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This is your teen brain on drugs: In search of biological factors unique to dependence toxicity in adolescence

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ABSTRACT

Response variability across the lifespan is an important consideration in toxicology and risk assessment, and the toxic effects of drugs and chemicals during adolescence need more research. This paper summarizes a workshop presented in March 2019, at the Society of Toxicology Annual Meeting in Baltimore, Maryland, that brought together experts in research on drug dependence and toxicity related to nicotine, cannabis, cocaine, and other illicit drugs during adolescence. The goal of the workshop was to address the following issues: (1) Do the effects of adolescent exposure differ from the same exposure in adults? (2) Are there unique biological markers of adolescent brain development? If so, what are they and how reliable are they? (3) Since multiple factors influence substance use disorder, can we disentangle risk factors for abuse and/or toxicity? What are the underlying biological susceptibilities that lead to dependence and neurotoxicity? What are the social, psychosocial and environmental factors that contribute to abuse susceptibilities? This paper reviews drug policy and national trends in adolescent substance use; the public health consequences of e-cigarettes; rat models of adolescent-onset nicotine self-administration and persisting effects of gestational nicotine; sex-dependent effects of delta-9-tetrahydrocannabinol on adolescent brain-behavior relationships; and translational approaches for identifying adolescent risk factors for transition to drug dependence. There is strong evidence that drug exposure prior to adulthood has longer lasting effects on behavior and the underlying neural circuitry. These effects, which are sex-dependent and influenced by stress, may be candidates as predictors of adolescent vulnerability. A major challenge to determining if adolescents have a unique susceptibility to dependence is whether and to what extent the human data allow distinction between the increased risk due to biological immaturity, an underlying biological susceptibility to dependence, or psychosocial and environmental factors for substance dependence. Factors important to consider for development of animal models include the timing and pattern of exposure as it relates to adolescence; age of assessment, and direct comparison with similar effects following exposures to adults to demonstrate that these effects are unique to adolescence. Here we provide a roadmap for further research into what makes adolescent brain development unique.

1. Introduction

Response variability across the lifespan is an important consideration in toxicology and risk assessment. This includes variability among individuals based on differences in genetic and environmental factors, as well as age- and sex-related differences across the lifespan. Substantial research has demonstrated considerable differences between toxicity in adulthood compared to early development (Felter

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et al., 2015). In contrast to these recognized developmental differences and despite some discussion and research on adolescent vulnerability for neurotoxicity of drugs of abuse (e.g., Abreu-Villaca et al., 2008; Achat-Mendes et al., 2003; Adriani et al., 2004; Cservenka and Brumback, 2017; Luikinga et al., 2018; Squeglia et al., 2014; Slotkin, 2002; Swartzwelder et al., 2019; Teixeira-Gomes et al., 2015), adolescents are generally considered to be smaller adults when considering the toxic effects of drugs and chemicals. However, adolescence is another life stage that needs to be examined when considering the toxic effects of drugs and chemicals.

Adolescence is the period when many first partake in drugs of abuse. Individual risk factors, the state of development, and the environmental context influence adolescents' decisions to use illicit drugs. Furthermore, emerging evidence from animal and human studies suggests that there are social, psychological, and neurobiological differences between adolescents and adults that increase vulnerability of adolescents to the dependence potential of central-nervous system–active substances (Casey and Jones, 2010 Casey et al., 2008; Giedd, 2008; IOM, 2015; Luna et al., 2004; Steinberg et al., 2009; Smith et al., 2013). Information on these factors comes largely from tobacco use, where more data are available (IOM, 2015). However, these factors can be generalized to other substances and other risk-taking behaviors.

First, adolescence is a period of continuous cognitive, psychosocial, and biological development. Cognitive development captures adolescents' capacities to perceive the risks or harms of substance abuse, which they weigh against the potential social benefits of using drugs (Albert and Steinberg, 2011; IOM, 2015; Millstein and Halpern-Felsher, 2002). Psychosocial development captures factors frequently associated with adolescence, such as heightened impulsivity and sensation seeking and the increased influence of social norms and peer pressure. These factors are also associated with increased risk behaviors (IOM, 2015; Steinberg, 2008). Biological development includes physical development (puberty), as well as neurobiological development. Evidence has shown that physically maturing earlier or later than peers is associated with risky behaviors (Cance et al., 2013; IOM, 2015; Mendle and Ferrero, 2012; Mendle et al., 2007).

Second, neurobehavioral development continues during adolescence, and dramatic changes in hormonal signaling during this period of life could present different vulnerability to toxic exposures. Toxic impacts on these processes can be particularly important. Neurobiological development during adolescence can be described as a series of transitions in plasticity in multiple domains of function that begins with puberty. The brain undergoes a period of synaptic overproduction, followed by synaptic pruning to adult levels. This period is when environmental impact has a significant effect on shaping brain circuits and function. Anatomically, brain gray matter peaks between 12 and 14 years of age, with differences in the timing of the pinnacle across regions. Functionally, the timing of peak gray matter generally corresponds to the role of a given region. For example, optimal motor learning occurs around 10 years of age coincident with maturation of the motor cortex. Cognitively, people reach adolescence when abstract thought and executive function are present in concert with the maturation of the frontal cortex (Oldham and Fornito, 2019). Predicting when a window of plasticity is open during adolescence therefore depends on the function and region.

The social and environmental context interacts with adolescent development to influence adolescent decision making around drug use. Key elements of the social and environmental context include policies, social norms, media, marketing and advertising. For example, evidence shows that the legalization of recreational marijuana in several states, and the introduction of e-cigarettes, has changed the perceptions of the risk of harm from cannabis and nicotine use, and these perceptions have the greatest impact on adolescent vaping (Ambrose et al., 2014; Cerda et al., 2017, 2018; Gorukanti et al., 2017; Keyes et al., 2016; Sarvet et al., 2018; Wall et al., 2011).

Animal studies suggest that there are behavioral, neurochemical, and/or anatomical changes following adolescent exposures that are potential biological indicators for dependence toxicity. Importantly, comparisons across species reveal some differences between rodents and people that are not fully comparable. For example, adolescence in all species begins with the onset of puberty. However, the beginning of adulthood differs. For humans, although the legal definition of adulthood is generally age 18 in the U.S, newer brain imaging data show that humans do not achieve full adulthood until between the ages of 25 and 30, (Dosenbach et al., 2010; IOM, 2015; Lynch et al., 2020). By contrast, in rodents, there is no agreed upon age that marks full adulthood, and this has resulted in different definitions in different experimental systems (Spear, 2000).

This workshop, presented at the 2019 Society of Toxicology Annual Meeting in Baltimore, Maryland, in March 2019, brought together experts in research on drug dependence and toxicity related to nicotine, cannabis, cocaine, and other illicit drugs during adolescence. The workshop participants evaluated the strengths and limitations of the experimental evidence for increased vulnerability in adolescents compared with adults and the extent to which proposed neural circuits and biological markers are unique to adolescent vulnerability and dependence. The panel discussed whether approaches used in drug abuse research are generalizable to toxicity testing to screen for effects of chemicals and drugs that may increase susceptibility of adolescents to substance use disorders (SUD). Participants discussed implications of the patterns of behavioral, neurochemical, and other factors unique to adolescents as related to public health and health policy.

The goal of the workshop was to address the following issues: (1) Do the effects of adolescent exposure differ from the same exposure in adults? (2) Are there unique biological markers of adolescent vulnerability? If so, what are they and how reliable are they? (3) Since multiple factors influence SUD, can we disentangle risk factors for abuse and/or toxicity? What are the underlying biological susceptibilities that lead to dependence and neurotoxicity? What are the social, psychosocial and environmental factors that contribute to abuse susceptibilities?

