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Epidemiological Associations of Various Substances and Multiple Cannabinoids with Autism in USA

Short Title:
US Drug and Cannabinoid Use and Autism Spectrum Disorder

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Abbreviations:

ASD	- Autism Spectrum Disorder
CB1R	- Cannabinoid type 1 receptor
DEA	- Drug Enforcement Agency
IDEA	- US Department of Education Individuals with Disabilities Act
NSDUH	- National survey of Drug Use and Health
Robo	- Roundabout, a guidance molecule receptor for axonal growth cones and arterial endothelial tips
SAMHSA	- Substance Abuse and Mental Health Services Administration
Slit	- Slits 1-3, arterial and axonal guidance molecule and ligand for Robo
+	- An additive operator for regression calculations

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~ - Tilde, a middle sign separating the two sides of a regression calculation
* - Asterisk, an operator used in regression calculations to include additive and interactive relationships

Table of Contents Summary:
Autism is growing fast in Colorado, Oregon, Maine and Massachusetts, but is actually falling in Iowa and Oklahoma. Epidemiology suggests cannabinoids could be the culprit.

What's known on this subject
The cause of ASD is not understood but maternal inflammation, parental age, epigenetics and affected siblings have all been implicated. Meanwhile caseload continues to climb rapidly and constitutes a major developmental anomaly. Geographical and temporal clusters suggest an environmental cause.

What this study adds
This study shows that alcohol and cannabinoids are the main epidemiological correlates of ASD and are robust to multivariate adjustment. Moreover multiple regression links non-psychoactive cannabinoids including cannabidiol and cannabinol to ASD, a relationship strengthened by many mechanistic pathways.

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Contributors Statement Page

Dr Reece designed the study, performed the statistical analysis and wrote the first draft.

Prof Hulse reviewed the manuscript for important intellectual content and revised the draft. He also provided administrative, research support and assisted with statistical advice and oversight.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Abstract

Objective
Autistic Spectrum Disorder (ASD) is increasing in across USA. Pediatricians and physicians in both Colorado and Australia continue to see high caseloads however this prevalence uptick remains largely unexplained. The present study was undertaken to study drug-ASD associations at state level.

Methods
Existing datasets from the US Department of Education Individuals with Disabilities Act, the Substance Abuse and Mental Health Services Administration National Survey of Drug Use and Health, and the Drug Enforcement Agency cannabinoid concentration in seizures were re-analyzed.

Results
ASD rates are high and rising fastest in Colorado, Maine, Massachusetts, Oregon, Rhode Island and New Jersey but falling in Oklahoma and Iowa. When the nine highest cannabis use states are grouped together ASD is rising significantly faster there than elsewhere (time:status interaction in quadratic mixed effects model $P<0.0001$). On univariate regression ASD rate was significantly positively associated with alcohol and cannabis exposure and with the cannabinoids Δ9-tetrahydrocannabinol, cannabinol, cannabichromene, cannabigerol and tetrahydrocannabivarin. These effects remained after multivariate adjustment for Δ9-tetrahydrocannabinol and cannabidiol (from $P<0.0001$). Cannabidiol correlated with ASD rate when a three year lag was introduced ($R=0.7483$, $P=0.0032$).

Conclusion
These data show that increased cannabinoid exposure explains on bivariate and multivariate regression much of the recent rise in ASD across USA, and in the context of other reports, also at some local cluster levels. Together with numerous mechanistic reports these data argue powerfully for causality and strongly indicate a large case-control study. ASD-like neurobehavioural toxicological syndromes likely represent the commonest form of cannabis-related teratology following peri-gestational exposure.

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Introduction

Autistic spectrum disorder (ASD) is one of the commonest development abnormalities of children affecting a mean of 1.68% of 8 year old boys across USA and up to 4.5% of 8 year old boys in New Jersey [1]. Surveys show substantial rates of rise in ASD incidence of 20% over two years in New Jersey and 30% in Colorado. Indeed there has been recent movements in Colorado to have autism declared an epidemic [2].

Whilst many previous epidemiological studies have been conducted the cause/s remain largely elusive. Higher socioeconomic status, having a previous autistic child, advanced parental age, gestational inflammation, twin associations, diabetes, bleeding and drugs [3-5] including cannabinoids [6, 7] have been previously implicated.

Three of three longitudinal studies of cannabis use have confirmed impairments of executive and cortical functioning and ADHD- and autistic- like cognitive deficits following prenatal cannabis exposure (PCE) [8-11].

A large Hawaiian study of 300,000 deliveries found 21 major birth defects were elevated after PCE [12]. Since ASD is commoner than the commonest of these defects, and since neurobehavioural toxicology is likely to be a power or pseudo-exponential function of exposure to multiple cannabinoids, in the context of rising cannabinoid use, rising Δ9-tetrahydrocannabinol concentrations, and cannabinoids now entering the US food chain, it would appear that ASD-like neurobehavioural toxicology will increasingly become the leading manifestation of increased gestational cannabinoid exposure. In 2017 161,000 women in USA used cannabis whilst pregnant and for 69,000 use was near-daily [13].

Many reports of cannabinoid toxicology and genotoxicity suggest an asymptotic pseudo-exponential dose-response relationship [14-18]. The implications of this on neurobehavioural neurotoxicology and general toxicology may prove to be most profound as the population moves into a higher cannabis use paradigm.

Since the USA regularly surveys drug use in a nationally representative sample [13] and is undergoing a period of social change in relation to the use of cannabis and other drugs we

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investigated the extent to which extant epidemiological evidence of drug and cannabinoid use relate to ASD incidence.

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Methods

Data sources. Data on the US National Database from the US Department of Education Individuals with Disabilities Act (IDEA) was used [19]. Data on state level use of various addictive drugs was sourced from the Substance Abuse and Mental Health Administration (SAMHSA) National Survey of Drug Use and Health (NSDUH) including shapefiles and SAS database files [13]. Data on cannabinoid concentration of US Drug Enforcement Agency (DEA) Seizures was from published sources [20, 21]. State based levels of individual cannabinoids was derived by multiplying the monthly cannabis use by the Federal concentration of each cannabinoid separately.

Statistics. Data was analyzed in "R" from Central R Archive Network version 3.5.2 and R Studio 1.1.463 2018. Parameters were log transformed to optimize normality assumptions where appropriate. Models were compared using analysis of variance procedures. Time was not transformed. Full regression models were reduced following the classical procedure of elimination of the least significant term. Maps and graphs were plotted in ggplot2. Bivariate maps were drawn using colorplaner 0.1.4. Time series analysis was performed with the stats package. Two-tailed t-tests of statistical significance were used throughout. $P<0.05$ was considered significant.

Ethics. These studies were approved by the Human Research Ethics Committees of Southcity 7-Day Family Medical Centre in Brisbane, Australia and the University of Western Australia in Crawley, Perth, Western Australia.

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Results

Data from the IDEA database was combined with SAMHSA NSDUH and DEA published data on levels of cannabinoid concentration identified in Federal seizures of drugs to analyze the relationship between autism rates and drug use at US state level [13, 19-21].

Figure 1A shows a map of US states by rates of last month cannabis consumption, Figure 1B shows a US state-based map of ASD rates, and Figure 1C shows a bivariate colorplane map of both parameters together on the same plot. Purple shading in Figure 1C indicates that both last month cannabis use and autism are high in California.

Supplementary Figures 1A and 1B shows the rate of ASD in 25 and 26 states respectively.

Figure 2 shows the published concentration of seven different cannabinoids over time in Federal seizures at the national level [20, 21].

Figures 3 and 4 illustrate the rates of ASD in 25 and 26 states respectively. The data has been split into two sets to assist with overplotting. A combined plot appears as Supplementary Figure 2. Figure 4 shows high levels of ASD in Minnesota, Colorado, Maine and Massachusetts and low and decreasing levels in Iowa. Figure 5 shows high ASD levels in Oregon, Rhode Island and New Jersey but low levels and flattening out levels in Oklahoma.

The relationship of the ASD rate to time appears to concave upwards. Plotting of the square root of the ASD rate against time linearizes this relationship as shown in Supplementary Figure 3. This comparison can be formalized by conducting an analysis of variance (ANOVA) study of models of ASD rate linear and quadratic in time. The AIC of the linear model is 1726.158 and that of the quadratic model is 1557.113 ($F=185.63$, $df=1$, $P<2.0\times10^{-16}$). These results confirm the model quadratic in time to provide the superior fit.

When the ASD rate is regressed against quadratic time and state using Iowa as the baseline comparator 128 terms are significant (Supplementary Table 1).

States may be divided into average and high cannabis use rates based on the most recent results of the SAMHSA NSDUH. When Colorado, Alaska, Washington state, Oregon,

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Montana, Massachusetts Washington DC, Rhode Island and Vermont are classed as high use states the graph shown in Figure 5 is obtained. Table 1 shows the outcome of linear regression procedures on this data for both linear models and for mixed effects models with the state as a random effect, for models linear and quadratic in time in both cases. As shown in Table 1, many terms are highly significant and the time: cannabis_use_status interaction is significant in all models from $P<0.0001$. Models quadratic in time were superior to the linear-only models for both linear and mixed effects models (linear models: $F=189$, $dF=1$, $P<2.0\times10^{-16}$; random effects models log ratio = 333, $P<0.0001$).

The mean ASD rates in high v low states was 100.11 ± 14.21 v 93.64 ± 5.39 ($P=0.6791$).

Figure 6 plots the ASD rate against the use by state of the various addictive agents. Positive slopes of lines are obtained for alcohol and cannabis. These relationships are quantified by regression studies in the upper half of Table 2. There one notices highly significant and positive β-estimates for cannabis by both monthly and annual measures of use.

For this reason cannabinoids were broken down by the major ones for which data is available at the time of writing. The data is presented graphically in Figure 7. One notes a positive slope for five of the cannabinoids, but an apparently slightly negative slope for cannabidiol.

These effects are quantitated in the lower half of Table 2 which confirms strongly positive relationships for these five cannabinoids. The analysis shows that the slope of the curve for cannabidiol is not significantly different from zero.

