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Chronic Pubertal, but not Adult Chronic Cannabinoid Treatment Impairs Sensorimotor Gating, Recognition Memory, and the Performance in a Progressive Ratio Task in Adult Rats

Miriam Schneider*[,1] and Michael Koch[1]

[1]*Brain Research Institute, Department of Neuropharmacology, University of Bremen, Bremen, Germany*

There is evidence from studies in humans and animals that a vulnerable period for chronic cannabinoid administration exists during certain phases of development. The present study tested the hypothesis that long-lasting interference of cannabinoids with the developing endogenous cannabinoid system during puberty causes persistent behavioral alterations in adult rats. Chronic treatment with the synthetic cannabinoid agonist WIN 55,212-2 (WIN) (1.2 mg/kg) or vehicle was extended over 25 days either throughout the rats' puberty or for a similar time period in adult rats. The rats received 20 injections intraperitoneally (i.p.), which were not delivered regularly. Adult rats were tested for object recognition memory, performance in a progressive ratio (PR) operant behavior task, locomotor activity, and prepulse inhibition (PPI) of the acoustic startle response (ASR). PPI was significantly disrupted only by chronic peripubertal cannabinoid treatment. This long-lasting PPI deficit was reversed by the acute administration of the dopamine antagonist haloperidol. Furthermore, we found deficits in recognition memory of pubertal-treated rats and these animals showed lower break points in a PR schedule, whereas food preference and locomotion were not affected. Adult chronic cannabinoid treatment had no effect on the behaviors tested. Therefore, we conclude that puberty in rats is a vulnerable period with respect to the adverse effects of cannabinoid treatment. Since PPI deficits, object recognition memory impairments, and anhedonia/avolition are among the endophenotypes of schizophrenia, we propose chronic cannabinoid administration during pubertal development as an animal model for some aspects of the etiology of schizophrenia.

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INTRODUCTION

Cannabis is the most widely used illicit drug after nicotine and alcohol (Watson *et al*, 2000; Ehrenreich *et al*, 1999). This popularity may result from the fact that the derivatives of the hemp plant marijuana and hashish are widely tolerated and often considered as relatively harmless. Regular intake of cannabinoids by adolescents, however, has been associated with disruptive effects on family life, social integration, job motivation, and also job stability (Bourque *et al*, 1991; Kandel *et al*, 1986; Ehrenreich *et al*, 1999).

*Correspondence: Dr M Schneider, Brain Research Institute, Department of Neuropharmacology, University of Bremen, PO Box 33 04 40, 28334 Bremen, Germany, Tel: +42 121 872 89,
E-mail: miriam.schneider@uni-bremen.de
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There is evidence from studies in humans and animals that a vulnerable period exists during certain phases of development, in particular during puberty, where persistent brain and behavioral alterations are induced by chronic cannabinoid administration. It has been shown in rats that chronic exposure of immature animals to Δ^9-THC caused more irreversible residual effects on different behaviors than chronic treatment of mature rats (Stiglick and Kalant, 1985). However, treatment periods in this study were relatively long (3–6 months) so that it remained difficult to isolate the specific vulnerable period in detail. Furthermore, a behavioral study in humans showed attentional deficits in adults only after cannabis abuse in early-onset users (onset before age 16) (Ehrenreich *et al*, 1999). These data indicate that vulnerable periods during human brain development exist up to the age of 16 years, during which cannabis use permanently impairs attentional functions. These results suggest that immature organisms may be more susceptible to the physiological effects of chronic cannabinoid administration in a certain vulnerable period than mature organisms.

Cannabinoid receptor density varies during developmental stages, specifically during puberty (Rodriguez de Fonseca *et al*, 1993). Additionally, cannabinoid receptors undergo downregulation after long-term cannabis abuse (Breivogel *et al*, 1999) and irreversible effects on brain morphology, for example, in the hippocampus, have been reported in response to chronic cannabinoid stimulation in adult rats (Landfield *et al*, 1988; Lawston *et al*, 2000).

Recent evidences suggested a connection between cannabis use and schizophrenia. Acute cannabis use can induce attentional deficits in humans similar to those observed in acute schizophrenia and is associated with schizotypal personality (Skosnik *et al*, 2001). Several reports have shown that cannabis consumption can induce schizophrenic-like symptoms in healthy individuals (Schneider *et al*, 1998; Emrich *et al*, 1997; Leweke *et al*, 1999b). Preliminary results showed that the intravenous administration of Δ^9-THC leads to the induction or worsening of psychotic symptoms among healthy volunteers and also in schizophrenic patients (D'Souza *et al*, 2000). A recent SPECT single-case study on the effects of cannabis use on a drug-free schizophrenic patient revealed that shortly after cannabis ingestion, this person developed positive psychotic symptoms (Voruganti *et al*, 2001). There is also evidence from follow-up studies, which showed that schizophrenic patients with previous cannabis abuse had significantly more rehospitalizations, tended to display worse psychosocial functioning, and scored significantly higher on psychopathological syndromes such as thought disturbance and hostility (Caspari, 1999). Furthermore, recent studies suggested that pubertal cannabis consumption may precipitate schizophrenia in vulnerable individuals (Hambrecht and Häfner, 2000; Hall and Degenhardt, 2000). These results indicate that cannabis abuse has an important impact on the long-term outcome of schizophrenic patients.

Recent findings even suggested that a dysregulation of the endogenous cannabinoid system may be associated with the pathogenesis of schizophrenia (Emrich *et al*, 1997; Leweke *et al*, 1999a; Dean *et al*, 2001). The concentrations in the cerebrospinal fluid of two endogenous cannabinoids, anandamide and palmitylethanolamide, are significantly higher in schizophrenic patients compared to nonschizophrenic controls (Leweke *et al*, 1999a). Furthermore, Dean *et al* (2001) found post-mortem changes in the density of CB1 receptors in the dorsolateral prefrontal cortex of schizophrenic patients, independent of recent cannabis ingestion.

