Dorsey *et al*. SUPPLEMENTAL DISCUSSION:

The bases for including the genes in Table 3, (excluding those in the "Intracellular Signaling" "Neurogenesis" and "Excitation-Inhibition" categories, which are discussed in the main text), are provided below with references.

Calcium signaling. Ca^{2+} is an intracellular messenger in a wide variety of cellular responses, including the generation of neural diversity during embryonic development [1]. Calmodulin, encoded by ***Calm1*** **(+1.4)**, acts as the Ca^{2+}-sensor in many responses including Ca^{2+}-calmodulin dependent kinases [***Camk1*** **(−1.6)**, ***Camk2a*** **(+1.9)**, ***Camkk1*** **(+2.6)**, ***Camkv*** **(+2.4)**] and calcineurin [***Ppp3cb*** **(−1.3)** and ***PPP3cc*** **(−1.3)**]. Four genes encoding subunits of voltage-gated Ca^{2+} channels are on the SFARI list [2,3] (Table S3) were dysregulated by VPA in the fetal brain: ***Cacna1b*** **(+1.4)**, ***Cacna1c*** **(−1.4)**, ***Cacna2d1*** **(−1.7)** and ***Cacnb2*** **(−1.9)**. Like *Cacnb2*, ***Cacnb4*** **(−1.5)** is an auxiliary subunit, however, it is not linked to autism by GWAS. *Cacna1c* is associated with Timothy syndrome [4], an ASD characterized by ID, as well as long-QT syndrome, a frequently lethal heart rhythm defect [5,6].

Retinoic acid signaling. Retinoic acid (RA) is a diffusible derivative of vitamin A that has been reported to regulate the fetal development of the prefrontal cortex and the dopaminergic system in the striatum; dysregulation of RA signaling in mice can lead to autistic-like behavior. ***Rarb*** **(−2.2) and** ***Rxrb*** **(−1.3)**, which encode the RA receptors, RARβ and RXRB, are downregulated 55% and 23% by VPA in the E12.5 mouse brain. In addition, four additional genes dysregulated by VPA **[*Megf10* (−1.9)**, ***Cntnap2***, **(+1.3)** ***Meis2*** **(−1.4)**, ***Cbln2*** **(−1.8)]** are involved in RA signaling; three of these (*Megf10*, *Cntnap2*, *Meis2*) are on the SFARI list. RA-dependent developmental processes in the fetal brain include generation of GABAergic striatal projection neurons and interneurons [7], expression of D2 dopamine receptors in the ventral striatum [8] and regulation of cortical neurogenesis and cortical plate thickness [9]. Shibata et al. [10] reported that genetic deletion of *Rarb* and *Rxrb* disrupts proper molecular patterning of prefrontal and motor areas, development of prefrontal cortex (PFC)-mediodorsal thalamus connectivity and development of intra-PFC dendritic spinogenesis. RA signaling may also regulate the development of the dopamine (DA) system [8]; deletion of *Rxrb* and *Rarb* resulted in locomotor defects related to dysfunction of the mesolimbic DA signaling pathway, as well as expression of D1 and D2 DA receptors in the ventral striatum. Interestingly, VPA reduced the expression the D2 DA receptor, ***Drd2*** **(−1.9)** by nearly half. Thus, these findings suggest that VPA could reduce the strength of RA signaling in the fetal brain, particularly in the dopaminergic system, resulting in altered numbers and/or connectivity of cortical or striatal neurons.

