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Exploring the impact of adolescent exposure to cannabinoids and nicotine on psychiatric risk: insights from translational animal models

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Invited Review

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Abstract

Adolescence represents a highly sensitive period of mammalian neurodevelopment wherein critical synaptic and structural changes are taking place in brain regions involved in cognition, self-regulation and emotional processing. Importantly, neural circuits such as the mesocorticolimbic pathway, comprising the prefrontal cortex, sub-cortical mesolimbic dopamine system and their associated input/output centres, are particularly vulnerable to drug-related insults. Human adolescence represents a life-period wherein many individuals first begin to experiment with recreational drugs such as nicotine and cannabis, both of which are known to profoundly modulate neurochemical signalling within the mesocorticolimbic pathway and to influence both long-term and acute neuropsychiatric symptoms. While a vast body of epidemiological clinical research has highlighted the effects of adolescent exposure to drugs such as nicotine and cannabis on the developing adolescent brain, many of these studies are limited to correlative analyses and rely on retrospective self-reports from subjects, making causal interpretations difficult to discern. The use of pre-clinical animal studies can avoid these issues by allowing for precise temporal and dose-related experimental control over drug exposure during adolescence. In addition, such animal-based research has the added advantage of allowing for in-depth molecular, pharmacological, genetic and neuronal analyses of how recreational drug exposure may set up the brain for neuropsychiatric risk. This review will explore some of the advantages and disadvantages of these models, with a focus on the common, divergent and synergistic effects of adolescent nicotine and cannabis exposure on neuropsychiatric risk.

Introduction

The developing adolescent brain is exquisitely sensitive to neurotoxic insults, particularly, those associated with exposure to recreational drugs such as cannabis and nicotine. Concurrently, adolescence represents the most common developmental window for drug-related experimentation. As testament to this, over 90% of smokers report becoming dependent on tobacco during adolescence (Saddleson et al., 2016). Similarly, adolescent cannabis use is increasingly common in developed countries and how legalisation in many of these jurisdictions may ultimately affect these usage rates remains to be seen. While adolescence represents a life period wherein experimentation with multiple recreational drug classes is more likely (including alcohol, psychostimulants and hallucinogenics), cannabis and nicotine in particular, have received a tremendous degree of both clinical and pre-clinical research attention.

Several reasons account for this selective focus on nicotine and cannabis. First, both tobacco and cannabis use have been associated with a range of neuropsychiatric symptoms following either acute or long-term use. Second, nicotine and cannabis have exceptionally high rates of comorbid use patterns, such that cannabis is often cut with nicotine in cannabis cigarettes. Indeed, this tendency for tobacco and cannabis co-exposure has been a complicating factor in disentangling their neurodevelopmental effects in clinical studies. Third, both nicotine and cannabis share overlapping clinical co-morbidity with mood and anxiety disorders as well as schizophrenia (Andréasson, Allebeck, Engström, & Rydberg, 1987; Arseneault et al., 2002; Breslau, Kilbey, & Andreski, 1993; Goodwin, Zvolensky, Keyes, & Hasin, 2012). Finally, a growing body of clinical and pre-clinical evidence points to common underlying neuropathological effects of adolescent exposure to nicotine and cannabinoids. Indeed, exposure to both compounds is increasingly linked to shared neuropathological disturbances in a variety of molecular, neuronal and pharmacological substrates in the mesocorticolimbic system, as well as in their associated neural input and output regions. Specifically, brain regions such as the prefrontal cortex (PFC), hippocampal formation (HIPP) and the mesolimbic dopamine (DA) pathway, comprising the ventral tegmental area (VTA) and nucleus accumbens

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(NAc) appear to be particularly vulnerable to the neuropathogenic effects of cannabis and nicotine exposure (Jobson et al., 2019; Renard et al., 2016, 2017; Velakoulis et al., 2006; Yücel et al., 2008). In this review, recent evidence highlighting this neural circuitry and associated molecular and neurochemical pathways will be examined, to reveal how translational animal models of neurodevelopmental nicotine and cannabis exposure are informing our understanding of how these drugs selectively target the adolescent brain and increase vulnerability to mood, anxiety and schizophrenia-related disorders.

