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A Systematic Review of the Effects of Perinatal Alcohol Exposure and Perinatal Marijuana Exposure on Adult Neurogenesis in the Dentate Gyrus

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Abstract

Background: Marijuana and alcohol are both substances that, when used during pregnancy, may have profound effects on the developing fetus. There is evidence to suggest that both drugs have the capacity to affect working memory, one function of the hippocampal formation; however, there is a paucity of data on how perinatal exposure to alcohol or cannabis impacts the process of adult neurogenesis.

Methods: This systematic review examines immunohistochemical data from adult rat and mouse models that assess perinatal alcohol or perinatal marijuana exposure. A comprehensive list of search terms was designed and used to search 3 separate databases. All results were imported to Mendeley and screened by 2 authors. Consensus was reached on a set of final papers that met the inclusion criteria, and their results were summarized.

Results: Twelve papers were identified as relevant, 10 of which pertained to the effects of perinatal alcohol on the adult hippocampus, and 2 pertained to the effects of perinatal marijuana on the adult hippocampus. Cellular proliferation in the dentate gyrus was not affected in adult rats and mice exposed to alcohol perinatally. In general, perinatal alcohol exposure did not have a significant and reliable effect on the maturation and survival of adult born granule neurons in the dentate gyrus. In contrast, interneuron numbers appear to be reduced in the dentate gyrus of adult rats and mice exposed perinatally to alcohol. Perinatal marijuana exposure was also found to reduce inhibitory interneuron numbers in the dentate gyrus.

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SUPPORTING INFORMATION

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Conclusions: Perinatal alcohol exposure and perinatal marijuana exposure both act on inhibitory interneurons in the hippocampal formation of adult rats. These findings suggest simultaneous perinatal alcohol and marijuana exposure (SAM) may have a dramatic impact on inhibitory processes in the dentate gyrus.

Keywords

Hippocampus; Dentate Gyrus; Neurogenesis; Alcohol; Marijuana; FASD

With the relaxation of cannabis restrictions across North America, a growing proportion of young adults (19 to 30 years of age) are reporting the simultaneous use of alcohol and marijuana (SAM), and indications are that this trend will continue to rise (Terry-McElrath and Patrick, 2018). This age demographic also coincides with the peak fertility period for males and females (Dunson, Colombo and Baird, 2002), and SAM significantly increases the risk of unplanned pregnancies (Finer and Zolna, 2014). Moreover, the use of illicit drugs in this age group is more common, with cannabis being the most commonly used drug by pregnant women (Chasnoff, Landress and Barrett, 1990). Approximately half of all marijuana users also report alcohol use (Goldschmidt et al., 2004; Jackson, Sher and Schulenberg, 2008; Subbaraman and Kerr, 2015), with recent statistics indicating that over 30% of pregnant females regularly consume alcohol and marijuana (Goldschmidt et al., 2004; Government of Canada, 2017). Although the prevalence rates for SAM are likely to rise (Jackson, Sher and Schulenberg, 2008), the effects of combined perinatal ethanol (EtOH) exposure and THC (Δ9-tetrahydrocannabinol) exposure on the developing brain are not well understood.

Previous studies have shown that working memory is impaired by perinatal alcohol exposure (Goodfellow and Lindquist, 2014; Livy et al., 2003) and similar deficits have been observed in adults exposed to marijuana (Kafaee Razavi et al., 2010; Wright et al., 2017). EtOH is a teratogen and so alcohol consumption during pregnancy can disrupt development leading to facial dysmorphology, pre- and postnatal growth deficiencies, and central nervous system (CNS) dysfunction (May et al., 2013; Riley et al., 2011). Heavy drinking in the second trimester, particularly the tenth to twentieth weeks of human pregnancy, when the brain is growing dramatically, is associated with an increase in the severity of many clinical features (Brocardo et al., 2017; Renwick and Asker, 1983). In rodent models, a portion of the developmental stages that are congruous with the human third trimester occurs up to postnatal day 9 (Livy et al., 2003). For the purpose of this review, postnatal day 10 and older will be considered to be postnatal while rodents at postnatal day 9 and younger will be taken as perinatal. Perinatal alcohol literature contains experiments using multiple exposure paradigms. E1 to E20 and P4–9 are both common models (Kleiber et al., 2013; Livy et al., 2003). A benefit of the E1 to E20 model is that alcohol can be integrated into the mother's diet without the need for gavage or injection. P4–9 exposure isolated effects to the brain growth spurt and with recent advancements pups can be exposed using vaporized EtOH, which can also decrease the stress related to injection or gavage. For our review, we have chosen to discuss pre-and postnatal exposure paradigms that range from the first gestational day to the ninth postnatal day in rodent models, as these dates coincide with the first to third trimester equivalent in humans (Maier et al., 1999).

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It has long been known that the hippocampus is a brain area that is particularly sensitive to the effects of perinatal alcohol exposure (PAE). PAE induces significant cell loss in the hippocampus (Ikonomidou et al., 2000; Redila et al., 2006; Hamilton et al., 2011), and even brief periods of binge exposure can produce significant changes in hippocampal structure and function (Bonthius and West, 1990; Guerri et al., 2009; Patten, Fontaine and Christie, 2014). Both $GABA_A$ and NMDA receptors have been implicated in the mechanism of alcohol-related neurodegeneration; $GABA_A$ receptors have been shown to become hyperexcitable while NMDARs are blocked (Olney et al., 2002). Since GABA signaling is thought to be integral to spatial and temporal integration of new neurons, it is logical that aberrations of this system lead to severe developmental consequences (Akerman and Cline, 2007). Long-term potentiation deficits have also been reported, and the histamine H_3 receptor has been implicated (Varaschin et al., 2018).

