Ontogeny of sensorimotor gating and short-term memory processing throughout the adolescent period in rats

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ABSTRACT

Adolescence and puberty are highly susceptible developmental periods during which the neuronal organization and maturation of the brain is completed. The endocannabinoid (eCB) system, which is well known to modulate cognitive processing, undergoes profound and transient developmental changes during adolescence. With the present study we were aiming to examine the ontogeny of cognitive skills throughout adolescence in male rats and clarify the potential modulatory role of CB1 receptor signalling. Cognitive skills were assessed repeatedly every 10th day in rats throughout adolescence. All animals were tested for object recognition memory and prepulse inhibition of the acoustic startle reflex. Although cognitive performance in short-term memory as well as sensorimotor gating abilities were decreased during puberty compared to adulthood, both tasks were found to show different developmental trajectories throughout adolescence. A low dose of the CB1 receptor antagonist/inverse agonist SR141716 was found to improve recognition memory specifically in pubertal animals while not affecting behavioral performance at other ages tested. The present findings demonstrate that the developmental trajectory of cognitive abilities does not occur linearly for all cognitive processes and is strongly influenced by pubertal maturation. Developmental alterations within the eCB system at puberty onset may be involved in these changes in cognitive processing.

1. Introduction

Adolescence, the transitional stage between childhood and adulthood, is characterized by highly dynamic processes of neuronal and behavioral adjustment. These developmental alterations are essential across species to allow for the maturation of adult social and cognitive skills, required to increase independence (Spear, 2000; Casey et al., 2008; Schneider, 2013). At the same time, adolescent neurodevelopment also comprises a critical period of vulnerability for unintentional injuries, suboptimal choices, and the emergence of various neuropsychiatric disorders (Merikangas et al., 2009; Paus et al., 2008; Kessler et al., 2007). Understanding the basis of psychiatric disorders therefore requires a comprehensive knowledge of how neurodevelopmental processes affect and modulate behavioral characteristics during adolescence. The onset of schizophrenia for example, a disorder which typically manifests during late adolescence or early adulthood, coincides with cognitive maturation of cortical areas and concomitant refinement of cognitive executive processes. A failure in these maturational events may thus play a critical role in the pathophysiology of schizophrenia (Catts et al., 2013).

In humans, the development of cognitive abilities displays heterogeneous trajectories. Some cognitive skills are already established in early childhood while other more complex functions continue to develop well into adolescence (Catts et al., 2013). Moreover, certain learning and cognitive processes seem to decline with the onset of puberty (Chung and Thomson, 1995; McGivern et al., 2002). Puberty refers to the restricted time period around mid-adolescence when sexual maturation is completed (Schneider, 2013). But notably, gonadal alterations in puberty and adolescent behavioral maturation are intimately linked in timing through multiple and complex interactions between neuronal developmental processes and gonadal steroid hormones (Sisk and Foster, 2004; Schneider, 2013).

Most ontogenetic investigations of cognitive processes in laboratory rodents have focused on the pre-weanling and weanling period. However, in recent years adolescence has also gained increasing attention, although longitudinal studies covering the

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complete adolescent period are still scarce. When testing adolescent animals for cognitive skills, one needs to consider that adolescent individuals differ notably from adults in their brain reward neuro-circuitry and in the way they process and respond to rewarding and aversive stimuli (Doremus-Fitzwater et al., 2010; Friemel et al., 2010). For the present study we therefore decided to focus on cognitive skills that can be assessed independently from negative/positive reinforcement in rodents and can be measured repeatedly in the same test animal, such as recognition memory and sensorimotor gating.

The spontaneous object recognition test was chosen for assessing the developmental trajectory of short-term memory processing. Recognition memory is generally regarded as the ability to discriminate the familiarity of items previously encountered. The test is based on the natural tendency of rodents to investigate and prefer novel objects, odors or social partners over familiar ones (Ennaceur and Delacour, 1988; Goepfrich et al., 2013; Schneider et al., 2008). Short-term recognition memory for objects has been shown to emerge quite early during postnatal development and appears to be completely functional in rats from postnatal day (pd) 17 on (Westbrook et al., 2014; Anderson et al., 2004). However, the development of recognition memory from pre-weaning ages through puberty until late adolescence has only been examined in part and still requires more detailed clarification.

Prepulse inhibition (PPI) is the natural reduction in the magnitude of the acoustic startle reflex (ASR) if a weaker, non-startling pre-stimulus is presented shortly before the startling stimulus. Since the ASR can be assessed equally well in humans as in laboratory rodents, modulations of the ASR show a high degree of face validity and translational value (Koch, 1999; Fendt and Koch, 2013). PPI has been suggested to provide a measure of sensorimotor gating, since it reflects the degree to which a motor reflex is gated by a preceding sensory stimulus (prepulse) (for review see Fendt and Koch, 2013). Sensorimotor gating is a fundamental protective mechanism that momentarily prevents or attenuates an overload of higher cortical areas with irrelevant information and hence protects ongoing cognitive processes against external interferences (Cromwell et al., 2008). Similar to recognition memory, comprehensive studies investigating the ontogeny of PPI throughout the complete adolescent period are still missing, since most studies investigated only selective time points during postnatal development and report partially contradictory findings. However, there is some evidence from studies in mice and rats that the ASR, as well as PPI increase with age (Pietropaolo and Crusio, 2009; Rybalko et al., 2015; Schwabe et al., 2007; Fendt et al., 2008).

The endocannabinoid (eCB) system is considered to be an ubiquitous regulator of synaptic transmission in the brain that mediates various central and peripheral processes (Kano et al., 2009; Castillo et al., 2012). The eCB signalling system comprises the G-protein coupled cannabinoid receptors (CB1 and CB2 receptor), the two main endogenous ligands N-arachidonoylethanolamide and 2-arachidonoylglycerol, as well as their synthetic and metabolic enzymes. This evolutionarily ancient and widely distributed neuromodulatory system is crucial for sustaining and restoring neuronal homeostasis (Kano et al., 2009) and in particular CB1 receptor signalling has emerged as a critical mechanisms for mediating cognitive behavior and neuroplasticity (Wotjak, 2005; Castillo et al., 2012). Moreover, a transient increase in CB1 receptor signalling has been reported to occur during adolescent brain development, which may provide increased plasticity and behavioral flexibility required specifically during this developmental stage (Schneider et al., 2015). In line with these observations, pharmacological interference with the eCB system during puberty induces pronounced and persistent deficits in cognitive skills (e.g. Schneider and Koch, 2003; Schneider and Koch, 2007; Schneider et al., 2008). Given the modulatory role of CB1 receptor signaling on adolescent behav-

ior, neuroplasticity, and cognition, the eCB system represents an interesting target system for mediating potential developmental changes in cognitive abilities such as object recognition and prepulse inhibition.

