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Outlining the Diverse Etiologies of Autism Spectrum Disorder

A Thesis submitted in partial satisfaction of the requirements for the degree of Master of Science

in

Cognitive and Information Sciences

by

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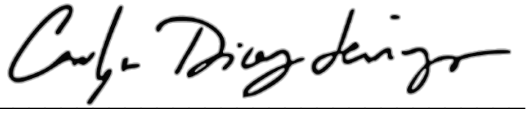

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Abstract

Outlining the Diverse Etiologies of Autism Spectrum Disorder

By Aramis D. Munoz-Valverde for the partial satisfaction of the requirements for the degree of Master of Science in Cognitive and Information Sciences, University of California, Merced, 2022
Dr. Carolyn Dicey Jennings, Chair

Autism Spectrum Disorder (ASD) is a disorder defined by the heterogeneity of its presentations, making diagnosis and treatment for those who need it difficult. Here I examine a set of causal mechanisms which lead to the development of ASD. I support the idea that within and between those mechanisms, what may look like a singular causal tale may instead account for a large variety of individual presentations. I examine how an understanding of the low-level neurobiological mechanisms underlying ASD allows us to begin unraveling the nature of the heterogeneity found in individual and sub-group presentations of ASD.

Understanding the diverse etiologies of ASD can facilitate diagnosis and treatment. It is therefore critical that our understanding of ASD is as nuanced as possible. Here I explore three precipitants of ASD: SHANK3 haploinsufficiency, pre-natal organophosphate exposure, and pre-natal neonicotinoid exposure. I explore why the precipitant is important, how disruption in the mechanisms relevant to the precipitant can lead to ASD, and how mitigating or agitating ancillary factors affect the likelihood of precipitation, severity of effect, and phenotype of presentation of ASD within subgroups and individuals.

Introduction

ASD is a developmental disorder of the mind characterized by the heterogeneity of its presentation. As a diagnostic measure, ASD is typically defined by the presence of a combination of the following symptoms; impaired social communication, repetitive behaviors, sensory anomalies, and/or highly restricted interests, typically beginning in early development (Lord, et al., 2020). These characteristics are not detrimental in and of themselves. Some individuals are affected positively by their ASD, in that their ASD presents in a manner useful to themselves or society. While others can experience significant decreases in their quality of life due to seizures (Liu, et al., 2021), intellectual disability, significant incompatibility with the people around them, or significant incompatibility with their social environment at large (Lipinski, Boegl, Blanke, Suenkel, & Dziobek, 2021).

For those individuals in which ASD presents detrimentally, there exists a pressing need for treatments and interventions. While psychosocial interventions can undeniably ameliorate deficits within language, social engagement, and joint attention for some, there remains a significant need for biochemical interventions for those with the most deleterious presentations of ASD (Lord, et al., 2020). As of the writing of this paper, there exist no pharmacological treatments for the core symptoms of ASD, and all pharmacological treatments which were evaluated within the last two years have shown either negative or ambiguous results in large randomized control trials (Baribeau, Vorstman, & Anagnostou, 2022).

The development of treatments and interventions for ASD has proven to be a non-trivial undertaking, due to the heterogeneity of the disorder and the sheer number of people who have it. The current estimate for ASD prevalence in 8-year olds in the United States is around 1 in 44, with prevalence being 4.2 times higher in boys than in girls (Maenner, Shaw, Backian, Bilder, & Durkin, 2021). The presentation of the disorder also varies widely with the population. A separate meta-analysis within the same study concluded that in those studies which evaluated IQ, 35.2% of individuals with ASD were classified as having an I.Q. below 70. This is significant as I.Q. scores below 70 are present in only 2.5% of the general population, and the observed decrease in I.Q. was not a uniform drop, indicating that I.Q. reductions are not consistently observed among individuals with ASD, but is instead a feature driven by a subset of the ASD population at large. This is also significant because intellectual disability, a condition with multiple deleterious effects on quality of life, is typically defined as having an I.Q. below 70, combined with deficits in intellectual functions affecting everyday life (American Psychiatric Association, 2013).

The current clinical and etiological understanding of ASD is not sufficient for the development of biochemical treatments for those who need them (Sauer, Stanton, Hans, & Grabrucker, 2021). Our lack of understanding here is not due to a shortage of research. There is an abundance of research characterizing ASD. The issue instead lies with the conflicting characterizations of ASD derived from that research. Findings on the etiology, prevalence, and general phenotype of ASD have consistently remained inconsistent, with results sometimes in outright opposition to each other (Pellicano, 2020). MRI examinations of temporal cortical thickness have shown that thickness is decreased (Wallace, Danker, Kenworthy, Geidd, & Martin, 2010), increased (Hardan, Muddasani,

Vemulapalli, Keshavan, & Minshew, 2006), and similar but more wrinkled (Ohta, et al., 2015) in people with ASD compared to controls. Neuroimaging studies have found that, in people with ASD, long range connectivity is increased (Supekar, et al., 2014), decreased (Yao, et al., 2021), and a mix of increased and decreased (Doyle-Thomas, et al., 2015).

The resolution of these inconsistencies in research findings is critical in the development of our understanding of and ability to treat ASD. A possible approach to resolving these inconsistencies is reducing the heterogeneity of ASD, by grouping subtypes of ASD along etiological or phenotypic lines, the underlying idea being that the seemingly conflicting information is actually reflective of distinct subtypes of ASD (Hertz-Picciotto, Schmidt, & Krakowiak, 2018). Research along these lines has seen some success, especially in finding diagnostic predictors for ASD severity (Wu Nordahl, et al., 2022). This type of research aims to determine clinically relevant discrete subgroups of ASD, allowing for the distinction, between etiological lines, of developmental pathways in ASD progression, and subsequent treatment along those lines (Emberti Gialloreti, et al., 2019).

In this paper I support the idea that what may appear to be conflicting evidence on the unifying characteristics of ASD may instead be evidence of a more complex, disparate structure within it. That it is not the case that ASD has indeterminate characteristics within it, but rather that there are multiple sub-groups within ASD, and that phenotypes within those subgroups are dependent on a myriad of factors which combined determine the nature of ASD within those subgroups and affected individuals.

To do so I will examine the etiology of ASD attributable to three precipitants, SHANK3 haploinsufficiency, pre-natal chlorpyrifos exposure, and pre-natal neonicotinoid exposure. I have decided to focus on these due to their well validated roles in precipitating ASD, the relative abundance of research examining their causal biomechanical pathways, and their clinical relevance when compared to other models of ASD. In the process I will touch upon factors which account for the significant variability in phenotypes within those mechanisms, and I will note how the phenotypes attributable to those precipitants varies generally.

Approach

In this paper I have taken an integrative biochemical approach, focusing on the interactions between chemicals and the networks of causal pathways which determine the overall function of neurons. I have taken this approach as it provides us with the ability to examine complex low-level interactions and allows us to examine nuances which prove to be important in the determination of ASD development. Furthermore, this approach is reflective of developing thought within ASD research, as the progress of research has made evident the importance of reducing the heterogeneity of ASD along mechanistic lines, and the examination of singular ASD precipitant mechanisms has been deemed to be “manifestly more tractable” than other approaches (Lord, et al., 2020).

