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SPECIAL ISSUE

Cannabis use in pregnancy and early life and its consequences: animal models

Miriam Schneider

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Abstract Cannabinoid receptors and their endogenous ligands have been detected from the earliest stages of embryonic development. The endocannabinoid system appears to play essential roles in these early stages for neuronal development and cell survival, although its detailed involvement in fundamental developmental processes such as proliferation, migration and differentiation has not yet been completely understood. Therefore, it is not surprising that manipulations of the endocannabinoid system by cannabinoid exposure during early developmental stages can result in long-lasting neurobehavioural consequences. The present review will summarize the possible residual behavioural effects of cannabinoid administration during pre- and perinatal as well as early postnatal development, derived from animal studies.

Keywords Cannabinoids · Prenatal · Postnatal · CB_1 receptor · Pregnancy · Animal models

Abbreviations

BGS	Brain growth spurt
CBD	Cannabidiol
CPP	Conditioned place preference
CP	CP 55,940
EPM	Elevated plus maze
FST	Forced swim test
GD	Gestational day
PD	Postnatal day
PR	Progressive ratio
THC	Δ^9-Tetrahydrocannabinol
USV	Ultrasonic vocalization
WIN	WIN 55,212-2

M. Schneider (✉)
Department of Psychopharmacology, Central Institute of Mental Health (ZI), J5, 68159 Mannheim, Germany
e-mail: miriam.schneider@zi-mannheim.de

Introduction

There is growing evidence from animal research but also human studies for the existence of specific vulnerable developmental periods during which exogenous manipulations of the endocannabinoid system might lead to deleterious consequences in later life. Beside puberty, which has been indicated a highly susceptible developmental phase [54], stages of early neuronal maturation during pregnancy and early childhood appear to represent additional sensitive periods for cannabinoid exposure. Cannabis preparations are among the illicit drugs most widely abused by pregnant women in Western societies [19]. Ingestion of cannabis derivatives during pregnancy might result in negative consequences for the offspring in later life, since cannabinoids are able to cross the placental barrier during gestation [26] and can also be transferred through maternal milk during lactation [28]. Due to the still maturing blood brain barrier cannabinoids can reach the foetal and neonatal brain in substantial amounts during these developmental periods.

In the past decade more and more information has been gained about the specific role of the endocannnabinoid system during early stages of development. It has been indicated that during early pregnancy successful embryonic passage through the oviduct and implantation into the uterus both require optimal endocannabinoid levels (for review, see [18]). Furthermore, endocannabinoid signalling is crucial for the normal onset of labour and its absence is involved in preterm birth via alterations in ovarian steroid hormones [67]. During foetal life, functional active

cannabinoid CB_1 receptors show an "atypical" distribution pattern compared to adult CB_1 receptor location and play a major role in brain development by regulating neural differentiation and guiding axonal migration and synaptogenesis ([16, 24] see also article by Galve-Roperh et al. in this special issue).

Although cannabis consumption during pregnancy does not produce overt birth defects, with the exception of a moderate reduction in birth weight [69], prenatal exposure to marijuana and hashish might indeed cause subtle long-term neurobehavioural disturbances, evident beyond the infant developmental stage [19]. Human studies, in particular those using retrospective evaluations, have some limitations because of the vast heterogeneity of cannabis intake. Hence, research with laboratory animals offers an important opportunity to collect further knowledge about specific effects of cannabinoids during early stages of development.

Studies using rodent models were able to detect a multitude of lasting neurobiological alterations in the offspring of cannabinoid treated mothers, such as changes in different neuroendocrine and neurotransmitter systems, with the most pronounced effects reported in the dopaminergic system (for detailed review see [16, 17, 40]). More recently, long-term disturbances in glutamatergic signalling and long-term potentiation were also shown [4, 36]. Concomitant with these neurochemical and molecular implications, early cannabinoid exposure has been reported to have an impact on behaviour in later life. The present article will focus on these possible long-lasting behavioural consequences of cannabinoid exposure during pregnancy and early postnatal life that have been observed in animal studies. The review will be restricted to observations obtained from laboratory rats after cannabinoid exposure during pre- and perinatal development as well as the early postnatal period before weaning.