Having demonstrated in single variable analyses an apparent relationship between the use of various substances and the ASD rate, a natural consideration related to the relative importance of the various substances on linear regression. Table 3 explores this question. When all cannabinoids are regressed against ASD rate in a simple linear additive model only cannabidiol is significant. When a model employing interactive terms in cannabinoid use is used the results are as shown in the second part of Table 3.

When all the different substances were compared in a simple additive model the results obtained in the middle of Table 3 are derived.

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When all the substances (excluding cannabis) and all the individual cannabinoids are regressed against the ASD Rate the results shown in the lower part of Table 3 are obtained. It is interesting to note that cannabidiol survives model reduction in nine terms from P<0.00001, and terms including Δ9-tetrahydrocannabinol occur six times (from P=0.0005).

When this exercise is repeated in a random effects mixed interactive model with state as a random effect, similar results are obtained (Table 4). Here Δ9-tetrahydrocannabinol is seen in four terms, cannabidiol in eight terms and cannabinol in one term.

The above analysis seems to present mixed evidence relating to the effect of cannabidiol on the ASD rate. For this reason its relationship was explored further.

One notes from Figure 2 that cannabidiol has a biphasic relationship with time having risen and then fallen since it is understood to be regulated in a manner opposite to Δ9-tetrahydrocannabinol in the cannabis sativa plant. It is conceivable that this biphasic rise and fall is creating some confusion in the analysis since cannabidiol alone of all the cannabinoids assessed, does not display a unidirectional trend.

For this reason Figure 8 charts the ASD-cannabidiol data by individual year. Visual inspection suggests that there appears to be a rise in the slope of the least squares regression line till about 2007 and a subsidence thereafter. Careful inspection of Figure 2C shows that the cannabidiol concentration curve of Federal; seizures apparently maximized in about 2002 which suggests a possible time lagged effect (Supplementary Table 2).

Figure 9A shows the slopes of the regression lines of Figure 8 against time and compares it to the curve for the cannabidiol concentration. An apparent time lag effect is seen.

Supplementary Figure 4 shows autocorrelation and partial autocorrelation plots of this data. Supplementary Figure 5 presents a cross-correlation graph of the cannabidiol and ASD-cannabidiol slope data which indicates that the correlation maximizes around a lag of 3-4 years.

Figure 9B re-draws the plot of Figure 9A with a three-year lag added to the cannabidiol concentration curve. This has the effect of raising the cannabidiol – ASD-cannabidiol slope

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correlation from R = 0.0165 (95%C.I. -0.5393 – 0.5624), t=0.0549, df=11, P=0.9572 up to R=0.7483 (95%C.I. 0.3357-0.9199), t=3.7414, df=11, P=0.0033.

Hence this more detailed analysis suggests that notwithstanding the declining cannabidiol content of US cannabis seizures, cannabidiol is indeed associated with the ASD rate, albeit after a lag period of 3-4 years or thereabouts. The salience of cannabidiol in the several multiple regression tabulations described above is also clear.

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Discussion

Despite clarion calls by at least two US Surgeons General [22, 23] on the risks associated with drug use during pregnancy, and strong warnings by the American College of Obstetricians and Gynecologists [24] the issue of drug use in pregnancy and possible increases in neonatal morbidity has not been fully investigated. In the light of recent emerging data identifying drug-related causal pathways for neonatal morbidity, this study investigated the relationship between epidemiological data on US state-based drug use and the incidence of ASD, particularly to investigate the apparently mystifying rise in many parts of the USA and elsewhere.

This study confirmed that ASD is rising in all states except Iowa and Oklahoma. The overall trend shows a quadratic time-dependent increase. When the high cannabis use states of Colorado, Alaska, Washington state, Oregon, Montana, Massachusetts Washington DC, Rhode Island and Vermont are grouped together the ASD rate is rising faster than in the remainder of the country (time: status interaction P= 0.0049 in a quadratic-time model). In the period 1995-2011 ASD rose with alcohol and cannabis use but not with opioid pain relievers, tobacco and cocaine. Upon multivariate testing terms including alcohol, cigarettes, Δ9-tetrahydrocannabinol, cannabidiol and cannabinol remained significant in final models. The effect of cannabidiol was complex due to its inverted U-shaped time trend but was confirmed to be associated with ASD rates on lagged analysis.

These findings have far reaching significance. Data demonstrate clear evidence of association between alcohol, tobacco, Δ9-tetrahydrocannabinol and cannabidiol with ASD incidence. However the present study does not occur in a vacuum, but in the context of a wealth of mechanistic studies suggesting pathways by which many cannabinoids have been shown to interfere with brain growth and maturation and fundamental neuronal physiology. This potent combination of high level epidemiological evidence and a plethora of mechanistic pathways raises a potentially causal relationship as a major issue.

The cause of ASD is not understood. Heightened startle reflex, inability to concentrate, impaired IQ and difficulty of visual processing well described in the PCE neurobehavioural toxicology literature all bear close resemblance to various autistic features [8-11, 25]. Abnormalities of cortical structure, white matter connections, and subcortical nuclei have

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been reported in ASD [26, 27]. Similar changes have been seen after cannabis exposure [28, 29] and indeed long term deficits of cortical and executive functioning have been reported in all three longitudinal studies of prenatally exposed children performed in Pittsburgh USA, Netherlands and Ottawa [8-11].

It is interesting to consider that the congenital cannabis exposure literature describes a range of defects from smaller heads, to microcephaly to anencephaly including immediate postnatal and intrauterine death [8, 12, 30, 31]. This implies a spectrum of post-PCE neurological disorders from mild to moderate to severe neuroteratology.

Whilst it is of considerable interest to consider the mechanisms by which cannabinoids impact upon brain physiology this is a large subject and can only be summarized here. Cannabinoids negatively affect neurogenesis a process critically important in the developing brain for the long distance migration of human neocortical neurons and the formation of the large and exuberant human cortex [32, 33]. By interfering with the formation of actin and tubulin which forms the microtubules of the mitotic spindle THC interferes with cell division [34]. THC interferes with notch signalling [35, 36] which is a key body morphogen and especially important for brain and heart morphogenesis [37-40]. The endocannabinoid system is a key regulator of synaptogenesis [41, 42]. The neurexin-neuroligin scaffolding pair is a key transsynaptic membrane complex governing synapse development and stabilization which is impeded by exogenous phytocannabinoids [43]. Cannabinoids affect immune and microglial function and thus synaptic pruning and ability to focus, concentrate and learn from experience [44]. Cannabinoids interfere with stathmin which is a key molecular pathfinder for growth cone steering and guidance [45] and has also been shown to be involved with synaptogenesis, neurogenesis and NMDA dependent memory [46].

The ratio of the guidance molecules slit to robo has been shown to be a key regulator of human and mammalian cortical development and diverts foetal subventricular neurogenesis from a small-capacity direct pathway to a slower but more prolific indirect pathway and is immediately responsible for the large human neocortex [33]. This ratio is adversely affected by cannabinoids [32]. Robo/slit also guide axons [32]. Mitochondria carry a full complement of endocannabinoid signalling machinery [47, 48] and not only generate the energy for DNA protection and maintenance but also signal directly to the nuclear genome by several metabolic pathways and shuttles [49] and are impeded by cannabinoids [16-18, 50-52] in a manner

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which directly interferes with major neuronal functions including signalling and memory [47, 48]. Acting via type 1 endocannabinoid receptors (CB1R's) cannabinoids are proinflammatory [53, 54] which negatively impacts neurogenesis [47, 55-58]. Cannabinoids can have deleterious effects on macro and micro- vasculature and the stem cell niches to which they contribute [59-62]. Negative effects have been documented on both sperm [63-66] and ova [67]. Foetal alcohol syndrome is known to act epigenetically in part via CB1R's [68]. Exogeneous cannabinoids are also known to suppress the cortical oscillations which are increasingly being understood to be fundamental to many cortical functions [69].

Finally cannabinoids including small doses of Δ9-tetrahydrocannabinol, cannabidiol, cannabichromene and cannabidivarin are known to have substantial epigenetic effects [70-73] a finding which achieves particular significance in the light of the large literature on the epigenetic aetiopathogenesis of ASD [74, 75].

It should also be observed that the present findings could arise either from a generalized effect on a whole population, or from a high signal effect from tiny communities. Evidence of spatial clustering of autism in parts of Utah and California suggest that this latter effect may be of public health importance [76-78]. Such clustering is consistent with local cannabis-based subcultures and this has indeed been documented in northern California [13].

Findings raise special concerns in relation to introduction of cannabidiol, cannabinol and many other cannabinoids into the US food chain as is understood to be in process following the US Farm Act. Indeed in this regard the recent experience near Ain in France near the Swiss border is relevant. Press reports disclose a 58-fold elevation of upper limb phocomelia locally [79, 80] along with a flourishing cannabis industry [81, 82]. Micromelia was seen also in cattle – suggesting a food chain effect – but not in nearby Switzerland where cannabinoids had previously been banned. An epidemiological association of PCE with micromelia has been described [12]. The French investigation into this outbreak has since been re-opened.

Strengths of the present study include its relatively long duration, its national level datasets, and access to what are likely the best state-based figures of population drug use and ASD available internationally. Limitations relate to its ecological and epidemiological design and include its lack of access to individual level data and the approximations involved in approximating state level cannabinoid exposure. Both these issues can be corrected by a

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large case-controlled study with an objective measurement of drug exposure such as hair analysis [83].

Conclusion

This study confirms an association at the epidemiological-ecological level between ASD with cannabinoids Δ9-tetrahydrocannabinol and cannabidiol, and to a leser extent tobacco and alcohol use. Since tobacco and alcohol use are declining US nation-wide [13] this implicates both psychoactive and non-psychoactive cannabinoids in the quadratically rising ASD epidemic across 49 US jurisdictions, an association which is robust to multivariate adjustment for other drug use. The relationship between cannabidiol and ASD lags by several years. Particular concerns apply to contamination of the national food supply with cannabinoids and their asymptotic neurotoxicity and genotoxicity. In the context of multiple previously established mechanistic pathways these association-level findings are consistent with a casual pathway and position cannabis as a major suspect driving the present epidemic. We advocate a large case-controlled study be undertaken including objective measures of drug exposure to investigate dose-response and putatively causal effects.