This review summarizes the workshop presentations that sought to address these questions. The review begins with an overview of the drug policy context and national trends in adolescent substance use in the United States. The paper then summarizes key findings from the National Academies of Sciences, Engineering, and Medicine (NASEM) report on the public health consequences of e-cigarettes, with special focus on effects of e-cigarette use among youth and young adults. Next, the review describes evidence from rat models of adolescent-onset nicotine self-administration and persisting effects of gestational nicotine and sex-dependent effects of marijuana compounds on adolescent brain behavior. The subsequent section describes translational approaches for identifying behavioral and biological indicators of adolescent risk for transition to drug dependence. The paper closes with a discussion of research issues in adolescent brain development in relation to drug abuse liability.

2. Shifting policy landscape and national trends in adolescent substance use (Leslie Kwan)

The broader context in which adolescent substance use occurs is important for understanding about adolescent substance use and its effects on adolescent neurobiology. This context includes shifting substance use policies, national trends in substance use, and adolescent substance use trajectories. Of note, the increasing regulation of tobacco products contrasts with the increasing deregulation of marijuana. Although causal effects of these policies on adolescent use are unclear, the presentation described national substance use trends from survey data.

2.1. The shifting policy landscape

Tobacco policies are changing at the local, state, and federal levels.

In 2009, President Barack Obama signed into law the Family Smoking Prevention and Tobacco Control Act (Tobacco Control Act), which granted the Food and Drug Administration (FDA) the authority to regulate the manufacture, distribution, and marketing of tobacco products.[1] The Tobacco Control Act excluded some tobacco products, notably e-cigarettes. However, in 2016, the FDA issued a regulation deeming its authority over e-cigarettes as a tobacco product (HHS, 2016). The Tobacco Control Act also established a number of federal youth access restrictions already implemented in many states—e.g., requiring face-to-face sales and age verification. The Act established 18 as the minimal legal age for sales, while also prohibiting the FDA from raising the age nationally. However, states and localities actively raised the age in their jurisdictions until December 2019, when Congress raised the minimum age to 21 nationally through a provision in their annual spending bill.[2]

In contrast to tobacco policies, marijuana policy changes occur primarily at the state and local levels. Importantly, at the federal level, the FDA lacks authority to regulate marijuana or its components (e.g., cannabinoids). (An exception to this is EPIDOLEX®, a prescription formulation of plant derived cannabidiol for treatment of certain types of seizure.) The authority over cannabis and cannabinoids currently falls on the Drug Enforcement Administration. States and localities have been loosening marijuana regulations through the legalization of marijuana for medical and/or recreational uses. As of July 1, 2019, 11 states (Alaska, California, Colorado, Illinois, Maine, Massachusetts, Michigan, Nevada, Oregon, Vermont, and Washington) and the District of Columbia have legalized marijuana for medical and recreational use (Governing, 2019). An additional 22 states (Arizona, Arkansas, Connecticut, Delaware, Florida, Hawaii, Iowa, Louisiana, Maryland, Minnesota, Missouri, Montana, New Hampshire, New Jersey, New Mexico, New York, North Dakota, Oklahoma, Pennsylvania, Rhode Island, Utah, and West Virginia) have legalized medical marijuana (Governing, 2019).

2.2. National adolescent substance use trends and trajectories

Data on adolescent substance use come from several nationally-representative, annual, cross-sectional surveys (MTF, 2018; SAMHSA, 2018; Wang et al., 2018). Tobacco has seen steep declines in adolescent use, owing largely to past tobacco control policies, including not only youth access restrictions, but also product regulation, taxation, indoor smoking bans, marketing and advertising restrictions (IOM, 2015). Trends are similar among older adolescents (high school) and younger adolescents (middle school). In 2017, 19.6% of high school students (2.95 million high school students) reported currently using any tobacco product (past 30-day, Wang et al., 2018). Among middle schoolers, 5.6% (670,000 middle school students) reported any current tobacco use (Wang et al., 2018). Two notable exceptions to the general decrease in tobacco product use are e-cigarettes and hookah. Both are newer products to the market and have seen recent increases in use among adolescents (MTF, 2018; Wang et al., 2018).

Current patterns of use of marijuana use are similar to tobacco use. Some declines have been observed, but they are not as steep as for tobacco. Stratified data show much higher rates among older adolescents compared with younger adolescents (MTF, 2018). In 2017, 6.5% of adolescents age 12 to 17 (approximately 1.6 million adolescents) reported currently using marijuana (past month, SAMHSA, 2018). Importantly, this national data hides heterogeneity across states and localities with different marijuana policies. In states with legalized medical only or medical and recreational marijuana, evidence is mixed on the influence of legalization on adolescent use (Cerda et al., 2018; Keyes et al., 2016; Sarvet et al., 2018). Major challenges to determining the effect of marijuana legalization on adolescent marijuana use include poor baseline data and artifacts of measurement and reporting.

Rates of cocaine use are much lower compared with rates of tobacco and marijuana use. In addition, rates of cocaine use have been fairly steady over time. Similar to tobacco and marijuana, younger adolescents use at lower rates compared with older adolescents (MTF, 2018). In 2017, 0.1% of adolescents age 12 to 17 (26,000 adolescents) reported currently using cocaine (SAMHSA, 2018).

For adolescents who take up drug use, evidence suggests that this early exposure influences their subsequent use. Drawing primarily from studies of adolescent tobacco use, evidence has shown that nearly all adult smokers begin in adolescence (IOM, 2015). Additionally, smoking earlier is associated with greater nicotine dependence and more intense smoking in adulthood (Chassin et al., 1996; IOM, 2015). Similar patterns have been observed with other substances (Wagner and Anthony, 2002).

3. Public health consequences of e-cigarettes: a focus on special concerns for youth and young adults (David Eaton)

As mentioned in the previous section, the FDA extended its regulatory authority to all tobacco products, including e-cigarettes, in 2016. This “Deeming Regulation” gave the FDA authority to regulate the manufacture, distribution, and marketing of e-cigarettes and included provisions that would allow restrictions on sales. The use of e-cigarettes has increased dramatically in the past 5+ years, with significant changes in the types of devices, the e-liquids used in them, and the nature of the population that chooses to use them (Levy et al., 2019; NASEM, 2018). Unlike combustible tobacco cigarettes, e-cigarettes do not burn and do not contain most of the estimated 7000 chemicals found in tobacco smoke. Thus, it is generally believed that e-cigarettes are safer than combustible tobacco cigarettes, although exposures to nicotine and a variety of other potentially harmful constituents are still present. One key public health issue surrounding the burgeoning use of e-cigarettes among youth/young adults is whether youth who initiate e-cigarette use are at increased risk for transitioning to combustible tobacco cigarettes. Alternatively, there is growing evidence that e-cigarettes may be an effective means for tobacco cigarette users to stop smoking, providing a potential public health benefit. In order to inform the public about the consequences of e-cigarettes and support potential future FDA and congressional action, the FDA asked the National Academies of Sciences, Engineering, and Medicine to conduct a thorough and objective analysis of the state of scientific evidence relating to e-cigarettes and public health that can inform the understanding of public health risks and benefits of e-cigarettes. Some of the specific questions addressed by the committee were:

1) What are the short- and long-term health risks of regular use of e-cigarettes?
2) What variables of the numerous types of devices and use patterns are important determinants of risk?
3) Are e-cigarettes an effective means to quit smoking combustible tobacco cigarettes?
4) Are e-cigarettes an “initiation pathway” of youth to smoking combustible tobacco cigarettes?

