Valproate Exposure as an *In Vitro* Model for Studying Morpho-Molecular Features of ASD: A Systematic Review

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Abstract. Background: Autism Spectrum Disorder (ASD) is a complex neurodevelopmental disorder with a strong genetic and environmental basis. It frequently causes social and communication deficits, as well as repetitive behaviors. Valproic acid (VPA) has been shown to induce autistic-like features in animal models when administered during critical development periods. However, not much is known about its effect in cells to replicate ASD characteristics *in vitro*. **Objective:** This review explores *in vitro* VPA models to elucidate the molecular and morphological characteristics of ASD, emphasizing their potential and proposing directions for future research. **Methods:** PubMed, SciELO, Embase, Web of Science, and Scopus databases were searched, and 11 studies were included after screening. **Results:** The studies explored VPA's effects on various cell cultures, including human neural cell lines, primary adult neurons, and primary embryonic neurons. VPA was found to be neurotoxic in a dose- and time-dependent manner, with greater toxicity in immature and undifferentiated cells. *In vitro*, VPA can influence gene expression, increase oxidative stress, disrupt neurogenesis and synaptogenesis, affect the GABAergic system, and alter critical signaling pathways for brain development and cell differentiation, such as Wnt/β-catenin. **Conclusion:** *In vitro* models provide valuable insights into the morpho-molecular alterations induced by VPA and their connection to ASD. These findings highlight the need for further research into VPA's cellular effects to deepen our understanding of its role in ASD pathology.

Keywords: valproic acid; autism spectrum disorder; *in vitro*; neuronal cell culture; neurotoxicity; morpho-molecular alterations.

1. INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by social deficits, communication issues, and stereotyped behavior, which is usually diagnosed in early childhood [1]. Its pathophysiology remains incompletely understood. However, growing evidence suggests that environmental and epigenetic factors may play a role in its development [2–5]. With ASD prevalence increasing globally, understanding the mechanisms of this disorder remains a paramount challenge in both clinical and research settings [6–8].

Many preclinical studies have tried to replicate key features of ASD, either *in vivo* or *in vitro*. A well-established ASD animal model involves the use of valproic acid (VPA) [9–11]. VPA is widely used as a

mood stabilizer for bipolar disorder, for migraine prophylaxis, and as an anticonvulsant [12–14]. VPA mechanisms of action are not fully detailed. Yet, suggested mechanisms include enhancement of inhibitory neurotransmission, the modulation of voltage-gated ion channels, and inhibition of histone deacetylases (HDAC) [15–17]. These effects may potentially alter the expression of genes involved in cell growth, differentiation, and maturation [18].

Studies indicate that VPA exposure during critical neurodevelopmental periods may induce molecular and behavioral phenotypes resembling ASD in animal offspring [9,19–21]. Clinical findings corroborate these results, demonstrating that prenatal VPA exposure during the first trimester can disrupt brain development, resulting in structural and functional deficits associated with ASD [22–25]. Nonetheless, there is a notable lack of established protocols and standardized methodologies for applying VPA *in vitro*, highlighting the need for further research to refine and adapt VPA-based models for cellular and molecular studies [23,26].

While the VPA-rodent model of ASD has been widely used, it is resource-intensive and subject to ethical constraints [27,28]. In contrast, *in vitro* approaches provide a more accessible, controlled, reproducible approach for investigating specific neurobiological mechanisms associated with ASD [29–32]. Additionally, researchers working with *in vivo* VPA models may seek to transition toward *in vitro* approaches while maintaining continuity in their studies. Establishing a reliable VPA-based *in vitro* model could facilitate this transition and expand the toolkit available for studying morpho-molecular alterations in ASD [33–35]. Alternative *in vitro* approaches, such as neurospheres and brain organoids, although valuable, present challenges related to high costs, lengthy differentiation protocols, and variability between batches [36–39]. VPA-based *in vitro* models, rather than serving as a replacement, could act as a complementary tool, offering a simpler and more cost-effective method for studying ASD-related cellular and molecular alterations [40,41].

Thus, this systematic review aims to evaluate and synthesize the existing literature on *in vitro* approaches using VPA to replicate the morpho-molecular characteristics associated with ASD. By integrating findings from diverse studies, we seek to offer valuable insights into the potential applications, limitations, and future directions of VPA-based *in vitro* research, advancing our understanding of morpho-cellular features in ASD.

2. MATERIALS AND METHODS

This systematic review adhered to a modified version of the Preferential Reporting Requirements for Systematic Review (PRISMA) guidelines [42] and was registered with OSF registries (doi: 10.17605/OSF.IO/NJXVA).

We aimed to answer the following question: which *in vitro* strategies involving VPA are currently being used to "mimic" morpho-molecular autistic-like outcomes in cell culture?

2.1 *Search strategy and study selection*

We conducted searches across five electronic databases: Medline (via Pubmed), SciELO (via Scientific Electronic Library), Embase (via Ovid), Web of Science (via Core collection), and Scopus (via Core collection). The complete search strategy is available in the supplementary materials (Table S1).

The chosen articles were published between 2014 and 2023, with searches conducted in both Portuguese and English. The search period extended from October 2023 to December 2023. The full text of each potentially eligible study was obtained and independently assessed for eligibility by two team members (QDJSV and MAS). Any disparities in eligibility were addressed through discussion with two additional reviewers (VBJ and GFA).

2.2 *Data extraction*

Data collection was carried out using the *Rayyan*® website. Each task was independently performed by three human reviewers (QDJSV, JVC, and MAS), with the exception of the deduplication process. The articles included were divided into three subgroups: human neural cell lines, primary adult neurons, and primary embryonic neurons.

2.3 *Eligibility criteria*

Study inclusion was decided by a consensus between all authors. Before the selection process, a meeting was held to discuss our study objectives and predefined the inclusion criteria in accordance with Population, Intervention, Comparison, Outcome, and Study type (PICOS) approach [43]. Thus, the PICOS criteria was used as the primary framework for determining eligibility, ensuring that only studies aligned with the research focus were included. The final selection was made after a discussion and agreement on the inclusion of studies that met all PICOS criteria.

The study selection criteria were as follows: Population: *in vitro* models that can include, but are not limited to neurons, microglia, astrocytes, and other cell types; Intervention: valproic acid and/or sodium valproate; Comparator: control groups; Outcome: increased inflammation, cell death, mitochondrial dysfunction, oxidative stress, or any other characteristic typically observed in an *in vivo* model of autism; Study design: only *in vitro* studies (original research). The PICOS strategy is described in Table 1.

[Table 1 here]

The exclusion criteria were as follows: articles that used drugs or chemicals other than VPA; any type of review, gray literature, animal model study, clinical trial, letter to the editor, uncontrolled study, or articles that did not align with the PICOS criteria. A common reason for exclusion was that many studies investigated the effects of VPA on teratogenicity or in cancer cell models. While these studies assessed the impact of VPA, they did not examine outcomes relevant to autism-related morpho-molecular alterations as outlined in our PICOS framework. Additionally, studies that cultured cells derived from VPA-exposed offspring were excluded, as they did not meet the Population criteria in our inclusion criteria. Our aim was to analyze studies using cell models directly exposed to VPA, without prenatal ASD models. Therefore, they were excluded.

In vivo models of VPA-induced autism typically exhibit key neurobiological and cellular characteristics, including increased neuroinflammation, oxidative stress marked by reduced antioxidant enzyme activity and heightened oxidative damage, mitochondrial dysfunction with impaired energy metabolism and altered mitochondrial dynamics, and increased neuronal cell death, leading to structural and functional deficits [44–47]. Therefore, we focused on *in vitro* studies that reported similar outcomes to ensure relevance to our research framework.

A narrative synthesis was undertaken to examine the results extracted from the included studies, along with the data compiled from various cell experiments conducted.

2.4 *Quality assessment*

The quality of the included studies was collectively assessed by three reviewers (QDJSV, JVC, and MAS) using a standardized questionnaire developed by all authors. The content of the questionnaire was adapted from the CAMARADES *in vivo* quality assessment, with modifications tailored for *in vitro* research [48].

Key parameters evaluated included the clarity of study objectives and hypotheses, the specifics of cell culture conditions (including cell lines, medium, passage number, and authentication), and the documentation of experimental procedures. It also assessed the appropriateness of statistical analysis, the clarity of data presentation, and the accuracy of discussions and conclusions. Additionally, methodological transparency, adherence to ethical guidelines, compliance with reporting standards, and whether the study was peer-reviewed were considered. Studies were evaluated based on these criteria, with a minimum score of five required for inclusion. All included studies met this threshold.

3. RESULTS

3.1 *Summary of findings*

Initially, a total of 2550 articles were retrieved from the literature search, which was subsequently narrowed down to 1163 articles after removing duplicates. After this, the review authors (QDJSV, JC, and MAS) screened titles and abstracts to identify studies that met the inclusion criteria, narrowing the selection to 50 relevant papers. Applying the exclusion criteria led to the removal of 39 articles for various reasons, as shown in Figure 1. Ultimately, this systematic review yielded a final sample of 11 English-language documents. A flow chart, adapted from the PRISMA diagram, is presented below in Figure 1.

[Figure 1 here]

3.2 *Study characteristics*

In our findings, four studies used human neural cell lines [26,49–51], two articles used primary adult neurons [52,53], and five used primary embryonic neurons [54–58]. The studies employed cells from both human and animal sources, with human-derived neurons being the most utilized [26,49–51,56].

Methodologies and outcomes exhibit considerable variance across the literature. Most papers examined cell viability, morphology, and neurite outgrowth [26,51–53,56,58]. Others analyzed changes in messenger RNA (mRNA) and protein expression in neuronal cells [49,50,54,55]. Two studies analyzed VPA's influence on neuronal electrophysiology [53,57].

Dosage ranged widely, with most studies applying multiple doses [26,50–58], while one paper used a single dose [49]. Treatment durations also varied from 24h [50,51,54,58] to 15 minutes [57], and 6 days [53,56].

