/+ (blue) centroids (Fig. 3N). Together, these data indicate that Ev2 regulates chromosome topology in the 27 Mb region by altering Dhs5/6UCE-Umad1-Akr1b8 gene-distance relationships in a heterogeneous manner among interneuron subpopulations.
long-range target genes (Umadl, Lsm8, Rbmr28, and Akr1b88) reveals that differences between (+) (red region), (-) (yellow region) in Ev12-** and Ev12-** are gene specific (FIG. 9D-E). However, chr6-wide analysis indicates that active marks (H3K4me3/1 and H3K27ac), but not inactive H3K27me3 marks, are enriched at (+) sites compared to (-) sites (FIG. 4F). Surprisingly, enrichment of active marks at (+) sites occurs in both Ev12-** and Ev12-** (FIG. F, G), supporting that active marks precede Ev12-regulated Dlx5/6 UCE interactions. Direct comparisons of H3K4me3 and H3K27ac profiles in Ev12-** and Ev12-** indicate very few changes (see subtle line shifts, FIG. 4I), supporting Ev12 independence at the majority of (+) sites. Furthermore, although Ev12 does not regulate the overall histone modification differences between (+), (-) and (I) sites, these differences support the involvement of differential mechanisms in (+), (-) and (I) site selection (FIG. 4I, J). Thus, in permissive regions, active histone modifications selectively mark the majority of (+) sites, prior to Ev12 regulation, while in the instructive 27 Mb region containing Dlx5/6 UCE-Ev12-chr6 target genes, Ev12 regulates active repression of histone methylation of (+) and (-) sites in a gene-specific manner.

[0110] Discussion

In this work, Applicant shows that Ev12-chr6 targets are asymmetrically positioned across 27 Mb, 5' to the Ev12 transcription start site, with the closest (Dlx6) and furthest genes (Akr1b88) regulating interneuron subtype genes (FIG. 5A). Analysis of multiple genetic models supports in vivo dosage relationships between Dlx6-Calb1, Umadl-Sst, Umadl-Slt3a, and Lsm8-Npy (FIG. 6B). Together with published reports that Dlx5/6 dosage controls the development of parvalbumin interneurons (Cho et al., 2015; Wang et al., 2010), Applicant’s genetic experiments link five of six Ev12-chr6 target genes to interneuron diversity. Thus, Ev12 regulates a small group of megalobase distant genes with biochemical and genetic roles in interneuron diversity, functionally organizing a 27 Mb region of chr6.

[0112] While involvement of Ev12-Dlx6 in regulating interneuron subtype genes may not be surprising, identification of Dlx6-regulated enhancers at multiple interneuron subtype genes (Calb1, Npy, and Sst) is unexpected, and supports a major role for Dlx6 dosage. In addition, the Ev12-Akr1b88-Slt3a axis links the mevalonate pathway to Akr1b88 regionally controlled enhancer activity, revealing a novel role for lncRNA regulation of lipid metabolism in interneuron diversity. This is important, as the embryonic Slt3a interneuron population gives rise to a major subclass of adult interneurons (vasoactive intestinal peptide, VIP+) involved in disinhibition, and control of adult brain circuitry and behavior (Lee et al., 2013; Letzkus et al., 2011; Pi et al., 2013). In addition, Slt3a itself controls the migration of interneuron progenitors from CGE to adult destinations (Murthy et al., 2014). Thus, developmental effects on Gad1 expression combined with changes in multiple interneuron subtype genes may contribute to adult brain GABAergic circuitry defects in mice lacking Ev12 (Bond et al., 2009).

[0113] Regulation of Dlx5/6 UCE-Gene Interactions Across Mouse Chr6: Biological Significance and Human-Mouse Conservation

[0114] In subpopulations of interneurons, Ev12 localizes to both activated and repressed target genes, regulating Dlx5/6 UCE-target gene distances, and supporting a role in spatial organization of genes across ~27 Mb. In addition to human chr7 synteny within the 27 Mb region of mouse chr6, evidence that Ev12 is enriched 6000-fold in human embryonic brain interneurons compared to other lncRNAs, supports Ev12 significance in human brain development (Lu et al., 2016). Across chr6 (~150 Mb), Ev12 regulates the number, density, and position of Dlx5/6 UCE-chr6 interactions (FIG. 4A-E, 5B-C), indicating that topological effects extend beyond the transcriptionally targeted 27 Mb region (instructive region). However, there is evidence to support that Dlx5/6 UCE-chr6- wide interactions outside the 27 Mb region (permissive region) are biologically significant, despite the absence of transcriptional effects? Gene ontology analysis (GO) of Dlx5/6 UCE-chr6-wide gene interactions shows that specific biological processes are associated with (-) development, transcription, metabolic/biosynthetic process and (I) stimulus response (FIG. 5B-D), and that Ev12 regulates the majority of Dlx5/6 UCE-chr6 interactions, with only 4.3% independent of Ev12 (FIG. 5D, compare gray circle). Analysis of human development brain Dlx5/6 UCE-chr7 gene interactions (Won et al., 2016) indicates that ~65% of mouse E13.5 GE Dlx5/6 UCE-chr6 gene interactions (Ev12-**), orange, FIG. 5E) are conserved. Conversely, ~44% of human Dlx5/6 UCE-chr7 gene interactions are conserved with mouse Dlx5/6 UCE-chr6 gene interactions (FIG. 5E). Furthermore, ~51% of human Dlx5/6 UCE-chr7 gene interactions are subject to Ev12 regulation in mice (FIG. 5E, deep yellow, red and green overlap). Thus, human-mouse conserved Dlx5/6 UCE-chr6 gene interactions and GO analysis support the potential biological significance of UCE-inlncRNA topological control across chr6, even at genes that do not have detectable changes in gene expression.

[0115] Selective Regulation of Megabase Distant Genes Through Complex Effects on Chromosome Topology

[0116] Although the majority of Ev12-regulated Dlx5/6 UCE-chr6 sites do not cause detectable changes in gene expression, it is possible that cellular heterogeneity masks transcriptional changes. DNA/RNA and DNA/DNA FISH analysis supports heterogeneity in chromosome topology among interneuron progenitors. In addition, E13.5 GE interneuron progenitors can be divided into three categories of Ev12 RNA cloud expression (zero, one, or two/nucleus) (FIG. 3B-C), contributing to heterogeneity. Although Ev12 regulation of Umad1 (~6-fold increase) and Akr1b88 (~7-fold decrease) is dramatic, Dlx5/6:Umad1 and Dlx5/6:Akr1b88 co-localization occurs in ~10% of nuclei (FIG. 3I, J). Recent evidence supports highly heterogeneous chromosome 3D structures in single cells (Stevens et al., 2017; Nagano et al., 2017). It remains to be determined whether heterogeneity results from transient, unsynchronized interactions that occur in the majority of cells, or interactions that are limited to specific GE subpopulations. In support of dynamic or transient mechanisms is the surprising result that the Ev12 RNA cloud does not co-localize at Dlx5/6 UCE, despite co-localization with target genes Umad1 and Akr1b88 (FIG. 3B, C, H). Given that only one-two Ev12 RNA clouds are detected per nucleus, it is likely that the Ev12 RNA cloud forms away from the site of Ev12 transcription initiation, and moves along chr6 to regulate target genes.

