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Consequences of adolescent use of alcohol and other drugs: Studies using rodent models

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Highlights

Rodent studies reveal persisting neurobehavioral effects of adolescent drug exposure

Ethanol, nicotine and cannabinoid vulnerability is greater in adolescents than adults

MDMA and methamphetamine show a converse age-related vulnerability

Affected brain regions include those undergoing developmental change at this time

Alterations include disruptions in cognition, affect, and drug self-administration

Abstract

Studies using animal models of adolescent exposure to alcohol, nicotine, cannabinoids, and the stimulants cocaine, 3,4-Methylenedioxymethampethamine and methamphetamine have revealed a variety of persisting neural and behavioral consequences. Affected brain regions often include mesolimbic and prefrontal regions undergoing notable ontogenetic change during adolescence, although it is unclear whether this represents areas of specific vulnerability or particular scrutiny to date. Persisting alterations in forebrain systems critical for modulating reward, socioemotional processing and cognition have emerged, including apparent induction of a hyper-dopaminergic state with some drugs and/or attenuations in neurons expressing cholinergic markers. Disruptions in cognitive functions such as working memory, alterations in affect including increases in social anxiety, and mixed evidence for increases in later drug self-administration have also been reported. When consequences of adolescent and adult exposure were compared, adolescents were generally found to be more vulnerable to alcohol, nicotine, and cannabinoids, but generally not to stimulants. More work is needed to determine how adolescent drug exposure influences sculpting of the adolescent brain, and provide approaches to prevent/reverse these effects.

Key words:

Adolescent
Affect
Alcohol
Animal models
Brain
Cannabinoids
Cocaine
Cognition
Ethanol
MDMA
Methamphetamine
Nicotine
Rat

1. Introduction

Drug use is frequently initiated during adolescence. The latest Monitoring the Future survey reported that 13, 20 and 31% of 8th, 10th and 12th graders endorse having tried cigarettes, with over 10% of 12th graders affirming that they have smoked cigarettes within the past month (Johnston et al, 2016). Annual prevalence rates for use of alcohol and marijuana/hashish across these age ranges were 21, 42 and 58% and 12, 25 and 35%, respectively. Some of this use reaches high levels with, for instance, >10% of 12th grade students reporting consumption of 10 or more alcohol drinks in a row in the past 2 weeks, and >5% reporting consumption of >15 drinks per occasion over the same 2 week interval (Patrick et al, 2013). Illicit drugs other than marijuana used by adolescents include psychostimulants such as cocaine and the "club drugs" 3.4-methylenedioxymethamphetamine (MDMA, commonly called "ecstasy") and methamphetamine (METH), with 2.5% of high school seniors in the United States reporting annual use of cocaine, 3.6% reporting MDMA use, and 0.6% endorsing use of METH (Johnston et al, 2016).

Among the likely contributors to the elevation of alcohol and drug use during adolescence are maturational changes occurring in the brain at this time – many of which are described in detail in other reviews in this special issue. Although notably oversimplified for the purposes here, prominent among these brain changes is a developmental dissociation between earlier maturation or even enhanced reactivity to rewarding and motivationally relevant stimuli in subcortical regions during adolescence that contrasts with relatively delayed maturation throughout adolescence of frontal brain regions that are critical for control of these subcortical regions (e.g., see Casey et al, 2011; Doremus-Fitzwater et al, 2010). That is, on the one hand, reactivity and cross-reactivity appears to be enhanced during adolescence in mesolimbic regions such as the nucleus accumbens (nAc) and amygdala (AMYG) that are critical for processing and responding to rewarding, aversive, and emotionally arousing stimuli, including social stimuli (e.g., Ernst & Fudge, 2009; Spear, 2011). This enhanced reactivity contrasts with

delayed development of prefrontal cortex (PFC) and other frontal regions that mature only slowly during adolescence and are critical for cognitive control (Casey et al, 2011). These and other developmental changes occurring in the adolescent brain, such as developmental declines in cortical gray matter, and increases in myelination and in the portion of the brain partitioning as white matter, appear to have been highly conserved evolutionarily. That is, similar developmental alterations are evident during this ontogenetic transition across a variety of mammalian species, despite notable species differences in overall brain complexity and the relative length of the adolescent period (see Spear, 2000, 2016a, for review). For instance, in rats, the two week period between postnatal days (P) 28-42 is thought to roughly subsume the early-mid adolescent period (~ 12-17 years in humans), with the interval from ~ P43-55 more comparable to the late adolescence/emerging adulthood period in humans (~18-25 years) (see Spear, 2015). The conserved nature of the brain transformations of adolescence support the use of animal models to explore some of the critical questions regarding contributors to drug use and consequences of that use that may be challenging to study empirically and ethically in underage humans (see Spear, 2016a).

Alcohol and other drugs exert many of their effects through action in mesolimbic and frontal cortical regions undergoing particularly marked remodeling during adolescence. Hence, the maturational state of these regions could potentially influence the propensity for experimentation and continued drug use during adolescence. To the extent that initiation/escalation of alcohol/drug use during adolescence has a biological basis, and because the developmental timing is similar across species (see Spear, 2000; Spear, 2016a, for review), one would predict that enhanced propensities for drug initiation and elevated use seen in human adolescents would also be evident in adolescents of other species. Indeed, studies in laboratory animals have shown that voluntary self-administration of and sensitivity to the effects of alcohol and other drugs differs during adolescence from that seen in adulthood. Using alcohol as an example, adolescent rats (like their human counterparts) (SAMHSA, 2013) often drink 2-3 times

more per drinking occasion than do adults (e.g., Doremus et al, 2005; Vetter et al, 2007; but see also Bell et al, 2006). Along with this elevation in intake, studies with adolescent rats have shown them to be less sensitive to many of the effects of alcohol (ethanol [EtOH]) that presumably serve as cues to limit intake (such as EtOH-induced motor impairing, sedative, aversive, and socially impairing effects), along with an enhanced sensitivity to desired effects of EtOH, including its rewarding and social facilitating effects (e.g. Spear et al, 2016b). Adolescents show an enhanced sensitivity to the rewarding effects not only of alcohol, but also nicotine and cocaine, with aversive effects attenuated during adolescence not only to alcohol, but also to nicotine, cocaine, amphetamine and delta-9-tetrahydrocannabinol ([THC] – one of the major cannabinoids in marijuana) (see Doremus-Fitzwater et al, 2010, for review). Collectively, these age-related propensities for accentuated appetitive and attenuated aversive properties of drugs could contribute to the enhanced susceptibility for initiation and escalation of drug use during adolescence.

That alcohol and drug use is often initiated (and sometimes escalates into high levels of use) during the time of the notable brain remodeling during adolescence leads to the question of whether drug exposure during this time may influence that development and exert persisting neural and behavioral alterations. For an extensive review of human imaging studies of adolescent alcohol and drug users, see Silveri et al in this special issue. Studies using animal models have also proven useful in examining this question empirically and with a rapidity of data accumulation that is often not possible in cross-sectional or time-consuming longitudinal studies with human adolescents (see Spear, 2016a, for review). From studies in laboratory animals, the evidence is mounting that indeed adolescent exposure to a variety of drugs influences neural, behavioral, affective and cognitive function long after adolescence. The present review will focus on studies in rodents and consequences of the three primary drugs used by adolescents – alcohol, nicotine, and marijuana/cannabinoids (CBs) – as well as cocaine and other stimulants, particularly MDMA and METH. Although not a focus of the current review, it should be noted

that, to the extent that roughly comparable data are available in human research and studies with rodents, signs of across-species consiliences have emerged in the consequences of adolescent exposures to drugs such as alcohol, nicotine, and CBs (for reviews, see Spear, 2016a; Counotte et al, 2011a; Jager & Ramsey, 2008, respectively). These across-species consiliences are evident despite a major difference in exposure-to-test timing between investigations in humans and laboratory animals. In studies of drug use in human adolescents, drug-using individuals are typically assessed during adolescence or shortly thereafter, either while still using the drug or after a relatively short abstinence period. In contrast, lasting consequences of repeated drug exposure during adolescence in animal studies are typically examined in adulthood – well after exposure termination – rather than during or immediately following the exposure period.

