

# Acute and chronic cannabinoid treatment differentially affects recognition memory and social behavior in pubertal and adult rats

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## ABSTRACT

Although cannabis belongs to the most widely used drugs among adolescents, little is known about its acute and lasting neurobehavioral effects during critical developmental periods. In the present study we investigated acute and long-term behavioral effects of the cannabinoid agonist WIN 55,212-2 (WIN) in pubertal and adult rats. Chronic WIN (1.2 mg/kg)/vehicle treatment was extended over 25 days throughout puberty, from postnatal day (pd) 40 to pd 65, or for a similar time period in adult rats (> pd 80). All animals were tested at three time points for object/social recognition memory, social interaction and spontaneous social behavior. First, acute cannabinoid effects were investigated directly after the first injection. Additionally, behavioral performance was retested 24 hours and 15 days after cessation of WIN treatment. Chronic pubertal WIN treatment induced persistent object/social recognition deficits, indicating a general impairment in short-term information processing. Lasting disturbances in social behavior, social play and self-grooming were also found. Furthermore, behavioral deficits seen after acute WIN administration were more pronounced in pubertal than in adult rats. These results confirm our recent findings that chronic pubertal cannabinoid treatment leads to lasting behavioral alterations in adulthood, and they show that acute cannabinoid administration induces more severe behavioral deficits in pubertal rats than in mature animals. We therefore conclude that an immature brain is more susceptible to the acute and chronic effects of exogenous cannabinoids than an adult organism, which might be explained by an overactive endocannabinoid system and concomitant maturational disturbances in further neurotransmitter systems during pubertal development.

**Keywords** Cannabinoid, puberty, recognition memory, social behavior, social interaction, WIN 55,212-2.

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## INTRODUCTION

An intricate and dynamic developmental process, brain development extends from early *in utero* events to late adolescence. In addition to early neurodevelopmental processes, such as cell migration and differentiation, puberty is a highly important developmental time span where many dynamic cellular and anatomical modifications are taking place, contributing to the formation of the adult state. Various neuronal maturation and rearrangement processes occur during this period, such as myelination, synaptic pruning and dendritic plasticity (De Bellis *et al.* 2001). Furthermore, final maturational processes of neurotransmitter systems such as the

glutamatergic, the dopaminergic, and also the endogenous cannabinoid system, are taking place during this sensitive period (Rodriguez de Fonseca *et al.* 1993; Spear 2000). Thus it is clear that the pubertal brain is very different from both the child and adult brain, and may display particular vulnerability to disruptions by drugs and stress (Chambers, Taylor & Potenza 2003). Unfortunately, the initial use of psychoactive agents like tobacco, alcohol and cannabis is generally linked to the onset of puberty (Adriani & Laviola 2004). Marijuana and hashish, derivatives of the hemp plant *cannabis sativa*, belong to the most widely used drugs among adolescents. Despite the increasing use of cannabis, little is known about its lasting neurobehavioral consequences,

especially during critical developmental periods, such as pubertal maturation (Schneider 2008).

We have shown recently that chronic treatment with the synthetic cannabinoid full receptor agonist WIN 55,212-2 (WIN) during pubertal development leads to long-lasting behavioral disturbances in adulthood (Schneider & Koch 2003, 2005, 2007). A comparable treatment in adult and prepubertal rats induces no or only minor lasting impairments on behavioral performance, identifying puberty as the highest vulnerable period for the adverse effects of exogenous cannabinoids (Schneider & Koch 2003; Schneider, Drews & Koch 2005). We therefore suggested that exogenous cannabinoids may induce lasting neurobiological alterations during sensitive periods of brain maturation, such as pubertal development. Further evidence that the neuropharmacological and behavioral effects of exogenous cannabinoids depend highly on the developmental state, has been provided by various studies in humans (Ehrenreich *et al.* 1999; Wilson *et al.* 2000; Patton Hall *et al.* 2002; Pope *et al.* 2003; Arseneault *et al.* 2004) and rats (Stiglick & Kalant 1985; Biscaia *et al.* 2003; O'Shea *et al.* 2004; Pistis *et al.* 2004).

The purpose of the present study was a further evaluation of the lasting neurobehavioral consequences of chronic pubertal WIN treatment with a focus on social behavioral skills and short-term mnemonic processing in a social and non-social context. Apart from the persistent long-term behavioral impairments we were additionally interested in the behavioral performance of pubertal and adult animals during (acute effects) and directly after chronic cannabinoid treatment.

## METHODS

### Subjects

A total of 48 first generation offspring male Wistar rats from our own breeding colony were used for the present study. Adult male and female Wistar rats were imported from Harlan-Winkelmann (Borchen, Germany) and housed together in pairs under standard conditions on a 12-hour light–dark schedule (lights on 7:00–19:00). 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. In order to avoid litter effects we attempted to assign equal proportions of rats of each litter to the different treatment groups. After weaning on postnatal day (pd) 21, male pups were housed in a different room in groups of six (Macrolon cage type IV) under standard conditions on a 12-hour light–dark schedule (lights on 6:00–18:00). They received free access to tap water and were fed *ad libitum* until they reached a body

weight of at least 250 g. Then they were maintained on a body weight of approximately 90% of their free-feeding weight by a mild food restriction schedule. Food (15 g/animal/day) was always provided in the evening at the beginning of the dark phase. This feeding schedule was used to keep the animals more agile during behavioral testing.

The experiments were done in accordance with the National Institutes of Health ethical guidelines for the care and use of laboratory animals for experiments, and were approved by the local animal care committee (Cologne, Germany).

### Drugs

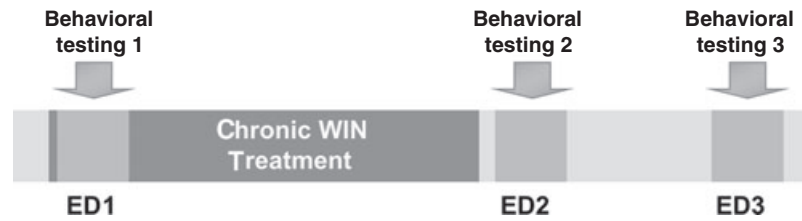
WIN 55,212-2 (WIN) (Sigma-Aldrich, Taufkirchen, Germany) was dissolved in 0.1% Tween 80 and diluted in saline (0.9%). The drug was administered intraperitoneally (i.p.) in a dose of 1.2 mg/kg. Injection volumes were 1 ml/kg. The experimenter was blind to the drug treatment of the animals.

### Experimental design

The pubertal chronic treatment of either the synthetic cannabinoid WIN or vehicle lasted 25 days from pd 40 to pd 65 throughout the rats puberty (Schneider 2008). During this period the rats received 20 injections i.p. which were not delivered regularly (for detailed description see Schneider & Koch 2003). Behavioral performance was assessed during and after cannabinoid treatment on pd 40, pd 66 and pd 80 (Fig. 1). First, the acute effects of WIN were investigated 30 minutes after the first cannabinoid injection on pd 40 [experimental day (ED) 1]. Additionally, the animals' behavioral performance were retested 24 hours (ED2) and 15 days (ED3) after the cessation of chronic WIN treatment on pd 66 and pd 80, respectively.

