Journal of Psychiatric Research 53 (2014) 1–7

Contents lists available at ScienceDirect

Journal of Psychiatric Research

journal homepage: www.elsevier.com/locate/psychires

CRHR1 links peripuberty stress with deficits in social and stress-coping behaviors

Vandana Veenit, Orbicia Riccio, Carmen Sandi*

Laboratory of Behavioral Genetics, Brain Mind Institute, School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

ARTICLE INFO

Article history:
Received 14 November 2013
Received in revised form
16 February 2014
Accepted 19 February 2014

Keywords:
Peripuberty stress
Corticotropin releasing hormone
NBI30775
Social behavior
Anxiety-like behavior
Stress-coping behavior

ABSTRACT

Stressful life events during childhood and adolescence are important risk factors for the development of psychopathologies later in life. The corticotropin releasing hormone (CRH) and the CRH receptor 1 (CRHR1) have been implicated in the link between early life adversity and adult anxiety and depression, with rodent studies identifying the very early postnatal period as highly susceptible to this programming. Here, we investigated whether stress exposure during the peripubertal period – comprising juvenility and puberty – is effective in inducing long-lasting changes in the expression of CRHR1 and CRHR2 in the hippocampus and amygdala, and whether treating animals with a CRHR1 antagonist following stress exposure could reverse behavioral alterations induced by peripuberty stress. We show that peripuberty stress leads to enhanced expression of the *Crhr1*, but not *Crhr2*, gene in the hippocampal CA1 and the central nucleus of the amygdala, in association with social deficits in the social exploration test and increased stress-coping behaviors in the forced swim test. Treatment with the CRHR1 antagonist NBI30775 (10 mg/kg) daily for 1 week (from P43 to P49), immediately following peripuberty stress exposure, prevented the occurrence of those psychopathological behaviors at adulthood. These findings highlight peripuberty as a period of plasticity for the enduring modulation of the CRHR1 system and support a growing body of data implicating the CRHR1 system in the programming effects of early life stress on eventual psychopathology. They also support recent evidence indicating that temporarily tackling CRHR1 during development might represent a therapeutic opportunity to correct behavioral trajectories linking early stress to adult psychopathology.

1. Introduction

Corticotropin releasing hormone (CRH) is critically implicated in the regulation of the physiological stress response and in the development of stress-related psychopathological behaviors (Steckler and Holsboer, 1999; Risbrough and Stein, 2006; Holsboer and Ising, 2008; Mathew et al., 2008; Binder and Nemeroff, 2010) including those induced by early adversity (Coplan et al., 1996; Nemeroff, 2004; Heim et al., 2008). The CRH family comprises several ligands (CRH and urocortins 1, 2 and 3) and two receptors, CRH receptor 1 (CRHR1, to which CRH and urocortin 1 bind preferentially) and the CRH receptor 2 (CRHR2), both present in the hypothalamus as well as in limbic brain areas including the amygdala and hippocampus (Binder and Nemeroff, 2010). In addition to its key role in modulating peripheral responses through the activation of the hypothalamus–pituitary–adrenal (HPA) axis, the CRH/CRHR1 system plays a critical role in mediating some types of anxiety and depression responses (Dunn and Berridge, 1990; Contarino et al., 1999; Steckler and Holsboer, 1999; Muller et al., 2003; Muller and Wurst, 2004; Todorovic et al., 2005; Ivy et al., 2010; Wang et al., 2012). Application of CRHR1 antagonists exerts anxiolytic and antidepressant-like effects in animals with high levels of anxiety and in animals that have been pre-exposed to stressors (Keck et al., 2001; Lancel et al., 2002; Sandi et al., 2008).

In rodents, early life stress – induced during the very early postnatal period by either maternal separation or unstable maternal care – was found to lead to increased anxiety-like behaviors and cognitive deficits in association with increased central CRH and CRHR1 expression (Plotsky et al., 2005; Fenoglio et al., 2006; Ivy et al., 2010; Wang et al., 2011, 2012). Manipulation of the CRHR1 system during development during the aftermath of early life stress exposure was found to prevent some of the long-term behavioral alterations observed at adulthood. Thus, in a model of unstable maternal care, either the administration of a CRHR1 antagonist in the developmental period following the maternal stress manipulation (Ivy et al., 2010) or a conditional

* Corresponding author.
E-mail address: carmen.sandi@epfl.ch (C. Sandi).

http://dx.doi.org/10.1016/j.jpsychires.2014.02.015
0022-3956/

forebrain CRHR1 deficiency occurring once the stressful episode has elapsed (Wang et al., 2011, 2012) were found to prevent the emergence of anxiogenic effects and cognitive impairments induced by unstable maternal care. These findings suggest that early interventions tackling the CRHR1 system following exposure to early life stress might be an effective approach to prevent the incidence of psychopathologies in later life. However, so far, these studies have involved very early stress manipulations occurring within the first two weeks of postnatal life and there is no information regarding the involvement of the CRHR1 system and the potential therapeutic value of CRHR1-targeted treatments when stress exposure takes place slightly later in development.