Marijuana is one of the most commonly used recreational drugs during pregnancy, yet little is known about how it effects the development of the brain (Vargish et al., 2017). THC is the major psychoactive ingredient in marijuana and is known to readily cross the placental barrier impacting fetal development (Grotenhermen, 2003). Evidence is emerging that perinatal THC, like perinatal alcohol, can impair cognitive functioning of offspring—possibly throughout the lifespan (Huizink and Mulder, 2006). Cannabinoid receptors and their endogenous ligands have been detected at the earliest stages of embryonic development; this indicates that maternal marijuana use can impact the developing brain (Fernández-Ruiz et al., 2000; Harkany et al., 2007). The 2 primary cannabinoid receptors, known as CB_1 and CB_2, can both act to reduce adenylyl cyclase activity in cells (Galiègue et al., 1995). CB_2 receptors are expressed sparsely in microglia, macrophages, and some neurons in the central nervous system, but are more ubiquitous in the peripheral nervous system (Roche and Finn, 2010). There is evidence that alcohol acts to reduce endogenous cannabinoid levels through a CB_2 receptor–mediated pathway and that this mechanism is important in alcohol use disorders (Basavarajappa et al., 2019; Martín-Sánchez et al., 2019). CB_1 receptors are expressed in both inhibitory and excitatory neurons, at perinatal timepoints in the rodent cortex, basal forebrain, and telencephalon (Scheyer et al., 2019). Due to the fact that CB_1 receptors are expressed perinatally and in the hippocampus (part of the telencephalon), it is likely that CB_1 receptors will be the major players when it comes to developmental THC exposure (Berrendero et al., 1999). CB_1 receptors can impact interneuron development, neuronal proliferation, migration, morphogenesis, synaptogenesis, and the balance of excitation and inhibition in the hippocampus (Berghuis et al., 2005, 2007; Mulder et al., 2008). A recent paper found that parental THC exposure can cause altered hippocampal oscillations, brain hyperexcitability, and spatial memory impairment (de Salas-Quiroga et al., 2020). In this review, we will systematically explore what is known of the effects of perinatal alcohol and marijuana exposure in the dentate gyrus. The entire hippocampus was included in the search parameters; however, the papers returned mainly concerned the dentate gyrus. The dentate gyrus is a good target of this research as it is 1 of 2 sites in the rodent brain that has adult neurogenesis (Praag et al., 2002). Adult neurogenesis in the dentate gyrus is thought to be a mechanism responsible for spatial memory (Clelland et al., 2009). It is worth noting that, while wellestablished in rodents, the existence of adult neurogenesis is still debated in humans due to the type and parameters of assays available

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for use in humans (Snyder, 2019). Spatial memory is affected by both THC and alcohol consumption in humans and so its corresponding brain structure a logical place to assess deficits caused by these drugs (Green et al., 2009; Mouro et al., 2019).

It is important to consider the actions of both substances alone, as well as in combination, as some work has suggested that the detrimental effects of perinatal alcohol and perinatal marijuana may be synergistic (Boa-Amponsem et al., 2019; Breit, Zamudio and Thomas, 2019; Janisse et al., 2014). Our initial systematic search to investigate documented changes in adult neurogenesis following SAM exposure returned a single result in the hippocampal formation, indicating there is a paucity of data for understanding the cellular consequences of perinatal SAM exposure. This singular paper found that perinatal cannabinoid exposure causes birth defects similar to perinatal alcohol exposure and implicated CB1–Hedgehog interactions as the cause (Fish et al., 2019). While the Fish paper is worth mentioning, it did not satisfy all of the inclusion/exclusion criteria in this review and will not be part of the final result tables. This paper also succinctly discusses the differences between THC and CBD, the 2 main cannabinoids present in marijuana, versus synthetic cannabinoids, which can be hundreds of times more potent and much longer lasting than THC and CBD (Fish et al., 2019). And while it is also worth mentioning that there are many other cannabinoids and terpenes in cannabis, this review will focus on THC and synthetic cannabinoids that bind with the CB_1 receptor (Berrendero et al., 1999).

This review will compare cellular data in adult offspring of rats or mice perinatally exposed to alcohol or marijuana. Our goal is to identify how perinatal SAM exposure impacts the structure and function of the adult hippocampus in hopes of directing future research.