With the present study we aim to clarify the trajectory of short-term recognition memory and sensorimotor gating throughout the complete period of adolescence, including pubertal time points, in male rats. In male rats a peripubertal period can be defined from ∼pd 38, shortly before the physiological onset of puberty (around pd 40), until pd 60 (Schneider, 2013). In contrast to puberty, the exact timing of adolescence is rather difficult to define. Per definition, adolescence covers the complete time span from childhood (shortly before puberty) to adulthood, including the pubertal period and hence, pubertal timing represents so far the only clear reference point. The adolescent period should start before the onset of puberty, shortly after weaning, and should extend well into young adulthood, after completion of sexual maturity (Schneider, 2013; Schneider, 2008). Therefore, behavioral performance in male rats was assessed repeatedly every 10th day from pd 30 until late adolescence on pd 70, and again in adult animals on pd 130. Additionally, we examined if pharmacological inhibition of CB1 receptor signaling by the antagonist/inverse agonist SR141716 at different developmental stages would affect behavioral performance differentially. Since CB1 receptor signaling and availability appears to be enhanced during adolescence, we here selected a sub-threshold dose of SR141716 (0.3 mg/kg) which has been previously shown not to affect behavior in adult animals (Schneider et al., 2015).

2. Material and methods

2.1. Animals

120 male Wistar Han (Wistar) rats with known birth dates (date of birth considered as pd 0) were purchased from Harlan Laboratories (Netherlands). They were delivered shortly after weaning and then housed in groups of four to six in MakrolonTM cages (Euro-standardtype IV) under a 12/12 h light-dark cycle. Animals had ad libitum access to food and tap water. All experiments were conducted in accordance with the ethical guidelines of the National Institutes of for the care and use of laboratory animals and were approved by the local animal care committee (Regierungspräsidium Karlsruhe, Germany).

2.2. Experimental design

To investigate the developmental trajectory of cognitive abilities during adolescence, one cohort of animals (n = 16) was tested repeatedly for object recognition memory and prepulse inhibition (PPI) of the acoustic startle reflex (ASR) every ten days throughout the entire period of adolescence from pd 30 to pd 70 as well as in adulthood (pd 130) (Schneider, 2013). 24 h prior to the first test session on pd 30 all animals were habituated once for 15 min to the open field arena. On the test day, animals were first tested for object recognition memory, followed 10 min later by PPI assessment. Preliminary findings in our lab indicated no confounding effects of this testing sequence. After a break following pd 70, animals were then re-tested again in the same test sequence after reaching adulthood at pd 130. A second cohort of adult animals (n = 16; >pd 100) was used as a control group to test for a potential impact of repeated testing on behavioral performance. This group was tested for object recognition memory and PPI of the ASR three times every ten days at the same intervals as the adolescent cohort.

In a second experiment we examined developmental effects of pharmacological CB1 receptor inhibition on cognitive performance on three selected time points: pd 30, 40, and 130. These

time point were chosen based on the findings in the basal development study described above. First we performed a dose-response experiment to identify a subthreshold dose of the CB1 receptor antagonist/inverse agonist SR141716 (SR), which would not affect behavioral performance in adult animals. We therefore tested one cohort of adult (>pd 100) animals for changes in the ASR amplitude (VEH n = 10; SR 0.3, 0.6, and 1 mg/kg n = 8 each). Here, 0.3 mg/kg SR was identified as the most suitable dose. Subsequently, three cohorts of animals, one for each age of interest, were tested for object recognition memory and PPI (as described above) after receiving either an acute injection of SR or the respective vehicle (VEH) (pd 30: VEH n = 12; SR n = 11; pd 40: VEH n = 15; SR n = 17; pd 130: VEH n = 18; SR n = 14).

2.3. Behavioral testing

Behavioral testing was performed between 10:00 am and 5:00 pm during the light cycle. Behavioral performance during the object recognition test was recorded and rated offline by a trained observer blinded to group assignment.

2.3.1. Object recognition test

Short-term memory for objects was assessed with the object recognition test in an open field (50 cm × 50 cm × 45 cm) as described previously (Goepfrich et al., 2013). Briefly, the test consisted of an initial 3 min sample (P1) and a 3 min discrimination phase (P2) which were separated by an inter-trial interval (ITI) of 15 min. 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 min for object discrimination in P2 and now exposed to the familiar object (A', an identical copy of the object presented in P1) and a novel test object (B). Duration of exploration time of the objects (sniffing, touching with whiskers, and licking) was recorded [s] during P1 and P2. Sitting beside or standing on top of the objects was not scored as object investigation. For the calculation of percentage object discrimination the exploration time of the novel object was expressed as percentage of the total exploration time of both objects during P2 [100/((A' + B) × B)].

2.3.2. Prepulse inhibition (PPI) of the acoustic startle reflex (ASR)

PPI was measured in a startle chamber (SR-LAB; San Diego Instruments, San Diego, USA) as described in detail before (Goepfrich et al., 2013). Shortly, a white noise pulse with an intensity of 115 dB sound pressure level (SPL) and a duration of 40 ms (0 ms rise/fall times) was used as a startle stimulus. Four pulses with different intensities (72, 76, 80, 84 dB SPL, duration 20 ms) were used as prepulses, and were presented 100 ms before the startle stimulus. The background noise was a white noise of 65 dB SPL. The PPI program consisted of an acclimatization period (background noise for 5 min), an initial startle exposure (5 trials) and the test period. The test program comprised six different trial types in a pseudorandomized order: startle pulse alone, startle pulse preceded by prepulses of different intensities (see above) or background noise alone. The ITI varied between 10 and 20 s. All combinations were presented 10 times. PPI was calculated as the percentage decrease of the ASR magnitude in trials when the startle stimulus was preceded by a prepulse: 100 − (100* mean ASR amplitude on prepulse-pulse trials/mean ASR amplitude on pulse alone trials).