The aim of the present study in rats was to investigate the long-term effects on sensorimotor gating, mnemonic processes, and on the performance in an operant behavior task of peripubertal or postpubertal chronic treatment with the synthetic cannabinoid full agonist WIN 55,212-2 (WIN). We chose this aminoalkylindole, because of its high cannabinoid receptor affinity and its superior solubility compared with Δ^9-THC (Lawston *et al*, 2000).

Prepulse inhibition (PPI) is the natural reduction in the magnitude of the acoustic startle response (ASR) if a weaker, nonstartling prestimulus is presented 30–500 ms before the startling stimulus. PPI is thought to be a preattentive filter mechanism, which is regulated by a cortico-limbic-striatopallidal circuit where dopamine, glutamate, and serotonin are the most important regulatory transmitters (Koch, 1999; Geyer *et al*, 2001). We have previously demonstrated that the synthetic cannabinoid WIN induces a PPI deficit in adult rats, when administered directly before testing (Schneider and Koch, 2002). Since PPI is disrupted in schizophrenic patients, animal models of PPI offer the possibility of investigating the neural mechanisms related to some characteristic impairments in schizophrenia (Swerdlow *et al*, 1994). Therefore, in the present study we were interested in the long-term outcome in sensorimotor gating in drug-free rats after chronic cannabinoid treatment during different developmental stages.

The spontaneous object recognition test was chosen for assessing the effects of chronic WIN treatment on short-term memory (Everts and Koolhaas, 1997). Recognition memory is generally regarded as the ability to discriminate the familiarity of things previously encountered (Mumby, 2001). This test is based on the natural tendency of adult rats to investigate novel objects. When exposed to the same object on a second encounter shortly after the first exposure, investigatory behavior is considerably reduced. This drop in interest is absent when the rat is exposed to a different object, or when the interexposure time exceeds 1–2 h. It was shown by Terranova *et al* (1996) that the CB1 receptor antagonist SR141716 facilitates short-term memory in the social recognition test and we previously showed an acute impairment of social and object recognition memory by the cannabinoid agonist WIN (Schneider and Koch, 2002).

Furthermore, object recognition memory is impaired in schizophrenic patients (Doniger *et al*, 2002; Crespo-Facorro *et al*, 2001; Heckers *et al*, 2000) and neuroimaging studies showed altered activation of frontal/limbic regions and the thalamus in schizophrenic patients during recognition memory tasks (Crespo-Facorro *et al*, 2001; Heckers *et al*, 2000). Therefore, object recognition in animals may be a useful test to investigate the pathological basis for memory impairments in schizophrenia.

We also examined the effects of chronic cannabinoid treatment on the performance in an operant behavior task, where the animals had to press a lever for palatable food. In addition to measuring the total food intake or the total number of lever-presses in an operant task, one can also measure the instrumental effort that a rat is willing to invest in order to obtain reward in a progressive ratio (PR) schedule. In this paradigm, rats are first trained to press a lever in order to obtain reward. Once they have reached stable performance, the number of lever presses required for the reward is progressively increased. At a certain degree of instrumental effort, animals cease responding. This 'break point' is considered an operational measure for a shift in motivation, where the rewarding value is lower than the effort that the animal is willing to invest to obtain this reward (Ellenbroek and Cools, 2000). The PR schedule therefore provides a probe to determine a reinforcement value that is independent of response rate. It was shown before that the PR schedule provides a valuable method to assess the influence of manipulations that might affect the perceived reinforcement value of gustatory stimuli (Reilly, 1999), and Ellenbroek and Cools (2000) proposed that a lower performance and therefore a lower break point in this

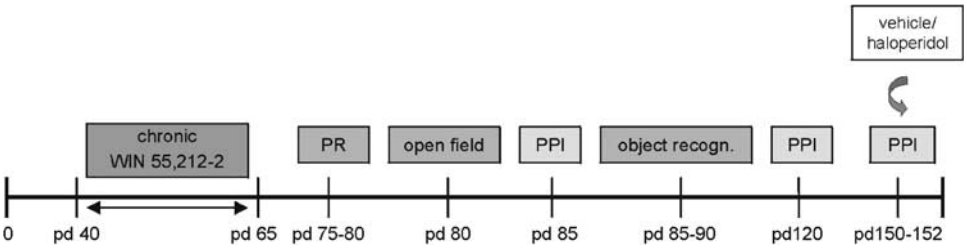

Figure 1 Time schedule of peripubertal chronic cannabinoid treatment and behavioral testing in adult rats.

PR schedule might serve as an animal model for anhedonia, one of the negative symptoms in schizophrenia.

METHODS

Subjects

A total of 47 rats were used in this study. For peripubertal cannabinoid treatment, 27 naive first-generation offspring male Wistar (Hannover) rats from our own breeding colony were used. Adult male and female Wistar (Hannover) rats were imported from Harlan–Winkelmann (Borchen, Germany) and housed together in pairs under standard conditions on a 12-h light–dark schedule (lights on 700–1900). They received free access to tap water and were fed *ad libitum*. After 3 weeks, male rats were removed from the breeding cages. The litters were culled to eight pups directly after birth (all male if possible). In order to avoid litter effects, equal proportions of rats of each litter were assigned to the different treatment groups.