MATERIALS AND METHODS

This review was carried out using the stylistic criteria for Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA), with minor amendments to the traditional process (e.g., exclusion of bias scoring) due to the nature of cellular studies (Moher et al., 2009). The literature on adult neurogenesis changes in the hippocampal formation after perinatal marijuana exposure is limited; for this reason, marijuana papers selected were compared to selected perinatal alcohol exposure instead of being separately analyzed. This method was used to provide an objective starting point for SAM research aimed at targeting the cellular basis for any developmental changes. In particular, this study was designed to target all papers that investigate adult neurogenic changes in the hippocampal formation under perinatal alcohol conditions or perinatal cannabis conditions. PRISMA search terms were designed to broadly include any cellular study in any age of exposed offspring, and then, papers were selected based on the inclusion and exclusion criteria (S1). Five blocks of search terms were used. Each block included similar terms that contained “OR” as an operator. Between search blocks an “AND” operator was used. The blocks of search terms used here required papers to (i) include perinatal drug exposure, (ii) mention the hippocampal formation, (iii) provide immunohistochemical and other cellular results, (iv) specify alcohol use, and (v) specify marijuana use (see Table S1). Three databases were selected based on their ability to return cellular-level research. The first and second authors individually performed 2 searches, one for perinatal alcohol (search blocks 1,

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2, 3, 4) and the other for perinatal marijuana (search blocks 1, 2, 3, 5) using the same terms, then exported all the citations to Mendeley. Duplicates were removed, and all papers were screened using the inclusion/exclusion criteria (S2). Papers selected in the screening process were read in full and assessed for eligibility as defined by the search criteria, and final papers selected were compared between authors. Any discrepancies in paper selection were resolved by discussion. Although outside the scope of this review, the main methods and findings of short-listed papers have been included as a supplementary table (Table S2). The final article numbers for each step of this review are included in Fig. 1. The search period included was January 1, 2000–March 13, 2020.

RESULTS

A total of 12 studies were identified using a predefined criteria (S1), with 10 studies focused on perinatal alcohol exposure and 2 on perinatal marijuana exposure. Eleven of the 12 studies identified included an evaluation of the dentate gyrus subfield of the hippocampus. Datasets for papers that did not satisfy the inclusion and exclusion criteria are not reported in this table.

Perinatal Alcohol Exposure

Effects on Cellular Proliferation.—The protein Ki-67, an endogenous marker for cell proliferation in the brain, can be used in conjunction with the administration of BrdU, and exogenous marker that is taken up by the DNA of dividing cells during mitosis, to quantify cell proliferation in the brain (Cameron and Mckay, 2001; Christie and Cameron, 2006). As is depicted in Fig. 2, there were 11 experiments across 10 papers that used Ki-67 or BrdU alone, or in combination, to label proliferating cells in the adult dentate gyrus (Table 1). Seven experiments used the intrinsic marker for cell proliferation, Ki-67, to study how perinatal EtOH exposure affected cell proliferation in the adult hippocampus, but none of the studies showed any change in the number of Ki-67-immunopositive cells. Similarly, the 4 studies that used bromodeoxyuridine (BrdU; 50–200 mg/kg), a thymidine analogue that is injected and incorporated into the DNA of actively dividing cells, failed to document any changes following perinatal EtOH exposure. Thus, whether endogenous or exogenous markers for cell proliferation were quantified, the results are in agreement that BrdU does not induce significant changes in this process in young adult animals.

Cell Maturation.—Doublecortin (DCX) is a microtubule-associated protein expressed by neuronal precursor cells and immature neurons. Thus, DCX-positive cells represent a set cells across a broad developmental spectrum, ranging from immature neural progenitor (INP) cells (also known as type 2B cells) to immature granule neurons (IGN) (Kronenberg et al., 2003). Four papers assessed DCX immunoreactivity in the dentate gyrus (Table 2) following perinatal EtOH exposure (Elibol-Can et al., 2014; Gil-Mohapel et al., 2011, 2014; Olateju et al., 2018). Two papers showed no change in DCX immunoreactivity (Elibol-Can et al., 2014; Gil-Mohapel et al., 2014), one showed a significant decrease in DCX-positive cells that was restricted to females (Gil-Mohapel et al., 2011), and one paper found a decrease in DCX cells in both males and females (Olateju et al., 2018) (see Fig. 2). To better elucidate changes in cellular maturation following perinatal EtOH exposure, we also

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examined papers that examined the basic helix loop helix transcription factor NeuroD (neurogenic differentiation factor 2). NeuroD is a marker expressed continuously by type 2b immature granule neurons once they begin to mature. We found that only one paper used NeuroD as a marker of maturation, and this work found that there was a decrease in the number of NeuroD-positive cells in both male and female rats perinatally exposed to alcohol (Hamilton et al., 2011). This work provides some convergent evidence to support the conclusion that perinatal EtOH exposure does negatively impact neuronal maturation.

Cell Survival.—BrdU can be used to examine cell survival if it is injected 3 to 6 weeks prior to tissue being collected for histology (van Praag et al., 1999). This allows sufficient time for new cells to develop and become functional (Praag et al., 2002). BrdU is only available to be incorporated into dividing cells within 2 to 3 hours of being injected (Cameron and Mckay, 2001), so it does not stain cells that are born after this timepoint, allowing researchers to compare the number of cells stained initially (in the immediate perfusion group) to the number of cells present after a given amount of time (a second experimental group). There were 7 papers where BrdU assays were conducted on brain samples collected to study cell survival (see Table 3). In one study, where rats were injected with BrdU (200 mg/kg) at postnatal day 80 (P80) and their brains were collected at P115, a decrease was found in the number of BrdU-positive cells in animals exposed to alcohol perinatally (Hamilton et al., 2011). In a second paper, 2 BrdU experiments were reported. In this work, BrdU (50 mg/kg) was injected every second day from P30 to P50, and then, brains are collected at either P50 or P80 (Klintsova et al., 2007). The number of BrdU-positive cells was found to be equivalent in animals assessed at P50, but a decrease in numbers was observed at P80. One study was performed where BrdU was injected at P60 and brains collected at P90, and no change in BrdU immunoreactivity was found (Gil-Mohapel et al., 2011). Two studies injected BrdU between P60 and P65 and analyzed the brains between P81 and P86 (3 weeks later) and found no change in the number of BrdU-labeled cells relative to controls (Sliwowska et al., 2010; Uban et al., 2010). One study utilized double labeling to assess the survival of new glial cells (GFAP/BrdU) as well as the survival of new granule neurons (NeuN/BrdU) and found no change in the proportion of each, relative to control, in either condition (Uban et al., 2010). Thus, the majority of studies indicate that perinatal alcohol exposure does not have a significant impact on cell survival.