Table 2 provides a structured summary of key information from the 11 selected studies, enabling a clear comparison of study characteristics and findings. The first column categorizes cell models (human neural cell lines, primary adult neurons, and primary embryonic neurons), followed by study authors, cell types, treatment duration, VPA dose, and key findings. This structure facilitates trend identification across studies and helps interpret experimental conditions and outcomes.

[Table 2 here].

3.2.1. *Human neural cell lines*

Kaushik *et al*. (2016) [49] and Peltier *et al*. (2024) [50] investigated VPA's role in ASD pathophysiological mechanisms in SK-N-SH and SH-SY5Y cells. Kaushik *et al*. examined environmental triggers for idiopathic autism by exposing differentiated SK-N-SH neurons to 34 mM of VPA for 48h. The authors observed upregulation of gene sets linked to neurotransmitter binding and synapse regulation, while downregulating others involved in neurodevelopmental processes, such as axonogenesis and neuron projection. They found that VPA's effects on gene expression *in vitro* were comparable to those induced by psychoactive pharmaceuticals (fluoxetine, venlafaxine, and carbamazepine). In contrast, Peltier *et al*. examined VPA's impact on cholesterol homeostasis in SH-SY5Y and HMC3 cells, revealing dose- and time-dependent changes in cholesterol transporter proteins (ABCA1 and ABCG1) and increased cholesterol efflux (SH-SY5Y cells/24h treatment). This study highlights VPA's potential to disrupt cholesterol pathways critical for neural development, whereas Kaushik *et al*. focused on neurodevelopmental gene regulation [49,50].

Chanda and colleagues (2019) [26] expanded on these findings by examining VPA's effects on embryonic stem cell-derived neurons at different developmental stages. Early exposure (day 1) reduced neurite length and branching dose-dependently, while later stages (day 21) showed milder effects, and mature neurons (days 50–56) were unaffected. VPA-mediated disruptions were linked to GSK-3β and HDAC pathways, with specific inhibitors mimicking these effects, and to MARCKSL1 downregulation, affecting cell motility in early neurons. Similarly, Zhang *et al*. (2023) [51] studied SH-SY5Y cells, showing that VPA increased reactive oxygen species (ROS), reduced cell survival, and impaired autophagy by altering LC-3B, Beclin-1, and p62 protein levels. They also observed activation of the Notch-1/Hes-1 pathway, critical for autophagy regulation. Together, these studies demonstrate VPA's capacity to disrupt neurodevelopment and autophagy, emphasizing dose- and timing-dependent impacts on neuronal health [26,51].

3.2.2 *Primary adult neurons*

Kumamaru *et al*. (2014) [52] and Takeda *et al*. (2021) [53] used cells from primary cortical neurons to assess VPA's impact. Kumamaru *et al*. studied cortical neurons from newborn rats. They found that VPA (1 mM and 5 mM) reduced vesicular GABA transporter (VGAT) expression from day *in vitro* (DIV) 1 to 7. At DIV4, VPA selectively inhibited GABAergic synapse formation. Their results highlighted VPA's HDAC inhibitory activity as a key mechanism, distinguishing it from TSA, a potent HDAC inhibitor, and VPM, a VPA analog lacking HDAC inhibition [52,53].

Conversely, Takeda *et al*. explored neuron-astrocyte co-cultures from newborn mice and found that VPA-exposed astrocytes (1 and 3 mM) significantly decreased miniature inhibitory postsynaptic currents (mIPSCs) and VGAT-positive puncta (neurons at DIV14). Moreover, these neurons exhibited reduced protein tyrosine phosphatase receptor type D (PTPRD) expression, critical for GABAergic synapse differentiation, without affecting dendritic morphology or axonal growth. These findings suggest that VPA disrupts synapse development both directly and via astrocytic influence during neuron maturation [53].

3.2.3 *Primary embryonic neurons*

Studies involving embryonic neurons provide insights into VPA's impact on early neurodevelopment, with differences emerging across species and cellular contexts. Ko *et al*. (2018) [55] observed that VPA (0.2–0.5 mM) increased brain-derived neurotrophic factor (BDNF) mRNA and protein levels in neural progenitor cells (NPCs) but not mature cortical neurons, revealing differential effects. Qi *et al*. (2022) [58] showed VPA (100 μM) promoted neurosphere formation and neuronal stem cells (NSC) proliferation via the TGFβ1 pathway, which could be blocked by specific inhibitors. Similarly, Zhang *et al*. (2015) [54] linked VPA (1–10 mM) to increased oxidative stress and dysregulated Wnt/β-catenin signaling. Nissen *et al*. (2016) [57] found a dose-dependent biphasic response in mouse embryonic neurons, with low VPA concentrations enhancing ATP levels and action potential (AP) frequency, while higher doses caused cytotoxicity. Al-Rubai *et al*. (2017) [56] reported that therapeutic VPA doses (500–750 μM) minimally affected human neural cells, whereas higher concentrations impaired cell migration, neuronal process length, and astrocyte integrity. These studies highlight that VPA's effects are dose-dependent and context-specific. While low doses may promote cellular activity, higher doses induce toxicity, affecting neurons and glial cells differently.

3.3 *Quality assessment findings*

The qualitative assessment revealed that while most studies provided well-described methodologies, appropriate result analyses, and thorough discussions [26,49,52,53], some lacked essential details about *in vitro* methodology [55,56,58]. Specifically, a few studies did not report passage numbers or clearly define negative controls [55,56]. Additionally, certain papers failed to acknowledge potential limitations in their experimental design [51,54]. Despite these gaps, the majority of included studies were published in peer-reviewed journals and adhered to high methodological standards, ensuring reliability and reproducibility.

4. DISCUSSION

This review offers a comprehensive analysis of the literature on central nervous system (CNS) cell models using VPA exposure to simulate ASD-like morpho-molecular changes *in vitro*. The studies employed various CNS cell types, including primary adult and embryonic neurons, neural stem cells, and neuronal cell lines from human and animal sources. VPA's effects were found to depend on its concentration, cell type, and developmental stage during exposure, with early-stage exposure and concentrations of 5 mM or higher causing the most significant damage. In humans, VPA is used therapeutically for conditions like epilepsy and bipolar disorder at plasma concentrations of 0.3–0.7 mM, but these doses are strongly linked to an increased risk of ASD when administered during pregnancy [59–61]. Animal models of ASD often require higher, toxic doses to induce autistic-like traits [62–64].

4.1. *VPA's pathophysiological mechanisms and implications for ASD research*

In vitro studies of VPA can provide critical insights into the molecular and morphological changes associated with ASD, offering a way of understanding its underlying mechanisms and potential pathophysiological implications [50,51,58]. Unlike purely genetic models, which focus on hereditary factors [32,65,66], VPA exposure can provide a well-established environmental model of ASD, aligning with growing evidence that ASD arises from a complex interplay between genetic susceptibility and environmental influences [62,64].

VPA is known to induce epigenetic modifications, which can alter gene expression, impacting neuronal differentiation, synaptic plasticity, and inflammatory pathways—key mechanisms implicated in ASD pathophysiology [18,67,68]. Epigenetics refers to changes in gene expression that do not involve alterations in the DNA sequence itself but are instead influenced by environmental factors such as chemical exposure, diet, and stress [69,70]. Therefore, using VPA *in vitro* allows researchers to investigate ASD-related cellular and molecular abnormalities without the genetic variability inherent in patient-derived induced pluripotent stem cells (iPSCs) or other genetically derived ASD *in vitro* models [29,38,39].

While ASD is a behaviorally diagnosed neurodevelopmental disorder that cannot be fully replicated *in vitro* or *in vivo*, the VPA model is recognized for its strong construct, face, and predictive validity, as it mimics key ASD-related biological mechanisms, behavioral traits, and pharmacological responses. While the *in vivo* effects of VPA are well-documented, its impact on cellular models remains less explored [11,20,71]. However, emerging evidence suggests that VPA's effects in cell culture studies may be as relevant as those observed *in vivo*, particularly in its influence on gene expression in neural development, neuronal communication, and calcium and potassium signaling—processes fundamental to ASD pathophysiology [22,57,72].

No comprehensive review has thoroughly examined whether the VPA's molecular and cellular changes observed *in vivo* are also replicated in simplified cell culture systems. Traditional *in vitro* models of ASD, such as iPSC-derived neurons or 3D cultures, offer valuable insights but can be cost-prohibitive and time-consuming,

making simpler models, like VPA-treated neuron cultures, a practical alternative for screening mechanistic hypotheses [32,73–75].

Key pathological features of ASD include mitochondrial dysfunction [41,44,76], neuroinflammation characterized by altered microglial activation [77–79], oxidative stress [4,80,81], GABAergic dysfunction [82–84], and disruptions in neuronal morphology and connectivity [85,86]. The studies analyzed in this review identified many of these alterations in VPA-treated neuron cultures, supporting the relevance of this model in studying ASD-related morpho-molecular mechanisms [22,54,57].

Rather than positioning VPA as a superior *in vitro* model over other approaches, this review highlights its continued use as a relevant and accessible tool for investigating ASD's characteristics, especially for researchers with limited resources for more advanced methods. The VPA model not only helps find new treatments but can also be important in reusing current medicines. Some available drugs, including antioxidants and anti-inflammatory drugs, have shown promise in reducing the effects of neurodevelopmental issues from VPA in lab tests. By studying how these drugs act on neuronal cells treated with VPA, researchers can identify which ones are most likely to move on to early testing for ASD treatment [72,75].

Additionally, combining the VPA model with high-throughput screening could speed up the development of new drugs. Recent advances in transcriptomic and proteomic studies offer a better way to analyze how various drugs affect the pathways disturbed by VPA exposure. Using these tools, scientists might find new treatment options faster, getting us closer to effective therapies for ASD.