In some, but not a majority, of the studies reviewed here, consequences of adolescent exposure were compared with equivalent exposure at some other point in life, most typically, adulthood, to assess whether adolescence is a particularly vulnerable period. As outlined in the sections to follow, for 3 of the 4 drug classes reviewed, when consequences of adolescent exposure were compared with exposure in adulthood (stimulants being the exception), effects were generally found to be more pronounced after exposures during adolescence. When considering differences in exposure age, it is important to note that there may be ontogenetic differences in pharmacokinetics that could influence differential drug vulnerabilities across age. Overall metabolic activity is typically greater in adolescence than in adulthood, and may extend to drugs of abuse as well (see Spear, 2000, for discussion and references). Thus, for those drugs where consequences of adolescent exposure are typically more pronounced than in adulthood, greater adverse consequences may be evident despite perhaps the tendency for these drugs to be metabolized more quickly during adolescence than in adulthood. In the case of stimulants where consequences of adolescent exposures may be attenuated relative to

exposure in adulthood, potential age differences in metabolism are of particular concern, an issue that will be revisited later.

Note in the sections to follow that in some cases, studies examining neurobehavioral alterations after adolescent exposures have been complemented by pharmacological studies to assess the efficacy of manipulations of the altered neural system(s) for reversing observed deficits. These studies are not only crucial for testing hypotheses regarding neural contributors to observed consequences of adolescent exposure, but also may provide data of potential translational use for future prevention/intervention efforts.

Finally, although not a focus of this review, occasional examples of sex differences in the persisting effects of adolescent drug exposure are included. These sex-specific findings are consistent with evidence for notable sex differences in maturation of the brain during adolescence (see Viveros et al, 2011) as well as data showing "significant differences in the phenotypes of females and males in the domain of addiction" (Becker & Koob, 2016, p.243). It should be noted, however, that at this point the data are not plentiful enough for systematic characterization of sex differences in vulnerability to lasting effects of adolescent drug exposure, let alone for assessment of the neural substrates underlying these differential sex vulnerabilities (although progress in this area is beginning to emerge – e.g., see Viveros et al, 2011

2. Alcohol

There has been a rapid escalation of research using laboratory animals to investigate effects of repeated exposure to EtOH during adolescence. Evidence is building for a variety of lasting behavioral, cognitive, affective, and neural alterations after such exposure. These consequences have typically (although not always – e.g., see Schindler et al, 2014) been explored after adolescent exposures producing blood EtOH concentrations [BECs] above the threshold defining "binge" use (BECs > 80 mg%). In such studies, BECs often average around 150-200 mg% -- well within the range observed in field studies of late adolescents (e.g., Day et al, 2013), with little if any effort to date focused on determination of potential dose-dependency

of effects. When studied, adolescent exposures to EtOH appear to induce more pronounced effects than equivalent EtOH exposures in adulthood, with some evidence to suggest that early-mid adolescence may be a time of particular vulnerability to lasting EtOH exposure effects (Spear, 2015).

2.1. Cognition, Behavior and Affect. Later effects on cognition after adolescent EtOH exposure are highly specific, with learning of a variety of less cognitive-challenging tasks largely unaffected (e.g., Popović et al, 2004; Risher et al, 2013). Reports of deficits on hippocampal-dependent tasks are mixed, with reports of performance decrements on trace conditioning (Yttri et al, 2004), spatial memory (Sircar & Sircar, 2005) and spatial-temporal object recognition (Swartzwelder et al, 2015) tasks contrasting with other findings of no alterations in spatial memory performance (Silvers et al, 2006; Acheson et al, 2013). Deficits have been suggested to be more likely to emerge on tasks tapping various "executive functions" (see Crews et al, 2007) such as conditional discrimination and object recognition tasks (Pascual et al, 2007). Task circumstances that demand some degree of cognitive and behavioral flexibility – i.e., the ability to modify behavior with changing environmental demands (see Hamilton & Brigman, 2015) – have been found to be particularly vulnerable to performance decrements after adolescent EtOH exposure, including reversal acquisition (Coleman et al, 2011), extinction, and set-shifting (Gass et al, 2014). Greater risk preferences in a probability discounting task have also been observed (Nasrallah et al, 2009; Boutros et al, 2014), along with signs interpreted to reflect increases in impulsivity and/or greater disinhibition (indexed, for example, via increased center time on an open field conflict test [Ehlers et al, 2011], more rears in the light side of a light-dark box [Desikan et al, 2014] and increased open arm time in an elevated plus maze under low light conditions [Gass et al, 2014]).

Alterations in affective measures have also been observed in adulthood after adolescent EtOH exposure. Depressive-like signs have emerged when indexed via decreases in sucrose consumption (used as a measure of anhedonia) or increased immobility in the Porsolt swim test

(Slawecki et al, 2004; Ehlers et al, 2011; Briones & Woods, 2013). Reliable increases in social anxiety have been observed in adult males after EtOH exposure during early/mid adolescence, an effect that is sex-specific and not evident in females (Varlinskaya et al, 2014; Varlinskaya & Spear, 2015). Increases in general anxiety indexed via decreased open arm time in the elevated plus maze [EPM] (Slawecki et al, 2004; Montesinos et al, 2015a; Pandey et al, 2015) or increased defecation when in a novel environment (Popović et al, 2004) have also been reported in male rats, although these effects are not ubiquitous (Ehlers et al, 2011; Gilpin et al, 2012; Quoilin et al, 2012; White et al, 2000). Part of the challenge in interpreting data from EPM and similar tests of anxiety is that increased time in more "risky" environments (such as the open arms) could potentially reflect elevated "disinhibition" rather than an anxiolytic profile (see Desikan et al, 2014; Gass et al, 2014, for discussion). To the extent that adolescent EtOH exposure may be characterized by profiles both of increased anxiety as well as disinhibition, competition between these propensities might well lead to varied outcomes, depending on variables such as perceived stressfulness of the situation, prior handling or other test experiences.

2.2. Retention of adolescent phenotypes into adulthood. In a number of studies, adolescent EtOH exposure resulted in the retention of adolescent-typical phenotypes into adulthood (e.g., Fleming et al, 2012). Although these effects were sometimes evident in baseline behavior, cognition and electrophysiology, they appear particular pronounced in terms of the retention of adolescent-typical EtOH sensitivities into adulthood (see Spear & Swartzwelder, 2014, for review). For instance, adolescent EtOH exposure has been shown to disrupt normal developmental declines in sensitivity to EtOH-induced deficits in spatial working memory that typically occur between adolescence and adulthood; thus, adults exposed as adolescents to EtOH remain "adolescent-like" in being more vulnerable to EtOH-induced memory impairments than adults without a history of adolescent EtOH exposure (White et al, 2000). Adolescent-typical enhanced sensitivity to EtOH's locomotor stimulant and rewarding

effects were also retained into adulthood after adolescent EtOH exposure (Maldonado-Devincci et al, 2010; Quoilin et al, 2012; Toalston et al, 2014). Enhanced sensitivity to these desired effects of EtOH could serve to promote EtOH consumption in adulthood. Similar retentions of adolescent-like responses to EtOH after adolescent EtOH exposure are also evident in terms of adolescent-typical insensitivities to sedative, motor impairing, and aversive effects of EtOH (White et al, 2002; Diaz-Granados & Graham, 2007; Matthews et al, 2008; Quiolin et al, 2012; Saalfield & Spear, 2015). To the extent that these measures serve in part as feedback cues to moderate intake, retention of adolescent-typical insensitivities to these cues could serve to increase capacity for the maintenance of elevated drinking in adulthood after adolescent EtOH exposure. Later expression of adolescent-like phenotypes was specific to EtOH exposure during adolescence (and was not evident following comparable exposure in adulthood) (e.g., White et al, 2002; Diaz-Granados & Graham, 2007) and was more pronounced after episodic than continuous EtOH exposure (e.g., Diaz-Granados & Graham, 2007). The production of multiple "withdrawal" episodes of EtOH exposure and clearance associated with episodic exposure may be of particular importance, given that adolescents have been shown to be more sensitive than adults to escalation in withdrawal signs emerging over repeated withdrawals (Wills et al, 2008, 2009).

2.3. Later alcohol consumption. The data are mixed as to whether adolescent EtOH exposure increases later EtOH consumption in adulthood. Alcohol-preferring (P) rats given voluntary access to EtOH in their home cages throughout adolescence later acquired operant self-administration of ETOH more quickly than animals not given prior EtOH access; these animals also showed greater resistance to extinction of self-administration, more spontaneous recovery and more rapid reacquisition of the operant task than animals without a history of prior EtOH intake in adolescence (Rodd-Henricks et al, 2002a). None of these effects were evident when home cage EtOH access was not provided until adulthood (Rodd-Henricks et al, 2002b). Similarly, mice given voluntary access to EtOH in their home cages from weaning through

adolescence and into adulthood consumed more EtOH than mice whose home cage access was delayed until adulthood (Ho et al, 1989). Intermittent intraperitoneal administration of EtOH during early-mid adolescence has been reported by two groups to increase later EtOH consumption in 2-bottle choice tests (Alaux-Cantin et al, 2013; Pandey et al, 2015), an effect that was not evident with comparable exposure late in adolescence (Alaux-Cantin et al, 2013).