Pros and cons of translational animal models of adolescent drug use

Clinical investigations into the effects of adolescent, recreational drug exposure and neuropsychiatric risk have several important limitations. First, many studies rely on retrospective self-reports of recalled drug use episodes which may be compromised in their historical accuracy. Second, it is impossible to determine precise drug exposure concentrations over these time periods due to the fact that concentrations will be highly variable across drug products (either illicit or legally acquired). Nicotine and cannabis provide excellent cases-in-point. For example, relative concentrations of delta-9-tetrahydrocannabinol (THC), the primary psychoactive constituent in cannabis, is known to vary widely depending on the specific plant strain. Furthermore, different routes of drug administration (e.g. vaping, e-cigarettes, Juuling, use of concentrated THC extracts such as 'shatter') will invariably expose the individual to highly divergent concentrations of either nicotine or THC. This sampling variability is unlikely to be adequately controlled for in retrospective clinical studies. Finally, establishing precise causal and mechanistic relationships between actual adolescent drug exposure and its resulting pathological sequelae is not possible. Thus, the underlying relationships between adolescent drug exposure and psychopathological outcomes remain correlative in nature, leaving many open questions. For example, do individuals exposed to nicotine during adolescence have a predisposition to developing mood and anxiety disorders? Does the use of cannabis during adolescence represent a form of self-medication to attenuate prodromal symptoms of schizophrenia? Importantly, ongoing innovations in prospective clinical trials examining the long-term impacts of adolescent drug exposure (e.g. the ABCD study currently underway at the NIMH) will hopefully be able to circumnavigate several of these issues while controlling for neurodevelopmental directionality of drug exposure effects. Nevertheless, often times questions of neurobiological mechanisms are more easily addressed using translational animal models, which allow for the precise control of drug dosing, temporal control of exposure and follow-up mechanistic interventions aimed at preventing or reversing the effects of neurodevelopmental drug exposure.

Nevertheless, despite many advantages in terms of experimental and causal precision, translational models of adolescent drug exposure suffer from several significant drawbacks. Perhaps most crucially, drug exposure protocols in rodents typically depend on experimenter administration of drugs as opposed to voluntary self-administration by the animal. While this allows for high experimental precision in terms of controlling dose and timing, it excludes the face validity of human drug-seeking and addiction behaviours. While voluntary drug self-administration is certainly possible in species such as rodents, this can be challenging in terms of time and cost as well as inherent difficulties in getting reliable self-administration for certain drug classes and in delivery formats that are comparable to human consumption. Nevertheless, Kruse, Cao, Viray, Stella, and Clark (2019) recently reported that rats will reliably engage in voluntary consumption of THC-containing gelatin format 'edibles', which led to several physiological and neurobiological molecular adaptations linked to THC exposure. Similarly, Freels et al. (2019) recently reported that rats would reliably self-administer vapourised cannabis extracts which closely resembled inhaled constituents in traditional smoked marijuana formats, further demonstrating the feasibility of employing volitional modes of cannabis-related exposure in rodent models of neurodevelopment.

In addition, differences in metabolic parameters between humans and rodent species in terms of required experimental drug dosing and timing of delivery, may render translational interpretations difficult, particularly when comparing dosing ranges during critical periods of human *v.* non-human neurodevelopment, wherein understanding exposure magnitudes is of critical importance. Irrespective of delivery route and dosing differentials, pre-clinical animal models can never capture all of the genetic/environmental interactions that underlie predispositions towards adolescent drug seeking behaviours and cannot account for complex socioeconomic environmental factors linked to adolescent drug taking behaviours. Finally, all complex neuropsychiatric diseases are ultimately human in nature. Schizophrenia and mood/anxiety disorders in particular, comprise highly complex collections of symptoms which cannot be wholly modelled in pre-clinical animal paradigms. Despite these obvious caveats on both sides of the pre-clinical/clinical research equation, rodent models overall have been remarkably successful in modelling many of the neuropsychiatric phenomena and endophenotypes associated with adolescent drug exposure, particularly in the realms of cannabis and nicotine use. This is due in large part to the remarkable similarities between human and rodent brain architecture, homologous neurochemical pathways and similar genotypic landscapes.

Nevertheless, how can we improve our pre-clinical neurodevelopmental models to better translate into corresponding effects following human adolescent drug exposure? In the following sections, we will review and contrast recent translational evidence using rodent adolescent models of cannabinoid and nicotine exposure and in so doing, review parallels between this pre-clinical data with relevant clinical and epidemiological findings.

