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AMPed-up adolescents: the role of age in the abuse of amphetamines and its consequences on cognition and prefrontal cortex development

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Abstract

Adolescent use of amphetamine and its closely related, methylated version methamphetamine, is alarmingly high in those who use drugs for nonmedical purposes. This raises serious concerns about the potential for this drug use to have a long-lasting, detrimental impact on the normal development of the brain and behavior that is ongoing during adolescence. In this review, we explore recent findings from both human and laboratory animal studies that investigate the consequences of amphetamine and methamphetamine exposure during this stage of life. We highlight studies that assess sex differences in adolescence, as well as those that are designed specifically to address the potential unique effects of adolescent exposure by including groups at other life stages (typically young adulthood). We consider epidemiological studies on age and sex as vulnerability factors for developing problems with the use of amphetamines, as well as human and animal laboratory studies that tap into age differences in use, its short-term effects on behavior, and the long-lasting consequences of this exposure on cognition. We also focus on studies of drug effects in the prefrontal cortex, which is known to be critically important for cognition and is among the later maturing brain regions. Finally, we discuss important issues that should be addressed in future studies so that the field can further our understanding of the mechanisms underlying adolescent use of amphetamines and its outcomes on the developing brain and behavior.

Adolescence, estimated to begin around 12 years old and continue into the mid-twenties in humans (Dahl, 2004), is the transitional period between childhood and adulthood characterized by considerable maturation in the brain and behavior. This developmental period may constitute a window of potential vulnerability to the adverse consequences of

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environmental experiences such as exposure to drugs of abuse, which often begins during adolescence (Casey and Jones, 2010; Gulley and Juraska, 2013). In fact, compared to those who initiate later in life, adolescent-onset substance users typically experience worse outcomes, which include a greater likelihood of developing clinical features of substance use disorders (SUDs; Chen et al. 2009). Furthermore, the suboptimal effectiveness of existing SUD treatments is especially problematic in adolescent-onset users, with ~50% relapsing to drug use after initial treatment compared to a ~30% initial relapse rate in adult-onset users (Poudel and Gautam, 2017). Females also tend to experience worse outcomes of drug use, including a more rapid transition from initial to problem drug use (Brecht et al., 2004; Haas & Peters, 2000; Piazza et al., 1989). For example, one report estimated the transition latency from initiation to problem cocaine use was over 50% shorter in females compared to males (Haas and Peters, 2000).

Taken together, this evidence suggests that age-of-onset and sex are factors that confer heightened susceptibility to problems with substance use. Importantly, adolescents' neurobehavioral development is often sex-specific (Juraska and Willing, 2017), which raises the possibility that these vulnerability factors may interact. Elucidating the underlying behavioral and neurobiological mechanisms of vulnerability may provide new avenues to improve current SUD treatments and, ultimately, outcomes for at-risk populations including adolescent-onset users and females. Here, we review studies that investigate the use and associated consequences of amphetamine (AMP) and its more frequently abused, methylated derivative, methamphetamine (METH), in adolescent humans and laboratory animals, with a focus on those studies that include both sexes and directly compare adolescents to adults.

Drug Use by Adolescents

It is important to acknowledge that in addition to their potential for abuse, amphetamines have long been used in the treatment of attention-deficit/hyperactivity disorder (ADHD), especially in children and adolescents. Amphetamines and methylphenidate are the most commonly prescribed psychotropic medications in children (Zito et al., 2003) and the incidence of ADHD diagnosis has increased dramatically since the 1990s (Scheffler et al., 2007). Both AMP and METH are FDA-approved treatments for ADHD in doses given orally of up to 40 mg (20 mg for adults) or 25 mg, respectively (Berman et al., 2009). In this review, we differentiate studies utilizing therapeutically applicable doses from those using higher, abuse-related doses that are associated with recreational (i.e., non-therapeutic) use. The bulk of our discussion is focused on frequent, high dose exposure and it is notable that some evidence suggests age differences in the effects of amphetamines in both humans and animal models are dose dependent.

Epidemiological studies

Amphetamine-type stimulants are among the most frequently abused illicit drugs worldwide (UNODC, 2016). The largest contributor to global law enforcement seizures of amphetamine-type stimulants is METH, and North America reported the highest seizures of METH compared to other regions (UNODC, 2016). Prescription rates for stimulants have also risen in recent years, in both young people and adults, which increases the potential for

nonmedical use (Compton et al., 2018). Notably, self-reported misuse of prescribed stimulants has been suggested to be between 15 and 35% in college students of the late adolescent/young adult age range (DeSantis et al., 2008; Lakhan and Kirchgessner, 2012). This population reports their primary source of these drugs is prescription diversion from friends at low or no cost (Bavarian et al., 2017), suggesting alarmingly easy access to amphetamine-type stimulants in this population.

AMP and METH use in adolescent populations may lead to an array of adverse consequences. Adolescents who use METH more frequently exhibit problem behaviors, including antisocial behavior, polydrug use, risky sexual behavior, and adolescent pregnancy (Zapata et al., 2008; Embry et al., 2009). This can lead to physical and mental health issues, including seizures/convulsions, depression, hallucinations, and paranoia (Sommers et al., 2006). The majority (~60%) of users of psychostimulants other than cocaine began their use before age 18, and were 70% more likely to develop clinical symptoms within 2 years of initiation compared to adult-onset (age 18+) users (Chen et al., 2009). METH use may be especially problematic for adolescents as those who use METH as their primary drug of abuse experience higher relapse rates than adolescents who abuse other drugs (Rawson et al., 2005). Studies on METH use patterns also have reported that individuals who maintain high METH use ten years after initiation tend to have an earlier mean age of onset for METH use (17 years old) compared to those who maintain lower use levels (19–20 years old; Brecht et al., 2013). In populations seeking treatment for a METH-related SUD, those who entered their first treatment program as young adults (18–25 years old) were more likely to have initiated their METH use prior to age 15, to rapidly escalate this use in the years leading up to treatment, and to maintain higher levels of use after treatment compared to those entering these programs after age 25 (Evans et al., 2013).

