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## Social deficits induced by peripubertal stress in rats are reversed by resveratrol

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## ABSTRACT

Adolescence is increasingly recognized as a critical period for the development of the social system, through the maturation of social competences and of their underlying neural circuitries. The present study sought to test the utility of resveratrol, a dietary phenol recently reported to have mood lifting properties, in modulating social interaction that is deficient following early life adversity. The main aims were to 1) pharmacologically restore normative social investigation levels dampened by peripubertal stress in rats and 2) identify neural pathways engaged by this pharmacological approach. Following peripubertal (P28–42) stress consisting of unpredictable exposures to fearful experiences, at adulthood the subjects' propensity for social exploration was examined in the three-chamber apparatus, comparing time invested in social or non-social investigation. Administered intraperitoneally 30 min before testing, resveratrol (20 mg/kg) normalized the peripubertal stress-induced social investigation deficit seen in the vehicle group, selectively altering juvenile but not object exploration. Examination of prefrontal cortex subregion protein samples following acute resveratrol treatment in a separate cohort revealed that while monoamine oxidase A (MAOA) enzymatic activity remained unaltered, nuclear AKT activation was selectively increased in the infralimbic cortex, but not in the prelimbic or anterior cingulate cortex. In contrast, androgen receptor nuclear localization was increased in the prelimbic cortex, but not in the infralimbic or anterior cingulate cortex. This demonstration that social contact deficits are reversed by resveratrol administration emphasizes a prosocial role for this dietary phenol, and evokes the possibility of developing new treatments for social dysfunctions.

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The quality of social relations plays a pervasive, though often underestimated, role in individuals' physical and mental health having effects far beyond the actual social interactions (House et al., 1988; Taborsky and Oliveira, 2012; Uchino, 2006). Social anhedonia or social avoidance is commonly observed in association with psychiatric disorders, both in patients (e.g. in individuals diagnosed with depression, anxiety, autism, bipolar disorder, or schizophrenia; Chevallier et al., 2011; Blanchard et al., 2001; Cannon et al., 1997) and even in relatives of diagnosed subjects (Docherty and Sponheim, 2008). Therefore, the development of therapeutic interventions tackling deficient social relations could be clearly beneficial to both individuals and society.

A key vulnerability factor for disturbed adult social relations is exposure to adversity during early life, including adolescence. This vulnerability of adolescence to stress seems to be rooted in the key maturational processes occurring during this period in the social brain (Blakemore, 2012). Although cultural and social learning factors frequently preclude identifying the role of biological factors in the long-term consequences of stress in human studies, we have recently shown in rats that exposure to stress during the peripubertal period leads to abnormal social interactions at adulthood (Márquez et al., 2013); the same pattern of social alterations was found when stress was substituted by administration of the glucocorticoid stress hormone, corticosterone (Veenit et al., 2013). Specifically, peripubertally stressed rats showed reduced motivation to explore a juvenile conspecific versus an object in the three-chambered test (Márquez et al., 2013), a task used in non-human primates and rodents (Bauman et al., 2013; Moy et al., 2004) that can be considered akin to social approach-avoidance tasks used in humans (Heuer et al., 2007; Roelofs et al., 2009). Particularly susceptible to the long-term effects of this form of peripubertal stress

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is the prefrontal cortex (Márquez et al., 2013), the engagement of which has been shown to be critically involved in social behaviors in humans (Dichter et al., 2009; Ho et al., 2012) and in animal models (Avale et al., 2011; Covington et al., 2010; Stack et al., 2010).

The present study sought to explore the effectiveness of acute treatment with the dietary compound resveratrol in normalizing alterations in social motivation observed in peripubertally stressed rats as adults, and to investigate relevant molecular targets affected by the treatment in selected prefrontal cortex subregions. Resveratrol, coming to prominence for its detection in red wine by investigations of certain paradoxical health benefits of alcohol consumption, can be found in red grapes, berries, among various sources. It is a natural phenol and phytoalexin that is blood–brain barrier permeable (Juan et al., 2010; Vitrac et al., 2003). Resveratrol exhibits numerous properties, with recent evidence suggesting that it can improve depressive-like behaviors, as seen in reduced immobility in rodents in the forced-swim test and tail-suspension tests, as well as in the sucrose consumption test (Samardzic et al., 2013; Xu et al., 2010; Yu et al., 2012).