The effects of cannabinoid exposure on adolescent neurodevelopment: comparing clinical and pre-clinical evidence for schizophrenia risk

Decades of clinical research have revealed a strong correlation between adolescent exposure to cannabis and an increased risk of neuropsychiatric illness, specifically, schizophrenia-related psychoses. A watershed report by Andreasson et al., in 1987, studied >45 000 Swedish military conscripts and positively correlated frequency of retrospective self-reports of cannabis use during adolescence to the increased likelihood of a schizophrenia diagnosis in young adulthood. These findings have now been replicated by numerous groups across the globe (Arseneault et al., 2002, 2004; Stefanis et al., 2004). Furthermore, similar studies have linked adolescent cannabis exposure to enduring cognitive deficits (Harvey, 2019; Orr et al., 2019) and mood disorders (Gobbi et al., 2019; Osuch et al., 2016). At the neuroanatomical level, numerous brain regions and associated neurochemical pathways have been

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associated with cannabis-related psychopathology. For example, chronic and acute cannabis use has been linked to significant structural alterations in the HIPP, amygdala, thalamus, cerebellum, striatum and prefrontal cortical regions (Chye et al., 2018; Koenders et al., 2015; Maple, Thomas, Kangiser, & Lisdahl, 2019; Orr et al., 2019), neural regions which are all implicated in schizophrenia-related aetiology and underlying neurodevelopmental trajectories (Fornito et al., 2008; Wood et al., 2010). It is important to note, however, that structural imaging studies can often include confounds such as co-morbid nicotine and/or alcohol exposure or be limited to populations with cannabis dependence rather than recreational users. While many of these human studies linked these neuropathological features to specific cognitive and neuropsychiatric symptom clusters and vulnerability, the underlying neurobiological mechanisms responsible for the effects of cannabinoid exposure on schizophrenia-related endophenotypes have largely been identified with basic, pre-clinical studies performed in rodent models.

For example, several post-mortem studies in schizophrenia populations have revealed strongly increased expression levels of cannabinoid CB1 receptors in frontal cortical regions such as the dorsolateral prefrontal and anterior cingulate cortices (Dalton, Long, Weickert, & Zavitsanou, 2011; Zavitsanou, Garrick, & Huang, 2004). This suggests that dysregulation or overdrive of cannabinoid signalling within these cortical regions may be linked to schizophrenia-related phenotypes. However, the mechanistic interpretation of what these adaptations might mean for schizophrenia-related psychopathology cannot be addressed in post-mortem analyses. To examine the potential pathological effects of aberrant cannabinoid receptor signalling in the frontal cortex, an early pre-clinical study reported in 2006 (Laviolette & Grace, 2006) revealed that pharmacological overstimulation of CB1 receptors with a synthetic CB1R agonist in analogous regions of the rat PFC, induced strong distortions in emotional salience attribution and associative memory formation, heightening sensitivity to fear-related associative cues that would normally be non-salient. Cannabinoid receptor hyper-stimulation similarly caused distortions in associative neuronal response patterns within isolated pyramidal neurons in the PFC, revealing for the first time a precise neuronal mechanism by which cannabinoid exposure might trigger schizophrenia-like emotional processing disturbances at the cortical level. This study further revealed that functional inputs from the basolateral nucleus of the amygdala (BLA) was required for these cannabinoid-related effects in the PFC, suggesting that cannabinoid signalling abnormalities within the BLA > PFC circuit may underlie the effects of cannabinoids on these affective processes between cortical and sub-cortical limbic regions.

In addition, pre-clinical research has revealed that the extent of cannabinoid receptor stimulation in regions such as the PFC can directly control emotional perception and memory salience through functional interactions with the mesolimbic DA system. For example, Draycott et al. (2014) demonstrated that while relatively lower levels of pharmacological CB1R stimulation in the PFC was capable of inducing overdrive of sub-cortical DA neurons (both in terms of the their tonic and phasic firing modes) and inducing schizophrenia-like distortions in emotional processing, higher levels of stimulation actually blunted DAergic activity and induced affective flatness and inability to form salient associative emotional memories, measured in a fear conditioning protocol. This suggested that the relative levels of cannabinoid activity within the mammalian PFC is a strong modulator of emotional salience and processing and that the observed dysregulation of CB1R levels in the frontal cortical regions of schizophrenia subjects (Dalton et al., 2011; Zavitsanou et al., 2004) may be related to similar affective abnormalities, found in both positive and negative schizophrenia symptom clusters. Clinical imaging studies have reported similar dysregulation of mesolimbic DAergic signalling following long-term use. Interestingly, in most clinical studies, chronic cannabis use was associated with significant downregulation in DA content within the striatum (Bloomfield, Ashok, Volkow, & Howes, 2016; Urban et al., 2012; van de Giessen et al., 2017). While such findings may seem incongruent with rodent models reporting hyperactive DAergic phenotypes following adolescent cannabis use, these studies did not focus on adolescent uses exclusively, which may suggest that with continued chronic use into adulthood, DAergic signalling may be blunted as a compensatory response to earlier hyper DAergic phenotypes. Future pre-clinical studies will need to examine the longer-term effects of chronic THC exposure into early adulthood and beyond to more closely examine these differences.