[0117] In addition to cellular heterogeneity, another possible explanation is that topological changes precede gene expression changes, and are part of a dynamic process during development. Evidence that chromosome topology is dynamic and changes during developmental and or cell
cycle transitions has been reported (Ilug et al., 2017; Nagano et al., 2017; Neordermeier et al., 2011; Phillips-Creamer et al., 2013). Therefore, Evf2 may be permissive and establish a topology required for future regulatory events, similar to that proposed for ZRS-Shh interactions in the zone of polarizing activity in the limb (Williamson et al., 2016). Furthermore, the finding that (1) gene interactions are grouped with stimulus response genes (GO analysis, FIG. 5D) suggests the possibility that transcriptional changes may be revealed in response to specific stimuli. Thus, it will be important to determine how heterogeneity, transient vs. stable associations, stimulus response, and developmental timing mechanisms relate transcriptional control and Evf2-regulated Dlx5/6UCE-chr6 gene interactions.

Data in this application support the idea that Evf2-Dlx5/6UCE interactions spatially and functionally organize megabase distant genes involved in interneuron diversity across a 27 Mb region of chr6. The requirement of the UCE-containing region (Evf2 3') in regulating neuronal diversity through repression of Dlx6 and Akr1b8, supports a role for ultraserved sequences that function through both RNA and DNA mechanisms. However, Evf2 gene activation requires the Evf2 5' end, and in the case of Lsm8, occurs through a trans-mechanism (FIG. 3H, 5A), supporting functional constraints outside of the ultraserved sequence. Thus, Evf2 3'- and 3'-distinguish between long-range repression (Akr1b8, Rbm28) and activation (Umad1, Lsm8) within the 27 Mb region. FIGS. 4E and 9D-G show that (+) and (-) sites are identified at both activated and repressed target genes (Umad1, Lsm8, Rbm28). Thus, Evf2 regulated Dlx5/6UCE interactions within the 27 Mb region do not follow a general rule where (+)/(-) sites correlate with transcriptional activation/repression. Furthermore, although Evf2 regulates Dlx5/6UCE: Umad1:Akr1b8 topology, such effects appear heterogeneous, and also do not follow simple correlations between gene-distances and transcriptional effects (FIG. 3I-K). Evf2 control of specific Dlx5/6UCE gene distance relationships are best revealed using SOMs, showing that Evf2 increases the number of nuclei that are in clusters bound by closer distances (FIG. 3O-P). Together, these results support that Evf2 and Dlx5/6UCE selectively regulate megabase distant genes through complex spatial effects on chromosome topology, with distinct roles of the Evf2 3'- and 3'-regions in transcriptional activation and repression.

Conservation of Dlx5/6UCE-chr6/7 gene interactions in mice and humans, and association of (+) and (-) with specific biological processes, suggest that interactions are part of a selective rather than stochastic process (FIG. 5A, D). Surprisingly, chr6-wide increase of active histone modifications at (+) compared to (-) sites is Evf2-independent (FIG. 4E-I, FIG. 5B), supporting that H3K4me3/1 and H3K27ac marks precede lncRNA-dependent enhancer interactions in permissive regions. Future experiments to define the mechanism of Evf2-Dlx5/6UCE-chr6 site selection specifically through studies of individual components of the Evf2-4NP complex (Cajigas et al., 2015) will be important to understanding how instructive and permissive topological domains are established.

Materials and Methods

Mouse Strains

Generation of Evf1 T575 Mice

The Evf1 targeting construct was generated using lambda phage based recombining in E. coli as described (Liu et al. 2003). The retrieval vector was constructed as follows. Using high fidelity Taq (Roche), homology arms of approximately 500 bp were PCR amplified (with restriction sites added) from BAC DNA. Using a three-fragment ligation, homology arms were cloned into Cgl and NheI sites of PL253, with a HindIII site engineered between them. A 19.4 kb region (corresponding to position 6,809,651-6,825,742 on mouse chromosome 6, NCBI assembly) was retrieved from pBAC c3.6 M8 (M. Eker, U. Ottawa) into the retrieval plasmid using recombination-induced EL250 cells (Liu et al., 2003). Further targeting was performed on the retrieved plasmid. The polycladenylation targeting vector was constructed in PL452, a floxed-Neon containing plasmid. The triple polycladenylation signal (Soriano 1999) was cloned into EcoRI and SalI sites of PL452. Approximately 500 bp of targeting homology arms were cloned sequentially on either side of the polyA-floxed-Neo insert. Briefly, fragments were PCR amplified as above and cloned into either Cgl and KpnI sites or NotI and SalI sites. This triple polya-floxed-Neo cassette was targeted into the retrieved 19.4 kb region using recombination-induced EL250 cells. Successful targeting was confirmed by Southern blot analysis of the completed construct using internal probes (NEBlot kit, NEB).

Mouse ES cells were targeted by homologous recombination using standard procedures. Successful targeting in ES cells was confirmed by Southern blot, verifying proper recombination at both the 5' and 3' ends. Probes were generated outside the 19.4 kb homologous region. EL250 cells and recombining plasmids PL253 and PL452 were provided by Dr. Neal Copeland.

Evf1 T575 (floxed neo)+ heterozygotes were verified by Southern, crossed to ElAcre (Jackson Labs) for two generations, and crossed to the Evf2 T575 background. Neo removal was verified by PCR (not shown). Mice are maintained on the same mixed background as Evf2 T575 strain; all mice are housed according to IACUC guidelines.

Additional Mouse Strains

1. Evf2 T757 (Bond et al. 2009) were crossed to C57/B16 for one generation, and maintained on a mixed background (C57/B16, 129/sv, FVB). Source: Kohls lab

2. Evf2 T575 R (Berghoff et al. 2013), maintained on the same background as Evf2 T575, source: Kohls lab

Akr1b8 +/ - (Akr1b8 +/ - KOMP/129SvJ) source: Jackson (strain 024334).