Changes in Inhibitory Neuron Numbers——EtOH is known to directly impact inhibitory cells in the brain; however, only a few studies have examined the impact of perinatal EtOH exposure on these cells in the dentate gyrus (see Table 4). In one study, a transgenic mouse model (Venus-VGAT) was used that allowed them to directly visualize inhibitory (GABAergic) interneurons (Bird et al., 2018). This paper found a decrease in the number of interneurons in the granule cell layer (GCL) of the dentate gyrus. The other paper took a more traditional histological approach and labeled cells with NeuN, a mature neuron marker, a marker for the excitatory neurotransmitter glutamate, or with a GAD67, a marker of inhibitory interneurons. This study only assessed males, but in these they found an increase in neurons double labeled with NeuN and glutamate, and an decrease in cells double labeled with NeuN and GAD67 (Lu et al., 2018). Thus, both studies assessing

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inhibition found perinatal alcohol exposure to result in decreased numbers of interneurons in the granule cells layers.

Perinatal Marijuana

Two studies assessing the effects of perinatal marijuana exposure in adult rats and mice were found. One perinatal marijuana study assayed 2 types of interneuron counts in transgenic mouse lines. They found that CCK-positive caudal ganglionic eminence–derived interneurons decreased in adult mice perinatally exposed to THC but Medial Ganglionic Eminence Derived Interneurons showed no change (Vargish et al., 2017). The other perinatal marijuana study assayed CB1 receptor levels and found an increase in the CA1 area of the Hippocampus (Tortoriello et al., 2014).

DISCUSSION

Perinatal Alcohol in the Dentate Gyrus

This review found that in adult rats and mice perinatally exposed to alcohol, most components of adult neurogenesis do not appear to be significantly affected, but that there is evidence for changes in interneurons in the hippocampus (Table 5). The intention of this review was to investigate changes in the hippocampus caused by perinatal alcohol and marijuana exposure; however, a paucity of papers devoted to this topic required a focus on the review of perinatal alcohol exposure effects alone, although 2 papers on perinatal marijuana exposure did meet our criteria. To date, only one paper has been published that assess the interaction of perinatal administration of these substances at the cellular level, but no papers have assessed this in the developing hippocampus (Fish et al., 2019). As this area of research is in its infancy, this review hopes to shed light on possible directions for future SAM and perinatal cannabis research, based upon likely points of interaction.

DCX is first expressed in type 2b immature neural progenitor cells but is produced continuously until the cell is an immature granule neuron (Kempermann et al., 2004). BrdU and Ki-67 are both markers of proliferation, and the results of one are often used to validate the other (Kee et al., 2002). To this end, it can be seen that in rats and mice perinatally exposed to alcohol, there is no large long-lasting effect in the numbers of actively dividing cells in the dentate gyrus. NeuroD is also a marker of maturation, and in the prior study that utilized it as a marker, a decrease was found.

BrdU is injected before the animal is euthanized. Cells that incorporate BrdU are actively dividing at the time of injection (Kee et al., 2002). Therefore, when collecting tissue at advanced timepoints, BrdU can assay temporally discrete populations of cells undergoing DNA synthesis (Kee et al., 2002). The studies that used BrdU to assess proliferation (injection immediately before euthanasia) found no change in proliferation; however, half of the studies which followed an adult population of cells over a month-long window found a decreased number of BrdU-stained cells. This indicates that the population of cells dividing at the time of BrdU injection is not surviving in the same proportions of survival rates observed in control animals.

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In perinatal alcohol exposure, research suggests that most damage happens due to cell loss early in development, and although some recovery occurs in terms of medically observable phenotype, this is caused by a slow recovery of the affected cell population throughout the individual's life (Bonthius and West, 1991). A second mechanism proposed suggests that cell death is caused by a loss of inhibitory interneurons and subsequent excitotoxicity or aberrant dendritic pruning (Khaspekov et al., 2005). Two papers were found on this subject: one showed a statistically significant change in the balance of inhibitory and excitatory neurons in the dentate gyrus, and the other suggested that the one specific type of interneuron is decreasing (Lu et al., 2018; Vargish et al., 2017).

Perinatal Marijuana in the Dentate Gyrus

Two papers that assessed the adult effects of perinatal marijuana exposure were identified. One found a decrease in cholecystokinin (CCK)-positive interneurons that arise from the caudal ganglionic eminence, and no change in interneurons arising from the medial ganglionic eminence in the dentate gyrus of mice (Vargish et al., 2017). This suggests that moving forward, more research is required to understand the effects on interneuron subtypes and their implications in disease. The second paper discussed the cannabinoid receptor type 1 (Tortoriello et al., 2014). This receptor is expressed in inhibitory neurons, so an increase in CB1 expression could indicate either that the number of interneurons is increasing, or that CB1 is being upregulated (Han et al., 2012). A new paper found a significant decrease in CB1R expression in males but not females, which is opposite to the previous finding of an increase in CB1-positive boutons (de Salas-Quiroga et al., 2020; Tortoriello et al., 2014). De Salas-Quiroga *et al.* also found that there was a marked decrease in the number of CCK-positive interneurons in the CA1 region of the hippocampus, which agrees with the paper published by Vargish *et al.*; however, the effect was only significant in males (de Salas-Quiroga et al., 2020; Vargish et al., 2017).