After weaning on postnatal day (pd) 21, male pups were housed in a different room in groups of three to six under standard conditions on a 12-h light–dark schedule (lights on 700–1900). They received free access to tap water and were fed *ad libitum* until pd 40 after reaching a body weight of 180 g. Then they were maintained on a body weight of 250–300 g by restricted feeding of 12 g rodent chow/rat/day (subjects were reduced to approximately 85% of their free-feeding weights).

The control subjects for adult cannabinoid treatment were 20 adult naive male Wistar (Hannover) rats ($>$pd 70). Rats were housed under similar conditions as described above.

The experiments were performed in accordance with the NIH ethical guidelines for the care and use of laboratory animals for experiments, and were approved by the local animal care committee (Senatorische Behörde, Bremen, Germany).

Drugs

WIN 55,212-2 (WIN) (Sigma-Aldrich, Taufkirchen, Germany) was dissolved in 0.1% Tween 80 and diluted in saline (0.9%). The drug was administered intraperitoneally (i.p.) at a dose of 1.2 mg/kg. Haloperidol (Haldol Janssen, Neuss, Germany) was dissolved in saline (0.9%) and was also administered systemically (0.1 mg/kg i.p.) 30 min prior to testing. The injection volumes were 1 ml/kg. The experimenter was not aware of the drug treatment of the animals.

Experimental Design

The peripubertal chronic treatment of either the synthetic cannabinoid WIN or vehicle lasted 25 days from pd 40 to pd 65 throughout the rats' puberty (Figure 1). In this period, the rats received 20 injections i.p.. These injections were not delivered regularly throughout this period. The rats received either one, two each day or no injection at all (10 times one injection, 5 times two injections, and 10 times no injection per day). This protocol was chosen in order to mimic the irregular consumption practice in humans (Lamarque *et al*, 2001). In order to exclude withdrawal effects after chronic treatment, there was a rest period for all animals for 10 days before behavioral testing was started.

PR test was performed between pd 75 and pd 80. PPI was tested on pd 85, on pd 120, and again on pd 150 following an injection of saline and, after a rest period of 1 day, following a single i.p. injection of haloperidol. The object recognition test was conducted between pd 85 and pd 90.

Adult (pd$>$70) chronic cannabinoid treatment was conducted as described above (20 injections over a period of 25 days) and all experiments were performed in a similar period of time as in pubertal-treated rats. The PR test was performed between 10 and 15 days after cannabinoid treatment. PPI was tested on day 20 after the last cannabinoid administration, on day 55, and again on day 85 after chronic treatment and after a single i.p. injection of vehicle/haloperidol. Object recognition was tested between days 20 and 25 after chronic treatment.

Behavioral Testing

PPI of the ASR. PPI was measured using the Startle Response System (TSE, Bad Homburg, Germany). The startle response of the rats was registered using a movement-sensitive measuring platform and transmitted to a personal computer with an analog to digital (AD) converter. During the measurement, the animals were placed in closed wire mesh cages (24.5 cm $\times$ 9 cm $\times$ 10 cm). For acoustic stimulation two loudspeakers were used, mounted on both sides of the test cage at a distance of 4 cm. A white noise pulse was used as the startle stimulus, with an intensity of 100-dB sound pressure level (SPL) and a duration of 20 ms (0 ms rise/fall times); three different pure tones (10 kHz, intensity 64, 68, and 72 dB SPL, duration 20 ms, 0 ms rise/fall times) were used as the prepulses. The background noise level was a white noise of 60 dB SPL.

An acclimatization time of 5 min, during which the rats received no stimulus except the background noise, was followed by presentation of 10 initial startle stimuli. Directly after this habituation program, the test program was started

with seven different trial types presented in a pseudorandom order: (1) trial: pulse alone, (2) trial: control (no stimulus), (3) trial: prepulse alone (72 dB SPL), (4) trial: prepulse alone (68 dB SPL), (5) trial: pulse with preceding prepulse (prepulse 72 dB SPL 100 ms before pulse), (6) trial: pulse with preceding prepulse (prepulse 68 dB SPL 100 ms before pulse), (7) trial: pulse with preceding prepulse (prepulse 64 dB SPL 100 ms before pulse). A total of 10 presentations of each trial type was given with an interstimulus interval randomized between 20 000 and 30 000 ms.

Object recognition. All adult rats were kept individually 6 h prior to testing in small Makrolon cages in which the test was conducted, and they were exposed to three different test settings (1 day between each test session). The main test consisted of an initial 5-min exposure (P1) to an object (mortar bowl), followed by a second exposure (P2) to the same object after a delay of 30 min. Two additional control experiments were performed, in which P1 was followed by the presentation of the same object 120 min later (transparent measuring beaker), or by a second exposure to a different object after 30 min (P1: drinking bottle cover cap, P2: transparent measuring cylinder). All objects were cleaned with 70% alcohol and thoroughly dried 1 h before testing. Sniffing, dragging, pushing, and gnawing of the objects were recorded. The time that the adult rats spent on these behaviors (investigating time) was taken as a measure of object recognition as described before (Schneider and Koch, 2002).

Progressive ratio. The PR test was conducted in an operant chamber ($24 \times 28 \times 28$ cm, Operant Behavior System, TSE, Bad Homburg, Germany). First, subjects were habituated 1 day to the test chamber, the palatable casein pellets, and the magazine response (shaping). After shaping, rats were trained 3 days for lever-pressing in sessions of 30 min on a continuous reinforcement schedule (CRF). After lever-response training was completed, one PR session (for 30 min) was conducted on the subsequent day. The PR schedule was changed every second minute according to the following exponential progression: 1, 2, 4, 6, 9, 12, 15,..., derived from the formula $5.e^{0.2n}-5$, where n is the position in the sequence of ratios (Mobini *et al*, 2000). The so-called 'break point', the conventional index of performance on a PR schedule of reinforcement (Reilly, 1999), was defined as the first PR sequence where lever-presses decreased $\leqslant 50\%$ relative to the previous sequence without increasing $\geqslant 100\%$ in the following sequence.