In fact, stress during childhood and puberty – termed hereafter as the peripubertal period – has been associated with psychopathology later in life (Heim and Nemeroff, 2001; Watt et al., 2009). The peripubertal period is a biological transitional phase involving adaptations in hormonal systems and neural circuits, including those related to stress and the development of emotionality (Spear, 2009; Romeo, 2010). In our lab, exposure of rats to stressful experiences (e.g., synthetic fox odor and exposure to an elevated platform) on scattered days during the peripuberty period (P28–P30, P34, P36, P40 and P42) was found to induce long-lasting effects on anxiety and stress-coping behaviors, including deficits in social behaviors (Toledo-Rodriguez and Sandi, 2011; Cordero et al., 2012; Marquez et al., 2013), as well as changes in metabolic responses in limbic brain regions including the hippocampus and the amygdala (Toledo-Rodriguez et al., 2012; Marquez et al., 2013). Here, we sought to investigate whether peripuberty stress would lead to alterations in the CRH system in limbic areas and whether pharmacological treatment with a CRHR1 antagonist during the immediate post-stress developmental period (i.e., adolescence) would reverse the long-term behavioral consequences of peripuberty stress.

2. Materials and methods

2.1. Animals

The study was conducted on male offspring of Wistar Han rats purchased from Charles River Laboratories, France, and bred in our animal house. After weaning at postnatal day P21, male rats from different litters were mixed throughout the different home cages (three per cage). Equivalent numbers of animals from each litter were placed in four groups (see below) and the placement of siblings within the same home cage was avoided. All animals were kept in constant conditions of humidity and temperature (22 ± 1 °C) with a 12-h light–dark cycle (lights on at 7:00 AM). Food and water were available *ad libitum*. All the procedures described were conducted in conformity with Swiss National Institutional Guidelines on Animal Experimentation and approved through a license granted by the Swiss Cantonal Veterinary Office Committee for Animal Experimentation.

2.2. Experimental design

Two experiments were performed (see experimental design in Fig. 1). The first one was conducted to evaluate the long-term consequences of peripuberty stress in the expression levels of CRHR1 and CRHR2 in the hippocampus and amygdala at adulthood. At adulthood, control and peripuberty stressed animals were decapitated under basal conditions. After decapitation, the brains were fast frozen in isopentane and then stored at −80 °C. The dissection of the different hippocampal subregions [dentate gyrus (DG), CA1 and CA3] and amygdala nuclei [central amygdala (CeA) and basolateral amygdale (BLA)] for gene expression analyses was performed by tissue punching in 200 μm slices sectioned on cryostat.

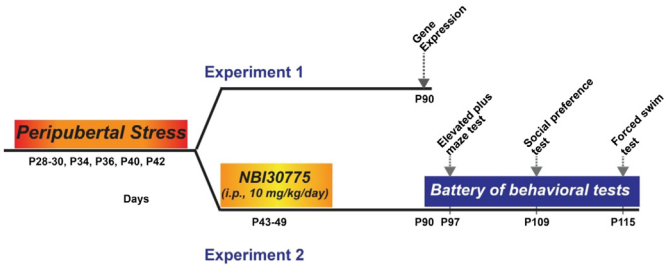

Fig. 1. Layout of the general experimental design: stressors were applied during the peripuberty period in both experiments 1 and 2. In experiment 1 (shown in upper panel), at adulthood (P90+), animals were sacrificed under basal condition and the brains were later utilized for gene expression study. In experiment 2 (shown in the lower panel), animals (both control and peripubertal stress) were injected (i.p.; vehicle or NBI30775) from P43 to P49. In adulthood (P90+), batteries of behavioral tests were performed in the order shown in the schematic.