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Table 1.: Linear Regression Models

Linear Models

Parameter	Parameters				Model			
	Est.	Std.Error	t value	Pr(>|t|)	Adj. R Squ	F	df	P
*Autism ~ Time * factor(Cannabis_Exposure)*								
Time	0.1960	0.0073	26.69	<2e-16	0.781	1210	3,1019	<2.0E-16
Cannabis_High	-36.0000	16.1000	-2.239	0.0250				
Time: Cannabis_High	0.0179	0.0080	2.237	0.0260				
*Autism ~ (Time)^2 * factor(Cannabis_Exposure)*								
Time	40.2236	1.3512	29.77	<2e-16	0.815	1120	4,1018	<2.0E-16
(Time)^2	-9.1136	1.3561	-6.72	3.0E-11				
Time: Cannabis_High	4.1692	1.4778	2.82	0.0049				

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Mixed Effects Models

Parameter	Parameters					Model		
	Value	Std.Error	dF	t-value	P-value	AIC	BIC	LogLik
2005-2013								
*Autism ~ Time * factor(Cannabis_Exposure), State as Random*								
Time	0.1782	0	970	34	0.0000	1386	1415	-687
Cannabis_High	-38.3913	13	49	-3	0.0038			
Time: Cannabis_High	0.0192	0	970	3	0.0024			
*Autism ~ (Time)^2 * factor(Cannabis_Exposure), State as Random*								
Time	33.3050	0.4064	968	81.9506	0.0000	1031	1065	-508
(Time)^2	-6.9954	0.4065	968	-17.2098	0.0000			
Time: Cannabis_High	4.1333	0.9955	968	4.1521	0.0000			
(Time)^2: Cannabis_High	-2.1249	0.9978	968	-2.1295	0.0335			

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Table 2.: Univariate Relationship of ASD Rate to Substance and Cannabinoid Exposure

Parameter	Parameters				Model			
	Est.	Std.Error	t value	Pr(>|t|)	Adj. R Squ	F	df	P
Autism Rate ~ Substance Use								
Alcohol Monthly	2.8724	0.3081	9.322	<2.0E-16	0.1156	86.90	1,656	<2.0E-16
Binge Alcohol Monthly	4.0727	0.7451	5.466	6.6E-08	0.0421	29.87	1,656	6.55E-08
Cigarettes Monthly	-6.7990	0.7468	-9.104	<2.0E-16	0.1108	82.88	1,656	<2.0E-16
Cocaine Annual	-0.2388	0.0861	-2.773	0.0057	0.0101	7.69	1,656	0.0057
Pain Releivers Annual	0.0049	0.2026	0.024	0.9806	-0.0057	0.00	1,174	0.9806
Cannabis Monthly	0.9099	0.0839	10.84	<2.0E-16	0.1507	117.60	1,656	<2.0E-16
Cannabis Annual	0.7780	0.1093	7.115	3.47E-12	0.0821	50.63	1,544	3.47E-12
Autism Rate ~ Cannabinoid Use								
THC Monthly	0.9768	0.0418	23.36	<2.0E-16	0.4533	545.80	1,656	<2.0E-16
Cannabidiol Monthly	-0.1215	0.0749	-1.623	0.1050	0.0025	2.63	1,656	0.1051
Cannabinol Monthly	0.6155	0.0713	8.637	<2.0E-16	0.1007	74.59	1,656	<2.0E-16
Cannabichromene Monthly	1.0752	0.0598	18.00	<2.0E-16	0.3295	323.90	1,656	<2.0E-16
Cannabigerol Monthly	0.8907	0.0475	18.75	<2.0E-16	0.3480	351.70	1,656	<2.0E-16
Tetrahydrocannabivarin Monthly	0.8959	0.0697	12.85	<2.0E-16	0.1998	165.00	1,656	<2.0E-16

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Table 3.: Linear Models of Relationship of ASD Rate to Substance and Cannabinoid Exposure

Parameter	Parameters				Model			
	Est.	Std.Error	t value	Pr(>|t|)	Adj. R Squ	F	df	P
1991-2011								
Linear Additive Model w All Cannabinoids								
Time	0.1320	0.0045	29.54	<2.0E-16	0.571	438	2,655	<2.0E-16
CBD	10.2000	1.9100	5.32	0.0000				
Interactive Model w All Cannabinoids								
Time:THC:CBD	-0.0215	0.0051	-4.19	0.0000	0.582	184	5,652	<2.0E-16
THC	1.3300	0.3300	4.03	0.0001				
Time:CBD	2.3500	0.6870	3.43	0.0007				
CBD	-4680.0	1370.0	-3.41	0.0007				
1995-2011								
All Drugs Additive Model								
Alcohol	3.4918	0.7474	4.67	0.0000	0.432	23.2	6,169	<2.0E-16
Binge_Alcohol	-8.2679	1.8225	-4.54	0.0000				
Tobacco	-12.5854	3.7767	-3.33	0.0011				
Cigarettes	13.7276	4.7634	2.88	0.0045				
Cocaine	16.3232	6.6386	2.46	0.0149				
All Drugs Interactive Model								
Cigarettes:Alcohol	-256	45	-5.73	0.0000	0.497	10.6	18,157	<2.0E-16
Cigarettes:CBD:Alcohol	9280	2020	4.59	0.0000				
CBD:Alcohol	-1450	339	-4.29	0.0000				
Cigarettes:CBD	-160000	41900	-3.82	0.0002				

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Time:Cigarettes:CBD	78	21	3.75	0.0003				
CBD	711	192	3.71	0.0003				
Cigarettes:THC:CBD	583000	165000	3.54	0.0005				
Time:Cigarettes:THC:CBD	-290	82	-3.55	0.0005				
Time:Cigarettes:THC	0.0208	0.0060	3.45	0.0007				
Time:Cigarettes:THC:CBD:Alcohol	449	131	3.44	0.0007				
Cigarettes:THC:CBD:Alcohol	-903000	263000	-3.44	0.0008				
Cigarettes	6520	2090	3.11	0.0022				
Time	0.8940	0.2900	3.08	0.0024				
Time:Cigarettes	-3.2000	1.0400	-3.08	0.0024				
Time:THC	-0.0027	0.0013	-2.16	0.0325				
Alcohol	1750	817	2.14	0.0338				
Time:Alcohol	-0.8480	0.4060	-2.09	0.0382				
Binge_Alcohol	-4.0100	1.9500	-2.05	0.0417				

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Table 4.: Mixed and Random Effects Models of Relationship of ASD Rate to Substance and Cannabinoid Exposure

Parameter	Parameters					Model		
	Value	Std.Error	dF	t-value	P-value	AIC	BIC	LogLik
1995-2011								
Alcohol	-364	40	592	-9	0.0000	-5	75	20
CBN	-11	3	592	-4.4	0.0000			
Time:Alcohol	0	0	592	9.1	0.0000			
THC:CBD:Alcohol	-103059	22471	592	-4.6	0.0000			
Time:THC:CBD:Alcohol	51	11	592	4.6	0.0000			
Cigarettes:THC:CBD:Alcohol	204251	49651	592	4.1	0.0000			
Time:Cigarettes:THC:CBD:Alcohol	-102	25	592	-4.1	0.0000			
THC	-1770	435	592	-4.1	0.0001			
Time:THC	1	0	592	4.1	0.0001			
Time:CBD	0	0	592	4	0.0001			
Time:THC:Alcohol	-2	0	592	-3.8	0.0001			
THC:Alcohol	3114	819	592	3.8	0.0002			
Cigarettes:CBD:Alcohol	-527	157	592	-3.4	0.0008			
Time:THC:CBD	-13	5	592	-2.4	0.0181			
THC:CBD	25439	10781	592	2.4	0.0186			

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Figure Captions

Figure 1.: Maps of Cannabis Use, Autism Spectrum Disorder nad Cannabis and Autism together. A.: Monthly cannabis use by State. B: Annual Austism Spectrum disorder by US State, 2011. Bivariate colorplane choropleth map of cannais and autism co-variance.

Figure 2.: Cannabinoid concentration of US DEA Cannabis seizures 1990-2011. Data derived from ((Elsohly 2000, 2016)). A: Δ8-tetrahydrocannabinol; B: Δ9-tetrahydrocannabinol; C: cannabidiol; D: Cannabichromene; E: Cannabinol; F: Cannabigerol; G: tetrahyrocannabivarin.

Figure 3.: Autism Spectrum Disorder Rates in States Alabama – Mississippi. (Data from IDEA dataset).

Figure 4.: Autism Spectrum Disorder Rates in States Missouri - Wyoming. (Data from IDEA dataset).

Figure 5.: Autism Spectrum Disorder rates over time by High v Average Cannabis use states.

Figure 6.: Autism Spectrum Disorder rates by Substance Exposure levels.

Figure 7.: Autism Spectrum Disorder Rates by Cannabinoid Exposure Levels

Figure 8.: Autism Spectrum Disorder by Cannabidiol Exposure Rate by Year, US National data.

Figure 9.: Lag Analysis of Slope of Autism Spectrum Disorder Rate – Cannabidiol Concentration Association by Time and Group.