General remarks on cannabinoid exposure during early development in animal studies

The use of animal models has proven effective and very helpful for investigating many drug-related biomedical problems in humans, since clinical research is often limited to epidemiologic and retrospective studies and case reports. Thus, clinical studies might be confounded to some extent by issues in reporting and confirming drug use, concurrent use of other drugs, as well as non-standardized drug intake between users (e.g. different quantities of intake at different times during pregnancy). To be most useful, any animal model must allow the results to be extrapolated with confidence to other species. Unfortunately, dealing with questions related to brain development, is not a trivial task. Although the course of foetal development or drug metabolism in humans do not closely resemble those of laboratory animals [40], the information obtained in valid animal models is still crucial for a better understanding of the underlying neurobiological mechanisms and possible deleterious consequences. However, some issues have to be considered very carefully when cannabinoids are administered during early developmental stages.

Timing of treatment and cannabinoid doses

An important consideration for the validity and significance of the effects of maternal cannabinoid administration obtained from animal studies should be made regarding cannabinoid dosage and especially timing of treatment. Cannabinoids have been shown to act as teratogenic compounds under certain conditions in animal research. Early cannabis administration (i.e. before or during organogenesis) as well as the use of very high cannabinoid doses produce morphological abnormalities that are even comparable to the foetal alcohol syndrome. However, this is a neurotoxic effect with little relation to the activation of the endocannabinoid system [11, 16, 17]. Therefore, for the present review, only studies using moderate cannabinoid doses reflecting human cannabis use were considered.

Disturbances of maternal behaviour and feeding

Especially when long-term effects of prenatal cannabinoid exposure are studied, two further basic methodological aspects must be considered carefully (for review see [1, 40]). It has been shown that it is of high importance to monitor the effects of cannabinoid administration during pregnancy on maternal food intake, weight gain and offspring growth. Owing to the marked reduction in food and water consumption produced by cannabinoids in animals, any effects on postnatal growth and behaviour could be the result of maternal under-nutrition during pregnancy and not in utero drug exposure. There is evidence from both animal and human studies that an early exposure to under-nutrition is frequently associated with low birth weight and alterations of the hypothalamic-pituitary-adrenal axis throughout the lifespan [32]. Therefore, weight gain of treated pups and dams should be monitored very carefully. The second major consideration is that a drug may adversely affect postnatal maternal behaviour and/or lactation performance, which might influence the normal development of the pups. It has been shown for example that pups that were born to drug-naive rats showed impairments in later life when they were fostered to dams that had been treated with marijuana extract (150 mg/kg) throughout pregnancy [3]. Compared to offspring raised by control dams, those raised by marijuana-treated dams weighed significantly less at postnatal days (PD) 7, 14, and 21, experienced significantly greater

postnatal mortality, and reared less in the open field when tested at 28–30 days of age. Thus, even though offspring were not exposed to marijuana during pre- or postnatal development, their growth and behaviour were significantly affected by being raised by mothers that were exposed to cannabis during pregnancy. Notably, the dose of marijuana extract used in this study was quite high, and unfortunately no information is available so far on the consequences of lower cannabinoid doses on maternal behaviour. Acute administration of 10–20 mg/kg Δ^9-tetrahydrocannabinol (THC) to lactating dams was shown to depress nursing and pup-retrieving behaviour, however, tolerance to this effects developed after chronic administration [2]. Therefore, the impact of moderate pre- and perinatal cannabinoid administration on maternal behaviour should definitely be further clarified in future studies.

Neonatal handling stress

Different problems appear when the cannabinoid exposure takes place in neonatal or infantile rats. It has been shown that neonatal handling of rat pups on PD 1 [20] and PD 9 [15] for a short period of only 15 min, increases maternal care immediately upon return to the nest and also increases c-fos activity in different brain regions (e.g. hippocampus, amygdala). Handling-evoked augmentation of maternal care of pups results in long-lasting reduction of hypothalamic corticotropin releasing hormone expression and upregulates hippocampal glucocorticoid receptor levels, which promotes lifelong alteration of stress responses [15]. Neonatal handled pups also show some behavioural differences in later life compared to unhandled controls [e.g. 60, 62]. Therefore, even though the cannabinoid injection takes less then 15 min, it should be taken into consideration that this short but possible stressful interaction with the rat pup during postnatal development might already induce behavioural changes independent from cannabinoid-specific effects or might even mask behavioural alterations induced by cannabinoid administration. Non-injected, naive control groups might be helpful to completely dissect the possible behavioural consequences of early handling stress and those related specifically to cannabinoid exposure.