The second experiment was performed to investigate the potential efficacy of treatment with the CRHR1 antagonist, NBI30775, during adolescence to counteract long-term behavioral alterations induced by peripuberty stress. We followed a 2×2 experimental design, with Stress and Drug as factors, which led to the following four groups: (1) Control-Vehicle, (2) Control-NBI30775, (3) Stress-Vehicle, and (4) Stress-NBI30775. Drug treatment was given intraperitoneally (i.p.) and daily from postnatal day P43 to P49. At adulthood, all animals were tested for anxiety-like and stress-coping behaviors, which included the elevated plus maze task; the social preference test, and the forced swim test. A resident–intruder test was also included but due to the corruption of a large number of video recordings of the encounters, data from the resident–intruder task are not included here. The minimum time elapsed between tests was 1 week.

2.3. Peripuberty stress paradigm

This protocol is based on exposure to fear-induction procedures (Marquez et al., 2013). Following exposure to an open field for 5 min on P28, the stress protocol consisted of presenting two different fear-inducing stressors (each one lasting 25 min): (1) exposure to the synthetic fox odor trimethylthiazoline (9 μl) (Phero Tech Inc., Delta, BC, Canada) released through a small cloth, in a plastic box (38 cm length, 27.5 cm width and 31 cm height) placed under a bright light (210–250 lx); and (2) exposure to an elevated platform (12 × 12 cm, elevated 95 cm from the ground) under direct bright light (470–500 lx). Following each stress session, the animals were returned to their home cages where, for 15 min, a transparent Plexiglas wall with holes separated each animal. The stressors were applied during the peripubertal period (a total of 7 days across postnatal day P28 to P42, i.e., on P28–P30, P34, P36, P40 and P42); during the light phase; and according to a variable schedule. The order and timing of the stressors were changed on different days. On some stress days, only one stressor was presented, while on other days, the two stressors were given consecutively. The control animals were handled on the days that their experimental counterparts were exposed to stress.

2.4. CRHR1 and CRHR2 mRNA analyses

Total RNA was isolated using the RNAqueous® Micro kit (Ambion) and cDNA was synthesized using the Superscript VILO kit (Life Technologies) according to the supplier's recommendations. For quantitative PCR, PCR reactions were performed in triplicate

using SYBR Green PCR Master Mix (Applied Biosystems) in an ABI Prism 7900 Sequence Detection system (Applied Biosystems). Two genes were used as internal controls: gamma-actin (actg1) and eukaryotic elongation factor-1 (eef1). *Crhr1* and *Crhr2* primers were designed using the Assay Design Center software from Roche Applied Science. The primer sequences are provided in Table S1. Gene expression was analyzed with qBase 1.3.5 software using the comparative cycle threshold method (Livak and Schmittgen, 2001).

2.5. Drug administration

The CRHR1 antagonist NBI30775 was a gift from Neurocrine Inc. It was administered intraperitoneally (i.p.) at 10 mg/kg in 4% v/v dimethyl-sulfoxide/0.5% w/v methocellulose (Sandi et al., 2008) daily from P43 to P49. The corresponding vehicle treatment was administered to control animals on the same days and times.

2.6. Elevated plus maze

Anxiety levels were evaluated using the elevated plus maze (EPM) test (Pellow and File, 1986). Briefly, the test consists of two opposing open arms (50 × 10 cm) perpendicular to two enclosed arms (50 × 10 × 50 cm) that extend from a central platform (10 × 10 cm), elevated 65 cm above the floor. The rats were placed individually on the central platform facing a closed arm and allowed to explore the maze for 5 min. Their behavior was monitored using a video camera and analyzed with a computerized tracking system (Ethovision 3.1.16, Noldus IT, The Netherlands). The percent time spent and the number (frequency) of entries in the center, open and closed arms were recorded. The entire apparatus was cleaned (with 1% acetic acid solution) and dried between each test.

2.7. Sociability test

The sociability test was adapted from the protocol described by Crawley and collaborators to investigate social affiliation in male mice (Moy et al., 2004). The test was carried out in a rectangular, three-chambered gray opaque polycarbonate box (a center 20 × 35 × 35 cm; a left and a right compartment 30 × 35 × 35 cm). Dividing walls had retractable doorways allowing access to each chamber. Left and right compartments contained a central Plexiglas cylinder (15 cm diameter), transparent and with small holes, where either a social (unfamiliar juvenile rat around 34 days old) or a non-social stimulus (yellow plastic bottle) was placed. The cylinder permits visual, tactile, auditory and olfactory communication. The juvenile rats used as social stimuli were first habituated to the three-chambered apparatus by placing them individually in the box within the Plexiglas cylinder for 10 min during the 3 consecutive days preceding the social test.