Some studies, however, found no subsequent increases in consumption among rats exposed as adolescents to EtOH vapor (Slawecki & Bentacourt, 2002) or in mice or rats given voluntary access to EtOH in the home cage (Tambour et al, 2008; Vetter et al, 2007). In some cases, although adolescent-exposed animals did not differ from controls in their operant responding for EtOH in adulthood, they nevertheless demonstrated exacerbated dependence-induced increases in operant intake (Gilpin et al, 2012) or increased resistance to extinction of operant responding for EtOH (Gass et al, 2014). Critical variables that may influence whether later consumption is increased include sex, genetic background, amount and mode of adolescent EtOH exposure, and how intake is examined in adulthood (e.g., Blizard et al, 2004; Siciliano & Smith, 2001; Strong et al, 2010; Walker & Ehlers, 2009; Pascual et al, 2009). When rats were given voluntary access to sweetened EtOH or the sweetener alone during adolescence, animals increased their later intake of only the solution that they received as adolescents, suggesting that in some cases increases in later intake may be related to familiarity and enhanced solution acceptability rather than an effect specific to EtOH (Broadwater et al, 2013); such controls are rarely included. More remains to be learned regarding the constraints and circumstances that influence EtOH consumption in adulthood after adolescent exposure to EtOH.

2.4. Neural alterations. A variety of replicable and persisting alterations in brain have been observed after repeated EtOH exposure during adolescence in brain regions such as the PFC, hippocampus (HPC), AMYG and nAc that undergo notable developmental transformations during adolescence. Among the consistent findings reported after adolescent EtOH exposure

are decreases in neurogenesis along with the induction of regionally-specific brain damage and cell death in regions such as the frontal cortex, HPC and cerebellum (Crews et al, 2000; Ehlers et al, 2013; Broadwater et al, 2014; Vetreno & Crews, 2015). Although binge-like levels of EtOH exposure can also induce brain damage in adult rats, the regions affected are different, with generally more pronounced effects evident after exposure during adolescence (Crews et al, 2000). A similar enhanced vulnerability of adolescents was also reported by Broadwater et al (2014) who observed lasting disruptions in neurogenesis and increases in cell death in adult rats after EtOH exposure during adolescence but not following equivalent exposure in adulthood. At the synaptic level, there is recent evidence that adolescent exposure to EtOH results in expression of a greater proportion of immature, more "plastic" synaptic spines in the adult HPC (Risher et al, 2015). Alterations in brain activity in this region were evident in terms of long-term potentiation (LTP), with adults exposed to EtOH during adolescence exhibiting more robust LTP than non-exposed animals, suggesting a surprising enhanced state of synaptic plasticity (Risher et al, 2015). Electrophysiological studies have revealed long-lasting decreases in slow-wave sleep, as well as attenuations in the P3 component of evoked potentials (Ehlers & Criado, 2010).

Adolescent EtOH exposure targets functioning of a number of neurotransmitter systems. Replicable and long-lasting decreases have been observed in the number of neurons in the basal forebrain displaying choline acetyltransferase (ChAcT) activity, a marker of cholinergic neurons (Coleman et al, 2011; Ehlers et al, 2011; Boutros et al, 2014; Vetreno et al, 2014; Swartzwelder et al, 2015). This effect is suggestive of a likely disruption in acetylcholine (ACh) function that is not evident after equivalent exposure in adulthood (Vetreno et al, 2014). In contrast to apparent ACh hypofunction, apparent elevations in dopamine (DA) function have often been reported in DA projections to reward-critical regions undergoing ontogenetic change during adolescence such as the nAc. These indices of elevated DA function include elevated basal levels of DA neurotransmission (Sahr et al, 2004) and extracellular DA levels (Badanich et

al, 2007; Pascual et al, 2009), along with greater DA responses to EtOH challenge (Sahr et al, 2004; Pascual et al, 2009; Philpot et al, 2009). Alterations in the major excitatory (glutamate) and inhibitory (gamma aminobutyric acid [GABA]) neurotransmitter systems in brain have also been reported after adolescent EtOH exposure. Included are alterations in HPC $GABA_A$ receptor expression and increases in their EtOH sensitivity (Fleming et al, 2012), as well as increases in glutamate N-methyl-D-aspartate (NMDA) receptors (GluN) along with increased expression and alterations in phosphorylation of the GluN2B subtype of this receptor (Pascual et al, 2009; Sircar & Sircar, 2006). Administration of a positive allosteric modulator of the glutamate receptor mGlu5 (CDPPB) was found to reverse cognitive deficits induced by adolescent EtOH exposure (Gass et al, 2014).

EtOH exposure during adolescence has also been shown to influence epigenetic regulation through alterations in histone acetylation, thereby influencing DNA methylation and hence regulating propensity for DNA transcription at specific portions of the genome (Pascual et al, 2009, 2012; Pandey et al, 2015). For instance, persisting increases in histone deacetylase (HDAC) were seen in the AMYG of adolescent-exposed rats, along with decreases in levels of histone acetylation (see Pandey et al, 2015). Blockade of histone deacetylation with a HDAC inhibitor, trichostatin A (TSA), normalized regional deficits in histone acetylation and restored transcription of genes such as brain-derived neurotrophic factor (BDNF), while also attenuating the anxiety-like behavior and elevated EtOH intake seen in adulthood after adolescent EtOH exposure while the drug was “on board” (Pandey et al, 2015).

One other notable target of EtOH is neuroinflammation. Research in adult animals and humans has shown that neuroimmune signaling can influence ethanol intake and ethanol actions, with ethanol exposure conversely inducing proinflammatory signaling in brain (Robinson et al, 2014). Hence, it is not surprising that alcohol exposure during adolescence induces lasting increases in expression of neuroimmune genes, including a variety of proinflammatory signaling molecules, along with activation of the innate immune system

receptor, Toll-like receptor 4 (TLR4) (see Crews & Vetreno, 2014, for review). Activation of TLR4 receptors by EtOH triggers transcription factors such as NFκB and AP-1 to induce cytokine production and mediators of inflammation that are thought to lead to the brain damage produced by EtOH (see Montesinos et al, 2015b). Evidence supports the suggestion that immune activation via TLR4 may be critical for the production of certain neural and behavioral consequences of EtOH exposure during adolescence. Induction of TRL4 activation with an agonist (lipopolysaccharide) was found to induce lasting reductions in neurogenesis similar to that observed after adolescent EtOH administration (Vetreno & Crews, 2015). Conversely, TLR-4 deficient (TLR4-KO) mice exposed to EtOH during adolescence did not show the inflammatory response nor cognitive and behavioral alterations characteristic of adolescent EtOH exposure (Montesinos et al, 2015a,b). Likewise, administration of an anti-inflammatory agent (indomethacin) was found to block the pattern of cell death and behavioral deficits associated with EtOH exposure during adolescence (Pascual et al, 2007). Thus, anti-inflammatory agents join other possible pharmacotherapeutic agents, including positive allosteric modulators of the glutamate receptor mGlu5 and HDAC inhibitors, as possible strategies for preventing or reversing some of the lasting consequences of EtOH exposure during adolescence.

3. Nicotine

Substantial evidence has been obtained in rodent studies that adolescent nicotine exposure induces persisting neural consequences. Although less studied, cognitive and behavioral effects have also been reported, including increases in later self-administration of nicotine and other drugs.

3.1. Cognition, Behavior and Affect. Although not extensively explored, several persisting alterations in cognitive functioning, attention, impulsivity, and affective behavior have been reported after adolescent nicotine exposure (see Counotte et al, 2011a). Cognitive alterations reported in adulthood after adolescent nicotine exposure include alterations in serial

pattern learning (Fountain et al, 2008) and attentional disruptions (Counotte et al, 2009, 2011b). Adult rats exposed to nicotine during adolescence (but not in adulthood) also exhibited increases in impulsive action (indexed by responding prematurely in a serial reaction time test) but not impulsive choice (indexed via delay discounting – i.e., the willingness to wait longer to receive a larger reward) (Counotte et al, 2009). These findings are consistent with other evidence that impulsivity indexed via action versus choice behavior are partially separable in their neural substrates, and hence sometimes differentially vulnerable to pharmacological manipulations (see Counotte et al, 2009, for discussion) , Increases in anxiety-like behavior have been reported in the open field and EPM (indexed via decreased time in the center of the field and the open arms, respectively) (Slawecki et al, 2003, 2005). Likewise, after adolescent nicotine exposure, adult rats acquired a conditioned fear response more rapidly, and extinguished this fear more slowly than control animals (Smith et al, 2006), findings also consistent with an anxiogenic profile.