Neuronal fate specification. ***Tbr1*** **(−2.8)** encodes T-brain-1, a brain-specific T-box transcription factor that has been linked to autism in multiple GWAS (see Table 1). VPA reduced *Tbr1* expression by 63% in the fetal mouse brain (Table S3). *Tbr1* has been described as a "master regulator of ASDs" because it regulates the expression of multiple genes with links to autism [11], thereby controlling brain connectivity in ASDs [12,13]. *Tbr1* has been reported to regulate differentiation of the preplate and layer 6 of the fetal cortex [14] as well as the connectivity of cortical layer 6 [15]. Recently, different *Tbr1* mutations found in individuals with ASD, intellectual disability and developmental delay had differing effects on cortical development [16]; one such mutation (K228E) caused significant upregulation of *Tbr1* but similar behavioral phenotypes to those of *Tbr1* KO mice [17]. Thus, it appears that both positive and negative deviations from an optimal level of *Tbr1* expression are associated with an autism-like behavioral phenotype. Considering its links to autism in GWAS and its central role in regulating fetal brain development, a VPA-induced reduction in *Tbr1* expression in the fetal brain could contribute to the behavioral abnormalities observed in animals exposed to VPA *in utero*.

Nr2f1 **(*Coup-tf1*) (−1.8)** encodes an orphan nuclear receptor that has been implicated in a range of neurodevelopmental functions including coordinating cortical patterning, neurogenesis, and laminar fate [18–20]; *Nr2f1* has also been reported to control subtype and laminar identity of cortical interneurons

derived from the medial ganglionic eminence [21] as well as regional dynamics of neuroprogenitors in the cortex, possibly being responsible for abnormal gyrification [22]. *Nr2f1*, together with *Nr1f2*, regulates cell migration in the basal forebrain [23] and is required for forebrain commissural projections [24].

Neuronal differentiation and regional specification in the CNS are regulated by the *Ebf* family of genes including ***Ebf3* (−1.6)** [25]. Variants of *EBF3* are associated with hypotonia, developmental delay, intellectual disability, and autism [26]. The *Olig* genes (*Olig1/2/3*), which encode members of the basic helix-loop-helix (bHLH) family of transcription factors, were originally identified as regulators of oligodendrocyte production in the CNS. More recently, the *Olig* genes have been found to regulate the specification and differentiation of neuronal phenotype during fetal brain development [27,28]. ***Olig1* (+2.1**, significant in females only**)** expression was more than doubled in fetal brains exposed to VPA. The basic Helix-loop-Helix gene, ***Neurod1* (−1.5)**, regulates multiple steps in cortical development including neuronal fate specification, differentiation, and migration [29].

Axon growth/guidance. Myristoylated Alanine-Rich C-kinase Substrate, encoded by ***Marcks* (−2.2),** plays a wide variety of roles in brain development and function [30,31]**.** These may be due at least in part to the regulation of axon growth in which MARCKS mediates membrane targeting of plasmalemmal precursor vesicles during axon development [32]. Although total deletion of *Marcks* in mice is embryonic lethal, the 55% reduction in *Marcks* expression induced by VPA may be less severe and cause subtle changes in brain connectivity that could lead to autistic-like behavior. ***Syn2* (+1.7)** (synapsin-2) has been reported to be required for normal axon formation [33]. ***Draxin* (−1.5)** (dorsal inhibitory axon guidance protein) is essential for the axon guidance underlying development of the corpus callosum and thalamocortical projections [34–36]. Heterogeneous nuclear ribonucleoprotein A/B encoded by ***Hnrnpab* (−1.6)** facilitates olfactory sensory neuron maturation and axon projections by regulating the local expression of its target genes at axon terminals [37].