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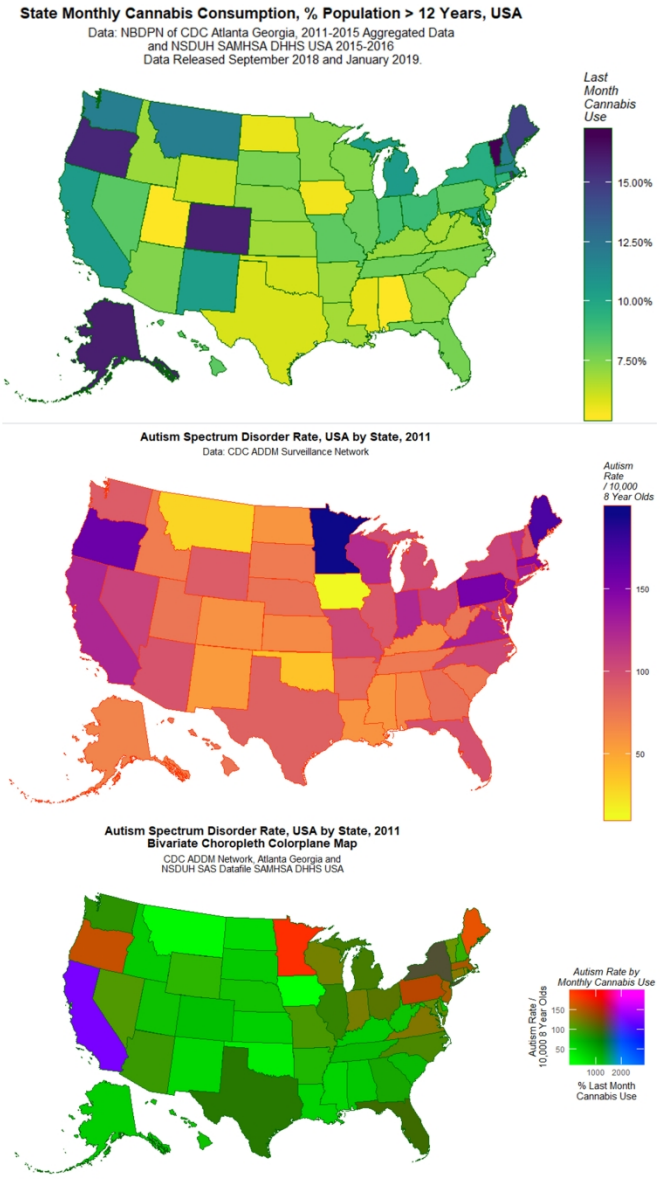

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Figure 2.: Cannabinoid concentration of US DEA Cannabis seizures 1990-2011. Data derived from ((Elsohly 2000, 2016)). A: Δ8-tetrahydrocannabinol; B: Δ9-tetrahydrocannabinol; C: cannabidiol; D: Cannabichromene; E: Cannabinol; F: Cannabigerol; G: tetrahyrocannabivarin.

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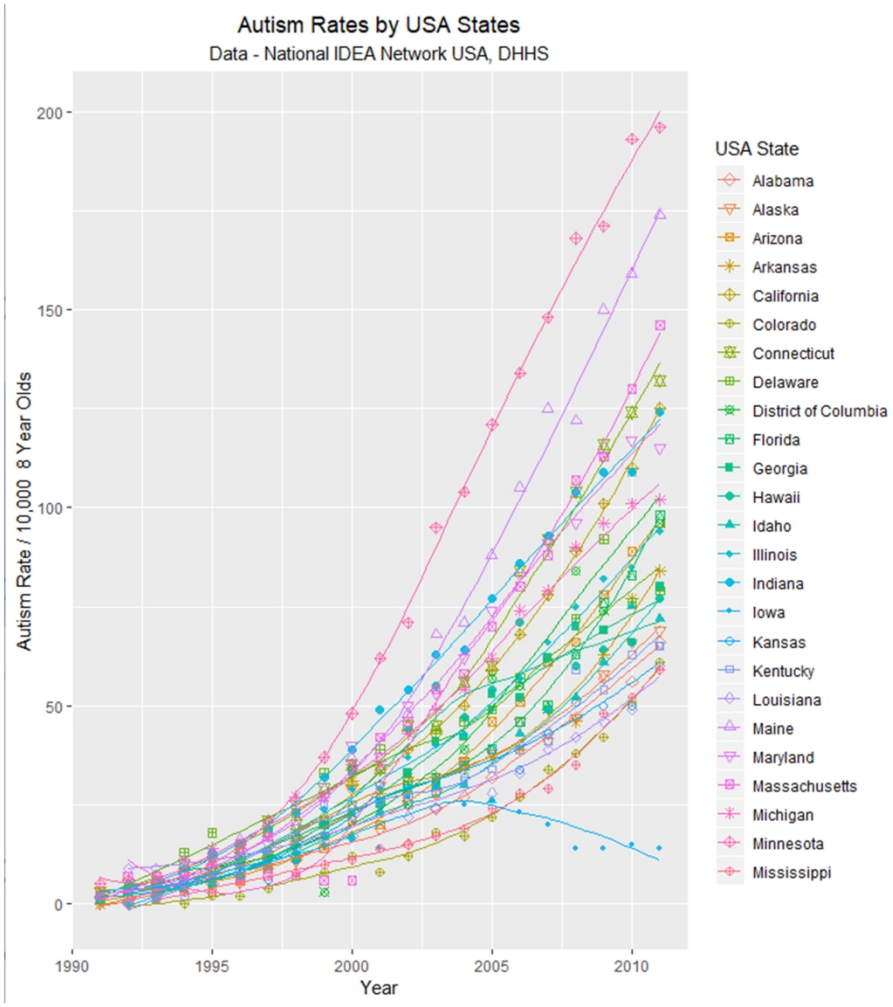

Figure 3.: Autism Spectrum Disorder Rates in States Alabama – Mississippi. (Data from IDEA dataset).

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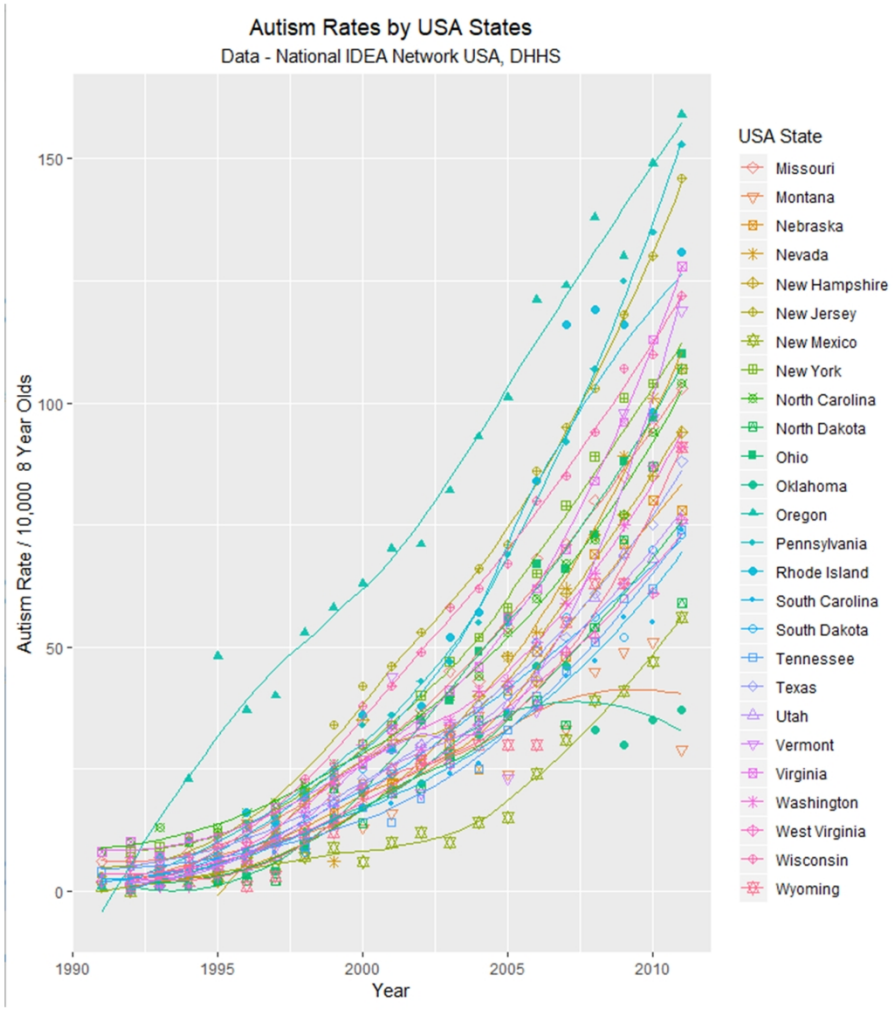

Figure 4.: Autism Spectrum Disorder Rates in States Missouri - Wyoming. (Data from IDEA dataset).

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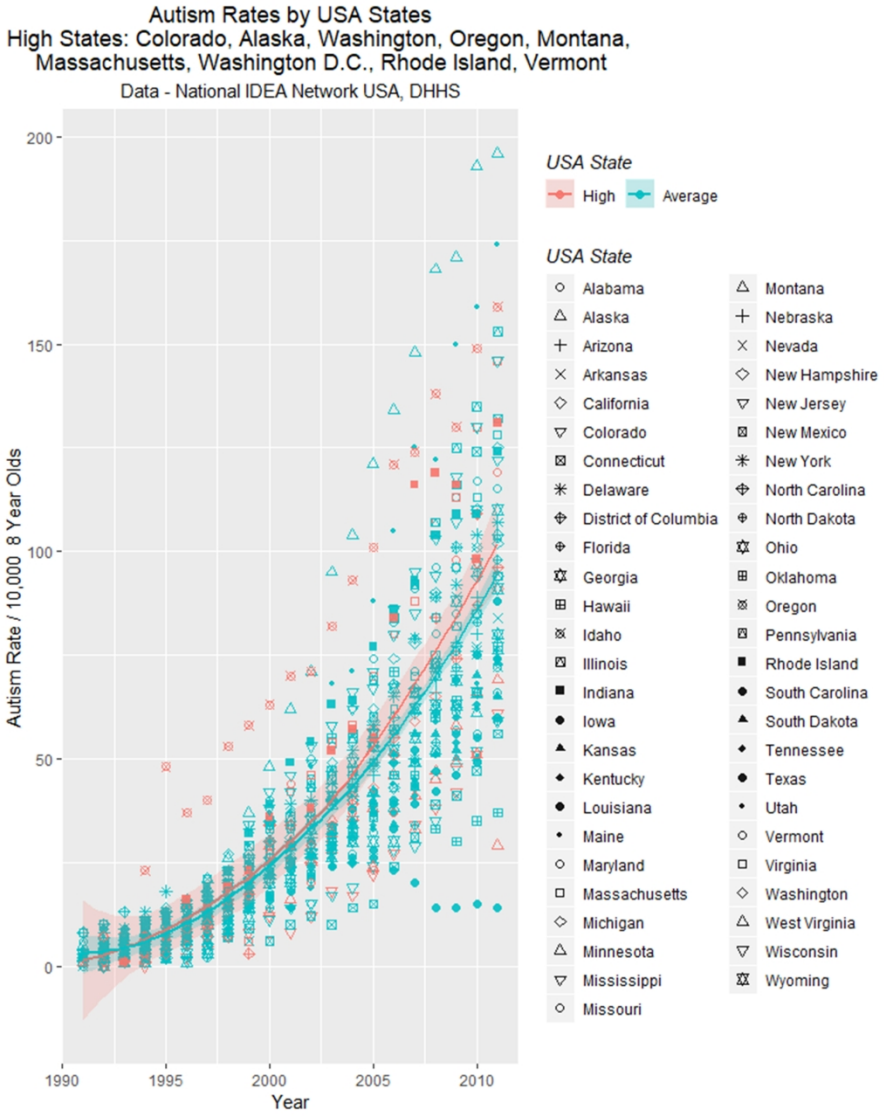

Figure 5.: Autism Spectrum Disorder rates over time by High v Average Cannabis use states.