We examined candidate protein activity in order to gain insight into the potential mechanisms of the engagement by resveratrol of the prefrontal cortex, where binding sites abound (Han, 2006). There, it has been shown to increase serotonin and to decrease monoamine oxidase A (MAOA; Xu et al., 2010; Yu et al., 2012), of potential relevance as previously peripubertal stress was shown to enhance gene expression of the latter in this region, with chronic MAOA inhibitor treatment reverting the induced social exploration deficiencies (Márquez et al., 2013). We also considered other proteins previously implicated in the effects of this phenol (Benitez et al., 2007; Patel et al., 2011): AKT, a serine/threonine-protein kinase (also known as protein kinase B) noted for its functions in diverse pathways including cellular survival and energy metabolism, as well as the androgen receptor, activated by the steroid hormone testosterone or its metabolite dihydrotestosterone. Androgen receptor functionality has been positively associated with social extraversion (Lukaszewski and Roney, 2011), while AKT have been associated with social information processing (Lin et al., 2012). Our results confirm the validity of resveratrol to revert social deficiencies induced by early life and identify molecular targets modified by resveratrol treatment in specific prefrontal cortical areas.

## 1. Methods

### 1.1. Animals

The experimental subjects were the offspring of Wistar Han rats (Charles River Laboratories, L'Arbresle, France), bred in our animal house (behavioral experiment,  $n = 36$ ; neurochemical assays,  $n = 20$ ). As is our usual practice, at weaning male rats from different litters were mixed throughout the different home cages by placing equivalent numbers of animals from each litter into the different experimental groups and by avoiding having siblings in the same home cage. They were housed three per standard plastic cage on a 12 h light–dark cycle (lights on at 0700 h). Food and water were available ad libitum. Another experiment was performed to examine a potential prefrontal site of action for resveratrol, using a separate cohort of standard Wistar Han subjects. All procedures were conducted in conformity with the Swiss National Institutional Guidelines on Animal Experimentation and approved by a license from the Swiss Cantonal Veterinary Office Committee for Animal Experimentation.

### 1.2. Peripubertal stress

The unpredictable environmental stress protocol was applied as previously described (Cordero et al., 2012; Márquez et al., 2013;

Toledo-Rodríguez and Sandi, 2011). The stressors were administered during the light phase on 7 days across the period spanning postnatal days 28–42, a transitional period culminating in puberty onset and associated with diverse behavioral and brain maturational processes, notably in the prefrontal cortex (Brenhouse and Andersen, 2011; Korenbrot et al., 1977; Spear, 2000). The protocol started with an exposure to an open field on P28 (5 min), after which subjects experienced repeated stress exposures (25 min) as described next, to an elevated platform or predator odor, either exclusively (P34, P36, P42) or one after another (P28–30, P40) in a pre-determined variable order. Synthetic trimethylthiazoline (9  $\mu$ l; Phero Tech, Delta, BC, Canada), a volatile molecule from the fox anal gland, was absorbed onto a piece of tissue and placed out of reach in a ventilated plastic box (38  $\times$  27.5  $\times$  31 cm; 200–250 lux). Elevated platforms were 95 cm above the ground (12 cm<sup>2</sup>), and exposures conducted under bright light (500–550 lux). After each daily session, the rats were returned to their home cage, but remained separate for a further 15 min during which a transparent perforated Plexiglas divider (MSPLAST, Pampigny, Switzerland) was in place. The control animals were handled on the days that their experimental counterparts were exposed to stress. Upon reaching adulthood, males were separated and paired with an adult virgin Wistar female rat, according to the previously reported protocol that yielded the sought social preference deficit (Márquez et al., 2013).

### 1.3. Three-chamber apparatus for social motivation

In adult subjects (see Fig. S1 for timeline), social tendencies were evaluated using an adaptation of the paradigm introduced by Crawley and collaborators (Moy et al., 2004), with animals in the control and peripubertal stress groups assigned to either a resveratrol or vehicle group (4 groups,  $n = 9$  each). Briefly, the test was conducted in a rectangular, three-chambered apparatus fabricated from gray opaque polycarbonate (center chamber, 20  $\times$  35  $\times$  35 cm; adjacent target investigation areas, 30  $\times$  35  $\times$  35 cm), with each dividing wall comprising a retractable door. Central to each target area was a transparent Plexiglass (15 cm diameter) receiving either the social (unfamiliar juvenile rat approximately 34 days old) or non-social stimulus (yellow plastic bottle). Small perforations covered each cylinder, permitted visual, auditory, olfactory, as well as limited tactile communication, while preventing potential confounds to the evaluation of social tendencies that could arise from more elaborate physical, aggressive or sexual interactions (Moy et al., 2004). The juvenile rats were first habituated to the three-chambered apparatus by placing them individually in the box within a cylinder for 10 min during the 3 days preceding the test.