This section briefly summarizes the major findings of the committee, with an emphasis on the evidence that addressed question 4 above—Does e-cigarette use lead teens and young adults to begin smoking cigarettes? In addition, new data from recent publications are examined relative to the committee findings.

The committee took the following approach to assess causality between e-cigarette exposures and various potential outcomes. First, the committee examined evidence on distal health outcomes, then moved

[1] *Family Smoking Prevention and Tobacco Control Act* (111th Congress, 2009–2010) https://www.congress.gov/bill/111th-congress/house-bill/1256.

[2] *Further Consolidated Appropriations Act*, 2020 (116th Congress, 2019–2020) S603 https://www.congress.gov/bill/116th-congress/house-bill/1865/text.

up the causal chain to intermediate/short-term outcomes, mechanisms/modes of action, and exposures. The committee considered human data most relevant and animal data supportive. Finally, in vitro data was useful for hypothesis generation and understanding mechanisms, but the relevance for establishing human health risk was uncertain.

Where feasible, the committee attempted to follow the guidelines provided for systematic review of over 800 peer-reviewed publications that addressed aspects of e-cigarette use, retrieved between February 1, 2017, and August 31, 2017. The committee assessed the quality of each study and the strength of causal association between e-cigarette use and various endpoints. Descriptors of the strength of evidence include: no available evidence, insufficient, limited, moderate, substantial, and conclusive. The committee made decisions about the strength of the evidence base based on the number and quality of studies available (i.e., randomized control trials, vs. observational studies; and the confidence that study limitations such as chance, bias, and confounding factors can be ruled out). After evaluation of the literature relating to the nature and toxicity profiles of constituents of e-cigarette vapors, the committee found "***conclusive evidence*** *that … most e-cigarette products contain and emit numerous potentially toxic substance …* [*and that*] *the number, quantity, and characteristics of potentially toxic substances emitted from e-cigarettes is highly variable and depends on product characteristics … and how the device is operated*." The committee found "***substantial evidence*** *that … under typical conditions of use, exposure to potentially toxic substances from e-cigarettes is significantly lower compared with combustible tobacco cigarettes*" (NASEM, 2018).

After evaluation of the existing literature on issues of nicotine dependence and use liability and considering toxicology and other aspects of e-cigarette constituents, the committee found "***substantial evidence*** *that e-cigarette use results in symptoms of dependence on e-cigarettes*" and "***moderate evidence*** *that risk and severity of dependence are lower for e-cigarettes than combustible tobacco cigarettes …* [*and*] *that variability in e-cigarette product characteristics* (*nicotine concentration, flavoring, device type, and brand*) *is an important determinant of risk and severity of e-cigarette dependence*" (NASEM, 2018).

To address the key question of whether e-cigarette use is a pathway to long-term combustible tobacco use among adolescents/young adults, the committee developed a conceptual framework for illustrates the various paths that non-smoking, non-e-cigarette users might take upon first introduction to e-cigarettes (see Fig. 1). The committee then assessed the strength of causal evidence for the various steps leading from first e-cigarette use to either tobacco use, e-cigarette use, no use of either one, or dual use for both intermediate (near term), and end-state (long-term use). The committee identified 10 published studies available at the time of review (from February 1, 2017, to August 31, 2017) addressing one or more aspects of these pathways and found that 7 of them were appropriate for use in a meta-analysis. The results of the meta-analysis and assessment of the strength of evidence of these studies led the committee to conclude that there was "***substantial evidence*** *that e-cigarette use increases risk of ever using combustible tobacco cigarettes among youth and young adults*" (NASEM, 2018). Further, the committee concluded that, among youth and young adult e-cigarette users who ever use combustible tobacco cigarettes, there was "***moderate evidence*** *that e-cigarette use increases the frequency and intensity of subsequent combustible tobacco cigarette smoking*" and "***limited evidence*** *that e-cigarette use increases, in the near term, the duration of subsequent combustible tobacco cigarette smoking*" (NASEM, 2018).

Since the time of the committee's final literature review in mid-2017, several new studies were published. At the time of the Committee's literature review, there was a paucity of clinical studies or case reports related to direct adverse health effects of e-cigarettes on youth or young adults. However, several recent clinical studies and case reports suggest that acute lung injury from e-cigarette use can occur, a process now referred to as "e-cigarette, or vaping, product use-associated lung injury (EVALI)" (Cao et al., 2020; Cherian et al., 2020; Chidambaram et al., 2020; Messina et al., 2020; Wang et al., 2020). As of February 2020, the US Centers for Disease Control and Prevention has identified 2807 cases of EVALI, with many in young adults (CDC, 2020). However, it should be noted that many of these clinical reports involved electronic delivery systems used with cannabis-based products and/or e-liquids that contained Vitamin E Acetate. Indeed, Blount et al. (2020) found evidence of Vitamin E acetate in bronchiolar lavage fluid from 48 of 51 EVALI patients, but not in any of 99 healthy matched controls, providing compelling evidence that Vitamin E acetate was the causative factor. However, it should be noted that the CDC stated that "Evidence is not sufficient to rule out the contribution of other chemicals of concern, including chemicals in either THC or non-THC products, in some of the reported EVALI cases."

Several other epidemiological studies have recently evaluated whether e-cigarette use is associated with smoking initiation among youth. The results of these studies are generally consistent with the committee's findings. Vogel et al. (2020) examined e-cigarette dependence and tobacco use progression. In a cohort study of high school seniors (ages 16 to 18), e-cigarette dependence at baseline was associated with higher odds of continuing vaping, number of nicotine vaping days in the past 30 days, number of vaping sessions per day, and puffs per session at six-month follow-up, after adjusting for baseline vaping and e-cigarette dependence risk propensity scores (Vogel et al., 2020). The authors concluded that these findings suggest that e-cigarette dependence may be associated with future persistence and escalation of tobacco use (Vogel et al., 2020). Two studies examined e-cigarette use and subsequent combustible tobacco cigarette initiation. Watkins et al. (2018) analyzed data from the Population Assessment of Tobacco and Health (PATH) Cohort Study and found that ever use and past 30-day use of e-cigarettes was associated with cigarette initiation within one year. This study also reported that youth whose first tobacco product was an e-cigarette had greater risk of initiating traditional combustible cigarettes use over a two-year follow-up period. Also using PATH data, Berry and colleagues (2018) found that the association of prior e-cigarette use with cigarette initiation was stronger among low-risk youth (defined using a 9-variable scale) than intermediate- or high-risk youth (Berry et al., 2019). One study investigated e-cigarette use and subsequent smoking progression. In a cohort study of adolescents ages 16 to 18, Barrington-Trimis et al. (2018) found that smoking progression was similar among those who used e-cigarettes at baseline and those who used only combustible tobacco cigarettes at baseline. Specifically, this study reported that those who used combustible tobacco cigarettes and dual users of e-cigarettes and combustible cigarettes had substantially higher risk of past 30-day combustible tobacco cigarette use and lower likelihood of smoking cessation at follow up. The authors concluded that exclusive e-cigarette use at baseline was associated with greater odds of subsequent experimental (initiation but no past-30-day use), infrequent (1–2 of the past 30 days), and frequent (3–5 or more of the past 30 days) cigarette use, compared to no e-cigarette use at baseline (Barrington-Trimis et al., 2018). In summary, these studies support the committee's findings in 2018 that youth e-cigarette use is associated with combustible tobacco cigarette initiation and progression.