There is some evidence suggesting vulnerability to initiate METH use may be influenced by both age and sex. More adolescent female users reported METH as their primary drug of choice compared to adolescent male users (Rawson et al., 2005) and adolescent females tend to initiate METH use earlier than males (Hser et al., 2005; He et al., 2013). The growing accessibility of amphetamines creates an urgent need to better understand the underlying mechanisms contributing vulnerability to these adolescent and female populations at higher risk of developing problems with the use of these drugs. In the next section, we discuss what existing work in this area has demonstrated and highlight the importance of continuing to expand our understanding of the potential intersection of these risk factors.

Laboratory studies in humans

Although relatively limited in number, some laboratory studies have investigated the effects of amphetamines in healthy human subjects. In one such study (Cavallo et al., 2016), young adult subjects (mean age = 20 years old) underwent classical conditioning of a cue with oral administration of placebo or a METH dose on the upper end of the therapeutic range (20 mg), followed by extinction training. After conditioning, subjects displayed preference for the drug-paired cue in a forced choice task and this preference diminished across extinction sessions. Interestingly, the extinction of this behavioral preference was blunted in individuals who reported greater positive subjective effects to METH during conditioning. These

findings suggest that individuals who experience more positive drug effects may not respond as well to extinction-based drug treatment programs. This study had a wide age range (18–35 years old) that extends beyond the adolescent period, so it is unclear whether adolescents and adults differ in their subjective responses to amphetamines.

Although direct comparison of subjective effects of amphetamines in adolescent and adult humans is currently lacking in the literature, a more recent study (Schepers et al., 2019) examined the subjective effects of AMP in a specifically adolescent population (18–19 years old). Participants reported their subjective effects following oral intake of AMP within a therapeutic dose range (10 or 20 mg) or placebo. Administration of AMP led to rewarding subjective effects, including reports of liking the drug, wanting more of the drug, feeling high, increased elation, and increased positive mood. No gender differences were observed, but a previous study from this same lab (Mayo et al., 2019) found that females gave higher ratings of vigor and reduced sedation following 20 mg METH compared to males. An important distinction between these two studies is the ages of the participants. Mayo et al. (2019) used participants ranging in age from 18–35 years old (average: 24.8), whereas Schepers et al. (2019) had a smaller age range of 18–19 years old that is more specific to adolescence. Sex differences in the subjective response to amphetamines in humans may emerge during late adolescence or young adulthood, but this distinction is impossible to confirm without a direct comparison between separate groups of adolescents and adults. Future studies that do this are clearly needed.

Laboratory studies in animals

One of the challenges with laboratory studies in humans is the ethical constraint of administering amphetamines to adolescents younger than 18 years old, thus limiting these studies to later adolescent ages. Tapping into the effects of AMP and METH use in earlier age ranges therefore requires the use of animal models. Many of the characteristic developmental changes that occur during adolescence are conserved across species (Spear, 2000), making animal models a useful tool to address these issues and further our understanding of the use and consequences of amphetamines taken during adolescence. While there remains no firm age range agreed on by the field, one widely-used definition of rodent adolescence is from postnatal (P) day 27–60 (Spear, 2000); however, this period encompasses a very broad range from post-weaning to well past onset of sexual maturity. The onset of puberty is a hallmark of adolescence, associated with not only the surge in gonadal hormones that underlies sexual maturation, but also adolescent neurobehavioral development in both humans and rodents (Juraska and Willing, 2017). The physical markers of pubertal onset in rats—namely, vaginal opening in females and preputial separation in males (Korenbrodt et al., 1977; Castellano et al., 2011)—occur earlier in females (~P35) than males (~P45), further complicating the definition of adolescence. For the purpose of this review, we will focus on a commonly accepted range of P27–P60 to represent rodent adolescence and indicate ages that were tested in the studies that are discussed.

Rodent models have been crucial in expanding our understanding of both the behavioral and neural mechanisms underlying adolescent vulnerability to amphetamines. The three most common rodent experimental models that have been used to assess the outcomes of AMP

and METH use are locomotor sensitization, conditioned place preference (CPP), and self-administration. In this section, we will discuss the evidence for age differences from each of these models.

As apparent by their classification as psychostimulants, amphetamines have potent locomotor activating effects. With repeated exposure, animals can become sensitized to these behavioral effects. This paradigm has been used in multiple investigations into age-dependent differences in response to both AMP and METH. The early postnatal ontogeny of psychostimulant sensitization and CPP has been reviewed elsewhere (Tirelli et al., 2003). Here, we focus on the studies that directly compare exposure to AMP-type stimulants in adolescence and utilize an adult-exposed comparison group.

Acute administration of amphetamines stimulates locomotor activity compared to saline in both adolescents and adults; however, these acute effects are typically blunted in adolescent compared to adult rats across a range of adolescent ages (P30–45; Bolanos et al. 1998; Mathews and McCormick 2007; Mathews et al. 2009, 2010; Zakharova et al. 2009; but see Sherrill et al., 2013). Notably, studies using mice have reported increased (P45; Kameda et al. 2011), equal (P33; Niculescu et al. 2005), or decreased (P30; Zombeck et al. 2009) activation in adolescent compared to adult mice, suggesting that the specific age during adolescence may be more important for determining adolescents' response to the motor stimulating effects of acute exposure to amphetamines in mice compared to rats.