Chronic WIN/vehicle treatment in adult rats (> pd 80) was conducted as described earlier (20 injections over a period of 25 days) and all experiments were performed in a similar period of time as in pubertal-treated rats. Behavioral performance of adult treatment groups was assessed after the first injection (ED1), 24 hours after the last injection (ED2) and again 15 days later (ED3) (Fig. 1).

Behavioral testing included the analysis of social behavior, social interaction and the object and social recognition test. Behavioral performance was videotaped (digital handycam, Sony, Berlin, Germany) and evaluated offline by a trained experimenter blind to group assignment. Constant background noise was provided by a radio during behavioral testing.



**Figure 1** Experimental design. The behavioral performance of pubertal and adult animals was assessed at three time points during and after cannabinoid treatment: (1) directly after the first acute WIN injection [experimental day (ED) 1]; (2) 24 hours after the cessation of chronic WIN administration (ED2); and finally (3) 15 days after the last cannabinoid injection (ED3). ED = experimental day; WIN = WIN 55,212-2

## Behavioral testing

### Object recognition

Short-term memory for objects was assessed with the object recognition test. The recognition test was performed in an open field (50 cm × 50 cm × 50 cm) made of plastic. The objects to be discriminated were made of metal or glass and existed in duplicate. All objects and the test arena were cleaned with 70% alcohol and thoroughly dried before and during testing. Preliminary investigations had ascertained that the rats showed a comparable interest in all different objects chosen for this test. The rats were habituated to the open field for 30 minutes, 24 hours before behavioral testing. The test consisted of an initial 3-minute sample phase (P1) and a 3-minute discrimination phase (P2) that were separated by an intertrial interval of 15 minutes. During P1, the rat was placed in the center of the open field and exposed to an unknown object (A). After cessation of P1 the rat was returned to the homecage and the object was removed. The rat was placed back in the open field after 15 minutes for object discrimination in P2 and was now exposed to the familiar object (A', an identical copy of the object presented in P1) and a novel test object (B). Exploration of the objects (sniffing, dragging, pushing and gnawing) was recorded during P1 and P2. Sitting beside or standing on top of the objects was not scored as object investigation.

### Social recognition

Social memory was assessed with the social recognition test. The design of the test was comparable with the object recognition paradigm in time course and testing conditions. For social recognition young male rats were used as social stimuli to exclude confounding effects of aggression and sexual behavior (Everts & Koolhaas 1997; Schneider & Koch 2002). They were marked on head and tail for later identification and were kept individually 1 hour prior to and during testing to assure a characteristic body odor. Juveniles were habituated to the test arena for 10 minutes, 24 hours before testing. As described above, the experimental rats were placed in the open field and exposed to an unknown social partner (A) for 3 minutes

during P1. After the 15-minute intertrial interval the familiar (A') and a novel social partner (B) were presented to the experimental animal in P2. Social investigation (anogenital exploration, non-anogenital exploration and approach/following) was recorded during P1 and P2. Other social behaviors, such as grooming, crawl over or social play, were not scored as social investigation.

### Social interaction

Social interaction was assessed in the open field during the first sample phase (P1) of the social recognition test where the rat to be tested was exposed to an unfamiliar social partner for 3 minutes. The following behavioral elements were quantified only for the experimental rats.

- 1 Social behavior: contact behavior, social exploration, tail manipulation and approach/following were scored as social behaviors (Pellis *et al.* 1997; Vanderschuren, Niesink & Van Ree 1997). (1) Contact behavior: contact behavior includes (a) grooming (chewing and licking the partner's fur) and (b) crawling over/under the partner; (2) social exploration: (a) anogenital investigation (sniffing or licking the anogenital area of the social partner) and (b) non-anogenital investigation (sniffing at any part of the partner's body, except the anogenital area); (3) tail manipulation: grabbing, biting and pulling the partners tail (mainly seen in juvenile rats); and (4) approach/following: approaching or following the social partner in the test arena.
- 2 Evade: running, leaping or swerving away from the social partner. Evade, which is normally defined as a defensive behavior in the context of social play, was scored in the social interaction test as an active withdrawal from social contact. Social play behaviors (pinning, attack, defense) occurred so rare during the 3-minute social interaction period that they were not evaluated.
- 3 Self-grooming behavior: licking or biting the own fur and rubbing the forepaws over the head.

### Spontaneous social behavior

Besides the examination of social behavior with an unknown conspecific during the social interaction test,

the natural occurrence of spontaneous social activities was also assessed in a group of familiar animals. Rats of one group were divided in groups of three (three WIN and three control animals) before videotaping on test days. All groups were videotaped for 1 hour in their homecage. The same behavioral elements as described above for the social interaction test were quantified for all rats (social behavior, self-grooming and evade in a social context).

Playful activities did occur more often during the 1-hour video recording than during the 3-minute social interaction test, and therefore the frequency of pinning, attacks initiated and received and defensive behavior were evaluated as well (for detailed description see Schneider & Koch 2005). Since play behavior, which is a typical behavior for the dark phase of the animals' light/dark cycle, occurs only sporadically and in a less complex manner during the light phase, the frequency of all playful activities (pinning, attacks initiated, attacks received and defensive behavior) observed during 1 hour of video recording were summed up to one single play behavior score.

### Statistical analysis

In the recognition test the exploration time was recorded during P1 (A), and for the two objects/conspicifics in P2 (A' and B). Beside the discrimination index (B-A'), object/social recognition and object/social discrimination were also evaluated. Object/social recognition was calculated as the percentage decrease in investigation time of the familiar object/conspicific from P1 to P2  $\{100 - [100/(A \times A')]\}$ . For the calculation of object/social discrimination the exploration time of the novel object/conspicific was expressed as percentage of the total exploration time of both objects/conspicifics during P2  $[100/(A' + B) \times B]$ . Different behaviors were quantified in the social interaction test and during homecage recording as described before. Additionally, during videotaping of spontaneous social behavior the occurrence of playful activities was evaluated and added up to one play behavior score. Differences between treatment groups (WIN or vehicle) in behavioral performance were evaluated using Student's *t*-tests. A value of  $P < 0.05$  was considered to represent a significant effect.