Cannabis versus THC

It is important to note that the effects of cannabis preparations (hashish or marijuana) cannot be considered completely equivalent to those observed after the administration of natural cannabinoids alone (e.g. THC, cannabidiol (CBD) or cannabinol) or even synthetic cannabinoid receptor agonists (e.g. WIN 55,212-2, CP 55,940). Cannabis contains beside THC over 70 different natural cannabinoids and many other chemicals that might contribute to some of the developmental effects observed, or that might even help to prevent some deleterious cannabinoid effects. The relative proportion among various cannabinoids which may have antagonistic or synergistic actions and the action of other non-cannabinoid ingredients present in cannabis extracts should be considered because they might contribute to some of the biological effects [34, 40]. In particular, the content of CBD in cannabis preparations, i.e. the ratio of CBD and THC, might be of specific interest, since the neuroprotective, as well as anti-psychotic and anxiolytic properties of this cannabinoid have been shown in various studies [e.g. 23, 35, 68] and might therefore counteract, to some extent, possible adverse consequences of cannabis exposure.

However, the composition of cannabis preparations (e.g. THC and CBD contents) is highly variable depending on regional factors as well as origin and preparation form (e.g. "sinsemilla", cannabis resin etc.), and therefore, no laboratory mixture will ever completely reflect the cannabinoid compositions ingested by different human cannabis users [47, 49]. Furthermore, by the use of natural cannabinoids alone or in combination and even by the application of synthetic cannabinoids, distinct information can be gained about the precise effects on CB1 receptor stimulation during specific phases of development. Therefore, studies using different cannabinoid agonists are crucial and absolutely necessary for a better understanding of the specific role of the endocannabinoid system during vulnerable developmental periods.

Behavioural consequences of pre- and perinatal cannabinoid exposure

Studies investigating possible deleterious effects of cannabinoids during pregnancy are not only extremely important from a general health perspective, but do also provide essential information on the importance and involvement of the endocannabinoid system during these early brain development processes.

A complete overview over the long-term consequences of pre- and perinatal cannabinoid exposure is given in Table 1. In order to exclude behavioural alterations due to possible teratogenic effects, only studies using moderate doses of cannabinoids (e.g. doses lower then 10 mg/kg of THC, or comparable doses of synthetic cannabinoid agonists) were included in the present review.

Pre- and perinatal cannabinoid treatment was found to have subtle effects on locomotor behaviour during later life. Especially the ontogeny of motor behaviours seems to be affected [36, 41] and was mainly characterized by an increase in locomotor activity during early postnatal ages. Long-lasting effects were found to be highly sex-specific.

Table 1 Long-term behavioural consequences of pre- and perinatal cannabinoid exposure