Additional pre-clinical evidence has pointed to similarities between deficits in inhibitory GABA signalling in the frontal cortex and the long-term effects of adolescent THC exposure. Thus, considerable evidence from post-mortem neural analyses has revealed striking deficits in GABAergic molecular markers from the brains of schizophrenia patients (Guidotti et al., 2000; Hashimoto et al., 2003). These phenotypes extend from losses in specific sub-units of the GABA-A receptor subtype (Huntsman *et al*,. 1998; Maldonado-Avilés et al., 2009) to dysregulated levels of glutamate decarboxylase (GAD-67, 65) levels, which are the primary cellular precursors for GABA by catalysing the decarboxylation of glutamate to GABA and CO_2 (Hashimoto et al., 2003). Nevertheless, evidence for a selective loss of GABAergic inhibitory mechanisms following adolescent cannabis exposure in human populations has not been reported. Using a translational rodent model of adolescent THC exposure, Renard, Rushlow, and Laviolette (2018) reported that adolescent THC exposure not only caused a significant loss of GAD-67 (but not GAD-65) in the PFC, but that the associated schizophrenia-like behavioural and cognitive endophenotypes could be reversed by pharmacologically restoring GABA-A signalling in the PFC, even in adulthood. More remarkably, restoration of GABA-A transmission was able to reverse adolescent THC-induced hyperactivity of sub-cortical DAergic neuronal activity states, revealing a potential translational mechanism by which the deleterious and long-term effects of adolescent cannabis exposure may be reversible, even in the mature brain. Interestingly, the selective loss of frontal cortical GAD-67 in the rat PFC was remarkably consistent with post-mortem schizophrenia phenotypes showing selective losses in cortical GAD-67 but not GAD-65 (Curley et al., 2011; Volk, Austin, Pierri, Sampson, & Lewis, 2000), again underscoring the successful translational modelling of human adolescent brain vulnerability to THC in rodent models and revealing novel mechanistic insights into established human post-mortem evidence.

At the neurochemical level, disturbances in DA signalling and downstream molecular pathways associated with DA receptor transmission, have perhaps been the most strongly linked to both acute and neurodevelopmental cannabis-related pathology. For example, as previously discussed, CB1R transmission directly in the mammalian PFC can strongly modulate downstream activity states of sub-cortical DAergic neurons (Draycott et al., 2014). In addition, recent evidence has demonstrated that THC administered directly into the NAc, can similarly (and dose-dependently)

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regulate the firing and bursting states of DAergic neurons in the VTA (Fitoussi, Zunder, Tan, & Laviolette, 2018). Both cannabinoid signalling in the PFC and NAc concomitantly regulate affective salience and associative emotional memory formation through these DAergic modulatory roles. In terms of neurodevelopmental effects, adolescent THC exposure has been shown to dramatically increase baseline levels of VTA DAergic activity levels (Friend et al., 2017; Renard et al., 2017, 2018). Recently, Corongiu, Dessì, and Cadoni (2019) reported that the adolescent rat brain showed heightened release of striatal DA specifically in response to systemic nicotine or THC exposure relative to the adult brain, suggesting that adolescence may represent a selectively vulnerable time period for DAergic vulnerability to psychostimulant or cannabinoid-derived drugs. Interestingly, both the acute and chronic effects of THC on mesolimbic DAergic activity states are mediated by reducing inhibitory feedback mechanisms onto VTA DAergic neurons (Fitoussi et al., 2018; Friend et al., 2017).