On testing day, the experimental rat was first placed in the middle chamber and allowed to explore for 5 min. The doorways into the two side chambers were closed during this habituation phase. After the habituation period, the unfamiliar juvenile was placed in one of the side chambers and the object on the other side. The location of the juvenile and the object in the left vs. right side chamber was counterbalanced. Next, both doors to the side chambers were carefully removed and the subject rat was allowed to explore the entire apparatus for a 10 min session. The session was video-recorded and the time spent sniffing each cylinder was manually scored by an experimenter blind to treatments to evaluate the level of preference for the unfamiliar juvenile compared to the object. A rat's exploration of object or juvenile conspecific (sniffing behavior) was defined by nose proximity to the cylinders at a distance less than 2 cm with the nose oriented toward the cylinders' contents (i.e. juvenile or object) as detected by a computerized tracking system (Ethovision 3.1.16, Noldus IT, The Netherlands) and confirmed with a computer software (Clicker, EPFL, Switzerland) by an experimenter unaware of animals' treatments. The entire apparatus was cleaned (with 1% acetic acid solution) and dried between each test.

2.8. Forced swim test

Rats were subjected to a forced swim test to evaluate stress-coping behavior (Porsolt et al., 1977) following the same conditions as in our previous study (Marquez et al., 2013). Briefly, animals were individually placed in a plastic beaker (25 cm diameter, 46 cm deep) containing 30 cm of water (25 °C) in two sessions. The first one lasted 15 min and the second one, performed 24 h later, lasted 5 min. Behavior was recorded with a video camera and the time spent floating (i.e., immobile with only those movements necessary to keep the snout above water) was quantified by an experimenter unaware of animals' treatments using a computer software (Clicker, EPFL, Switzerland).

2.9. Statistics

The SPSS 14.0 (SPSS, Chicago, IL) statistical package was used for statistical analyses. Results are expressed as the mean ± standard error of the mean. Data of animals with mean value beyond 3 standard deviations (SDs) from its respective group mean were removed from further analyses. Student's *t*-tests were performed between control and stress groups to assess differences in gene expression in specific brain regions. Data from Experiment 2 were analyzed using two-way ANOVA with Stress (control vs. stress) and Drug (vehicle vs. NBI30775) as factors. *Post hoc* analyses involved Student's *t*-tests as appropriate. Significance was set at $p < 0.05$.

3. Results

3.1. Peripuberty stress leads to increased Crhr1 expression in hippocampal CA1 and central nucleus of the amygdala

Student's *t*-tests revealed higher expression of *Crhr1* mRNA in the CA1 subfield of the hippocampus in peripuberty stress animals as compared to controls (Fig. 2A; $t = -2.24$, $df = 14$, $p < 0.05$), while no significant changes were observed in the CA3 (Fig. 2B; $t = -0.55$,

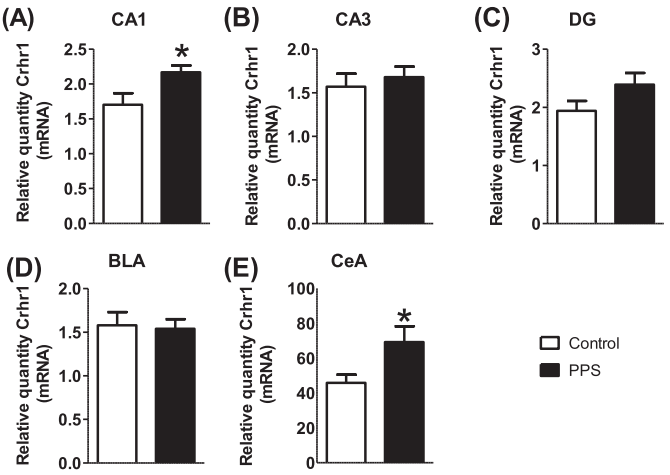

Fig. 2. Impact of peripubertal stress on *Crhr1* gene expression in various hippocampal areas [CA1 (A), CA3 (B) and DG (C)] and amygdala nuclei [BLA (D) and CeA (E)]. The relative mRNA quantity of *Crhr1* was significantly different between control and PPS in CA1 (A) and CeA (E). *$p < 0.05$. PPS, peripubertal stress. $N = 6–9$/group.

df = 13, p = n.s.) or dentate gyrus (Fig. 2C; $t = -1.75$, df = 13, p = n.s.). In the amygdala, Student's t-tests indicated no effect of peripuberty stress on *Crhr1* mRNA expression in the basolateral amygdala (Fig. 2D; $t = 0.20$, df = 12, p = n.s.), but a significant increase in the central nucleus of the amygdala (Fig. 2E; $t = -2.44$, df = 12, $p < 0.05$). No difference between groups was found for *Crhr2* mRNA expression in any of the analyzed hippocampal subfields or amygdala nuclei (Table S2).