As such, I will make heavy use of cellular, biochemical, and rodent models of ASD. Rodent models in particular must be cautiously examined when extrapolating to human diseases. There are disparities to be found, especially in scenarios where the complexity of the human brain plays a major role (Lacreuse, Bennet, & Dettmer, 2022). However, results from rodent models employed in human disease research are typically

somewhat analogous to the results expected from human models, especially when the manipulations of the experiment are to highly preserved systems like those involved in neural regulation (Perlman, 2016). Furthermore, most research utilizing rodents typically accounts for the expected disparities. Either though the selection of mice bred or genetically engineered for that use, or through one off manipulations to match the relevant bio-mechanical pathways (Navabpour, Kwapis, & Jarome, 2020). Most importantly, there is a vast amount of information to be derived from rodent models. The use of rodent models in neuroscience geared towards human disorders is widespread, due to ethical concerns, the similarities in low-level biomechanical systems between rodents and humans, and similarities in neuro-anatomical specializations in function found across most mammals. Nonetheless, I have made efforts to include only rodent models which are directly relevant to the topic of discussion and have not evaluated data from generalized mouse models of ASD or other animal models of ASD.

The selection of the three precipitants, SHANK3 haploinsufficiency, pre-natal chlorpyrifos, and pre-natal neonicotinoid exposure, must also be justified. I want to make clear that there are a large set of factors and mechanisms which are associated with the development of ASD, and that my decision to address these three is not due to a belief that those other factors and mechanisms are not important or valid.

These precipitants were instead selected in accordance with the aims of this paper. There are three reasons for the decisions underlying the selection of this particular set of precipitants. The first reason is that the main idea of this paper, that ASD is not a single disorder caused by the dysfunction of a single underlying mechanism, is best demonstrated by the contrast between ASD attributable to a genetic disorder and ASD attributable to chemical exposures. As such, the inclusion of at least one genetic precipitant was necessary.

The second reason is that the importance of considering the etiology of ASD in the development of treatments and interventions requires both that the precipitant be well understood mechanistically, and that the resulting ASD constitutes a disease state. Meaning that we must only consider those precipitants which reliably contribute to harmful outcomes, which are amenable to mechanistic characterizations, and would benefit from pharmacological treatments.

The third reason is that, in order to demonstrate how the heterogeneity of ASD arises, there should be evidence that heterogeneity caused by other factors is also attributable to the changes induced by the ASD precipitant. This is a necessary as the heterogeneity in ASD phenotypes should be attributable to the causes underlying ASD in order to be useful. For example, it would be pointless to show that hemlock consumption in people with ASD leads to death, as hemlock consumption leads to death for all people, not just those with ASD. This requires that the ASD attributable to the precipitant demonstrate some degree of heterogeneity, that there are factors which can reliably cause that heterogeneity, and there exists a causal chain by which the heterogeneity caused by those factors can be explained.

ASD Precipitants and Factors in ASD Phenotype

SHANK3 Haploinsufficiency

SHANK3 is a scaffolding gene whose protein localizes in the postsynaptic density of neurons in the brain. SHANK3 proteins, which are the end result of the transcription of

SHANK3 genes, have a positive effect on the genesis and maturation of dendritic spines in neurons and are therefore an important component in the development of neurons and neural networks. They interconnect receptors in the postsynaptic membrane and play a role in the growth and maintenance of the neural actin cytoskeleton (GeneCards: The Human Gene Database, 2022). SHANK3 mutations are present in 0.69% of patients with ASD and up to 2.12% of patients with ASD and moderate to severe intellectual disability, making these mutations a significant, if not large, determinant of detrimental ASD development.

In those patients with genomic rearagements of SHANK3, all demonstrated SHANK3 haploinsufficiency[1] (Leblond & et.al., 2014), and of those with SHANK3 haploinsufficiency, more than 80% demonstrated ASD or ASD-like behavior (Betancur & Buxbaum, 2013). The deletion or mutation of one (or both) of the copies of SHANK3, and the subsequent reduction in the overall amount of fully functioning SHANK3 protein at the synapse, is highly correlated with the development of ASD (Lipiainen, Song, & Lin, 2022). The reliability with which SHANK3 haploinsufficiency leads to ASD is important to note, as there are more than 1000 genes that have been implicated in the development of ASD, but very few of those genes can be said to directly precipitate ASD independent of other factors (Ayhan & Konopka, 2019).

The direct line between SHANK3 haploinsufficiency and the presentation of particularly debilitating presentations of ASD must be noted, as that is one of the major reasons for its inclusion here. The majority of SHANK3 haploinsufficient patients display neo-natal hypotonia, moderate to severe intellectual disability, absent to severely delayed speech, and minor dysmorphic features (Phelan & McDermid, 2011). SHANK3 haploinsufficiency also leads to brain malformations and seizures (Betancur & Buxbaum, 2013).

Function and Relevant Mechanisms

The effect of SHANK3 mutations on neurons is highly variable, with extracellular nutrients (Schoen, et al., 2019) (Serret S, 2015), deficit localization in the brain (Bey, et al., 2018) (Wang, Xu, Bey, Lee, & Jiang, 2014), development conditions (Peixoto, Wang, Croney, Kozorovitskiy, & Sabatini, 2016), hormones (Berkel, et al., 2018), cell type (Bey, et al., 2018) (Taesun, Heejin, Haram, Jiseok, & Eunjoon, 2019), mutation type (Durand, et al., 2012) (Durand, et al., 2007) (Wang, et al., 2011) (Yong-Hui & Michael D, 2013), and other genes (Duffney, et al., 2015) (Ivashko-Pachima, Ganaiem, Ben-Horin-Hazak, Lobyntseva, & Fischer, 2022) playing a significant role in the resulting phenotype of SHANK3 mutations.

Generally, SHANK3 haploinsufficiency appears to precipitate its deleterious effects through reductions in the developmental capacity of growth cones, reductions in synaptic receptors, reduced spine maturation, and the elimination of synaptic scaling and intrinsic homeostatic plasticity.

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[1] Haploinsufficiency refers to conditions where a single fully functioning and typical copy of a gene is not sufficient to produce the typical set of observable characteristics or function in an organism. SHANK3 haploinsufficiency here is shorthand for the lack of normal functioning caused by the deletion or loss of function mutation of one copy of the gene.

Homeostatic Regulation: Synaptic Scaling and Homeostatic Plasticity

A lack of homeostatic regulation, in this case meaning the elimination of a neurons ability to regulate it's own excitability through synaptic scaling and homeostatic plasticity, has clear implications for the development of seizures (Mulroe, et al., 2022). This is because seizures are fundamentally runaway synchronous propagations of electrical discharges through neural networks (World Health Organization, 2022), and a network composed of neurons which are incapable of downregulating their activity in response to prolonged firing, are likely more liable to exhibit runaway propagations.

In mouse V1 cortical neurons, SHANK3 haploinsufficiency precipitates the loss of homeostatic compensation functions. A loss of 50% of operational SHANK3 protein abolishes synaptic scaling, the ability of a neuron to react to prolonged firing or lack of firing by decreasing or increasing the strength of its synapses all at once by the same multiplicative factor. It also abolishes intrinsic homeostatic plasticity, meaning that neurons with SHANK3 haploinsufficiency are not able to increase or decrease their own excitability via dendritic or somatic changes. The reason for this is that the accumulation of AMPAr, a protein/receptor whose accumulation drives activity induced changes to synaptic strength (and is therefore critical for both intrinsic homeostatic plasticity and synaptic scaling functions), is functionally inhibited. Reinstatement of SHANK 3 function rescues these functions overall, as does the administration of lithium, (Tatavarty, et al., 2010) which is discussed in the lithium section below.

The Role of Zinc

In order to understand how zinc, an elemental nutrient, can affect the severity of presentation of ASD caused by SHANK3 mutations, we must first appreciate that the mutations only matter insofar as they cause an overall reduction in functional SHANK3 proteins. Mutations in SHANK3 genes lead to the transcription, and subsequent production, of SHANK3 proteins which are not fully functional. As such, SHANK3 genes, in this context, are only a factor relevant to ASD due to the protein end product of their transcription, SHANK3 protein. SHANK3 protein is only relevant to ASD due to the functions that SHANK3 protein plays a role in. So, it is a reduction of the fulfilment of that functioning that is the real problem. Zinc is important because it permits SHANK3 proteins to fulfill their functions, and zinc deficiency reduces the functioning of SHANK3 proteins.