Treatment	Treatment period	Behavioural effects caused by pre- or perinatal cannabinoid exposure	References
Locomotor behaviour and sensorimotor gating			
THC 5 mg/kg (p.o.)	GD 5–PD 24	Altered behaviour in an open field (increased rearing, sniffing, grooming) (M and F); increased locomotor activity (F) (>PD 70)	Rubio et al. [53]
THC 5 mg/kg (p.o.)	GD 5–PD 24	Ontogeny of spontaneous locomotor activity was affected (F and M) (PD 15–PD 70); increased activity most evident at preweanling ages and also in adult females (PD 70)	Navarro et al. [41]
WIN 0.5 mg/kg (s.c.)	GD 5–GD 20	Hyperactivity at PD 12 and PD 40 (M)	Mereu et al. [36]
THC 0.1, 0.5, 2 mg/kg (p.o.)	GD 5–PD 24	Alterations in locomotor habituation and exploratory activity (F); biphasic and sex-specific effects on spontaneous locomotor activity (PD 70)	Moreno et al. [39]
WIN 0.5 and 1 mg/kg (s.c.)	GD 5–GD 20	No effects on startle magnitude and prepulse inhibition on different postnatal ages (PD 40, 60 or 80) (M)	Bortolato et al. [8]
Emotional behaviour			
THC 5 mg/kg (p.o.)	GD 5–PD 24	Increased open arm entries and open arm time in the EPM (>PD 70) (M)	Rubio et al. [53]
THC 2.5 and 5 mg (p.o.)	GD 15–PD 9	Highest THC dose increased separation-induced ultrasonic vocalization of rat pups (PD 12), inhibited social interaction and play behaviour (PD 35) and increased anxiety-related behaviour in the EPM (PD 80) (M)	Trezza et al. [63]
WIN 0.5 mg/kg	GD 5–GD 20	Decrease in separation-induced ultrasonic vocalization (PD10) (M)	Antonelli et al. [4]
THC 2 mg/kg (s.c.)	GD 1–GD 22 PD 2–PD 10	Decreased center time in the open field (PD 90) (M)	Newsom and Kelly [42]
THC 5 mg/kg (p.o.)	GD 5–PD 24	Increased emergence latency in the dark-light emergence test (F); increased self-grooming under novelty conditions (F and M) (PD 70)	Navarro et al. [41]
Cognitive performance			
Cannabis resin (~55% THC), 4.2 mg/kg (i.p.)	GD 2–GD 6	Learning deficits in the morris water maze (PD 22); but: significant lower bodyweight compared to controls (sex not indicated; presumably M)	Kawash et al. [29]
THC 5 mg/kg (p.o.)	GD 15–PD 9	Long-term memory impairment in the inhibitory avoidance test and impaired olfactory short-term memory in the social discrimination task (PD 80) (M)	Campolongo et al. [10]
WIN 0.5 mg/kg (s.c.)	GD 5–GD 20	Poorer performance in homing behaviour (simple form of learning) (PD10-12), and impaired active avoidance performance (PD 80) (M)	Antonelli et al. [4]
WIN 0.5 mg/kg (s.c.)	GD 5–GD 20	Disruption of memory retention in the passive avoidance task (PD 40 and PD 80) (M)	Mereu et al. [36]
Sensitivity towards drugs of abuse			
THC 5 mg/kg (p.o.)	GD 5–PD 24	Enhanced sensitivity towards the rewarding effects of morphine in a CPP paradigm (F and M) (>PD 70)	Rubio et al. [53]
THC 5 mg/kg (p.o.)	GD 5–PD 24	Tolerance to analgesic effects of morphine in adulthood (PD 75); naloxone-induced withdrawal symptoms in weaned pups (all effects specific for M)	Vela et al. [64]
THC 1, 5 mg/kg (p.o.)	GD 5–PD 24	Increased response to moderate doses of morphine (CPP) (M and F)	Rubio et al. [52]

Table 1 continued

Treatment	Treatment period	Behavioural effects caused by pre- or perinatal cannabinoid exposure	References
THC 5 mg/kg (p.o.)	GD 5–PD 24	Increased rate of acquisition of morphine self-administration under a fixed ratio schedule (F) in adulthood (~PD 70); no effect in M	Vela et al. [65]
THC 0.15 mg/kg (i.v.)	GD 15–PD 2	No differences in basal heroine self-administration; however, shorter latency to the first active lever press, higher responding to lower doses of heroin, increased rates of responding following mild stress and higher levels of heroin-seeking during drug extinction (PD 62) (M)	Spano et al. [61]
THC 5 mg/kg (p.o.)	GD 5–PD 24	No effect on food or morphine self-administration in a progressive ratio schedule in adulthood (F and M)	Gonzalez et al. [22]
THC 5 mg/kg (p.o.)	GD 15–PD 9	No effects on ethanol self-administration (~PD 60) (M)	Economidou et al. [13]

THC: Δ^9-Tetrahydrocannabinol; WIN: WIN 55,212-2; *GD* gestational day, *PD* postnatal day, EPM: *EPM* elevated plus maze, *CPP* conditioned place preference, *M* male, *F* female

Hyperactivity as well as alterations in locomotor habituation and exploratory behaviour in adulthood were most pronounced in female rats [39, 41, 53], although some marginal behavioural alterations were also reported in males [53]. No lasting effects were found on startle magnitude and sensorimotor gating in male rats on different ages tested [8].