Neuronal migration. ***Robo4* (−2.2)** has been reported to regulate the radial migration of newborn neurons in the developing neocortex [38,39]; *Robo4* expression was reduced by more than half in the fetal brain by VPA. Dysregulation of *Robo4* expression at E12.5 could affect the developing connectivity of the fetal brain leading to behavioral abnormalities in the adult. ***Mllt11* (−1.5)** regulates migration and neurite outgrowth of cortical projection neurons during development [40]. ***Ptk2b* (−1.7)** (*Pyk2*) is a non-receptor cell-adhesion kinase and scaffold protein which, if either overexpressed or knocked down, impairs cortical neuron migration by altering growth cone dynamics [41]. *Ptk2b* is regulated by ***Ptpn5* (−1.5)** (STEP), a tyrosine phosphatase [42]. None of these three genes has been linked to autism in GWAS but downregulation of *Ptk2b*, *Ptpn5* and/or *Mllt11a* by VPA has the potential to alter neuronal migration at a critical time when circuits are forming in the fetal brain. ***Otp* (−2.5)** encodes a homeodomain transcription factor that is associated with the development of the hypothalamus in vertebrates. *Otp* is necessary for the migration of diencephalic neurons to the amygdala [43] and affects neuropeptide switching in oxytocin neurons [44–46]. A loss of function mutation in ***Apc2* (−1.7)** causes Sotos syndrome which is characterized by ID and characteristic facial features; this is because *Apc2* is a downstream effector of ***Nsd1* (−1.4)** in regulating the migration and laminar positioning of cortical neurons [47]. Thus, VPA can disrupt this critical developmental pathway at two steps. ***Mboat7* (+1.9)** (also known as *Lpiat1*) deficiency has multiple effects on the developing brain including disordered cortical lamination and delayed neural migration [48].

Synaptogenesis. ***Slc17a7* (+1.6)**, which encodes the vesicular glutamate transporter VGLUT1, promotes the development of cortical presynaptic terminals [49]. The VPA-induced increase in *Slc17a7* would be consistent with the abnormal acceleration or premature initiation of this process. ***Slc17a6* (−2.4)**, which encodes the transporter homolog, VGLUT2, was downregulated by 60%. *SLTRKs* are single-pass transmembrane proteins of the leucine-rich repeat (LRR) superfamily that have been proposed as candidate genes for neuropsychiatric disorders [50]. There are 6 members of this family in humans and mice, encoded by *Slitrk1−6*. ***Slitrk1* (−1.5)** has been shown to modulate neurite outgrowth

[51] and to promote the development of excitatory synapses [52], *Slitrk2* (not significantly affected by VPA) is on the SFARI list and has been linked to schizophrenia and X-linked NDDs [53] and ***Slitrk5* (−1.6)** deficiency impairs corticostriatal circuitry [54] and synaptogenesis [55]. *Slitrk1* and *Slitrk5* expression in the fetal brain was reduced 33% and 37% by VPA; ***Slitrk4* (+1.7)** expression was increased 67% by VPA (Table S4).

Activity-dependent immediate early genes (IEGs) have been shown to regulate synaptogenesis [56]. The IEG, ***Arc* (+1.6)** (*Arg 3.1*), has been described as a flexible hub for synaptic plasticity and cognition [57] and has been reported to mediate activity-dependent synapse elimination in the developing cerebellum [58]. The cerebellins (CBLNs) are secreted glycoproteins that link presynaptic neurexins with postsynaptic δ1 glutamate receptors to form trans-synaptic adhesion complexes and promote the formation and stability of excitatory synapses [59]. The formation of these complexes is deficient in the ***Cbln2* (−1.8)** knockout mouse [60] resulting in the destabilization of excitatory synapses. Consequently, the 45% reduction in *Cbln* expression induced by VPA in the fetal mouse brain would be predicted to compromise excitatory synaptogenesis.

***Top3b* (−1.5)** encodes an RNA topoisomerase that works with FMRP to promote synapse formation [61] and is a high-confidence gene on the SFARI list (Table S3). *Top* family members have been reported to facilitate transcription of long genes linked to autism [62]. ***Syncrip* (−1.4)** (SFARI list) (also known as *Hnrnpg*) encodes the synaptotagmin-binding cytoplasmic RNA-interacting protein, which is a candidate gene for ASD and ID [63–65]. Bannai et al. [66] reported that *Syncrip* is a component of mRNA-containing granules in dendrites, possibly regulating local protein synthesis in developing dendritic spines.