Studies examining age differences in AMP-induced behavioral sensitization appear to be more nuanced. The most common behavioral sensitization that has been assessed is sensitization to locomotor activation. Reports using rats and mice have found that adolescents have greater (Adriani et al., 1998; Mathews and McCormick, 2007; Mathews et al., 2009, 2011; Kameda et al., 2011), equal (Niculescu et al., 2005; Hankosky et al., 2013; Sherrill et al., 2013; Kang et al., 2016a), or reduced (Mathews and McCormick, 2007; Richetto et al., 2013) sensitization relative to adults. Many factors could contribute to these disparate findings, such as dose, withdrawal period, and sex. Brief or low, therapeutic-like doses (e.g. 0.5 mg/kg) of AMP can induce locomotor sensitization more readily in adolescent compared to adult rats (Mathews and McCormick, 2007; Mathews et al., 2009, 2011; Kameda et al., 2011). In two studies using higher, abuse-like doses of AMP (2–3 mg/kg), this age difference was eliminated (Niculescu et al., 2005; Sherrill et al., 2013); however, another study using a very high 10 mg/kg dose saw greater sensitization in adolescents (Adriani et al., 1998). In addition to dose, the withdrawal period after exposure to AMP may impact the expression of age differences in locomotor sensitization. Our lab reported similar AMP-induced locomotor sensitization in adolescent- and adult-exposed rats 3–4 months after exposure (Sherrill et al., 2013). Richetto and colleagues (2013) found that rats exposed to high doses of AMP (2.5 mg/kg) during adolescence demonstrated sensitization after 4 weeks of withdrawal, while those treated with a lower dose (1 mg/kg) did not display significant locomotor sensitization. Adult-exposed rats in this same study showed locomotor sensitization after the long withdrawal, regardless of the AMP dose.

Sex is another factor that can influence age differences in AMP-induced locomotor sensitization. Interestingly, locomotor sensitization in adolescents seems to be sex specific,

with adolescent females displaying greater sensitization to the locomotor activating effects of AMP than adolescent males (Brown et al., 2011). Moreover, sensitization can be induced in adolescent females at lower doses of AMP than adult female and male rats regardless of age (Mathews and McCormick, 2007). Adolescent neurobehavioral development is often sex-dependent (Juraska and Willing, 2017), which makes it important to include both sexes when investigating vulnerability to long-lasting disruption by exposure to amphetamines. Future studies should examine both males and females to determine potential sex differences in response to repeated exposure to these drugs.

In addition to a relative dearth of studies in females, most of the aforementioned studies examining sensitization have focused on the locomotor activating effects of amphetamines; however, stereotypy—repetitive movements localized to a small area—is another behavior that can become sensitized following repeated exposure to these drugs. Several reports have demonstrated blunted sensitization of AMP-induced stereotypy in adolescents compared to adults (Adriani et al., 1998; Bolanos et al., 1998; Hankosky et al., 2013; Sherrill et al., 2013; Kang et al., 2016a). Notably, our lab has shown that this age-of-exposure dependent difference in stereotypy persists, albeit attenuated, when the rats are challenged up to 3–4 months later, well into adulthood (Hankosky et al., 2013; Sherrill et al., 2013; Kang et al., 2016a). These findings provide evidence that AMP exposure during adolescence detrimentally impacts the development of an adult-like sensitization response to AMP-induced stereotyped behavior as these rats mature into adulthood. As our work only included male subjects, it is currently unknown whether the sex-specific effects of AMP-induced locomotor sensitization seen in adolescents also exhibits a long-lasting change into adulthood.

Another paradigm that assesses drug effects is conditioned place preference, which has been used with AMP-type stimulants to examine age differences in conditioning of a drug-associated contextual memory. The findings with this paradigm have been mixed. One study in mice found that adolescent males (P30–49) demonstrated a blunted AMP CPP relative to adult males (Adriani and Laviola, 2003). Another study with AMP found that both adolescent (P45–52) and adult rats of both sexes established significant AMP CPP, but this effect did not differ by age or sex (Mathews and McCormick, 2007). These disparate findings may be due to differences in the conditioning procedures or they could be another case of species differences. In support of this notion, adolescent male rats (P34–38) display significant METH CPP after a short 3-day training protocol, while adult males do not significantly prefer the METH-paired chamber (Zakharova et al., 2009). In this same study, this age difference was eliminated after a longer 5-day conditioning procedure after which both adolescent and adult groups show significant METH CPP, demonstrating that the length of the training in CPP can influence reported age differences.

Only two studies have used adolescent rats of both sexes in examining CPP with AMP. Mathews and McCormick (2007) reported no significant sex difference in adolescent or adult rats, while another study found that adolescent females established a significant AMP CPP but adolescent males failed to do so (Brown et al., 2011). A potential explanation for these inconsistent findings of sex differences in adolescence may be the age of the rats. Brown et al. tested rats on P32–39, while Mathews and McCormick used an older adolescent

age of P45–52. Notably, the pubertal status of males and females differ between these age ranges (Korenbrodt et al., 1977; Castellano et al., 2011; Juraska and Willing, 2017). Females would likely be peri-pubertal onset in the Brown et al. experiment, while they would be post-pubertal in the other study. Just like in humans, puberty onset in males occurs later compared to females, with the males in Brown et al. likely being pre-pubertal and the Mathews and McCormick study's males being peri-pubertal onset. More research is needed to determine whether puberty onset plays a role in observed sex differences between adolescent males and females in drug-paired contextual memory using amphetamines.

Most work on age differences in response to AMP and METH has employed behavioral sensitization and conditioned place preference paradigms; however, more recent research has begun investigating potential age differences in an animal model with greater face validity—self-administration. Many of the clinical features of SUDs can be modeled using self-administration paradigms. The diagnostic criteria for SUD outlined in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V) include, but are not limited to, spending a great deal of time obtaining and using the drug, craving the drug, taking more drug than intended, failure to cease drug use, and continued drug use despite negative consequences. Few studies have examined these domains in adolescents and even fewer have included an adult group for direct assessment of potential adolescent vulnerability.