In addition, lasting consequences of cannabinoid exposure during pregnancy on emotional behaviour have been reported, although most of the findings are contradictory. During early postnatal development, cannabinoid treatment in different studies induced both, an increase in separation-induced ultrasonic vocalization (USV) in rat pups [63], which would indicate an increase in anxiety-related behaviour, and also a reduction in USV [4], indicating the opposite. Similarly, in adulthood, cannabinoid pre-treated rats were shown to display anxiolytic effects [41, 53], whereas other studies reported an increase in anxiety-related behaviours [42, 63]. Cannabinoids are well known to have biphasic effects on emotional behaviour, depending inter alia on dosage and testing conditions [38]. Therefore, the high variability in treatment protocols (e.g. timing of treatment, choice of cannabinoid agonists) might account for behavioural differences observed after pre- and perinatal cannabinoid treatment. Thus, further studies are needed to clarify the residual consequences on emotional behaviour.

The most striking consequences of pre- and perinatal cannabinoid exposure have been reported regarding lasting cognitive impairments as well as an altered sensitivity towards drugs of abuse. Deficits in learning and memory have been found in pre- and perinatal cannabinoid treated offspring throughout the early postnatal period and adolescence [4, 29, 36], and those impairments persisted into adulthood [4, 10, 36]. Effects of cannabinoid pre-exposure include disturbances in learning capacity, short- and long-term memory retrieval as well as memory retention. Sex-specific effects on cognitive performance have not been reported so far (studies were done with male animals only).

Finally, pre- and perinatal cannabinoid administration has a clear impact on drug-sensitivity in later life, which seems to be highly sex-specific. Especially, the reactivity towards morphine at different postnatal ages has been thoroughly investigated. A naloxone injection to cannabinoid-exposed rats shortly after weaning resulted in the development of an opioid-like withdrawal syndrome [64]. This effect was specific to male animals and was not detected in females. In adulthood, animals of both sexes show enhanced sensitivity towards the rewarding effects of morphine in a conditioned place preference paradigm (CPP) [52, 53], although one study found this effect to be more pronounced in males [52]. Only adult male animals were also shown to display tolerance against the analgesic effects of morphine [64].

For morphine self-administration, an increased acquisition rate was detected in cannabinoid-treated adult female rats, but not in males [65]. However, a different study on heroine self-administration using male young adult rats, reported shorter latencies to first lever press, higher responding to lower doses, increased responding after mild stress, as well as higher levels of heroine-seeking during extinction, although no differences were detected on basal self-administration [61] (females were not tested). In addition, no effects of perinatal cannabinoid treatment were found in both sexes on food and morphine self-

administration, when a progressive ratio schedule was used [22]. Interestingly, the alterations in drug sensitivity seem to be particularly distinct for opioids, since cannabinoid exposure during pregnancy did not affect ethanol self-administration in young adult rats [13]. Unfortunately, the sensitivity towards other drugs of abuse, such as psychostimulants or cannabinoids, has not been investigated so far. Despite the sometimes inconsistent findings, especially regarding sex-specific effects on drug sensitivity, it seems obvious that early cannabinoid exposure has an impact on drug effects in later life and might therefore increase addiction vulnerability.

Taken together, the findings reported from different studies on the behavioural consequences of pre- and perinatal cannabinoid exposure for the offspring in later life, do add up to a very heterogeneous picture. Although it seems obvious that cannabinoid treatment during pregnancy does induce more than only subtle behavioural changes in adulthood, which seems to be most clear for disturbances of mnemonic processing, more studies are needed to estimate the extent of the actual risk for lasting perturbations. One major problem which is compromising the comparability of different studies, is the high variability of cannabinoid treatment protocols used (e.g. different doses, different ligands), with the inconsistency of treatment range and duration as the most confounding variables. For example, prenatal treatment onset can vary between gestational day (GD) 1 or 2, up to GD 15 in different studies (see Table 1). In addition, a direct comparison of prenatal and perinatal cannabinoid treatment is quite difficult, since a completely different developmental period is involved if cannabinoids are administered during early postnatal ages, as it will be discussed in the next chapter. In conclusion, studies that will clearly dissect the role of the endocannabinoid system and the vulnerability of different treatment periods for cannabinoid treatment during gestational and neonatal periods are highly needed for the future.