Akr1b8 +/ - : Evf2 T575, crossed to Evf2 T575 for three generations, and maintained on the Evf2 T575 mixed background, source: Kohls lab

5. Dlx5/6KO/TS: Dlx5/6KO/+ mice (Merlo et al. 2002) were maintained on Evf2 T575 background, and crossed to Evf2 T575 mixed background, source of Dlx5/6KO/+ (A. Bendall).

Microarray Data and Validation

E13.5 medial and caudal ganglionic eminences were isolated from embryos using fine microdissection scissors (Lumsden biosciences), in L1.5 medium. In FIG. 7A, a schematic of E13.5 mouse brain shows ganglionic eminences (LGE, MGE, CGE, based on schematic (Gelman and Marin 2010)), and dorsal/ventral and rostral/caudal axes. Dotted red line shows the boundary between MGE/LGE and CGE where tissues are dissected. At E13.5, the sulcus between MGE and LGE is well defined, allowing precise definition of LGE/MGE/CGE regions under a dissecting microscope. RNA isolation, cDNA production, qPCR were
performed, as previously described (Berghoff et al. 2013). For microarray analysis, 5 pools of E13.5 MGE’s from two brains/genotype from males (5) and females (5) were hybridized to 10 Affymetrix GeneChipMouse430_2 arrays, and the results analyzed using GeneSpring software. Genes showing a minimum of 2-fold differences, and p-values of ≤0.05 were validated further by TaqMan qRT-PCR. Probes for TaqMan qPCR:

0134] Dlx5 (Mm00438430_m1)
0135] Dlx6 (Mm01162601_m1)
0136] Acnb (Mm00607939_s1)
0137] Akr1b8 (Mm004841314_m1)
0138] Calb (Mm00486647_m1)
0139] Gad1 (Mm00420743_s1)
0140] Npy (Mm01410146_m1)
0141] Som (Mm00436671_m1)
0142] Shh (Mm00442874_m1)
0143] Vip (Mm0060234_m1)

[0144] Custom LIF probe

Svfl probe: (0.1 μM): 5'-AGAGCTATGGAGTCCTTCCGC-3' [SEQ ID NO: 7]
5'-GATGAGTTTGGTCCCTTGCCTG-3', [SEQ ID NO: 8]
Svfl-R: (0.1 μM): 5'-GCTTCTGAGATGGAACCCAT-3' [SEQ ID NO: 9]
5'-CTTCTGAGATGGAACCCAT-3' [SEQ ID NO: 10]

[0145] SYBR-Green Primers for qPCR:

Svfl-F: (0.2 μM, 5'-GCTTCTGAGATGGAACCCAT-3') [SEQ ID NO: 10]
5'-AGAGCTATGGAGTCCTTCCGC-3' [SEQ ID NO: 11]
Svfl-R: (0.2 μM, 5'-GATGAGTTTGGTCCCTTGCCTG-3') [SEQ ID NO: 12]
5'-GCTTCTGAGATGGAACCCAT-3' [SEQ ID NO: 13]
Umoud1-F: (1.2 μM, 5'-CACACCGCCACCCCTAGGTAAG-3') [SEQ ID NO: 14]
5'-GCTTCTGAGATGGAACCCAT-3' [SEQ ID NO: 15]
Umoud1-R: (1.2 μM, 5'-GCTTCTGAGATGGAACCCAT-3') [SEQ ID NO: 16]
5'-GCTTCTGAGATGGAACCCAT-3' [SEQ ID NO: 17]
Lm48-F: (0.8 μM, 5'-CACACCGCCACCCCTAGGTAAG-3') [SEQ ID NO: 18]
5'-GCTTCTGAGATGGAACCCAT-3' [SEQ ID NO: 19]
Lm48-R: (0.7 μM, 5'-GCTTCTGAGATGGAACCCAT-3') [SEQ ID NO: 11]
5'-GCTTCTGAGATGGAACCCAT-3' [SEQ ID NO: 12]
Rbm28-F: (1 μM, 5'-GTGGACACCGCTGAACCCAT-3') [SEQ ID NO: 20]
5'-GCTTCTGAGATGGAACCCAT-3' [SEQ ID NO: 21]
Rbm28-R: (1 μM, 5'-GCTTCTGAGATGGAACCCAT-3') [SEQ ID NO: 22]
5'-GCTTCTGAGATGGAACCCAT-3' [SEQ ID NO: 23]

[0146] Transfections into Primary E13.5 GE
[0147] Luciferase Reporter and Expression Vectors
[0148] For all luciferase experiments, enhancers were cloned into the pGL3 promoter vector (Promega) using the KpnI and Nhel restriction sites on the 5' and 3' sites, respectively. The Calb enhancers (site 1, site 2, and site 3), Akr1 enhancers (site 1 and site 2), NPY enhancers (site 1 and site 2), and SST enhancer (site 1) were identified by MACS2 peak analysis of ChIP-seq (DNA sequences obtained from the UCSC genome browser). Primers were designed to PCR amplify enhancer sequences from C57BL/6J mouse genomic DNA into expression plasmids.

[0149] Expression plasmids for pCMV6-Akr1b8, pCMV3-EGFP, or pNTR223.1-Dlx6, were purchased from Origene, Addgene, or DNASU, respectively. Subsequently, Dlx6 was amplified by PCR and cloned into the BamHI and EcoRI restriction sites on the pCMV3 backbone. To generate the pCMV6-EGFP control plasmid, EGFP was PCR amplified and cloned into the pCMV6 empty vector using the restriction sites Ascl and NotI.

[0150] Primary Embryonic Brain Ganglionic Eminence Transfections

[0151] MGE and CGE tissues were dissected from E13.5 Swiss Webster embryos, dissociated in L15 media by pipetting several times, and spun through a cell strainer for single cell preparations. Briefly, cells were seeded at a density of 2.5×10^6 cells per cm^2 (Flandin et al. 2011). One day prior to seeding cells, 24-well plates were coated with poly-L-lysine (30 μg/mL; Sigma) and laminin (5 μg/mL; Sigma), while 96-well plates were coated with poly-L-lysine (3 μg/mL; Sigma) and laminin (5 μg/mL; Sigma). Initially, cells were seeded in neurobasal medium (DMEM:F12 supplemented with L-glutamate, B-27 (Gibco), N2 supplement (Gibco), bovine pituitary extract (35 μg/mL; Life Technologies), mito- serum extender (BD Biosciences), penicillin (100 U/mL; Gibco), streptomycin (100 μg/mL; Gibco), and glutamax (0.8 mM; Gibco)).