## RESULTS

### Object recognition

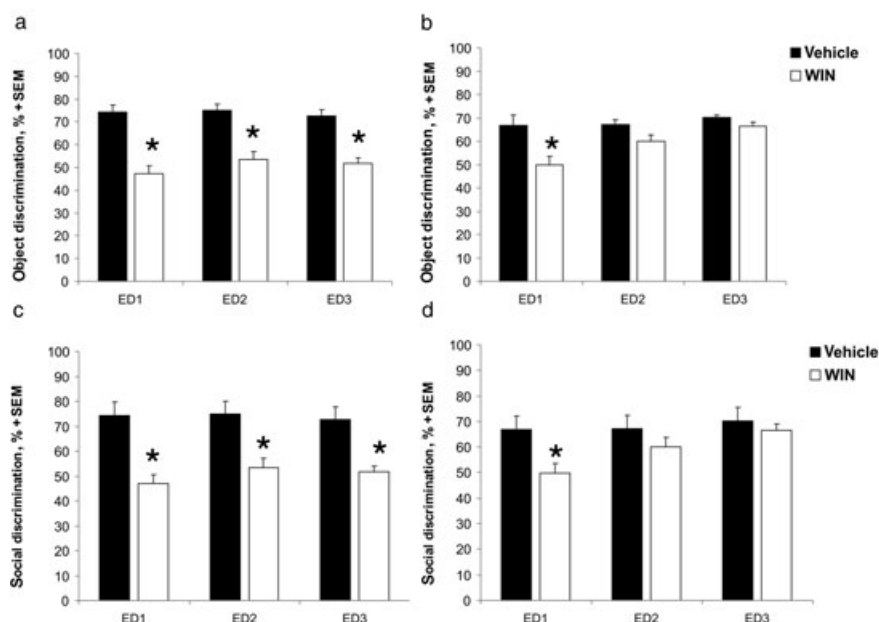
#### Pubertal WIN treatment

Acute cannabinoid administration on pd 40 (ED1) significantly increased the investigation time of the familiar object (A') ( $P = 0.007$ ) and decreased the exploration of

the new object (B) ( $P = 0.039$ ) during P2 compared with vehicle-treated controls. The discrimination index ( $P < 0.001$ ), object recognition ( $P = 0.001$ ) and object discrimination ( $P < 0.001$ ) (Fig. 2a) were also significantly reduced compared with controls (Student's *t*-test) (values for discrimination index [s]  $\pm$  standard error of the mean (SEM): WIN group =  $-0.2 \pm 1.0$ ; vehicle group =  $6.4 \pm 1.3$ ; values for object recognition [%]  $\pm$  SEM: WIN group =  $35.7 \pm 9.3$ ; vehicle group =  $75.7 \pm 3.5$ ). No effects of cannabinoid administration were seen on object investigation during P1 (Student's *t*-test,  $P > 0.05$ ) (data not shown). Similar impairments were seen on pd 66, 24 hours after the last WIN injection (ED2). The investigation time of B ( $P = 0.002$ ), the discrimination index ( $P = 0.048$ ), object recognition ( $P = 0.016$ ) and object discrimination ( $P < 0.001$ ) (Fig. 2a) were significantly decreased in cannabinoid-treated rats (Student's *t*-test) [values for discrimination index (s)  $\pm$  SEM: WIN group  $P = 1.7 \pm 1.1$ ; vehicle group =  $5.8 \pm 1.7$ ; values for object recognition (%)  $\pm$  SEM: WIN group =  $41.6 \pm 5.9$ ; vehicle group =  $63.8 \pm 6.5$ ]. No effects of WIN were seen on investigating time of A during P1 (Student's *t*-test,  $P > 0.05$ ; data not shown). Lasting deficits on object recognition memory were observed on pd 80 (ED3), 15 days after the cessation of chronic pubertal WIN treatment. Discrimination index ( $P < 0.001$ ), object recognition ( $P = 0.003$ ) and object discrimination ( $P < 0.001$ ) (Fig. 2a) were persistently reduced in cannabinoid-treated rats compared with controls [Student's *t*-test; values for discrimination index (s)  $\pm$  SEM: WIN group =  $1.2 \pm 1.0$ ; vehicle group =  $7.1 \pm 1.0$ ; values for object recognition (%)  $\pm$  SEM: WIN group =  $35.4 \pm 7.4$ ; vehicle group =  $65.4 \pm 5.2$ ]. The investigation of A during P1 and of B during P2 did not differ significantly between the treatment groups (Student's *t*-test,  $P > 0.05$ ).

#### Adult WIN treatment

Acute WIN treatment in adult rats on ED1 significantly reduced the discrimination index ( $P = 0.024$ ) and object discrimination ( $P = 0.038$ ) (Fig. 2b) compared with the vehicle-treated controls. A trend was observed for a decrease in object recognition [ $P = 0.057$ , Student's *t*-test, values for discrimination index (s)  $\pm$  SEM: WIN group =  $-0.2 \pm 0.7$ ; vehicle group =  $4.5 \pm 1.2$ ; values for object recognition (%)  $\pm$  SEM: WIN group =  $45.2 \pm 4.6$ ; vehicle group =  $63.4 \pm 4.6$ ]. No cannabinoid effects were found on exploration time in P1 (Student's *t*-test,  $P > 0.05$ , data not shown). On ED2 no effects of the chronic cannabinoid treatment were found on the discrimination index and object discrimination (Fig. 2b, Student's *t*-test,  $P > 0.05$ ). However, a trend for reduced object recognition ( $P = 0.074$ ) was observed in



**Figure 2** Effects of pubertal and adult cannabinoid treatment on object (a: pubertal, b: adult treatment) and social (c: pubertal, d: adult treatment) discrimination (%) are shown for ED1, ED2 and ED3. Pubertal WIN treatment significantly affected object/social discrimination at all ages. WIN also acutely impaired object/social discrimination in adults, but no persistent effects were seen ( $P < 0.05$  is indicated by asterisks; pubertal: WIN:  $n = 17$ , vehicle:  $n = 14$ ; adult: WIN:  $n = 9$ , vehicle:  $n = 8$ ). ED = experimental day; SEM = standard error of the mean; WIN = WIN 55,212-2

cannabinoid-treated rats [Student's  $t$ -test; values for discrimination index ( $s$ )  $\pm$  SEM: WIN group =  $2.9 \pm 0.6$ ; vehicle group =  $5.3 \pm 0.8$ ; values for object recognition (%)  $\pm$  SEM: WIN group =  $42.0 \pm 4.0$ ; vehicle group =  $57.5 \pm 4.4$ ]. No differences were seen on exploration time in P1 (Student's  $t$ -test,  $P > 0.05$ , data not shown). Chronic cannabinoid treatment in adult rats did not induce any permanent alterations on ED3 in the discrimination index, object recognition and object discrimination (Fig. 2b) and on the exploration time of A during P1 [Student's  $t$ -test,  $P > 0.05$ ; values for discrimination index ( $s$ )  $\pm$  SEM: WIN group =  $4.0 \pm 0.5$ ; vehicle group =  $7.4 \pm 1.3$ ; values for object recognition (%)  $\pm$  SEM: WIN group =  $56.0 \pm 4.2$ ; vehicle group =  $54.2 \pm 5.1$ ].