A preference test was carried out additionally 1 day before lever-press training in order to exclude drug effects on food preference of the animals. For this test, the rats were placed in a standard Makrolon cage and the amount of freely available pellets and lab chow consumed was measured for 10 min. The caloric content of the chow and pellets was similar.

Open field. Locomotor activity was measured in an infrared-beam-operated open field ($44.7 \times 44.7 \times 44$ cm, ActiMot-System, TSE, Bad Homburg, Germany) for 35 min. At the beginning of the test sessions, each rat was placed in the middle of the open field. Rearings and distance traveled (m) were recorded.

Statistical Analysis

PPI was calculated as the percent decrease in the ASR following a startle stimulus preceded by a prepulse ($100 \times$ (mean ASR amplitude on pulse alone trials$-$mean ASR amplitude on prepulse-pulse trials)/mean ASR amplitude on pulse alone trials). The effects of chronic treatment on PPI were evaluated using repeated measures two-way analysis of variance (ANOVA), followed by *post hoc* Tukey's *t*-tests for pairwise comparisons.

Object recognition was calculated as the percent decrease in investigating time from the first presentation (P1) to P2. Student's *t*-tests were used for the evaluation of ASR amplitude (pulse-alone trials), object recognition memory, operant responding in the PR schedule, preference tests, and open field behavior.

A value of $P<0.05$ was considered to represent a significant effect.

RESULTS

Peripubertal Treatment Group

PPI of the ASR. PPI was significantly disrupted by chronic peripubertal cannabinoid treatment on pd 85 (Figure 2a) ($F_{1,40}=12.1$, $P<0.05$, $n=11$), pd 120 (Figure 2b) ($F_{1,32}=15.3$, $P<0.05$, $n=9$), and also on pd 150 (Figure 2c) ($F_{1,24}=22.6$, $P<0.05$, $n=7$) for all three different prepulse intensities. This PPI deficit was reversed by the acute administration of the dopamine antagonist haloperidol after pd 150 (Figure 2d) ($F_{1,24}=2.9$, $P>0.05$, $n=7$). A direct comparison between the WIN and control groups (PPI-intensities lumped together) under saline (pd 150) and under haloperidol (pd 152), respectively, revealed that haloperidol significantly improved PPI in WIN-treated rats ($F_{3,80}=14.4$, $P<0.05$; $n=7$). No significant differences between the ASR amplitudes (pulse-alone trials) of controls and the WIN group were found on any test day (Table 1) ($P>0.05$, Student's *t*-test).

Object recognition. Chronic WIN treatment during puberty impaired spontaneous object recognition in adult rats (Figure 3). WIN pretreated animals showed a significant lower reduction of investigation time from the first exposure (P1) to the second exposure (P2) of the same object carried out 30 min later ($P<0.01$, Student's *t*-test; WIN group, $n=14$; control group, $n=10$).

There were no significant differences in either control experiment in the reduction of investigatory behavior from P1 to P2 between the control and WIN groups (data not shown) ($P>0.05$, Student's *t*-test; WIN and control group, $n=9$).

Progressive ratio. The break point in a PR schedule was significantly reduced in cannabinoid pretreated rats compared to controls (Figure 4) ($P<0.01$, Student's *t*-test; $n=13$).

In a control experiment, the effects of WIN on food preference (casein pellets *vs* lab chow) were tested, but no