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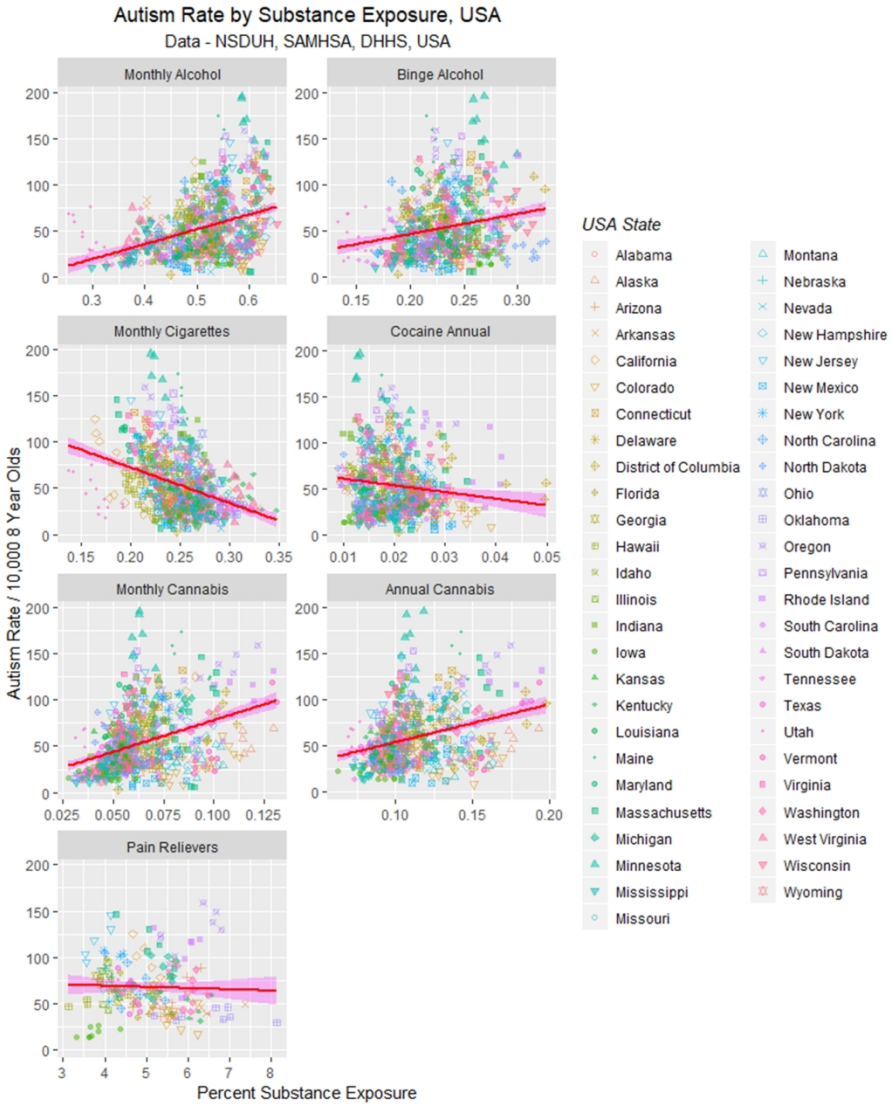

Figure 6.: Autism Spectrum Disorder rates by Substance Exposure levels.

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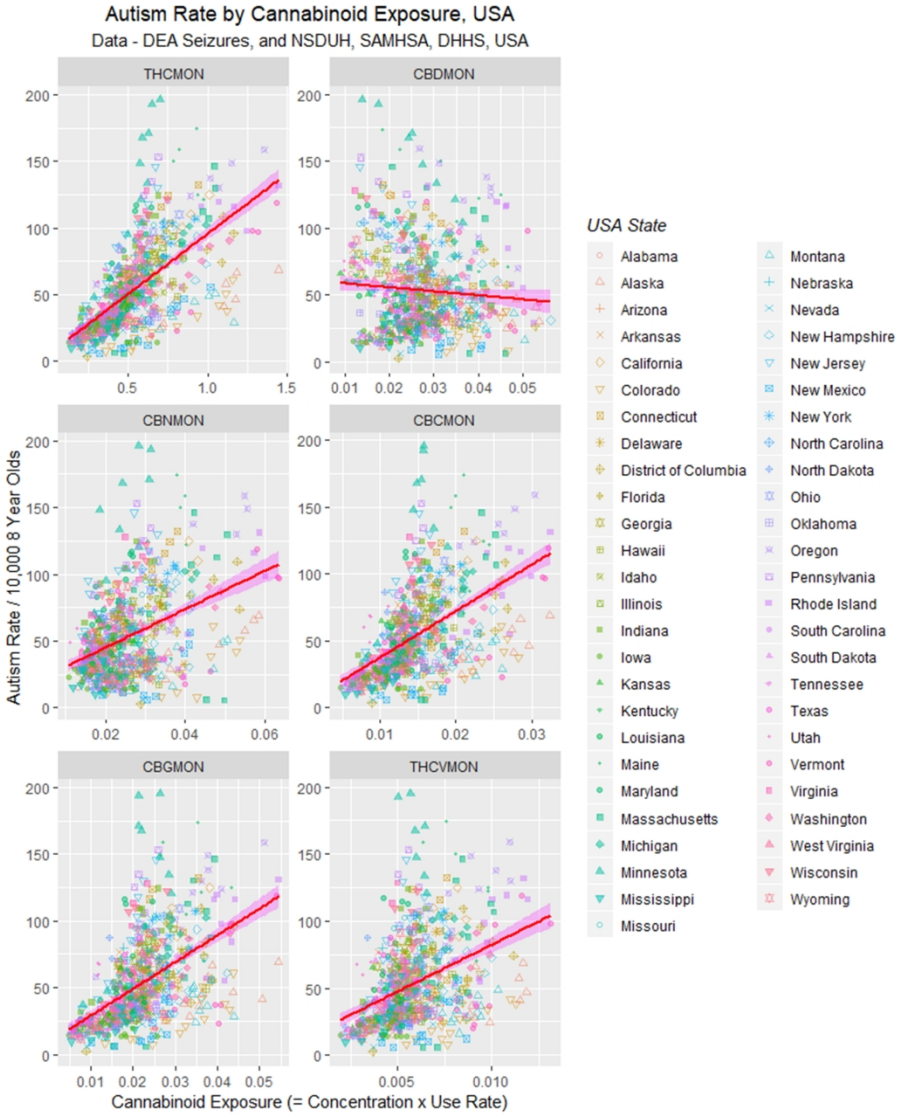

Figure 7.: Autism Spectrum Disorder Rates by Cannabinoid Exposure Levels

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Figure 8.: Autism Spectrum Disorder by Cannabidiol Exposure Rate by Year, US National data.

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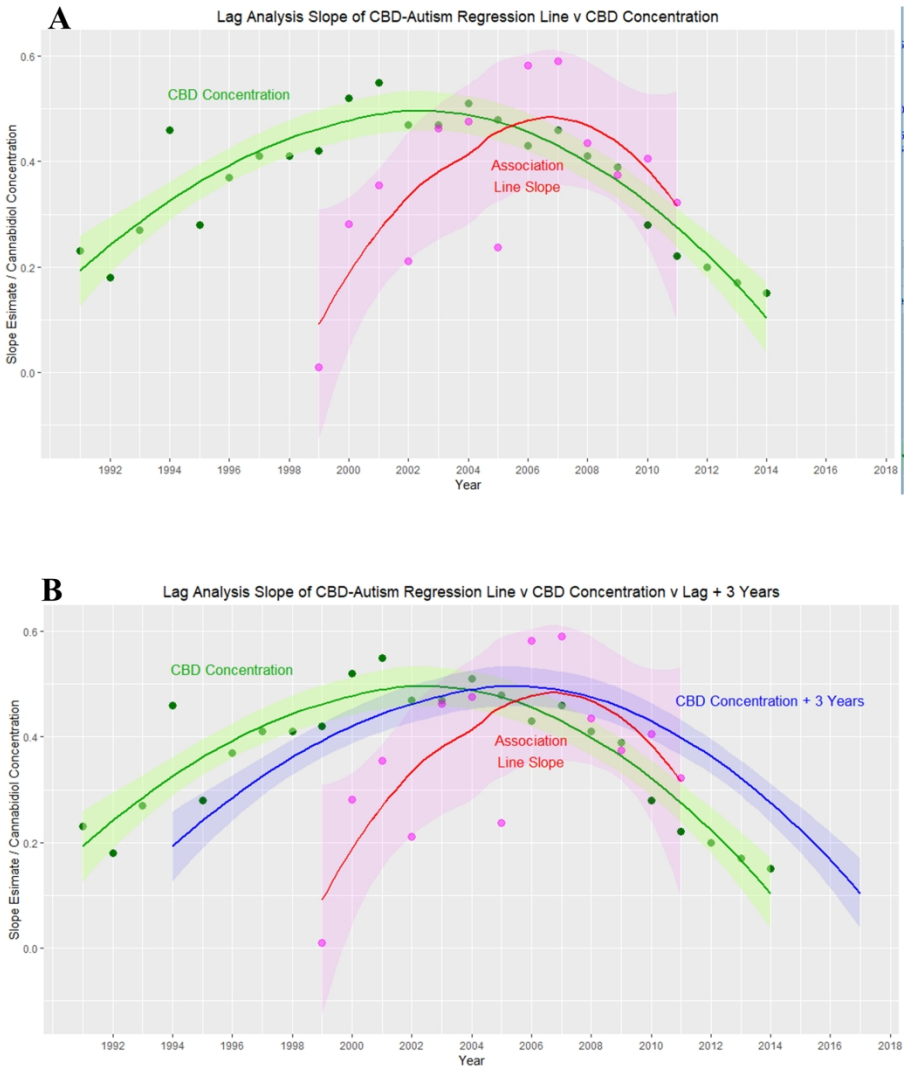

Figure 9.: Lag Analysis of Slope of Autism Spectrum Disorder Rate – Cannabidiol Concentration Association by Time and Group.