## Social recognition

### Pubertal WIN treatment

Acute WIN administration in pubertal rats on ED1 significantly reduced the investigation of the unknown social partner (B) in P2 ( $P = 0.002$ ), the discrimination index ( $P < 0.001$ ), social recognition ( $P = 0.020$ ) and social discrimination ( $P < 0.001$ , Fig. 2c) [Student's  $t$ -test; values for discrimination index ( $s$ )  $\pm$  SEM: WIN group =  $2.0 \pm 1.0$ ; vehicle group =  $13.8 \pm 1.6$ ; values for social recognition (%)  $\pm$  SEM: WIN group =  $57.3 \pm 5.8$ ; vehicle group =  $74.6 \pm 3.4$ ]. However, the initial explora-

tion of the social partner (A) during P1 was also significantly reduced in WIN-treated rats ( $P = 0.007$ ) compared with controls (Student's  $t$ -test, data not shown). On ED 2 WIN-treated rats showed a significant reduction in discrimination index ( $P < 0.001$ ), social recognition ( $P = 0.002$ ) and social discrimination ( $P < 0.001$ ) (Fig. 2c) compared with vehicle-treated controls [Student's  $t$ -test; values for discrimination index ( $s$ )  $\pm$  SEM: WIN group =  $-1.5 \pm 2.8$ ; vehicle group =  $21.1 \pm 2.6$ ; values for social recognition (%)  $\pm$  SEM: WIN group =  $53.2 \pm 5.1$ ; vehicle group =  $74.8 \pm 2.8$ ]. No effects were seen on investigating A during P1 (Student's  $t$ -test,  $P > 0.05$ ) (data not shown). Similar effects were seen 15 days after cannabinoid treatment on ED 3. Chronic pubertal WIN treatment permanently decreased the discrimination index ( $P = 0.001$ ), social recognition ( $P = 0.002$ ) and social discrimination [ $P < 0.001$ , Student's  $t$ -test; shown in Fig. 2c are the values for discrimination index ( $s$ )  $\pm$  SEM: WIN group =  $0.7 \pm 2.2$ ; vehicle group =  $16.0 \pm 3.5$ ; values for social recognition (%)  $\pm$  SEM: WIN group =  $59.2 \pm 3.9$ ; vehicle group =  $76.1 \pm 2.9$ ]. The exploration of A during P1 did not differ significantly between the treatment groups (Student's  $t$ -test,  $P > 0.05$ , data not shown).

### Adult WIN treatment

Acute WIN administration in adult rats on ED 1 significantly reduced the discrimination index ( $P = 0.006$ )

**Table 1** Social interaction test (pubertal WIN treatment).

Social interaction (pubertal WIN treatment) ± SEM	ED1		ED2		ED3	
	Vehicle	WIN	Vehicle	WIN	Vehicle	WIN
Grooming	1.0 (±0.2)	0.2 (±0.1)*	0.3 (±0.1)	0.1 (±0.1)	0.3 (±0.1)	0.1 (±0.1)*
Crawl over	1.6 (±0.4)	0.6 (±0.2)*	1.7 (±0.3)	0.9 (±0.3)	2.0 (±0.4)	0.4 (±0.1)*
Following	4.0 (±0.5)	3.6 (±0.6)	7.3 (±0.5)	4.1 (±0.4)*	6.1 (±0.3)	4.1 (±0.5)*
Tail manipulation	0.1 (±0.1)	0.1 (±0.1)	0.2 (±0.1)	0.1 (±0.1)	0.4 (±0.1)	0.0 (±0.0)
Anogenital exploration	8.3 (±0.7)	5.2 (±0.5)*	10.9 (±0.6)	12.9 (±0.6)*	10.5 (±1.0)	13.4 (±1.0)*
Non-anogenital exploration	5.6 (±0.5)	6.4 (±0.5)	8.9 (±0.8)	6.3 (±0.5)*	10.1 (±0.7)	4.8 (±0.5)*
Entire social behavior	20.6 (±1.5)	16.1 (±1.4)*	29.2 (±0.8)	24.4 (±1.1)*	29.5 (±1.8)	22.7 (±1.7)*
Self-grooming	2.8 (±0.3)	2.7 (±0.3)	1.4 (±0.3)	2.4 (±0.3)*	1.1 (±0.2)	1.9 (±0.3)*
Evade (social context)	0.0 (±0.0)	0.4 (±0.2)	0.3 (±0.1)	0.9 (±0.4)	0.2 (±0.2)	1.1 (±0.4)*

Effects of pubertal WIN treatment on social interaction testing ( $P < 0.05$  is indicated by asterisk; WIN:  $n = 17$ , vehicle:  $n = 14$ ). ED = experimental day; SEM = standard error of the mean; WIN = WIN 55,212-2.

and social discrimination ( $P = 0.017$ ) (Fig. 2d). Social recognition was not affected by cannabinoid treatment [Student's  $t$ -test,  $P > 0.05$ ; values for discrimination index (s) ± SEM: WIN group =  $5.7 \pm 1.5$ ; vehicle group =  $20.4 \pm 4.7$ ; values for social recognition (%) ± SEM: WIN group =  $57.3 \pm 8.3$ ; vehicle group =  $71.1 \pm 2.9$ ]. However, as described before in pubertal-treated rats, the initial exploration of the social partner (A) during P1 was also significantly reduced in adult cannabinoid-treated rats ( $P = 0.017$ ) as compared with controls (Student's  $t$ -test, data not shown). On ED2, a trend for a reduced discrimination index ( $P = 0.076$ ) was found in WIN-treated rats. The chronic cannabinoid administration affected neither social recognition nor social discrimination as shown in Fig. 2d [Student's  $t$ -test,  $P > 0.05$ ; values for discrimination index (s) ± SEM: WIN group =  $12.3 \pm 2.2$ ; vehicle group =  $19.4 \pm 3.0$ ; values for social recognition (%) ± SEM: WIN group =  $63.3 \pm 6.1$ ; vehicle group =  $73.8 \pm 3.5$ ]. Initial exploration times during P1 did not differ between the treatment groups (Student's  $t$ -test,  $P > 0.05$ ; data not shown). Chronic WIN treatment in adult rats did not induce any lasting alterations in social recognition memory. No effects were found on the discrimination index, social recognition and social discrimination as seen in Fig. 2d [Student's  $t$ -test,  $P > 0.05$ ; values for discrimination index (s) ± SEM: WIN group =  $21.3 \pm 2.0$ ; vehicle group =  $18.3 \pm 3.0$ ; values for social recognition (%) ± SEM: WIN group =  $63.7 \pm 3.0$ ; vehicle group =  $68.9 \pm 3.2$ ]. Exploration during P1 was also not affected (Student's  $t$ -test,  $P > 0.05$ , data not shown).