2.4. Drugs

SR 141716 (SR) (generously provided by NIMH) was dissolved in ethanol and Tween80 and then diluted with saline (1:1:18). SR was administered intraperitoneally (i.p.) at a dose of 0.3 mg/kg 30 min before behavioral testing with an injection volume of 1 ml/kg.

2.5. Statistical analysis

In experiment 1 no age differences were detected for the different prepulse intensities and hence, PPI data were lumped together and calculated as mean PPI values. Differences in the cognitive development of adolescent rats and repeated testing in adult animals for object exploration times, mean PPI and ASR amplitudes were analyzed by repeated measure (RM) ANOVA and by subsequent time point comparisons using Fischer LSD tests. The percentage object discrimination of each assessment was tested against chance level (i.e. 50%) using a oneway ANOVA, followed by a Fischer LSD test for post-hoc comparisons. Dose-response effects of SR were analyzed by a one-way ANOVA followed by post hoc Dunnett's tests for pairwise comparisons. Effects of SR on recognition memory were analyzed for each age and drug treatment group by using oneway ANOVAs that each included a control group which was set to 50, followed by a Fischer LSD test for post-hoc comparisons. Effects on exploration times were analyzed separately for each age group with Student's *t*-tests. Effects on PPI were assessed by one-way RM ANOVA for each cohort separately. All data are expressed as means ± SEM. The overall level of statistical significance was defined as p ≤ 0.05 and p < 0.1 for a statistical trend. All statistical analyses were run on IBM SPSS 22 (IBM, Armonk, USA).

3. Results

3.1. Ontogeny of short-term memory in the object recognition task

The statistical analysis of object recognition revealed significant differences throughout adolescent development in the animal's ability to discriminate a novel from a familiar object (Fig. 1A). Testing the percentage object discrimination of each time point against chance level with Fischer LSD test (subsequent to ANOVA F6,105 = 2.6) revealed successful discrimination performance (above chance level) at pd 30 (p = 0.04) and pd 130 (p = 0.03) but impaired recognition performance at all other ages. Deficient object recognition which did not differ from chance level was observed throughout puberty on pd 40 (p = 0.46), pd 50 (p = 0.94), and pd 60 (p = 0.19). During young adulthood short-term memory performance was still impaired, although less pronounced than during puberty, since the difference in behavioral performance from chance level reached only a statistical trend at pd 70 (p = 0.09).

Exploration times of the objects during P1 also differed significantly over the time course of the experiment (Fig. 1B; F4,59.4 = 10.48 p ≤ 0.001). Object exploration was highest during early adolescence, lower during late adolescence, and lowest exploration times were detected in adulthood (pd 130). Post-hoc analysis confirmed significantly lower scores in adulthood compared to all other time points measured (p ≤ 0.001). Throughout adolescence, exploration was highest at pd 30 (p ≤ 0.05) and 40 (p ≤ 0.04) compared to late adolescence (pd 60 and 70).

Repeated testing in adult control animals revealed normal and unchanged object recognition behavior with percentage object discrimination indices significantly above chance level across all testing days (Fig. 2A) (F3,60 = 2.5; Fischer LSD p = 0.05; p = 0.02; p = 0.04, respectively). Exploration times did also not differ between the three repeated test sessions (Fig. 2B) (F2,30 = 1.93; p = 0.16).

3.2. Ontogeny of sensorimotor gating

No age-specific effects in percent PPI with respect to the 4 different prepulse intensities tested were detected, and hence PPI
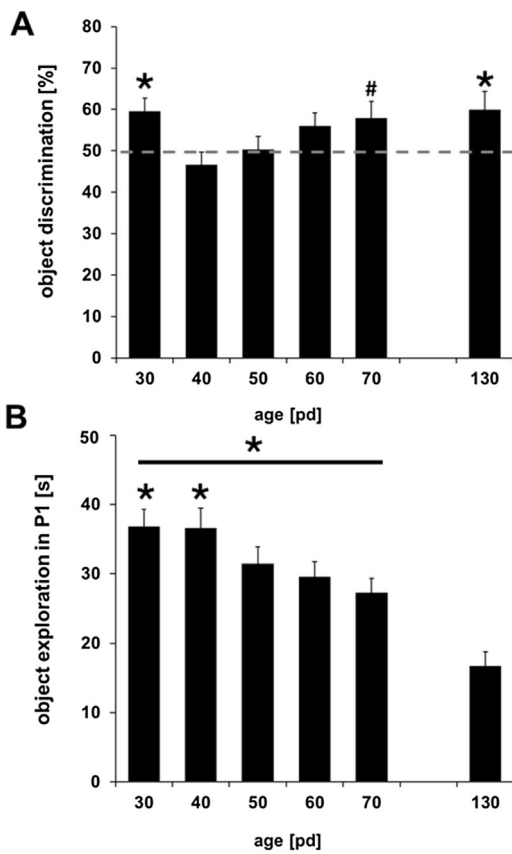
Fig. 1. Developmental trajectory of short-term object recognition memory throughout adolescence (pd 30–70) until adulthood (pd 130). The ability for successful object discrimination (above chance level 50%) was already fully developed on pd 30. However, performance levels declined afterwards and remained disturbed throughout puberty from pd 40 to 60 and recovered again at young adulthood around pd 70 where only a trend for deficient discrimination was observed (A). Object exploration times were highest around pd 30 and 40 and gradually declined until pd 130 (B). Data are expressed as mean + S.E.M. (★ p < 0.05, # p < 0.1; n = 16).