[0152] Specifically, for the Akr1b8 gene expression study, cells were seeded at 470,000 cells per well in a 24-well plate. 24 hours after culturing cells, the medium was changed to neurobasal media without antibiotics and 1.4 μg of expression vector (pCMV6-Akr1b8) or control vector (pCMV6-EGFP) was transfected using Fugene 6 (Promega), as recommended in the user manual. Cells were harvested 48 hours after transfection for RNA isolation (PicoPure RNA isolation kit; Applied Biosystems) and RT-PCR to quantify Akr1b8 (Assay ID: Mm00484314 and Shh-R (Assay ID: Mm00442874) normalized to β-actin.

[0153] For all luciferase experiments, cells were cultured at a density of 78,300 cells per well in a 96-well microplate treated for tissue culture. Cells were allowed to attach for 24 hours before changing the medium to neurobasal media without antibiotics. Transfections using Fugene 6 (Promega) were performed as recommended. Cells were harvested 48 hours after transfection with 1x passive lysis buffer (Promega) supplemented with 0.1% Digitonin (Sigma) for cell lysis. To ensure thorough cell lysis, lysates were subjected to two freeze-thaw cycles prior to performing Dual Luciferase Reporter assays (Promega). All transfections were normal-
ized to the internal control expressing Renilla luciferase, performed at least in triplicate and a minimum of two times.

[0154] For Calb1 enhancer transfections, Applicant used five concentrations ranging from 20 ng to 240 ng of pcDNA3-Dx6, where the total amount of expression plasmids was maintained at 240 ng using pcDNA3 EGFP as the control; 50 ng of pGL3 luciferase reporter containing Calb1 site 1, site 2, or site 3; and 5 ng of pRL-null. For NPY and SST transfections, three concentrations ranging from 40 ng to 160 ng of pcDNA3-Dx6 were tested, where the total amount of expression plasmids was maintained at 280 ng using pcDNA3 EGFP as the control, along with 50 ng of pGL3 luciferase reporter containing NPY site 1, NPY site 2, or SST site 4, and 5 ng of pRL-null. For AkrR-enhancer transfections, optimal effects were obtained with 160 ng of pCMV6-AkrR8 for CGE AkrR1/2 and MGE AkrR1, and 80 ng of pCMV6-AkrR8 for MGE AkrR2. The total amount of expressed plasmid DNA was maintained at 240 ng using pCMV6 EGFP as the control. For reporters, Applicant used 50 ng of pGL3 luciferase reporter containing AkrR1/2 and 5 ng of pRL-null.

[0155] For Farnesol (FOH; Sigma) and Geranylgeraniol (GGOH; Sigma) treated cells, 50 ng of each AkrR enhancer reporter plasmid and 5 ng of pRL-null were used. FOH and GGOH were freshly prepared in DMSO (Sigma) at varying concentrations using serial dilutions. Neurobasal media without antibiotics was supplemented with a final concentration of 0.01, 0.1, 1, 10, or 100 μM for GGOH and 0.1, 1, 10, or 100 μM for FOH. Prior to adding transfection reagent/DNA mixture, the media was changed to that containing the respective concentration of metabolite.

[0156] Primer and Enhancer Sequences

[0157] The DNA sequences obtained from the UCSC genome browser are listed below. Enhancer sequences were PCR amplified from C57BL/6J mouse DNA using the following primers:

[0158] Calbindin1 enhancers: Site 1 (476 bp):

5’ primer: (SEQ ID NO: 24)
GAATTATAGGAAAACACAATCTAAGACGGG

3’ primer: (SEQ ID NO: 25)
CAGAGAAGATTTCTTCTTGATG

[0159] Site 2 (776 bp):

5’ primer: (SEQ ID NO: 27)
CTTTCACACGGAATAAACCCTAAGAC

3’ primer: (SEQ ID NO: 28)
GCTGCTCATTTTGAGCCTTTGATGGG

[0160] Site 3 (364 bp):

5’ primer: (SEQ ID NO: 30)
CGCAACCACCTCTGATATGAGATTGATG

3’ primer: (SEQ ID NO: 31)
GACTGATGATGGTAAAACAC

[0161] Site 4 (648 bp):

5’ primer: (SEQ ID NO: 32)
CGCAACCACCTGATATGAGATTGATG

3’ primer: (SEQ ID NO: 33)
GACTGATGATGGTAAAACAC
[0161] AkrR enhancers: Site 1 (1062 bp):

5' primer:  
ATACAGCGGATCTGGGCA

3' primer:  
AGGTCGACCTTGGCCAAG

[0162] Site 2 (1667 bp):

5' primer:  
GCTCTCTCCCGGC

3' primer:  
ACGGATCTCTTTTCCTGGTGAQ}

[0163] NPY Enhancers: Site 1 (535 bp):

5' primer:  
CTCAATCTCGGCACATGATAGA

3' primer:  
CATGATCGATGAGATTTAAGTTT

SEQ ID NO: 39

SEQ ID NO: 40

SEQ ID NO: 41

CGGTTCTCTCTTGTAAGCTATCTCAAC

AAAGAAGAATTCG
[0169] Ark Site 2 from Human

(SEQ ID NO: 56)

[0170] DLX Binding Site in Human Chromosome 11

(SEQ ID NO: 57)
digested in 2 U/μl Micrococcal nuclease (M0247S, NEB) at 37°C for 7 min. The reaction was quenched with EDTA (10 mM final concentration). Triton X-100 and Sodium Deoxycholate were added (0.1% final concentration). Samples were incubated on ice for >15 minutes. Immunoprecipitation buffer (20 mM Tris-HCl pH 8.0, 2 mM EDTA, 150 mM NaCl, 0.1% Triton X-100, 1× Protease inhibitor cocktail, 1 mM PMSF) was added to a final volume of 200 μl and the samples were rotated at 4°C for 1 hour. The chromatin was pre-cleared by rotating at 4°C with 15 μl of Protein G-Agarose beads for 1 hour. After centrifugation to pellet the beads, the supernatant was further pre-cleared by rotating at 4°C with 15 μl rabbit IgG conjugated Protein G-Agarose beads for 1 hour. The pre-cleared chromatin was incubated with rabbit IgG (1 μg), or antibodies targeting histone modifications (1 μl) at 4°C for 1-2 hours with rotation. 15 μl of Protein G-Agarose beads blocked with 1% BSA in 1×PBS were added to each sample and incubated at 4°C overnight with rotation. The beads were pelleted by centrifugation and washed twice with 200 μl Low Salt Wash buffer (20 mM Tris-HCl pH 8.0, 2 mM EDTA, 150 mM NaCl, 1% Triton X-100, 0.1% SDS) and twice with 200 μl High Salt Wash buffer (20 mM Tris-HCl pH 8.0, 2 mM EDTA, 500 mM NaCl, 1% Triton X-100, 0.1% SDS). Immunoprecipitated DNA was eluted in 100 μl of ChIP elution buffer (100 mM NaHCO3, 1% SDS) at 65°C for 1-1.5 hour. The DNA was purified using phenol chloroform extraction and ethanol precipitated. The pellet was resuspended in 10 mM Tris-HCl pH 8.5. The DNA was incubated with 20 μg of RNAase A at 55°C for 1 hour. 40 μg Proteinase K were added and incubated at 55°C for 1 hour. The immunoprecipitated DNA was purified using the Qiagen PCR Purification Kit.