On the testing day, the experimental rat was introduced into the central chamber and with the compartment doors in place was allowed a 5 min habituation. After this habituation phase, the social and inanimate cues were placed in either adjacent compartment, in a counterbalanced position across sessions. Next, both doors to the side chambers were carefully removed, opening up the entire apparatus for a 10-min video-recorded exploration session. The apparatus was cleaned with 5% acetic acid solution between each test. The time spent sniffing each cylinder (Fairless et al., 2011) was manually scored by an experimenter blind to the treatments to evaluate the level of preference for the unfamiliar juvenile compared with the object.

### 1.4. Resveratrol administration

The subjects were administered an acute intraperitoneal injection of vehicle or resveratrol [20 mg/kg, *trans*-3,5,4'-trihydroxystilbene; Sigma–Aldrich, Buchs, Switzerland; in 5% (2-Hydroxypropyl)- $\beta$ -cyclodextrin (Sigma–Aldrich) with 10% ethanol

to increase solubility and stability (Delmas et al., 2011)]. It should be noted that at this amount ethanol was sufficiently limited that no effect would be expected on social investigation (Varlinskaya and Spear, 2002). The experiments took place between 10 h and 12 h, with injections 30 min prior to behavioral testing or tissue sampling, following previously reported procedures (e.g. Gupta et al., 2012; Renthal et al., 2009; Xu et al., 2010).

### 1.5. Protein extraction and quantification

In order to help minimize variability that could result from anxiety-like differences between the groups, one month prior the subjects were pre-screened for anxiety-like behaviors in the elevated plus maze, [5 min; 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]. Their behavior was monitored using a video camera and analyzed with a computerized tracking system (Ethovision 3.1.16; Noldus IT), focusing on the percent time spent in open arm. The entire apparatus was cleaned with 5% acetic acid solution and dried thoroughly between each animal. Next, the subjects were assigned to the vehicle or the resveratrol group ( $n = 10$  each) to ensure equivalent reactivity to elevated plus-maze exposure.

Thirty 30 min following the intraperitoneal injection of either resveratrol or vehicle, the subjects were sacrificed, brains were extracted, frozen in isopentane on dry ice, and stored at  $-80^{\circ}\text{C}$  until processing. In order to evaluate prefrontal cortex activity, tissue from separate subregions was obtained by selectively punching samples of prelimbic, infralimbic, and pregenual anterior cingulate cortex (Harris Uni-Core, Ted Pella, Redding, California, US) from 50 to 200  $\mu\text{m}$  slices obtained on a cryostat (Leica CM3050S) covering the extent of each subregion according to atlas landmarks (Paxinos and Watson, 1997).

Mitochondrial, cytoplasmic and nuclei-enriched fractions were obtained using an adaptation of a previously published differential centrifugation protocol (Simon-Arces et al., 2012). Sample lysates were obtained using a Teflon pestle in ice-cold IM homogenization buffer 20 mM Tris HCl, 10 mM KOAc, 1 mM EDTA, 0.25% NP40, containing freshly added 1 mM dithiothreitol (DTT) and a proteinase and phosphatase inhibitor cocktail (Complete EDTAfree, Roche Diagnostics GmbH, Rotkreuz, Switzerland). The samples were then centrifuged (20 min at 700 g,  $4^{\circ}\text{C}$ ). While the nuclei-enriched pellet was held back, the supernatant was collected and centrifuged again (15 min at 10,000 g,  $4^{\circ}\text{C}$ ). The supernatant and the pellet obtained were cytoplasmic- and mitochondrial-enriched fractions, respectively. The mitochondrial pellet was resuspended (75 mM mannitol, 25 mM sucrose, 5 mM  $\text{KH}_2\text{PO}_4$ , 20 mM Tris HCl, 0.5 mM EDTA, 100 mM KCl, 0.1% bovine serum albumen; MSK buffer) and used fresh while nuclei and cytoplasmic fractions were stored at  $-80^{\circ}\text{C}$  until subsequent analyses.