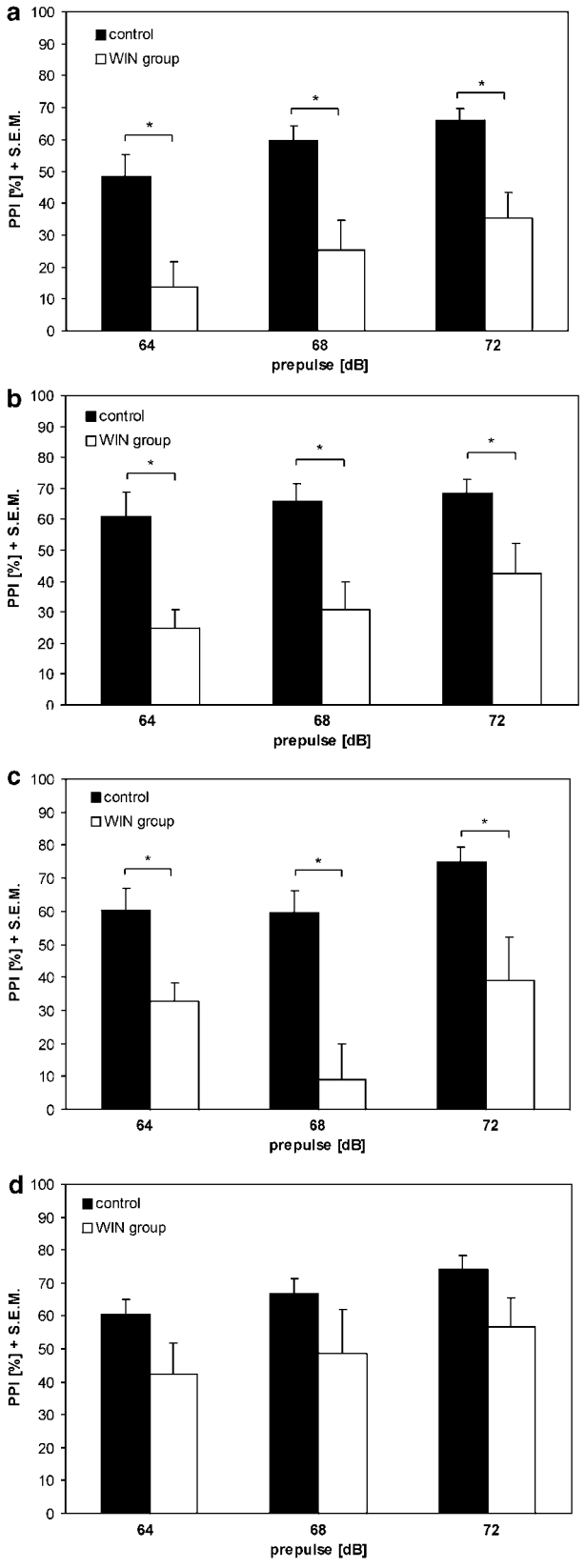

Figure 2 Effects of chronic pubertal WIN administration on mean PPI on pd 85 (a), pd 120 (b), and pd 150 (c), 20, 55, and 85 days after cannabinoid treatment, respectively. PPI was reduced significantly for all three different prepulse intensities (64, 68, and 72 dB) on all test days ($P<0.05$ is indicated by asterisks). No significant reduction of PPI was found on pd 152 (d) after a single acute injection of haloperidol (0.1 mg/kg).

significant differences between the WIN and control groups were observed, that is, both treatment and control groups preferred casein pellets (data not shown) ($P>0.05$, Student's *t*-test; WIN group, $n=11$; control group, $n=10$).

Open field. No differences between controls and WIN pretreated rats in rearings or in distance traveled (m) were found after pubertal treatment in the open field (data not shown) ($P>0.05$, Student's *t*-test; $n=9$).

Adult Treatment Group

PPI of the ASR. Chronic treatment of adult rats with the synthetic cannabinoid WIN had no significant effect on PPI (Figure 5) ($F_{1,36}=0.07$, $P>0.05$, $n=10$) on day 20 after chronic treatment. Since no effect was obtained 20 days after the last cannabinoid administration, no further PPI tests were conducted. There were no significant differences between the ASR amplitudes (pulse-alone trials) of controls and the WIN group (Table 1) ($P>0.05$, Student's *t*-test; $n=10$).

Object recognition. Adult cannabinoid treatment had no effect on object recognition (Figure 6), since there was no significant difference in the reduction of investigation time from P1 to P2 between controls and the WIN group ($P>0.05$, Student's *t*-test; WIN group, $n=10$; control group, $n=9$).

There were also no significant differences in either control experiment in the reduction of investigatory behavior from P1 to P2 between the control and test groups (data not shown) ($P>0.05$, $n=8$).

Progressive ratio. No differences were obtained between controls and WIN pretreated animals in the PR schedule (Figure 7). There were no significant differences in break points for both groups ($P>0.05$, Student's *t*-test; WIN group, $n=9$; control group, $n=8$).

Additionally, no effects on food preference (casein pellets *vs* lab chow) between the WIN and control groups were found on a preference test (data not shown) ($P>0.05$, Student's *t*-test; WIN and control groups, $n=13$).

Open field. No differences in rearings or in distance traveled (m) were found after adult treatment in the open field between controls and WIN pretreated rats (data not shown) ($P>0.05$, Student's *t*-test; WIN group, $n=7$; control group, $n=6$).

DISCUSSION

The present data show that chronic peripubertal, but not adult treatment, with the synthetic full cannabinoid agonist WIN leads to long-lasting deficits in sensorimotor gating, object recognition memory, and the performance in an appetitive instrumental task in adult rats. Therefore, we conclude that puberty in rats is a vulnerable period for the effects of cannabinoids.

Our findings are consistent with results from studies in humans (Ehrenreich *et al*, 1999) and rats (Stiglick and Kalant, 1985). A behavioral study in humans showed

Table 1 ASR Magnitudes in Pulse-Alone Trials

		Pubertal treatment			
	Adult treatment	**pd 85**	**pd 120**	**pd 150**	**Haloperidol**
Control group ± SEM	97.8 (± 23.3)	78.3 (± 7.2)	86.3 (± 18.9)	51.9 (± 11.3)	43.7 (± 11.2)
WIN group ± SEM	106.6 (± 21.8)	76.0 (± 11.8)	98.6 (± 23.1)	59.8 (± 12.4)	56.1 (± 9.9)

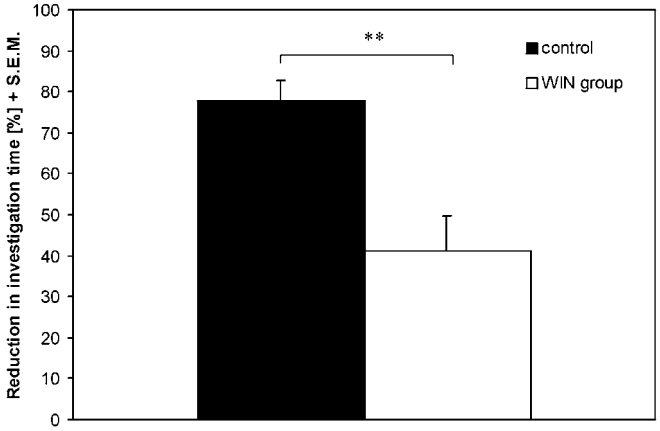

Figure 3 Effects of pubertal WIN treatment on object recognition memory. WIN pretreated animals showed a significant lower percent reduction of investigation time from the first exposure (P1) to the second exposure of the same object carried out 30 min later (P2) ($P<0.01$ is indicated by asterisks).