3.2. Later Drug Self-administration. Adolescent nicotine exposure has also been shown to influence later responses to, and self-administration of, nicotine and other drugs of abuse. Indeed, nicotine has been suggested to be a gateway drug, often leading to the use of other drugs such as marijuana and cocaine (e.g., see Kandel & Kandel, 2014). For instance, exposure to nicotine during adolescence has been reported to increase locomotor sensitization to later amphetamine, cocaine, and amphetamine (Collins et al, 2004; Kelley & Rowan, 2004; Nolley & Kelley, 2007), effects that, where studied, were sex specific (evident only in adolescent males) and not apparent in animals who received equivalent nicotine exposure in adulthood (e.g., Collins et al, 2004). In contrast, adolescent nicotine exposure has been reported to decrease the rewarding properties of cocaine and methylphenidate in adulthood when indexed via conditioned place preferences (i.e., development of a preference for a location paired with drug administration)(Nolley & Kelley, 2007). Given that the nAc is thought to modulate both locomotor activity and the rewarding value of stimuli, with particular involvement of the core and

shell regions, respectively, Kelley and Rowan (2004) have suggested that nicotine exposure during adolescence may differentially influence these regions to accentuate motor activation while conversely attenuating rewarding effects. Generally speaking, decreases in drug reward sensitivity are often associated with compensatory increases in drug self-administration under relatively low levels of response effort (see Koob & LeMoal, 2006). Indeed, age-specific enhancement of self-administration of nicotine (Adriani et al, 2003, 2006) and cocaine (McQuown et al, 2007; Dao et al, 2011) has sometimes (albeit not always – see Weaver et al, 2012; Levin et al, 2011) been reported after adolescent exposure to nicotine, with these effects generally restricted to exposures during early-mid adolescence, and not late adolescence or adulthood. This enhanced self-administration does not extend to all types of rewards given that similar effects were not evident when animals were given the opportunity to self-administer sucrose pellets (McQuown et al, 2007).

3.3. Age-dependent vulnerabilities. Where studied, persisting effects of adolescent exposure are generally more pronounced than observed after comparable nicotine exposure in adulthood. As one example, genome-wide microarray analyses were used to compare effects of repeated nicotine exposure during adolescence versus adulthood on gene expression in the VTA both immediately following the end of the 2 week exposure period as well as 1 month later (Doura et al, 2010). In this study, most nicotine-induced changes in gene expression in adults were observed immediately following the exposure period and had returned to control levels 1 month later, whereas persisting expression changes were most commonly seen in adolescents, with these genes being over-represented in networks involved in developmental processes, brain plasticity and various other neural functions. Effects of adolescent nicotine exposure have been shown to vary not only from adult exposures, but also from nicotine exposure earlier in life– i.e., during the prenatal or early postnatal period (see Slotkin, 2002; Dwyer et al, 2009). The timing specificity of developmental nicotine exposures has been suggested to be dependent on maturational changes in expression of nicotinic ACh receptors (nAchRs) and their

subunit composition, as well as the structures undergoing nicotine-regulated development at that time. For instance, nAChRs are thought to be critical for regulating catecholamine and autonomic development during the prenatal period but to influence limbic and later stages of catecholamine development during the adolescent period (Dwyer et al, 2009). Indeed, alterations in limbic regions and other forebrain targets of the catecholamine, DA, are among the regions shown to be vulnerable to nicotine exposure during adolescence, as discussed in the following section.

3.4. Neural consequences. Cell losses in the brain (indexed via decreases in DNA) along with enlargement of surviving cells (indexed via increases in protein/ DNA ratios) have been reported after adolescent nicotine exposure, effects that were particularly pronounced in the cortex (Abreu-Villaça et al, 2003). Reported structural changes after adolescent nicotine exposure include increases in the number of spines on medium spiny neurons in the nAc and greater dendritic branching in regions such as the medial PFC, nAc shell, basolateral nucleus of the AMYG, bed nucleus of the stria terminalis, and HPC dentate gyrus (see Smith et al, 2015, for review). These consequences were found to persist into adulthood and were largely specific to adolescence exposure, with different regions affected and higher doses generally required to produce effects with adult exposure. Such dendritic remodeling was blocked by co-administration of a DA D1 receptor antagonist during the adolescent exposure period, suggesting that these dendritic modifications are D1 receptor dependent (Ehlinger et al, 2016). Indeed, a variety of neurotransmitter systems, including the DA system, show persisting alterations after exposure to nicotine during adolescence.

Nicotine acts in the brain through activation of nAChR, and hence it is not surprising that the cholinergic system upon which nicotine exerts direct effects is affected by adolescent nicotine exposure. Transient age- and subunit- dependent up- and down-regulation of nAChRs have been reported at the end of the nicotine exposure periods in adolescence as well as in adulthood (Doura et al, 2008), although these effects largely resolve with time post-exposure

(e.g., see Slotkin et al, 2004; Counotte et al, 2009). In contrast, longer-term decreases in cholinergic markers (ChAcT and binding to the ACh transporter) have been reported in the forebrain after adolescent nicotine exposure (Slotkin et al, 2007), a pattern reminiscent of that seen after adolescent EtOH exposure as well. Increases in these cholinergic markers were observed, however, in hippocampus and midbrain, suggesting regional specificity in observed effects (Slotkin et al, 2007). Other persisting alterations reported after adolescent nicotine exposure include an increase in electrically stimulated DA efflux from PFC slices (Counotte et al, 2009), elevated cortical levels of the serotonin (5HT) transporter, as well as enhanced binding to $5HT_{1A}$ and $5HT_2$ receptors (Slotkin et al, 2007). Lasting glutaminergic alterations in PFC have also been reported, characterized by a downregulation of mGlu2 glutamate receptors along with an associated reduction in mGlu2-mediated presynaptic inhibition (Counotte et al, 2011b). These disruptions in mGlu2 were not evident after comparable nicotine exposure in adulthood and appear critical for the deficits in attention observed after adolescent nicotine exposure, given that restoration of mGlu2 activity via local infusion of a mGlu2 agonist reversed the attentional deficit (Counotte et al, 2011b).

4. Cannabinoids

There has been a recent onslaught of research using animal models to assess potential neurobehavioral consequences of adolescent exposure to CBs. Findings of long-lasting neural and behavioral consequences of adolescent exposure to THC and a variety of synthetic CB agonists have emerged with a variety of measures; these findings have been reviewed recently in several excellent reviews to which the reader can refer for additional information, critique and references (Trezza et al, 2008; Renard et al, 2014; Higuera-Matas et al, 2015; Lubman et al, 2015). Although not commonly assessed, where examined, observed effects of adolescent CB exposure have sometimes been found to be sex-specific (e.g., Lee et al, 2014; Higuera-Matas et al, 2015; see Viveros et al, 2011, for review) and generally not evident after comparable exposures in adulthood (e.g., Viveros et al, 2012). Periods of particular vulnerability during

adolescence have been little investigated to date, with work suggesting that early/mid adolescence (e.g., P35-40 and P40-45) is a time of greater vulnerability than during late adolescence (P50-55 or later) (Cass et al, 2014) contrasting with other findings that effects are more pronounced with exposures during late adolescence/emerging adulthood (P40-65) than during the juvenile-to-mid-adolescent period (P15-40) or in adulthood (>P70) (see Schneider, 2008).

4.1. Cognition, Behavior and Affect. Particularly well studied have been tests of working memory, including object recognition tests along with some work examining object location and social recognition; such tests expose animals briefly to a particular social or non-social stimulus (or object location) and then, shortly thereafter, give animals access to both the familiar and an unfamiliar stimulus (or location) and assess the extent to which animals direct their attention to the novel stimulus, thereby displaying evidence of remembering the initially presented stimulus. The most typical findings are long-lasting disruptions in these tests of working memory after repeated adolescent CB exposure (see Renard et al, 2014, for review). Effects were often, although not always (O'Tuathaugh et al, 2010) found to be specific to adolescent exposures. Cognitive effects of adolescent CB exposure appear highly specific, with working and spatial memory performance on a water maze task (location of a hidden platform) reported to be unaffected (e.g., see Higuera-Matas et al, 2009).