Fig. 2. Repeated testing for short-term object recognition memory in adult control animals. Repeated testing had no significant effect on behavioral performance in adult rats for object discrimination (A). Discrimination values differed significantly from chance level at all time points tested. No differences were found for object exploration times (B). Data are expressed as mean + S.E.M. (n = 16).

values were calculated as mean PPI. The mean PPI of the ASR varied significantly over the time course of development ($F_{5,75} = 33.21$, p < 0.001) (Fig. 3A). PPI increased over time with lowest performance levels being detected on pd 30. PPI at pd 30 differed significantly from all other time points tested ($p \leq 0.001$), while highest PPI performance was reached in adulthood on pd 130 ($p \leq 0.001$) compared to all adolescent ages. PPI at pd 40 differed significantly from pd 30, 50, 60, and 70 (p < 0.05), while PPI on pd 50 differed from pd 40 and 70 ($p \leq 0.037$), as well as pd 60 from 70 (p = 0.046).

The ASR amplitude also differed during development ($F_{5,75} = 20.74$, p < 0.001) and increased with age (Fig. 3B). ASR on pd 30 as well as on pd 40 was significantly lower than ASR at all other ages tested ($p \leq 0.001$). Highest ASR amplitudes were measured on pd 130 in adulthood ($p \leq 0.001$).

Adult control animals displayed no significant alterations of mean PPI values (Fig. 4A) ($F_{2,30} = 0.54$, p = 0.53) nor ASR amplitude (Fig. 4B) ($F_{2,30} = 0.54$, p = 0.86) over three repeated test sessions.

3.3. Effects of pharmacological inhibition of CB1 receptor signaling on cognitive performance during adolescence and adulthood

In a first experiment we were aiming to identify a suitable subthreshold dose of the CB1 receptor antagonist/inverse agonist SR which would not affect behavioral performance in adult rats. Adult animals were tested for effects of various doses of SR on the
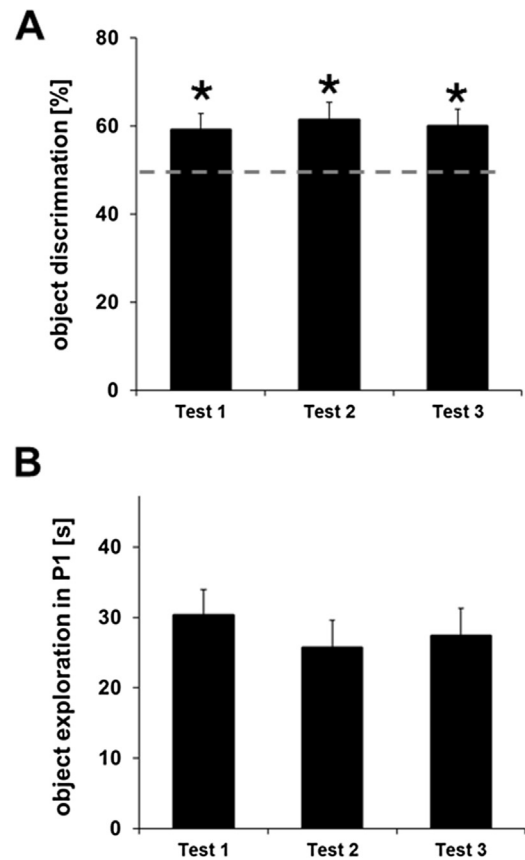
ASR amplitude (Fig. 5). SR affected the ASR in a dose-dependent manner ($F_{3,30} = 6.0$, p = 0.002). Post-hoc comparisons revealed the most pronounced ASR deficit after acute injection of 1 mg/kg SR compared to vehicle treated controls (p = 0.002), and a trend for an attenuated ASR in animals treated with 0.6 mg/kg (p = 0.067). Behavioral performance after injection of 0.3 mg/kg SR did not differ significantly from vehicle treated rats (p > 0.05).

Since a dose of 0.3 mg/kg SR was found not to affect the amplitude of the startle reflex, and has also been shown not to affect other behaviors in our previous work (Schneider et al., 2015), this dose was deemed suitable for further cognitive performance testing. Acute SR injections did not affect object recognition memory in prepubertal or adult rats as percentage object discrimination was significantly above chance level in both groups at pd 40 ($F_{2,30} = 10.2$; Fischer LSD p < 0.001; p = 0.003 for vehicle and SR, respectively) and at pd 130 ($F_{2,39} = 13.0$; Fischer LSD p < 0.001; p < 0.001 for vehicle and SR, respectively) (Fig. 6). However, deficient object discrimination on pd 40 ($F_{2,39} = 9.0$; Fischer LSD p = 0.31) could be improved significantly by application of 0.3 mg/kg SR (p < 0.001) (Fig. 6 B). Exploration time during P1 was significantly reduced on pd 30 in SR compared to vehicle treated animals (Fig. 6D) ($T_{21} = 5.736$; p < 0.001). No differences in exploration time during P1 were observed for any other time point ($T_{30} = 0.609$; p = 0.55 on pd 40 and $T_{30} = 0.771$; p = 0.45 on pd 130).

SR treatment did not alter PPI performance at any time point tested (Fig. 7). As expected, the PPI response differed significantly across various prepulse intensities (pd 30: $F_{3,66} = 23.4$, $p \leq 0.001$; pd 40: $F_{3,93} = 45.6$, $p \leq 0.001$; pd 130: $F_{3,90} = 80.1$, $p \leq 0.001$) but we did not detect an interaction effect for the treatment condition
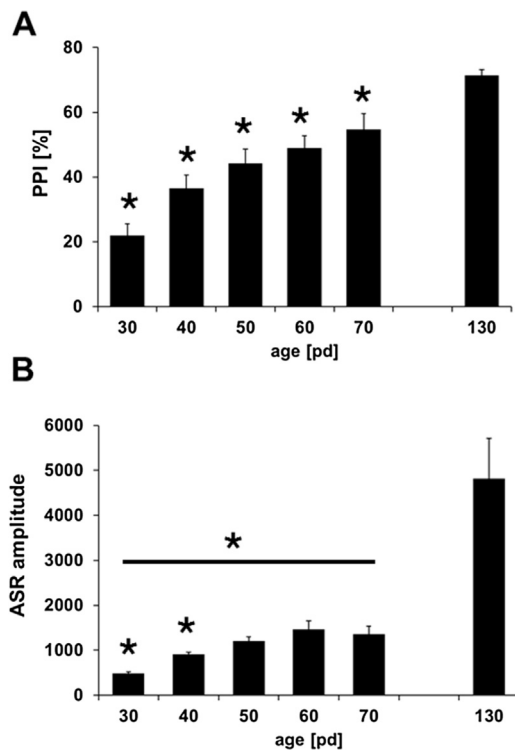
Fig. 3. Developmental trajectory of PPI of the ASR throughout adolescence (pd 30–70) until adulthood (pd 130). PPI values, calculated as mean PPI over the 4 pre-pulse intensities assessed, showed a gradual increase throughout adolescence until adult performance levels were reached at pd 130 (A). Similarly, ASR amplitudes were significantly lower during the adolescent period than after reaching adulthood. Data are expressed as mean + S.E.M. (★ $p < 0.05$, n = 16).