4.2. *VPA effects on neurons from different origins in vitro: Variations and similarities*

VPA is a well-known HDAC inhibitor, which acts by preventing the removal of acetyl groups from histones, resulting in a more decondensed chromatin and active gene transcription [87]. One proposed mechanism suggests that VPA-induced HDAC inhibition alters the expression of genes involved in cholesterol transport [50]. Additionally, when administered at high doses, particularly during critical periods of brain development, VPA increases oxidative stress, leading to elevated ROS. This rise in ROS can damage cellular components and impair autophagy, a key process that regulates the degradation and recycling of damaged proteins and organelles. In the developing brain, disrupted autophagy results in cellular waste accumulation, ultimately compromising neuronal health and survival [22].

Studies with primary embryonic neurons reveal that VPA's effects vary depending on the cell type, ranging from negative to negligible, compared to human neural cell lines. Ko *et al*. (2018), Kumamaru *et al*. (2014), Al-Rubai *et al*. (2017), and Nissen *et al*. (2016) reported stronger negative effects in NPCs and NSCs compared to fully mature neurons. NPCs are precursor cells that can differentiate into various CNS cell types, with the key difference between NPCs and cortical neurons being their maturity—cortical neurons are more developed [52,55–57]. VPA tends to have a stronger impact on less mature, undifferentiated cells like NPCs and NSCs compared to fully mature cells. This is likely because immature cells are in a highly dynamic phase of proliferation, differentiation, and structural formation in the brain. VPA-induced epigenetic changes, alterations

in cell cycle regulation, and increased oxidative stress tend to negatively affect immature cells more severely than mature ones [88,89].

VPA's impact on neurons is dose-dependent, regardless of cell type. For example, Qi *et al*. (2022) [58] observed that in rat cortical neurons, low VPA doses promoted neurosphere formation, while higher doses reduced cell proliferation. Similarly, Zhang *et al*. (2015) [54] found that VPA induced a dose-dependent decrease in GSK-3β levels, a key regulator of neuroplasticity and synapse formation. Nissen *et al*. (2016) [57] reported that higher VPA concentrations significantly reduced action potential frequencies, whereas lower doses had no measurable effect. At neurotoxic doses, VPA disrupts neuronal morphology, leading to cell shrinkage, apoptosis, and abnormal growth patterns. Additionally, excessive VPA exposure impairs ion channel function and neurotransmitter systems, ultimately altering neuronal signaling and excitability [22,87,88].

Two studies specifically examined VPA's effect on VGAT and the GABAergic system. Kumamaru *et al*. (2014) [52] found that VGAT expression decreased after 12h of VPA exposure, while Takeda *et al*. (2021) [53] reported that VPA not only reduced VGAT levels in astrocytes but also downregulated PTPRD, a protein essential for GABAergic synapse differentiation. Since VGAT is responsible for storing GABA, the brain's primary inhibitory neurotransmitter, disruptions in GABAergic transmission—a hallmark of ASD—could impair neural communication and contribute to behavioral deficits [90]. While VPA is known to increase GABA availability by inhibiting its degradation (a mechanism that provides therapeutic effects in epilepsy), its exposure during early neurodevelopment or at high concentrations may induce adverse neurodevelopmental changes [82,91].

Regarding neuronal morphology, findings remain inconsistent. Kumamaru *et al*. (2014) [52] reported reduced axonal growth at DIV4, while Takeda *et al*. (2021) [53] found no significant changes in dendritic length, branching, or axonal growth after six days of VPA exposure in co-cultured neurons and astrocytes. The difference in findings likely stems from variations in cell type: while one study examined rat cortical neurons, the other used astrocytes co-cultured with neurons. Astrocytes, unlike neurons, do not generate action potentials but instead provide structural and metabolic support for CNS neurons. Since astrocytes modulate synaptic function and neuroinflammation, their presence could influence VPA’s effects on neuronal development [22,87].

VPA’s impact also varies by exposure duration, with comparable effects observed between human neural cell lines and primary neurons [26,52,53]. As noted, VPA is particularly detrimental when administered during early neurodevelopment, a critical window when the brain undergoes neurogenesis, cell maturation, and synaptogenesis. During this period, the developing brain is highly sensitive to environmental influences, making it susceptible to irreversible morphological and functional alterations induced by VPA [22,92].

4.3. *In vitro x in vivo: VPA's impact on cultured neurons compared to ASD models*

Impaired neurogenesis is considered a key feature in the pathophysiology of ASD [7]. *In vivo*, the ASD model involves administering VPA to pregnant rats between embryonic days (E)12.5 and E14.5—a mid-gestational period in rodents that corresponds to the first trimester in humans, when neurogenesis is most

active [21]. *In vitro*, VPA's negative effects on neuronal maturation appear to be mediated by its HDAC inhibitory properties [26,52]. Supporting this, prenatal VPA exposure altered histone acetylation levels in the cerebellum of postnatal day 14 rats, alongside behavioral and inflammatory changes (Kazlauskas et al., 2016). Rats exposed to VPA on E12.5 exhibited reduced neuron numbers in the prefrontal cortex at both postnatal day 4 and 8 weeks of age [93].

Another study using the same model found that VPA enhanced embryonic neurogenesis but depleted the neural precursor cell pool, ultimately downregulating adult hippocampal neurogenesis [94]. Similarly, subcutaneous VPA administration on postnatal day 4 reduced neurogenesis in the hippocampus of 8-week-old mice by decreasing the number of proliferating neural precursor cells [95]. While *in vitro* studies mainly report effects on neural differentiation and cell morphology, *in vivo* studies suggest more severe disruptions in neurogenesis and neural proliferation in the adult brain.

VPA's impact *in vitro* appears to depend on the stage of cellular differentiation, with the most pronounced effects observed in early-stage neurons between DIV1 and DIV21 [26,52]. This aligns with *in vivo* findings, where VPA induces autistic-like features when administered in early pregnancy—corresponding to the first trimester in humans and gestational days 11–13 in rodents. During this period, the first neurons of the cerebral cortex are generated and begin differentiating into mature neurons [96].

A key molecule in early neuronal development is BDNF, which plays a critical role in neuritogenesis and synapse formation [97]. Accordingly, dysregulation of BDNF expression and activity may significantly contribute to abnormal development in ASD. Almeida and collaborators (2014) [96] observed that VPA administration to pregnant mice, at embryonic day 12.5, led to a six-fold increase in BDNF levels within 6h. Similarly, *in vitro* study found that short-term VPA exposure (6h) elevated BDNF levels in NPCs derived from rat fetal brains at E14, but had no effect on cortical neurons collected at E18. This highlights the importance of neuronal maturation stage in determining VPA's effects [55].

VPA-exposed neurons *in vitro* showed a decreased number of GABAergic synapses and mIPSC frequency. These effects were observed in primary neurons derived from postnatal day 1 [52] and newborn mice [53]. Consistent with these findings, *in vivo* studies have reported widespread GABAergic dysfunction in VPA-induced ASD models. Prenatal VPA exposure reduced GABA protein levels and downregulated GABA receptor mRNA expression in the medial prefrontal cortex (mPFC) at postnatal day 21 [82]. Additional studies found that VPA exposure decreased GABA B1 receptor levels in the mPFC at postnatal day 35 [91], lowered GABA B2 receptor levels in the hippocampus at postnatal day 90 [98], and downregulated GABA expression in the retina at postnatal day 30 [99]. Notably, children with ASD have been found to exhibit reduced GABA concentrations in the sensorimotor cortex, as detected via magnetic resonance spectroscopy [84], further emphasizing the importance of GABAergic signaling in ASD.

The Wnt/β-catenin pathway, which regulates cell proliferation, is also disrupted by VPA exposure. Although an *in vitro* study reported upregulation of this pathway following VPA treatment, it did not result in increased cell proliferation; rather, it was associated with decreased cell viability [54]. *In vivo*, prenatal VPA exposure upregulated Wnt/β-catenin signaling in the cerebellum, prefrontal cortex, and hippocampus [100].

Additionally, VPA-induced Wnt/β-catenin activation promoted neural progenitor cell proliferation, leading to macrocephaly in rats [101]. Notably, inhibition of this pathway mitigated the effects of VPA, indicating its critical role in the VPA model of ASD.

5. STUDY LIMITATIONS

VPA has been widely used to replicate ASD-like features in *in vivo* models. In this review, we aimed to identify studies utilizing VPA in neuronal cells as a potential tool to investigate the molecular and morphological features of ASD *in vitro*. However, we deliberately avoided labeling cell culture studies as an "ASD model". *In vitro* research examines isolated cellular phenomena without the broader physiological context needed to capture the complex interplay of genetic, epigenetic, environmental, and neurodevelopmental factors underlying ASD. Furthermore, most reviewed studies focused on VPA's effects on general neurodevelopmental processes, such as cell differentiation and survival, rather than explicitly addressing ASD-related outcomes. This underscores the challenge of linking VPA-induced cellular changes directly to the broader ASD phenotype.

Despite searching five major databases with multiple strategies, we identified only 11 eligible studies. This limited number reflects the scarcity of research specifically using *in vitro* VPA models to investigate ASD-related mechanisms. While many studies utilized VPA in cell cultures, they often did so for unrelated purposes or relied on cells derived from VPA-exposed animals, making them ineligible for inclusion. Expanding the number of databases or refining search strategies could potentially yield a broader selection of relevant studies in future reviews. Another challenge was the heterogeneity of methods and cell types among studies, which made direct comparisons difficult. To address this, we categorized studies based on cell type and structured our discussion accordingly, contextualizing findings within both *in vitro* and *in vivo* VPA models of ASD.

Nevertheless, investigating the molecular and morphological effects of VPA in *in vitro* systems remains highly relevant for advancing ASD research. Cell-based studies offer a controlled environment to dissect the biological pathways influenced by VPA. Our review revealed that VPA alters gene expression, oxidative stress levels, and other cellular and molecular characteristics. These findings highlight the potential of *in vitro* studies to identify molecular targets, refine therapeutic strategies, and enhance the design of more complex *in vivo* models. By consolidating existing knowledge, this review underscores the importance of leveraging *in vitro* research to bridge critical gaps in ASD studies.