The committee also evaluated the strength of evidence that e-cigarettes are useful as smoking cessation aids among those already addicted to tobacco products. The committee found "***moderate evidence*** *from randomized controlled trials that e-cigarettes with nicotine are more effective than e-cigarettes without nicotine for smoking cessation,*" but "***insufficient evidence*** *from randomized controlled trials about the effectiveness of e-cigarettes as cessation aids compared with no treatment or to FDA–approved smoking cessation treatments*" (NASEM, 2018). Since the committee's review, one randomized control trial comparing e-cigarettes to other FDA-approved smoking cessation devices (e.g., nicotine patches and gums), Hajek et al. (2019) found that "e-cigarettes were more effective for smoking cessation than [NRT, 18.0% vs. 9.9%], when both products were accompanied by behavioral support." The study further reported that, among participants with 1-year abstinence, those

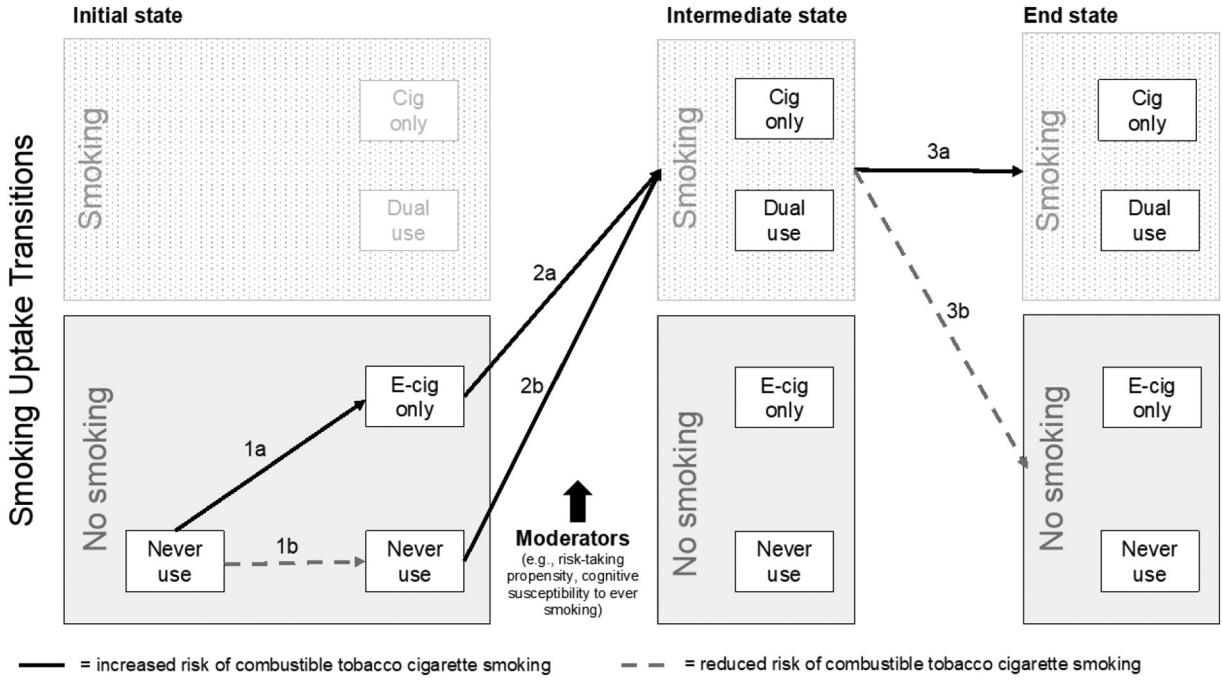

Fig. 1. NASEM committee conceptual framework for transition from e-cigarette use to combustible tobacco cigarette use initiation and progression.
Note: Cig = combustible tobacco cigarette; E-cig = e-cigarette; 1a. E-cigarette initiation (no prior use of cigarette); 1b. no use of tobacco products; 2a. transition to ever smoking following vaping; 2b. ever smoking as first tobacco product; 3a. progression to combustible tobacco smoking; 3b. temporary experimentation with smoking.
Adapted from NASEM, 2018.

in the e-cigarette group were more likely than those in the nicotine-replacement group to use their assigned product [80% vs. 9%] (Hajek et al., 2019). This study thus reinforces the committee's finding.

Finally, the NASEM committee report (2018) did a series of modeling exercises to evaluate the long-term public health implications of e-cigarettes, using a series of variables for: intrinsic harm of e-cigarettes alone (0, 10, 25 or 50% as harmful as tobacco cigarettes), the magnitude of the 'initiation' effect (0, 10, 25% of e-cigarette users become long-term smokers), and the magnitude of the effectiveness in smoking cessation (complete stopping; 5% increase in combustible tobacco cigarette smoking; 5, 0, 5, 10, and 15% decrease in tobacco smoking). Because of the magnitude of negative health consequences of long-term tobacco use, the model found net public health benefits to e-cigarettes over the next 35 years even if e-cigarettes are only modestly effective for smoking cessation (e.g., 5% decrease). However, the magnitude of that effect was much less at 50 years, as that much time is needed to fully realize the negative public health consequences of teens who first begin smoking via e-cigarette use (NASEM, 2018).

4. Rat models of adolescent-onset nicotine self-administration and persisting effects of gestational nicotine (Edward Levin)

Addiction to drugs is a particularly nefarious form of neurotoxicity, inasmuch as one of the principal neurotoxic effects of these chemicals is to cause those exposed to repeatedly dose themselves with more of the toxic chemical. As with many neurotoxic chemicals, exposure to drugs of abuse has differential effects depending on the life stage of exposure. For example, in a variety of studies across the lifespan, there have been findings of long-term adverse cognitive and emotional effects of early developmental nicotine exposure (Levin and Abreu-Villaça, 2018). In contrast, there are some possibly beneficial effects of nicotine exposure in adulthood on cognitive and emotional function (Levin et al., 2006; McClernon et al., 2006). Adolescent exposure to drugs of abuse can be particularly problematic for this is when most people begin using these drugs.

Adolescence is an especially vulnerable period of exposure to addictive drugs. Adolescence is a common time for the initiation of drug addiction, and many neural systems are still developing, including those involved with addiction. A prominent example of adolescent vulnerability to drug addiction is with tobacco. The great majority of addicted tobacco users begin during adolescence. The earlier one starts using tobacco, the greater the likelihood of long-term tobacco addiction (Chassin et al., 1996). However, it is quite difficult to determine with human studies the causative relationship between adolescent drug use and persistent addiction because of predisposition bias. Individuals who have predisposing characteristics—genetic, environmental or both—may also be likely to start using drugs earlier. That is, early initiation and persistent addiction could both result from a common set of predisposing factors. However, it also may be the case that exposure to addictive drugs, particularly self-exposure, during adolescent brain development could disrupt this late phase of neurodevelopment to potentiate the liability to persistent addiction. Trials randomly assigning individuals to early vs late onset of drug taking cannot be done in humans. Experimental animal models can be quite informative for determining the impacts of adolescent drug taking on persistent addiction.

Nicotine is the principal neuroactive chemical in tobacco and is central to the addictiveness of tobacco. The great majority of people who become addicted to tobacco start during adolescence, when the brain is still undergoing important phases of development (HHS, 2014). It is difficult to determine in humans whether adolescent onset potentiates addiction since there are a number of different causative factors underlying the substantial adolescent onset drug use problem. The same genetic/environmental conditions that predispose individuals to tenacious addiction also could induce them to start using early; drug use during adolescence could shape brain development around the addictive behavioral pattern; or the increased plasticity of adolescence could be a more fertile ground for the growth of addiction. However, animal models facilitate determining the causative nature of adolescent- compared with adult-onset nicotine self-administration with

random assignment to drug-taking groups that is not possible in humans.