The data found in this review suggest that the balance between inhibition and excitation may be where the largest effect will be seen in emerging SAM models. It is tempting to hypothesize that the actions of simultaneous perinatal alcohol and marijuana exposure will be synergistic because it appears these substances target 2 different sites. Specifically, alcohol appears to primarily target postsynaptic GABA receptors, whereas cannabinoids seem to target presynaptic CB1 receptors (Kawamura et al., 2006; Sheng and Kim, 2011). Studies done on these receptors in the absence of SAM conditions also support this finding (Chevaleyre and Castillo, 2004; Huang, Lo and Hsu, 2001; Losonczy, Biro and Nusser, 2004; Selvam, Yeh and Levine, 2019).

CONCLUSIONS

This systematic literature review, conducted using PRISMA-style search criteria, suggests that an interaction of alcohol and marijuana in a SAM model of exposure could influence inhibitory interneurons of the dentate gyrus. This study found that in adult rats and mice perinatally exposed to alcohol, within the dentate gyrus, proliferation is not affected but migration, maturation, survival, and interneurons are all affected. The papers pertaining to marijuana exposure suggested differences in interneurons, and thus, interneurons are the

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likely point of convergence of these 2 drugs. Specifically, CB_1 receptors are expressed largely in the second trimester in the hippocampus, are presynaptic, and lead to decreased GABA release. GABA is integral to spatiotemporal integration of developing neurons. Perinatal THC exposure and perinatal alcohol exposure overlap in their ability to affect maturation and integration of pyramidal neurons in the dentate gyrus. Therefore, future studies may show that circuit integration and cell survival in pyramidal neurons in the dentate gyrus of SAM exposed animals. As research begins to acknowledge the patents exposed to both alcohol and marijuana perinatally, an understanding of the underlying mechanism will allow clinicians to better diagnose, and hopefully treat, this understudied population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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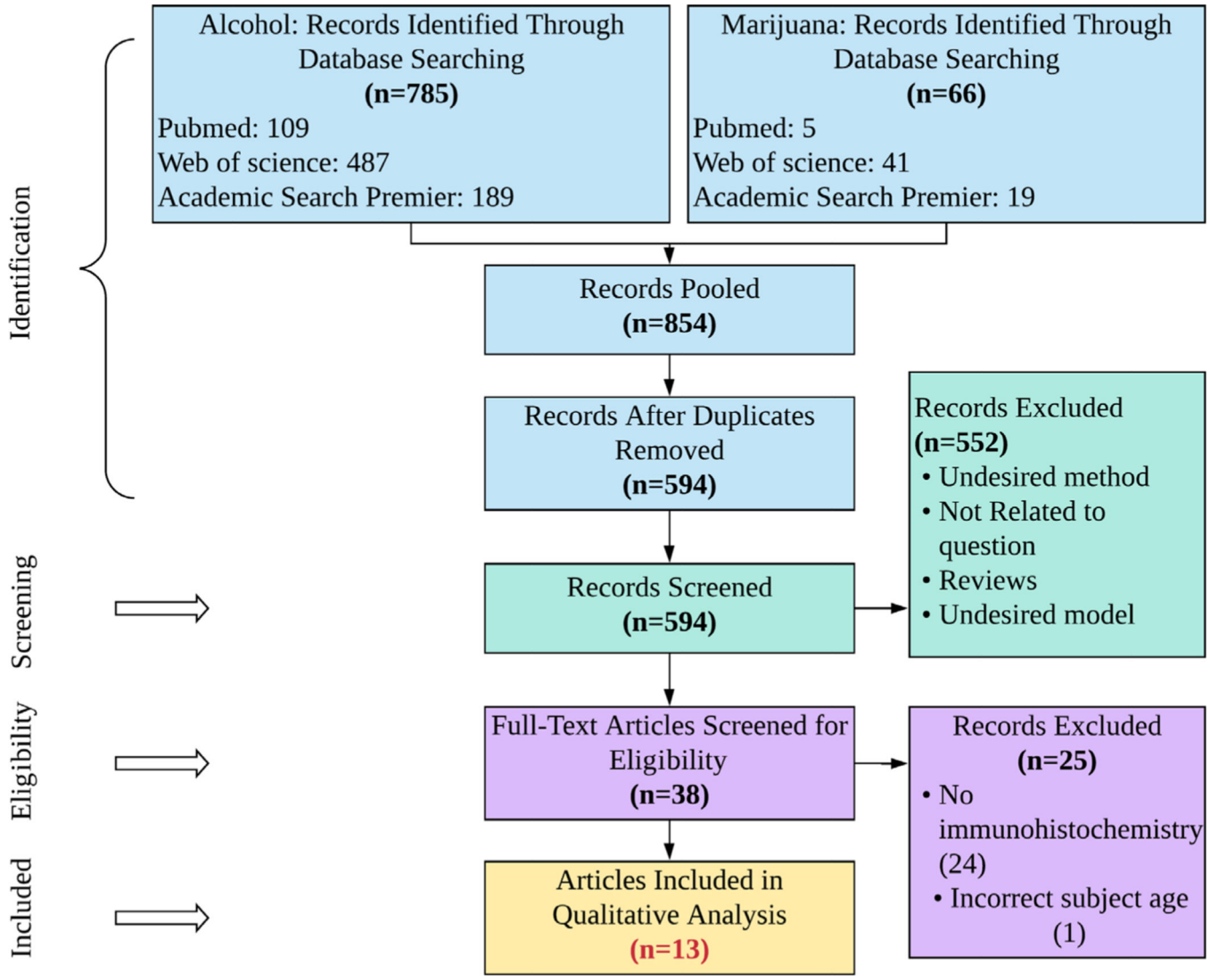