SHANK3 proteins contain a protein interaction module which is critical to its capacity to localize at the postsynaptic density of neurons. This module also binds zinc. Zinc is generally important in neurons; it is densely localized in dendritic spines and governs synaptic transmission and plasticity (Grabrucker, et al., 2011). Zinc is important to the proper function of the SHANK3 protein, as it appears to be necessary for SHANK3 to properly form complexes with other proteins like HOMER1 and AMPAr, possibly due to structural changes induced in SHANK3 proteins by zinc binding to it. Without zinc, SHANK3 proteins fall into an inactive state, where they do not perform their necessary structural functions, and so deleteriously affect the mechanisms which are dependent on that structure, namely the interactions with other proteins which that structure facilitates (Arons, et al., 2016).

If we recall that the main issue in SHANK3 haploinsufficiency is the reduction of functional SHANK3 proteins, it becomes evident that ASD caused by SHANK3

haploinsufficiency can be exacerbated by zinc deficiencies. This is because SHANK3 protein requires zinc in order to function, and if there is a deficiency of it, the overall amount of *operational* SHANK3 protein is further reduced. The first reduction being caused by the loss of function mutation, and the second reduction being caused by deficiency related inactivity on otherwise functioning proteins. Further loss of operational protein can also result from zinc depletion during development, as SHANK3 is downregulated in the synapses of mice deprived of zinc in-utero. This downregulation is ameliorated by the administration of zinc post-delivery (Grabrucker, et al., 2011).

It is worthwhile to note that twins born under the same roof, with slightly different food preferences, could present with ASD in significantly different ways, with the twin preferring, for example, white meat (which is low in zinc) presenting with more significant ASD symptoms than the one preferring red meat (which is high in zinc). One could also extrapolate to communities where malnutrition is common. ASD in SHANK3 haploinsufficient people in this setting would likely present more severely than it would in individuals with no such malnutrition.

The Effects of Lithium Administration

In patients with certain subtypes of SHANK3 haploinsufficiency and ASD, progressive loss of learned abilities and catatonia were reversed by the administration of lithium (Serret, et al., 2015). Lithium appears to have induced this reversal by increasing SHANK3 synthesis. SHANK3 synthesis is increased with the administration of lithium in neurons, with an up to six-fold increase in SHANK3 at synapse from a baseline of haploinsufficiency (Hélène, et al., 2016), the mechanism by which this occurs has not yet been fully outlined. In this section I will outline what is known about the effects of lithium and its role in SHANK3 production and propose a possible mechanism by which it occurs.

Lithium administration results in increased mTOR phosphorylation[2] (activation) (Malhi, Tanious, Das, Coulston, & Berk, 2013). The mechanisms by which lithium causes that increase of mTOR phosphorylation are understood, but will not be covered here (Tye, et al., 2022).

mTOR is an important, evolutionarily conserved, protein which regulates protein synthesis in response to a wide range of stimuli (Hay & Sonenberg, 2004). In neurons, increases in mTOR activity via increased phosphorylation have been shown to impair autophagy, impair developmental spine pruning, and increase dendritic spine density.

Recent studies have determined that SHANK3 is a regulator of mTOR phosphorylation. As SHANK3 activity increases, so does mTOR phosphorylation (Chaudry & Vasudevan, 2022). Consequently, SHANK3 haploinsufficiency results in significant decreases in mTOR phosphorylation.

So, I have not yet gotten to how lithium increases the overall amount of SHANK3 protein. This is where the current understanding of SHANK3, lithium, and mTOR stands as of the writing of this paper. Related in some manner, with the mechanism by which

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[2] Phosphorylation is the chemical addition of a phosphoryl group to a molecule. The phosphorylation of a molecule enables that molecule to induce changes in other molecules. As such, the phosphorylated version of a molecule is generally considered to be the “active” version of said molecule (Cohen, 2000).

they are related remaining unclear. Due to the many possible pathways by which lithium affects neurons, a unifying factor is required in order to suggest how these all may fit together.

A paper examining the role of mTOR in the chain of events leading to the detrimental effects on neural development stemming from ethanol use in mice, evaluated the mechanism by which ethanol causes changes in protein expression, among those SHANK3 expression. Ethanol was known to lead to increases in SHANK3 expression. In evaluating the importance of mTOR in these increases, these authors determined that mTOR regulates SHANK3 expression, finding that ethanol induced increases in SHANK3 expression were dependent on mTOR phosphorylation, as the deactivation of mTOR proteins led to the negation of SHANK3 protein increases in neurons treated with ethanol (Montesinos, Pascual, Millan-Esteban, & Guerri, 2018). The demonstration of a role for mTOR in the regulation of SHANK3 expression indicates a possible mechanism by which lithium increases SHANK3 expression. That mechanism is outlined in figure 1 below.

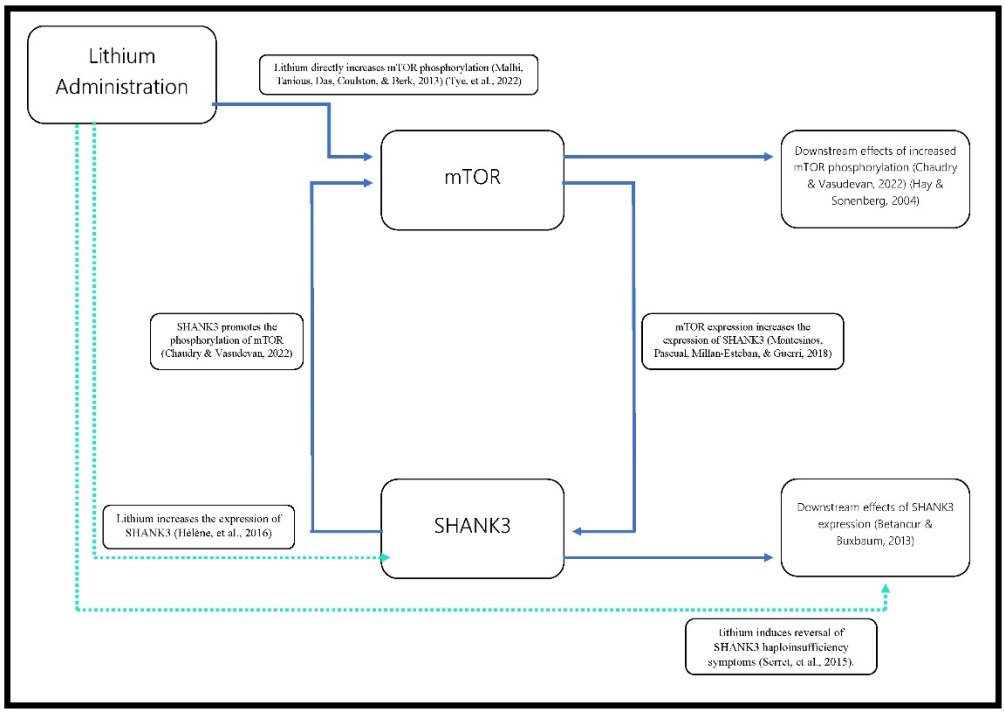

Figure 1: A possible mechanism by which the administration of lithium could induce increases in SHANK3 protein expression and reverse the symptoms of SHANK3 haploinsufficiency seen in the reversal cases. Causal mechanisms which are hypothesized to constitute the mechanism are in solid blue, while evidence suggesting those mechanisms are in dotted teal.