6. CONCLUSION

The present work aimed to summarize and discuss the different *in vitro* studies using VPA to mimic morpho-molecular autistic-like features. Our findings revealed a diverse range of methodologies, leading to varied reported effects. VPA has complex, time- and concentration-dependent effects on neural cells, impacting not only cellular homeostasis but also modulating gene expression, protein levels, signaling pathways, cell differentiation, and electrophysiology. Importantly, VPA's *in vitro* effects are strongly influenced by the stage of neurodevelopment, with the most severe impairments occurring in early-stage neurons. Additionally, doses

between 5 mM and 12 mM have been shown to cause greater cellular damage, affecting neuronal morphology, synapses, and reducing cell viability.

While *in vitro* models cannot fully replicate the complexity of ASD, they provide a controlled environment to dissect specific neurobiological mechanisms. In particular, *in vitro* models using VPA offer a cost-effective and less technically demanding alternative for investigating ASD-related physiological alterations and identifying potential drug targets, compared to other *in vitro* strategies. Nonetheless, the variability in experimental designs, cell types, and treatment conditions underscores the need for standardized protocols to improve data comparability.

In summary, VPA-cell exposure can serve as a useful tool to study molecular and morphological ASD-related cellular features. Altogether, these findings provide valuable insights into the mechanisms underlying VPA's influence on neurodevelopment, with potential implications for understanding ASD pathophysiology. Future studies should focus on exploring different applications of VPA in CNS cell cultures, investigating a wider range of doses, and analyzing ASD-related outcomes more comprehensively. Unlike approaches that rely solely on cells derived from VPA-exposed animal models, directly treating cultured CNS cells with VPA provides a more controlled environment to examine its morpho-molecular effects while also reducing animal use, aligning with the principles of ethical and reproducible research.

AUTHORS' CONTRIBUTION

Conceptualization: VASCONCELOS, BAMBINI-JUNIOR; Methodology: VASCONCELOS; Data curation: VASCONCELOS, CARLETTI, SERAFINI; Writing – Original Draft: VASCONCELOS, CARLETTI, SERAFINI; Supervision: ARAGÃO, BAMBINI-JUNIOR, GOTTFRIED; Writing – Review & Editing: ARAGÃO, BAMBINI-JUNIOR, GOTTFRIED. All authors read and approved the final version of the manuscript.

LIST OF ABBREVIATIONS

ASD = Autism Spectrum Disorder
VPA = Valproic acid
HDAC = Histone deacetylases
PRISMA = Preferential Reporting Requirements for Systematic Review
ROS = Reactive oxygen species
VGAT = Vesicular GABA transporter
DIV = Day *in vitro*
mIPSCs = Miniature inhibitory postsynaptic currents
PTPRD = Phosphatase receptor type D
BDNF = Brain-derived neurotrophic factor
NPCs = Neural progenitor cells
NSCs = Neuronal stem cells
AP = Action potential

CNS = Central Nervous System

GABA = Gamma-aminobutyric acid

E = Embryonic day

mPFC = Medial prefrontal cortex

CONFLICT OF INTEREST

The authors have no conflicts of interest to report.

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SUPPORTIVE/SUPPLEMENTARY MATERIAL

OSF repository: https://osf.io/njxva/

REFERENCES

[1] American Psychiatric Association. *Diagnostic and statistical manual of mental disorders*; 5th ed., text rev.; American Psychiatric Association Publishing: Washington, DC, 2022.

[2] Guo, Z.; Tang, X.; Xiao, S.; Yan, H.; Sun, S.; Yang, Z.; Huang, L.; Chen, Z.; Wang, Y. Systematic review and meta-analysis: multimodal functional and anatomical neural alterations in autism spectrum disorder. *Mol Autism*, **2024**, *15* (1), 16. https://doi.org/10.1186/s13229-024-00593-6.

[3] Rajabi, P.; Noori, A. S.; Sargolzaei, J. Autism spectrum disorder and various mechanisms behind it. *Pharmacol. Biochem. Behav.*, **2024**, *245*, e173887. https://doi.org/10.1016/j.pbb.2024.173887.

[4] Długosz, A.; Wróblewski, M.; Błaszak, B.; Szulc, J. The role of nutrition, oxidative stress, and trace elements in the pathophysiology of autism spectrum disorders. *Int. J. Mol. Sci.*, **2025**, *26* (2), 808. https://doi.org/10.3390/ijms26020808.

[5] Love, C.; Sominsky, L.; O'Hely, M.; Berk, M.; Vuillermin, P.; Dawson, S. L. Prenatal environmental risk factors for autism spectrum disorder and their potential mechanisms. *BMC Med.*, **2024**, *22* (1), 393. https://doi.org/10.1186/s12916-024-03617-3.

[6] Centers for disease control and prevention (CDC). Data & statistics on autism spectrum disorder https://www.cdc.gov/ncbddd/autism/data.html (accessed May 29, 2023).

[7] Styles, M.; Alsharshani, D.; Samara, M.; Alsharshani, M.; Khattab, A.; Qoronfleh, W.; Al-Dewik, N. Risk factors, diagnosis, prognosis and treatment of autism. *Front. Biosci. (Landmark Ed),* **2020**, *25* (9), 1682–1717. https://doi.org/https://doi.org/10.2741/4873.

[8] Ostrowski, J.; Religioni, U.; Gellert, B.; Sytnik-Czetwertyński, J.; Pinkas, J. Autism spectrum disorders: etiology, epidemiology, and challenges for public health. *Med. Sci. Monit.*, **2024**, *30*, e944161. https://doi.org/10.12659/MSM.944161.

[9] Chen, B.; Xu, X.; Wang, Y.; Yang, Z.; Liu, C.; Zhang, T. VPA-induced autism impairs memory ability through disturbing neural oscillatory patterns in offspring rats. *Cogn. Neurodyn.*, **2024**, *18* (4), 1563–1574. https://doi.org/10.1007/s11571-023-09996-2.
[10] Tian, Y.; Xiao, X.; Liu, W.; Cheng, S.; Qian, N.; Wang, L.; Liu, Y.; Ai, R.; Zhu, X. TREM2 improves microglia function and synaptic development in autism spectrum disorders by regulating P38 MAPK signaling pathway. *Mol. Brain*, **2024**, *17* (1), 12. https://doi.org/10.1186/s13041-024-01081-x.
[11] Nicolini, C.; Fahnestock, M. The valproic acid-induced rodent model of autism. *Exp. Neurol.*, **2018**, *299*, 217–227. https://doi.org/10.1016/j.expneurol.2017.04.017.
[12] Angus-Leppan, H.; Arkell, R.; Watkins, L.; Heaney, D.; Cooper, P.; Shankar, R. New valproate regulations, informed choice and seizure risk. *J. Neurol.*, **2024**, *271* (8), 5671–5686. https://doi.org/10.1007/s00415-024-12436-8.
[13] Mari, J.; Dieckmann, L. H. J.; Prates-Baldez, D.; Haddad, M.; Rodrigues da Silva, N.; Kapczinski, F. The efficacy of valproate in acute mania, bipolar depression and maintenance therapy for bipolar disorder: an overview of systematic reviews with meta-analyses. *BMJ Open*, **2024**, *14* (11), e087999. https://doi.org/10.1136/bmjopen-2024-087999.
[14] Collins-Yoder, A.; Lowell, J. Valproic acid: special considerations and targeted monitoring. *J. Neurosci. Nurs.*, **2017**, *49* (1), 56–61. https://doi.org/10.1097/JNN.0000000000000259.
[15] Zhang, L. Y.; Zhang, S. Y.; Wen, R.; Zhang, T. N.; Yang, N. Role of histone deacetylases and their inhibitors in neurological diseases. *Pharmacol. Res.*, **2024**, *208*, 107410. https://doi.org/10.1016/j.phrs.2024.107410.
[16] Mishra, M. K.; Kukal, S.; Paul, P. R.; Bora, S.; Singh, A.; Kukreti, S.; Saso, L.; Muthusamy, K.; Hasija, Y.; Kukreti, R. Insights into structural modifications of valproic acid and their pharmacological profile. *Molecules*, **2022**, *27* (1), 104. https://doi.org/10.3390/molecules27010104.
[17] Safdar, A.; Ismail, F. A comprehensive review on pharmacological applications and drug-induced toxicity of valproic acid. *Saudi Pharm. J.*, **2023**, *31* (2), 265–278. https://doi.org/10.1016/j.jsps.2022.12.001.
[18] Csoka, A. B.; El Kouhen, N.; Bennani, S.; Getachew, B.; Aschner, M.; Tizabi, Y. Roles of epigenetics and glial cells in drug-induced autism spectrum disorder. *Biomol.*, **2024**, *14* (4), 437. https://doi.org/10.3390/biom14040437.
[19] Chen, O.; Tahmazian, I.; Ferrara, H. J.; Hu, B.; Chomiak, T. The early overgrowth theory of autism spectrum disorder: insight into convergent mechanisms from valproic acid exposure and translational models. *Prog. Mol. Biol. Transl. Sci.*, **2020**, *173*, 275–300. https://doi.org/10.1016/bs.pmbts.2020.04.014.
[20] Bambini-Junior, V.; Baronio, D.; MacKenzie, J.; Zanatta, G.; Riesgo, R. dos S.; Gottfried, C. Prenatal exposure to valproate in animals and autism. In *Comprehensive guide to autism*; Springer New York, 2014; pp 1779–1793. https://doi.org/10.1007/978-1-4614-4788-7_108.
[21] Bambini-Junior, V.; Rodrigues, L.; Behr, G. A.; Moreira, J. C. F.; Riesgo, R.; Gottfried, C. Animal model of autism induced by prenatal exposure to valproate: behavioral changes and liver parameters. *Brain Res.*, **2011**, *1408*, 8–16. https://doi.org/10.1016/j.brainres.2011.06.015.
[22] Taleb, A.; Lin, W.; Xu, X.; Zhang, G.; Zhou, Q. G.; Naveed, M.; Meng, F.; Fukunaga, K.; Han, F. Emerging mechanisms of valproic acid-induced neurotoxic events in autism and its implications for pharmacological treatment. *Biomed. Pharmacother.*, **2021**, *137*, 1–8. https://doi.org/10.1016/j.biopha.2021.111322.
[23] Zarate-Lopez, D.; Torres-Chávez, A. L.; Gálvez-Contreras, A. Y.; Gonzalez-Perez, O. Three decades of valproate: a current model for studying autism spectrum disorder. *Curr. Neuropharmacol.*, **2023**, *22* (2), 260–289. https://doi.org/10.2174/1570159x22666231003121513.
[24] Chaliha, D.; Albrecht, M.; Vaccarezza, M.; Takechi, R.; Lam, V.; Al-Salami, H.; Mamo, J. A systematic review of the valproic-acid-induced rodent model of autism. *Dev. Neurosci.*, **2020**, *42* (1), 12–48. https://doi.org/10.1159/000509109.
[25] Zhao, H.; Wang, Q.; Yan, T.; Zhang, Y.; Xu, H.-J.; Yu, H.-P.; Tu, Z.; Guo, X.; Jiang, Y.-H.; Li, X.-J.; et al. Maternal valproic acid exposure leads to neurogenesis defects and autism-like behaviors in non-human primates. *Transl. Psychiatry*, **2019**, *267* (9), 1–23. https://doi.org/10.1038/s41398-019-0608-1.
[26] Chanda, S.; Ang, C. E.; Lee, Q. Y.; Ghebrial, M.; Haag, D.; Shibuya, Y.; Wernig, M.; Südhof, T. C. Direct reprogramming of human neurons identifies MARCKSL1 as a pathogenic mediator of valproic acid-induced teratogenicity. *Cell Stem Cell*, **2019**, *25* (1), 103-119. https://doi.org/10.1016/j.stem.2019.04.021.