Disruption of *POGZ* is associated with ID, ASDs and impaired cortical development [67–69]. Markenscoff-Papadimitriou et al. [70] reported that ***Pogz* (−2.2)** promotes chromatin accessibility and expression of clustered synaptic genes; moreover, brain-specific conditional knockout of *Pogz* results in gene expression changes associated with synaptic function as well as an autistic-like behavioral phenotype [71]. The X-linked gene, ***Ddx3x* (−1.6),** encodes an RNA helicase that functions in corticogenesis and synaptogenesis [72,73]. Mutations in *DDX3X* account for approximately 2% of intellectual disability in females (DDX3X syndrome) [74,75]. Knockdown of the RNA binding protein, ***Csde1* (−2.0)** has been reported to cause abnormal dendritic spine morphology and synapse formation [76].

Wnt signaling plays an important role in the development and maturation of the nervous system. ***Wnt5a* (−2.7)** modulates hippocampal postsynaptic development by increasing the length of the postsynaptic density and eliciting new synaptic contacts [77].

Fn14, encoded by ***Tnfrsf12a* (+4.8)**, and its microglia-derived ligand TWEAK, regulate the number of synapses with bulbous spine s at retinogeniculate connections, thereby strengthening the circuit that is established in response to visual stimulation [78]. Whether this signaling mechanism plays a role in shaping other neural pathways during embryonic cortical development is not known.

Aggrecan, encoded by ***Acan* (−6.1)** is a proteoglycan and a key component of the extracellular matrix and perneuronal nets (PNNs) (see “Excitation-Inhibition” section of main article). Aggrecan removal in the adult brain causes a loss of PNNs and the reversion to juvenile ocular dominance plasticity [79]. The potential consequences of the 84% reduction in *Acan* expression induced by VPA in the fetal brain has not been investigated but might be expected to result in disrupted development of synaptic plasticity within and beyond the visual system.

Dendrite development. ***P2rx7* (−2.0)** (ionotrophic P2X7 receptor, which is activated by extracellular ATP) has been described as the “central hub of brain diseases” [80]. Inhibition of P2X7 receptors reduced dendritic spine pathology under pathological conditions (in *Mecp2*-deficient mice) [81], while P2rx7 deficiency caused dendritic branching deficits on otherwise normal mice [82]. Interestingly, ***Tmem163* (−1.7)** is required for full function of P2X7 receptors [83]. ***Sema3a* (−2.0)** regulates dendritic complexity via ***Farp1***

(−1.7) in an activity dependent manner [84]. *Farp1* links postsynaptic cytoskeletal dynamics and transsynaptic organization to coordinate synaptic development [85]. In contrast, ***Itpka* (+14.1)**, which has been reported to regulate dendrite morphology [86] was overexpressed by 14-fold in fetal brain exposed to VPA. Thus, the reduction in expression of *P2rx7*, *Tmem163*, *Sema3a* and *Farp1* and the increase in *Itpka* induced by VPA may work together to alter the initial steps in dendritic branching in the E12.5 brain. ***Icam5* (+16.6)** (intercellular adhesion molecule 5) is overexpressed in the brain of the *Fmr1* knockout mouse (which models fragile X syndrome, an ASD), leading to dendritic spine abnormalities. The massive increase in *Icam5* expression induced by VPA has the potential to severely disrupt the connectivity of the developing brain, which could lead to autism-like behavior. ***Gas7* (−1.7)** encodes a spine initiation factor that responds to neuronal activity; *Gas7* knockdown decreased spine density in hippocampal neurons [87]. ***Dlg5* (−3.0)** encodes a membrane-associated guanylate kinase (MAGUK) protein, which is required for dendritic spine formation and synaptogenesis [88], raising the possibility that synaptic development and organization may be impacted by the 67% reduction *Dlg5* expression in fetal brain exposed to VPA at E12.5. Normal expression levels of ***Disc1* (−1.9)** (Disrupted in schizophrenia-1) are required for microtubule function and depletion of *Disc1* or dominant negative *Disc1* constructs impairs neurite outgrowth *in vitro* and proper development of the cortex *in vivo* [89]. Increased expression of Twinfillin-2 which is encoded by ***Twf2* (+1.6)**, increases thin dendritic spine length in hippocampal neurons [90].