In a series of studies, Levin and colleagues found that male and female rats first given access to nicotine self-administration during adolescence self-administer significantly more nicotine than rats first given access in adulthood (Levin et al., 2003, 2007, 2011). Male adolescent rats have higher rates of nicotine self-administration than female adolescent rats. However, female rats that start nicotine self-administration during adolescence have a more persistent elevation of self-administration as they mature into adulthood (Levin et al., 2011). Adolescent-onset nicotine self-administration in female rats also causes long-term impairment in regulation of nicotine intake (Levin et al., 2011). These randomized controlled rodent experiments showed that access to nicotine caused higher levels of self-administration of nicotine among adolescent rats than adult rats. This effect persisted into adulthood among female rats. This type of experimental study can provide a way to discover the mechanisms for adolescent vulnerability to drug abuse.

5. Sex-dependent effects of delta-9-tetrahydrocannabinol on adolescent brain-behavior relationships (Diana Dow-Edwards)

Marijuana contains up to 80 different compounds which are derived from the plant *Cannabis sativa*. These cannabinoids include tetra-hydrocannabinol (THC), the major psychoactive cannabinoid (CB) and cannabadiol which generally antagonizes THC. THC primarily acts through the CB receptor 1 (CB1) which is widely distributed throughout the brain—especially in the cortex, cerebellum, striatum, amygdala and hippocampus. The endocannabinoid system, which is the natural endogenous system comprised of endocannabinoids, such as 2AG and anandamide, and their receptors serve to regulate activity at gamma aminobutyric acid (GABA) and glutamate synapses (Fig. 2; Bossong and Niesink, 2010). Since GABA and glutamate ultimately control synaptic activity and thus plasticity through long term potentiation and long term depression, they are critical to normal synaptic maturation which occurs throughout development including adolescence. Cass et al. (2014) demonstrated that CB exposure during postnatal day (P)35–40 or 40–45 (but not 50–55 or thereafter) produces long term functional downregulation of the prefrontal cortex GABAergic transmission. Extrapolations from these rodent models suggest that early/mid adolescence is a vulnerable period for the normal maturation of the GABA inhibitory system of the prefrontal cortex. During adolescence, synaptic maturation (pruning) occurs within several important functional domains such as the cognitive, social and emotional neural networks that utilize the prefrontal cortex (Fig. 2). Therefore, exogenous cannabinoids that act through the CB1 receptor can disrupt the delicate balance between GABA and glutamate as the neural networks mediating cognitive, social and emotional domains undergo maturation via synaptic pruning and increased white matter efficiency.

Animal studies in rats have shown that CB1 receptors peak in cortex, striatum and mesencephalon between 30 and 40 days postnatal (Rodriguez de Fonseca et al., 1993) which suggests that exogenous cannabinoids can have maximal impact on brain maturation and function during this period of adolescence. A more recent study by Heng et al. (Heng et al., 2011) illustrated that the medial prefrontal cortex showed the greatest degree of pruning between 25 and 70 days of age in rats compared to more lateral and caudal regions of cortex. Again, the normal pruning of synapses (as suggested by a loss of CB1 receptors located at synapses) can be disrupted by the presence of exogenous CB (Kim et al., 2008). An additional layer of complexity is imparted by the fact that early stress has significant, sexually dimorphic effects on CB1 receptor binding throughout the rat brain (Dow-Edwards et al., 2016). That is, early rearing adversity increased CB1 receptor densities in many regions including the prefrontal cortex in male Sprague-Dawley rats and decreased these same receptors in the same regions in females. This suggests that early rearing conditions can modify the basal tone within the endocannabinoid system and potentially alter drug responses in a sexually dimorphic way. Undoubtedly,

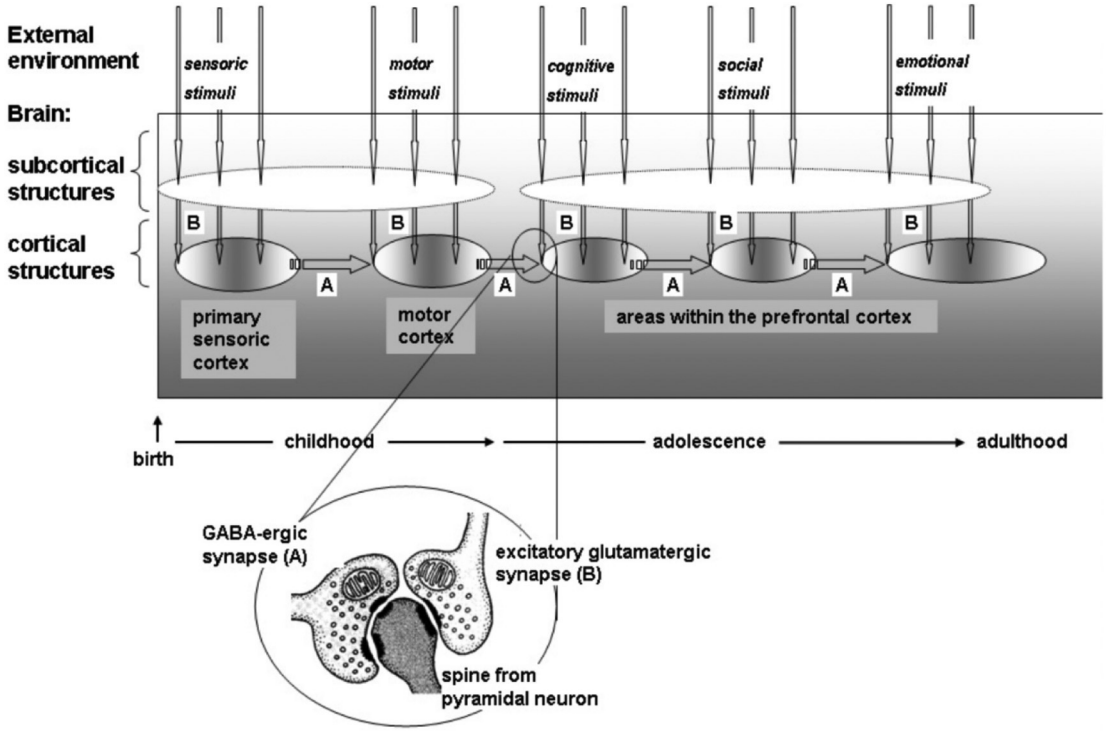

Fig. 2. Synaptic maturation during adolescence involves pruning in cognitive, social and emotional domains which are dependent on the prefrontal cortex. CB1 receptors located on GABA and glutamate terminals regulate synaptic activity and thus long term potentiation and long term depression which determine the fate of each synapse.
Modified from Bossong and Niesink, 2010.

early rearing conditions, as well as prenatal conditions, vary from lab to lab thus impacting the behavioral and biochemical responses to drug challenges and the reproducibility of findings across labs.