To the best of my knowledge, this is the first time that this specific mechanism has been outlined. While the relevance and existence of this mechanism is speculative and should be taken as such, the need to constrain the search space within the

experimental sphere makes its outline worthwhile (Lord, et al., 2020). Moreover, the fact that there is experimental evidence for each individual interaction, as well as experimental results which could be explained by this mechanism[3], warrants some degree of cautious speculation.

Due to the large amounts of lithium necessary to produce the dramatic reversal of symptoms seen in the reversal cases, the ameliorating effects are not liable to be induced via dietary consumption of lithium, and are more likely to be a factor in those people who were diagnosed with other mental illnesses linked to SHANK3 haploinsufficiency and subsequently treated with lithium. As the utility of lithium in treating SHANK3 related ASD has only been demonstrated in very recent case studies, the availability and access to capable doctors is also likely to play a role in differential outcomes, where those without access to capable doctors are less likely to be adequately treated and adequately diagnosed.

Brain Area Dependent Changes and Complicating Factors

The effects of SHANK3 haploinsufficiency are not the same across all neurons. The heterogeneity of SHANK3 haploinsufficiency induced changes in the brain are important, as that heterogeneity can be used to explain the heterogeneity of findings found in ASD generally construed. This heterogeneity must also be accounted for when evaluating symptom etiology, possible interventions, and interactions with other factors in the environment. The following are examples of such factors and are meant to illustrate the heterogeneity possible within a monogenetic precipitant of ASD, SHANK3 haploinsufficiency.

When SHANK3 is mostly knocked out in mice, neurons exhibit less receptors in general and decreased spinal density. Dendritic length is longer for knockout mice neurons in the basal ganglia striatum and cortical striatal synapses. There is reduced postsynaptic signaling, meaning signals propagate through neurons normally, but the post-synaptic neuron isn't likely to continue propagating the signal. The signal amplitude for fields of neurons is significantly lower for SHANK3 knockout rats, as is the frequency of firing. This change is present only in striatal synapses and is absent in cortical-striatal synapses (Peça, et al., 2011). This is to be contrasted with the increases in signal propagation in V1 neurons discussed in the section on homeostatic regulation above.

Increased caudate volume has been proposed to be linked with repetitive behaviors and there was a small but significant increase in volume for the structure in the same SHANK3 knockout mice. (Peça, et al., 2011) These mice were anti-social and groomed excessively. It is important to note that one of the factors of ASD diagnosis is the presence of repetitive behaviors, and that not all cases of ASD present with repetitive behaviors. As such, it is important to distinguish precipitants which reliably lead to the development of such symptoms.

It is also important to note that not all of the effects of SHANK3 haploinsufficiency are described here, and that our understanding remains lacking in some areas, namely those attributable to effects on long term development. SHANK3

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[3] The ability of lithium to increase SHANK3 expression (Hélène, et al., 2016).

haploinsufficiency causes developmental issues not immediately attributable with its deregulation, due to its indirect impacts on neural developmental pathways. For example, early cortical hyperactivity induced by complete SHANK3 deletions can cause a compensatory increase in the synaptogenesis of striatal spiny projections in neurons during development. This increase leads to cortico-striatal hyperconnectivity, and does not appear to generalize to other, non-coupled brain areas. The tight coupling of separate brain areas during early development can cause circuit dysfunction later in development, even in brain areas that are less liable to exhibit the detrimental effects of SHANK3 haploinsufficiency, due to lower basal expression of the gene (Peixoto, Wang, Croney, Kozorovitskiy, & Sabatini, 2016). This is important as it illustrates the complex developmental pathways by which ASD develops, an important consideration given that these experiments were conducted in mice. As mentioned before, mice models can offer an incomplete picture of the development of disorders, when there are complex long-term effects involved, effects which a mouse model is not liable to completely capture (Lacreuse, Bennet, & Dettmer, 2022).

Chlorpyrifos

Chlorpyrifos (CPF), an organophosphate pesticide, was the most widely used agricultural pesticide in the United States, with 6 to 10 million pounds of the pesticide being sprayed on fruits and vegetables across American farms per year (Lerner, 2017). Its use in agriculture in the United States was banned in 2021 after more than a decade of litigation and governmental reversals (Romo, 2021). Despite the prevalence of regulations against its use, CPF is still used in developing countries (Bayoumi, 2022).

The residential ban in 1998 and subsequent agricultural ban in 2021 were due to the significant developmental risks associated with early fetal exposure (Centers for Disease Control and Prevention, 2017). Perinatal exposure to organophosphates is a significant risk factor to the development of ASD and other neurodevelopmental disorders. A landmark study of maternal residence, organophosphate pesticide application, and likelihood of ASD development, found that any chronic fetal exposure to organophosphate pesticides leads to a 60% increase in ASD likelihood. If exposure is early in pregnancy, the increase in likelihood is 330%. If the fetus resides within 500 meters of an agricultural site, there is a 760% increase in the likelihood of ASD (Roberts, et al., 2007). Exposure in the first year of life increases the odds of ASD with comorbid intellectual disability by up to 30 percent (von Ehrenstein, et al., 2019).

CPF exposure does not affect neural systems in a straightforward way. The agrichemical industry has typically denied the toxicity of CPF in humans by ignoring its downstream effects (Lerner, 2017). Scientists working for the agrichemical industry claim that CPF primarily damages neurons by the direct inhibition of the enzymatic activity of acetylcholine esterase and the subsequent accumulation of acetylcholine to toxic doses, and is therefore only detrimental at occupational levels[4] or when mishandled (Gibson, Peterson, & Shurdut, 1998). This is incorrect. A closer examination reveals a

[4] It is worthwhile to note that the EPA is required to set tolerance levels considering the overall exposure of a chemical from sources including drinking water, residential settings, and food, but not occupational exposure (Centner, 2018).

multiplicity of causal chains, the whole of which is responsible for the detrimental effects attributable of CPF exposure. The enzymatic disruption of cholinergic activity is not the whole story. The toxicity of CPF is due to a variety of concurrent effects and disruptions on neural systems and sub-systems, cholinergic disruption included.

Function and Relevant Mechanisms

Acetylcholine

While CPF's effects on neural systems are not solely due to its effects on cholinergic activity, it is still worthwhile to examine how CPF affects cholinergic activity. As even within this mechanism there are important nuances governing CPF's effects on neural function. Here I examine the downstream effects of CPF exposure at low doses, and some important nuances which make exposure to low doses of CPF relevant to neural development in humans. I will examine the effects of CPF metabolization, and non-enzymatic inhibition of AChE. The examination of those will also allow us to understand the role that some genes have to play in determining the effects of CPF exposure later on.

At large doses, CPF directly inhibits the enzymatic activity of acetylcholine esterase (AChE), an enzyme which promotes axonal growth in developing neurons, and breaks down acetylcholine[5]. The accumulation of acetylcholine caused by CPF leads to the hyperactivation of neurons by acetylcholine and subsequent organism death, that is why it is useful as a pesticide. In humans, acute poisoning by CPF can lead to seizures, paralysis, and death by lung failure. However, for this to occur in humans requires massive doses of CPF, and while accidental acute exposures of this nature have led to human deaths in some cases, these are very rare.