[0178] Antibodies: ChIP antibodies targeting histone modifications are Encode verified: H3K4me3 (Abcam ab8580), H3K4me1 (Abcam ab8895), H3K27me3 (Active Motif 39155).

[0179] Cross-Linked Chromatin

[0180] For anti-DLX and anti-H3K27ac ChIP cells were fixed in 1% paraformaldehyde for 10 min then lysed in SDS lysis buffer (1% SDS, 50 mM Tris-HCl pH 8, 10 mM EDTA) with protease inhibitors (11836153001, Roche). The lysates were sonicated with a Bioruptor Pico (Diagenode) for 10 cycles (30 sec On, 30 sec Off). The lysates were then centrifuged to pellet cellular debris and the supernatant collected for ChIP. 25 μg of chromatin were diluted 1:10 in RIPA Buffer (10 mM Tris pH 7.6, 1 mM EDTA, 0.1% SDS, 0.1% Sodium Deoxycholate, 1% Triton X-100) with protease inhibitors (B14020, Biolog). The chromatin was pre-cleared by rotating at 4°C with 50 μl of Protein G-Agarose beads (11719416001, Roche) for 1 hour. After centrifugation to pellet the beads, the supernatant was further pre-cleared by rotating at 4°C with 50 μl rabbit IgG conjugated Protein G-Agarose beads for 1 hour. The pre-cleared chromatin was incubated with rabbit IgG (2.5 μg), previously validated anti-pan-DLX (2.5 (Feng et al. 2006; Bond et al. 2009; Cajigas et al. 2015)) or anti-H3K27ac (1 Abcam Ab4729) at 4°C for 4 hours with rotation. 50 μl of Protein G-Agarose beads blocked with 1% BSA in 1×PBS were added to each sample and incubated at 4°C overnight with rotation. Beads were pelleted by centrifugation and washed twice with Low Salt Buffer (20 mM Tris-HCl pH 8.1, 2 mM EDTA, 150 mM NaCl, 0.1% SDS, 1% Triton X-100), three times with High Salt Buffer (20 mM Tris-HCl pH 8.1, 2 mM EDTA, 500 mM

[0174] Chromatin Immunoprecipitation (ChIP)

[0175] For ChIP experiments whole ganglionic eminences were dissected from 10 Evf2+/− and 10 Evf2+/+ E13.5 embryos. Tissues were pooled for each genotype, triturated by pipetting, and filtered through a cell-strainer capped 5 ml polystyrene round-bottom tube (BD Falcon) to make single-cell suspensions. Duplicate ChIP experiments were performed to determine reproducibility, generating libraries as described below.

[0176] Native Chromatin

[0177] Native ChIP protocol has been described in detail previously (Brind’Amour et al. 2015), and detailed for E13.5 GE cells as follows. Cells from the single cell suspension described above were split into 1×10^6 cell aliquots, and pelleted through centrifugation at 1000g for 10 min. Cell pellets were flash frozen in liquid nitrogen, and stored at −80°C. Nuclei were isolated using EZ Nuclei Isolation Lysis Buffer (N3408, SIGMA). Chromatin was
NaCl, 0.1% SDS, 1% Triton X-100), four times with LiCl buffer (0.25M LiCl, 10 mM Tris-HCl pH 8.1, 1 mM EDTA, 1% sodium deoxycholate and 1% NP-40), twice with 0.1% Tween-20 in 1×PBS, and once with TE buffer (10 mM Tris-HCl pH 8.1 and 1 mM EDTA). Immunoprecipitated DNA was eluted from the beads by centrifugation and DNA crosslinking was reversed at 65°C for 4 hours. The DNA was incubated with 20 mg of RNase A at 55°C for 1 hour. 40 μg Proteinase K (3115879001, Roche) were added and incubated at 55°C for 1 hour. The Immunoprecipitated DNA was purified using the Qiagen PCR Purification Kit (Qiagen).

[0181] ChIP-Seq Library Preparation, Sequencing and Analysis

[0182] Quality of ChIP’d DNA was determined using Picogreen Reagent (Quant-IT™ PicoGreen dsDNA Assay Kit, Thermo Fisher P11496) and a fluorometer instrument. 150 ng to 1 μg of DNA was prepared into Illumina libraries, according to manufacturer’s instructions, and using the TruSeq Nano DNA Library Prep Kit (Illumina, FC-121-4003). Resulting libraries were deep sequenced, using the Illumina HiSeq2500 system in Rapid Run mode, obtaining between 10M and 15M of 100-bp length, single-end reads per library.

[0183] ChIP-Seq Read Alignment

[0184] Raw sequencing reads for all the individual ChIP-seq datasets were aligned using bwa (Li and Durbin 2009) (version 0.7.12) mapper with the following settings ‘-a 8 -s amin’. Applicant allowed two mismatches relative to the reference and only retained the unique alignments with Phred quality score greater than 30 as done in the previous study (Marinov et al. 2014). The datasets were mapped against mm9 version of the mouse genome.

[0185] ChIP-Seq Data Analysis

[0186] Quality Assessment

[0187] ChIP-seq quality assessment was carried out using the strategy described by ENCODE ChIP-seq data analysis guidelines (Landt et al. 2012). Cross-correlation analysis was performed using SPP package (Kharchenko et al. 2008) using the parameter ‘-a —1005:600’. The analysis is essential to assess the NSC (Normalized Strand Correlation) and RSC (Relative Strand Correlation) values as recommended by ENCODE (Landt et al. 2012). As per the guideline, all of our selected ChIP-seq datasets are above NSC value (>0.05) and RSC value (>0.8) threshold, and subsequent QC scores equal to or above 1 (Landt et al. 2012; Marinov et al. 2014).