[27] Pastorino, P.; Prearo, M.; Barceló, D. Ethical principles and scientific advancements: in vitro, in silico, and non-vertebrate animal approaches for a green ecotoxicology. *GAC*, **2024**, *8* (100096). https://doi.org/10.1016/j.greeac.2024.100096.
[28] Andersern, M. L.; Winter, L. M. F. Animal models in biological and biomedical research - experimental and ethical concerns. *An. Acad. Bras. Cienc.*, **2019**, *91* (1), 1–14. https://doi.org/10.1590/0001-3765201720170238.
[29] Gordon, A.; Geschwind, D. H. Human in vitro models for understanding mechanisms of autism spectrum disorder. *Mol. Autism*, **2020**, *11* (1). https://doi.org/10.1186/s13229-020-00332-7.
[30] Berendsen, S.; Frijlink, E.; Kroonen, J.; Spliet, W. G. M.; van Hecke, W.; Seute, T.; Snijders, T. J.; Robe, P. A. Effects of valproic acid on histone deacetylase inhibition in vitro and in glioblastoma patient samples. *Neurooncol. Adv.*, **2019**, *1* (1), 1–23. https://doi.org/https://doi.org/10.1093/noajnl/vdz025.
[31] Dorsey, S. G.; Mocci, E.; Lane, M. V.; Krueger, B. K. Rapid effects of valproic acid on the fetal brain transcriptome: implications for brain development and autism. *Transl. Neurosci.*, **2024**, *14* (482). https://doi.org/10.1101/2023.05.01.538959.
[32] Hohmann, S. S.; Ilieva, M.; Michel, T. M. In vitro models for asd-patient- derived ipscs and cerebral organoids. In *Progress in molecular biology and translational science*; Academic Press: Cambridge, Massachusetts, 2020; Vol. 173, pp 355–375. https://doi.org/10.1016/bs.pmbts.2020.04.019.
[33] Khoram-Abadi, K. M.; Basiri, M.; Nemati, M.; Nozari, M. Agmatine ameliorates valproic acid-induced depletion of parvalbumin-positive neuron. *Int. J. Dev. Neurosci.*, **2024**, *84* (2), 134–142. https://doi.org/10.1002/jdn.10314.
[34] Zhu, M. M.; Li, H. L.; Shi, L. H.; Chen, X. P.; Luo, J.; Zhang, Z. L. The pharmacogenomics of valproic acid. *J. Hum. Genet.*, **2017**, *62*, 1009–1014. https://doi.org/https://doi.org/10.1038/jhg.2017.91.
[35] Chatterjee, D.; Maparu, K.; Singh, S. Exploring pathological targets and advancing pharmacotherapy in autism spectrum disorder: contributions of glial cells and heavy metals. *Histol. Histopathol.*, **2025**, *3*, 18870. https://doi.org/10.14670/HH-18-870.
[36] Qian, X.; Song, H.; Ming, G. L. Brain organoids: advances, applications and challenges. *Development*, **2019**, *146* (8), 166074. https://doi.org/10.1242/dev.166074.
[37] Luo, D.; Xu, J.; Liu, F.; Gu, Z. Advances and challenges in cerebral organoids research. *Adv. Nanobiomed. Res.*, **2024**, *4*, 2300126. https://doi.org/10.1002/anbr.202300126.
[38] Nie, L.; Irwin, C.; Geahchan, S.; Singh, K. K. Human pluripotent stem cell (HPSC)-derived models for autism spectrum disorder drug discovery. *Expert. Opin. Drug Discov.*, **2024**, *20* (2), 233–251. https://doi.org/10.1080/17460441.2024.2416484.
[39] Sabogal-Guaqueta, A. M.; Mitchell-Garcia, T.; Hunneman, J.; Voshart, D.; Thiruvalluvan, A.; Foijer, F.; Kruyt, F.; Trombetta-Lima, M.; Eggen, B. J. L.; Boddeke, E.; et al. Brain organoid models for studying the function of IPSC-derived microglia in neurodegeneration and brain tumours. *Neurobiol. Dis.*, **2024**, *203*, 106742. https://doi.org/10.1016/j.nbd.2024.106742.
[40] Kowalski, T. W.; Lord, V. O.; Sgarioni, E.; Gomes, J. do A.; Mariath, L. M.; Recamonde-Mendoza, M.; Vianna, F. S. L. Transcriptome meta-analysis of valproic acid exposure in human embryonic stem cells. *Eur. Neuropsychopharmacol.*, **2022**, *60*, 76–88. https://doi.org/10.1016/j.euroneuro.2022.04.008.
[41] Jang, E. H.; Lee, J. H.; Kim, S. A. Acute valproate exposure induces mitochondrial biogenesis and autophagy with Foxo3a modulation in SH‑SY5Y Cells. *Cells*, **2021**, *10* (10), 2522. https://doi.org/10.3390/cells10102522.
[42] Page, M. J.; Mckenzie, J. E.; Bossuyt, P. M.; Boutron, I.; Hoffmann, T. C.; Mulrow, C. D.; et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*, **2021**, *372*. https://doi.org/10.1186/s13643-021-01626-4.
[43] Liberati, A.; Altman, D. G.; Tetzlaff, J.; Mulrow, C.; Gøtzsche, P. C.; Ioannidis, J. P. A.; Clarke, M.; Devereaux, P. J.; Kleijnen, J.; Moher, D. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *PLoS Med.*, **2009**, *6* (7). https://doi.org/10.1371/journal.pmed.1000100.
[44] Mehra, S.; Ahsan, A. U.; Sharma, M.; Budhwar, M.; Chopra, M. Gestational Fisetin Exerts neuroprotection by regulating mitochondria-directed canonical Wnt signaling, BBB integrity, and apoptosis in prenatal