Some critical downstream pathways linked to DAergic dysregulation include the glycogen-synthase-kinase-3 (GSK-3), protein kinase B (Akt), mammalian target of rapamycin (mTOR) as well as p70-S-6-kinase (p70S6K). These signalling pathways are similarly dysregulated in a variety of neuropsychiatric diseases including schizophrenia and mood and anxiety disorders (Jernigan et al., 2011; Kozlovsky, Belmaker, & Agam, 2001). Several clinical reports have also linked these pathways to underlying genetic susceptibility to cannabis-induced psychosis. For example, Di Forti et al. (2012) reported that genetic variation in Akt (Threonine 308) was associated with increased risk of cannabis-related psychosis. Furthermore, this biomarker was linked to functional regulation of the DA D2 receptor (Colizzi et al., 2015a, 2015b), suggesting that dysregulation of the Akt-D2 receptor signalling pathway may confer increased risk of cannabis-related neuropsychiatric side-effects. Using a translational rodent model of adolescent THC exposure, this functional link was demonstrated by Renard et al. (2017), who showed that, in addition to a hyperactive DAergic phenotype, adolescent THC exposure caused significant dysregulation of the phosphorylation state of Akt at Threonine 308 (but not at Serine 472), directly in the PFC. Indeed, this alteration in Akt levels is consistent with molecular evidence linking states of hyperDAergic D2 receptor transmission and concomitant down-regulation of the Akt signalling pathway (Sutton & Rushlow, 2012). Thus, using a mechanistic rodent neurodevelopmental model it was possible to functionally link, with high neuroanatomical and molecular precision, a previously reported genetic susceptibility marker to a specific expression pattern and phosphorylation site in the mammalian PFC. Beyond markers for GABA and Akt, pre-clinical evidence has found also that adolescent THC exposure can cause strong down-regulation of GSK-3, mTOR and p70S6K in the PFC.

Interestingly, these same molecular adaptations have been reported in several post-mortem analyses. For example, Kozlovsky et al. (2001) reported a 41% reduction in the levels of GSK-3 in the frontal cortex of schizophrenia patients, consistent with findings in the rodent brain (Renard et al., 2017). Similarly, Jernigan et al. (2011) found significant reductions in both mTOR and p70S6K in post-mortem samples from frontal cortex in patients with major depression, consistent with findings in rodent brain and with reported abnormalities in anxiety and affective processing (Renard et al., 2017, 2018). These results are also interesting from a translational perspective given recent clinical findings demonstrating increased risk of mood disorders, anxiety and suicide following adolescent THC exposure (Gobbi et al., 2019). Thus, despite the complexity of human neuropsychiatric diseases such as schizophrenia and mood/anxiety disorders, rodent translational models can successfully model a variety of neuropsychiatric endophenotypes by using well-chosen and selective behavioural assays and combining these with high level correlational or mechanistic experimental designs aimed at reversing or preventing these neuroadaptations. These sorts of integrative translational approaches can offer excellent explanatory power over non-causative clinical correlational analyses.

The effects of nicotine exposure on adolescent neurodevelopment: comparing clinical and pre-clinical evidence for mood/anxiety disorder risk

As noted previously, nicotine and cannabis, particularly in the context of adolescent neurodevelopmental exposure, share numerous neuropathological effects, as revealed in both clinical and pre-clinical studies. Similar to cannabis, chronic tobacco use has been associated with increased vulnerability to psychosis and is associated with an earlier age of onset for psychotic episodes (Bhavsar et al., 2018; Gurillo, Jauhar, Murray, & MacCabe, 2015; Mustonen et al., 2018). In addition, the strong co-morbidity between schizophrenia and smoking behaviours is well-established, as some reports indicate that upwards of 90% of schizophrenia patients are heavily dependent upon nicotine products (Dalack & Meador-Woodruff, 1996; Hughes, Hatsukami, Mitchell, & Dahlgren, 1986). Nevertheless, there is considerably less evidence to suggest a direct correlation between adolescent tobacco use and an increased likelihood of schizophrenia-related psychotic symptoms in later life. Both cannabis and tobacco use typically begin in adolescence and >90% of tobacco-dependent individuals begin smoking during their teen years. With the advent of new forms of nicotine delivery such as e-cigarettes, hookah pipes, vaping and Juuling, these adolescent usage rates are likely to rise in the coming years.