[0188] Peak Calling and Differential ChIP-Seq Analysis

[0189] After quality assessment, Applicant used “irreproducible discovery rate” (IDR) frame-work to call the peaks against their respective input ChIP libraries using MACS2 program (Feng et al. 2011) as described in the ENCODE guidelines (Landt et al. 2012). MACS2 peak calling was performed using the following settings ‘-p 1e-3-to-large-nomodel-shiftsize’ while rest of the parameters were set to their default mode. The final conservative set of peaks for all the samples were called across technical replicates with an IDR threshold of 0.01.

[0190] Differential ChIP-Seq Analysis

[0191] Differential ChIP-seq analysis between two conditions was performed using MACS2 program (Feng et al. 2011) by treating one of the samples as the control for the other. The peak identification by MACS2 was carried out using the same parameter settings as previously described in ChIP-seq data analysis part. The cross-correlation analysis step (Kharchenko et al. 2008) was also performed on the respective datasets to determine the ‘shiftsize’ parameter essential for peak identification by MACS2.

[0192] Chromosome Conformation Capture Using Ddx5/6UCE as Bait (4Cseq)

[0193] Whole ganglionic eminences (GE’s) were dissected from 10 E16.5 and 10 E17.5 E13.5 embryos (schematic in Fig S2, GE=LG2+MGE+CGE). Tissues were pooled for each genotype, triturated by pipetting, and filtered through a cell-strainer capped 5 ml polystyrene round-bottom tube (BD Falcon) to make single-cell suspensions. Cells were fixed in 2% paraformaldehyde/10% Formaldehyde Serum (FBS) at room temperature for 10 min with rotation. 125 mM glycine was used to quench the formaldehyde. The 4C method used has been described in detail (van de Werken et al. 2012). EcoR1 was used for the primary restriction digestion and DpnII was used for the secondary restriction digestion.

[0194] The following steps were performed to generate the 4C library for sequencing. First, overhangs were added to the 4C template using PCR amplification with primers containing the bisulfite sequence.

[0195] Primers:

\[ \text{Dix5/6UCE Forward:} \]
\[ 5'\text{TGTCGCGACGCGATCTGTATGATAACGAGCTGCACACCTGCT} \]
\[ \text{GAGST3 } \]
\[ 5'\text{TGTGCTGCGCGATGAGATGATGACACAGCCCGCACCGCTCGAA} \]

[0196] PCR reaction: 200 ng 4C template, 0.2 mM dNTPs, 35 pmol Primer Dix5/6UCE-Fwd, 35 pmol Primer Dix5/6UCE-Rev, 1.75 U Expand Long Template Enzyme Mix (Roche), 1× Buffer 1. PCR cycles: 94°C—2 min, 94°C—10 sec, 55°C—1 min, 68°C—3 min, 29 cycles, 68°C—5 min. The PCR product was purified using the High Pure PCR Product Purification Kit (Roche). Then, the 4C DNA containing the overhangs was used as template for a second PCR that adds index sequences and Illumina sequencing adapters to generate the 4C library for sequencing. PCR reaction (50 μl): 225 ng DNA template, 0.5 mM dNTPs, 5 μl Nextera XT Index1 primer (N7XX, Illumina), 5 μl Nextera Index 2 primer (S5XX, Illumina), 3.5 U Expand Long Template Enzyme Mix (Roche), 1× Buffer 1. PCR cycles: 94°C—5 min, 94°C—10 sec, 55°C—30 sec, 68°C—1 min, 8 cycles, 68°C—7 min. The PCR product was purified using the High Pure PCR Product Purification Kit (Roche).

[0197] 4Cseq Reads Mapping

[0198] 4C sequencing reads for all the samples were aligned on a reduced mm9 version of mouse genome using bowtie2 alignment program (Langmead and Salzberg 2012). The reduced genome consists of only EcoR1 (+/−50 base-pair) cut-sites. These EcoR1 sites were selected based on the presence of a second restriction enzyme cut-site i.e. DpnII, within its +/−50 base-pairs. Applicant trimmed the 5’ end of the raw reads to remove the bait sequence before mapping to the reduced genome. Applicant allowed two mismatches outside the EcoR1 sequence in the reduced genome.
during mapping and only retained chromosome 6 specific unique alignments with Phred quality score greater than 30. [0199] 4Cseq Differential Data Analysis
[0200] 4C reads mapped at the EcoR1 restriction site resolution on chromosome 6, were further filtered based on their reproducibility in each pair of replicates. An EcoR1 cut-site was deemed reproducible if the two replicates in a given condition (Etv2 T2/TS) have either both non-zero counts or both zero counts. By applying this criteria, Applicant retained a total of 997 reproducible EcoR1 restriction cut-sites across the replicates of the two conditions. Applicant then performed a DESeq2 (Love et al. 2014) based differential contact count analysis on these sites to obtain condition specific significantly higher (p-adjusted values<0.05) and a log 2 fold changes>2 for Etv2 T2/TS and conserved (p-adjusted value<0.05) 4C interaction sites.

[0201] Histone Lysine Methylation (ChIP-seq) Determination at Dlx5/6UCE Interaction Sites
[0202] (4Cseq)
[0203] To interrogate the interplay between changes in chromatin contacts and changes in local chromatin landscape (e.g., histone modifications). Applicant computed ChIP-seq signal density of three different histone marks (H3K4me3, H3K4me1 and H3K27me3) near each reproducible EcoR1 cut-site. Using “bedtools intersect” (Quinlan and Hall 2010) in both Etv2 T2/TS and Etv2 T2/TS conditions, surrounding regions of each cut-site were scanned from +/-1 kb to +/-10 kb at every 125 base-pair interval to gather average ChIP-seq signal for each mark. For each window size, the distributions of sequencing depth normalized ChIP-seq read counts from each condition were compared against each other. Depending on whether the same or different sets of 4C sites are being compared between the two conditions, either a paired (FIG. 4G, H, J) or an unpaired (FIG. 4F) T-test was performed for window sizes of +/-2 kb, 6 kb and 10 kb to test the difference in histone modifications between the two conditions.

[0204] Human/Mouse specific Dlx5/6UCE—Gene Interaction and Conservation Analysis
[0205] Applicant has used the preprocessed hiclib ("hiclib.org/mirnylab/hiclib") normalized human cortex Hi-C data (Wen et al. 2016) (GSE77565; ftp.ncbi.nlm.nih.gov/g综合整治/GSE77565; suppl/GSE77565_FBD_IC-heatmap-chr-10k/idls.gz) at 10 kb resolution to first extract all the Evf2 (chr7:96,594,838-96,643,377 in hg19) interacting genetic regions (genes/>-500 Kb) from human chromosome 7. At 10 kb resolution the human Evf2 region is distributed within five Hi-C bins (9660 to 9664), and any of the Evf2 bin with non-zero normalized interaction count with a genomic region was considered for further processing in the downstream analysis. Applicant also extracted the Evf2 T2/TS and Evf2 T2/TS 4C interacting genetic regions from mouse chromosome 6 in the similar manner. In the next step, Applicant used "hitOver" (Kent et al. 2002) tool to get the list of conserved Evf2 interacting genetic regions among human cortex (H-C), mouse-Evf2 T2/TS (4C) and mouse-Evf2 T2/TS (4C) conditions.