A relatively limited number of studies have examined age differences in the acquisition of and motivation for AMP or METH self-administration. With AMP available as the reinforcer during a 14-day acquisition period, adolescent rats of both sexes that began training on P35 met acquisition criteria faster than adult rats that started on P90 (Shahbazi et al., 2008). Moreover, the adolescent rats earned significantly more AMP infusions, which led to higher cumulative intake compared to the adult group. When motivation for the drug was assessed by changing to a progressive ratio (PR) schedule of reinforcement, our lab found that adult-onset (P90) rats acquire self-administration of a low dose of METH (0.02 mg/kg/inf) more readily than adolescent-onset (P40) rats (Hankosky et al., 2018a). At all doses we tested, adolescent-onset males were the group with the lowest proportion of rats meeting acquisition criteria. This finding is in line with a previous study showing adolescent male rats (~P35–45) self-administer a low dose of METH (0.03 mg/kg/inf) at the same rate they respond for saline in 2-h sessions (Motbey et al., 2013). While the findings with AMP and METH self-administration appear to be in conflict, the latter are generally consistent with the findings from the sensitization and CPP literature that suggest there is a reduced sensitivity to the stimulant, rewarding, and reinforcing effect of amphetamines in adolescent compared to adult males.

One explanation for this age difference in acquisition of self-administration could be lower motivation for the drug in adolescents. Indeed, adolescent males earned fewer AMP infusions under a PR schedule than adult males (Shahbazi et al., 2008). Interestingly, the opposite was true for females: young adult females had higher PR breakpoints than their older adult counterparts as well as age-matched males that also acquired during adolescence. When METH is the reinforcer, adult-onset rats reach higher breakpoints across a range of doses compared to adolescent-onset rats, regardless of sex (Hankosky et al., 2018a). With only two comprehensive investigations of age and sex differences in self-administration of

amphetamines, it is challenging to draw firm conclusions regarding the potential vulnerability of adolescent females and the potential resilience seen in adolescent males. In addition, these studies analyzed behavior under short access conditions (2 h/day) that tend to engender stable drug intake across sessions. Long access conditions (6 h/day), in contrast, are associated with escalated intake across time, which is a more common drug-taking pattern in human users.

Only a few reports have included adolescent and adult groups in studies of long access self-administration (LgA) of amphetamines and these used METH as the reinforcer. In the first, it was reported that adolescent males beginning self-administration at ~P27 had greater METH intake than adults during 6-h sessions, but had similar intake to adults during 2-h sessions (Anker et al., 2012). Adolescents were also reported to escalate their METH intake more than adults as evidenced by greater average intake over the last three days compared to intake on the first three days of LgA (Anker et al., 2012). Typically, escalation is assessed as an increase in intake compared to the first LgA session (Ahmed and Koob, 1998; Kitamura et al., 2006). Our lab has extended the findings of Anker and colleagues by including females in both age groups. We have found that adolescent-onset rats (P40) of both sexes earn more METH during LgA sessions than their adult counterparts (P90) and escalate their intake relative to the first LgA session (Westbrook and Gulley, 2020; Westbrook et al., 2020). There are at least three potential explanations for this age-of-onset difference in METH intake: (1) individual differences in escalation, (2) differences in sensitivity to METH, and/or (3) differences in the development of compulsive drug-taking.

Differences in escalation between age-of-onset groups may be due to individual differences in the proportion of rats within a group that escalate their intake. The adolescent-onset group may have higher METH intake because more of the animals in that group escalated their intake relative to adult-onset groups. In order to address the issue of individual differences, which requires a large sample size, we combined data from our two recent studies that used identical procedures for LgA self-administration of METH (Westbrook and Gulley, 2020; Westbrook et al., 2020). The proportion of rats in each age-of-onset and sex group that escalated their METH intake (>5% increase) from the first to the last LgA session is shown in Figure 1. These data show that individual differences in the propensity of rats to escalate their METH intake are unlikely to contribute to the greater METH intake by adolescent-onset rats because the proportion of adolescent-onset rats that escalate is not statistically higher than adult-onset rats.

An alternative explanation for the reported age-of-onset difference in METH intake during LgA is a reduced sensitivity to METH in adolescents. Less sensitivity to METH in adolescents would mean that adolescents would need to take more METH in order to achieve a similar effect to that in adults. Indeed, we have previously reported a reduced sensitivity to several doses of METH in adolescent-onset rats compared to their adult-onset counterparts using a PR schedule (Hankosky et al., 2018b). One challenge to this interpretation is that when trained to self-administer different doses of METH (0.05, 0.08 or 0.1 mg/kg/inf) under ShA conditions there were no significant age-of-onset differences in acquisition (Hankosky et al., 2018a) or METH intake (Westbrook and Gulley, 2020; Westbrook et al., 2020).

If reduced METH sensitivity in adolescents were to be at least partly responsible for the increased METH intake seen under long access conditions, adolescents would likely take more METH throughout the 6-h session compared to adults. If this age difference occurs in the early LgA sessions, then it is likely due to reduced sensitivity. However, if it only occurs in the later LgA sessions, it could be due to either tolerance or compulsive drug-taking. We used our combined study data to analyze METH intake per hour in the first and last LgA sessions. We found that all rats increased their METH intake from the first to the last LgA session in each hour of the session (Figure 2A), and adolescent-onset rats earn more METH than adult-onset rats during hours 2–5 when collapsed across session (Figure 2B). These findings provide some evidence for lower METH sensitivity in adolescent-onset rats as they take more METH throughout the session regardless of whether it is the first or last LgA session. One possibility is that adolescents may be less sensitive to METH leading them to take more drug to achieve the same effect as adults, and this heightened drug-taking experience may make this population more prone to developing compulsive drug-taking.