Still, the effects of CPF can be appreciated at very small doses. An examination of neurons exposed to CPF found that neurons treated with CPF exhibited the effects of inhibited AChE[6] at doses far lower than would be expected from CPF on its own (Eaton, et al., 2008). The reason for the discrepancy is that the primary metabolite (meaning the main product of a chemical transformed by metabolic chemical reactions) of CPF, chlorpyrifos-oxon (CPFO), is 100 times more potent in inhibiting AChE's enzymatic activity than CPF in developed neurons. CPFO's effectiveness in disrupting AChE's enzymatic function is further heightened in developing neurons (Monnet-Tschudi, Zurich, Schilter, Costa, & Honegger, 2000), where it is 1000 times more effective in disrupting AChE's enzymatic activity. CPFO is more effective in inducing changes within neurons because it is more chemically reactive and more water soluble than CPF[7] (Flaskos, 2012). The increased potency that the primary metabolite of CPF, CPFO, has in detrimentally affecting neural functioning, illustrates the importance of considering

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[5] Acetylcholine is a neurotransmitter with a diverse set of effects on neural plasticity and excitability. The primary mode of action of CPF in insects is neuron death precipitated by excess synaptic acetylcholine.

[6] The effects of inhibited AChE in neurons are the accumulation of acetylcholine, increased glutamate, a generally excitatory neurotransmitter, and increased calcium ions.

[7] CPFO is CPF, but with an oxygen atom at the place where a sulfur atom would be in CPF. Needless to say, oxygen is more chemically reactive than sulfur.

metabolites in determining the overall effect of a chemical within a given biological system.

CPF and CPFO disrupt more than just the enzymatic activity of AChE, they also inhibit the morphological functions of AChE, with even lower levels of CPF and CPFO inducing morphological deficiencies in AChE, leading to a reduction in axonal length by around 20-30 percent (Yang, et al., 2008). Moreover, the fact that CPF exhibits effects at such low doses makes evident the fact that CPF is not just toxic at the large doses typically seen in occupational exposure, and that CPF affects neural functioning by more than one mechanism.

The low doses at which CPF can detrimentally affect neural development suggests that non-residential exposure to CPF can also be a factor contributing to the development of ASD. Studies of CPF exposure through breastmilk in infants in India have found doses of CPF up to 150% higher than the highest allowable dose at the time, in mothers who were not employed at an agricultural site, with other pesticides known to cause developmental deficiencies also present at levels higher than the permissible levels at the time (Sanghi, Pillai, Jayalekshmi, & Nair, 2003). In the United States, levels of CPF in water and foods have occasionally been found to exceed permissible concentrations (Centner, 2018). Moreover, a plurality of studies evaluating ASD development in relation to pesticide exposure have shown that the presence of pesticide metabolites in urine during critical periods of development is positively correlated with the development of ASD, in a dose dependent manner (Roberts, Dawley, & Reigart, 2019). However, it must be noted that non-residential CPF exposure has not been adequately associated with ASD development along epidemiological lines. This is because studies which evaluated CPF metabolites in urine also found a variety of other pesticides known to cause neuro-developmental deficiencies, and various pesticides can metabolize into the same metabolite (Morgan, et al., 2011) (Buckley, et al., 2022). So, while a plurality of studies has found a correlation between ASD development and exposure to pesticides attributable to non-residential sources generally, these increases cannot and likely should not be solely attributed to CPF.

Toxicant Co-Exposure

As ASD almost always develops in conjunction with exposure to other chemicals, it is important to evaluate how toxicant co-exposure can determine the presentation of ASD, and how co-exposures can increase the heterogeneity attributable to ASD broadly. The exposure to other toxicants and mechanisms with similar effects can increase the severity of phenotypic presentation at the level of neurons above and beyond what each toxicant would do by itself. When CPF is co-administered with Lead (II) chloride (Pb^2+) and Bisphenol-A(BPA) at biologically realistic concentrations[8], the detrimental effects on cell viability, neurite outgrowths, and number of synapses are heightened (Pisollato, et al., 2020). On its own, BPA, a common additive in plastics, reduces neurite outgrowth, neuron viability, and neurite outgrowths (at concentrations of 10^-7 M to 10^-

[8] Biologically relevant concentrations means at levels that could and have been found in developing humans, as determined by concentrations found in umbilical cord blood, children's blood, breast milk, and other such samples.

5 M). In terms of signaling, BPA upregulates glutamate and increases intercellular Calcium (Ca^2+) levels, leading to neurons which are more excitable and release more glutamate, a generally excitatory neurotransmitter, at the post-synaptic density (Wang, Change, Aguilar, Dong, & Hong, 2019). Lead on its own leads to reductions in synaptic efficiency, impaired synaptic morphology, and increases neural Ca^2+ concentrations by disrupting synaptic vesicle dependent transport (Gąssowska, et al., 2016). The synergistic effects of co-administrated Pb^2+, BPA, and CPF are due to their similar effects on Ca^2+ and synaptic efficacy, albeit via somewhat different avenues.

The effect of toxicant co-administration can also ameliorate CPF's effects. If polychlorinated biphenyl-138 (PCB-138), an environmental toxicant, is co-administered with CPF and Methylmercury (another developmental toxin), neurite growth is instead promoted (by about 17%), as is the total number of neurons (by about 55%) (Pisollato, et al., 2020). While it is clearly not advisable to prescribe neurotoxin cocktails to patients, it is useful to understand that the detrimental effects of developmental toxins like CPF can be ameliorated by counterbalancing with other chemicals. This implies that treatments can indirectly remediate the overall phenotype of ASD attributable to a single precipitant by counterbalancing against its downstream effects. This possibility was also noted by the authors (Pisollato, et al., 2020).

cAMP response element-binding protein and ASD symptoms

cAMP response element binding protein (CREB) is a cellular transcription factor, meaning that it up-regulates or downregulates the expression of genes, mainly those important to neural development and synaptic function, by binding to DNA. CPF increases the amount of pCREB, the active (phosphorylated) form of CREB, without increasing CREB itself, by 300-400% (Schuh, Lein, Beckles, & Jett, 2002). The precise mechanism by which CPF increases pCREB is not yet properly understood. (Narasimhamurthy, Andrade, & Mumbrekar, 2022) (Eaton, et al., 2008).

Regardless of how CPF upregulates pCREB, the fact that pCREB is upregulated can possibly account for a significant portion of CPF induced ASD symptoms. While there is a deficiency in studies examining pCREB on its own, transgenic studies regulating the overall expression of CREB genes into CREB protein can be used as a proxy for the regulation of pCREB. This is because the very fact that there are changes induced by the manipulation of CREB implicates the involvement of pCREB (Lopez de Armentia, et al., 2007), as CREB primarily induces downstream changes through the interactions induced by its phosphorylated form (Cohen, 2000). As such, those studies examined below which note the effects of the manipulation in CREB can be cautiously interpreted to reflect the manipulation by proxy of pCREB.

Upregulation of CREB increases CA1 pyramidal neuron excitability, leading to sporadic epileptic seizures, significant loss of hippocampal neurons, and significant loss of CA1 neurons in mice. Reversal of CREB upregulation ameliorated the hyperexcitability of the remaining neurons in said mice (Lopez de Armentia, et al., 2007).

Around 67-89% of children with ASD report sleep issues, usually delayed sleep onset and fragmented sleep patterns (Wu, et al., 2020). pCREB is involved in sleep wake cycle dependent transcriptional changes. pCREB induces transcriptional changes dependent on sleep wake cycles in the suprachiasmatic nuclei, peaking during the mid to late night, and reacting to light stimulation only during the subjective night (Obrietan,

Impey, Smith, Athos, & Storm, 1999). Although there is a severe need for further investigation, there is some preliminary evidence suggesting the ratio between pCREB, CREB, and other signaling proteins is a significant driver of sleep disturbances. Mice genetically modified to not produce pCREB demonstrated difficulties in adapting to novel light cycles, fragmented sleep, reduced adaptation to light generally, and when light cues were removed, the modified mice delayed sleep onset for longer into the subjective night than controls (Wheaton, et al., 2018). Mice genetically modified to produce more pCREB also demonstrated difficulties adapting to novel light cycles, fragmented sleep, and delayed sleep onset generally (Lee, et al., 2018). The similarities in sleep dysregulation caused by both increased and decreased pCREB alludes to the possibility that the ratio between the two is somehow responsible for the effects on sleep. The role of CREB and pCREB in dysregulating sleep is consistent, however there remains a lot of work to be done to fully explain how the dysregulation in pCREB leads to the dysregulation of sleep.