Protein in the sample lysates was quantified using the detergent-compatible Bio-Rad DC protein assay (Bio-Rad Laboratories AG, Reinach, Switzerland). Protein samples were prepared in order to obtain equal concentrations by  $\text{H}_2\text{O}$  dilution, and for Western blotting mixed with 33% SDS blue loading buffer (New England Biolabs Inc, USA) containing DTT (nuclei pellets were first re-suspended in IM buffer with DTT). The average amount of protein obtained per punch for the prelimbic, infralimbic and anterior cingulate subregions was respectively, for nucleus and cytoplasm 250 and 200  $\mu\text{g}$ , 140 and 160  $\mu\text{g}$ , and finally 170 and 185  $\mu\text{g}$ .

### 1.6. Monoamine oxidase A assay

Monoamine oxidase A (MAOA) activity was evaluated in 5  $\mu\text{g}$  of mitochondrial protein extracts (volume adjusted for concentration)

using the luminescent MAOGlo Kit (Promega #V1401, Dübendorf, Switzerland), according to the manufacturer's instructions. Samples were processed in duplicates, and after a 30 min incubation with the luciferin detection reagent in a white opaque-wall small volume Greiner plate (HuberLab, Aesch, Switzerland), the luminescent signal was detected using the Infinite F500 detection platform managed with iControl 1.7.1.12 (Tecan, Männedorf, Switzerland), integrated over 200 ms. The sensitivity of the assay was confirmed using monoamine oxidase A and B inhibitors, respectively clorgyline and deprenyl (Sigma–Aldrich, data not shown). A standard curve was produced, representing luminescence according to amount of monoamine oxidase A enzyme (ng, Promega #V1452). For each sample the luminescence value was converted to an equivalent MAOA quantity, obtained by interpolation in the linear range of the standard curve of activity.

### 1.7. Western blotting

The samples for western blot were prepared as previously described (Kohl et al., 2013). Eight micrograms of protein was loaded and resolved on a 10% polyacrylamide gel and transferred onto a nitrocellulose membrane (Whatman Protran, Dassel, Germany). After blocking with 5% non-fat dry milk in phosphate-buffered saline containing 0.2% Triton-X (PBS-T), membranes were incubated with a primary antibody in 5% milk/PBS-T overnight at  $4^{\circ}\text{C}$  on a shaker [Androgen receptor, 1:500, Millipore #06-680, Zug, Switzerland; AKT and Phosphorylated AKT threonine 308, 1:1000, ThermoScientific, respectively #PA1-14033 and PA1-14030, Perbio, Lausanne; Zif268/Egr-1, 1:2500, Cell Signaling #4154; Bioconcept, Allschwil, Switzerland; nuclear pore proteins (NPP), 1:1000, Abcam #ab50008, LucernaChem, Lucerne, Switzerland; Actin, 1:5000, Sigma–Aldrich #A3853; concentrations previously tested to be within the linear range of detection). Subsequently, membranes were incubated with the secondary horseradish peroxidase-linked antibodies [goat-anti rabbit (Invitrogen #G-21234, LuboScience, Lucerne, Switzerland) for androgen receptor, Egr-1, AKT and pAKT; goat anti-mouse (Calbiochem #401215, USA) for NPP and actin] diluted in 5% milk/PBS-T for 1 h. Immunocomplexes were visualized using a chemiluminescence peroxidase substrate (SuperSignal West Dura Extended Duration Substrate, ThermoScientific, Perbio, Lausanne, Switzerland). The immunoreactivity was detected using the ChemiDoc XRS system (Bio-Rad Laboratories AG). For the densitometrical analysis of the bands, Quantity One 4.6.3 software (Bio-Rad Laboratories AG) was used. Absorbance values were normalized to actin and average densitometric results are presented. Visualizing of NPP confirmed the nuclear enrichment.

### 1.8. Statistical analysis

Statistical analyses were conducted using the Statistics Package for the Social Sciences (SPSS; Zürich, Switzerland). The object and juvenile target exploration percentage levels were analyzed together in a three-way, mixed between-within subjects analysis of variance (ANOVA; Stress × Drug × Target, two levels each). Total exploration percent as well as an index of sociability [object %/(object % + juvenile %)] was also analyzed using a two-way between-subjects ANOVA (Stress × Drug). Before running the analyses, the assumptions of normal data distribution and equivalence of variance and covariance were verified using the Shapiro–Wilk, Levene and Box tests, respectively, and Box plots were examined for extreme outliers (lying more than three times the interquartile range beyond the box boundaries, the first and third quartiles). For results from protein analyses, comparisons were made using  $t$ -tests. For the androgen receptor localization and AKT activation, analyses were conducted on ratios, cytoplasmic over nuclear

fractions of androgen receptor protein, and phosphorylated over total AKT protein.