Alterations in social and affective measures have also been observed after adolescent CB exposure. Several studies have observed reductions in social behavior (O'Shea et al, 2004, 2006; Schneider, 2008; Schneider et al, 2008; Realini et al, 2011), perhaps associated in part with decreases in social motivation (Renaud et al, 2016) and increases in social anxiety (O'Shea et al, 2004, 2006). Social anxiety has occasionally (O'Shea et al, 2006; Quinn et al, 2008) but not typically (O'Shea et al, 2004; Schneider, 2008; Schneider et al, 2008) been reported after equivalent CB exposure in adulthood. Observed alterations in social behavior do not appear to be solely related to increases in social anxiety given that disruptions in the

microstructure of social behavior are evident after adolescent CB exposure both when animals interact with an unfamiliar (and hence potentially stressful) social partner as well as when they interact with a familiar cage-mate (Schneider et al, 2008). Studies using non-social measures of anxiety have reported mixed results. Some studies, for instance, have reported increased anxiety after adolescent CB exposure in the light-dark box (indexed via fewer entries and longer latencies to enter the light side - Renaud et al, 2016) and in the EPM (Stopponi et al, 2014). Others, in contrast, have reported no notable alterations in anxiety (e.g., Higuera-Matas et al, 2009; O'Tuathaigh et al, 2010; Keeley et al, 2015) or in some cases even decreases in anxiety (e.g., Biscaia et al, 2003; also, see O'Tuathaigh et al, 2010; Keeley et al, 2015) in EPM tests – a diversity that, as discussed earlier, could potentially reflect the same tests reflecting anxiety versus "disinhibitory" profiles, depending on the specific pre-test or test conditions. Induction of a depressive-like phenotype (indexed via increased immobility in the forced swim test) has also been reported after adolescent CB exposure, along with signs of anhedonia (as indexed via a decreased preference for sucrose or palatable foods - e.g., Rubino et al, 2008; Bambico et al, 2010; Realini et al, 2011). Females may be somewhat more vulnerable to these depressive-like effects, with effects sometimes (Rubino et al, 2008; Realini et al, 2011), although not always (e.g., Rubino et al, 2008; Bambico et al, 2010) restricted to females.

4.2. Endophenotypes of schizophrenia. Given evidence from human studies that adolescent use of marijuana may be one factor that increases risk for later emergence of schizophrenia among otherwise vulnerable individuals (e.g., Semple et al, 2005), one focus of studies using animal models of adolescent CB exposure has been on assessment measures thought to reflect endophenotypes of schizophrenia (see Malone et al, 2010). These include the deficits in working memory, disruptions in social behavior, anxiety, and anhedonia discussed above, although it should be noted that these endophenotypes are not specific to schizophrenia and are evident with other conditions as well. One alteration particularly characteristic of schizophrenia is a disruption in sensory-motor gating. This type of cognitive filtering is often

indexed in rodent studies via assessing the reduction in startle response to a loud tone when that stimulus is immediately preceded by a weaker, non-startle-inducing tone – a phenomenon known as pre-pulse inhibition (PPI). Long-term deficits in PPI have been frequently observed in rodent studies after CB exposure during adolescence, but not when animals received equivalent exposure in adulthood (e.g., Renaud et al, 2016), supporting the association of CB action on the adolescent brain with the later induction of schizophrenia-like alterations.

Although little studied to date, some studies have found that emergence of schizophrenia-relevant endophenotypes after adolescent CB exposure are even more pronounced among genetically vulnerable organisms or after early brain damage. For instance, adolescent CB exposure-induced anxiolytic effects were not evident in wild-type male mice but were evident in male mice with a knock-out of the catechol-O-methyl-transferase (COMT) gene (responsible for the breakdown of DA and norepinephrine); a similar synergism was not evident in males exposed to CB in adulthood, suggesting a critical genotype X exposure X age interaction in establishing vulnerabilities to lasting consequences of CB exposure. Likewise, in animals with early lesions of the medial PFC, disruptions in working memory and social behavior induced by adolescent CB exposure were exacerbated relative to animals that were not subjected to this early neural perturbation (Schneider & Koch, 2007). These findings are reminiscent of human data suggesting that separate vulnerabilities may interact or synergize to exacerbate risk for later emergence of schizophrenia and other neurodevelopmental disorders.

It should be noted, though, that in contrast to the idea that CB exposure may increase risk of schizophrenia in individuals with pre-existing vulnerabilities, there are reports that under certain circumstances adolescent exposure to cannabinoids may *reverse* adverse effects of exposure to other perturbations that include drugs (e.g., Lopez-Rodriguez et al, 2014), stressors (Abush & Akirav, 2013), or early maternal deprivation (López-Gallardo et al, 2012), leading some researchers to suggest that “cannabinoid exposure during adolescence may… have a protective role when individuals have faced severe life challenges, such as intense stress or

maternal deprivation" (Higuerra-Matas et al, 2015, p.140). For example, administration of a cannabinoid agonist 1 hr following daily restraint stress during late adolescence (P45-60) reversed the stress-associated disruptions in LTP and short-term memory in a spatial location task seen one month later (Abush & Akirav, 2013). However, CB reversal effects are often highly measure-specific (e.g., see Abush & Akirav, 2013), with the CB agonist sometimes producing alterations alone or exacerbating effects of the other manipulation (e.g.,Schneider & Koch, 2005, 2007; Zamberletti et al, 2012). These reversal effects also do not appear to be adolescent-specific; similar effects have been reported in a number of cases following CB manipulations in adulthood (e.g., Schulz et al, 2013; Segev et al, 2014), with adult CB activation even found to reverse some effects of adolescent CB exposure (Realini et al, 2011). Certainly, although consequences of CB exposure may interact with other perturbations, potential beneficial vs. detrimental effects of these manipulations need careful further study before contemplating their potential translational relevance.

4.3. Later drug self-administration. A number of studies have used animal models to explore the notion of marijuana as a "gateway" drug – i.e. the propensity for adolescent CB exposure to increase the later probability of use of other illicit drugs. These studies have generally found evidence for increases in the later self-administration of cocaine, heroin and other opiates after repeated CB exposure during adolescence (e.g., Biscaia et al, 2008; Ellgren et al , 2008; Higuera-Matas et al, 2008), although observed effects were sometimes evident only in one sex or emerged only with stress-induced relapse (Stopponi et al, 2014). The nature of the sex-specificity varied across studies, with for instance adolescent CB exposure reported to increase self-administration of morphine in males but not females (Biscaia et al, 2008), whereas females (but not males) showed elevations in cocaine self-administration (Higuera-Matas et al, 2008). Later increases in self-administration after adolescent CB exposure may not reflect alterations in the processing of all rewards given that under the same circumstances where exposure-induced increases in cocaine self-administration were observed, no increases were

evident when food was used as the reinforcer (Higuera- Matas et al, 2008). Whether elevations in later drug self-administration would also be evident when initial CB exposure is delayed until adulthood have yet to be explored systematically.

4.4. Neural effects. Neural consequences of adolescent CB exposure have been observed in a variety of brain regions critical for social/emotional behavior, working memory, cognitive filtering, and drug-self-administration, including regions such as the PFC, AMGY, nAc, HPC and other forebrain regions. A variety of neurotransmitter systems and signaling systems have been studied, although effects observed often vary across studies. Even when looking at effects of adolescent CB exposure on later functioning of the endogenous CB system, observed effects vary widely from no alterations in binding or functional activation of CB receptors to CB receptor 1 (CBR1) up-regulation or down-regulation (see Higuera-Matas et al, 2015, for review). As pointed out in that review, some of the variation may depend in part on whether adolescents were repeatedly exposed to THC or synthetic CB agonists, with these substances often producing opposite effects on CBR1 density and function (decreases with THC and increases with synthetic agonists).