Paraoxonase-1/arylesterase (PON1)

In examining why some people exposed to CPF develop ASD related symptoms and others don't, it is useful to examine how genetic factors can determine the overall effect of exposure. The overall effect of exposure to CPF and CPFO can be determined by genetic factors like the expression of PON1. We can think about exposure as the total amount of change caused by a given toxicant. And so, if a toxicant is metabolized and excreted by the body in an hour, that exposure can be considered to be lower, in a sense, than if it took a month for the toxicant to be metabolized and excreted.

Paraoxonase-1/arylesterase (PON1) is an enzyme responsible for the elimination of CPF and CPFO. Increased sensitivity to CPF toxicity in newborns can be partially attributed to their deficient levels of PON1, on average 4 orders of magnitude lower than adults. There is significant variation in PON1 expression across individuals generally, with newborns demonstrating variance in PON1 expression levels as wide as 26 order of magnitude, and mothers varying by up to 14 orders of magnitude, with further variations on the efficiency of PON1 in the elimination of CPF depending on the genotype of PON1 exhibited (Furlong, et al., 2006). Mice exposed to the same levels of CPF demonstrated susceptibility to its adverse effects inversely correlated to the amount of PON1 expressed and the efficiency of the variant of PON1 expressed (Furlong, et al., 2005). Accordingly, children exposed to organophosphate pesticides in-utero showed lower adverse effects if they had more effective PON1 genotypes, or if their mothers exhibited more effective PON1 genotypes (Eskenazi, et al., 2010).

Many factors significantly affect PON1 expression. PON1 is upregulated epigenetically in response to a maternal high-fat diet (Strakovsky, Zhand, Zhou, & Pan, 2014). PON1 expression in male mice was increased by 170% following castration (bin Ali, et al., 2002) implicating a role for hormonal regulation. And smoking, diet, age, medication and exercise all exert considerable effects on PON1 expression in humans (Costa, Vitalone, Bole, & Furlong, 2005).

PON1 expression is therefore an important factor in the development of the detrimental effects of CPF exposure, and the multiplicity of factors which regulate the expression of PON1 are also liable to affect the resulting developmental toxicity of CPF and subsequent presentation of ASD.

Cytochromes P450

Cytochromes P450 (CYP450) are enzymes responsible for a wide variety of metabolic processes (Stavropoulou, Pircalabioru, & Bezirtzoglou, 2018), crucially for us, they are responsible for the initial step in the metabolic elimination of CPF (Carr, Alugubelly, & Mohammed, 2018). Like PON1, CYP450's effectiveness in eliminating CPF is modulated by a wide range of mechanisms, from genetic polymorphism[9], to medication, to disease states (Chang & Kam, 2002). The availability of precursor proteins has an especially significant effect on the effectiveness of CYP450. Since CYP450's are a family of proteins, inflammation, stress responses, and infection can cause the body to prioritize the production of one set of CYP450 enzymes over others (Morgan E. , 1997). Furthermore, since CYP450's are mostly made in the liver, poor liver function can possibly potentiate CPF's adverse effects in the same manner as an inefficient version of PON1 potentiates CPF's effects (Morgan E. , 1997).

Since the effectiveness of CYP450 plays a role in the determination of the severity of CPF's effects (Carr, Alugubelly, & Mohammed, 2018), the variety of factors affecting P450 expression may also play a role in determining the severity of CPF exposure's effects.

Brain-derived neurotrophic factor

CPF causes increases in brain-derived neurotrophic factor (BDNF)[10] (Pisollato, et al., 2020), possibly as a neuroprotective response to dysregulation (Di Consiglio, Pistollato, Mendoza-De Gyves, Bal-Price, & Testai, 2020).

The observed increases in BDNF are interesting due to the idiosyncratic effects BDNF can have on neuron development. BDNF exerts opposing effects dependent on neuron type (McAlister, Katz, & Lo, 1997), and general increases in BDNF during development are liable to result in significant synaptic and morphological disruptions (de los Angeles Robinson-Agramonte, et al., 2022), which in turn are liable to induce network level abnormalities as the brain matures and adapts to the low-level disruptions (Rauti, et al., 2020). This effect is likely to increase the heterogeneity of neural phenotypes between individuals exposed to CPF, and so is important to highlight.

The CPF induced upregulation of BDNF and its possible downstream effects are also to be contrasted with CPF's neurotoxic effects. One can imagine how these two effects may conflict or concur in causing the phenotype of a given neuron. In those places where BDNF causes a reduction in neurite growth, there may a further reduction in the overall length of neurites, and in those places where BDNF increases neurite outgrowth, the neurotoxic effects may instead be ameliorated due to a counterbalancing between the two effects.

Neonicotinoids

Neonicotinoids (Neonics) are a diverse set of pesticides sharing structural similarities with nicotine. Their primary mechanism of action is the binding of nicotinic

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[9] More CYP450 is generally better, however people with a variant of CYP450, CYP2B6, more readily turn CPF into the more neurotoxic CPFO, making people with that variant especially susceptible to the adverse effects of CPF exposure (Imaishi & Goto, 2018).

[10] 53%+/-9% increase with CPF alone, at lowest observable effect concentrations.

acetylcholine receptors (nAChR) which also function as ion channels important for the production of action potentials. Typically considered less neurotoxic than organophosphate pesticides[11] their use has grown to levels similar to organophosphates, and they've become the primary replacement for organophosphate pesticides in places where those have been outlawed. Their use went from none in the year 2000 to around 30 percent of the market as of 2018 (Selenrich, 2017) (Ihara & Matsuda, 2018). This means that exposure levels for people born earlier will be lower. As CPF was banned in the United States in 2021, neonic exposure levels for those born from 2021 onwards will be higher as neonics continue to replace CPF, and the numbers of neonic exposed people, as well as the degree of exposure, will grow. Due to the capacity of insects to adapt to pesticides by natural selection, there is a significant likelihood of compensatory increases in pesticide application by the agricultural industry. A study sampling environmental toxicant exposure through urinary metabolites in pregnant people across the United States showed that the number of such people exposed to neonics, as well as the concentration of neonics detected, was significantly higher[12] in samples taken from 2017-2020 compared to samples taken from 2015-2016 (Buckley, et al., 2022). Moreover, all samples of cerebrospinal fluid taken from children treated for leukemias and lymphomas tested positive for at least one neonic, and 93% tested positive for more than one (Laubscher, et al., 2022).

Due to the relatively recent widespread adoption and development of neonics, their effects, both molecularly and developmentally, are not as well understood as in organophosphates. The development of this understanding is important, as neonics are persistent in the environment and within the food chain, affecting foods not traditionally associated with pesticides, like wild and farmed fish (Lu, Chang, Palmer, Zhao, & Zhang, 2018). Neonics are nearly completely resistant to conventional water treatment[13], and as such make their way into tap water at the same levels as if one were drinking directly from untreated surface water, with the effect particularly pronounced in agricultural areas (Klarich, et al., 2017). Moreover, as systemic pesticides, they incorporate into every part of the target crop, meaning that unlike pesticides like CPF, the rinsing of crops treated with neonics will not significantly reduce the amount of neonic a person eating that crop is exposed to. Their prevalence is further increased by their long half lives in soil, typically exceeding 1000 days. When neonics do break down, the metabolites are generally more toxic (Bonmatin, et al., 2015).