Behavioural consequences of neonatal and early postnatal cannabinoid exposure

Only very few animal studies have so far investigated the effects of cannabis administration exclusively during the early postnatal stages of development. The neonatal period, reaching from birth until PD 7, and the infantile period before weaning (PD 8 until ~PD 21) [46] are considered important since they are coinciding with a highly important period of brain development—the brain growth spurt (BGS). The BGS is a period during which the brain grows at an accelerated rate and it is characterized by a series of rapid fundamental developmental changes, such as maturation of dendritic and axonal outgrowth, the establishment of neural connections, and synaptogenesis and proliferation of glial cells with accompanying myelinization. In addition, most of the different neurotransmitter systems undergo qualitative and quantitative changes during this developmental period [12, 30, 66]. This is also the period when animals acquire many new motor and sensory abilities [7] and when spontaneous motor behaviour peaks [9].

In mammals, the period of BGS varies from species to species. In humans, it begins during the third trimester of pregnancy and continues throughout the first 2 years of life. The rat is born very immature, at an age equivalent to 150 days of human gestational life [46]. Therefore, the BGS in rodents is postnatal, spanning the first 3–4 weeks of life, reaching its peak around PD 7 [12]. Hence, considering these neurodevelopmental changes during the early postnatal period, cannabinoid administration during this specific time span is most likely to produce long-lasting effects on the central nervous system [43].

The behavioural consequences observed after cannabinoid exposure during the neonatal and infantile period are summarized in Table 2. Short-term neonatal THC exposure was found to alter pain sensitivity of adult animals in later life [31, 37]. Furthermore, chronic cannabinoid treatment from PD 4 to PD 14 induced learning impairments and was found to increase the rewarding effects of morphine in late puberty [43, 59]. A similar chronic treatment from PD 4 to 25 disrupted short-term memory and social interaction during puberty, but did not affect emotional behaviour [44]. Remarkably, a single injection of a very low dose of the synthetic cannabinoid agonist WIN 55,212-2 (0.1 mg/kg) on PD 10—near the peak of BGS in rodents [12]—was able to induce lasting behavioural changes in later life [33]. Treated animals showed a reduction in locomotor and exploratory activity, and also an increase in anxiety-related and depressive-like behaviour during early puberty 24 days later.

During the periods of BGS, blockade of the NMDA receptor for a period of hours has been found to trigger widespread apoptotic neurodegeneration in the rodent brain. NMDA antagonists are most effective in inducing apoptosis in the rat forebrain on PD 7. Thus, during this developmental period survival of NMDA receptor-bearing neurons is depending on glutamatergic input being regulated within narrow bounds [27]. Cannabinoid agonist are well known to affect glutamatergic signalling [21, 25], and therefore, cannabinoid induced alterations in glutamate levels might contribute to the lasting effects observed after one or only few cannabinoid injections during this specific vulnerable period.

Finally, we could show that even chronic cannabinoid treatment at the end of the infantile and during the juvenile period (PD 15–PD 40) induced residual, albeit subtle, effects in adulthood [55]. Treated rats showed deficient sensorimotor gating and higher anxiety-related behaviour. However, no lasting effects on short-term memory processing and

Table 2 Long-term behavioural consequences of neonatal and early postnatal cannabinoid exposure