## 2. Results

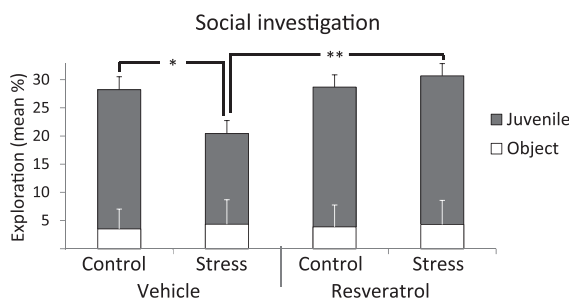
### 2.1. Resveratrol normalizes the social investigation deficit induced by peripubertal stress

The beneficial, normalizing effect of resveratrol on the social investigation deficit can be seen in Fig. 1. A three-way ANOVA with Stress and Drug as between-subjects variables and Target as a within-subjects variable was conducted to determine whether there was an effect of resveratrol administration on the exploration of the social and inanimate target cues, according to peripubertal experience. Prior to running the ANOVAs, it was found that some of the assumptions were not met [normality (Shapiro–Wilk,  $p < 0.001$  for Object and Social cue exploration percent;  $p = 0.006$  for the Sociability ratio); mild heterogeneity of covariance (Box's Test,  $M = 19.82$ ,  $F(9, 11734.84) = 1.95$ ,  $p = 0.041$ ); Levene Tests of homogeneity of variance acceptable,  $p > 0.05$ ]. Two extreme outlier subjects were identified (one subject each for Control- and Stress-Vehicle subgroups) and their exclusion recovered the normal distribution and covariance homogeneity (Shapiro–Wilk and Box's Test, both  $p > 0.05$ ) allowing for the ANOVA.

As expected, exploration of the social cue was largely favored to the inanimate object [main effect of Target,  $F_{(1,30)} = 387.5$ ,  $p < 0.001$ ]. While there were also a main effect of Drug [ $F_{(1,30)} = 6.4$ ,  $p = 0.017$ ], and significant two-way interactions [Stress  $\times$  Drug,  $F_{(1,30)} = 4.6$ ,  $p = 0.040$ ; Drug  $\times$  Target,  $F_{(1,30)} = 4.9$ ,  $p = 0.034$ ], these were superseded by a significant three-way interaction [Stress  $\times$  Drug  $\times$  Target,  $F_{(1,30)} = 4.8$ ,  $p = 0.037$ ; main effect of Stress,  $F_{(1,30)} = 1.1$ ,  $p > 0.3$ ; and Stress  $\times$  Target,  $F_{(1,30)} = 2.3$ ,  $p = 0.142$ ]. The significant three-way Stress  $\times$  Drug  $\times$  Target interaction and other interactions noted above were followed up with simple effects analyses. As seen in Fig. 1, the detrimental effect of peripubertal Stress was selectively present for the Social cue in the Vehicle condition [Stress-Vehicle lower than Control-Vehicle mean values ( $\pm$ s.e.m.), respectively = 20.4 ( $\pm$ 2.3) and 28.2 ( $\pm$ 2.3),  $p = 0.023$ ; all other comparisons,  $p > 0.1$ ]. Meanwhile, resveratrol administration only affected Social exploration levels of the Stress group [Stress-Drug = 30.7 ( $\pm$ 2.2), vs Stress-Vehicle,  $p = 0.003$ ; all other comparisons,  $p > 0.5$ ].

### 2.2. Resveratrol does not increase prefrontal cortex monoamine oxidase A activity

The results of the luminescence assay for monoamine oxidase activity are presented in Fig. 2. Resveratrol did not significantly alter



**Fig. 1.** Exploration of social and non-social cues. Peripubertal stress reduced the propensity of rats to spend time exploring a social cue (left). A single peripheral injection of resveratrol successfully increased social investigation back to normal levels, without affecting exploration of an inanimate object. Mean values are presented, with error bars representing the standard error of the mean. \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ .

the MAOA enzymatic activity in either of the prefrontal cortex areas examined [Fig. 2, prelimbic cortex:  $t(18) = -0.48$ ,  $p > 0.6$ ; infralimbic cortex:  $t(18) = 0.67$ ,  $p > 0.5$ ; anterior cingulate cortex:  $t(18) = 1.02$ ,  $p > 0.3$ ].