Fig. 1.

PRISMA flowchart showing the databases used, papers found per database, and number of papers excluded at each review stage.

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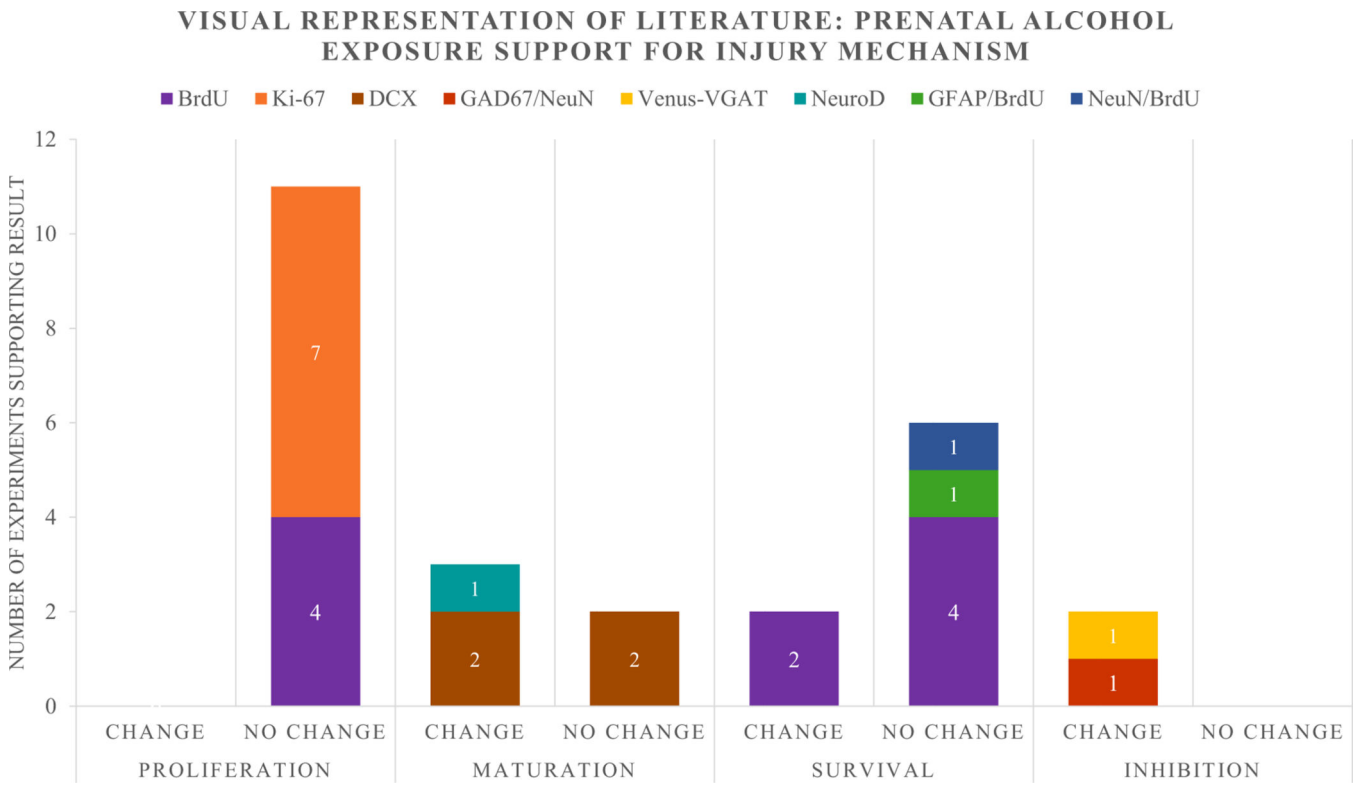

Fig. 2.

Visual representation of the number of experiments in the above studies that showed an increase and decrease, or showed no change in proliferation, maturation, survival, and inhibition studies.

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Table 1.

Summary of Perinatal Alcohol Exposure Experiments Concerning Proliferation. In the Exposure Age and Test Age Column, “P” Indicates Postnatal Day and “E” Indicates Embryonic Day

Paper	Exposure Age	Model	Sex	Test Age	Marker/ Stain Used	Main Finding
Gil-Mohapel and colleagues (2011)	E1-P9	Rat	M	P78–82	BrdU	No Change
					Ki-67	No Change
Gil-Mohapel and colleagues (2014)	E1–21	Rat	MF	P386	Ki-67	No Change
Hamilton and colleagues (2011)	E1-P10	Rat	MF	P60	BrdU 2 hours	No Change
					Ki-67	No Change
				P90	Ki-67	No Change
Klintsova et al. (2007)	P4–9	Rat	M	P50	BrdU	No Change
					Ki-67	No Change
Olateju et al. (2018)	E7-E17	Mouse	MF	P56	Ki-67	No Change
Sliwowska et al. (2010)	E1–21	Rat	M	P60–65	BrdU	No Change
Uban et al. (2010)	E1–21	Rat	F	P60–65	BrdU	No Change

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Table 2.