Treatment	Treatment period	Behavioural effects caused by early postnatal cannabinoid exposure	References
THC 2 mg/kg (s.c.)	PD 1–PD 4	Higher pain sensitivity (lower baseline tail-flick values) at PD 50 but not PD 87; increased pain sensitivity following morphine administration (PD 87) (M)	Kumar et al. [31]
THC 10 mg/kg (s.c.)	PD 4, 6 and 8	No effect on acute stress-induced analgesia, but delayed development of conditioned analgesia after stress exposure; no effect on habituation to a novel environment (PD 129) (M)	Mokler et al. [37]
THC 5 mg/kg (s.c.)	PD 4–PD 14	Impaired learning in a Y-maze test (delayed alternation task) during late puberty (PD 56); no effects in a spatial discrimination task (M)	O'Shea and Mallet [43]
THC 5 mg/kg (i.p.)	PD 4–PD 14	Enhanced sensitivity towards the rewarding effects of morphine in a CPP paradigm ($\sim$ PD 56) (M)	Sigh et al. [59]
WIN 0.1 mg/kg (s.c.)	PD 10	Reduced locomotor and exploratory activity in the holeboard, reduced percentage open arm time and entries in the EPM (M), depressive-like behaviour in the FST (F) (PD 34-36)	Llorente et al. [33]
CP, increasing doses: 0.15, 0.20 and 0.30 mg/kg	PD 4–PD 25	Impaired object recognition (PD 53) and reduction in social interaction (PD 55); no effect in the light/dark emergence test (PD 57) (M)	O'Shea et al. [44]
WIN 1.2 mg/kg (i.p.)	PD 15–PD 40	Impaired prepulse inhibition, reduced number of rearings and center time in the open field (>PD 75); no effects on object recognition memory, PR performance and locomotor activity (M)	Schneider et al. [55]

THC: Δ^9-Tetrahydrocannabinol; *CP* CP 55,940, *WIN* WIN 55,212-2, *PD* postnatal day, *EPM* elevated plus maze, *FST* forced swim test, *CPP* conditioned place preference, *PR* progressive ratio, *F* Female, *M* male

locomotor activity in general were detected. Especially the finding of an undisturbed object recognition memory capability, which has been found to be affected by cannabinoid exposure during an earlier age [44], seems to point towards a heighten susceptibility of the neonatal rat brain to cannabinoid exposure in comparison to later postnatal development during the infantile and juvenile developmental periods. In particular, the finding of long-lasting alterations after a single cannabinoid administration on PD 10 emphasizes the specific vulnerability to cannabinoid exposure at the neonatal time period around the peak of the BGS.

Behavioural consequences of pubertal cannabinoid exposure

The effects of acute and chronic cannabinoid exposure during pubertal maturation have been reviewed in detail elsewhere [54]. Therefore, only a short overview of the most important effects will be provided here for a direct comparison with the consequences of earlier cannabinoid exposure. The time span of puberty reaches in female rats from around PD 28 to $\sim$PD 40 (onset indicated by vaginal opening), and from PD 40 to PD 60 in males (indicated by balanopreputial separation) [54]. Studies reporting persistent consequences of chronic cannabinoid exposure during the time period of pubertal maturation are summarized in Table 3.

Concluding remarks

The endocannabinoid system is present and functional in the central nervous system from early stages of brain development on and plays an important role in brain organization during pre- and postnatal life. The studies reviewed in this article point out the high vulnerability of the endocannabinoid system to exogenous manipulations during these early stages of development. Beside the pubertal period, which has been indicated before as highly susceptible for cannabinoid administration [54], the immature brain seems to be specifically vulnerable to cannabinoid exposure during foetal development and the early neonatal and infantile periods.

The administration of cannabinoids during early development, at physiological relevant doses, has been reported to result in several persistent behavioural consequences, mainly affecting mnemonic processing and drug sensitivity, and also to some extent emotional behaviour and the development of locomotor activity. Interestingly, many behavioural effects of pre- and perinatal cannabinoid exposure seem to be highly sex-related, and more studies using both sexes, especially during postnatal development, are needed for a further detailed clarification of sex-specific effects. An additional problem results from the high heterogeneity of different developmental cannabinoid studies. Therefore, in future research more comparative studies are