VPA-induced rodent model of autism. *Mol. Neurobiol.*, **2024**, *61* (7), 4001–4020. https://doi.org/10.1007/s12035-023-03826-6.
[45] Farbin, M.; Hejazi, A.; Fakhraei, N.; Azizi, Y.; Mehrabi, S.; Hajisoltani, R. Neuroprotective effects of apigenin on prenatal valproic acid-induced autism spectrum disorder in rats. *IBRO Neurosci. Rep.*, **2024**, *17*, 493–502. https://doi.org/10.1016/j.ibneur.2024.10.003.
[46] Lu, X. Y.; Li, M. Q.; Li, Y. T.; Yao, J. Y.; Zhang, L. X.; Zeng, Z. H.; Yu-Liu; Chen, Z. R.; Li, C. Q.; Zhou, X. F.; et al. Oral edaravone ameliorates behavioral deficits and pathologies in a valproic acid-induced rat model of autism spectrum disorder. *Neuropharmacology*, **2024**, *258*, 110089. https://doi.org/10.1016/j.neuropharm.2024.110089.
[47] Campos, J. M. B.; de Aguiar da Costa, M.; de Rezende, V. L.; Costa, R. R. N.; Ebs, M. F. P.; Behenck, J. P.; de Roch Casagrande, L.; Venturini, L. M.; Silveira, P. C. L.; Réus, G. Z.; et al. Animal model of autism induced by valproic acid combined with maternal deprivation: sex-specific effects on inflammation and oxidative stress. *Mol. Neurobiol.*, **2024**, *62* (3), 3653–3672. https://doi.org/10.1007/s12035-024-04491-z.
[48] Bahor, Z.; Liao, J.; Currie, G.; Ayder, C.; MacLeod, M.; McCann, S. K.; Bannach-Brown, A.; Wever, K.; Soliman, N.; Wang, Q.; et al. Development and uptake of an online systematic review platform: the early years of the CAMARADES systematic review facility (SyRF). *BMJ Open Sci.*, **2021**, *5* (1), e100103. https://doi.org/10.1136/bmjos-2020-100103.
[49] Kaushik, G.; Xia, Y.; Yang, L.; Thomas, M. A. Psychoactive pharmaceuticals at environmental concentrations induce in vitro gene expression associated with neurological disorders. *BMC Genom.*, **2016**, *17* (Suppl 3), 435. https://doi.org/10.1186/s12864-016-2784-1.
[50] Peltier, M. R.; Behbodikhah, J.; Renna, H. A.; Ahmed, S.; Srivastava, A.; Arita, Y.; Kasselman, L. J.; Pinkhasov, A.; Wisniewski, T.; De Leon, J.; et al. Cholesterol deficiency as a mechanism for autism: a valproic acid model. *JIM*, **2024**, *72* (1), 80–87. https://doi.org/10.1177/10815589231210521.
[51] Zhang, Y. H.; Wang, T.; Li, Y. F.; Deng, Y. N.; He, X. L.; Wang, L. J. N-acetylcysteine improves autism-like behavior by recovering autophagic deficiency and decreasing Notch-1/Hes-1 pathway activity. *Exp. Biol. Med.*, **2023**, *248* (11), 966–978. https://doi.org/10.1177/15353702231179924.
[52] Kumamaru, E.; Egashira, Y.; Takenaka, R.; Takamori, S. Valproic acid selectively suppresses the formation of inhibitory synapses in cultured cortical neurons. *Neurosci. Lett.*, **2014**, *569*, 142–147. https://doi.org/10.1016/j.neulet.2014.03.066.
[53] Takeda, K.; Watanabe, T.; Oyabu, K Tsukamoto, Shuntaro Oba, Yuki Nakano, Takafumi Kubota, Kaori Katsurabayashi, S.; Iwasaki, K. Valproic acid-exposed astrocytes impair inhibitory synapse formation and function. *Sci. Rep.*, **2021**, *23*. https://doi.org/10.1038/s41598-020-79520-7.
[54] Zhang, Y.; Yang, C.; Yuan, G.; Wang, Z.; Cui, W.; Li, R. Sulindac attenuates valproic acid-induced oxidative stress levels in primary cultured cortical neurons and ameliorates repetitive/stereotypic-like movement disorders in wistar rats prenatally exposed to valproic acid. *Int. J. Mol. Med.*, **2015**, *35* (1), 263–270. https://doi.org/10.3892/ijmm.2014.1996.
[55] Ko, H. M.; Jin, Y.; Park, H. H.; Lee, J. H.; Jung, S. H.; Choi, S. Y.; Lee, S. H.; Shin, C. Y. Dual mechanisms for the regulation of brain-derived neurotrophic factor by valproic acid in neural progenitor cells. *Korean J. Physiol. Pharmacol.*, **2018**, *22* (6), 679–688. https://doi.org/10.4196/kjpp.2018.22.6.679.
[56] Al-Rubai, A.; Wigmore, P.; Pratten, M. Evaluation of a human neural stem cell culture method for prediction of the neurotoxicity of anti-epileptics. *ATLA*, **2017**, *45* (2), 67–81. https://doi.org/10.1177/026119291704500202.
[57] Nissen, M.; Buehler, S. M.; Stubbe, M.; Gimsa, J. Neuronal in vitro activity is more sensitive to valproate than intracellular atp: considerations on conversion problems of IC50 in vitro data for animal replacement. *BioSystems*, **2016**, *144*, 35–45. https://doi.org/10.1016/j.biosystems.2016.04.009.
[58] Qi, C.; Zhang, J.; Wang, Y.; Lin, M.; Gao, J.; Lu, H. Valproic acid enhances neurosphere formation in cultured rat embryonic cortical cells through TGFβ1 signaling. *J. Biomed. Res.*, **2022**, *36* (2), 127–140. https://doi.org/10.7555/JBR.36.20210109.
[59] Battino, D.; Tomson, T.; Bonizzoni, E.; Craig, J.; Perucca, E.; Sabers, A.; Thomas, S.; Alvestad, S.; Perucca, P.; Vajda, F. Risk of major congenital malformations and exposure to antiseizure medication monotherapy. *JAMA Neurol.*, **2024**, *81* (5), 481–489. https://doi.org/10.1001/jamaneurol.2024.0258.

[60] Ornoy, A.; Echefu, B.; Becker, M. Valproic acid in pregnancy revisited: neurobehavioral, biochemical and molecular changes affecting the embryo and fetus in humans and in animals: a narrative review. *Int. J. Mol. Sci.*, **2024**, *25* (1), 390. https://doi.org/10.3390/ijms25010390.
[61] Bjørk, M. H.; Zoega, H.; Leinonen, M. K.; Cohen, J. M.; Dreier, J. W.; Furu, K.; Gilhus, N. E.; Gissler, M.; Hálfdánarson, Ó.; Igland, J.; et al. Association of prenatal exposure to antiseizure medication with risk of autism and intellectual disability. *JAMA Neurol.*, **2022**, *79* (7), 772–781. https://doi.org/10.1001/jamaneurol.2022.1269.
[62] Giona, F.; Pagano, J.; Verpelli, C.; Sala, C. Another step toward understanding brain functional connectivity alterations in autism: an editorial highlight for "neurobiological substrates underlying corpus callosum hypoconnectivity and brain metabolic patterns in the valproic acid rat model of autism. *J. Neurochem.*, **2021**, *159* (1), 12–14. https://doi.org/10.1111/jnc.15452.
[63] DeCoteau, W. E.; Fox, A. E. Timing and intertemporal choice behavior in the valproic acid rat model of autism spectrum disorder. *J. Autism Dev. Disord.*, **2022**, *52* (6), 2414–2429. https://doi.org/10.1007/s10803-021-05129-y.
[64] Smolinski, N. E.; Sarayani, A.; Thai, T. N.; Jugl, S.; Ewig, C. L. Y.; Winterstein, A. G. Prenatal exposure to valproic acid across various indications for use. *JAMA Netw. Open*, **2024**, *7* (5), e2412680. https://doi.org/10.1001/jamanetworkopen.2024.12680.
[65] Liao, C.; Moyses-Oliveira, M.; De Esch, C. E. F.; Bhavsar, R.; Nuttle, X.; Li, A.; Yu, A.; Burt, N. D.; Erdin, S.; Fu, J. M.; et al. Convergent coexpression of autism-associated genes suggests some novel risk genes may not be detectable in large-scale genetic studies. *Cell Genom.*, **2023**, *3* (4), 100277. https://doi.org/10.1016/j.xgen.2023.100277.
[66] Ilieva, M.; Fex Svenningsen, Å.; Thorsen, M.; Michel, T. M. Psychiatry in a dish: stem cells and brain organoids modeling autism spectrum disorders. *Biol. Psychiatry*, **2018**, *83* (7), 558–568. https://doi.org/10.1016/j.biopsych.2017.11.011.
[67] Mehra, S.; Ul Ahsan, A.; Seth, E.; Chopra, M. Critical Evaluation of valproic acid-induced rodent models of autism: current and future perspectives. *J. Mol. Neurosci.*, **2022**, *72* (6), 1259–1273. https://doi.org/10.1007/s12031-022-02033-7.
[68] Ornoy, A.; Weinstein-Fudim, L.; Becker, M. SAMe, choline, and valproic acid as possible epigenetic drugs: their effects in pregnancy with a special emphasis on animal studies. *Pharmaceuticals*, **2022**, *15* (2), 192. https://doi.org/10.3390/ph15020192.
[69] Fard, Y. A.; Sadeghi, E. N.; Pajoohesh, Z.; Gharehdaghi, Z.; Khatibi, D. M.; Khosravifar, S.; Pishkari, Y.; Nozari, S.; Hijazi, A.; Pakmehr, S. A.; et al. Epigenetic underpinnings of the autistic mind: histone modifications and prefrontal excitation/inhibition imbalance. *Am. J. Med. Genet. B Neuropsychiatr. Genet.*, **2024**, *195* (8), e32986. https://doi.org/10.1002/ajmg.b.32986.
[70] Masini, E.; Loi, E.; Vega-Benedetti, A. F.; Carta, M.; Doneddu, G.; Fadda, R.; Zavattari, P. An overview of the main genetic, epigenetic and environmental factors involved in autism spectrum disorder focusing on synaptic activity. *Int. J. Mol. Sci.*, **2020**, *21* (21), 1–22. https://doi.org/10.3390/ijms21218290.
[71] Mabunga, D. F. N.; Gonzales, E. L. T.; Kim, J.; Kim, K. C.; Shin, C. Y. Exploring the validity of valproic acid animal model of autism. *Exp. Neurobiol.*, **2015**, *24* (4), 285–300. https://doi.org/10.5607/en.2015.24.4.285.
[72] Meng, Q.; Zhang, W.; Wang, X.; Jiao, C.; Xu, S.; Liu, C.; Tang, B.; Chen, C. Human forebrain organoids reveal connections between valproic acid exposure and autism risk. *Transl. Psychiatry*, **2022**, *12* (1), 130. https://doi.org/10.1038/s41398-022-01898-x.
[73] Chan, W. K.; Griffiths, R.; Price, D. J.; Mason, J. O. Cerebral organoids as tools to identify the developmental roots of autism. *Mol. Autism*, **2020**, *11* (1), 58. https://doi.org/10.1186/s13229-020-00360-3.
[74] Rabeling, A.; Goolam, M. Cerebral organoids as an in vitro model to study autism spectrum disorders. *Gene Ther.*, **2023**, *30*, 659–669. https://doi.org/10.1038/s41434-022-00356-z.
[75] Cheffer, A.; Flitsch, L. J.; Krutenko, T.; Röderer, P.; Sokhranyaeva, L.; Iefremova, V.; Hajo, M.; Peitz, M.; Schwarz, M. K.; Brüstle, O. Human stem cell-based models for studying autism spectrum disorder-related neuronal dysfunction. *Mol. Autism*, **2020**, *11* (1), 99. https://doi.org/10.1186/s13229-020-00383-w.
[76] Frye, R. E. Mitochondrial dysfunction in autism spectrum disorder: unique abnormalities and targeted treatments. *Semin Pediatr. Neurol.*, **2020**, *35*, 100829. https://doi.org/10.1016/j.spen.2020.100829.