## Social interaction

### Pubertal WIN treatment

WIN administration on ED1 acutely reduced social grooming ( $P = 0.002$ ), crawl over ( $P = 0.034$ ),

anogenital exploration ( $P = 0.001$ ) and the total amount of social behavior ( $P = 0.039$ ; Student's  $t$ -test). A trend for increased evade of social contact ( $P = 0.062$ ) was also found. Non-anogenital exploration, tail manipulation, following and self-grooming did not differ significantly between the treatment groups as shown in Table 1 (Student's  $t$ -test,  $P > 0.05$ ). On ED2, non-anogenital exploration ( $P = 0.007$ ), following ( $P < 0.001$ ) and the total amount of social behavior ( $P = 0.002$ ) were reduced in WIN-treated rats, whereas self-grooming ( $P = 0.026$ ) and anogenital exploration ( $P = 0.024$ ) were increased (Student's  $t$ -test). Furthermore, cannabinoid treatment induced a trend for a reduction in crawl over ( $P = 0.061$ ). No effects were seen on social grooming, tail manipulation and evade (Table 1; Student's  $t$ -test,  $P > 0.05$ ). Chronic pubertal WIN administration persistently reduced grooming ( $P = 0.037$ ), crawl over ( $P < 0.001$ ), non-anogenital exploration ( $P = 0.007$ ) and the total amount of social behavior ( $P = 0.012$ ) on ED3 (Student's  $t$ -test). The social behaviors that are following ( $P = 0.001$ ), anogenital investigation ( $P = 0.042$ ), self-grooming ( $P = 0.032$ ) and evade ( $P = 0.036$ ) were persistently increased in cannabinoid-treated rats (Student's  $t$ -test). No effects were seen on tail manipulation (Table 1; Student's  $t$ -test,  $P > 0.05$ ).

### Adult WIN treatment

Acute WIN administration in adult rats on ED1 reduced anogenital exploration ( $P = 0.003$ ), following ( $P = 0.029$ ) and the total amount of social behavior ( $P = 0.007$ ; Student's  $t$ -test). Grooming, crawl over, non-anogenital exploration, tail manipulation, self-grooming and evade did not differ significantly between the treatment groups as shown in Table 2 (Student's  $t$ -test,  $P > 0.05$ ). No significant cannabinoid effects were observed on ED2 (Table 2; Student's  $t$ -test,  $P > 0.05$ ).

**Table 2** Social interaction test (adult WIN treatment).

Social interaction (adult WIN treatment) ± SEM	ED1		ED2		ED3	
	Vehicle	WIN	Vehicle	WIN	Vehicle	WIN
Grooming	0.1 (±0.1)	0.2 (±0.1)	1.0 (±0.3)	0.7 (±0.3)	0.6 (±0.4)	0.3 (±0.2)
Crawl over	0.9 (±0.2)	0.3 (±0.2)	1.0 (±0.5)	0.7 (±0.4)	1.1 (±0.5)	0.9 (±0.3)
Following	6.3 (±0.3)	4.4 (±0.6)*	4.6 (±0.8)	5.1 (±0.6)	4.5 (±0.4)	4.8 (±0.6)
Tail manipulation	0.1 (±0.1)	0.0 (±0.0)	0.0 (±0.0)	0.1 (±0.1)	0.0 (±0.0)	0.4 (±0.4)
Anogenital exploration	11.4 (±0.9)	7.0 (±0.9)*	10.1 (±1.0)	8.3 (±0.8)	9.9 (±0.6)	9.4 (±1.1)
Non-anogenital exploration	7.3 (±0.4)	6.9 (±0.4)	6.3 (±0.7)	5.7 (±0.4)	6.3 (±0.8)	6.9 (±0.4)
Entire social behavior	25.9 (±1.3)	18.9 (±1.8)*	23.0 (±2.2)	20.6 (±1.8)	22.4 (±1.3)	22.2 (±2.6)
Self-grooming	1.0 (±0.3)	1.4 (±0.5)	1.0 (±0.5)	1.6 (±0.5)	0.6 (±0.3)	1.3 (±0.5)
Evade (social context)	0.5 (±0.4)	0.9 (±0.4)	0.3 (±0.1)	0.7 (±0.3)	0.4 (±0.2)	0.3 (±0.1)

Effects of adult WIN treatment on social interaction testing ( $P < 0.05$  is indicated by asterisk; WIN:  $n = 9$ , vehicle:  $n = 8$ ). ED = experimental day; SEM = standard error of the mean; WIN = WIN 55,212-2.

**Table 3** Spontaneous social behavior (pubertal WIN treatment).

Spontaneous social behavior (pubertal WIN treatment) ± SEM	ED1		ED2		ED3	
	Vehicle	WIN	Vehicle	WIN	Vehicle	WIN
Grooming	2.0 (±0.5)	1.4 (±0.3)	3.9 (±0.6)	0.2 (±0.1)*	3.2 (±0.3)	1.4 (±0.3)*
crawl over	2.4 (±0.7)	2.1 (±0.4)	3.6 (±0.5)	2.0 (±0.4)*	4.2 (±0.5)	2.7 (±0.4)*
Following	0.1 (±0.1)	0.7 (±0.2)*	0.9 (±0.3)	1.0 (±0.3)	0.9 (±0.3)	1.9 (±0.3)*
Tail manipulation	0.3 (±0.1)	0.2 (±0.1)	0.1 (±0.1)	0.1 (±0.1)	0.3 (±0.1)	0.5 (±0.2)
Anogenital exploration	1.1 (±0.4)	2.5 (±0.5)	3.2 (±0.4)	3.6 (±0.4)	3.5 (±0.7)	8.5 (±1.0)*
Non-anogenital exploration	1.7 (±0.5)	2.1 (±0.5)	5.1 (±0.5)	2.7 (±0.4)*	3.7 (±0.5)	3.8 (±0.4)
Entire social behavior	7.7 (±1.6)	9.1 (±1.7)	16.9 (±1.4)	10.6 (±0.7)*	15.9 (±1.5)	18.9 (±1.4)
Self-grooming	4.8 (±0.7)	5.1 (±0.9)	6.6 (±0.6)	5.2 (±0.4)*	7.8 (±0.5)	6.6 (±0.7)
Evade (social context)	0.1 (±0.9)	0.3 (±0.1)	0.0 (±0.0)	0.1 (±0.1)	0.1 (±0.1)	0.1 (±0.1)
Play behavior	2.1 (±0.7)	15.1 (±4.5)*	4.1 (±1.4)	6.3 (±1.3)	5.3 (±1.4)	14.5 (±3.2)*

Effects of acute and chronic pubertal WIN administration on social behavior during homecage recording ( $P < 0.05$  is indicated by asterisks; WIN:  $n = 9$ , vehicle:  $n = 8$ ). ED = experimental day; SEM = standard error of the mean; WIN = WIN 55,212-2.

Chronic WIN administration in adult rats did not induce any persistent alterations on ED3 in social behavior, self-grooming and evade (Table 2; Student's  $t$ -test,  $P > 0.05$ ).