Summary of Perinatal Alcohol Exposure Experiments Concerning Maturation. In the Exposure age and Test Age Column, “P” Indicates Postnatal Day and “E” Indicates Embryonic Day

Paper	Exposure Age	Model	Sex	Test Age	Marker/ Stain Used	Main Finding
Elibol-Can et al. (2014)	E7-E20	Rat	M	P60	DCX	No Change
Gil-Mohapel et al. (2011)	E1-P9	Rat	M	P78-82	DCX	No Change
				P115	DCX	No Change
Gil-Mohapel et al. (2014)	E1-21	Rat	MF	P386	DCX	Decrease in F
Hamilton et al. (2011)	P4-P9	Rat	MF	P60	NeuroD	Decrease
				P90	NeuroD	Decrease
Olateju et al. (2018)	E7-E17	Mouse	MF	P56	DCX	Decrease

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Table 3.

Summary of Perinatal Alcohol Exposure Experiments Concerning Survival. In the Exposure Age and Test Age Column, “P” Indicates Postnatal Day and “E” Indicates Embryonic Day; in the Marker/Stain Used Column, “P” Indicates the Postnatal Day When Subjects Were Injected With BrdU

Paper	Exposure Age	Model	Sex	Test Age	What was Investigated?	Marker/ Stain Used	Main Finding
Gil-Mohapel and colleagues (2011)	E1-P9	Rat	MF	P90	Dividing Cells	BrdU, P60	No Change
Hamilton and colleagues (2011)	P4-9	Rat	MF	P115	Dividing Cells	BrdU P80	Decrease
Klintsova and colleagues (2007)	P4-9	Rat	M	P80	Dividing Cells	BrdU P50	Decrease
Sliwowska and colleagues (2010)	E1-21	Rat	M	P81-86		BrdU P60-65	No Change
Uban and colleagues (2010)	E1-21	Rat	F	P81-86	Dividing Cells	BrdU P60-65	No Change
					0- to 3-week-old Glia	GFAP/BrdU P60-65	No Change
					0- to 3-week-old Mature Granule Neurons	NeuN/BrdU P60-65	No Change

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Table 4.

Summary of Perinatal Alcohol Exposure Experiments Concerning Interneurons. In the Exposure Age and Test Age Column, “P” Indicates Postnatal Day and “E” Indicates Embryonic Day

Paper	Exposure Age	Model	Sex	Test Age	What was Investigated?	Marker/Stain Used	Main Finding
Lu et al. (2018)	E9-20	Rat	M	P84	GABAergic Mature Neurons	NeuN and GAD67	Increase
					Glutamatergic Mature Neuron	NeuN and Glutamate	Decrease
Elibol-Can et al. (2014)	E7-E21	Rat	MF	P60	Venus-VGAT	CA1	Decreased in M
						CA3	Decreased in M
						GCL	Decreased in MF
						Hilus	Decreased in M

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Table 5.

Results of PRISMA Showing the Impact of Perinatal Marijuana and Perinatal Alcohol in the Hippocampal Formation of Adult Rats and Mice. A/M Indicates if the Perinatal Exposure is Alcohol(A) or Marijuana(M). M and F in the "Main Finding" Column Indicate When There are Sex Differences in the Results. Exposure and Test Ages are Reported as Embryonic (E) Days of Age or Postnatal (P) Days of Age.

	A/M	Exposure Age	Model	Sex	Test Age	What was Investigated?	Marker/ Stain Used	Hippocampal Subregion	Main Finding
1	A	P2-P9	Mouse	MF	P90	GABA Interneurons	Venus-VGAT	CA1	Decreased in M
								CA3	Decreased in M
								GCL	Decreased in MF
								Hilus	Decreased in M
2	A	E7-E21	Rat	M	P60	Type 2b INP to Immature Granule Neuron	DCX	CA1 CA2 + 3	No Change No Change
								DG	No Change
3	M	E10.5–E17.5	Mouse	MF	P20-P60	Cannabinoid receptor type 1	CB1R	CA1	Decrease in M
						Cholecystokinin interneurons	CCK	CA1	Decrease in M
4	A	E1–21	Rat	M	P78–82	Dividing Cells	BrdU	DG	No Change
						Type 2b INP to IGN	DCX	DG	No Change
						Dividing Cells	Ki-67	DG	No Change
					P115	Dividing Cells	BrdU, P80	DG	Decrease
						Type 2b INP to IGN	DCX	DG	No Change
5	A	E1-P9	Rat	MF	P386	Type 2b INP to IGN	DCX	DG	Decrease in F
						Dividing Cells	Ki-67	DG	No Change
6	A	P4–9	Rat	MF	P60	Dividing Cells	BrdU 2 hours	DG	No Change
						Dividing Cells	Ki-67	DG	No Change
						Type 2b INP to Mature Granule Neuron	NeuroD	DG	Decrease
					P90	Dividing Cells	BrdU P60	DG	No Change
						Dividing Cells	Ki-67	DG	No Change
						Type 2b INP to Mature Granule Neuron	NeuroD	DG	Decrease
7	A	P4–9	Rat	M	P80	Dividing Cells	BrdU	DG	Decrease
					P50	Dividing Cells	BrdU	DG	No Change
					P50	Dividing Cells	Ki-67	DG	No Change
8	A	E9–20	Rat	M	P84	GABAergic Mature Neurons	NeuN and GAD67	CA3	Increase
								DG	Increase