Table 3 Long-term behavioural consequences of pubertal cannabinoid exposure

Treatment	Treatment period	Lasting behavioural effects caused by pubertal cannabinoid exposure	References
WIN 1.2 mg/kg (i.p.)	PD 40–PD 65 (puberty)	Deficient PPI, impaired object recognition memory, reduced break point in a PR test for food reward, no effects on general locomotor activity (M); testing took place between PD 75 and PD 150	Schneider and Koch [56]
WIN 1.2 mg/kg (i.p.)	PD 40–PD 65 (puberty)	Alterations in social and play fighting behaviour, reduced center time in the open field (M); >PD 75	Schneider and Koch [57]
WIN 1.2 mg/kg (i.p.)	PD 40–PD 65 (puberty)	Impaired object and social recognition memory, lasting disturbances in social behaviour, social play and self grooming (M); >PD 75	Schneider et al. [58]
CP 150, 200, 300 μg/kg (i.p) (increasing injections for 3, 8 and 10 days, respectively	PD 30–PD 50 (puberty)	Impaired object recognition memory and reduced social interaction (F); >PD 70	O'Shea et al. [45]
CP increasing doses 0.15, 0.20, 0.30 mg/kg (i.p.)	PD 30–PD 50 (prepubertal period/early puberty) and PD 56–PD 76 (late puberty)	Impaired object recognition and reduced social interaction (M); >P 78/104	O'Shea et al. [44]
THC 5 mg/kg (i.p.)	PD 32–PD 55 (prepubertal period/early puberty)	Impaired object recognition and reduced social interaction (M); >PD 67	Quinn et al. [50]
WIN 2, 4, 8 mg/kg (i.p.), injections twice per day for three days	Injections starting between PD 35/PD 42 (prepubertal period/early puberty)	Long-lasting tolerance to cannabinoids, morphine, cocaine and amphetamine (M); testing took place 14 days after treatment	Pistis et al. [48]
THC 1.5 mg/kg (i.p.)	PD 28–PD 49 (1 injection every third day) (prepubertal period/early puberty)	Enhanced heroin self-administration (M); >PD 57	Ellgren et al. [14]
CP 0.4 mg/kg (i.p)	PD 35–PD 45 (prepubertal period/early puberty in M; late puberty in F)	Less activity in the holeboard test (F), decreased anxiety-related behaviour in the EPM (F and M), >PD 75	Biscaia et al. [6]
CP 0.4 mg/kg (i.p.)	PD 35–PD 45 (prepubertal period/early puberty in M; late puberty in F)	Higher morphine self-administration rates under a FR1, but not under a PR schedule (M), no effects on self-administration (F), >PD 70	Biscaia et al. [5]
THC 2, 5, 10 mg/kg (i.p.) increasing doses; injections twice per day	PD 35–PD 45 (prepubertal period/early puberty in M; late puberty in F)	No effects on anxiety-related behaviour (elevated plus maze and open-field) but 'behavioural despair' response in the FST and reduced sucrose preference (F); no such effects in M; >PD 75	Rubino et al. [51]

THC: Δ^9-Tetrahydrocannabinol; *CP* CP 55,940, *WIN* WIN 55,212-2, *PD* postnatal day, *EPM* elevated plus maze, *FST* forced swim test, *PR* progressive ratio, *F* Female, *M* male

needed, which directly compare lasting effects of a cannabinoid treatment during different restricted developmental periods (e.g. prenatal, perinatal and neonatal). Such studies would be highly important, since they would offer a more precise delineation of vulnerable time windows for cannabinoid exposure during early development. Nevertheless, the results described in this review point out that lasting behavioural alteration are an assumable consequence of cannabis exposure during early neurodevelopment.

These findings are of high importance given the potential therapeutic application of cannabinoid-based drugs in children (e.g. cancer chemotherapy, ADHD etc.). Although, side effects of cannabinoid treatment have been reported to be minor when treatment took place in children between 3 and 14 years (for review see [18]), the possible long-lasting consequences of cannabinoid exposure during early postnatal development are not yet sufficiently investigated. Therefore, cannabinoid treatment during childhood would require a much better knowledge of the effects of these compounds on the brain of immature individuals.

In conclusion, although our knowledge of the detailed long-term consequences of maternal cannabinoid exposure is still incomplete, the anticipated effects might be more severe then previously expected, especially regarding

possible lasting cognitive deficits or increased addiction vulnerability. Therefore, further research is urgently needed to estimate the precise risk of cannabis intake during pregnancy, to clearly define specifically sensitive periods during early maturation and also to shed light on the underlying neurobiological mechanisms.

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