[0206] Circular Visualization, Density, Histone Peak Plots and Gene Ontology Analysis
[0207] Circular visualization of integrated 4C and histone mark data were generated using circo software package (Krzywinski et al. 2009). The density plots were generated using R “smoothScatter” and “bde2D” package (Wand 1994). Wash U Epigenome Browser (Zhou et al. 2011) was used to plot the histone peaks and their signal intensities. Gene ontology analysis of Evf2 T2/TS, Evf2 T2/TS (+, -) and (i) Dlx5/6UCE interacting sites were performed using AmiGO2 browser. An adjusted p-value threshold of 0.05 was used to filter out significant ontology enrichments of each gene set (Gene Ontology 2015).

[0208] Self-Organizing Maps

[0210] Fluorescent In Situ Hybridization (FISH) of E13.5 GE Nuclei
[0211] DNA FISH
[0212] The DNA FISH method was adapted from a detailed lab protocol provided by Dr. Jerold Chun (Scirpiss, LaJolla, Cal.) (Westra et al. 2008). Single cell suspensions from whole GE’s were made as described above. Cell pellets were gently resuspended in 500 μl Nuclear Extraction Buffer (0.32 M sucrose, 5 mM CaCl2, 5 mM Mg(AC)2, 0.1 mM EDTA, 20 mM Tris- HCl pH 8.0, 0.1% TritonX-100) and incubated on ice for 10 min. Cells were centrifuged at 1000 g for 2.5 min at 4°C and the supernatant was removed. Cells were washed gently with ice-cold 1×PBS with 2 mM EDTA. Cells were centrifuged at 1000 g for 2.5 min at 4°C. The supernatant was removed and cells were gently resuspended in 500 μl of ice-cold fixative (3 Methanol: 1 Glacial Acetic Acid). The cells were fixed for 10 min on ice. 5 μl of cells in fixative were transferred to Superfrost Plus microscope slides (Fisher Scientific) and allowed to air dry. The slides were transferred to a slide holder, vacuum-sealed and stored at -80°C.

[0213] Slides were incubated with 100 μg/mL RNase at 37°C for 30 min. Cells were washed twice with 2×SSC (0.30 M NaCl buffer. 0.030 M trisodium citrate) for 2 min, treated with 50 μg/mL pepsin in 0.01 M HCl at 37°C for 7 min, and washed twice with 2×SSC for 2 min. Cells were fixed in 1% paraformaldehyde for 10 min at room temperature and washed 3 times with 2×SSC for 5 min. The slides were dehydrated by incubation for 2 min in 70%, 80% and 100% ethanol. 200 μl denaturation solution (70 formamide in 2×SSC) was added and the slides were incubated at 85°C for 10 min. Slides were dehydrated in ice-cold 70%, 80% and 100% ethanol and allowed to air dry. 150 μl pre-hybridization buffer (50% formamide, 0.1% SDS, 300 ng/ml Salmon Spem DNA, 2×SSC) were added and the slides were incubated overnight at 37°C.

[0214] DNA FISH probes were generated by nick translation using the FISH Tag DNA Kit (Thermo Fisher Scientific) following manufacturer’s instructions. The templates for the nick translation reactions were obtained from the BAC PAC Resources Center (Children’s Hospital Oakland Research Institute): Dlx5/6 region: W11-1693 G2, Umdad region: W111946E1, Akr1b8 region: RP23-120H14. DNA probes in hybridization buffer (50% formamide, 10% dextran sulfate, 0.1% SDS, 300 ng/ml Salmon SpemDNA, 2×SSC) were denatured in the presence of 2 μg Mouse Hylbloc DNA (Applied Genetics Laboratories) at 80°C for 7 min and re-annealed at 37°C for 1 hour. Slides were
incubated for 5 min in 2xSSC with 50% formamide, 2 min in 4xSSC with 0.1% Tween-20 and 2 min in 2xSSC at 45°C. The slides were dehydrated in ethanol and denatured as described above. 10 µl of FISH probe solution were added, the coverslips were sealed with rubber cement and the slides were incubated overnight at 37°C. Slides were incubated in 2xSSC with 50% formamide for 10 min (3 times), in 2xSSC for 10 min and in 2xSSC with 0.1% NP40 for 5 min at 45°C. The slides were rinsed with 1xPBS, incubated with 5 mg/ml DAPI for 5 min, rinsed again and mounted using SlowFade Gold antifade reagent (Thermo Fisher Scientific).

**0215** Combined RNA and DNA FISH

**0216** Slides containing cell nuclei were prepared as described above. Slides were incubated with 50 µg/ml peptin in 0.01 M HCl at 37°C for 7 min, and washed twice with 2xSSC. Cells were fixed in 4% paraformaldehyde for 5 min at room temperature and washed 3 times with 2xSSC for 5 min. The slides were incubated in 1xPBS with 1% hydrogen peroxide for 30 min at room temperature and rinsed twice with 2xSSC. The slides were dehydrated by incubation for 2 min in 70%, 80%, and 100% ethanol. 200 µl denaturation solution (70% formamide in 2xSSC) were added and the slides were incubated at 85°C for 10 min. Slides were dehydrated in ice-cold 70%, 80% and 100% ethanol for 2 min and allowed to air dry. 150 µl pre-hybridization buffer (50% formamide, 0.1% SDS, 300 ng/ml Salmon Sperm DNA, 2xSSC) were added and the slides were incubated overnight at 37°C.

**0217** DNA FISH probes were generated as described above. The digoxigenin labeled RNA probe was generated as described previously (Feng et al. 2006). DNA probes and RNA probe in hybridization buffer (50% formamide, 10% dextran sulfate, 0.1% SDS, 300 ng/ml Salmon Sperm DNA, 2xSSC) were denatured in the presence of 2 µg Mouse Hybloc DNA (Applied Genes Laboratories) at 85°C for 7 min and re-annexed at 37°C for 1 h. Slides were incubated for 5 min in 2xSSC with 50% formamide, 2 min in 4xSSC with 0.1% Tween-20 and 2 min in 2xSSC at 45°C. The slides were dehydrated in ethanol and denatured as described above. 10 µl of FISH probe solution was added, and coverslips were sealed with rubber cement and the slides were incubated overnight at 37°C.