Postsynaptic cytoskeleton. The organization and assembly of glutamate receptors in the postsynaptic membrane are dependent on scaffold proteins including members of the HOMER and SHANK families [91]. ***Homer1* (+1.6)** and ***Shank2* (−2.3)** are associated with autism by GWAS (Table S3) and are responsible for rare syndromic cases of autism; ***Homer3* (−1.4)** is also dysregulated in the fetal brain [91−96]. Disruption of *Homer* and *Shank* gene expression by VPA would be expected to alter synaptic assembly at E12.5 and throughout development.

Dopaminergic system. Several lines of evidence link autistic symptoms with dysfunction in the mesencephalic DA system [97-99]. This can be explained by abnormal circuitry [100], notably in the indirect pathway downstream from D2-receptor expressing medium spiny neurons (MSNs). The subthalamic nucleus (STN), an excitatory nucleus in the indirect pathway downstream from the D2-MSNs, is a key factor in the normal functioning of the basal ganglia. ***Foxa1* (−1.6)**, which is reduced by 38% by VPA, has been reported to be essential for the development and functional integrity of the STN [101−103]. ***Lmx1a* (−1.6)**, which is reduced by about 40% in the E12.5 brain exposed to VPA (Table S4), has been shown to promote mesencephalic DA neuron development and diversity [104−108]. The basic helix-loop-helix transcription factor ***Srebf1* (+1.4** significant in males only) has been shown to be both necessary and sufficient for midbrain dopaminergic neurogenesis [109]. Engeln et al. [110] reported that transgenic mice with the BDNF receptor, trkB, knocked out specifically in D1 MSNs, displayed autism-like stereotypic behavior that was reversed by chemogenetic inhibition of D1-MSNs. Using RNA-seq of ribosome-associated mRNA, the authors found that 26 genes were dysregulated in the transgenic mouse D1-MSNs. Of these 17 were significantly dysregulated by VPA in the present study [***Slc9a3r1* (+3.0)**, ***Csf1r* (−1.4)**, ***Sparc* (−1.4)**, ***Mcam* (+1.7)**, ***Robo4* (−2.2)**, ***Emp2* (−1.6)**, ***Klf4* (−1.6)**, ***Rtn4rl1* (+2.0)**, ***Bdnf* (+2.8)**, ***C1qtnf1* (−1.4)**, ***Tmem108* (+1.4)**, ***Smyd2* (−1.3)**, ***Wnt7b* (−1.8)**, ***Palm3* (+1.9)**, ***Adora2a* (−1.3)**, ***Drd2* (+2.0)**, ***S1pr1* (−1.3)**]. Secreted frizzled-related proteins, encoded by *Sfrp1* and ***Sfrp2* (−1.8)**, regulate Wnt signaling to modulate midbrain dopaminergic neuron development [111]; indeed, the phenotype of *Sfrp1*/*Sfrp2* and ***Wnt5a* (−1.7)** knock-out mice is similar [112]. Another gene linked to the development of the DA system is ***Nr2f1* (−1.8)** [23], which was downregulated by 45% by VPA.

All three DA receptor subtypes (*Drd1*, *Drd2*, *Drd3*) are linked to autism by GWAS (SFARI List) and partial antagonists of the D2 DA receptor (encoded by *Drd2*) have demonstrated utility in reducing stereotypic behaviors in human subjects [113]; ***Drd2* (+2.2)** expression was doubled by VPA in the present

study. Tyrosine hydroxylase encoded by the ***Th*** **(−1.4)** gene is the rate limiting step in the biosynthesis of catecholamines including DA; *Th* is downregulated by about 35% in fetal brain exposed to VPA.

Ppp1r1b **(+3.7)**, encodes the dopamine- and cAMP-regulated neuronal phosphoprotein (DARPP-32). DARPP-32 amplifies and/or mediates many actions of cyclic AMP-dependent protein kinase at the plasma membrane and in the cytoplasm, with a broad spectrum of potential targets and functions within the dopaminergic system and throughout the brain [114].