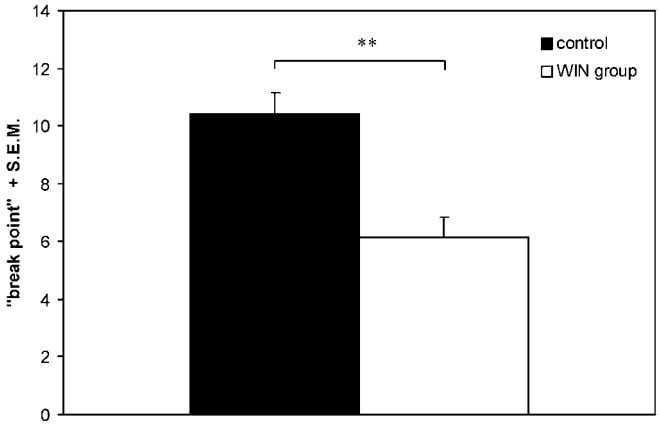

Figure 4 Effects of pubertal cannabinoid treatment on the performance in a PR schedule in adult drug-free rats. The bars indicate the last sequence in which the animals responded according to criterion. WIN pretreated rats showed a significant reduction in break points compared to controls ($P<0.01$ is indicated by asterisks).

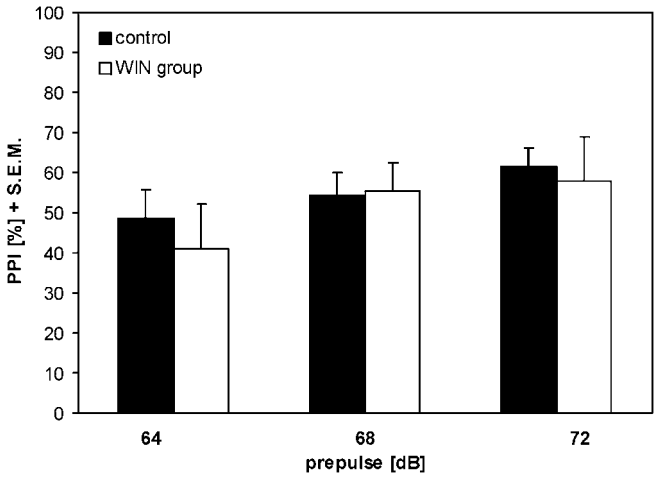

Figure 5 No effects were seen after adult chronic WIN administration on PPI—20 days after chronic treatment. There was no significant difference between WIN pretreated animals and controls.

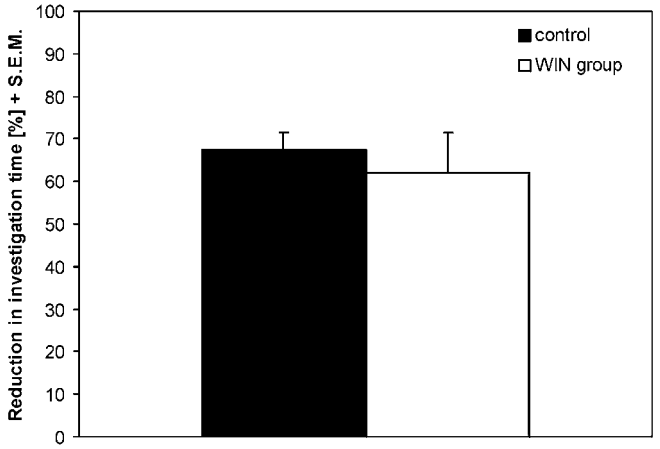

Figure 6 Adult chronic WIN administration had no effect on object recognition memory. There was no significant difference between the WIN group and controls.

attentional deficits in adults after cannabis abuse in early-onset users (onset before age 16) (Ehrenreich *et al*, 1999), suggesting that vulnerable periods exist during human brain development up to the age of 16 years, during which cannabis can permanently compromise attentional functions. Additionally, it has been shown that chronic exposure of immature rats to Δ^9-THC for 3–6 months caused more severe residual effects on behavior than chronic treatment of mature rats (Stiglick and Kalant, 1985).

There are considerable fluctuations in cannabinoid receptor density during development, specifically during puberty (Rodriguez de Fonseca *et al*, 1993). A progressive increase in cannabinoid binding in male rats was found from pd 10 to maximum values between pd 30 for the limbic forebrain and pd 40 for the striatum and the ventral mesencephalon. Binding decreases after pd 40 during puberty until it reaches adult values at about pd 70 (Rodriguez de Fonseca *et al*, 1993). Additionally, it was shown in female rats that the hypothalamic levels of the endocannabinoid anandamide peak immediately before the onset of puberty (Wenger *et al*, 2002). These findings support the contention that the endogenous cannabinoid system seems to be highly susceptible to the effects of

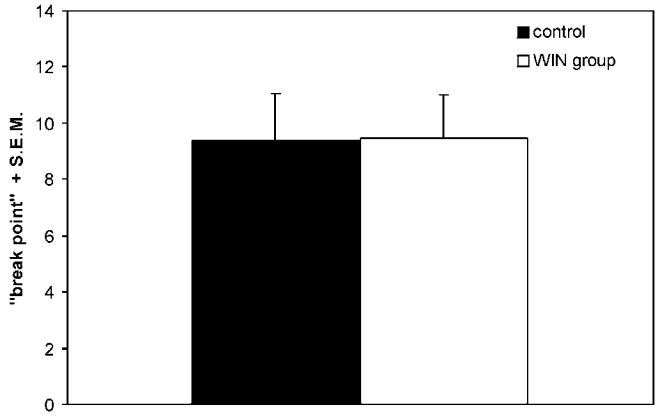

Figure 7 Adult chronic WIN treatment had no effect on the performance in a PR schedule. The bars indicate the last sequence in which the animals responded according to criterion. There was no significant difference in break points between controls and WIN-treated rats.

cannabinoid administration during pubertal development. Furthermore, chronic cannabinoid treatment leads to downregulation and desensitization of brain cannabinoid receptors (Breivogel *et al*, 1999; Rubino *et al*, 2000) and significantly increases cAMP levels and PKA activity (Rubino *et al*, 2000).