3.2. Post-stress treatment with the CRHR1 antagonist affects some behaviors in the elevated plus maze

In our previous study, peripuberty stress was found to lead to mild, though significant, changes in the elevated plus maze (Marquez et al., 2013). In contrast to Marquez et al., 2013, this study involved daily injections during the peripuberty period on both control and stress animals, which might have affected the programming of certain anxiety-like behaviors, as results show a tendency in the same direction that only reaches significance in some parameters (Fig. 3). For example, ANOVAs of the percent time spent by the animals in each of the relevant compartments (i.e., center, closed or open arms) of the apparatus reveal a lack of significant effect for each of the factors (i.e., Stress and Drug) or their interaction for all compartments (Fig. 3A; all $p > 0.5$), with a tendency toward significance for the Stress factor in the percent time spent in the center [$F(1, 31) = 3.21$, $p = 0.08$]. Similar results are obtained for the distance traveled in each of the compartments (Fig. 3B), although for the distance moved within the center there is a significant Stress × Drug interaction [$F(1, 31) = 5.77$, $p < 0.05$]. Post hoc analyses revealed that Stress-Vehicle animals spent less time in the central compartment than Control-Vehicle ones ($p < 0.05$), with the difference between Stress-Vehicle and Stress-NBI30775 groups showing a tendency toward significance ($p < 0.1$). Values from Stress-NBI30775 and Control-Vehicle animals did not significantly differ (n.s.). No differences between groups were found in other parameters analyzed (e.g., transitions between zones or total distance); these data are displayed in Table S3.

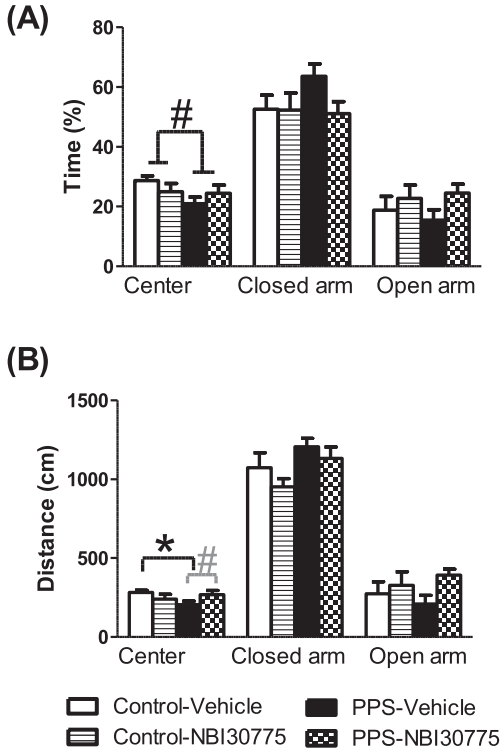

Fig. 3. Effects of NBI30775 injection from postnatal P43 to P49 on anxiety-like behavior during elevated plus maze in adulthood. (A) Percentage of time spent in various arms (center, closed arm and open arm). (B) Distance traveled in various arms (center, closed arm and open arm). The results are expressed as mean ± SEM; $^{*}p < 0.05$; $^{\#}p < 0.1$; $N = 9$ for Control-Vehicle, Control-NBI30775, PPS-NBI30775 and 8 for PPS-Vehicle.

3.3. Post-stress treatment with the CRHR1 antagonist reverses social deficits induced by peripuberty stress

A two-way ANOVA on total duration of juvenile exploration (Fig. 4) revealed a significant effect for the Stress × Drug interaction [$F(1, 31) = 7.64$, $p < 0.05$], but not for each of the factors, Stress [$F(1, 31) = 0.32$, n.s.] or Drug [$F(1, 31) = 0.08$, n.s.] separately. Post hoc analyses reveal that the Stress-Vehicle group spent significantly less time exploring the juvenile than did the Control-Vehicle ($p < 0.05$) or the Stress-NBI30775 ($p < 0.05$) groups. Control animals treated with NBI30775 show a non-significant trend to explore juveniles less than controls treated with vehicle ($p < 0.1$). A two-way ANOVA for total duration of object exploration indicated no effect of Stress [$F(1, 32) = 0.15$, n.s.], Drug [$F(1, 32) = 0.05$, n.s.] or Stress × Drug interaction [$F(1, 32) = 0.58$, n.s.]. Therefore, the results of this experiment suggest that administration of NBI30775 in peripuberty stress animals during a developmental period right after peripuberty alleviates the long-term effects of stress on sociability.