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Supplementary Table 1.:

Linear Regression of

*log(ASD Rate) ~ Time * State*

Model			
Adj. R Squ	F	df	P
0.9435	113.4	152,870	<2.0E-16

Parameter	Parameters			
	Est.	Std.Error	t value	Pr(>|t|)
Year	18.52111	2.22618	8.32	3.39E-16
(Year)^2	-16.22251	2.29552	-7.067	3.25E-12
StateAlaska	0.37091	0.08936	4.151	3.64E-05
StateArizona	0.48102	0.08936	5.383	9.44E-08
StateArkansas	0.40574	0.08936	4.54	6.41E-06
StateCalifornia	0.76522	0.09094	8.414	<2.0E-16
StateColorado	-0.46441	0.09336	-4.974	7.90E-07
StateConnecticut	0.96039	0.08936	10.747	<2.0E-16
StateDelaware	0.79471	0.09271	8.572	<2.0E-16
StateDistrict of Columbia	0.63511	0.10116	6.278	5.39E-10
StateFlorida	0.61185	0.09094	6.728	3.12E-11
StateGeorgia	0.50015	0.09094	5.5	5.01E-08
StateHawaii	0.61717	0.09094	6.786	2.13E-11
StateIdaho	0.35477	0.08936	3.97	7.78E-05
StateIllinois	0.46341	0.09094	5.096	4.26E-07
StateIndiana	1.01891	0.08936	11.402	<2.0E-16
StateKansas	0.40124	0.09094	4.412	1.15E-05
StateKentucky	0.259	0.09094	2.848	0.004504
StateLouisiana	0.5777	0.09094	6.352	3.42E-10
StateMaine	1.00518	0.08936	11.248	<2.0E-16
StateMaryland	0.75822	0.09094	8.337	2.95E-16
StateMassachusetts	0.7047	0.09094	7.749	2.58E-14
StateMichigan	0.87731	0.08936	9.817	<2.0E-16
StateMinnesota	1.3104	0.08936	14.664	<2.0E-16

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StateMissouri	0.83065	0.08936	9.295	<2.0E-16
StateMontana	0.21607	0.09188	2.352	0.018912
StateNebraska	0.40637	0.10065	4.038	5.88E-05
StateNevada	0.2466	0.09425	2.616	0.00904
StateNew Hampshire	0.2727	0.11217	2.431	0.015252
StateNew Jersey	1.05025	0.08936	11.753	<2.0E-16
StateNew Mexico	-0.28658	0.08936	-3.207	0.001391
StateNew York	0.95166	0.09094	10.464	<2.0E-16
StateNorth Carolina	0.94959	0.08936	10.626	<2.0E-16
StateOregon	1.31703	0.08936	14.738	<2.0E-16
StatePennsylvania	0.87929	0.09094	9.669	<2.0E-16
StateRhode Island	0.69	0.09094	7.587	8.41E-14
StateSouth Carolina	0.28578	0.08936	3.198	0.001434
StateSouth Dakota	0.42762	0.08936	4.785	2.01E-06
StateTennessee	0.39018	0.08936	4.366	1.42E-05
StateTexas	0.64041	0.09094	7.042	3.85E-12
StateUtah	0.33563	0.09094	3.691	0.000238
StateVermont	0.40357	0.09331	4.325	1.70E-05
StateVirginia	0.93286	0.08936	10.439	<2.0E-16
StateWashington	0.42881	0.09094	4.715	2.81E-06
StateWest Virginia	0.40826	0.08936	4.569	5.62E-06
StateWisconsin	0.70868	0.09336	7.59	8.20E-14
StateWyoming	0.2011	0.09251	2.174	0.029986
Year: Alabama	23.16522	3.01326	7.688	4.04E-14
Year: Alaska	17.84855	3.01326	5.923	4.54E-09
(Year)^2: Alaska	5.27944	3.06484	1.723	0.08532
Year: Arizona	17.9368	3.01326	5.953	3.82E-09
(Year)^2: Arizona	13.94352	3.06484	4.55	6.14E-06
Year: Arkansas	20.55062	3.01326	6.82	1.70E-11
Year: California	19.623	3.1483	6.233	7.13E-10
(Year)^2: California	9.56445	3.24635	2.946	0.003303
Year: Colorado	32.60208	3.29528	9.894	<2.0E-16
Year: Connecticut	16.97698	3.01326	5.634	2.38E-08
(Year)^2: Connecticut	10.05801	3.06484	3.282	0.001073
Year: Delaware	13.02494	3.22113	4.044	5.73E-05
Year: District of Columbia	14.38563	3.83666	3.75	0.000189

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(Year)^2: District of Columbia	16.51202	3.77657	4.372	1.38E-05
Year: Florida	13.79746	3.1483	4.383	1.32E-05
(Year)^2: Florida	11.52304	3.24635	3.55	0.000407
Year: Georgia	19.32476	3.1483	6.138	1.27E-09
(Year)^2: Georgia	6.81849	3.24635	2.1	0.035985
Year: Hawaii	15.45595	3.1483	4.909	1.09E-06
(Year)^2: Hawaii	7.47594	3.24635	2.303	0.021521
Year: Idaho	17.23331	3.01326	5.719	1.47E-08
(Year)^2: Idaho	8.95443	3.06484	2.922	0.003572
Year: Illinois	27.16754	3.1483	8.629	<2.0E-16
Year: Indiana	17.37134	3.01326	5.765	1.13E-08
(Year)^2: Indiana	6.1813	3.06484	2.017	0.044019
Year: Kansas	12.77052	3.1483	4.056	5.43E-05
(Year)^2: Kansas	9.32734	3.24635	2.873	0.004163
Year: Kentucky	22.26272	3.1483	7.071	3.15E-12
(Year)^2: Louisiana	16.23259	3.24635	5	6.92E-07
Year: Maine	23.93269	3.01326	7.942	6.11E-15
(Year)^2: Maine	8.17685	3.06484	2.668	0.007774
Year: Maryland	26.72503	3.1483	8.489	<2.0E-16
Year: Massachusetts	20.77978	3.1483	6.6	7.13E-11
(Year)^2: Massachusetts	19.59134	3.24635	6.035	2.35E-09
Year: Michigan	16.86518	3.01326	5.597	2.92E-08
(Year)^2: Michigan	6.01115	3.06484	1.961	0.05016
Year: Minnesota	21.39765	3.01326	7.101	2.57E-12
(Year)^2: Minnesota	9.07836	3.06484	2.962	0.003139
Year: Mississippi	19.74009	3.38571	5.83	7.79E-09
(Year)^2: Mississippi	9.78299	3.47687	2.814	0.005007
Year: Missouri	12.54726	3.01326	4.164	3.44E-05
(Year)^2: Missouri	13.41273	3.06484	4.376	1.35E-05
Year: Montana	13.48666	3.17938	4.242	2.45E-05
Year: Nebraska	18.14064	3.7782	4.801	1.85E-06
(Year)^2: Nebraska	10.84901	3.77089	2.877	0.004112
Year: Nevada	30.20513	3.38571	8.921	<2.0E-16
(Year)^2: Nevada	7.66277	3.47687	2.204	0.027791
Year: New Hampshire	30.29094	4.39234	6.896	1.03E-11
Year: New Jersey	16.82538	3.01326	5.584	3.15E-08

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(Year)^2: New Jersey	9.55946	3.06484	3.119	0.001874
Year: New Mexico	20.45319	3.01326	6.788	2.11E-11
(Year)^2: New Mexico	12.46387	3.06484	4.067	5.20E-05
Year: New York	11.24563	3.1483	3.572	0.000374
(Year)^2: New York	13.73906	3.24635	4.232	2.56E-05
Year: North Carolina	6.6465	3.01326	2.206	0.027662
(Year)^2: North Carolina	15.40161	3.06484	5.025	6.10E-07
Year: North Dakota	25.38383	3.1483	8.063	2.46E-15
(Year)^2: North Dakota	9.04935	3.24635	2.788	0.005427
Year: Ohio	39.22575	3.1483	12.459	<2.0E-16
Year: Oklahoma	16.3106	3.1483	5.181	2.75E-07
Year: Oregon	21.05384	3.01326	6.987	5.58E-12
Year: Pennsylvania	22.23269	3.1483	7.062	3.36E-12
(Year)^2: Pennsylvania	7.6986	3.24635	2.371	0.017935
Year: Rhode Island	27.95825	3.1483	8.88	<2.0E-16
Year: South Carolina	15.32255	3.01326	5.085	4.50E-07
(Year)^2: South Carolina	12.19256	3.06484	3.978	7.52E-05
Year: South Dakota	16.71716	3.01326	5.548	3.84E-08
(Year)^2: South Dakota	8.61742	3.06484	2.812	0.005039
Year: Tennessee	10.79004	3.01326	3.581	0.000361
(Year)^2: Tennessee	16.83085	3.06484	5.492	5.23E-08
Year: Texas	11.32636	3.1483	3.598	0.000339
(Year)^2: Texas	13.08558	3.24635	4.031	6.04E-05
Year: Utah	20.70804	3.1483	6.578	8.25E-11
(Year)^2: Utah	7.40697	3.24635	2.282	0.022752
Year: Vermont	24.38156	3.24698	7.509	1.48E-13
(Year)^2: Vermont	7.42617	3.25079	2.284	0.022587
Year: Virginia	10.41223	3.01326	3.455	0.000576
(Year)^2: Virginia	16.59357	3.06484	5.414	7.97E-08
Year: Washington	23.33216	3.1483	7.411	2.97E-13
(Year)^2: Washington	6.29793	3.24635	1.94	0.052702
Year: West Virginia	14.3677	3.01326	4.768	2.18E-06
(Year)^2: West Virginia	12.18968	3.06484	3.977	7.55E-05
Year: Wisconsin	28.44684	3.29528	8.633	<2.0E-16
Year: Wyoming	22.9849	3.22196	7.134	2.06E-12
(Year)^2: Wyoming	12.0022	3.33822	3.595	0.000342