Alterations in other neurotransmitter systems have also be observed. Diverse alterations in activity of the endogenous opioid system after adolescent CB exposure include complex patterns of sometimes opposing sex differences (Biscaia et al, 2008). Glutamate and GABA activity in a variety of brain regions is also altered after adolescence CB exposure (see Higuera-Matas et al, 2015). For instance, decreases in HPC glutamate tone (along with decreases in number of HPC dendritic spines receiving glutamate input – Rubino et al, 2009b) have been suggested to be related to the cognitive impairments evident after adolescent CB exposure (Higuera-Matas et al, 2015). Downregulated GABA transmission in PFC has also been observed after adolescent CB exposure. This GABA downregulation was linked to enduring PFC disinhibition in adulthood, a pattern of PFC disinhibition reminiscent of that seen normally early in adolescence (Cass et al, 2014). PFC dysregulation consistent with cortical

disinhibition has likewise been reported by Renaud and colleagues (2016) after CB exposure during adolescence (but not in adulthood). In this case, however, they linked the cortical disinhibition to a hyper-DA state reflected by notable increases in basal firing and burst firing of DA neurons in the ventral tegmental area that project to the PFC.

Studies at the molecular level have proved to be a sensitive approach for assessing persisting consequences of adolescent CB exposure. Molecular signatures of compromised synaptic efficacy have been reported in the PFC (Rubino et al, 2009a), along with abnormalities in Akt, Wnt, and mTOR signaling cascades that "bear… remarkable similarity to adaptations observed in neuropsychiatric populations" (Renaud et al, 2016, p. 2). Adolescent CB exposure has also been reported to disrupt regulation of transcription factors induced by DA manipulations in the AMYG, and to alter receptor expression, transcription factors, opiate- and CB-sensitive signaling cascades and immediate early gene expression in forebrain DA terminal regions such as the nAc (Ellgren et al, 2008; Rubino et al, 2008; Wegener & Koch 2009). Although many of the molecular studies to date have focused only on consequences of adolescent exposures, in recent studies where exposure age has been varied, the adolescent brain has been shown to be either particularly vulnerable or to reveal different persisting alterations following repeated CB exposure than that observed in the adult brain (e.g., see Renaud et al, 2016).

5. Cocaine

After marijuana, the next most frequently used illicit drug globally is cocaine (Karila et al, 2014), although as discussed earlier the incidence of its use among adolescents is far lower than that of marijuana. It is a psychostimulant that, like other psychostimulants to be discussed later, inhibits reuptake of dopamine, norepinephrine, and serotonin, enhancing availability of these monoamine neurotransmitters in the synaptic region where receptors for these neurotransmitters are located (e.g., Koob & LeMoal, 2006). Reminiscent of the rodent data with alcohol, although the literature is somewhat mixed, systematic investigation of cocaine

intravenous self-administration in adolescent and adult rats have shown that adolescent rats are more prone to cocaine self-administration than are adults when indexed via levels of intake at low doses of cocaine, escalation of cocaine intake and resistance to increases in "price" of the cocaine (Wong et al, 2013). Intriguingly, this effect was evident in mid-adolescent (~P42-49) but not early adolescent (~P35-42) rats, reminiscent of the increase in cocaine use seen across this age span in human adolescents (Johnston et al, 2016). As outlined below, various lasting consequences of repeated cocaine exposure during adolescence have been reported in rats and mice, although in most cases comparable adult exposure groups have not been studied, and hence little is known as to whether these effects are more or less pronounced than comparable exposures in adulthood.

5.1. Cognition, Behavior and Affect.

Although studies are limited, the work available suggests that adolescent cocaine exposure induces a number of modest and sometimes unexpected behavioral and affective alterations in adulthood consistent with a decrease in threat evaluation and attenuated cautiousness. Evidence for alterations in spontaneous activity in adulthood are mixed, with observations of increases in spontaneous locomotor activity (Mateos-García et al, 2015) contrasting with other findings of decreased activity in a novel environment (Black et al, 2006). No effect of cocaine during adolescence on adult social interactions was observed (Mateos-García et al, 2015). Likewise, no increases in anxiety were observed in either the EPM or resident-intruder testing in adult rats after adolescent ethanol exposure (Alves et al, 2014). However, these animals exhibited several atypical behavioral alterations interpreted as suggesting possible impairment in the recognition and evaluation of potentially threatening stimuli. In the EPM, this was evinced via decreased time in the center of the maze (the relatively safe area between the open and closed arms) and in the resident-intruder test, as a longer latency to retreat from the aggressive "resident" (Alves et al, 2014). Intriguingly similar evidence for decreases in "cautious" behavior in adulthood after adolescent cocaine exposure

were reported by Sillivan et al (2011) as indexed in their work via increased open arm time in the EPM, along with attenuated fear conditioning, increased center time in the open field and hole board tests, and increased hole poke behavior when no food was present in the holes (used as an index of novelty seeking).

Cognitive functions have been little investigated, with few notable differences reported to date. No evidence of adolescent exposure-associated alterations in working or reference memory was seen during acquisition or reversal of a hole board food search task and Morris water maze (Sillivan et al, 2011). Performance on an attentional set-shifting task revealed no performance deficits after adolescent cocaine exposure, with adolescent exposed rats conversely exhibiting more rapid shifts in performance on the set-shifting task than controls, perhaps due to alterations in attentional mechanisms; this effect was evident when exposed animals were tested in adulthood (P70) but not late adolescence (P56) (Black et al, 2006). These findings differ somewhat from those of Kantak et al, (2014) where both adolescent (P37-55) and adult (P77-95) rats were allowed to either self-administer cocaine or were given cocaine non-contingently, and tested >2 weeks later on a strategy set-shifting task and reversal. Adolescent cocaine exposure did not disrupt performance on the set-shifting task, although non-contingent (but not self-administered) cocaine during adolescence impaired reversal acquisition. In contrast, animals exposed to cocaine in adulthood exhibited faster acquisition on the set-shifting task and its reversal than controls, regardless of whether cocaine was delivered contingently or non-contingently. This lack of notable effects of adolescent cocaine exposure on "executive function"-type tasks that rely in part on circuitry involving the PFC contrast with compelling evidence for neural alterations in these regions, as discussed below. Indeed, Kantak and colleagues (2014) suggested that the immaturity of the PFC during adolescence may exert a protective effect against cognitive alterations from repeated cocaine exposure. Clearly, more studies in this area are warranted.

5.2. Later drug-self-administration. Repeated adolescent exposure to cocaine has been reported to induce sensitization to the locomotor stimulant effects of cocaine in rats that is evident when the animals are tested in adulthood (P70) but not during late adolescence (P56) (Black et al, 2006). In one of the few studies comparing repeated exposure to cocaine during adolescence with analogous exposure in adulthood, latter locomotor sensitization to cocaine was found to be more marked after cocaine exposure during adolescence (Wheeler et al, 2013). Cross-sensitization to METH has also been reported in both male and female adult mice exposed to cocaine as adolescents (Shanks et al, 2015). In the latter study, similar cross-sensitization to METH was also seen when the adolescents were exposed repeatedly to amphetamine or methylphenidate, although the former effect was only evident in males and the latter was specific to females (Shanks et al, 2015). Evidence for enhanced later sensitivity to cocaine after adolescent cocaine exposure has also been reported for the rewarding effects of cocaine, with high (although not low) novelty seeking adolescents repeatedly exposed to cocaine showing greater CPP to both cocaine and MDMA in adulthood relative to their non-exposed counterparts (Mateos-García et al, 2015).

5.3. Neural effects. Using magnetic resonance imaging in mice, Wheeler and colleagues (2013) observed persistent structural alterations in a network of regions innervated by DA, including decreases in gray matter volume of the nAC, anterior cingulate cortex, amygdala and insula, increases in volume of the substantia nigra and the orbital frontal cortex, but no changes observed in the volume of PFC. This study was one of the few to include animals that were equivalently exposed to cocaine in adulthood, and observed that the effects in these dopaminergic-innervated regions were more pronounced after adolescent than adult exposure. Direct evidence for functional alterations in dopaminergic projections have also been reported. For instance, Alves et al (2014) observed greater levels of DA turnover (indexed via the ratio of the DA metabolite DOPAC to DA) in the amygdala following EPM testing (in the absence of any alterations in anxiety in this test, as discussed above) (Alves et al, 2012). In this

same study, adults exposed to cocaine as adolescents exhibited less notable increases in DA in the hippocampus following resident-intruder testing in adult rats following adolescent cocaine exposure, although the adolescent exposed animals showed greater corticosterone elevation post-test than did controls, findings they interpreted as evidence for greater stress responsivity in these animals (Alves et al, 2014). Greater stress sensitivity has also been reported in the PFC glutamatergic system, as discussed further below (Caffino et al, 2013).