During childhood, the neural networks subserving sensory and motor functions mature. A child learns to walk due to development of the motor circuits. During adolescence, the circuits mediating higher cognitive function, socialization and emotional regulation develop through a process of pruning and myelination. The CB1 receptor regulates these processes as described above, and the presence of exogenous cannabinoids is known to alter these processes. We know that smoking marijuana during adolescence has permanent effects on cognition. A recent review by Crane et al. (Crane et al., 2013) compared early onset smokers (< 16 years) with late onset smokers and found early onset smokers had poor inhibition, verbal fluency, visual attention, executive function and episodic memory. Overall, marijuana smokers showed decreased executive function, decision making, processing speed and attention compared to nonsmokers. These findings have been replicated in animal studies supporting the cause-effect relationship between marijuana smoking and poor cognitive function. While studies on marijuana and mental health are controversial, marijuana smoking is associated with anxiety, depression and suicidal thoughts. For example, there is a 5-fold increase in depression and anxiety in adolescent females and a twofold increase in males (Patton et al., 2002). A meta-analysis that included 112,000 cases from 10 countries concluded that there was a strong positive association between marijuana smoking and anxiety disorders (Kedzior and Laeber, 2014). Another recent meta-analysis of 12 prospective studies with about 12,000 adults and adolescents concluded that there was an association between recent/frequent CB use and anxiety and mood disorders (Mammen et al., 2018). A secondary analysis of the Youth Risk Behavior Study of 27,000 high school students concluded that marijuana use was associated with increased odds of reporting suicidal ideation and depressive symptoms (Chadi et al., 2019). Another large repeated cross-sectional survey of 43,000 Canadians over the age of 15 concluded that females using marijuana more than once/week reported higher psychological distress and suicidal thoughts than their male counterparts (Halladay et al., 2018). Again, since it is difficult to determine cause/effect relationships in clinical studies, even in longitudinal studies, we must turn to animal studies to permit sufficient control to determine causality. Many labs have reported that adolescent marijuana exposure alters affect and that early adversity enhances these effects of cannabinoids (Rubino et al., 2009; Rubino and Parolaro, 2016; Silva et al., 2016; Zamberletti et al., 2014; Pushkin et al., 2019).

Importantly, smoking marijuana in adolescence is associated with a 3- to 4-fold increased risk of psychosis (Marconi et al., 2016). While psychosis has a known genetic component, marijuana smoking lowers the age of onset of schizophrenia, makes treatment more difficult and lengthens the hospital stay all in a dose-response relationship. For example, the first psychotic episode occurred a full 6 years earlier among smokers of the potent cannabis extracts than among non-smoking controls (Di Forti et al., 2014). Animal studies have verified that cannabinoid exposure during adolescence impairs prepulse inhibition (PPI), a measure of sensory gating which is impaired in schizophrenics, and alters locomotor activity (Rubino and Parolaro, 2014).

Adolescent cannabis increases risk of future drug use. That is, adults who used before age 15 were 4 times more likely to be dependent on an illicit drug than adults who first used marijuana at age 21 or older (SAMHSA (Substance Abuse and Mental Health Services Administration), 2014). While overall, 9% of people who experiment with marijuana become dependent, this figure rises to 17% of teenagers. In fact, between 25 and 50% of teens who smoke daily become dependent (Piomelli et al., 2016). Others have reported that of those who start smoking between 14 and 15 years of age, the weekly smokers have a 60–fold increase in risk of becoming dependent on an illicit drug compared to those who never smoked or those who started smoking when they were in their twenties (Fergusson and Horwood, 2000). Many animal studies also find that adolescent exposure increases drug self-administration in adulthood (Higuera-Matas et al., 2008; Pushkin et al., 2019; Ellgren et al., 2007). The Ellgren study demonstrated clearly that a low-dose weekend model of marijuana smoking during adolescence in the rat results in increased heroin self-administration in adulthood (Ellgren et al., 2007). Therefore, these animal studies demonstrate that cannabis exposure during adolescence changes the biology of the developing reward circuit resulting in increased drug taking in adulthood. The increase in drug-taking in adulthood is not a function of friends/peer pressure or socio-economic status in animals.

In conclusion, adolescent cannabinoid exposure produces far greater effects than adult exposure in the domains of cognition, mental health and especially increased reward taking. Imaging studies illustrate the adverse effects of cannabis on the development of cognitive networks, social networks, connectivity and brain structure. There are many sex differences and these depend on the system being examined. Animal studies show effects of early stress on CB1 receptors and drug responses and support the sexually dimorphic nature of cannabis effects. Clinically among female marijuana smokers, there is a stronger association with depression, anxiety and suicidal thoughts than in males and compared to non-smokers. Throughout both the clinical and preclinical reports, dose-response relationships emphasize that the earlier one starts smoking, the more frequently they smoke, and the more potent the form of marijuana used, the greater the impact on cognition, mental health and tendency to use other illicit drugs. And, the decriminalization/legalization of marijuana across the world will certainly increase use due to decreased perceived risk.

6. Translational approaches for identifying behavioral and biological indicators of adolescent risk for transition to drug dependence (Susan Andersen)

Early adolescent use of drugs increases the risk of lifelong substance dependence more than the initiation of use at later ages (Anthony and Petronis, 1995). A number of animal models have been developed that allow researchers to examine and manipulate risk factors for addiction. For example, exposure to childhood adversity can be modeled by the maternal separation model (in which rodent pups are separated from their mothers in the first postnatal weeks), limited nesting model (in which rodent pups are reared in impoverished environments), and other paradigms involving social isolation (Andersen, 2015). Reduced cortical regulation, such as that found in attention deficit hyperactivity disorder (ADHD), can be found in the Spontaneously Hypertensive rat. Genetic models are prevalent for a number of conditions. In addition to animal studies, human studies of population variants also show elevated risk behaviors for addiction such as anxiety, novelty seeking, and impulsivity. These studies can be useful for the determination of drug effects (Belin et al., 2016). However, understanding when a risk behavior predicts that transition to addiction is not well-established for immature animals.

The approach needs to be easily accessible in order to predict who is at-risk for substance use and dependence. Behavior can be measured in various settings (homes, schools, doctor's offices) and by different individuals (parent, teacher, doctor). Working memory is consistently associated with an increased risk of drug use in humans (Khurana et al., 2015; Khurana et al., 2017). Impaired memory in male and female rats that are young adolescents is associated with increased relapse to take cocaine in adulthood (Jordan and Andersen, 2018). The novel object recognition task (NOR) measures a type of memory that is sensitive to age changes (Cyrenne and Brown, 2011; Heyser and Ferris, 2013; Heyser et al., 2004; Jablonski et al., 2013), drug changes and is a short (35 min), a drug-free assessment that is ideal for developmental studies. The object recognition has direct cross-species translation and is used in human studies (Fenske et al., 2006). NOR measures components of episodic memory (Ennaceur and Delacour, 1988) and does so without manipulating motivation levels (Blaser and Heyser, 2015). Changes in

motivational status present a possible confound for age-related studies.

Measures of impulsivity also predict vulnerability to addiction across species. In humans, the measure is defined as "acting without thinking" and describes motor impulsivity. Motor impulsivity is difficult to measure in young animals due to many weeks of training. However, motor impulsivity is predictive of elevated drug-taking in rats (Dalley et al., 2011). Impulsive choice is also predictive in both species and is measured by delay discounting. Initially known as the "marshmallow test," delay discounting occurs when a smaller reward is selected sooner rather than delay the response for a larger reward later. Delay discounting is associated with more cocaine intake in adult rats (Molander et al., 2011; Perry et al., 2005) and adolescent (Freund et al., 2019). Novelty-seeking behavior in adolescents and adults is a known risk factor for problematic drug use (Khurana et al., 2015; Palmer et al., 2013; Wills et al., 1994), and longitudinal studies suggest novelty-seeking plays a causal role in addiction (Foulds et al., 2017). Like humans (Steinberg, 2008), novelty-seeking in animals increases with age, peaks in adolescence (Adriani et al., 1998), and is higher in males than females (Palanza et al., 2001). Novelty-seeking is a stronger predictor in later adolescence (Flagel et al., 2014; Lukkes et al., 2016). Together, these three key behaviors, working memory, impulsivity, and novelty-seeking, are measures that are strong indicators of increased drug use.