Exposure to neonics has been associated with anencephaly, ASD, and a symptom cluster of memory loss and finger tremor (Cimino, Boyles, & Thayer, 2017). Maternal imidacloprid (a neonic) exposure, as measured by urinary metabolites, was observed to be correlated in a dose dependent manner with smaller head circumference in children

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[11] Neonics are considered less neurotoxic due to their lower affinity for mammalian acetylcholine receptors and higher affinity for insect acetylcholine receptors.

[12] Levels taken from 2016-2016 had 75th percentile amounts of .38 ng/ml of neonic metabolite N-desmethyl-acetamiprid, while samples taken from 2017-2020 had .59ng/ml.

[13] Only granulated activated carbon filtration reduced neonic prevalence in water.

(Pan, et al., 2022). The odds of a person diagnosed with ASD to have been exposed to imidacloprid (via pet tick medications) is around 30 percent higher than average, with a 100% increase in likelihood for in-utero exposure (Keil, Daniels, & Hertz-Picciotto, 2014). An approximately 2-point decrease in full scale IQ scores was observed in 7-year olds living within 1 kilometer of neonic agricultural application in-utero, namely imidacloprid (Gunier, Bradman, Harley, Kogut, & Eskenazi, 2017). Furthermore, exposure to agricultural neonics as determined by maternal residence within 1 kilometer of an neonic application site was observed 150% more in people with anencephaly and spina bifida than in controls (Yang, et al., 2014).

As with organophosphate pesticides, it has been claimed that neonics pose a low risk for non-target organisms and the environment. In neonics, this has been claimed due to their low affinity for mammalian nAChR receptors, and high affinity for insect nAChR receptors (Jeschke & Nauen, 2008) (Jeschke, 2020). And as with organophosphates, this claim is repudiated upon close examination.

Function and Relevant Mechanisms

Metabolites

Due to the diverse set of molecular configurations in neonics, the range of effects induced by exposure are similarly diverse, although all neonics mostly decompose into the same metabolites, the characteristics of those are harder to determine (Ford & Casida, 2006). Of particular interest to us is the primary metabolite for imidacloprid, desnitro-imidacloprid. Desnitro-imidacloprid is 1000 times more effective at binding nicotinic AChR (nAChR) in humans than its parent compound, and 100 to 1000 times more effective than nicotine in its ability to bind mammalian nAChR, depending on the particular subtype of nAChR being considered (Tomizawa & Casida, 2009). This is important as it refutes the claim that neonics pose a low risk to humans. While imidacloprid itself does not show an affinity for the mammalian versions of nAChR, its metabolite, desnitro-imidacloprid, shows an affinity for mammalian nAChR comparable to the parent compound's affinity for insect nAChR. Moreover, the potency of the primary metabolite of imidacloprid in affecting proteins important for the function of neurons again illustrates how the metabolites of a chemical must be considered if one wishes to properly characterize the effects of that chemical.

Acetylcholine and Effects on Neural Signaling

Mouse neurons with the neonics clothianidin or acetamiprid applied to them demonstrated acetylcholine induced amplitudes two times higher than controls. Furthermore, those same neurons were more sensitive to acetylcholine. Acetylcholine at low doses did not activate control neurons, but the same neurons did activate after clothianidin or acetamiprid were applied (Cartereau, Martin, & Thany, 2017). Similar effects were noted for imidacloprid at very low doses, with exposed neurons demonstrating higher sensitivity to acetylcholine and larger acetylcholine induced amplitudes. High levels of clothianidin and acetamiprid exposure instead inhibited acetylcholine induced amplitudes and the neurons sensitivity to acetylcholine (Loser, et al., 2021). As with clothianidin and acetamiprid, higher amounts of imidacloprid inhibited acetylcholine induced amplitudes and acetylcholine sensitivity (Li, Ann, & Akk, 2011). This goes to show the heterogeneity possible within a single mechanism. As the dose of neonic increases from a baseline of zero, the amplitudes induced by

acetylcholine increase, and then as the dose of neonic continues to rise they are reduced. If one were to attempt to evaluate neonic exposure by a simple EEG measure, say the amplitudes seen upon the administration of acetylcholine directly to the cortex, at low doses of neonic exposure, a researcher would see a very significant effect, evidenced by a massive increase in the resulting amplitude, and at high doses of exposure to the same neonic, that jump in amplitudes seen in the low dose cases would disappear.

Effects of Specific Neonics, Dose, and Gender on Rodent Behavioral Abnormalities

The following sections are meant to illustrate that, insofar as one constrains the heterogeneity of neonic exposure induced behavioral abnormalities by etiological lines, one can derive consistent results. The behavioral effects of varying doses of neonic exposure in these rodent models are consistent, even if somewhat counterintuitive at first glance, within the constraints of a single neonic. If we were to try and group these behaviors by any other measure, they would not make sense.

Imidacloprid

Male and female mice exposed to a single large dose (337 mg/kg) of imidacloprid in-utero developed motor deficiencies and neurobehavioral defects. Increases in AChE activity across the brain were widespread, with average increases of around 125%-145% in the brain, and 125% increase in plasma, compared to controls, with significant but not drastic differences according to gender and brain area (Abou-Donia, et al., 2008). Imidacloprid impaired cognitive functions and learning performance, as well as gene expression related to said performance in infant male rats exposed to 0.5 mg/kg per day (Kara, et al., 2015). Mice exposed in-utero to chronic low levels of imidacloprid at 0.5mg/kg/day showed elevated motor activity, enhanced social dominance, reduced depressive behavior, and lower social aggression, with differences between the female and male groups. The doses in this study were far lower than in prior studies, and demonstrated different effects on motor activity than those observed at higher doses, indicating that the degree of exposure causes differing outcomes at the level of behavior (Burke, et al., 2018).

Acetamiprid

Male mice exposed to acetamiprid in-utero and through breast milk demonstrated significantly lower anxiety levels at high and low exposure levels (10mg/kg/day and 1mg/kg/day respectively) and significantly higher sexual behavior only at the low dose, with high dose mice expressing similar amounts of sexual behavior as controls. Interestingly, there was no increase in testosterone, and female mice exposed at the same rates exhibited no differences when compared to controls (Sano, et al., 2016).

Clothianidin

Male mice administered clothianidin during early pregnancy (65mg/kg/day) developed more anxiety than those exposed during late pregnancy, and there were differential effects on neural development in those exposed earlier in pregnancy within the hippocampal dentate gyrus, attributed to an inhibition of neuron maturation (Maeda, et al., 2021)

Adult male mice exposed to a single 50 mg/kg dose of clothianidin showed higher anxiety than controls and mice exposed to 5 mg/kg, as measured by number of human

audible vocalizations[14]. The 50 mg/kg mice showed increased neural activity in thalamic and hippocampal regions, the neural activity of the 5 mg/kg group was not tested (Hirano, et al., 2018). Chronic administration of clothianidin (10 mg/kg/day to 250 mg/kg/day) and chronic stress led to a higher stress response in adult male mice when compared to mice exposed to chronic stress alone. Higher doses also lead to higher stress responses in a dose dependent manner (Hirano, et al., 2015).

Relevance of Effects of Specific Neonics, Dose, and Gender on Rodent Behavioral Abnormalities

The demonstration of seemingly contradictory changes in anxiety related behavior between acetamiprid, which reduces anxiety associated behavior in mice, and clothianidin, which increases anxiety related behavior in mice, reflects the contradictory findings we were concerned with at the beginning of this paper. If these neonics were not readily distinguishable, we would have a good deal of trouble understanding the results in these studies. We may say, "Results examining the effect of neonic exposure in the development of anxiety are consistently inconsistent. Experiments of neonic exposure on anxiety have found that, compared to controls, neonic exposure increases anxiety (Maeda, et al., 2021), decreases anxiety (Sano, et al., 2016), or doesn't affect anxiety, but does negatively affect learning performance (Kara, et al., 2015).". It is only with the etiological subgroup distinctions that we are able to understand these results. I point this out as what is true for neonics in this case is likely also true for ASD as a whole.