(pd 30: $F_{3,66} = 1.99$, $p = 0.12$; pd 40: $F_{3,93} = 0.17$, $p = 0.92$; pd 130: $F_{3,90} = 80.1$, $p = 0.97$). No statistical differences were detected on pd 30 ($F_{1,22} = 2.08$, $p = 0.163$), pd 40 ($F_{1,31} = 0.003$, $p = 0.95$) nor pd 130 ($F_{1,30} = 2.05$, $p = 0.16$) between the treatment groups. In line with the dose-response experiment, we also observed no statistical differences in ASR amplitudes between the treatment groups at any time point tested (Students's t-test for pd 30, 40, and 130 respectively, $p \geq 0.2$) (Fig. 7D–F).

4. Discussion

With the present study we assessed the ontogeny of two distinct cognitive abilities throughout the period of adolescence – sensorimotor gating and short-term recognition memory for objects. We observed pronounced differences in developmental trajectories of both processes in male rats. While the ability for sensorimotor gating increased gradually over adolescence, short-term recognition memory for objects was found to show a non-linear developmental pattern. Our findings further implicate the transient elevation of CB1 receptor signaling with beginning of puberty as potential modulator of the trajectory of short-term memory processing in pubertal rats, while developmental changes in the eCB system appear not to impact on the ontogeny of sensorimotor gating.

4.1. Developmental trajectory of short-term object recognition memory

We observed an interesting developmental pattern of short-term recognition memory processing in adolescent rats. In line with previous studies (Jablonski et al., 2013; Westbrook et al., 2014; Ainge and Langston, 2012; Heyser and Ferris, 2013; Reger et al., 2009) the ability to discriminate a familiar from a novel object
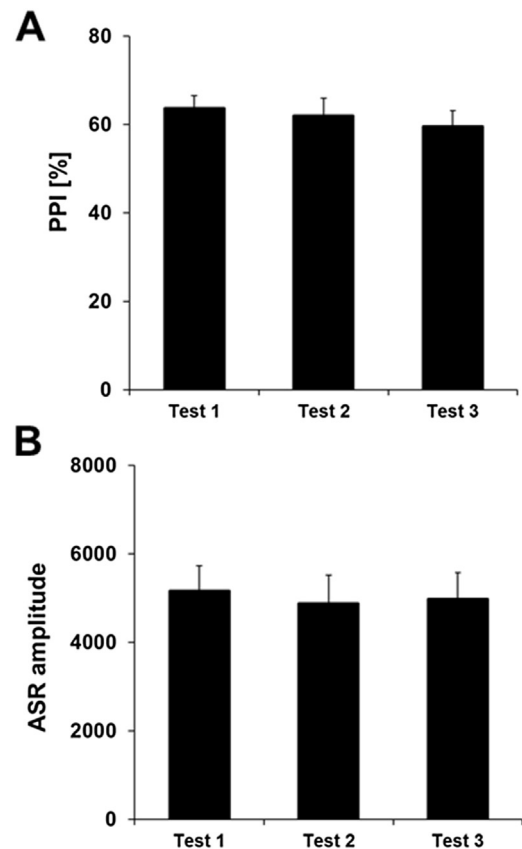

Fig. 4. Repeated testing for PPI of the ASR in adult control animals. Repeated testing had no significant effect on behavioral performance in adult rats for mean PPI (A), and ASR amplitudes (B). Data are expressed as mean + S.E.M. (n = 16).

over a short-term ITI was well established in juvenile male rats at pd 30. However, recognition memory performance dropped on pd 40, around the onset of puberty in male rats, was affected until the end of puberty around pd 60. A slight recovery with only a trend for unsuccessful discrimination (below chance level) was observed in young adulthood on pd 70. Successful object discrimination was detected again on pd 130. A control experiment in a different cohort of adult animals confirmed that repeated testing per se did not affect behavioral performance in the object recognition task, indicating that the performance deficits from pd 40 to pd 60 indeed represent specific age effects. Although object exploration times differed significantly throughout the time points tested, with animals showing enhanced object exploration rates as adolescents compared to adulthood, this effect appears to be unrelated to the memory deficit from pd 40–60, since exploration times were equally high on pd 30 and pd 40. Notably, a similar drop in memory functions around puberty onset as described here, has been reported previously in humans. Children's ability to recognize unfamiliar faces improves with increasing age, however, several studies observed a temporary disruption in face recognition memory at the onset of puberty (for review see Chung and Thomson, 1995). A similar dip in cognitive performance around puberty was also found in a match-to-sample task (McGivern et al., 2002). Moreover, a study in mice reported learning deficits in a spatial avoidance task to occur with puberty onset (Shen et al., 2010). The authors linked the behavioral finding to the pubertal emergence of inhibitory $\alpha 4\beta d$ γ-aminobutyric acid type A (GABAA) receptors, which appear to shape developmental plasticity by modulating hippocampal long-term depression.