Our behavioral assessment of pubertal/adult cannabinoid treatment was performed using several tests. The PPI paradigm was used in this study to test the effects of chronic peripubertal and adult treatment on sensorimotor gating. PPI is the reduction of the ASR magnitude when a weak, nonstartling prestimulus is presented shortly before the startle stimulus, and acute administration of the synthetic cannabinoid WIN significantly disrupted PPI in rats without affecting overall startle magnitude (Schneider and Koch, 2002). This result is largely consistent with other studies showing a PPI deficit induced by the cannabinoid agonist CP 55,940, although in these studies, the startle magnitude was decreased, compromising the interpretation that the PPI deficits reflect a genuine sensorimotor gating disturbance (Mansbach *et al*, 1996; Martin *et al*, 2003), although another recent study showed that CP 55,940 may enhance PPI under certain conditions (Stanley-Cary *et al*, 2002).

In the present study, we observed a long-lasting PPI deficit in drug-free adult rats who were treated during puberty, while adult chronic cannabinoid administration did not affect PPI. Additionally, we found no effect on startle magnitude.

PPI is thought to reflect the activity of a preattentive filter mechanism, which is regulated by a cortico-limbic-striato-pallidal circuit (Koch, 1999; Swerdlow *et al*, 2001). A disruption of this filter mechanism may produce sensory overstimulation and cognitive fragmentation, as well as attentional impairments which are core symptoms of schizophrenia. A disruption in PPI in schizophrenic patients has been reported in various studies (Braff *et al*, 2001; Hamm *et al*, 2001). Dopamine plays a central role in the regulation of PPI, and PPI deficits can be seen after overactivation of the mesoaccumbal dopaminergic system (Geyer *et al*, 2001; Wan and Swerdlow, 1993; Koch, 1999). It is conceivable that cannabinoids affect PPI via the dopaminergic system, since the activation of the dopaminergic system by acute cannabinoid treatment is already well established (French *et al*, 1997; Giuffrida *et al*, 2000), and acute PPI deficits induced by WIN can be reduced by the dopamine antagonist haloperdiol (Schneider and Koch, 2002; Nava *et al*, 2000; Navarro *et al*, 1993). Recently, it has been shown by microdialysis that Δ^9-THC increases the extracellular dopamine concentration preferentially in the shell of the nucleus accumbens (Tanda *et al*, 1997). Furthermore, systemic administration of Δ^9-THC or synthetic cannabinoids produces an increase in spontaneous firing of dopaminergic neurons within the ventral tegmental area (VTA) (French *et al*, 1997; Gessa *et al*, 1998). However, the specific mechanisms mediating these stimulatory effects upon the dopamine system are still unknown. Since cannabinoid receptors are not localized on dopaminergic neurons (Herkenham *et al*, 1991), a direct action of Δ^9-THC is unlikely. One possible mechanism of cannabinoid action in the VTA-nucleus accumbens pathway is a cannabinoid-induced inhibition of GABAergic interneurons within the VTA, which would lead to a disinhibition of dopamine cell firing. An alternative possibility is an interaction with the endogenous opioid system, which is known to modulate dopamine release (Ameri, 1999).

There are only few studies on the effects of *chronic* cannabinoid treatment on the mesolimbic dopaminergic system. Wu and French (2000) observed that following chronic cannabinoid treatment in rats, VTA neurons continued to show a robust increase in firing rate after acute Δ^9-THC stimulation, whereas a reduction in mesolimbic dopaminergic activity was found after adult chronic cannabinoid stimulation using extracellular single unit recordings from mesoaccumbal dopamine neurons (Diana *et al*, 1998). However, in this study chronic treatment lasted only 6.5 days and dopaminergic activity was measured 24 h after the last cannabinoid exposure, whereas in our study the chronic treatment lasted for 25 days and PPI was tested 20 days after chronic treatment in order to avoid withdrawal and carry-over effects. Furthermore, in the Diana *et al* study, chronic cannabinoid administration was carried out in adult rats. However, the findings that adult chronic cannabinoid administration reduces dopaminergic mesolimbic activity rather than enhances it are consistent with our data, since PPI was not affected in the adult treatment group. In our study, behavioral effects were only obtained in peripubertal-treated rats. To our knowledge, there are so far no studies on the long-term effects of chronic pubertal cannabinoid treatment on the dopaminergic system. A common phenomenon in brain development is the overproduction and subsequent pruning of synaptic connections during the period preceding adulthood (Andersen *et al*, 2000). During ontogeny changes in dopamine D1/D2 receptor occurrence in the rat striatum and prefrontal cortex run parallel to the developmental changes in the endogenous cannabinoid system. It has been shown in male rats that D1/D2 receptors increase in density in the first weeks of life and peak at approximately pd 40, followed by a decline by 58–75% from puberty to adulthood (Andersen *et al*, 2000, 2002). Due to these profound maturational processes within the endogenous cannabinoid and dopaminergic systems during puberty, studies on adult treat-

ment offer only limited information about the possible mechanisms underlying the effects of chronic pubertal treatment. However, since the PPI deficit observed in our study induced by chronic peripubertal WIN treatment was reversed by the dopamine D2/D1 antagonist haloperidol (85 days after the chronic treatment), one could consider at least an involvement of persistent alterations in dopaminergic system for this sensorimotor gating deficit.