Biochemically, research aimed at identifying measures associated with drug use has been less fruitful across all species. Low levels of brain-derived neurotrophic growth factor (BDNF) in saliva may reflect brain activity. For example, we found that salivary BDNF levels correlated with BDNF levels in the prefrontal cortex in juvenile rats (Jordan and Andersen, 2018). Saliva levels of BDNF were also significantly associated with impaired memory and relapse to cocaine-taking behavior (Jordan and Andersen, 2018). Salivary BDNF levels are stable across maturation in males and can serve as a biological indicator of addiction (Jordan and Andersen, 2018). The relationships between BDNF, memory, and drug use were not as clear in female rats. These rats were too young for puberty, so it is unlikely this resulted from hormone changes. However, the female brain is more mature than the male brain at this stage (Brenhouse and Andersen, 2011). More research needs to establish these inter-relationships, because they would be useful for predicting risk for addiction in individuals with ADHD, early child maltreatment (which reduces cortical BDNF levels) (Lukkes et al., 2018), high levels of aggression, and other factors.

The last measure that has direct translation is magnetic resonance imaging (MRI). MRI is a "picture that is worth a thousand words" and could be a useful and robust predictor of substance dependence, but MRI is not practical due to both cost and access. Changes in blood flow in cocaine addicts were one of the first uses of MRI in research (Maas et al., 1998). As clear as the image provided, increased blood flow is evident in the ventral striatum in response to a cocaine cue's presentation. Researchers at the National Institutes of Health have shown species-similar increases in blood flow in response to a conditioned cocaine cue in mice with a history of cocaine (Liu et al., 2013) or even dams with a history of cocaine use (Caffrey and Febo, 2014). Juvenile rats exposed to a cue associated with cocaine do not respond unless they are further manipulated to resemble adolescence. Juvenile rats, like humans (Rapoport et al., 1978), do not generally like how stimulants make them feel. When a virus that causes an increase in a dopamine receptor (D1) is injected into the prefrontal cortex, juvenile rats prefer cocaine-associated environments and cues. That same cocaine cue presented in the MRI increases blood flow in the same regions activated in human and mouse chronic users (Lowen et al., 2015). In adult humans and animals, changes in D2/D3 receptor are associated with impulsivity and drug use (Dalley et al., 2007; Volkow et al., 2006). These data and approaches provide ways to examine parallel mechanisms of vulnerability in future studies.

Additional measures that reflect the timing of the sensitive period can be used to identify optimal windows of opportunity to identify and intervene. A translational approach to identifying endophenotypes (or measurable biological indicators correlated with behavioral symptoms and underlying genetics) that are related to human disorders, coupled with a strong knowledge of sensitive periods and underlying mechanisms, can be used to prevent or reduce psychiatric symptoms by early adulthood. While there is evidence for a number of promising biological indicators for addiction risk, there is insufficient research to develop prevention programs at this stage. Greater understanding of the underlying mechanisms of underlying behavioral change is needed. In order to advance prevention science, more investment is needed in both the cause and consequences of drug use within developmental animal models.

7. Research issues in adolescent brain development in relation to drug abuse liability (all participants, led by Charles Vorhees and John Talpos)

Panelists agreed that the effects of adolescent exposure to substances of abuse differ from the same exposure of adults. Human data indicate that drug use initiation during adolescence poses a greater risk for addiction and long-term effects than when drug use starts later in life (HHS, 2014). Differentiating adolescent exposure from adult exposure is challenging, because most humans initiate substance use during adolescence and young adulthood. Factors critical to research aimed at differentiating adolescent from adult exposure include the timing of assessment, timing of exposure, dose and route of administration, and whether exposure is continuous or intermittent. Drug exposure during the course of development will fully express enduring changes after development is relatively complete. The peak of gray matter expression for many clinically relevant brain regions occurs during adolescence. Therefore, when drug exposure occurs earlier in the neuronal and synaptic overproduction phase, latent effects are likely to be expressed later in life as synaptic pruning occurs. As pruning is often protracted, this once latent effect will gradually increase in magnitude with age.

The timing of a drug exposure is critical to understanding how the adolescent brain will cope and respond to a potential "toxic" exposure. The nature of the change depends on the expression of signaling systems and their location in the brain. The three preclinical talks discussed the importance of receptor density and location in determining the likelihood of persistent effects of drug exposure. The main receptor for marijuana that confers the pleasurable effects is the cannabinoid receptor 1. This receptor appears early in life and is widely distributed in the brain. Exposure to THC during adolescence can increase the risk of subsequent drug abuse, including heroin; while exposure in vulnerable individuals can lead to psychosis. Nicotinic acetylcholine receptors similarly are found throughout the brain. Exposure to nicotine during early adolescence may increase the risk of dependence. The dopamine 1 receptor has a more localized distribution and shows transient overproduction during adolescence followed by pruning to adult levels. The phase of overproduction is the period when drug exposure may have a selective impact on adult outcomes. In some instances, early exposure may have a protective effect.

There are significant age differences in drug sensitivity across development. The "preferred" dose of a specific drug may be lower during adolescence when the underlying mechanisms of action are more sensitive. Pharmacokinetic differences also exist between different routes of administration that need to be taken into account in modeling later susceptibility to drug dependence. Injection of drugs of interest, versus combustion/inhalation, versus oral administration produce can different effects. Unfortunately, how pharmacokinetics change across age for different routes of administration are poorly understood. Finally, data show that the effects of intermittent versus continuous drug administration before adolescence differ than in adults. Intermittent delivery is more reflective of the brief environmental exposures that program brain responses. Continuous drug exposure before adolescence may elevate or attenuate baseline effects without challenging the

immature system to adapt.

The panelists agreed that there is emerging evidence supporting some behavioral, neurochemical, or other endpoints as unique biological markers (i.e., predictors) of adolescent vulnerability to dependence. For example, the last presentation on translational approaches showed how behavioral risk factors can be predictive of the need for eventual drug use intervention. Dopamine is involved in addiction, and dopaminergic cells undergo pruning during adolescence. However, the role of these processes in the vulnerability of the adolescent brain remains unclear. More evidence is needed to determine whether such risk factors and other biological markers are drug specific or whether there are consistent patterns across different drug classes.

A major challenge to determining if adolescents have a unique susceptibility to dependence is whether and to what extent the human data allow distinction between the increased risk due to biological immaturity, an underlying biological susceptibility to dependence, or psychosocial and environmental factors for substance dependence. A key issue for understanding biological susceptibility to dependence is the relationship between substance use and mental health and behavioral problems. In particular, understanding to what extent substance use causes mental health and behavioral problems, adolescents with underlying mental health and behavioral problems may seek out substances, and these two processes may be mutually reinforcing. Not surprisingly, baseline values are important for determining outcomes. Medications designed for a clinical population may be helpful for the group that needs them, but harmful to an abusing population. In most instances, medication is prescribed to normalize a pathological condition. Take for example the case of psychostimulants and ADHD. It has been well documented that individuals diagnosed with ADHD are significantly more likely to use, abuse, and become dependent upon alcohol and drugs (Biederman et al., 1998). Moreover, individuals with ADHD have a higher incidence of drug relapse and shorter period of remission than a comparable population (Wilens et al., 2010). Methylphenidate and amphetamine are two of the leading treatments for ADHD; both are also highly addictive drugs prone to abuse. Counter intuitively, individuals who have their ADHD treated with the drugs show a substantial *decrease* in the overall incidence of addiction (Wilens et al., 2003; Faraone and Wilens, 2003; Biederman et al., 2008). These data highlight the need to consider unique or vulnerable populations when evaluating the addictive and neurotoxic potential of drugs of abuse. When designing testing systems, knowing the baseline conditions for the target population could significantly influence the outcome. Additionally, owing to small sample sizes, there is a lack of epidemiological evidence on subpopulations, which hinders understanding heterogeneous effects across subpopulations.