Similar to cannabinoids, nicotine's psychoactive and dependence-producing motivational effects are strongly controlled by DAergic transmission variables, specifically within the mesolimbic system. For example, blockade of DA receptors systemically or directly in the NAc has been shown to dramatically potentiate the rewarding properties of nicotine (Laviolette & van der Kooy, 2003; Laviolette, Lauzon, Bishop, Sun, & Tan, 2008; Sun & Laviolette, 2014). In contrast, once nicotine dependence has developed, both the rewarding properties and aversive effects of nicotine withdrawal are dependent on DAergic transmission, as both effects are blocked by DA receptor antagonists (Tan, Bishop, Lauzon, Sun, & Laviolette, 2009; Grieder et al., 2012). Interestingly, the reinforcing effects of nicotine are similarly blocked by CB1R antagonists (Gueye et al., 2016), suggesting functional interactions between neurobiological substrates subserving both nicotine and cannabinoid reinforcement. In addition to DAergic regulation of nicotine's acute motivational properties, chronic nicotine exposure has been shown to potently sensitise the mesolimbic DA pathway to subsequent nicotine exposure (Tan et al., 2009).

Beyond nicotine's well-established associations with schizophrenia, a convergence of clinical and pre-clinical neurodevelopmental studies have highlighted the association between nicotine exposure and an increased likelihood of developing mood and anxiety-related disorders. Indeed, depression and smoking behaviours show strong co-morbidity, and both typically develop in adolescence (Fleming & Offord, 1990; Lewinshohn, Hops, Roberts, Seeley, & Andrews, 1993). For example, the TOPP

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study (Moylan et al., 2013) reported a prospective relationship between adolescent active smoking behaviours and the presence of early adult anxiety. Similarly, Johnson et al. (2000) reported a strong relationship between adolescent smoking behaviours and anxiety-related symptoms in later adolescence and early adulthood. Nevertheless, similar to issues with clinical studies into the neurodevelopmental effects of cannabis exposure on neuropsychiatric vulnerability, establishing causality between smoking behaviours and mood and anxiety disorders has been challenging, particularly in terms of determining whether smoking behaviours in prodromal phases represent attempts at self-medication and/or some other underlying predisposition rendering individuals more vulnerable to nicotine's reinforcing properties. Again, pre-clinical rodent studies have been able to circumvent many of these limitations by using defined, chronic nicotine exposure protocols during controlled windows of adolescent brain development.

Using a chronic systemic nicotine exposure protocol in adolescent rats, Counotte et al. (2009) reported long-term cognitive deficits, problems with attentional behaviours and increased impulsivity enduring into early adulthood. Interestingly, the window of nicotine vulnerability was selective for adolescence as nicotine exposure in the immediate post-adolescent developmental period had no long-term adverse effects. In addition, these deficits were correlated with a selective increase in DA release in the PFC, but not in the NAc, suggesting a long-term dysregulation of the mesocorticolimbic DA system, remarkably similar to that observed following adolescent THC exposure (Renard et al., 2017, 2018). In addition, adolescent nicotine exposure was shown to alter glutamatergic control of synaptic plasticity in the rodent PFC by altering levels of metabotropic glutamate type-2 receptors and to transiently increase the levels of nicotinic receptor expression on glutamatergic terminals in the PFC (Counotte et al., 2009, 2011). Subsequent translational studies have examined the underlying molecular and neuronal disturbances associated with adolescent nicotine exposure and mood/anxiety-related phenotypes. For example, using the same adolescent chronic nicotine exposure protocol in rats (Counotte et al., 2009), Jobson et al. (2018) reported that rats displayed profound anxiety-like and depressive-like behaviours when examined in early adulthood. In addition, these rats displayed heightened sensitivity to the acquisition of normally sub-threshold associative fear memories. Similar to previous reports (Counotte et al., 2009, 2011), these mood/anxiety behavioural endophenotypes were accompanied by dysregulation of the mesocorticolimbic DA system with strongly increased spontaneous levels of VTA DA neuron frequency and bursting states. In addition, this study found concomitant dysregulation of excitatory neuronal activity in the PFC and a corresponding down-regulation of DA D1 receptor expression levels (but no change in the D2 subtype).