Cholinergic system. Cholinergic projection neurons in the nucleus basalis and cholinergic interneurons in the striatum are specified in the embryonic forebrain beginning at about E10 in mouse [115]. This process is regulated by several transcription factors including ***Lhx8*** **(−2.7)**, ***Isl1*** **(−1.5)**, and ***Gbx1*** **(−2.3)** all of which are downregulated in response to VPA in the fetal mouse brain. In the developing forebrain, *Isl1-Lhx8* hexameric complexes promote cholinergic identity [116]. Conditional deletion of *Isl1* depletes cholinergic projection- and interneurons resulting in abolition of cholinergic innervation of the cortex [117]. ***Nkx2-1*** **(−2.5**, significant in females only), a homeodomain transcription factor, is required for the development of cholinergic septo-hippocampal neurons and large subsets of basal forebrain cholinergic neurons [118]. The 33−67% decrease in the expression of these transcription factors induced in the fetal brain by VPA has the potential to alter the proper development of circuits critical for normal brain function. Indeed, the loss of these neurons due to lack of *Nkx2-1* causes alterations in hippocampal theta rhythms and learning and memory defects [119]. In addition, *Nkx2-1* acting together with *Lhx6* (not affected by VPA), is necessary to specify pallidal projection neurons and forebrain interneurons in the medial ganglionic eminence [120].

Endocannabinoid system. ***Cnr1*** **(−2.0)**, encodes the primary endocannabinoid **(**eCB) receptor in the brain; *Cnr1* has been linked to autism in GWAS. eCBs have been reported to regulate a wide range of embryonic neurodevelopmental processes [121,122], including neural progenitor proliferation, long-range axon patterning, interneuron migration and morphogenesis, and neuronal fate specification [123−125]. A 50% decrease in expression of *Cnr1* would be predicted to alter the cellular responses to eCB and could disrupt normal fetal brain development [126].

Autism and Down syndrome (DS). While the cause of autism is unknown, DS is caused by triplication of human chromosome 21 (Hsa21), leading to the hypothesis that a 50% increase in gene dosage of one or more Hsa21 genes is the cause of DS symptoms. ASDs and DS share ID as a common feature; moreover, DS individuals are diagnosed with ASD at a higher frequency than the general population [127,128]. In fact, several genes triplicated in DS that have been linked to autism or to prenatal brain development are up- or downregulated by VPA in the fetal brain [129−131]; these include ***Dyrk1a*** **(−1.5)**, ***Brwd1*** **(−1.3)**, ***Cbs*** **(+5.0)** and ***Wdr4*** **(−1.4)**. ID in both ASDs and DS has been attributed to overexpression of *Cbs* (cystathionine beta synthase) [132−134]; dysregulation of *Cbs* and dihydrofolate reductase **(*Dhfr*, +1.5)** contributes to inborn errors of amino acid metabolism and is linked to ASDs [135,136]**.** *Dyrk1a* (which encodes dual specificity tyrosine phosphorylation regulated kinase 1A) is associated with autism in all five GWAS (Table 1) and may underlie intellectual disability in DS due to its increased gene dosage [128]. In contrast, haploinsufficiency of *DYRK1A* results in a (non-DS) syndrome characterized by intellectual disability including impaired speech development, autism spectrum disorder including anxious and/or stereotypic behavior problems, and microcephaly [137,138].

Circadian rhythms. Eleven genes involved in generating circadian rhythms were significantly affected by VPA in the fetal mouse brain (Table 3). Two of these (*Per1* and *Per1*) are SFARI risk genes. While ***Per1*** **(+1.9),** ***Per2*** **+1.7),** ***Cry1*** **(+2.5)*, Cry 2*** **(+2.0),** ***Arntl*** **(+2.0) and** ***Mef2d*** **(+1.5)** [139−141] were upregulated by VPA, *Clock* was not affected. ***Fbxl3*** **(−1.8)** a negative regulator of *Cry1* and *Cry2*, is downregulated by VPA whereas ***Fbxl21*** **(+3.2)**, which is upregulated by VPA, down-regulates *Fbxl3* [142]. Thus, these VPA-induced changes could plausibly lead to increased *Per* and *Cry* expression. ***Arntl*** **(+2.1)**, together with *Clock*, activates rhythmic transcription of *Per* and *Cry* genes; knockout of *Arntl*

(*Bmal1*) induces autistic-like behavior and cerebellar dysfunction which was ameliorated by mTORC1 inhibition [143].