Although the PFC was reported to be resistant to adolescent cocaine exposure in terms of gross structural alterations (Wheeler et al, 2013), such exposure has been reported to induce long-lasting increases in metabolic activity in the PFC, a “hypermetabolic PFC” than contrasts with the hypometabolic state reported after repeated cocaine exposure in adulthood (Cass et al, 2013). The consequence of both alterations, however, has been postulated to reflect decreased PFC functional capacity, consistent with an inverted U-shaped relationship between levels of PFC activity and efficacy of PFC functions (Cass et al, 2013).The increased PFC excitability after adolescent cocaine exposure is thought to be related in part to attenuated GABA activity in PFC (Caffino et al, 2013) associated with reduced GABAergic inhibition from the HPC to PFC as well as attenuated in GABA interneuron activity in the PFC (Cass et al, 2013). Such PFC hyperexcitability has also been to greater glutamatergic activity in the PFC, particularly in response to stressors. That is, adolescent cocaine exposure was found to sensitize mPFC glutaminergic synapses to a stress challenge three days later when indexed via increases in glutamate release, GluN1 responsivity, and spine hyperresponsiveness, along with decreases in glutamate transporter activity (involved in the clearance of glutamate) (Caffino et al, 2013).

Neural alterations induced by cocaine exposure in adolescence may be associated with transient drug-induced disruptions in gene expression. Alterations include increases in expression of genes encoding for cell adhesion molecules and declines in those encoding transcription factors observed in PFC after binge-like exposure to cocaine during adolescence, expression changes that were generally transient and declined with time following exposure

termination (Black et al, 2006). Transient expression changes associated with adolescent cocaine exposure have also been reported in the amygdala in genes critical for synaptic function, axonal guidance and Wnt signaling (Sillivan et al, 2011). Decreases in histone methylation in PFC were also observed (Black et al, 2006). How these drug-induced changes in gene expression and epigenetic regulation relate to lasting neurobehavioral changes after adolescent cocaine exposure remain to be determined.

6. Other Psychostimulants.

All drugs of the psychostimulant class, amphetamine (AMPHET), MDMA and METH, are indirect monoamine (MA) agonists, with AMPHET and METH having greater acute actions on DA, and MDMA having the most pronounced 5HT effects. The focus here will be on the "club" drugs METH and MDMA – stimulants used for recreational purposes, although it should be noted that the use of prescription stimulants such as methylphenidate as "cognitive enhancers" is increasing among some populations of youth (Clemow & Walker, 2014). Studies exploring consequences of adolescent exposure to METH and MDMA have been shown to produce a variety of persisting neural and behavioral alterations (see Teixeira-Gomes et al, 2015, for review and references) although, intriguingly, as reviewed below the adolescent period may not be a particularly vulnerable period for these effects, and in some cases may even be protective.

6.1. Cognition, Behavior and Affect. A number of persisting functional alterations have been observed after adolescent stimulant exposure. Exposure to MDMA has been observed to impair working memory (e.g., Piper & Meyer, 2004) and decrease social interactions (e.g., Bull et al, 2004). Increases in social anxiety-like behavior in the social interaction test contrast, however, with reports of no alterations in anxiety (Bull et al, 2004) or even attenuated anxiety (e.g., Piper & Meyer, 2004) when indexed via open arm time in the EPM. Stimulant exposure during adolescence has also been reported to sensitize animals to later stimulant challenge (McPherson & Lawrence, 2006; Klomp et al, 2012; although see also Piper et al, 2006). Measure-specific age differences have been reported, with for instance repeated AMPHET

exposure during adolescence inducing less persisting stereotypy to later AMPHET challenge than after equivalent exposure beginning in adulthood, although the adolescent-exposed animals were reported to exhibit greater persisting cognitive deficits than those exposed as adults in a working memory task and during acquisition of a delayed non-matching to sample task (Sherill et al, 2013).

6.2. Neurotoxicity. Neurotoxicity observed after adolescent stimulant exposure includes decreases in MA levels or levels of their transporters, damage to MA nerve terminals, apoptotic neuronal death and reactive gliosis indexed via increases in glial fibrillary acidic protein (see Teixeira-Gomes et al, 2015). Although there are scattered reports that adolescent exposure to these stimulants alters functioning of a variety of neurotransmitters, including ACh (Siegel et al, 2011) and glutamate (Kindlundh-Högberg et al, 2008), the DA and 5HT systems appear to be particularly targeted. For instance, persisting decreases in striatal levels of DA and 5HT (Cappon et al, 1997), along with occasional reports of decreases in 5HT levels in HPC, frontal regions of cortex (Bull et al, 2004) and AMYG (Faria et al, 2006) have been reported following adolescent exposure to METH or MDMA. Studies comparing consequences of adolescent versus adult stimulant exposure, however, have generally found neurotoxicity of adolescent stimulant exposure to be less severe than after exposure in adulthood, at least with some response measures. For instance, after METH exposure in adulthood, decreases in DA uptake and binding to the DA transporter were seen in striatum 1 week later, whereas neither DA alteration was seen in animals receiving METH exposure as adolescents (Riddle et al, 2002). In a study examining both short-term (1 day) and persisting (1 week) consequences of METH exposure, similar short-term DA alterations were seen following METH exposure in both adolescents and adults, whereas only adults exhibited persisting disruptions in the DA system, indexed via decreases in DA synthesis, uptake and transporter activity (Kokoshka et al, 2000). In this same study, however, no age differences in 5HT synthesis were observed, with short-term and persisting decreases evident after exposure at either age (Kokoshka et al, 2000).

Although Klomp and colleagues (2012) also reported persistent decreases in the serotonin transporter after MDMA exposure at both ages, the extent of this loss was less pronounced in frontal brain regions after exposure during adolescence than in adulthood.

6.3. Age-dependent vulnerabilities continued. The relative resistance of adolescents to persisting MA neurotoxicity may represent the later ontogenetic stages of an extended developmental delay in emergence of stimulant neurotoxicity. This conclusion was reached from rare and valuable research examining the ontogeny of stimulant neurotoxicity across a wide age span ranging from prenatal, pre-weanling, juvenile, adolescent and adult exposures. In this work, minimal MDMA neurotoxicity was observed after exposure at younger ages, with evidence of neural alterations (indexed via decreases in serotonin transporter binding sites) beginning to emerge after exposure during the late juvenile/early adolescent period (i.e., ~ P25-30), although toxicity at those ages was still attenuated relative to that seen after equivalent exposure in adulthood (Kelly et al, 2002). A similar ontogeny was revealed in a study of MDMA-induced decreases in serotonin transporter density and 5HT concentrations in frontal cortex 1 week following acute MDMA exposure where decreases were evident after exposure at P35, but not at P14, 21 or 28 (Aguirre et al, 1998). Similar ontogenetic delays in the emergence of neurotoxicity were reported with METH, with METH decreasing striatal DA, increasing glial fibrillary acidic protein and inducing hyperthermia only after administration at P40 and P60, but not P20 (Cappon et al, 1997). Monophasic ontogenetic increases in stimulant neurobehavioral toxicity have not always been observed, however, with for instance an inverted U-shaped curve peaking later in adolescence observed in a study where rats exhibited greater learning deficits after METH exposure at P41-50 than at P21-30, P31-40 or P51-60 (Vorhees et al, 2005).

A number of potential contributors to the attenuated toxicity of adolescents relative to adults to these stimulants has been suggested. One primary suspect has been the induction of drug-induced hyperthermia, given that METH-induced neurotoxicity has been reported to be exacerbated by hyperthermia and this hyperthermia emerges only gradually during ontogeny

(e.g. Riddle et al, 2002). Yet, while METH-induced hyperthermia could be environmentally facilitated at both P20 and P40 (by placement in a context with a temperature above room temperature), this hyperthermia precipitated METH-induced neurotoxicity only at P40 and not P20 (Cappon et al, 1997), suggesting that hyperthermia alone is insufficient for inducing neurotoxic effects, at least in very young animals. Pharmacokinetic factors may also contribute to the stimulant insensitivity of young animals, with higher brain levels of stimulants typically produced in adulthood after administration of the same doses to adults and younger animals (e.g., see Kokoshka et al, 2000). Yet, even when brain METH concentrations were equated by administering a higher dose to adolescents than adults, greater and longer-lasting METH neurotoxicity was evident in adults (Truong et al, 2005; Rau et al, 2006), suggesting that, as with other drugs of abuse, age differences in drug effects are unlikely to be due merely to across-age pharmacokinetic differences. Maturational stage of MA systems has also been suggested to contribute to the resistance of adolescents to MDMA (e.g., Klomp et al, 2012) and METH (Truong et al, 2005; Volz et al, 2009) neurotoxicity. For instance, adolescent animals exhibit both enhanced DA efflux as well as greater kinetic activity of MA transporters which could result in greater capacity to sequester DA in synaptic vesicles. Together these factors could protect against METH-induced elevations of cytoplasmic DA levels and subsequent neurotoxicity induced via production of DA-associated reactive oxygen species (see Truong et al, 2005; Volz et al, 2009). Thus, a variety of factors may contribute to the relative resistance of younger animals to stimulant neurotoxicity.