Our present findings in Wistar rats are further in line with a recent study in mice (Molenhuis et al., 2014), where male mice
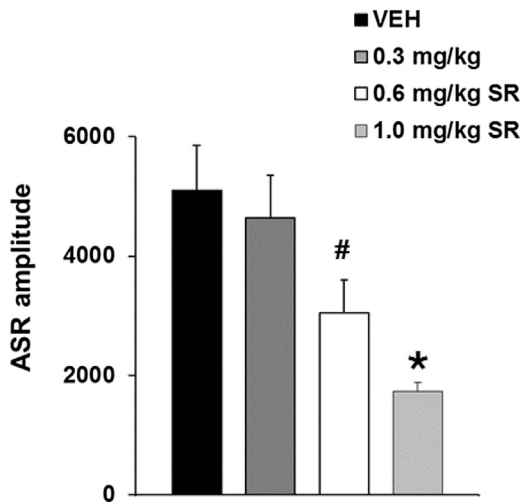
Fig. 5. Dose-response effects of the CB1 receptor antagonist/inverse agonist SR141716 on the ASR. Acute injections of 1 mg/kg significantly affected the ASR, while 0.3 mg/kg had no effect. Data are expressed as mean + S.E.M. (★ $p < 0.05$, # $p < 0.1$; VEH n = 10, SR 0.3, 0.6 and 1 mg/kg n = 8).

of different strains showed diminished object recognition memory around puberty (pubertal onset in male mice: pd 28–30) compared to adulthood. Of note, no such influence of puberty onset on discrimination performance was reported in other studies in rats (Reger et al., 2009; Heyser and Ferris, 2013; Cyrenne and Brown, 2011). Reger et al. (2009) failed to find any age differences in male rats, but used broad age classifications (pd 29–40 for adolescents; pd 50 for adults) which are likely to have masked more subtle age effects. Cyrenne and Brown. (2011) observed higher object novelty preference in male pubertal (pd 40) Lister Hooded rats compared to adult animals. However, object discrimination data are not provided and the ITI was rather short (2 min), compared to 15 min in our study and thus complicating direct comparisons. Finally, Heyser and Ferris (2013) report intact recognition memory around puberty onset (pd 42). However, for this study male and female Sprague Dawley rats were tested together at the same age. Since puberty in female rats is already completed at pd 42, mixing both sexes together might have covered potential impairments in males at this age. Additionally, the authors also used 2 sample trials before discrimination testing, which makes the results difficult to compare.

4.2. Developmental trajectory of PPI of the ASR

The longitudinal analysis of the development of PPI and baseline ASR revealed a gradual increase for both measures throughout adolescence until adulthood. Both, mean percentage PPI and the startle amplitude of adolescent animals (from pd 30–70) were significantly lower compared to pd 130, when the animals were retested in adulthood. Repeated testing in an adult cohort of rats did not reveal any variations in PPI and ASR magnitude, implicating that repeated testing every ten days and thus prolonged testing experiences neither influenced percentage PPI nor the startle amplitude per se. In contrast to humans, rat pups are born immature and their hearing develops postnatally. Auditory nerve-brainstem evoked responses have been recorded starting at about the age of pd 12–14 and adult thresholds are being reached on pd 22 (Geal-Dor et al., 1993). Accordingly, startle reactivity is already detectable in neonatal rats during the first weeks of life (Rybalko et al., 2015). Although various studies have addressed the ontogeny of PPI and the ASR (e.g. Engel et al., 2000; Romero et al., 2010; Pietropaolo and Crusio, 2009; Moran et al., 2016; Schwabe et al., 2007; Fendt et al., 2008), little is still known about the detailed

development of these measures throughout the complete adolescent period in rats. Most studies in rats and mice report a similar increase of ASR and PPI over the time points tested as reported in the present study. Engel et al. (2000) report a gradual increase in PPI over early adolescence (pd 18, 20, 22, 25, 29, and 40). The authors stated that adult PPI levels had been reached by pd 40. However, the adult levels, to which pd 40 PPI performance was compared to, were obtained from animals aged pd 58, thus from animals still categorized as mid-adolescent (Schneider, 2013). In contrast, our present findings indicate that PPI still continues to increase further until pd 130. In line with our findings, PPI levels have been found to increase from pd 35–70 in female and male Wistar rats, although no time points were assessed between pd 35 and 70 (Romero et al., 2010). Similarly, an increase in PPI and ASR magnitude was reported in female and male Sprague-Dawley rats from pd 34–38 to pd 84–86, although animals were not tested between pd 38 and 84 (Fendt et al., 2008). Schwabe et al. (2007) reported an increase in ASR magnitude over adolescence (pd 21, 35, 49, and 70), while PPI only increased in animals selectively bred for high PPI, whereas no significant increase was observed in animals bred for low PPI. Additionally, strain dependent effects on the gradual development of PPI throughout puberty (pd 21, 30, and 49) have been reported in mice, although the ASR amplitude was found to increase with age independent of the strain (Pietropaolo and Crusio, 2009). Notably, despite the basic robust finding of more pronounced PPI in adult relative to pre-weanling animals, studies have also pointed out the importance of the choice of sensory modalities (e.g. acoustic, tactile or visual), testing parameters and the test paradigm (gap PPI vs noise PPI), since each modality may display unique ontogenetic profiles (Moyer et al., 2015; Moran et al., 2016). The observed increase in ASR amplitude throughout adolescence might be linked at least to some extent to the physical development, increase in body weight and increasing muscle strength. But notably, Rybalko et al. (2015) report very different changes of ASR amplitude depending on the test frequency, and observed even a decrease in ASR amplitudes to high-frequency tones during ontogeny, indicating that body weight might not be the dominant factor influencing ASR amplitude. Instead, the authors suggested that frequency-dependent changes may result from the formation of behavioral salience associated with different acoustic stimuli during ontogeny.

From human studies it is well known that PPI is already present in human neonates and children and continuously matures in boys and girls from the 3rd to the 10th year of life (Gebhardt et al., 2012), although mid- and late-adolescent ages have not been further investigated.