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Supplementary Table 2.:

Cannabidiol Lines Slope v Cannabidiol Concentration

Year	Parameter					Model				Cannabidiol Concentration
	Parameter	Estimate	Std.Error	t value	Pr(>|t|)	Adj. R Squ	F	df	P	
1999	log(Cannabidiol_Exposure)	0.0085	0.2709	0.031	0.9750	-0.0204	0.001	1,49	0.9750	0.42
2000	log(Cannabidiol_Exposure)	0.2815	0.2632	1.070	0.2900	0.0029	1.144	1,49	0.2900	0.52
2001	log(Cannabidiol_Exposure)	0.3547	0.2471	1.435	0.1580	0.0208	2.060	1,49	0.1576	0.55
2002	log(Cannabidiol_Exposure)	0.2109	0.2651	0.796	0.4300	-0.0074	0.633	1,49	0.4301	0.47
2003	log(Cannabidiol_Exposure)	0.4634	0.2771	1.672	0.1010	0.0347	2.797	1,49	0.1008	0.47
2004	log(Cannabidiol_Exposure)	0.4758	0.2460	1.934	0.0589	0.0520	3.740	1,49	0.0589	0.51
2005	log(Cannabidiol_Exposure)	0.2376	0.2560	0.928	0.3580	-0.0028	0.861	1,49	0.3580	0.48
2006	log(Cannabidiol_Exposure)	0.5823	0.2228	2.613	0.0119	0.1063	6.828	1,49	0.0120	0.43
2007	log(Cannabidiol_Exposure)	0.5904	0.2294	2.574	0.0133	0.0646	4.382	1,49	0.0416	0.46
2008	log(Cannabidiol_Exposure)	0.4349	0.2078	2.093	0.0416	0.1049	6.623	1,49	0.0133	0.41
2009	log(Cannabidiol_Exposure)	0.3739	0.2074	1.802	0.0777	0.0430	3.248	1,49	0.0777	0.39
2010	log(Cannabidiol_Exposure)	0.4058	0.2064	1.966	0.0550	0.0553	3.867	1,49	0.0550	0.28
2011	log(Cannabidiol_Exposure)	0.3223	0.2159	1.493	0.1420	0.0240	2.228	1,49	0.1420	0.22

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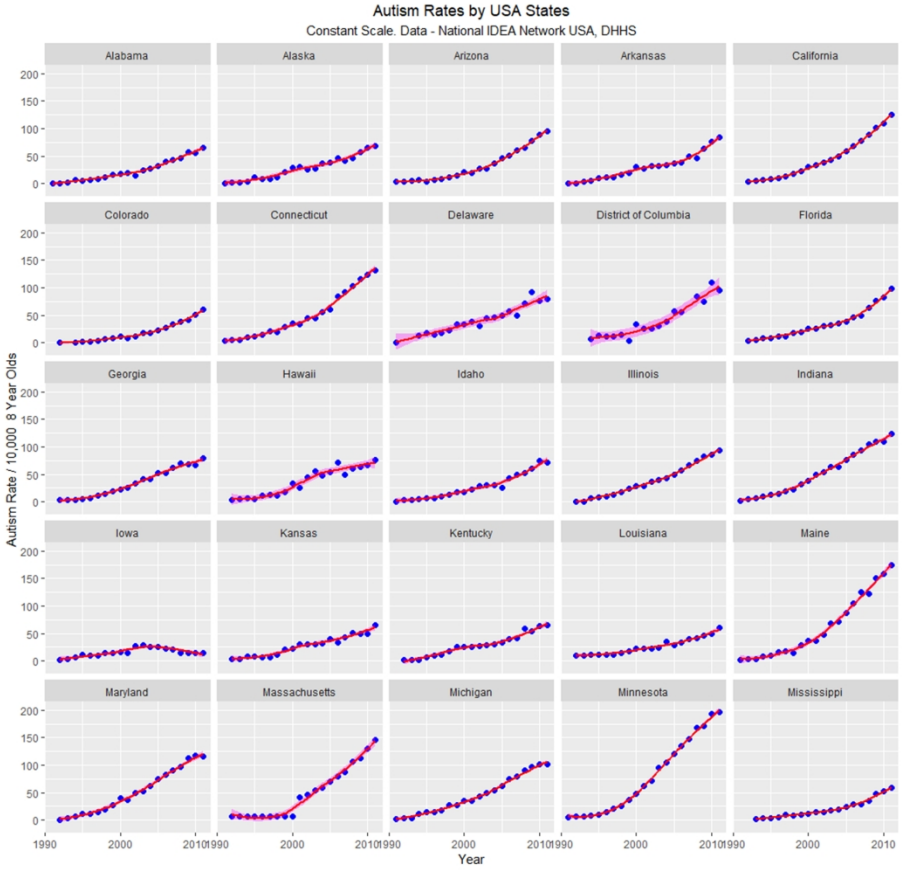

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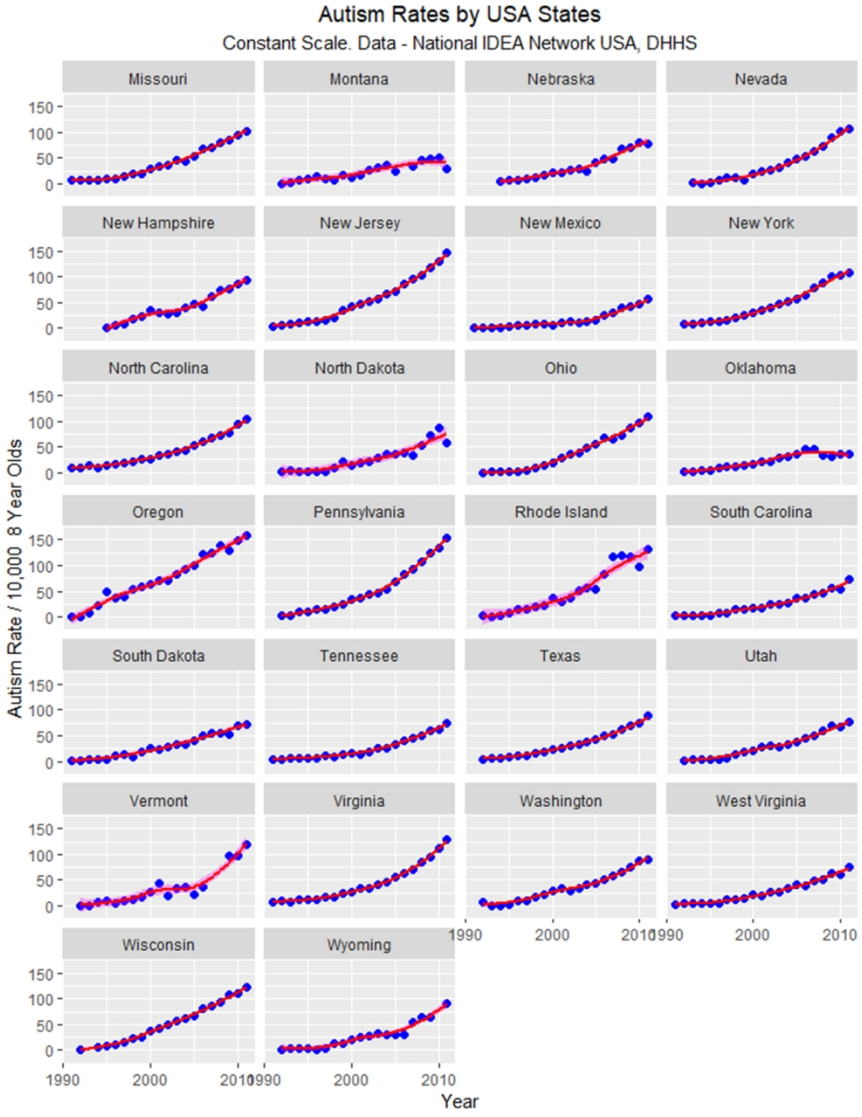