Daily rhythms in the fetal brain are thought to be entrained by the mother, however, whether there is a functional *Per/Cry/Clock* cycle in the fetal brain has not been explored. It is possible that disrupting the expression of these genes could alter early embryonic brain development through a mechanism unrelated to sleep. For example, *in utero* knockdown of *Per3* caused abnormal positioning of cortical excitatory neurons as well as impaired axon extension and dendritic arbor formation [144].

If VPA-induced changes in circadian rhythm gene expression were to persist postnatally, they could cause disrupted sleep patterns, a common occurrence in autism. Indeed, exposure of fetal rats to VPA on E12.5 caused sleep disturbances that were similar to those reported in autistic children [145]; moreover, a VPA-induced reduction in GAD-67 (*Gad1*) was also found in the juvenile rat brains. Zhang et al. [146] reported that *GAD1* and *GAD2* are downregulated in superior temporal gyrus of autistic subjects; in the present study, VPA exposure reduced the expression of ***Gad1* (−2.5)** and ***Gad2* (−3.3)** by 60% and 70%, while RNAs encoding GABA-A and -B receptors subunits, encoded by ***Gabra4* (+2.5)** and ***Gabbr2* (−2.4),** were found to be upregulated 2.5-fold and downregulated 60% by VPA, respectively.

Neuroinflammation. Immune dysregulation has been proposed to play a key role in the pathogenesis of autism [147]. Several heat shock proteins (HSPs) are upregulated in the ASD brain. Genes encoding neuroinflammatory molecules such as insulin-like growth factors [***Igf1* (−3.2), *Igf2* (−2.7)**], transforming growth factor-β isoforms [***Tgfb2* (−2.4), *Tgfb3* (−2.0)**], and the TGF-β receptor, ***Tgfbr2* (−2.5)**, are decreased in fetal brain in response to VPA exposure. Expression of several HSPs [***Hspa1a* (+5.9), *Hspa1b* (+5.6), *Hspa2* (+6.2) and *Dnajc12* (+4.5)**] was dramatically increased by VPA exposure; this may be a generalized response to the administration of a high, acute dose of VPA**.** *DNAJC12* encodes a chaperone protein responsible for the proper folding of phenylalanine hydrolase and *DNAJC12* deficiency causes hyperphenylalaninemia leading symptoms ranging from mild autistic features or hyperactivity to severe intellectual disability [148,149].

***Cd200* (−2.0)** is a surface glycoprotein expressed by neurons and its receptor, *Cd200r*, is expressed on microglia. Disturbances in this signaling pathway result in microglial activation and can lead to neuroinflammation [147] as is observed in MIA, a cause of autism.

Epigenetic regulation of gene expression in the action of VPA. The mechanism(s) by which VPA alters gene expression in the fetal brain is not known; however, VPA is a Class I histone deacetyase (HDAC) inhibitor, suggesting that many of the changes reported here may involve increased histone acetylation [150,151]. Indeed, Konopko et al. [152] reported that VPA increased the acetylation of several histones at the promoter regions of *Bdnf* 5' untranslated exons 1, 4, and 6 and that this was positively correlated with increased expression of exon 9, the protein-coding region.