4.3. Effects of pharmacological inhibition of CB1 receptor signaling during development on cognitive performance

In a final experiment we set out to examine a potential involvement of maturational processes in the eCB system on adolescent cognitive performance. Endocannabinoids act as retrograde messengers to suppress both excitatory and inhibitory synaptic transmission and thus, the eCB system is considered an ubiquitous neuromodulator in the central nervous system that is crucial for numerous forms of neuroplasticity (Kano et al., 2009; Castillo et al., 2012). Among the most renowned effects of plant derived and synthetic cannabinoids are alterations in learning and memory functioning, mediated mainly by the CB1 receptor (Wotjak, 2005). Interestingly, the CB1 receptor antagonist/inverse agonist SR 141716 was shown to reverse memory deficits caused by cannabinoids and even improve memory performance when administered alone (Terranova et al., 1996). Moreover, a transient increase in eCB signaling has been reported to occur during adolescent brain development, with peak levels of CB1 receptor expression in the striatum

and the medial prefrontal cortex being reached around puberty onset (Rodriguez de Fonseca et al., 1993; Klugmann et al., 2011; Schneider, 2008; Schneider et al., 2015). Accordingly, cannabinoid pharmacology exerts stronger effects in adolescent than adult animals (Schneider, 2008; Schneider et al., 2008; Cass et al., 2014) and sub-threshold doses of SR141716 were found to reduce behavioral characteristics of the pubertal period (e.g. high reward seeking behavior) to adult performance levels, implicating an involvement of the developing eCB system, and in particular the activity state and functionality of the CB1 receptor as a critical mediator of adolescent behavior (Schneider et al., 2015).

For the present study we examined the effects of a sub-threshold dose (0.3 mg/kg) of SR141716 on recognition memory and sensorimotor gating in pre-pubertal (pd 30), pubertal (pd 40), and adult (pd 130) animals. We have shown previously that treatment with cannabinoid agonists disrupts both cognitive processes in adult rats (Schneider and Koch, 2002), and even more pronounced in pubertal animals (Schneider and Koch, 2003; Schneider et al., 2008). If a transient increase in CB1 receptor signaling during puberty contributes to the specific developmental trajectory observed for short-term recognition memory and/or PPI, a controlled inhibition of this activity increase – to an extent that would not influence adult behavioral performance – should ameliorate age-dependent behavioral differences. In line with the developmental findings on recognition memory described in the first experiments, object discrimination did not differ from chance level in vehicle treated rats on pd 40. And indeed, we observed an improvement up to adult performance levels in pubertal rats in the ability to discriminate familiar from novel objects after acute injections of SR141716. As expected, sub-threshold CB1 receptor inhibition did not affect recognition memory performance in juvenile and adult animals. Although SR 141716 can be classified as a CB1 receptor antagonist, it has been well documented in various studies that SR behaves as an inverse agonist rather than a neutral antagonist. Thus, its biochemical or behavioral effects generally are opposite to the effects of cannabinoid agonists (Pertwee, 2005). Therefore, the higher availability of CB1 receptors in adolescent rats might well explain the increased behavioral effects of SR in these animals, considering its inverse cannabimimetic action at the receptor. We reported recently that increased sensitivity of CB1 receptors in Cnr1 mutant rats led to increased behavioral effects of a very low dose (0.3 mg/kg) of SR in these animals, while wild type rats with normal CB1 receptor activity remained unaffected by this low dose of SR (Schneider et al., 2015). Administration of SR141716 did also not affect object exploration during the sample phase in pubertal and adult rats, but notably exploration times were decreased at pd 30 in SR treated animals. This reduction in exploration times specifically on pd 30 was not related to adequate recognition performance, since the animal's capability to discriminate the objects remained unchanged, but points toward a pronounced pharmacological effect of SR 141716 in prepubertal rats on exploratory behavior. The detailed maturational changes of CB1 receptor expression throughout adolescence are still largely unknown. In our previous research we observed pronounced, region-specific changes in CB1 expression, sensitivity, and pharmacological reactivity, specifically around puberty onset (Schneider, 2008; Schneider and Koch, 2003; Schneider et al., 2015). However, previous studies also demonstrated a progressive increase in CB1 receptor binding during early adolescence, with region-dependent enhanced binding occuring already in prepubertal rats, although maximum values coincided with the approximate onset of puberty in female and male rats respectively (Rodriguez de Fonseca et al., 1993). Therefore, CB1 antagonism/inverse agonism may already induce stronger effects in prepubertal animals compared to adult rats. Why exploration times remained unaffected in pd 40 animals still remains to be clarified, but may depend on
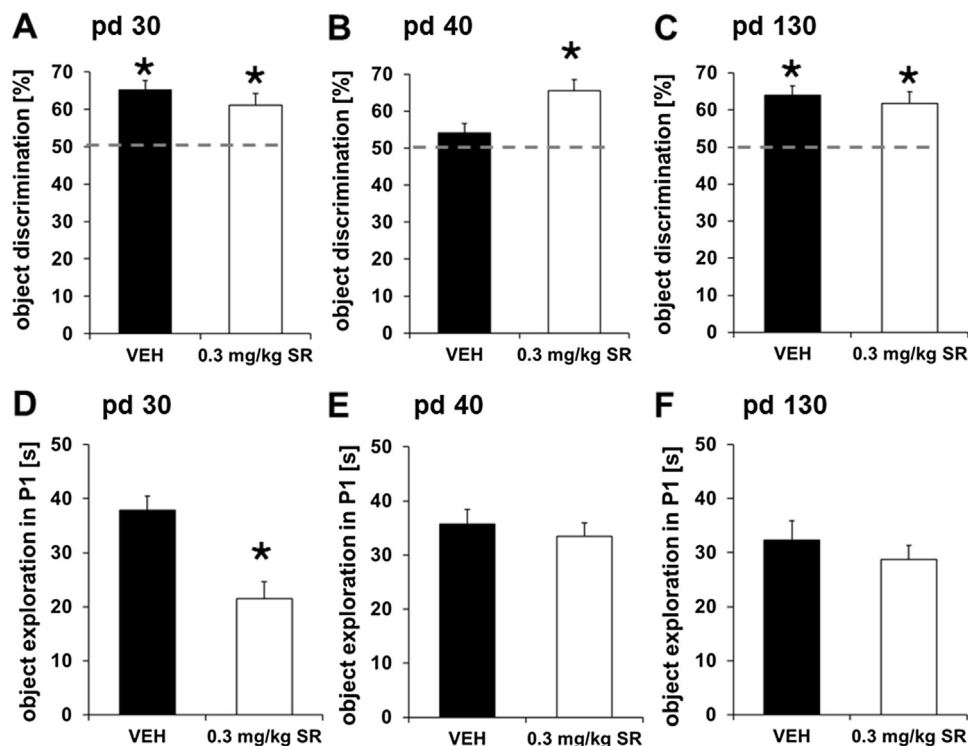