### Spontaneous social behavior

#### Pubertal WIN treatment

Acute WIN treatment on pd 40 (ED1) induced a significant increase in following ( $P = 0.031$ ) and social play behavior ( $P = 0.019$ ; Student's  $t$ -test). Additionally, a trend for enhanced anogenital exploration ( $P = 0.064$ ) was observed. No effects of cannabinoid treatment were found on grooming, crawl over, tail manipulation, non-anogenital exploration, the total amount of social behavior, evade and self-grooming as seen in Table 3 (Student's  $t$ -test,  $P > 0.05$ ). Grooming ( $P = 0.001$ ), crawl over ( $P = 0.001$ ), non-anogenital exploration ( $P < 0.001$ ), the total amount of social behavior ( $P < 0.001$ ) and self-grooming ( $P = 0.041$ ) were significantly reduced in

cannabinoid-treated rats on ED2 compared with controls (Student's  $t$ -test). No significant effect could be found on following, tail manipulation, anogenital exploration, evade and play behavior (Table 3; Student's  $t$ -test,  $P > 0.05$ ). Grooming ( $P = 0.001$ ) and crawl over ( $P = 0.016$ ) were still decreased 15 days later on ED3 in WIN-treated rats (Student's  $t$ -test). However, following ( $P = 0.037$ ), anogenital exploration ( $P < 0.001$ ) and play behavior ( $P = 0.036$ ) were significantly increased after pubertal cannabinoid treatment compared with controls (Student's  $t$ -test). No significant differences between the treatment groups were detected in tail manipulation, non-anogenital exploration, the total amount of social behavior, self-grooming and evade (Table 3; Student's  $t$ -test,  $P > 0.05$ ).

#### Adult WIN treatment

Acute WIN treatment in adult rats on ED1 did not induce any significant alteration in social behavior,

**Table 4** Spontaneous social behavior (adult WIN treatment).

Spontaneous social behavior (adult WIN treatment) $\pm$ SEM	ED1		ED2		ED3	
	Vehicle	WIN	Vehicle	WIN	Vehicle	WIN
Grooming	1.9 ( $\pm$ 0.4)	2.2 ( $\pm$ 0.6)	2.0 ( $\pm$ 0.4)	2.0 ( $\pm$ 0.4)	2.3 ( $\pm$ 0.4)	2.0 ( $\pm$ 0.3)
Crawl over	2.5 ( $\pm$ 0.3)	2.0 ( $\pm$ 0.5)	2.4 ( $\pm$ 0.4)	1.7 ( $\pm$ 0.2)	2.8 ( $\pm$ 0.7)	3.3 ( $\pm$ 0.4)
Following	0.6 ( $\pm$ 0.2)	0.4 ( $\pm$ 0.1)	0.3 ( $\pm$ 0.1)	0.6 ( $\pm$ 0.1)	0.4 ( $\pm$ 0.1)	0.2 ( $\pm$ 0.1)
Tail manipulation	0.3 ( $\pm$ 0.1)	0.1 ( $\pm$ 0.0)	0.0 ( $\pm$ 0.0)	0.2 ( $\pm$ 0.1)	0.1 ( $\pm$ 0.1)	0.0 ( $\pm$ 0.0)
Anogenital exploration	4.3 ( $\pm$ 0.9)	2.7 ( $\pm$ 0.4)	3.4 ( $\pm$ 0.8)	3.8 ( $\pm$ 0.6)	4.0 ( $\pm$ 0.6)	4.2 ( $\pm$ 0.7)
Non-anogenital exploration	3.9 ( $\pm$ 0.7)	3.3 ( $\pm$ 0.3)	3.4 ( $\pm$ 0.5)	3.2 ( $\pm$ 0.7)	2.9 ( $\pm$ 0.5)	2.9 ( $\pm$ 0.3)
Entire social behavior	13.4 ( $\pm$ 1.6)	10.8 ( $\pm$ 1.3)	11.4 ( $\pm$ 1.8)	11.4 ( $\pm$ 1.6)	12.4 ( $\pm$ 1.4)	12.7 ( $\pm$ 1.2)
Self-grooming	4.8 ( $\pm$ 0.5)	3.4 ( $\pm$ 0.6)	3.8 ( $\pm$ 0.6)	3.7 ( $\pm$ 0.6)	4.1 ( $\pm$ 0.5)	4.1 ( $\pm$ 0.4)
Evade (social context)	0.0 ( $\pm$ 0.0)	0.0 ( $\pm$ 0.0)	0.0 ( $\pm$ 0.0)	0.0 ( $\pm$ 0.0)	0.0 ( $\pm$ 0.0)	0.0 ( $\pm$ 0.0)
Play behavior	0.0 ( $\pm$ 0.0)	0.4 ( $\pm$ 0.1)	0.0 ( $\pm$ 0.0)	0.3 ( $\pm$ 0.1)	0.8 ( $\pm$ 0.3)	0.3 ( $\pm$ 0.1)

Effects of acute and chronic WIN administration in adult rats on social behavior during homecage recording ( $P < 0.05$  is indicated by asterisk; WIN:  $n = 9$ , vehicle:  $n = 8$ ). ED = experimental day; SEM = standard error of the mean; WIN = WIN 55,212-2.

self-grooming and evade in a social context as seen in Table 4 (Student's  $t$ -test,  $P > 0.05$ ). However, a trend for an increase in playful activities was observed ( $P = 0.059$ ). No significant effects of chronic WIN treatment in adult rats were found on ED2 and 15 days later on ED3 (Table 4; Student's  $t$ -test,  $P > 0.05$ ).

## DISCUSSION

The results of the present study confirmed recent findings that chronic cannabinoid treatment during pubertal development induces lasting behavioral alterations in adulthood, which are not seen after an identical treatment in adult rats (Schneider & Koch 2003; O'Shea *et al.* 2004). Pubertal, but not adult, chronic WIN administration induces persistent disturbances in object and social recognition memory and leads to social withdrawal and alterations in social behavior and self-grooming. Furthermore, we showed that acute administration of the synthetic cannabinoid receptor agonist WIN induces more severe effects on behavioral performance in pubertal than in adult rats.

### Recognition memory

The acute and chronic cannabinoid effects on short-term memory functioning were assessed using the object and social recognition test. Recognition memory is generally regarded as the ability to discriminate the familiarity of items or events previously encountered and presents a useful tool for the testing of working memory functioning (Mumby 2001). Unlike object recognition, where the shape of objects is of central importance, social recognition memory is based on olfactory cues and represents a form of olfactory short-term memory (Everts & Koolhaas 1997). Object and social recognition memory are

thought to be mediated by different neuronal substrates. In social recognition the action of vasopressin and oxytocin in limbic structures, like the hippocampus and the septum, appears to be critical, whereas object recognition is independent of these processes (Everts & Koolhaas 1997).