Intriguingly, a number of studies have reported that repeated stimulant exposure during adolescence may protect against later deficits induced by subsequent METH or MDMA exposure in adulthood (e.g., Piper et al, 2006, McFadden et al, 2011). For instance, periodic MDMA exposure from P35-60 attenuated the locomotor hypoactivity and decreases in serotonin transporters typically seen following MDMA exposure in adulthood (Piper et al, 2006). Similar attenuations in subsequent neurotoxicity, however, have been reported when the initial

stimulant exposure was delayed into adulthood. Consequently, these effects are not adolescent-specific and may be associated with the emergence of tolerance to stimulant-induced hyperthermia (McFadden et al, 2012) given that, as discussed previously, hyperthermia has been shown to exacerbate neurotoxicity.

Collectively, these data provide compelling evidence that stimulants show a markedly different pattern of ontogenetic sensitivity to drug neurotoxicity than the other drugs reviewed here, with young animals through adolescence being relatively resistant to the neurotoxic effects of METH and MDMA, perhaps due in part to the maturational state of DA systems that may confer partial protection against neurotoxicity. Despite this relative resistance, however, lasting neural, behavioral and cognitive consequences of adolescent exposure to these substances have nevertheless been observed, suggesting that adolescent exposures are not innocuous. For more detailed discussion of studies in laboratory animals examining the effects of adolescence stimulant exposure, see recent excellent reviews by Buck & Siegel (2015) and Teixeira-Gomes et al (2015).

7. Summary, conclusions and future directions

A summary of the major findings discussed in this review are provided in Table 1. Although these findings are not exhaustive (an unfeasibility given the rapid escalation of work in much of this area), the data reviewed provide convincing evidence that adolescent exposure to these drugs induces drug-specific patterns of sustained neurobehavioral alterations. Particularly common consequences evident across studies are disruptions in cognitive functions such as working memory, alterations in affect including increases in social anxiety, as well as increased cell death. Long-lasting alterations in forebrain neurotransmitter systems critical for modulating reward, socioemotional processing and cognition have also emerged, including some evidence for induction of a hyper-DA state after EtOH, CBs and nicotine (though not stimulants), and for attenuations in neurons expressing cholinergic markers after EtOH and

nicotine. Alterations in later self-administration of the same or other drugs have also been reported under some circumstances, although null findings are also common.

Empty cells in the Table could represent dependent measures that have yet to be systematically explored, or negative findings. As examples of potentially promising areas for further inquiry, note that although neuroimmune activation, alterations in neurogenesis, histone regulation, and sleep and other electrophysiological indices have been recently reported after adolescent exposure to EtOH, these sensitive measures have seemingly received little attention in the literature after adolescent exposure to other drugs. Likewise, alterations in cognitive flexibility have been reported in a number of instances after adolescent exposure to EtOH, but have seemingly been little explored after exposure to the other drugs. As another example, despite frequent reports of DA alterations after adolescent alcohol/drug exposure, and evidence that endogenous CBs play critical roles in regulation of DA neurons (e.g. Wang & Lupica, 2014), there has yet been little apparent investigation of alterations in endogenous CBs and their receptors after adolescent exposure to any of the drugs other than THC and other CBs. Thus, despite rapid progress to date, more work remains to determine whether apparent drug-selective findings reflect meaningful differences across drugs or merely areas of research focus to date, and to assess whether comparable findings observed across drugs reflect similarly altered neural substrates.

Another understudied area at present is that of sex differences. When both sexes have been investigated, sex differences have often emerged. Inclusion of both sexes in studies of lasting consequences of adolescent drug exposure has been sufficiently rare, though, to make it difficult to characterize the extent and nature of such sex differences. Even when sex-specific vulnerabilities in adolescent exposure effects have emerged, there often has been little exploration of the critical mechanisms that underlie these differential vulnerabilities. Assessment of sex differences in the functional and neural consequences of adolescent drug effects will undoubtedly be aided in the future by the rapid progress being made in the area of

puberty and the emergence of sex differences in neurobehavioral function (see reviews by Gur & Gur and by Shultz & Sisk in this Special Issue for examples of recent work in this area).

One type of measure where notable variations in findings has been observed is in the assessment of general anxiety-like behaviors. In contrast to generally consistent findings of persisting decreases in social behavior and increases in social-like anxiety after adolescent drug exposure, findings have been more variable when using tests such as the EPM, light/dark box and open field. As discussed earlier, increased time spent in the open could varyingly reflect low anxiety levels or greater "disinhibition". And the extent to which possible drug-induced increases in anxiety versus greater disinhibition would predominate in a particular test situation might well vary depending on prior experiences and the particular test circumstances. Development of test procedures to disentangle detection of these two constructs would be useful.

Only a modest amount of work has been conducted to assess whether adolescence is a time of particular vulnerability for the production of lasting drug effects. When other aged exposure groups have been studied, consequences typically were found to be more pronounced after exposures during adolescence than in adulthood for three of the four drugs/drug classes examined. The stimulants METH and MDMA were the exception, with neurotoxicity very low in young animals, more marked by adolescence, and continuing to increase into adulthood. Given this precedent for greater toxicity after adult than adolescent exposure for at least some drugs, it would appear premature to assume greater adolescent vulnerability and dismiss the importance of periodically including adult exposure groups to assess relative across-age vulnerabilities in programmatic research in this area.

Thus, there is compelling evidence that adolescent drug exposure can induce persisting neural, cognitive, affective and behavioral consequences. To date, brain regions undergoing notable development during adolescence appear to be particularly sensitive to lasting perturbations after adolescent drug exposure. Yet, it is not clear whether these regions

are truly more vulnerable or if they have merely received particular scrutiny due to their developmental "volatility" during adolescence. Work remains to determine the breadth of findings within drugs, consistencies across drugs, exposure-age specificity, underlying mechanisms, and consilience of findings with human drug-abusing adolescents, with a goal of identifying promising translational approaches for the development of effective prevention/intervention efforts.

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impaired/attenuated; ↑ enhanced; = no notable exposure effects; Y alterations reported (often complex)

Table 1. Overview of consequences of repeated adolescent exposure to ethanol (EtOH), nicotine (NIC), cannabinoids (CBs) and MDMA and methamphetamine stimulants (STIM)

						EtOH	**NIC**	**CB**	**COC**	**STIM**
General Age Vulnerability						Adol>Adult	Adol>Adult	Adol>Adult	?	Adol<Adult
Cognitive/behav.										
	Spatial memory					↓ =		=		
	Conditional discrimin./pattern learning					↓	↓			
	Attention						↓		(↓)	
	Obj. recognit./working memory					↓		↓	=	↓
	Pre-pulse inhibition							↓		
	Congitive flexibility					↓				
	Risk preference					↑				
	Impulsivity/disinhibition					↑	↑			
	Retent. of adoles-typical phenotypes					↑				
Affective/Social behavior										
	Depression-like behaviors					↑		↑		

	Social interactions				↓		↓	=	↓
	Social anxiety-like behaviors				↑		↑	↓	↑
	Other anxiety-like behaviors				↑, ↓	↑	↑, ↓, =		=, ↓
Later self-admint. (same/different drugs)					↑, =	↑, =	↑, =		
Neural									
	Neurogenesis				↓				
	Cell death				↑	↑			↑
	Spines/dendritic branching				↑				
	Electrophysiol. Alterations				Y			Y	
	Neuroimmune activation				Y				
	Histone acetylation/epigentic regulat.				Y			Y	
	Alterations in:								

		Ach				Y	Y			Y
		Glutmate/GABA				Y	Y	Y	Y	Y
		DA				Y	Y	Y	Y	Y
		5HT					Y	Y		Y
		CB						Y		
	Affected brain regions:									
		PFC				Y	Y	Y	Y	Y
		HPC				Y	Y	Y	Y	Y
		nAc				Y	Y	Y	Y	
		AMYG				Y	Y	Y	Y	Y