Notably, this apparently selective effect on the D1R subtype is consistent with human clinical findings which have found similar downregulation in the DA D1R system in various limbic neural regions (Cannon et al., 2009; Dougherty et al., 2006), suggesting that overdrive of the mesolimbic VTA > PFC DAergic pathway may be a contributing factor to adolescent nicotine-related mood and anxiety disorder vulnerability. Similarly, Iñiguez et al. (2009) reported that chronic nicotine exposure in adolescent rats (but not adults) induced a variety of long-term depressive-like behavioural phenotypes persisting into early adulthood and heightened sensitivity to anxiety-related stimuli. Interestingly, this report demonstrated that subsequent administration of either nicotine or anti-depressants (fluoxetine or bupropion) in adulthood, was sufficient to reverse these mood-related disturbances, providing a strong translational validation of the effects of adolescent nicotine exposure on increased mood/anxiety related endophenotypes in adulthood. In summary, translational rodent models of adolescent nicotine exposure have revealed numerous mechanistic insights into the underlying neuropathological features increasing the likelihood of developing mood and anxiety-related symptoms in later life. Interestingly, several of these features are similar to those observed following adolescent THC exposure, raising the critical question: might cannabinoids and nicotine act synergistically during adolescent neurodevelopment to trigger increased vulnerability to mental health problems later in life?

Cannabis and nicotine combined: synergistic effects on neuropsychiatric vulnerability?

The research highlighted in this review has thus far focused on several commonalities observed between the effects of cannabis and nicotine on adolescent neurodevelopment and their independent effects on neuropsychiatric risk. Importantly, cannabis and tobacco products are very often consumed in concert, with tobacco being cut with cannabis in marijuana cigarettes or co-occurring smoking of cannabis and tobacco products separately, in the same individual (Wetherill et al., 2015a, 2015b). Given the common neural mechanisms and molecular pathways that are modulated by neurodevelopmental exposure to both compounds, is there compelling evidence for a synergistic effect of their co-exposure on mental health related outcomes? From a clinical perspective, there is currently only limited evidence suggesting a synergistic effect between adolescent nicotine and cannabis co-use in terms of increased neuropsychiatric risk factors, particularly in terms of how exposure to one, may increase dependence liability for the other. For example, Audrain-McGovern, Stone, Barrington-Trimis, Unger, and Leventhal (2018) reported that adolescent use of e-cigarettes or hookah smoking was associated with higher rates of comorbid cannabis use. E-cigarette use is also associated with the initiation of cannabis consumption at younger ages, steeper use escalation and the development of nicotine and cannabis dependence (Agrawal et al., 2011, 2012; Peters, Budney, & Carroll, 2012). A meta-analysis by Gurillo et al. (2015) examining correlative links between tobacco use and psychosis risk concluded that while daily tobacco use was correlated with an increased risk of psychotic disorder and earlier age of onset, the overall effect of smoking appeared to be modest. Importantly however, this study did not directly examine adolescent smoking behaviours and specific psychotic risk variables, nor did it examine nicotine and cannabis co-usage as specific risk factors.

More recently, Tucker et al. (2019) performed an extensive clinical analysis comparing physical and mental health measures between subjects using cannabis or nicotine alone, *v.* those co-administering them via smoked formats. Interestingly, these authors reported that cannabis and tobacco co-administration was correlated with significantly worse mental and physical functioning overall when compared to cohorts using either substance independently. Subjects co-administering cannabis and tobacco also displayed significantly greater rates of anti-social behaviours and social maladjustment. Thus, evidence is emerging suggesting a potential synergistic effect of cannabis and nicotine exposure on mental health outcomes. To date, no existing clinical studies have reported any underlying neurobiological mechanisms which may

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underlie the effects of combining tobacco and cannabis nor how their combination might selectively increase neuropsychiatric risk for any particular mental health domain. However, Filbey, Gohel, Prashad, and Biswal (2018) recently reported differences in several resting state brain networks comparing cohorts using cannabis or nicotine in isolation *v.* combined use. In this case, subjects using nicotine and cannabis in concert displayed greater resting state network connectivity involving the frontal parietal network, visual network, insular cortex, posterior cingulate and middle temporal gyri and basal ganglia network. While these findings are correlative, they highlight several neural regions which may be of future interest when examining potential synergistic effects of nicotine and cannabis on neural processes associated with psychiatric risk.