In the present study pubertal cannabinoid treatment induced significant deficits in object recognition memory that did not differ much over the treatment period. Both the ability to discriminate between a familiar and an unknown object (object/social discrimination index and percentage of object/social discrimination) and the ability to recognize an object previously encountered were impaired in pubertal WIN-treated rats on all testing days (ED1, ED2, ED3). Similarly, chronic pubertal WIN administration reduced the rats' capability for social discrimination and social recognition on ED2 and ED3, indicating permanent impairments in social memory. However, the deficits in social recognition and social discrimination on ED1 after acute WIN administration probably do not relate to deficits in short-term memory functioning, since the initial exploration of the social partner during the first presentation (P1) was also significantly reduced. Social recognition tests rely in general on the intrinsic motivation of rodents for exploring novel conspecifics. This motivation seems to be impaired after acute WIN administration in pubertal rats indicated so by the decrease in investigating the unknown social partner during the initial exposure (P1). This effect is surprising as only juvenile male rats were used for this test in order to prevent aggression or other negative social experience. It is well known that interactions with unfamiliar conspecifics can lead to social stress (Blanchard, McKittrick & Blanchard 2001), which might have been the case with WIN-treated rats. A further behavioral evaluation of the social interaction test reveals that the loss of interest in

the social partner during the initial exposure of the social recognition test mainly derives from a decrease in anogenital investigation. A possible explanation for this reduced social interest and possible social stress might be increased anxiety of the unfamiliar conspecific. This assumption would also be in accordance with the social withdrawal observed in the social interaction test (see social behavior) after acute pubertal WIN administration.

Acute WIN administration in adult rats also affected object and social recognition memory on ED1, although the effects were less severe as observed after pubertal WIN administration. Object/social discrimination and the discrimination index for object and social recognition memory were reduced, but there was only a trend for reduced object recognition and no effect on social recognition in adult WIN pre-treated rats. Similar to pubertal WIN treatment, the acute cannabinoid effects on social recognition memory in adult rats seem to be related rather to increased anxiety than to deficient short-term memory, since the initial investigation of the unfamiliar conspecific in P1 was also decreased.

On ED2, object and social recognition memory functioning in adult cannabinoid-treated rats were almost restored. Only a trend for reduced object recognition and for a reduced social discrimination index remained.

No lasting cannabinoid effects on object or social recognition memory could be detected in adult rats on ED3, indicating that only pubertal chronic WIN administration leads to lasting deficits in object and social recognition memory functioning. Additionally, the fact that comparable lasting results on ED2 and ED3 were obtained in the two different forms of the recognition test (social and object) suggests that pubertal WIN treatment induces a rather general impairment of short-term mnemonic information processing.

Cannabinoid effects on learning and memory are well established (Hampson & Deadwyler 1999). Recognition memory impairments induced by acute cannabinoid administration have been reported previously (Schneider & Koch 2002; Kosiorek *et al.* 2003), and Terranova *et al.* (1996) demonstrated that the selective CB1 receptor antagonist SR141716 facilitates short-term memory in the social recognition test. Additionally, CB1 receptor knock-out mice show enhanced memory capabilities in the object recognition test (Reibaud *et al.* 1999). It has therefore been suggested that an endogenous cannabinoid tonus might be involved in modulations of memory processes (Terranova *et al.* 1996; Reibaud *et al.* 1999). Lasting effects on object recognition memory functioning as a specific consequence of pubertal/adolescent cannabinoid administration have been shown earlier (Schneider & Koch 2003; O'Shea *et al.* 2004; Quinn *et al.* 2008; Schneider & Koch *et al.* 2007) and are in accordance with our present results of deficient social and object

recognition memory after pubertal WIN administration. However, one single study reported similar residual deficits in object recognition memory after chronic cannabinoid treatment in adolescent (pd 30–50) and adult animals (pd 56–76) (O'Shea, McGregor & Mallet 2006). One explanation might be that animals that were used as adult controls in this study were still quite young and might not yet have reached sexual maturity (e.g. Clegg 1960). Therefore, these apparent adult controls might have still been in a susceptible developmental period for cannabinoid effects.

### Social behavior

The effects of cannabinoids on social behavioral skills were evaluated using the social interaction test and videotaping of spontaneous social behavior among a group of familiar conspecifics. The social interaction test is an ethologically based test that measures explorative and social behavior between two rodents meeting for the first time in an open field (File & Hyde 1978). In contrast, homecage recording of spontaneous social behavior evaluates the natural occurrence of social behavior among familiar conspecifics (Schneider & Koch 2005).

Acute WIN administration on ED1 in pubertal rats in the social interaction test induced a general decrease in the frequency of social behaviors (grooming, crawl over and anogenital exploration) and we observed a trend for increased evade upon social contact. No such decrease of social activities was found in the evaluation of social behavior during homecage recording among a group of familiar rats. However, acute cannabinoid treatment increased following and anogenital sniffing in the homecage—behaviors that are typically increased during social interaction with an unknown conspecific. The social withdrawal observed during social interaction after acute pubertal WIN administration points towards increased anxiety of the unfamiliar social partner. The social interaction test is classically used as a test for anxiety-related behavior because of its sensitivity to both anxiolytic and anxiogenic effects (File 2000; File, Cheeta & Akanezi 2001; Irvine *et al.* 2001) and is therefore thought to present a model of social anxiety in humans (File & Hyde 1978). Since no decrease in social activities was found during homecage recording, the cannabinoid-induced social withdrawal seems to be restricted to interaction with an unfamiliar social partner.

On ED2, 24 hours after the last cannabinoid injection, social behaviors in pubertal cannabinoid-treated rats differed considerably as compared with acute effects on ED1. In the social interaction test there was still a decrease in the total amount of social behaviors, but anogenital sniffing was now increased. In contrast, homecage recording revealed a strong decrease in social activities. This social

withdrawal during homecage recording was exclusively seen on ED2 and differs completely from acute and long-term cannabinoid effects in pubertal animals on spontaneous social behavior. We assume that this pronounced decrease in social behavior on ED2 could be a sign of withdrawal from chronic cannabinoid treatment. Although cannabis withdrawal symptoms are mostly subtle because of the lipophilic properties of cannabinoids, the existence of such symptoms has been demonstrated in humans (Budney & Hughes 2006) and animals (Maldonado & Rodriguez de Fonseca 2002; Gonzalez, Cebeira & Fernandez-Ruiz 2005; for review see Cooper & Haney 2008).

Several permanent effects of the chronic pubertal WIN treatment were found on ED3. In the social interaction test social grooming, crawl over, non-anogenital investigation, following and the total amount of social behaviors were reduced in cannabinoid-treated rats. However, anogenital exploration and evade in social context were increased. These findings are partially in accordance with earlier findings (O'Shea *et al.* 2004) that reported a persistent decrease in social interaction after adolescent cannabinoid exposure. However, in this study all social behaviors were summed up to a social interaction score and therefore no information is given on possible changes in single behaviors.