[77] Usui, N.; Kobayashi, H.; Shimada, S. Neuroinflammation and oxidative stress in the pathogenesis of autism spectrum disorder. *Int. J. Mol. Sci.*, **2023**, *24* (6), 5487. https://doi.org/10.3390/ijms24065487.
[78] Kwon, H. S.; Koh, S. H. Neuroinflammation in neurodegenerative disorders: the roles of microglia and astrocytes. *Transl. Neurodegener.*, **2020**, *9* (42), 1–12. https://doi.org/10.1186/s40035-020-00221-2.
[79] Ju, S.; Xu, C.; Wang, G.; Zhang, L. VEGF-C induces alternative activation of microglia to promote recovery from traumatic brain injury. *JAD*, **2019**, *68* (4), 1687–1697. https://doi.org/10.3233/JAD-190063.
[80] Liu, X.; Lin, J.; Zhang, H.; Khan, N. U.; Zhang, J.; Tang, X.; Cao, X.; Shen, L. Oxidative stress in autism spectrum disorder — current progress of mechanisms and biomarkers. *Front. Psychiatry*, **2022**, *13*, 1–20. https://doi.org/10.3389/fpsyt.2022.813304.
[81] Bakshi, V.; Sunand, K.; Begum, N.; Kakalij, R.; Tekula, M. Neuroprotective effect of resveratrol on valproic acid induced oxidative stress autism in swiss albino mice. *Int. J. Pharm. Sci. Drug Res.*, **2018**, *10*. https://doi.org/10.25004/IJPSDR.2018.100301.
[82] Yang, J. Q.; Yang, C. H.; Yin, B. Q. Combined the GABA-A and GABA-B receptor agonists attenuates autistic behaviors in a prenatal valproic acid-induced mouse model of autism. *Behav. Brain Res.*, **2021**, *403*, 113094. https://doi.org/10.1016/j.bbr.2020.113094.
[83] Di, J.; Li, J.; O'Hara, B.; Alberts, I.; Xiong, L.; Li, J.; Li, X. The role of GABAergic neural circuits in the pathogenesis of autism spectrum disorder. *Int. J. Dev. Neurosci.*, **2020**, *80* (2), 73–85. https://doi.org/10.1002/jdn.10005.
[84] Puts, N. A. J.; Wodka, E. L.; Harris, A. D.; Crocetti, D.; Tommerdahl, M.; Mostofsky, S. H.; Edden, R. A. E. Reduced GABA and altered somatosensory function in children with autism spectrum disorder. *AUTRES*, **2017**, *10* (4), 608–619. https://doi.org/10.1002/aur.1691.
[85] Takumi, T.; Tamada, K.; Hatanaka, F.; Nakai, N.; Bolton, P. F. Behavioral neuroscience of autism. *Neurosci. Biobehav. Rev.*, **2020**, *110*, 60–76. https://doi.org/10.1016/j.neubiorev.2019.04.012.
[86] Han, V. X.; Patel, S.; Jones, H. F.; Dale, R. C. Maternal immune activation and neuroinflammation in human neurodevelopmental disorders. *Nat. Rev. Neurol.*, **2021**, *17* (9), 564–579. https://doi.org/10.1038/s41582-021-00530-8.
[87] Phiel, C. J.; Zhang, F.; Huang, E. Y.; Guenther, M. G.; Lazar, M. A.; Klein, P. S. Histone deacetylase is a direct target of valproic acid, a potent anticonvulsant, mood stabilizer, and teratogen. *J. Biol. Chem.*, **2001**, *276* (39), 36734–36741. https://doi.org/10.1074/jbc.M101287200.
[88] Kim, J.; Park, S.-H.; Sun, W. The differential developmental neurotoxicity of valproic acid on anterior and posterior neural induction of human pluripotent stem cells. *Int. J. Stem Cells*, **2024**. https://doi.org/10.15283/ijsc24066.
[89] Hayashi, Y.; Ohnuma, K.; Furue, M. K. Pluripotent stem cell heterogeneity. *Adv. Exp. Med. Biol.*, **2019**, *1123*, 71–94. https://doi.org/10.1007/978-3-030-11096-3_6.
[90] Horder, J.; Petrinovic, M. M.; Mendez, M. A.; Al, E. Glutamate and GABA in autism spectrum disorder—a translational magnetic resonance spectroscopy study in man and rodent models. *Transl. Psychiatry*, **2018**, *8* (106). https://doi.org/10.1038/s41398-018-0155-1.
[91] Wang, X.; Zhao, Z.; Guo, J.; Mei, D.; Duan, Y.; Zhang, Y.; Gou, L. GABAB1 receptor knockdown in prefrontal cortex induces behavioral aberrations associated with autism spectrum disorder in mice. *Brain Res. Bull.*, **2023**, *202*, 110755. https://doi.org/10.1016/j.brainresbull.2023.110755.
[92] Tung, E. W. Y.; Winn, L. M. Valproic acid increases formation of reactive oxygen species and induces apoptosis in post implantation embryos: a role for oxidative stress in valproic acid-induced neural tube defects. *Mol. Pharmacol.*, **2011**, *80* (6), 979–987. https://doi.org/10.1124/mol.111.072314.
[93] Ishida, K.; Tatsumi, K.; Minamigawa, Y.; Mori, K.; Matsumaru, D.; Nagase, H.; Kanda, Y.; Takuma, K.; Nakanishi, T. Neuronal differentiation reporter mice as a new methodology for detecting in vivo developmental neurotoxicity. *Biochem. Pharmacol.*, **2022**, *206*, 115332. https://doi.org/10.1016/j.bcp.2022.115332.
[94] Juliandi, B.; Tanemura, K.; Igarashi, K.; Tominaga, T.; Furukawa, Y.; Otsuka, M.; Moriyama, N.; Ikegami, D.; Abematsu, M.; Sanosaka, T.; et al. Reduced adult hippocampal neurogenesis and cognitive impairments following prenatal treatment of the antiepileptic drug valproic acid. *Stem Cell Rep.*, **2015**, *5* (6), 996–1009. https://doi.org/10.1016/j.stemcr.2015.10.012.

[95] Willinger, Y.; Friedland Cohen, D. R.; Turgeman, G. Exogenous IL-17A alleviates social behavior deficits and increases neurogenesis in a murine model of autism spectrum disorders. *Int. J. Mol. Sci.*, **2023**, *25* (1), 432. https://doi.org/10.3390/ijms25010432.
[96] Almeida, L. E. F.; Roby, C. D.; Krueger, B. K. Increased BDNF expression in fetal brain in the valproic acid model of autism. *Mol. Cell. Neurosci.*, **2014**, *59*, 57–62. https://doi.org/10.1016/j.mcn.2014.01.007.
[97] Camuso, S.; La Rosa, P.; Fiorenza, M. T.; Canterini, S. Pleiotropic Effects of BDNF on the cerebellum and hippocampus: implications for neurodevelopmental disorders. *Neurobiol. Dis.*, **2022**, *163*, 105606. https://doi.org/10.1016/j.nbd.2021.105606.
[98] Jiang, S.; Xiao, L.; Sun, Y.; He, M.; Gao, C.; Zhu, C.; Chang, H.; Ding, J.; Li, W.; Wang, Y.; et al. The GABAB receptor agonist STX209 reverses the autism-like behaviour in an animal model of autism induced by prenatal exposure to valproic acid. *Mol. Med. Rep.*, **2022**, *25* (5), 154. https://doi.org/10.3892/mmr.2022.12670.
[99] Guimarães-Souza, E. M. Joselevitch, C.; Britto, L. R. G.; Chiavegatto, S. Retinal alterations in a pre-clinical model of an autism spectrum disorder. *Mol. Autism*, **2019**, *10*, 19. https://doi.org/10.1186/s13229-019-0270-8.
[100] Qin, L.; Dai, X.; Yin, Y. Valproic acid exposure sequentially activates Wnt and mTOR pathways in rats. *Mol. Cell. Neurosci.*, **2016**, *75*, 27–35. https://doi.org/10.1016/j.mcn.2016.06.004.
[101] Go, H. S.; Kim, K. C.; Choi, C. S.; Jeon, S. J.; Kwon, K. J.; Han, S. H.; Lee, J.; Cheong, J. H.; Ryu, J. H.; Kim, C. H.; et al. Prenatal exposure to valproic acid increases the neural progenitor cell pool and induces macrocephaly in rat brain via a mechanism involving the GSK-3β/β-catenin pathway. *Neuropharmacol.*, **2012**, *63* (6), 1028–1041. https://doi.org/10.1016/j.neuropharm.2012.07.028.

APPENDICES

Table **1**. PICOS criteria for *in vitro* studies: description of Population, Intervention, Comparison, Outcome, and Study Type used in the systematic review.

PICOS Criteria	Description
Population	*In vitro* models that can include, but are not limited to neurons, microglia, astrocytes, and other cell types.
Intervention	Valproic acid and/or sodium valproate.
Comparison	Control groups.
Outcome	Increase inflammation, cell death, mitochondrial dysfunction, oxidative stress, or any other feature that would be seen in a in vivo model of autism.
Studies type	Only in vitro studies (original research).