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	A/M	Exposure Age	Model	Sex	Test Age	What was Investigated?	Marker/ Stain Used	Hippocampal Subregion	Main Finding
						Glutamatergic Mature Neuron	NeuN and Glutamate	CA3 DG	Decrease Decrease
9	A	E7-E17	Mouse	MF	P56	Type 2b INP to Immature Granule Neuron	DCX	DG	Decrease
						Dividing Cells	Ki-67	DG	No Change
10	A	E1–21	Rat	M	P60–65	Dividing Cells	BrdU	GCL	No Change
								Hilus	No Change
					P81–86		BrdU P60–65	GCL	No Change
								Hilus	No Change
11	M	E5.5–E17.5	Rat	M	P120	CB1-positive Boutons	CB1R	CA1	Increase
12	A	E1–21	Rat	F	P60–65	Dividing Cells	BrdU	GCL	No Change
					P81–86	Dividing Cells	BrdU P60–65	GCL	No Change
						0- to 3-week-old Glia	GFAP/BrdU P60–65	GCL	No Change
						0- to 3-week-old Mature Granule Neurons	NeuN/BrdU P60–65	GCL	No Change
13	M	E10.5–E18.5	Mouse	MF	P30	Caudal Ganglionic Eminence–derived Interneurons	GFP (5HT3AR-GFP transgenic line)	DG/Hilar Border	Decrease
						Medial Ganglionic Eminence–derived Interneurons	GFP (NKX2.1-cre: RCE-GFP transgenic line)	DG/Hilar Border	No Change

CA1, cornu ammonis area 1; CA2, cornu ammonis area 2; CA3, cornu ammonis area 3; DG, dentate gyrus; GABA, gamma-aminobutyric acid; GCL, granule cell layer; IGN, Immature granule neurons; INP, Immature Neural Progenitor.

Marker purposes are as follows: BrdU, bromodeoxyuridine, a thymidine analogue that labels DNA synthesis; CB1R, cannabinoid receptor type 1; DCX, doublecortin, a neuronal migration protein; GAD67, glutamate decarboxylase 67 kilodalton isoform; GFP, green fluorescent protein; Ki-67, antigen Ki-67, a marker of proliferation; NeuN, neuronal nuclei, a mature neuron marker; NeuroD, neurogenic differentiation factor 2, a marker of type 2b immature up to mature granule neurons; Venus-VGAT, a transgenic construct that labels GABAergic neurons.

*1.*Bird et al. (2018); 2. Elibol-Can et al. (2014); 3. de Salas-Quiroga et al. (2020); 4. Gil-Mohapel et al. (2011); 5. Gil-Mohapel et al. (2014); 6. Hamilton et al. (2011); 7. Klintsova et al. (2007); 8. Lu et al. (2018); 9. Olateju et al. (2018); 10. Sliwowska et al. (2010); 11. Tortoriello et al. (2014); 12. Uban et al. (2010); 13. Vargish et al. (2017).

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Table 6.

Search Terms

Search Term Blocks	Search Terms
Specifies Prenatal	“Antenatal” OR “antepartum” OR “fetal” OR “prenatal”
Specifies brain region	“Hippocamp*” OR “dentate” OR “dentate gyrus” OR “CA1” OR “CA2” OR “CA3” OR “LPP” OR “MPP” OR “perforant pathway” OR “fimbria-fornix” OR “Schaffer collaterals” OR “Commisural pathway”
Specifies cytoarchitecture	“Genetic” OR “gene” OR “mRNA” OR “methylation” OR “hypermethylation” OR “acetylation”OR “hypomethylation” OR “methyl mark” OR “epigenetic” OR “NDMA” OR “nicotinic” OR “muscarinic” OR “CB2” OR “CB1” OR “GPCR” OR “G-protein” OR “G protein” OR “AMPA” OR “Calcium channel” OR “sodium channel” OR “chloride channel” OR “cAMP” OR “cyclic AMP” OR “PKA” OR “Signal transduction” OR “neurogenesis” OR “granule cell” OR “proliferation” OR “apoptosis” OR “neural stem cell” OR “neural stem cells” OR “cellular migration” OR “Immunohistochemistry” OR “IHC” OR “Ki-67” OR “PCNA” OR “Sox2” OR “BrdU” OR “DCX” OR “GFAP”
Specifies Alcohol	“PNEE” OR “Prenatal ethanol” OR “Prenatal alcohol” OR “PAE” OR “prenatal alcohol exposure” OR “FASD” OR “FAS” OR “fetal alcohol spectrum disorder” OR “fetal alcohol syndrome” OR “fetal alcohol exposure” OR “prenatal ethanol” OR “fetal ethanol” OR “ethanol” OR “alcohol”
Specifies Cannabis	“cannabis” OR “marijuana” or “THC” OR “Tetrahydrocannabinol” OR “Δ^9-tetrahydrocannabinol” OR “cannabinoid” OR “WIN 55,212–2” OR “PME”