**0218** Slides were incubated in 2xSSC with 50% formamide for 10 min (3 times), in 2xSSC for 10 min and in 2xSSC with 0.1% NP40 for 5 min at 45°C. The slides were rinsed with 1xPBS and incubated in 1% blocking solution (Tyraside Signal Amplification Kit, Thermo Fisher Scientific) for 1 h. Mouse monoclonal anti-Digoxigenin (Roche) was diluted 1:400 in blocking reagent, added to the slides and incubated at 4°C overnight. Slides were washed 3 times in 1xPBS for 3 min at room temperature, incubated with 1:100 HRP-goat anti-mouse IgG in blocking solution for 1 hour and tyramide labeled according to manufacturer's instructions (TSA Kit, Thermo Fisher Scientific). The slides were washed 3 times with 1xPBS for 3 min, incubated with 5 mg/ml DAPI for 5 min, rinsed with 1xPBS and mounted using SlowFade Gold antifade reagent (Thermo Fisher Scientific).

**0219** Confocal Microscopy

**0220** Cells were visualized using a Zeiss Laser Scanning Microscope 880 and a 100x immersion oil objective. Z-stacks of 0.3 µm intervals were taken using the Zen 2.1 software. To measure interprobe distances, a line was traced from the center of one probe to the center of the adjacent probe. Distances were measured between probes on the same z-slice.
[0221] This Example demonstrates the novel pathway to directly increase the level of serotonin receptor gene expression in neurons, providing a novel agent for treating neurological disorders and stress-induced conditions. Applicant has found that Ev2 long non-coding RNA up-regulates serotonin receptor expression by decreasing the expression of a specific enzyme, Akr1b8, in developing interneurons. Mice lacking Ev2 exhibit changes in behavior, including behavioral despair, learning and seizure susceptibility. Compositions and methods of treating neurological disorders and stress-induced conditions are contemplated by treating a subject with Akr1b8/B10 or an agonist thereof. Methods and compositions for treating neurological disorders and stress-induced conditions by a subject with a small molecule effectors or metabolites of the mevalonate pathway are also contemplated.

[0222] For example, a method of treating a neurological disorder or stress-induced condition in a subject, the method comprising the steps of: administering an effective amount of at least one aldo-keto reductase family 1, member b10 (Akr1b10), aldo-keto reductase family 1, member B8 (Akr1b8), an agonist of Akr1b10, or an agonist of Akr1b8 in order to alleviate, reduce or inhibit one or more symptoms of the neurological disorder or stress induced condition in the subject.

[0223] Each publication, patent, and patent publication cited in this disclosure is incorporated in reference herein in its entirety. The present invention is not intended to be limited to the foregoing examples, but encompasses all such modifications and variations as come within the scope of the appended claims.

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[0230] Caijigas, I., Leib, D. E., Cochrane, J., Luo, H., Swyter, K. R., Chen, S., Clark, B. S.,


[0233] Cobos, I., Calcañagnotto, M. E., Vilaythong, A. J., Tiwon, M. T., Noebels, J. L., Barnab, S. C.,


[0246] Development 125, 5079-5089.


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1. A method of treating a neurological disorder or stress-induced condition in a subject, the method comprising the steps of:

administering an effective amount of at least one of aldo-keto reductase family 1, member B10 (Akr1B10), aldo-keto reductase family 1, member B8 (Akr1B8), an agonist of Akr1B10, or an agonist of Akr1B8 in order to alleviate, reduce or inhibit one or more symptoms of the neurological disorder or stress induced condition in the subject.

2. The method according to claim 1, wherein the neurological disorder or stress-induced condition is a mood disorder.

3. The method according to claim 2, wherein the mood disorder is selected from the group consisting of depression, anxiety, and combinations thereof.

4. The method according to claim 3, wherein the mood disorder is selected from the group consisting of anxiety disorder, panic disorder, obsessive-compulsive disorder (OCD), eating disorder, chronic pain, and posttraumatic stress disorder (PTSD).

5. The method of claim 1, wherein the neurological disorder or stress-induced condition in a subject is a developmental neurological disorder.

6. The method of claim 5, wherein the developmental neurological disorder is selected from the group comprising a learning disorder, autism, and epilepsy.

7. The method of claim 1, wherein the neurological disorder or stress-induced condition is drug addiction.

8. The method of claim 1, wherein the subject is a human.

9. A method of increasing expression of 5-hydroxytryptamine receptor 3A (5Htr3a) in at least one neuron, the method comprising:

contacting the at least one neuron with at least one of Akr1B8, an agonist of Akr1B8, Akr1B10, or an agonist of Akr1B10, wherein the at least one neuron exhibits an increase expression of 5Htr3a.

10. The method of claim 9, wherein the increased expression of 5Htr3a increases the level of serotonin signaling by the neuron.

11. The method of claim 9, wherein the at least one neuron is an interneuron.

12. The method of claim 9, wherein the neuron is a human neuron.

13. The method of claim 9, wherein the neuron is contacted in vivo.

14. A method of increasing the serotonin level in a subject, the method comprising:

administering the subject at least one of Akr1B8, an agonist of Akr1B8, Akr1B10, or an agonist of Akr1B10 in an effective amount to increase the serotonin level in the subject.

15. The method of claim 14, wherein the subject is suffering from a neurological disorder or a stress-induced condition.

16. The method of claim 14, wherein the subject is a human.

17. The method of claim 15, wherein the neurological disorder or stress-induced condition is selected from the group consisting of depression, anxiety disorder, panic disorder, obsessive-compulsive disorder (OCD), eating disorder, chronic pain, posttraumatic stress disorder (PTSD), epilepsy, autism, autism spectrum disorder, and combinations thereof.

18. A method of inducing a pluripotent stem cell to differentiate into a neuron comprising:

culturing the pluripotent stem cell with at least one of Akr1B8, an agonist of Akr1B8, Akr1B10, or an agonist of Akr1B10, wherein the pluripotent stem cell differentiates into a neuron that expresses 5Htr3a.

19. The method of claim 18, wherein the pluripotent stem cell is a human pluripotent cell.

20. The method of claim 18, wherein the pluripotent stem cell is cultured in neuronal producing medium.

21. The method of claim 18, wherein the pluripotent stem cell is an embryonic stem (ES) cell or an induced pluripotent stem (iPS) cell.

22. A method of treating a neurological disorder or stress-induced disorder, the method comprising: administering a small molecule effector or metabolite of the mevalonate pathway, wherein administration of the small molecule effector or metabolite alleviates, reduce or inhibit at least one or more symptoms of the neurological disorder or stress-induced disorder.