3.4. Post-stress treatment with the CRHR1 antagonist reverses certain alterations induced by peripuberty stress in the forced swim test

The forced swim test included two sessions on two consecutive days (Fig. 5). On the first day, a two-way ANOVA on the time spent floating revealed significant effect of Stress × Drug interaction [$F(1, 32) = 9.74$, $p < 0.05$], with no effect observed for Stress [$F(1, 32) = 1.63$, n.s.] or Drug [$F(1, 32) = 2.60$, n.s.] separately. Post hoc analyses revealed that all experimental groups spent more time floating than the Control-Vehicle group (all $p < 0.05$). On day 2, a two-way ANOVA revealed significant effects of the Drug [$F(1, 32) = 5.93$, $p < 0.05$] and Stress × Drug interaction [$F(1, 32) = 13.30$, $p < 0.05$], while no effect for the Stress factor [$F(1, 32) = 0.93$, n.s.] was observed. Post hoc analyses revealed that floating duration in the Stress-Vehicle groups is significantly longer than in each of the other experimental groups (all $p < 0.05$). Together, these results indicate that NBI30775 treatment stems behavioral alterations

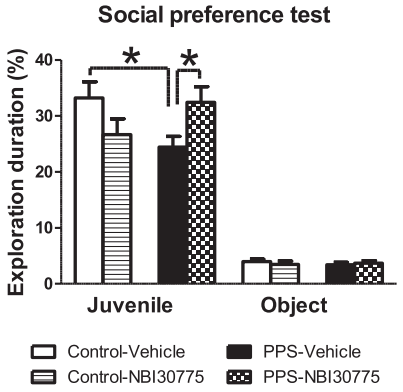

Fig. 4. Effects of NBI30775 injection from P43 to P49 on social preference test in adulthood. The graph shows the percent time of total juvenile exploration compared to total object exploration. The results are expressed as mean ± SEM. $^{*}p < 0.05$; $^{\#}p < 0.1$; $N = 9$/group.

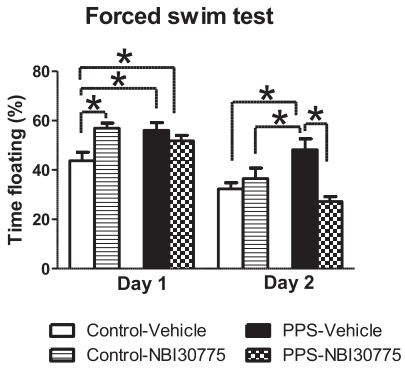

Fig. 5. Effects of NBI30775 treatment from P43 to P49 on depression-like behavior during forced swim test in adulthood. The graph shows the percent time of floating for day 1 and day 2. The results are expressed as mean ± SEM. $^{*}p < 0.05$; $N = 9$/group.

observed in peripuberty stress animals on the second session of the forced swim test, but not on the first. In addition, the treatment on its own, given to non-stressed animals during the peripuberty period leads to a differential reaction to the forced swim challenge as compared to vehicle-treated animals on the first exposure to the test.

4. Discussion

Existing evidence has identified alterations in the CRH system accompanying certain psychopathologies (Holsboer and Ising, 2008; Binder and Nemeroff, 2010) such as anxiety and depression, linked to early life adversity (Coplan et al., 1996; Nemeroff, 2004; Avital and Richter-Levin, 2005; Schmidt et al., 2007; Heim et al., 2008; Cordero et al., 2012). Although drug treatments tackling CRHR1 have been envisioned as potentially promising anxiolytic and antidepressive drugs (Kunzel et al., 2003; Refojo and Holsboer, 2009), recent evidence in rodents suggests that they could also be effective in modifying not only actual pathological manifestations at adulthood but also the developmental trajectories linking early adversity to adult psychopathology. These rodent studies showed that exposure to stress during the two first postnatal weeks leads to increased central CRH and CRHR1 expression, and altered a number of behaviors in tests of emotion and cognition (Plotsky et al., 2005; Ivy et al., 2010; Wang et al., 2011, 2012). Importantly, they also found that some of the long-term behavioral alterations could be reversed by inhibiting CRHR1 function (e.g., through CRHR1 antagonist treatment or conditional forebrain CRHR1 knockout) in the developmental period occurring immediately after early life stress (Ivy et al., 2010; Wang et al., 2011, 2012). So far, all those studies involved maternal stress during the first two postnatal weeks. Here, we show that exposure of rats to stress during the peripubertal period – comprising juvenility and puberty – is also effective in inducing long-lasting changes in the expression levels of the *Crhr1*, but not *Crhr2,* gene in specific hippocampus and amygdala subfields. Moreover, we show that treatment with the CRHR1 antagonist NBI30775 daily for 1 week (from P43–P49) immediately following peripuberty stress exposure can also be effective in reversing certain emotional and social behaviors observed at adulthood in peripubertally stressed animals.