In both our recent studies, adolescent-onset rats not only increased METH intake during the 6-h LgA sessions, but also they significantly escalated their first hour METH intake in the last LgA session compared to the first session, while adult-onset rats did not show this significantly increased drug-loading behavior (Westbrook and Gulley, 2020; Westbrook et al., 2020). These findings suggest that adolescents may be more susceptible to developing compulsive drug-taking. Anker and colleagues (2012) reported that adolescent males continued lever pressing during periods when drug was not available, which provides some evidence for greater compulsive or perseverative behavior in adolescents. The notion that adolescents more readily develop compulsive drug-taking needs to be further addressed in paradigms that tap into one of the key diagnostic criteria for SUDs, which is continued drug seeking and use despite negative consequences. For instance, self-administration studies using signaled non-availability periods or punishment with aversive stimuli (e.g., footshocks) are two approaches that future research could employ to assess age differences in the compulsivity of taking amphetamines. These paradigms have shown that adolescents more readily develop compulsive cocaine taking (Anker et al., 2011; Holtz and Carroll, 2015), but whether the same is true of AMP-type stimulants remains to be determined. Future studies should aim to determine the relative contribution and role of reduced sensitivity vs. developing compulsive drug-taking with regard to adolescent use of amphetamines.

One of the hallmark problems with SUDs is that they are chronic, relapsing disorders. Even after extended periods of abstinence from drug use, individuals can experience relapse in their drug taking. Furthermore, relapse can occur in response to multiple triggers, including stress and drug-related cues, even after individuals have completed treatment for SUDs. Exposure therapy is one treatment used in humans suffering from SUDs and this procedure is based on the principles of extinction learning. Despite promising results in adult rodent models, clinical applications of exposure therapy have provided minimal improvements in treatment outcomes, at best (Mellentin et al., 2017). One explanation for the lack of success of this treatment approach in clinical populations is that many human drug abusers initiate their use during adolescence, but rodent models of extinguished drug-seeking have mainly focused on adult-onset use. Adolescent drug use may delay the development of extinction

learning that typically occurs across adolescence (McCallum et al., 2010; Andrzejewski et al., 2011; Hammerslag and Gulley, 2014; Westbrook et al., 2018), which may contribute to relapse vulnerability. Studies using cocaine self-administration have provided some evidence in support of this notion with adolescent males showing worse extinction learning than adult males (Anker and Carroll, 2010; Zbukvic et al., 2016); however, less is known about adolescent extinction following use of AMP or METH. To our knowledge, our lab has reported the first investigation into age and sex differences in extinction of LgA METH self-administration. We found that adolescent-onset rats (P40) of both sexes did not differ from adult-onset rats in extinction learning after long access METH self-administration (Westbrook and Gulley, 2020). In addition, the only factor in our study that influenced relapse risk in response to a METH priming injection was sex. Females reinstated METH-seeking to a greater extent than males, regardless of age-of-onset. Adolescent-onset users of these drugs may be more susceptible to other triggers of relapse, such as cues, context, or stress, but this idea would need to be studied in future work.

Another contributing factor to relapse is that craving for the drug can in fact increase across abstinence—a phenomenon termed incubation of craving. While it is currently unknown if there are age differences in incubation of craving for AMP or METH, rats of both sexes that began METH self-administration during late adolescence/young adulthood (P56) display significant incubation of METH craving after 30 days of forced abstinence (Everett et al., 2020). This incubation effect has also been reported in older adult male and female rats (Daiwile et al., 2019). Future studies should directly compare incubation of craving for AMPs in adolescent and adult rats to determine if this mechanism could be contributing to the heightened relapse risk seen in adolescent human users.

Impact of repeated exposure to amphetamines

Toxicity of amphetamines

Long-term use of amphetamines has been associated with numerous changes in an individual's general health, including cardiovascular and respiratory complications, nutritional deficiencies and related metabolic dysfunction, and increased incidence of oral disease (Panenka et al., 2013). Adverse neurological consequences are also common in those who abuse these drugs and include an increased risk of stroke and cerebral hemorrhage, psychosis and cognitive dysfunction (Cadet and Bisagno, 2016). Additionally, there is growing evidence that AMP or METH abuse can induce significant epigenetic modifications — such as histone modification and DNA methylation — in a complex manner (Cadet et al., 2015, 2019). Because these effects have been studied primarily in adult populations, the potential age-dependency of these epigenetic changes remain unknown and is thus an important area for future research. It is important to recognize that toxic effects are typically seen with abuse-like doses of amphetamines, whereas therapeutic doses are much less likely to induce toxicity. Indeed, a meta-analysis suggested that therapeutic use of psychostimulants for ADHD in children and adolescents (age range: 4–20 years old) was associated with a normalization in measures of brain structure and function. Specifically, drug treated patients were more similar to healthy controls than unmedicated individuals with ADHD (Spencer et al., 2014).

Whether adolescence represents a period during which individuals are more susceptible to the toxic effects of high dose amphetamines has only received limited attention to date. In one notable study, adolescent (P40) and adult (P90) male rats were given four 10 mg/kg s.c. injections of METH at 2-h intervals and were sacrificed at 1 h or 7 days after the final METH injection. The treatment led to a 20% mortality rate in adults, whereas no adolescent rats died as a result of this high-dose exposure to METH. Additionally, both age groups displayed reductions in markers of dopaminergic and serotonergic function in the striatum at the 1-h withdrawal time and persistent reductions in tryptophan hydroxylase activity at 7 days of withdrawal. In contrast, deficits in dopamine transporter and tyrosine hydroxylase activity at the longer withdrawal period were only observed in the adult-exposed group (Kokoshka et al., 2000). Other studies have also shown that juvenile and adolescent animals may be less sensitive to METH-induced deficits in striatal dopamine and 5-HT function, particularly when the hyperthermic effects of high dose METH are minimized (Cappon et al., 1997).