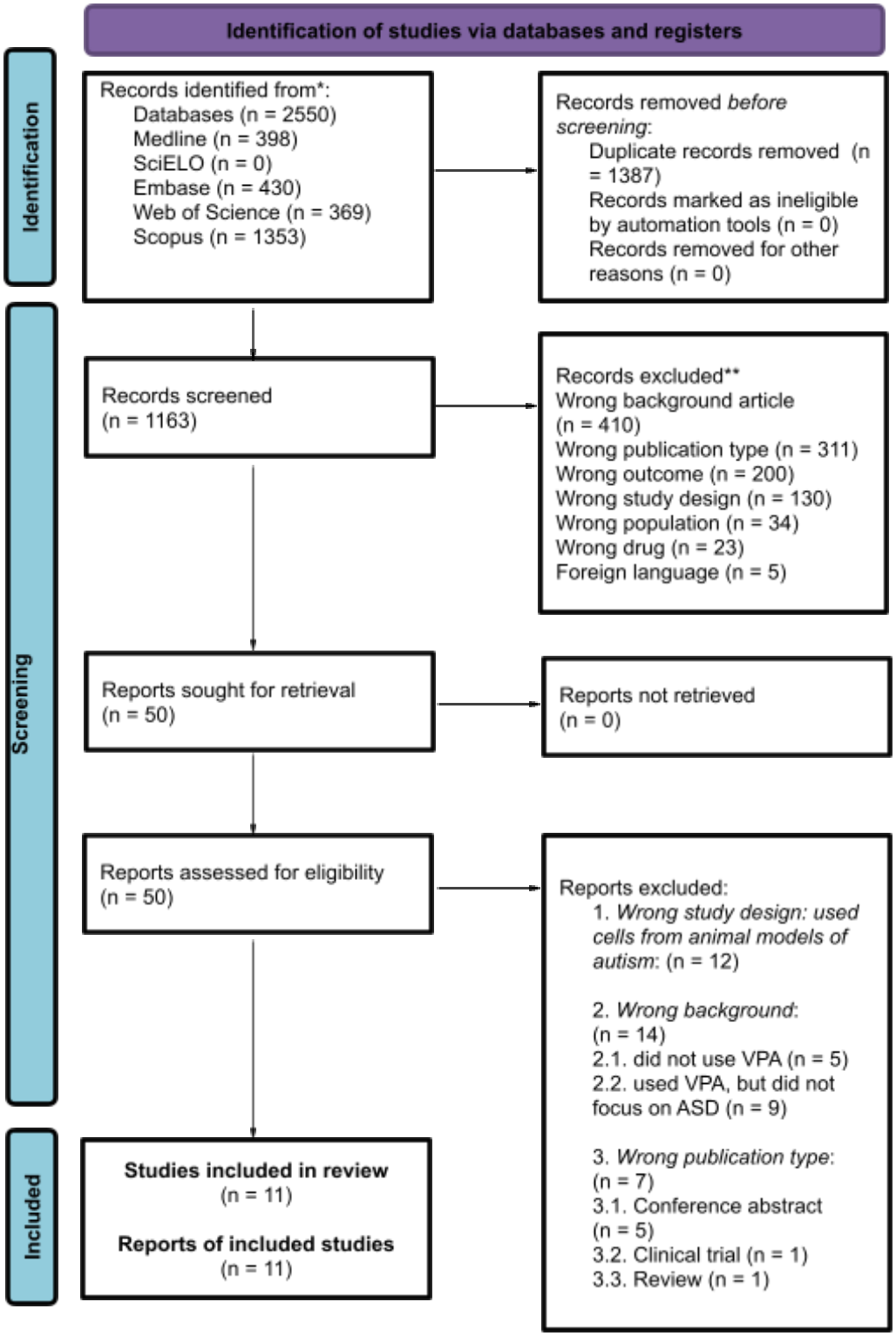
Table **2**. Summary of key data from the selected studies.

Cell category	Author(s) / Year	Cell type and source	Treatment Period	VPA dose(s)	Key findings
Human neural cell lines	Kaushik et al., 2016	Human SK-N-SH cell line	48h	4.9 mg/L	- VPA altered gene sets related to axonogenesis, regulation of neurogenesis and synapse. - VPA upregulated 8 gene sets related to neurotransmitter binding and synapse. - VPA downregulated 5 gene sets related to axon and neuron projection growth.
	Peltier et al., 2024	Human HMC3 (glia) and SH-SY5Y (neuron) cell lines	24h and 48h	250, 1000, and 5000 μM	- 24h VPA incubation in neurons increases mRNA expression of cholesterol transporters ABCA1 and ABCG1 and enhances cholesterol efflux. - 48h VPA incubation in neurons reduces ABCA1 and ABCG1 protein levels. - 24h and 48h VPA incubations in glial cells also altered mRNA and protein levels of cholesterol transporters, but did not affect cholesterol efflux. - VPA disrupts cholesterol homeostasis in neurons.
	Zhang et al., 2023	Human SH-SY5Y cell line	24h	2, 4, 8, 10, and 12 mM	- VPA increased ROS generation and reduced cell viability. - VPA induces autophagic dysregulation, by decreasing LC3B and Beclin-1, increasing p62 levels and activating the Notch-1/Hes-1 signaling pathway.
	Chanda et al., 2019	Human reprogrammed cells from H1 and H9 embryonic stem cell lines	72h	0.1 mM and 1 mM	- 0.1 mM VPA for 72h on day 1 neurons reduces neurite length and branching, and 1 mM VPA inhibits neurite outgrowth in these cells. - 1 mM VPA for 72h on day 21 neurons causes less severe dendritic arborization defects. - VPA downregulates MARCKSL1 mRNA and protein levels in day 1 and day 21 neurons. - VPA-mediated inhibition of neuritogenesis could be caused by the inhibition of HDAC, the downregulation of MARCKSL1 and inhibition of GSK-3.
Primary adult neurons	Kumamaru et al., 2014	Rat cortical neurons (postnatal day 1)	6h, 12h, 24h, 48h, 3 days	0.3, 1.0, and 5.0 mM	- VPA exposure during early development (before DIV7) reduces VGAT expression. - VPA exposure decreases the number of VGAT-positive puncta and the axonal length in growing neurons at DIV4. - VPA's effect on VGAT is likely due to its HDAC inhibitor activity. - A minimum 12h exposure to 1 mM VPA is required to downregulate VGAT expression.
	Takeda et al., 2021	Mouse astrocytes and cortical neurons (newborn mice)	6 days + 14 days co-culture	0.3, 1.0, and 3 mM	- VPA-exposed astrocytes reduce the number of VGAT-positive presynaptic puncta, decreasing GABAergic synapses and the frequency of mIPSCs in neurons. - VPA-exposed astrocytes downregulate the expression of PTPRD mRNA in neurons. - VPA-exposed astrocytes did not affect neuronal morphology.

Primary embryonic neurons	Ko et al., 2018	Rat cortical neural progenitor cells (embryonic day 14) and cortical neurons (embryonic day 18)	3h and 6h	0.2 and 0.5 mM	- In NPCs, 0.2 mM and 0.5 mM VPA for 3h and 6h elevates BDNF mRNA and tPA mRNA and protein, suggesting that processing of pro-BDNF into its mature form is enhanced. - In NPCs, 0.5 mM VPA for 6h reduced MeCP2 protein in the nuclei and elevated BDNF protein levels. - In NPCs, VPA promotes the processing and maturation of BDNF. - Cortical neurons didn't display any changes in the doses investigated.
	Qi et al., 2022	Rat cortical neurons (embryonic days 12.5–14.5)	24h, 48h, and 72h	1, 50, 100, 500, 1000, and 10.000 μmol/L	- 50, 100, 500, and 1000 μmol/L VPA increased cell viability after 48h and 72h. - 10,000 μmol/L reduced viability from 24h forward. - 100 μmol/L VPA for 48h increased cell proliferation, the number and the average diameter of neurospheres, and TGFβ1 mRNA expression. - VPA might enhance neurosphere formation and NSC proliferation by activating TGFβ1.
	Zhang et al., 2015	Rat cortical neurons (embryonic day 18)	24h	1, 5, and 10 mM	- VPA exposure increased β-catenin protein levels and decreased GSK-3β protein levels. - VPA exposure increased oxidative stress markers ROS and 4-HNE levels.
	Nissen et al., 2016	Mouse cortical neurons (embryonic day 18)	15 min	0.03 mM to 65.61 mM	- Low VPA doses (0.27 mM – 0.81 mM) slightly increased intracellular ATP levels. - Low VPA doses (0.09 mM and 0.27 mM) increased the AP frequencies. - Intracellular ATP levels IC50 value was estimated to be between 16 mM and 28 mM VPA, and AP frequencies IC50 value estimated between 0.3 mM and 2.0 mM VPA.
	Al-Rubai et al., 2017	Human neural stem cells from aborted fetuses (< 12 weeks' gestation)	6 days	250, 500, 750, 1000, 1500, 2000, and 2500 μM	- SV therapeutic doses (500-750 μM) reduced neurospheres' size after 24h. - SV in higher concentrations reduced cell viability and total cellular protein. - 1500 μM and 2000 μM SV decreased GFAP levels (astrocyte marker), but had no effect on tubulin III (neuronal marker). - 1000 μM, 1500 μM, and 2000 μM SV decreased neurosphere's migration distance. - 2000 μM SV reduced neuronal process length.

Abbreviations: VPA, valproic acid; mRNA, messenger RNA; ROS, reactive oxygen species; HDAC, histone deacetylase; GSK-3, glycogen synthase kinase 3; DIV, days *in vitro*; VGAT, vesicular GABA transporter; mIPSCs, miniature inhibitory postsynaptic currents; PTPRD, protein tyrosine phosphatase receptor type D; NPCs, neural progenitor cells; BDNF, brain-derived neurotrophic factor; tPA, tissue-type plasminogen activator; TGFβ1, transforming growth factor beta 1; NSCs, neural stem cells; 4-HNE, 4-hydroxynonenal; min, minutes; AP, action potentials; IC50, half-maximal inhibitory concentration; SV, sodium valproate; GFAP, glial fibrillary acidic protein.

Fig. (1). Flow Chart of studies included in this review.



Graphical abstract legend:

Graphical Abstract: The effects of *in vitro* valproic acid (VPA) exposure on neuronal cells. Key findings highlight dose- and time-dependent alterations in neuronal function, with undifferentiated cells being particularly

vulnerable. VPA exposure leads to changes in gene expression, cholesterol dysregulation, oxidative stress, impaired autophagy, and structural disruptions, all of which impact neuronal viability and function. These findings support the use of VPA as an *in vitro* model to investigate ASD-related cellular mechanisms, providing insights into neurodevelopmental alterations associated with the disorder.

This figure was created with the assistance of BioRender and Canva.