We found evidence for specific increases in *Crhr1* mRNA in the CA1 hippocampal subfield and in the central amygdala in adult rats that had been subjected to peripubertal stress, but no changes in *Crhr2*. These data are in alignment with other studies showing the modulation of the CRH system in response to early life stress across species. For example, increased cerebrospinal fluid (CSF) CRH concentrations were found in adult humans (Heim et al., 2008), non-human primates (Coplan et al., 1996) and rodents (Plotsky et al., 2005) that had been exposed to early life adversity and trauma. While we find no changes in *Crhr2* expression in our study, data from previous maternal stress studies in rodents yielded mixed results regarding changes in the central expression of this gene (Vazquez et al., 2003; Plotsky et al., 2005; Bravo et al., 2011). In line with our findings, increased *Crhr1* mRNA expression in the amygdala (Bravo et al., 2011) and hippocampus (Fenoglio et al., 2006; Ivy et al., 2010; O'Malley et al., 2011) was also found in adult rodents subjected to maternal stress during the early postnatal period. In addition, previous evidence focused in the dorsal raphe nucleus identified juvenility and adolescence as periods of vulnerability for the modulation of the CRH system by stress (Lukkes et al., 2009). Interestingly, genetic studies in humans have pointed to an interaction between genetic variants in the *Crhr1* gene and childhood maltreatment on the development of psychopathological alterations (Tyrka et al., 2009; Roy et al., 2012; Guillaume et al., 2013). Recently, a study in rhesus monkeys has shown that single nucleotide polymorphisms in the *Crhr1* gene affect both anxiety temperament and metabolic activity in the anterior hippocampus and amygdala (Rogers et al., 2013). Interestingly, our previous data have also highlighted changes in the metabolic activity of hippocampus and amygdala in peripubertally stressed rats (Toledo-Rodriguez et al., 2012; Marquez et al., 2013). Furthermore, increasing CRH drive occurring specifically in the central amygdala through lentiviral-induced neuropeptide overexpression was also found to enhance anxiety-like (Flandreau et al., 2012) and depression-like (Keen-Rhinehart et al., 2009) behaviors in rats. Enhanced CRHR1 expression in the hippocampus has been linked with impaired cognitive function induced by early life stress (Fenoglio et al., 2006; Ivy et al., 2010).

We also found that treatment with the CRHR1 antagonist NBI30775 during the week following exposure to peripuberty stress prevented deficits in social exploration and stress-coping behaviors displayed by peripuberty stressed animals at adulthood. Reduced social exploration in rodents has been related to both depressive-like (measured as a reduction in social interest and lack of motivation) and anxiety-like (Nestler and Hyman, 2010; Castro et al., 2012) behaviors. Peripuberty stressed animals showed a reduction in their exploration of the juvenile conspecific but no difference in the exploration of the object as compared to controls was found, and NBI30775 treatment specifically reversed the effects of stress on social exploration. These data fit with increasing evidence implicating the CRH system in the modulation of social behavior (Hostetler and Ryabinin, 2013). For example, pharmacological activation of CRHR1 centrally was found to decrease social exploration in rats (Dunn and File, 1987; Campbell et al., 2004; Gehlert et al., 2005), an effect that was also observed when the amygdala was directly targeted (Sajdyk et al., 1999; Gehlert et al., 2005; Spiga et al., 2006). In agreement with our pharmacological data, prior evidence has also shown that antagonizing CRHR1 has no effect on social behaviors (in our study, the antagonist led to a non-significant reduction in social interaction) but is effective in abolishing the deficits induced by enhanced CRH function on sociability (Sajdyk and Gehlert, 2000; Gehlert et al., 2005). In this context, it should be noted that CRH antagonists have been considered promising candidates for the treatment of social phobia in humans (van Ameringen et al., 2000).