Despite the relative deficit of research of neonics, we see the emergence of a pattern of associations, much like environmental toxicant combinations can ameliorate or potentiate the effects of CPF, imidacloprid, acetamiprid, and thiamethoxam showed synergistic increases in toxicity in neurons when in combination with each other, with the notable exception of acetamiprid and imidacloprid, the combination of which slightly ameliorated their respective effects (Cheng, et al., 2020). Cytochrome p450 also participates in the metabolism of neonics, and the efficiency by which it does so also varies between genotypes, with effects liable to be similar to those found for CPF (Khidkhan, et al., 2021).

Discussion

Here I have examined the etiologies and phenotypic characteristics relevant in the development of ASD as precipitated by SHANK3 haploinsufficiency, prenatal chlorpyrifos exposure, and prenatal neonic exposure. I have outlined some of the mechanisms determining the phenotypic characteristics within those and evaluated the variability possibly attributable to them. In doing so I hope to have shown that the development of ASD is complicated, that there is a wide variability in the phenotypes between different precipitants of ASD, and that the same variability found between the phenotypes of separate ASD precipitants can also be found within the phenotypes attributable to a single precipitant of ASD.

For SHANK3 haploinsufficiency, I examined the importance of zinc in the determination of SHANK3 haploinsufficiency severity, outlined a possible mechanism by

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[14] 70 human audible vocalizations in mice exposed to 50mg/kg, as opposed to 0 in mice not exposed and mice exposed to 5mg/kg.

which the administration of lithium leads to a reversal of regression in a subset of SHANK3 haploinsufficient patients, examined how seizures could possibly be attributed to the dysregulation of low-level neural homeostatic processes caused by SHANK3 haploinsufficiency, and I touched upon the heterogeneity possible within SHANK3 in terms of changes in neural function and phenotype.

For chlorpyrifos exposure, I highlighted the importance of considering the effect that metabolites like chlorpyrifos-oxon can have in determining the overall phenotype attributable to toxicant exposure. I outlined the effects of combined toxicants in the determination of how neurons react to chlorpyrifos, and I examined the role that genes like PON1 have to play in determining the severity of ASD precipitated by chlorpyrifos exposure.

For neonics, I examined the dose dependent effects of neonic induced increases in acetylcholine sensitivity highlighting the heterogeneity to be found there. Furthermore, I highlighted the similarities between the heterogenous behavioral anomalies attributable broadly to neonics, and the heterogenous anomalies attributable broadly to ASD. I suggested that the reduction of heterogeneity through etiological distinctions can clarify the effects of ASD on individuals in the same way that such information clarifies the behavioral anomalies attributable to neonics.

What I hope to have illustrated through this paper is the wide variability to be found within single ASD precipitants, the larger variability in phenotypes to be found across multiple ASD precipitants, and the variety of mechanisms that are affected in ASD. I hope to have illustrated that there are significant differences between subgroups of ASD based on their etiology, and the need to evaluate and develop treatments along those lines. I also hope to have shown through the gestalt of this paper that ASD is not a single disorder caused by the dysfunction of a single underlying mechanism.

Conclusion and Final Thoughts

One factor that has emerged as particularly important in the heterogeneity of presentation and development of ASD is the combined exposure to neurodevelopmental toxicants. The continuing rise in ASD diagnoses cannot be attributed to better testing or genetics alone. A rate increase from 1 in 150 in 2002, to 1 in 59 in 2008, and then 1 in 44 in 2018 is more likely attributable to an increase in fetal exposure to environmental toxicants (Pelch, Bolden, & Kwiatkowski, 2019). This suggests that genetics are not the driving cause of the rise of ASD prevalence, but are instead determinants of who gets ASD, and what kind of ASD they get, given certain kinds of exposures. Socioeconomic factors also play a role in the determination of ASD development. Poorer people tend to be exposed to higher levels of environmental toxicants and are more likely to live in areas with high pollution, be it from industrial, agrichemical, or pre-existing toxicant sources (Buckley, et al., 2022). Immigrant farmworker communities are uniquely vulnerable to agrichemical toxicants (Arcury & Quandt, 2011), as made evident by the higher rates of ASD in agricultural communities (Roberts, et al., 2007).

In the simplest case, I ask the reader to imagine a crowded clinic with three people in it, one with a complete SHANK3 deletion, one with prenatal chlorpyrifos exposure, and one with prenatal neonic exposure. What would they be diagnosed with at the end of the day?

The medical system in the United States, especially in rural areas, is not equipped to adequately serve the needs of people with the most deleterious kinds of ASD. I think that the need for detailed examination is especially well illustrated in the cases of SHANK3 deletion and CPF exposure. I invite the reader to consider how socioeconomic factors can affect the severity and overall deleteriousness of ASD. How the access to proper medical attention and treatment can ameliorate ASD, and who is likely to get that treatment. How factors like stress, pollution, and malnutrition could exacerbate ASD, and who is more likely to be affected by those.

Limitations

Despite the stated aim of outlining the precise mechanisms by which ASD develops, I have not managed to provide them for most of the precipitants of ASD outlined here. Our understanding of ASD is continually evolving, and gaps in our knowledge are closing, but they've not done so yet. The section on neonics exemplifies the need for further research on the mechanics attributable to individual ASD precipitants. In that section I only managed to outline the existence of factors which cause phenotypic variability, but not how those factors go about causing that variability.

Due to the rapid pace of development in this area of study, this work is also severely limited by the lack of definitive evidence for all the mechanisms doing explanatory work. There are many experiments to read and even more that remain undone. Indeed, in an effort to answer how a given factor affects the phenotype under consideration, I have engaged in some speculation of my own, specifically in examining the role of lithium in ameliorating SHANK3 haploinsufficiency. I have done so not because I wish to be speculative, but because I see value in adding to the stack of experiments in the collective "to do" pile. I have attempted to restrict speculation to what is at least mildly substantiated by experimental evidence and have only done so in heavy correspondence with the literature. In a field with so much research to sift through and so many ancillary findings, I think there is value in finding a chart hidden deep in a paper in a completely different area of study, insofar as one has good reason to believe that it can reasonably apply to the question at hand. In those areas where I have speculated, I hope that I have provided adequate reason for doing so, and I must note that the speculations in this paper are simply that, and that much more work is required in outlining how ASD develops.

It is also important to note that I have focused on a very limited range of ASD precipitants, and that this is not a comprehensive examination of all the effects of the precipitants examined. The entire range of factors affecting the development of ASD is significantly larger (Sauer, Stanton, Hans, & Grabrucker, 2021), and consequently the complexity of ASD development is even greater than what was outlined here. Another significant limitation of this work is the focus on ASD precipitants prevalent within the United States, specifically those relevant to ASD in California. Future work will examine the etiology of ASD in South East Asian and European countries. ASD prevalence in South Korea has been estimated to be as high as 1 in 38 (Qiu, et al., 2020), and ASD prevalence in Iceland has been estimated to be as high as 1 in 32 (Delobel-Ayoub, et al., 2020). The toxicant profiles for these countries are liable to be quite different, as South Korea depends very heavily on some of the pesticides outlined here (Park, et al., 2019), while pesticide use in Iceland is nearly negligible due to its adherence to stringent EU

standards (Tarazona, et al., 2022). Further research will account for the significant variance in toxicological and environmental profiles within and across countries.

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