In order to assess the effects of chronic WIN treatment on short-term memory, the spontaneous object recognition test was chosen, since an acute injection of WIN significantly disrupts social and object recognition memory in rats (Schneider and Koch, 2002). In the crucial experiment—the second exposure to a familiar object after a delay of 30 min—chronic pubertal, but not adult, treatment with the synthetic cannabinoid WIN resulted in a significant impairment of recognition memory in adult rats. In both control experiments (a second presentation of the same object after 120 min, and presentation of different objects within 30 min), no differences were obtained between control and WIN pretreated animals for neither pubertal nor adult chronic administration. These control experiments indicate that WIN selectively impaired recognition memory and that the increase of investigation time in the main experiment is not due to motor effects or enhanced exploratory behavior. This interpretation is further supported by the normal performance in the open field.

Since the recognition test is considered to be a model for short-term memory function (Everts and Koolhaas, 1997), our results suggest that chronic peripubertal treatment with cannabinoid agents induces a long-term deficit in short-term information processing in drug-free rats.

The object recognition deficit may also be due to enhanced dopaminergic activity, since it was shown that metamphetamine administration (Bisagno *et al*, 2002) and also prenatal cocaine exposure (Morrow *et al*, 2002) disrupt recognition memory in rats. Dopaminergic projections, including those to the frontal cortex, ventral hippocampus, and septum, have been proposed to be involved in the performance of short-term memory tasks and elevated dopamine turnover in the prefrontal cortex is associated with poor performance in object recognition tasks and other working memory impairments (Morrow *et al*, 2000, 2002; Murphy *et al*, 1996; Jentsch *et al*, 1997). Deficits in object recognition memory are also seen in schizophrenic patients (Crespo-Facorro *et al*, 2001; Doniger *et al*, 2002; Heckers *et al*, 2000) and are accompanied by abnormal and inadequate activation of the thalamus and the prefrontal cortex (Heckers *et al*, 2000; Crespo-Facorro *et al*, 2001). Acute administration of Δ^9-THC and synthetic cannabinoids like WIN increases dopamine transmission in the prefrontal cortex (Chen *et al*, 1990; Jentsch *et al*, 1997; Pistis *et al*, 2001, 2002). However, long-term exposure of adult rats to high doses of Δ^9-THC resulted in a reduced dopamine metabolism in the prefrontal cortex (Jentsch *et al*, 1998). As mentioned above, we cannot directly translate from these studies in adult rats to pubertal cannabinoid treatment, however, it is conceivable that persistent alterations in dopaminergic transmission are a possible cause for object recognition impairment.

The PR schedule is a valuable test to determine reinforcement value in an operant behavior task that is independent of total response rate (Reilly, 1999). By increasing the number of lever presses required for each successive reinforcement, animals eventually cease to respond according to criterion (break point). It was proposed by Ellenbroek and Cools (2000) that a reduced break point and hence a lower performance in a PR schedule might serve as an animal model for anhedonia, one of the core negative symptoms in schizophrenia. It has recently been shown that PCP and amphetamine, which are known to mimic psychotic symptoms, both reduce the break points in common marmosets and rhesus monkeys, whereas the atypical antipsychotic clozapine enhanced the break point in marmosets (Cilia *et al*, 2001).

Pubertal, but not adult, chronic cannabinoid administration impaired the performance in the PR schedule as shown by a significant reduction in break points. This result is consistent with earlier findings in monkeys of impaired break points in a PR task after acute administration of Δ^9-THC (Schulze *et al*, 1988). Interestingly, WIN treatment had no effect on the total number of lever-presses. Since food preference and locomotor activity of cannabinoid-treated animals was not affected, the disruptive effects on PR cannot be due to reduced food motivation or an increase/decrease in locomotor activity.

Taken together, our results indicate that chronic pubertal—but not adult—treatment with the synthetic cannabinoid agonist WIN leads to long-lasting disruptions in sensorimotor gating, object recognition, and the performance in a PR task. These enduring disturbances might be due to a persistent imbalance in various neurotransmitter systems, including the cannabinoid, the opioide, and the dopaminergic system, induced by chronic cannabinoid stimulation during pubertal development.

As mentioned before, there is evidence for a connection between cannabis use and schizophrenia (Schneider *et al*, 1998; Emrich *et al*, 1997; Leweke *et al*, 1999a; Skosnik *et al*, 2001; D'Souza *et al*, 2000; Voruganti *et al*, 2001), and for a possible dysregulation in the endogenous cannabinoid system being associated with the pathogenesis of schizophrenia (Emrich *et al*, 1997; Leweke *et al*, 1999a; Dean *et al*, 2001) Moreover, some of the highest densities of CB1 receptors have been found in regions of the human brain that have been implicated in schizophrenia including the frontal cortex, basal ganglia, and hippocampus (Glass *et al*, 1997).

Furthermore, it was shown in recent longitudinal studies that cannabis use in adolescence (until age 18 years) could be regarded as a high-risk factor for developing schizophrenia in adulthood (Zammit *et al*, 2002; Arseneault *et al*, 2002).

Since PPI deficits, object recognition memory impairments, and anhedonia/avolition are among the symptoms of schizophrenia, we propose chronic cannabinoid administration during pubertal development of rats as a new neurodevelopmental animal model for some aspects of the etiology of schizophrenia. This assumption is further supported by the fact that the cannabinoid-induced PPI deficit observed in our study was reversed by a clinically potent antipsychotic drug.

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