In the forced swim test, peripuberty stressed animals showed increased floating on the second test session, an effect classically interpreted as evidence for increased depression-like behavior though, following (Lu et al., 2008), we refer here to the results of this test as stress-coping behaviors. This effect was prevented by NBI30775 treatment. These data are in good agreement with previous reports showing that enhanced CRH drive is associated with

depressive behaviors in both humans (Arato et al., 1989; for a review see Binder and Nemeroff, 2010) and animals (Muller and Wurst, 2004; Kolber et al., 2010); specifically, increasing CRH drive in the central amygdala through lentiviral-induced neuropeptide overexpression was found to enhance floating behaviors in the forced swim test (Keen-Rhinehart et al., 2009). Decreasing CRHR1 levels produces antidepressant-like effects in animals as treatment with CRHR1 antagonists has been reported to do in humans (Zobel et al., 2000; Kunzel et al., 2003). It is worth noting, however, that the behavioral data obtained on the first session of the forced swim test depicted a slightly different picture. While enhanced floating was again observed in peripuberty stressed animals, reinforcing their impaired stress-coping behaviors and/or depression-like phenotype, this effect was not prevented by the NBI30775 treatment. Moreover, NBI30775 treatment on its own also led to increased floating behavior in this first session. Studies involving conditional forebrain CRHR1 knockout starting around weaning have reported effects of this manipulation at adulthood (Zobel et al., 2000; Kunzel et al., 2003), however, most studies administering CRHR1 antagonists to control, non-stressed animals during early life only reported mild and non-significant behavioral changes (Fenoglio et al., 2005; Ivy et al., 2010). Our findings, obtained at a slightly different timing of drug administration (i.e., post-puberty or late adolescence instead of pre-weaning regimes given in former studies) hint at the possibility that antagonizing CRHR1 in control individuals during adolescence might affect their developmental trajectory and in turn affect their subsequent emotional behavior.

Our results therefore suggest an important role of the CRHR1 system in the translation of the peripuberty effects of stress on adult behavioral alterations and highlight the potential of CRHR1 antagonists applied in the aftermath of stress to prevent the emergence of psychopathological behaviors. However, the data obtained in the elevated plus maze are not conclusive as the anxiety-like phenotype induced by peripubertal stress in the current study is much milder than in our former reports (Marquez et al., 2013), and only manifested as changes in the animal's behavior in the central compartment of the maze. No significant effects were observed in the time they spent in the open arms, which is classically considered as being the index for anxiety-like behaviors. A possible explanation for the difference in the strength of the anxiety-like phenotype between this and our former studies is the fact that the current study involved recurrent injections (also in controls) during the peripuberty period which might have affected the animals' development in the anxiety domain, while no injections were performed in earlier studies. Should the anxiety-like phenotype have emerged in peripuberty stressed animals, our prediction would have been on the effectiveness of the CRHR1 antagonist treatment as an anxiolytic (see Post et al., 2005). This hypothesis is supported by evidence showing that the increased anxiety-like behavior displayed at adulthood in animals subjected to early life stress was prevented in conditional forebrain CRHR1 knockout mice in which the gene deletion takes place from the 3rd–4th postnatal week (Wang et al., 2012). It should also be noted that although our study did not address potential long-term changes in gene expression induced by the CRHR1 antagonist treatment, previous studies with the same antagonist have shown changes in the expression levels of Crhr1 in the amygdala, when administered to animals under basal conditions (Post et al., 2005).

In conclusion, we show enduring changes in *Crhr1* expression in the hippocampal CA1 and the central amygdala of peripuberty stressed animals accompanying behavioral alterations in sociability and stress-coping behaviors. We also show that these behavioral alterations are reversed by CRHR1 antagonist treatment transiently given in the aftermath of stress exposure, during adolescence. Our data highlight peripuberty as a period of plasticity for the enduring modulation of the CRHR1 system and support a growing body of data implicating the CRHR1 system in the programming effects of early life stress on psychopathology, for a review, see (Regev and Baram, 2013).

Author contribution

Vandana Veenit is the lead author who performed the experiments, analyzed the data and wrote the manuscript. Orbicia Riccio carried out the PCR and did the analysis for gene expression study. Carmen Sandi is the senior corresponding author and contributed to the concept and design of all experiments, interpretation, wrote manuscript and provided financial support.

Conflict of interests

The authors declare that no conflict of interests exists.

Role of the funding source

This work was supported by grants from the Swiss National Science Foundation (31003AB-135710 and the NCCR Synapsy), Oak Foundation, and intramural funding from the EPFL.

Acknowledgments

We thank Céline Fournier, Olivia Zanoletti and Jocelyn Grosse for excellent technical assistance and M. Isabel Cordero for helpful discussions. We also thank Dr. Dimitri Grigoriadis, Neurocrine Biosciences Inc., for the donation of the CRHR1 antagonist NBI30775. This work was supported by grants from the Swiss National Science Foundation (31003AB-135710 and the NCCR Synapsy), Oak Foundation, and intramural funding from the EPFL.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jpsychires.2014.02.015.

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