Consequences on cognition and emotional behavior in users

The focus here will be on studies that evaluate effects of doses relevant to abuse of these substances (1–3 mg/kg in animal models, 40 mg/day in humans) on cognition as these are likely more relevant to the addiction process as well as in treatment success for SUDs (Perry and Lawrence, 2017) as compared to lower, therapeutically-effective doses (<1 mg/kg in animal models, 5–40 mg/day in humans). Moreover, we pay particular attention to investigations of adolescent-onset drug use as several more general reviews of AMP- or METH-induced changes in cognition have been published previously (Dean et al., 2012; Hart et al., 2012; Potvin et al., 2018).

In the small number of studies that have been published to date, use of METH in adolescents has been associated with deficits across multiple cognitive domains. For instance, individuals between the ages of 12 and 23 that met diagnostic criteria for METH abuse or dependence performed worse on a battery of neuropsychological tests than a group of age- and education level-matched participants (King et al., 2010a). These deficits were characterized as impairments in non-verbal reasoning, inhibitory control, and attention. In most cases, performance tended to improve in those who had experienced longer periods of abstinence. Notably, participants in the METH using group had a greater incidence of psychiatric symptoms such as depression, anxiety, mania, paranoia, and psychoticism, as well as a heightened salivary cortisol response to an acute social stress (King et al., 2010b). This study population of 54 METH users and 75 healthy controls was 63% and 50% female, respectively, and the youngest female METH users exhibited the most robust effects on psychiatric symptoms and stress reactivity (King et al., 2010b). In a smaller study of adolescents (mean age = 15.6 years old) that met criteria for combined cannabis and METH abuse/dependence (n=8), volume of the left putamen was significantly greater than the volume measured in age-matched controls. This volume increase, which was also seen (albeit not statistically significant) in a comparison group of subjects (n=9) that met criteria for abuse/dependence on METH alone, was associated with a significant increase in self-reported novelty seeking behavior (Churchwell et al., 2012). In a separate analysis of cognitive functioning and psychiatric symptomology in adolescents diagnosed with METH

dependence and those with METH and cannabis co-dependence, deficits in non-verbal reasoning, memory, planning and self-monitoring were observed in both groups compared to controls (Cuzen et al., 2015). The METH and cannabis co-dependent group was most affected, and significant improvements in performance were observed for all drug using groups at a 12-month follow up. In the only other adolescent study population described to date, METH-dependent participants with a mean age of 18.1 years old that had a mean onset age of METH use of 15 years old were found to have poorer inhibitory control compared to age-matched controls, regardless of gender (Kim et al., 2016). This deficit was associated with significant decreases in N-acetyl aspartate levels in the anterior cingulate cortex, compared to controls. Relative deficits in this amino acid in the brain have been associated previously with a number of disorders, and it has been suggested as a putative marker of neuronal health (Moffett et al., 2007). In an earlier publication describing results of cortical thickness and white matter fiber tract integrity analysis in this same study population, adolescent relative to adult METH users were found to have significantly reduced cortical gray matter thickness and compromised integrity of fiber tracts between the prefrontal cortex and striatum and the orbitofrontal cortex and nucleus accumbens (Lyoo et al., 2015).

Although limited in number, these studies of adolescent METH users are generally consistent with the much larger literature that has demonstrated AMP- or METH-associated deficits in cognitive and emotional behavior in adults. Unfortunately, clinical studies, whether in adolescent or adult users, suffer from multiple limitations that have been highlighted by others (e.g., Hart et al., 2012) and are important to recognize when evaluating the potential causal link between drug use and behavioral dysfunction. These include relatively low sample sizes and the use of cross-sectional designs that make it challenging to determine the potential impact of cognitive deficiencies that pre-date, co-exist, or ever contribute to drug use in vulnerable populations. In addition, it is often difficult to match control group participants on other indicators of intellectual capability such as years of education, or on the co-use of alcohol and/or tobacco. A recent empirical study (Fitzpatrick et al., 2020) and a meta-analysis (Potvin et al., 2018) concluded that there remains sufficient evidence for long lasting deficits in cognition when these factors are accounted for in METH abusers, but it has been valuable to turn to animal models of repeated drug exposure to begin addressing some of these limitations and obtain valuable information about potential neural mechanisms.

Laboratory animal studies of consequences on behavior and neurobiology

One of the key issues that has remained especially difficult to address in clinical populations is whether there is a unique effect of amphetamines in the still-developing, adolescent brain compared to the mature brain of the adult. Animal models are well-suited for such investigations, and there have been many important findings showing the impact of repeated drug exposure in adolescents. In addition to the work we highlighted in a previous review (Hankosky and Gulley, 2016), more recent studies have added to the evidence that adolescent exposure to AMP or METH has the potential to induce significant and enduring effects on the brain and behavior. For example, intermittent administration of these drugs at doses that model abuse-related exposure (1–3 mg/kg given i.p. or s.c.) and that are given during some or all of the periadolescent period in rats and mice (P27–60) have been shown

to increase risk taking behavior and induce deficits in learning and memory, attention, cognitive flexibility, inhibitory control and prepulse inhibition of startle (Kang et al., 2016b; Boomhower and Newland, 2017; Loxton and Canales, 2017; Swanepoel et al., 2017; Nazari et al., 2020). These types of treatment regimens do not always lead to AMP-induced behavioral dysfunction (Izquierdo et al., 2016), nor does exposure to a more therapeutically relevant dose (0.5 mg/kg) or route of administration (p.o.) in either rodent (Jordan et al., 2015) or non-human primates (Soto et al., 2012). Collectively, these studies clearly demonstrate the potential for AMP or METH exposure during adolescence to induce lasting, adverse consequences, but it is difficult for any of these studies to directly speak to the question of a unique vulnerability to the effects of these drugs in adolescents because they did not utilize comparison groups of subjects that were exposed to drug during adulthood.