As discussed previously, various pre-clinical translational studies have highlighted shared neural circuits and behavioural phenotypes that are common to both cannabinoid and nicotine exposure during adolescence. Most importantly, several rodent studies reviewed above have demonstrated that adolescent exposure to either compound can cause long-term hyperactive dysregulation of the mesocorticolimbic DA system, persisting into early adulthood (Counotte et al., 2009; Jobson et al., 2018; Renard et al., 2017, 2018). Given the profound importance of mesolimbic DAergic dysregulation as underlying neuropathogenic markers in both schizophrenia and mood/anxiety-related disorders, this common effect may serve as a critical mechanism related to how neurodevelopmental exposure to either compound or in combination, might increase neuropsychiatric risk factors. Indeed, given the high co-morbidity between schizophrenia and mood/anxiety disorders (Kiran & Chaudhury, 2016; Samsom & Wong, 2015) shared psychopathological features following chronic exposure to both nicotine and cannabis may be expected to be observed in both clinical and pre-clinical experimental analyses.

In addition to common disturbances in sub-cortical DAergic signalling, pre-clinical studies have revealed that both adolescent cannabinoid and nicotine exposure leads to long-term disturbances in frontal cortical activity states, synaptic plasticity and regulation of inhibitory GABAergic *v.* excitatory glutamatergic signalling mechanisms. As noted previously, adolescent THC exposure in rodents causes a long-term loss of GABAergic markers in the PFC concomitant with hyperactive pyramidal neuron activity and co-occurring increases in gamma-band oscillatory activity, consistent with dysregulation of inhibitory/excitatory balance within the PFC (Renard et al., 2018). Cass et al. (2014) similarly reported that overactivation of CB1 receptor stimulation during adolescence led to long-term impairments in the normal development of inhibitory GABA signalling in the rodent PFC. Similarly, adolescent nicotine exposure causes hyperactivity of the rodent PFC along with profound alterations in glutamatergic receptor expression levels and synaptic plasticity dynamics (Counotte et al., 2009, 2011; Jobson et al., 2018). Together, these two common neurodevelopmental adaptations within the mesocorticolimbic circuitry may represent a convergence point whereby cannabis and tobacco exposure might cause enduring increases in neuropsychiatric vulnerability. However, critical questions remain, particularly in terms of translating these basic neuroscience findings into the human clinical context. In addition, there are still considerable knowledge gaps concerning how THC and nicotine might act synergistically at the neurophysiological level to produce these common adaptations during adolescent brain development. Finally, future pre-clinical studies are needed to more clearly characterise how nicotine and THC may act in concert during neurodevelopmental windows to produce either common or divergent effects on neuronal, molecular and behavioural endophenotypes associated with schizophrenia, mood and anxiety disorders.

Future directions

As highlighted in this review, the use of translational animal models to model neurodevelopmental impacts of recreational drug exposure can provide a level of mechanistic insight not available with traditional epidemiological/clinical approaches in human populations. While animal models cannot completely capture the overwhelming complexity found in diseases such as schizophrenia and/or mood/anxiety disorders, the careful targeting of common affective or cognitive endophenotypes modelled in select animal-based behavioural assays can offer a window into a broad range of symptom clusters that may not be adequately captured in broad-based, retrospective studies examining the historical exposure to drugs during adolescent brain development.

How can the translational utility of rodent studies to human research be improved? In general, designing animal models and study designs that work backwards from the existing clinical literature can be a useful approach for neurodevelopmental translational research. The translational studies highlighted in this review examining the impact of adolescent cannabis exposure on select neural and genetic biomarkers linked to schizophrenia risk, are a good example of this approach, as in many cases, these rodent studies have been able to mechanistically verify the roles of several of these neuropathological markers in the context of adolescent THC exposure and schizophrenia-like endophenotypes.

To improve translatability, future pre-clinical research might aim to more specifically target clinically identified genetic and/or protein substrates known to be biomarkers for increased vulnerability to the effects of adolescent cannabis or nicotine exposure. Nevertheless, it is important to note that one of the strengths of translational research using animal models is the ability to dig down into underlying molecular signalling and neural mechanisms that would not be feasible (or beyond the scope of detection) in human studies. Thus, while a practical focus on clinical translation should always be a consideration, animal neurodevelopmental models that take more exploratory, proactive approaches to identifying the effects of neurodevelopmental drug exposure on previously unidentified pathways and mechanisms are equally important in moving clinical research forward. Particularly in the context of identifying novel pharmacotherapeutic interventions aimed at either shielding the developing adolescent brain from the deleterious effects of drugs such as cannabis or nicotine or even reversing the long-term impact and neuropsychiatric sequelae that may result following chronic adolescent drug use.

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