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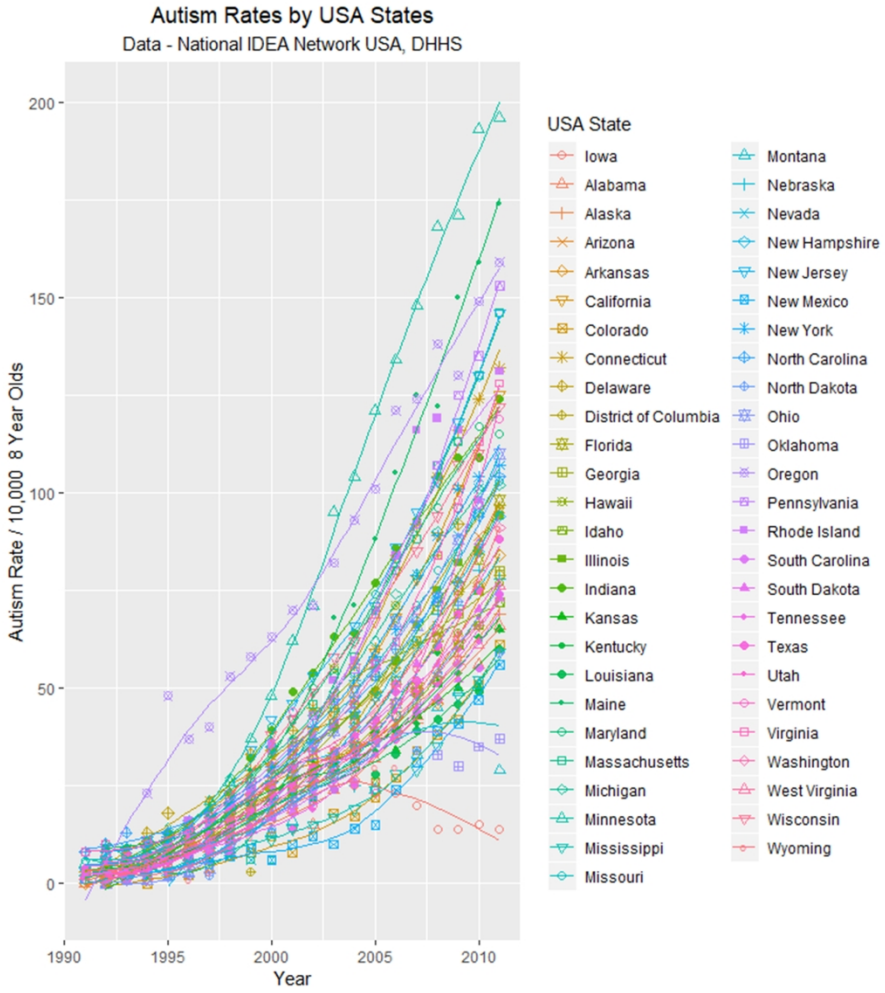

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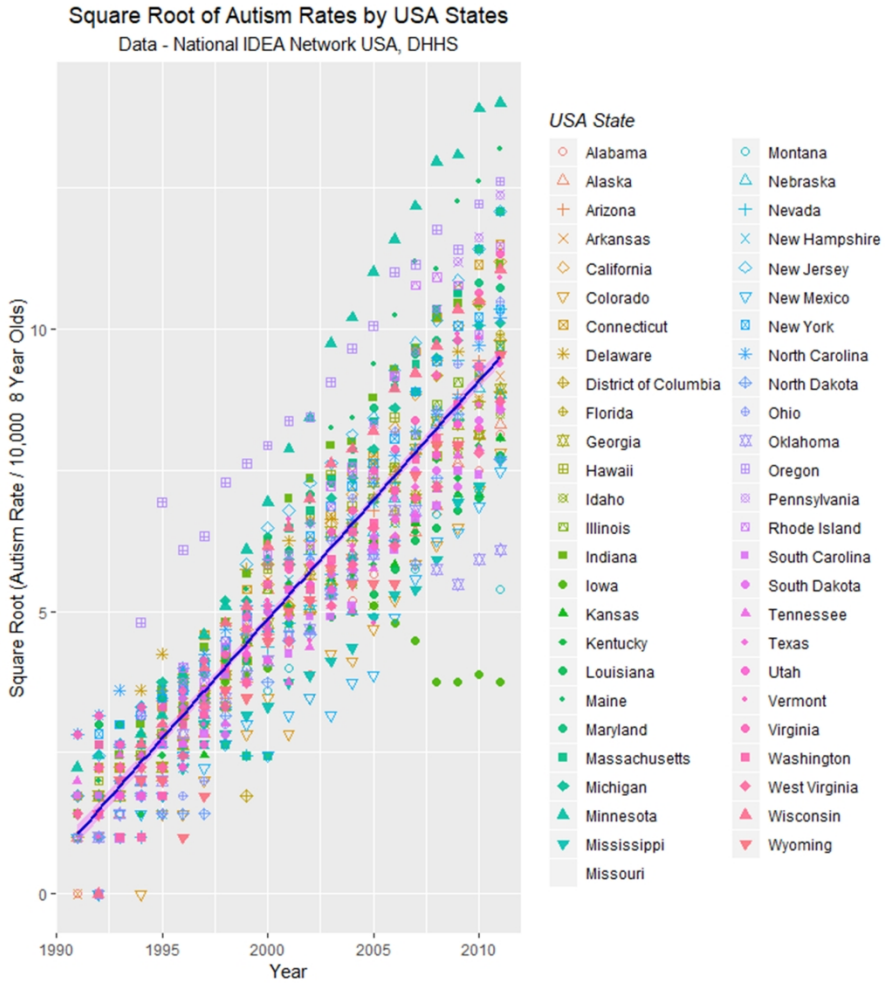

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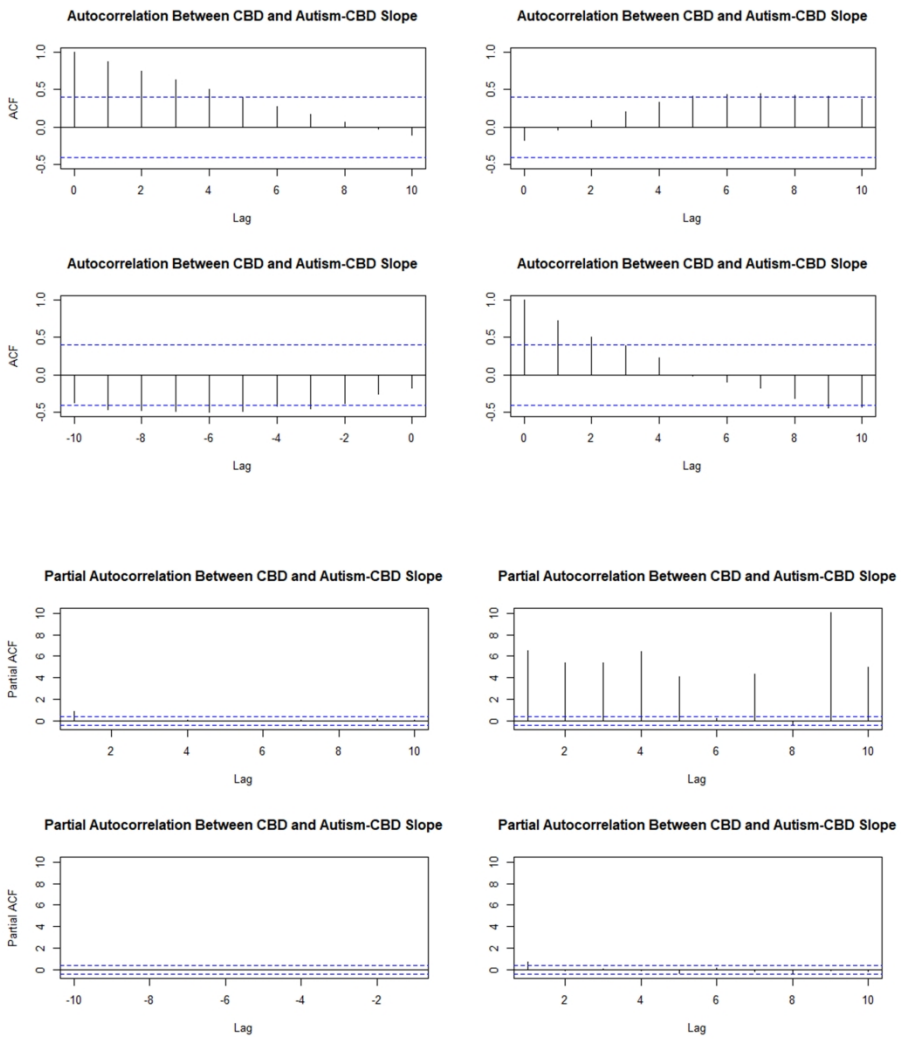

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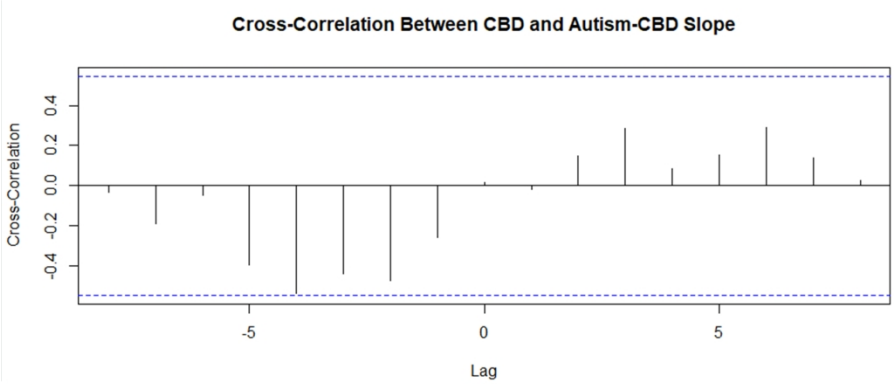

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STROBE Statement—checklist of items that should be included in reports of observational studies

Epidemiological Associations of Various Substances and Multiple Cannabinoids with Autism in USA

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract Title Page
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found Abstract Page
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported Introduction
Objectives	3	State specific objectives, including any prespecified hypotheses Introduction
Methods		
Study design	4	Present key elements of study design early in the paper Methods Section
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection Methods Section
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable Methods and Results
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group Methods and Results
Bias	9	Describe any efforts to address potential sources of bias Results
Study size	10	Explain how the study size was arrived at Results
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why Methods and Results
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding Results
		(*b*) Describe any methods used to examine subgroups and interactions Results
		(*c*) Explain how missing data were addressed Methods
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed Methods *Case-control study*—If applicable, explain how matching of cases and controls was

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	addressed
	Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy
	(*e*) Describe any sensitivity analyses Results

Continued on next page

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Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed Results and Tables
		(b) Give reasons for non-participation at each stage N/A
		(c) Consider use of a flow diagram N/A
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders N/A
		(b) Indicate number of participants with missing data for each variable of interest N/A
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) Results
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time Results
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included Results
		(*b*) Report category boundaries when continuous variables were categorized N/A
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses Results
Discussion		
Key results	18	Summarise key results with reference to study objectives Results, Discussion, Conclusion and Abstract
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias Discussion
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence Discussion and Conclusion
Generalisability	21	Discuss the generalisability (external validity) of the study results Discussion and Conclusion
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based Nil Funding supplied – mentioned in funding statement.

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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