In a series of studies from our lab that utilized non-contingent and self-administered exposure to abuse-like levels of AMP and METH, we have found evidence that adolescents are sometimes more sensitive to the disruptive effects of these drugs on cognition. For example, in male rats given 3 mg/kg AMP or saline every other day from P35-P55, we found adolescent AMP exposure was associated with significant deficits in working memory performance on a delayed-matching-to-position (DMTP) task administered ~2 months after the last AMP injection. This deficit, which was not observed in rats exposed in adulthood (P98-P116) or following exposure to 1 mg/kg, was associated with an increased susceptibility to proactive interference and a reduction in behavioral flexibility, as measured by decreased performance in the reversal learning component of the task (Sherrill et al., 2013). Subsequently, we demonstrated similar, long-lasting deficits in reversal learning in male rats performing an attentional set-shifting task. In this case, however, the effect was not more significant in adolescents as adult-exposed rats were similarly impaired (Hankosky and Gulley, 2013). In a study testing the impact of 3 mg/kg AMP exposure during an earlier adolescent time period (P27-P45), we found a significantly greater deficit in inhibitory control in adult-compared to adolescent-exposed females. In males, we observed moderate to no effect of AMP pretreatment during adolescence across two different experiments (Hammerslag et al., 2014). Interestingly, in a recent study in male mice that used a different measure of inhibitory control, adolescents (P22-P31) exposed to 4 mg/kg AMP every other day (5 injections total) were impaired in their ability to suppress responding when an inhibitory cue was present compared to similarly exposed mice given the drug during late adolescence (P35-P44) or young adulthood (P75-P84; Reynolds et al., 2019). In an operant strategy shifting task (Hankosky et al., 2018b), we found females that began ShA self-administration of METH during adolescence (P41) exhibited significant deficits in discrimination and reversal learning (i.e., behavioral flexibility) compared to females that began self-administration during young adulthood (P91). These effects were not observed in males. In a separate experiment with rats that underwent LgA METH self-administration, we found a significant, though modest deficit in recognition memory for males and females that began self-administration in adolescence, but not those that began in adulthood (Westbrook et al., 2020). Taken together, these studies highlight that the cognitive effects of AMP and METH that are more disruptive when exposure occurs during adolescence, compared adulthood, are primarily effects on memory, inhibitory control, and behavioral flexibility.

One similarity shared by all the cognitive assessments we have used to date is their sensitivity to disruption of normal functioning in the PFC. AMP use during adolescence has been shown to alter rodent neuronal morphology, with most changes demonstrated in the PFC. One such study reported that twice daily injections of a therapeutic-like dose (0.5 mg/kg AMP) from P22-P34 substantially increased both the length and branching of the basilar dendrites of pyramidal neurons in the medial PFC when rats were sacrificed at P48 (Diaz Heijtz et al., 2003). These structural changes were associated with an upregulation in tyrosine hydroxylase and calcium/calmodulin-dependent protein kinase II (CaMKII) expression within the medial PFC, though it should be noted that this was in a separate group of rats given the same treatment regimen. Tyrosine hydroxylase is the rate-limiting enzyme in the synthesis of dopamine, and the dopamine system, in particular, is a leading candidate mechanism for the unique effects of adolescent compared to adult exposure to amphetamines because of its delayed development in the PFC (Brenhouse et al., 2008; Naneix et al., 2012; Kang et al., 2018; Reynolds and Flores, 2019) and the potent effect amphetamines have on dopaminergic signaling. In fact, abuse-like levels of AMP exposure during adolescence has been shown to significantly reduce dopamine axon growth from the ventral tegmental area (VTA) to the PFC by drug-induced changes in the Netrin-1 receptor, DCC (deleted in colorectal cancer), that are caused by AMP's ability to recruit the DCC microRNA repressor, microRNA-218 (Reynolds et al., 2015; Cuesta et al., 2018; Hoops et al., 2018).

Using *in vitro* slice electrophysiology, we demonstrated an attenuation of dopamine-induced increases in inhibitory synaptic currents in PFC pyramidal cells in brain slices taken 3–5 weeks after rats were exposed to 10 injections of 3 mg/kg AMP every other day during adolescence (P27–45) or adulthood (P85–103; Kang et al., 2016a). These effects were mediated at least in part by reduced sensitivity of D1 receptors, which is in line with our work demonstrating that D1 receptor expression in the PFC, but not the nucleus accumbens, is reduced for at least 3 weeks following this same AMP exposure during adolescence (Kang et al., 2016b). We also showed that adult rats pre-treated with AMP during adolescence were more sensitive to the behavioral effects of D1 agonists, which is one predicted result of AMP-induced disruptions in D1 receptor expression (Sherrill and Gulley, 2018). However, it is important to note that we have also recently reported no reduction in D1 receptor expression in the PFC following LgA METH self-administration that began during adolescence (P41) or adulthood (P91; Westbrook et al., 2020). Although the effects in Kang et al. (2016a) did not depend on age of exposure (P27–45 vs. P85–103), it is notable that the impact of AMP exposure was apparent up to three months after the last AMP injection in one experiment, suggesting that at least in rats, adolescent AMP exposure can have long-lasting effects on PFC function that extends into adulthood. In our follow-up study (Paul et al., 2016), we reported an age-of-exposure dependent effect on inhibitory tone when neurons from pre-treated rats were re-exposed to AMP in adulthood. After 3–5 weeks of withdrawal from AMP exposure, slices from adult rats exposed to AMP from P85–103 did not display the increased inhibitory tone seen in control rat slices in response to AMP. Interestingly, in slices taken from adult rats exposed to AMP during adolescence (P27–45), bath application of AMP suppressed inhibitory tone in the PFC after 3–5 weeks of withdrawal, but this AMP-induced suppression was absent after a longer withdrawal period (11–14 weeks). Taken

together, our difference in findings in inhibitory tone after application of AMP (which releases endogenous dopamine) vs. exogenous dopamine suggests that exposure to AMP impacts the development of inhibitory signaling through presynaptic dopamine mechanisms in the PFC in an age-of-exposure dependent manner, but this difference in inhibitory tone can recover with a longer withdrawal. One of these presynaptic mechanisms that is impacted by AMP taken during adolescence are the presynaptic dopaminergic axon inputs into the PFC from the VTA as discussed earlier.