Fig. 6. Effects of SR141716 (SR) on percentage object discrimination in prepubertal (A), pubertal (B) and adult (C) animals and object exploration times (D–F). Object discrimination did not differ from chance level specifically on pd 40 (vehicle treated animals), while at all other ages animals showed successful object discrimination. Acute injections of a sub-threshold dose of SR on pd 40 were found to improve recognition memory significantly, while injections of the same dose had no effect on pd 30 and 130. Exploration time on pd 30 was significantly reduced in SR treated animals compared to vehicle-treated controls. No differences were observed for any other time point. Data are expressed as mean + S.E.M. (★ p < 0.05; pd 30: VEH n = 12, SR n = 11, pd 40: VEH n = 15, SR n = 17; pd 130 VEH n = 18, SR n = 14).
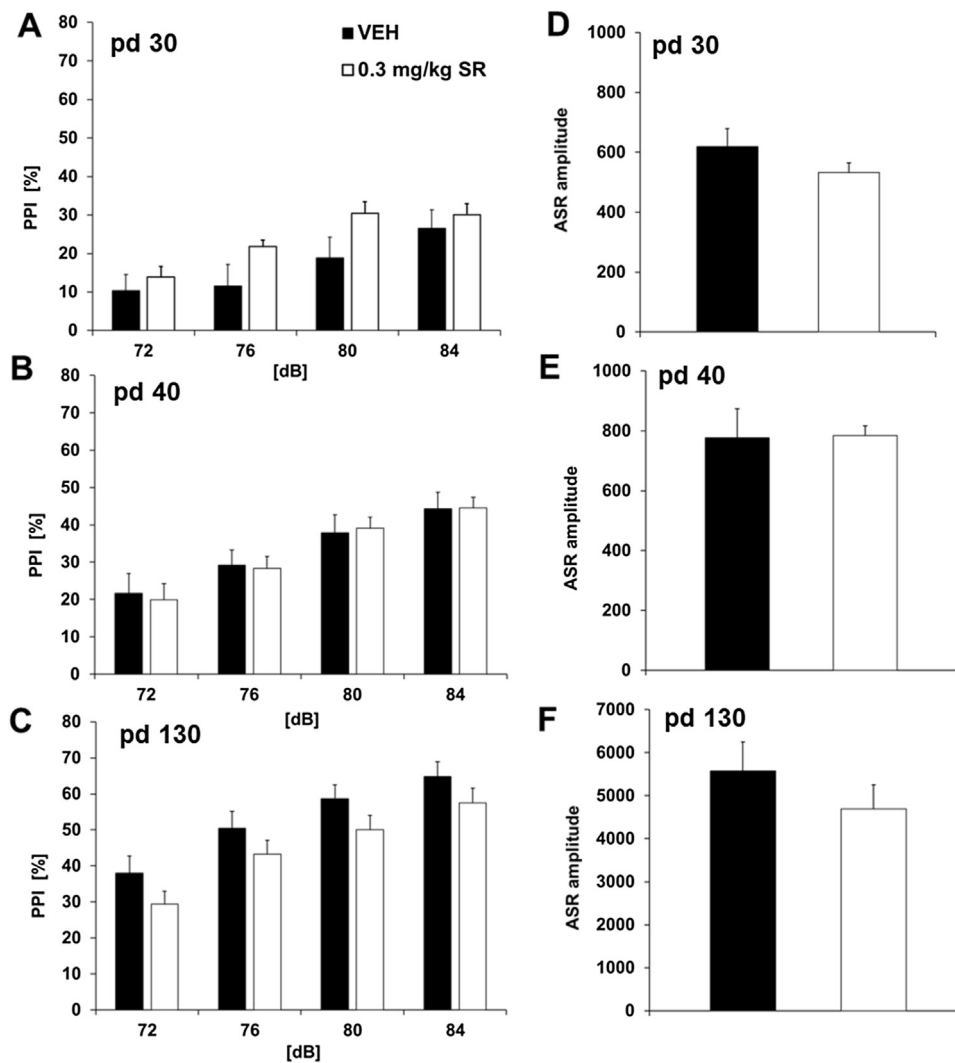
Fig. 7. Effects of SR141716 on PPI of the ASR in prepubertal (A), pubertal (B) and adult (C) animals and ASR amplitude (D-F). Acute injections of a sub-threshold dose of SR141716 did neither affect PPI nor ASR at any age tested. Data are expressed as mean + S.E.M. (pd 30: VEH n = 12, SR n = 11, pd 40: VEH n = 15, SR n = 17; pd 130 VEH n = 18, SR n = 14).

differences in the maturational timing of further neurotransmitter systems that are modulated by eCB signaling.

No significant effects of this subtle CB1 receptor inhibition were detected for sensorimotor gating and ASR amplitudes at any ages, indicating that sensorimotor gating appears to be less sensitive towards the maturational changes in eCB signaling with puberty.

4.4. Conclusion

We here report on different developmental trajectories for short-term memory processing and sensorimotor gating throughout adolescence in male rats. While recognition memory showed a non-linear trajectory with a transient performance decline around puberty onset, sensorimotor gating was shown to develop gradually. Although the ontogeny of both processes differs profoundly, the results highlight the importance of including the complete adolescent developmental period, including pubertal time points, in developmental studies, since behavioral performance may mature well into late-adolescence or early adulthood. Our findings further implicate maturational processes in the eCB system in the trajectory of short-term recognition memory, but not sensorimotor gating. Although both tests analyze cognitive abilities, each paradigm investigates different aspects of cognition and hence

involves different circuits and brain regions. Moreover, each test involves different sensory modalities (acoustic vs. visuo-tactile) that are processed by distinct sensory systems in the brain which also show divergent developmental trajectories (Moran et al., 2016). The present results imply that these two aspects of cognition appear to be differently influenced by eCB signaling during adolescence.

Conflict of interest

None.

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