The lasting cannabinoid effects on spontaneous social behavior during homecage recording were quite similar to the lasting cannabinoid effects in the social interaction test. Grooming and crawl over were permanently decreased, whereas following and anogenital exploration were increased among familiar conspecifics. It is not conceivable that those lasting cannabinoid effects on social behavior are also related to increased anxiety, since anogenital exploration was increased during social interaction with the unknown conspecific. Additionally, the decrease in social activities (grooming and crawl over) and the unusual increase in exploratory behavior (following and anogenital exploration) among familiar conspecifics during homecage recording indicate (together with the impaired social recognition memory) that WIN-treated rats show inadequate social behavior and deficient olfactory social recognition.

Acute cannabinoid treatment in adult rats in the social interaction test only reduced following, anogenital exploration and the total amount of social behavior, but had no effect on social grooming and crawl over. These acute effects on social behavior are in accordance with the reduced social exploration during social recognition and might also be related to increased anxiety as described before for pubertal-treated rats. No acute WIN effects were found in homecage recording of spontaneous social behavior in adult rats on ED1. Chronic WIN administration in adult rats had no lasting consequences at all,

neither on social behavior in the social interaction test, nor on homecage recording (ED2 and ED3). Therefore, cannabinoid-induced long-term alterations in social behavior seem to be completely restricted to cannabinoid administration during pubertal development.

These findings prove that social behavior in a forced situation expressed towards an unknown social partner differs highly from spontaneous social behavior among familiar conspecifics in the homecage. Therefore both tests provide different but subsidiary information about such a complex behavior as social activity.

Studies on cannabinoid actions on non-aggressive social behavior are rare, but indicate that acute and chronic administration of  $\Delta^9$ -tetrahydrocannabinol reduces social interaction in rodents (Sieber, Frischknecht & Waser 1980; Van Ree, Niesink & Nir 1984) and induces social withdrawal and isolation in baboons (Sieber 1982). Additionally, a recent study showed reduced social interaction in cannabinoid receptor (CB1) knock-out mice (Haller *et al.* 2004). The mechanisms by which these cannabinoid-induced social impairments are mediated are unknown so far. Given the fact that the stress hormone corticosterone clearly affects social behavior (Tang *et al.* 2003), an interaction with stress-responsive systems might be involved. Cannabinoids are capable of stimulating the hypothalamus-pituitary-adrenal (HPA) axis (Manzanares, Corchero & Fuentes 1999) and chronic perinatal cannabinoid treatment increases corticosterone levels in adult rats (Navarro, Rubio & de Fonseca 1994). Therefore, modulations of the HPA-axis might be involved in the alterations in social behavior, seen after pubertal cannabinoid treatment.

### Social play behavior

The occurrence of social play behavior was recorded in the light period of the animals' cycle during homecage videotaping of spontaneous social behavior. Social play behavior is an activity common to juveniles of many mammalian and avian species and is a complex and early form of (non-mother directed) social behavior (Vanderschuren *et al.* 1997; Pellis & Pellis 1998). Social play has a considerable incentive value especially in juvenile rats and is important for the development of social skills and the selection of appropriate behavioral patterns (Vanderschuren *et al.* 1997).

In the present study, acute WIN treatment in pubertal animals on ED1 significantly increased playful activities. This untypical high expression of social play behavior under light conditions was not found on ED2 directly after chronic pubertal WIN treatment, but again as a permanent consequence 15 days later on ED3. Interestingly, a trend for increased play behavior after acute WIN treatment was also seen in adult rats on ED1. No alterations

on social play were found on ED2 and ED3 in adult cannabinoid-treated rats, indicating that chronic WIN treatment in adult rats does not induce any lasting alterations in social play behavior. These findings are consistent with our recent observation that chronic pubertal cannabinoid treatment persistently increases the percentage of play behavior during the light period (Schneider & Koch 2005). This effect could not be related to a general increase in motor activity since WIN-treated rats did not show an increase in overall play behavior and activity in the open field. We suggested that this unusual occurrence of play during the light phase after pubertal WIN treatment may be because of changes in the sleep-waking cycle. However, the excessive increase in play fighting directly after WIN administration in pubertal rats and the trend for such an increase in adult rats point more towards a direct play soliciting mechanism of WIN than alterations in circadian rhythm.

### Self-grooming

Self-grooming behavior was recorded additionally during the social interaction test and evaluation of spontaneous social behavior. Self-grooming is a complex instinctive behavior that is affected by individual experience to very small extent only. Care of the skin and fur protects the animal from injury and infections from ectoparasites and other agents and plays an important role in thermoregulation (Robertson *et al.* 1999).

Self-grooming was increased after pubertal cannabinoid treatment during social interaction on ED1 and ED3, whereas a decrease was found in the recording of spontaneous social behavior on ED2. Neither acute nor chronic cannabinoid administration in adult rats had any effect on self-grooming behavior. Therefore, the lasting cannabinoid effects observed in the present study on self-grooming behavior were also restricted to pubertal WIN treatment. The increase of self-grooming after chronic pubertal WIN treatment during interaction with an unknown conspecific may be interpreted as a displacement activity (McFarland 1982). The contact with the unfamiliar social partner might be experienced by WIN-treated rats as social stress (Blanchard *et al.* 2001) and therefore self-grooming might have served as a coping strategy. The reduction in self-grooming during homecage recording, which was only seen on ED2 24 hours after the last cannabinoid injection, may in contrast reflect an aversive state of withdrawal from chronic cannabinoid administration. As described before, social behavior during homecage recording in pubertal cannabinoid-treated rats on ED2 (withdrawal from all social activities among familiar conspecifics and no increase of social play) greatly differed from social activities on ED1 and ED3. It has been shown that chronic

cannabinoid administration results in reduced mesocumbal activity of dopaminergic neurons 24 hours after the last cannabinoid exposure (Diana *et al.* 1998), and this decrease in dopaminergic activity might be involved in the observed reductions in self-grooming, social behavior and the absence of play soliciting on ED2.

### CONCLUSIONS

In the present study acute and chronic WIN administration differentially impaired object and social recognition memory, altered social behavior and led to an inadequate increase in social play and self-grooming behavior in pubertal rats. Similar, albeit less pronounced, acute cannabinoid effects were found in adult rats. However, chronic cannabinoid treatment had no lasting behavioral consequences in adult rats, indicating the specificity of the pubertal development period as a vulnerable phase for the adverse effects of cannabinoids. In addition, indications for withdrawal-related behavior shortly after cessation of chronic WIN administration were observed exclusively in pubertal animals.

The present data indicate that an immature brain is more susceptible to the acute and chronic effects of exogenous cannabinoids than an adult organism. These findings might be explained by an upregulated endocannabinoid system and concomitant developmental disturbances in the maturation of further neurotransmitter systems during pubertal development.

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