It is also likely that many of the genes targeted by VPA are themselves epigenetic writers, erasers, or readers; moreover, there is well-established crosstalk among epigenetic marks [153], including between DNA CpG methylation and covalent histone modifications, which could explain why HDAC inhibition by VPA could both increase and decrease gene expression. The diversity of epigenomic modifications provides a plausible explanation for the diversity and severity of ASD symptoms [154]. It is potentially relevant that the histone deacetylases ***Hdac1* (+1.6)**, ***Hdac3* (+1.3)**, ***Hdac5* (+1.6)**, ***Hdac6* (+1.5)**, ***Hdac7* (−2.1)**, ***Hdac9* (−1.3)**, ***Hdac10* (−1.3)** and ***Hdac11* (+1.5)** are all dysregulated by VPA.

***Setd1b* (−2.2)** encodes a lysine-specific methyltransferase which has been linked to autism in GWAS studies (Table S3). De novo variants of *SET1B* are associated with intellectual disability, ASD and epilepsy [155,156]. ***Kmt5b* (−6.5)** encodes the epigenetic writer, lysine methyl transferase 5B, which methylates lysine residues on histones, resulting in gene activation or silencing, depending on the histone and lysine residue. Loss of function mutations in *KMT5B* lead to developmental delay and ASD

[157] (SFARI List). *Kmt5b* expression is reduced by 85% in fetal mouse brains exposed to VPA (Table S3). Pathogenic mutations in ***Ehmt1* (−1.4)** (SFARI List), a lysine methyltransferase, and ***Smarcb2* (−1.7)**, an actin-dependent regulator of chromatin, are causative for Kleefstra Syndrome Spectrum, an ASD; reduced expression of these genes leads to increased neuronal excitability [158,159].

***Mecp2* (−1.6)** is an X-linked gene that encodes the epigenetic reader, methyl cytosine binding protein 2**.** Mutations in *MECP2* cause Rett syndrome, an ASD that affects mostly girls [160]; *MECP2* is on the SFARI list. MeCP2 binds to methylated CpGs in DNA mediating gene repression [161−163]. Either increased or decreased expression of MeCP2 can cause developmental brain disorders [164]. MeCP2 binds to CpGs throughout the genome; altered MeCP2 would be expected to alter the expression of many genes. One such gene is ***Bdnf* (+2.8)**. MeCP2 binds to CpGs in the 5'UTR of exon 4 of *Bdnf* leading to suppression of BDNF expression [165]. Reduction of MeCP2 as observed with VPA (Table S3), would be predicted to decrease normal epigenetic suppression of CpG methylated genes. Although reduced *Mecp2* expression induced by VPA would be expected to increase BDNF expression, VPA did not alter CpG methylation in the regulatory regions of *Bdnf*; instead, increased *Bdnf* expression was associated with increased histone acetylation and methylation [152].

Heavner and Smith [166] have noted that eight chromatin-modifying genes [*Chd4*, *Cdh8*, ***Adnp* (−1.6), *Arid1b* (−2.2), *Chd3* (−2.0)**, ***Chd7* (−1.3), *Smarca2* (−1.6), *Smarca4* (−1.6)**] function to regulate both embryonic cortical development and synaptic homeostasis during learning in the mature brain; six of these genes are downregulated by VPA. Paulsen et al. [167] reported that haploinsufficiency of the chromatin remodelers, ***Kmt5b* (−6.5)**, ***Arid1b* (−2.2)** and ***Smarcc2* (−1.6)**, increased the numbers of inhibitory GABAergic neurons in organoid models of human cortex leading to reduced spontaneous circuit activity. Li et al. [168] found that mutating the BAF chromatin remodeling complex subunit, *ARID1B*, enriches ventral telencephalon progenitors by affecting the fate transition of progenitors to oligodendrocyte and interneuron precursor cells. Tuoc et al. [169] reported that another BAF (BAF-170, encoded by *Smarcc2*) regulates cortical size with its deletion resulting in an increased pool of intermediated progenitors and an enlarged cortex. In the present study, *Kmt5b* and *Arid1b* were downregulated in the fetal brain by 85% and 50%, in response to VPA. Although *Chd8* expression was unaffected by VPA, *Chd3* and *Chd7*, which have similar chromatin remodeling functions [170], were reduced by 50% and 23%.

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