Gaps in current knowledge

While the field has made great strides, there are several issues that need to be addressed in future research in order to improve our understanding of the use and consequences of amphetamines taken during adolescence. One major challenge with the extant literature is the lack of a consensus for the definition of adolescent ages, particularly in the rodent literature. The adolescent time frame for rats of P27–60, as suggested by Spear (2000), was the focus for most of the studies reviewed here. However, this may not capture the early adolescent period in mice (Hoops and Flores, 2017). Moreover, pubertal onset appears to drive many of the developmental changes that occur during adolescence (Juraska and Willing, 2017) and is thus an important marker for adolescence in addition to chronological age. Further complicating the definition of adolescence is the fact that females undergo puberty earlier than males and adolescent development is sex specific (Juraska and Willing, 2017). Although cumbersome in practice, future research should include both sexes and consider the potentially distinct roles of pubertal status and chronological age in their study designs in order to determine the developmental mechanisms that may contribute to use of amphetamines and their disruptive effects on the brain and behavior.

Another key concern with the current literature on adolescents and amphetamines is that many studies do not include comparison groups at different times in the life cycle. Without an adult (or even juvenile) comparison group that underwent similar drug exposure procedures, it is impossible to determine whether the reported effects of drugs in adolescents are unique to this population. It is important to note that even in work that includes both adolescent- and adult-exposed groups, one potential confounding factor in interpreting the reported differences is that these different age groups often are tested after different lengths of withdrawal, or else at different ages in adulthood. We discussed earlier work from our lab that demonstrated the importance of withdrawal length vs. adult testing ages in the age-of-exposure dependency of AMP on inhibitory tone in the PFC (Paul et al., 2016). While including both sexes at different age-of-onsets and testing at matched withdrawal lengths *and* matched age is understandably time consuming and oftentimes entirely impractical to implement within the scope of a single experiment, future investigations should take into consideration and try to address some of these factors that contribute to the complexity in studying the multifaceted effects of adolescent drug exposure.

A final gap that we want to highlight is that most studies of the neurobehavioral consequences of amphetamines, including those from our own laboratory, have used non-contingent (i.e., experimenter administered) drug exposure. While a useful tool, this exposure model likely induces stress since the injection procedure is involuntary and

requires restraint. The effects it elicits may not be the same as those induced by volitional self-administration of amphetamines. Indeed, multiple studies in adult rodents have reported discrepancies in the neural and/or behavioral consequences of psychostimulants given in a contingent compared to non-contingent manner (e.g., Lominac et al., 2012; Reichel et al., 2012; Radley et al., 2015). Along with the greater face validity of self-administration models, these contingent drug delivery paradigms are better able to capture the motivational and cognitive facets of drug use that likely contribute to continued and uncontrolled drug use. In addition to using contingent drug delivery, future investigations into age differences in the use and consequences of amphetamines should be conducted using the emerging self-administration paradigms that are arguably more translationally relevant models of human drug-taking, such as intermittent access, punishment-induced abstinence, and/or choice procedures. Intermittent access, although typically leading to lower drug intake, is thought to better represent the pattern of drug use since the drug is not available continuously like it is in more traditional self-administration paradigms. Recently, intermittent access has been reported to be better suited to detect individual susceptibility to developing addiction-like behaviors compared to continuous access procedures (Garcia et al., 2020). Punishment-induced abstinence better captures drug use in the context of the concurrent negative consequences of that use (Krasnova et al., 2014). Finally, drug use does not occur in isolation, but in most self-administration paradigms animals only have the option of responding for the drug. Choice procedures provide the animals with an alternative option, such as responding for a food reinforcer. These procedures are thought to be more sensitive to detect individual vulnerability to developing problems with drug use (Lenoir et al., 2013). While use of these different types of self-administration paradigms is growing, these studies are almost exclusively limited to studies utilizing adult males. Age differences in the use and consequences of amphetamines in these more translationally relevant models remain to be elucidated.

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Highlights

- Nonmedical use of amphetamines by adolescents is a persistent problem
- Amphetamines may have lingering adverse effects that depend on age and gender/sex
- These consequences include disruption of cognition and prefrontal cortex function

Figure 1.

The proportion of rats per group that escalate their METH intake (>5% increase) or do not escalate from the first to the last (14th) LgA session. Chi square tests failed to reveal statistically significant differences between groups in the proportion of rats that escalate. Data were combined from two studies from our lab that used identical METH self-administration protocols (Westbrook and Gulley, 2020; Westbrook et al., 2020).

Figure 2.

Hourly intake of METH (0.1 mg/kg/infusion) during the first and last 6-h LgA sessions in rats that began self-administration during adolescence (P41) or adulthood (P91). Mixed ANOVA with age of onset, sex, session, and hour as factors revealed a significant main effect of session [$F(1,163)=197.35$, $p < 0.0001$] and interaction of age-of-onset and hour [$F(5,815)=4.56$, $p = 0.0004$]. (A) When collapsed across sex and age of onset, rats had significant escalation of METH intake during the last of 14 LgA sessions compared to the first session. $###p < 0.001$ first vs. last collapsed across hour. (B) Hourly intake with data collapsed across LgA session (14 sessions total). $@p < 0.05$, $@@p < 0.01$, $@@@p < 0.001$ vs. adult-onset group within hour. Data are from 40–45 rats/group that were combined from two of our previous studies that used identical METH self-administration protocols (Westbrook and Gulley, 2020; Westbrook et al., 2020).