Environmental factors important for shaping adolescent substance use include the influence of socioeconomic position and exposures to stress and trauma. A key challenge in this area of research is the difficulty of parsing the unique effects of environmental risk factors. One promising area of research pertains to stress “histories,” where adverse childhood experiences are cataloged to determine a total stress load. In animal models, stress significantly influences the expression of drug effects. In animal studies, differences in housing conditions of animals can significantly affect the outcome and need to be taken into account. Changes in maternal care of rats, for example, reduce responsiveness to reward whereas exposure to adolescent stressors (isolation housing, social defeat) increases drug sensitivity. Other approaches used to manipulate environmental influences include barren-cage housing or chronic variable stress, where the animal lives in a state of uncertainty. Exposure to stress further changes affect, which can also have secondary effects on a number of measures. All of these environmental events should be included in analyses given their influence on shaping development.

The panelists identified a number of considerations for future research. Box 1 lists specific research questions raised during the discussion. First, there currently is no agreement on how to define and operationalize adolescence. Key considerations include how to divide age, determining at what point researchers consider developmental periods to be stable, whether to focus on chronological age or a developmental domain approach (based on competencies) (e.g., Somerville, 2016), how adolescence is differentiated from puberty, and whether adolescence is the same in males and females (especially in rodents).

Important considerations for making inferences from rodent studies include: whether a standard definition of rodent adolescence is needed or if applying a range of models is just as informative; understanding how well current animal models are predictive of adolescent effects in humans. An ongoing issue in animal studies of drugs on adolescent brain is that most studies of this kind do not compare adolescent exposure to one of the same length administered prior to or after the adolescent period. Such evidence is essential to prove that the presumptive vulnerability of the adolescent brain is differentially affected. In rodents, the start of adolescence is usually denoted as the start of puberty (Korenbrot et al., 1977; Yoshimura et al., 2005; Thigpen et al., 2007). This can be done in humans too, but there are no defined markers in rodents or people for when adolescence ends. In people, adulthood is determined legally (e.g. 18 years of age). This is a social convention, but as brain imaging research has shown, the human cortex, particularly the prefrontal cortex, does not reach full maturity until 25 years of age, or in some studies even later (Dosenbach et al., 2010; Lynch et al., 2020; Tamnes et al., 2010). Neuroimaging combined with psychological tests for risk taking behavior and making judgments between competing choices, reveal that gray matter development correlates with higher cognitive function, indicating that legal definitions of adulthood are inaccurate (Weise et al., 2019). By convention, human adulthood is defined as 18 years of age, but 25 years of age would be closer to biologically-based adult cognition. In rodents, by contrast, there is no definitive age when adulthood is reached (Andersen, 2003). Postnatal day 60 is often used, but how accurate it is, is not known.

Second, there lacks consensus on endpoints. Behavioral endpoints measured in adolescent rodents and humans are different, but their relationship to one another is often unclear. In some cases, such as hippocampal involvement in spatial learning and memory, there appears to be good across species comparability among mammals, but in other areas, such as anxiety, depression, fear, and social behavior, equivalencies across species are more difficult and associations weaker. Another issue is when addiction is the endpoint of greatest interest versus other measures of neurotoxicity (e.g., cognitive decline) or toxicity. For example, inhalant abuse can cause acute neurotoxicity whereas the concerns around tobacco use are more centered on the elevated risks for cancers, pulmonary vascular, and respiratory disease.

Third, the panelists discussed the importance of examining potential sex effects. There are animal and human data showing sex differences in adolescent brain development and behavior, but few experiments investigated such differences or their implications in terms of how drugs of abuse differ between male and female rodents. Among studies that did examine sex differences, many found sex-specific effects of drug abuse or more severe effects in one sex compared with the other. To what extent are such effects found in other species? There are studies, such as with THC, where adolescent exposure in rats shows differential effects, but how prevalent this phenomenon is remains unclear. Sex-related hormone differences before puberty suggest underlying differences in the substrate upon which drugs act during brain development. In the case of stimulants, evidence suggests that differences in effect are the result of differences in the timing of development of specific subregions of the brain in males versus females. How best to account for such differences is not yet settled, and there are a number of unresolved issues. First, sex is typically treated as a binary variable. Second, gonadal hormones may act as covariates that interact with brain substrates to modify a drug's effects. Third, removal of sex hormones (castration or ovariectomy) coupled with replacement using estrogen, testosterone, or progesterone injections is one way to investigate these

Box 1
Questions for future research.

Defining and operationalizing adolescence

- How is adolescence defined in rodents compared with humans?
- Are or should there be standardized definitions?
- Do we know enough about the unique aspects of adolescent brain development to create more precise definitions?
- Can biological indicators be generated that would demarcate the adolescent stage of brain development?
- Does it make biological sense to define a point at which the brain stops changing with advancing age?
- Should we focus more on a developmental domain approach (i.e., when an organism is capable of performing a specific cognitive task) or should the focus be on a chronological age approach and markers of stages of brain ontogeny (e.g., Somerville, 2016)?
- To what extent is it possible to extrapolate from rat to human adolescence since in people adolescence is not only a stage of brain maturation it is also a phase of social development representing the transition from childhood to adulthood?

Biological endpoints

- How does one compare a drug-induced difference in different behaviors between species?
- Are there underlying structure-function relationships across mammals that can be used to map one type of behavior from a rat to humans?

Sex and gender effects

- How can research deal with gender that is not binary? Does or can it apply to animal models?
- Is it possible that a transgender individual might have a different drug and toxicity response profile than non-transgender people?

hormones as they impact how a drug affects brain development. Issues on the horizon might relate to gender and whether animal models can address nonbinary or transgender issues.

Finally, the panelists discussed regulatory considerations for research and especially focused on how science can contribute to recommendations for clinical practice or drug development evaluations to reduce or prevent adolescent drug dependence. Importantly, nicotine, THC, and methylphenidate are all subject to different regulations. Regulations occur at different levels of government (local, state, federal) and some regulate abuse liability (dependence potential) whereas others focus on therapeutic potential. This may pose a particular challenge for determining abuse liability. For example, the federal government regulates research on marijuana, and the material provided to researchers is not comparable to what is available to consumers in states where recreational use has been legalized. Thus, scientists cannot study the forms of marijuana to which people are most exposed. Researchers interested in translation need to understand the implications of different regulatory standards and of coordinating across different agencies to ensure they are modeling what teens are actually doing when they use addictive drugs. A knowledge of regulatory issues and concerns may enhance the real world impact of adolescence drug abuse research.

Disclaimer

Leslie Kwan conducted this non-sponsored research while a doctoral student at The George Washington University. The information in these materials is not a formal dissemination of information by the Food and Drug Administration (FDA) and does not represent agency position or policy.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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