### 2.3. Resveratrol increases nuclear localization of prelimbic cortex androgen receptor

Although no differences in prefrontal subregion activation were suggested by Zif268 immediate-early gene imaging (see Fig. S2), resveratrol nevertheless produced selective alterations in the candidate molecular pathways examined. As shown in Fig. 3, prelimbic cortex androgen receptor localization was affected by resveratrol administration, with increased translocation to the nucleus suggested by the reduction in the cytoplasm-to-nuclear ratio of the androgen receptor levels [ $t(18) = 2.11$ ,  $p = 0.050$ ; 1 outlier excluded in the Resveratrol group]. No such shift in balance was apparent in the infralimbic [ $t(18) = 0.6$ ,  $p > 0.5$ ] or the anterior cingulate cortex [ $t(18) = -0.25$ ,  $p > 0.8$ ]; also see supplemental material Fig. S3].

### 2.4. Resveratrol activates AKT in the infralimbic cortex

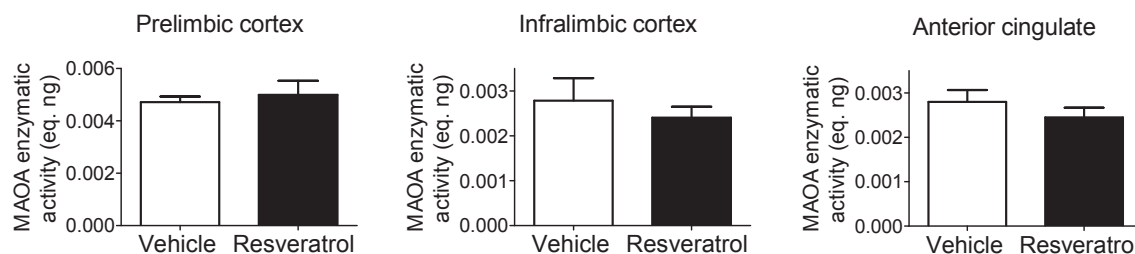
Next, the regulation of AKT was examined (cf. Fig. 4). AKT phosphorylation (ratio over total AKT) was increased in the infralimbic cortex nuclear fraction (Fig. 4, middle; non-parametric test; Resveratrol median = 0.095, Vehicle median = 0.078; Mann–Whitney  $U = 21.0$ ,  $p = 0.029$ ; for cytoplasmic fraction, phosphorylation ratio  $p > 0.7$ ). In contrast, no differential AKT activation was observed in the anterior cingulate cortex [nuclear ratio,  $t(17) = -0.42$ ,  $p > 0.6$ ; cytoplasmic ratio  $t(18) = 0.47$ ,  $p > 0.6$ ], or the prelimbic cortex [nuclear ratio,  $t(18) = 0.72$ ,  $p > 0.4$ ; cytoplasmic ratio  $t(18) = -0.22$ ,  $p > 0.8$ ]; also see supplemental material Fig. S4].

## 3. Discussion

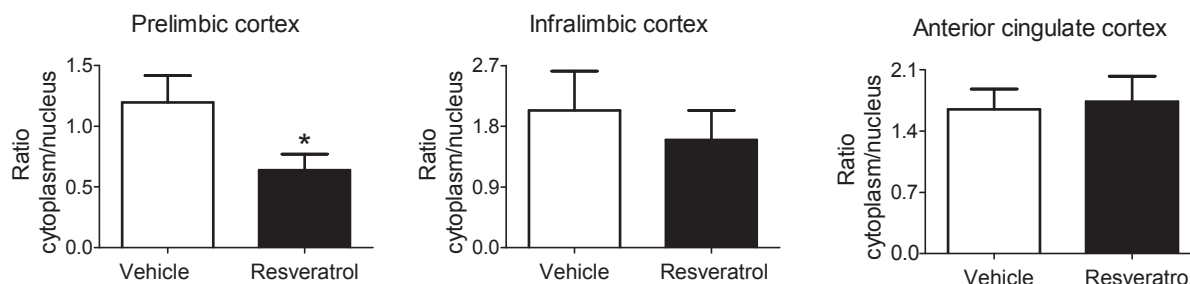
Social relations have pervasive repercussions on health, well beyond the social interactions themselves, and their quality can depend on environmental and temperamental factors, such as sociability (Berkman et al., 2000; Cohen, 2004; House et al., 1988; Uchino, 2006). Here, using rats as the model system, we confirm that exposure to stress during the peripubertal period results in deficient social motivation at adulthood, with exploration levels similar to those previously reported (Márquez et al., 2013). Strikingly, we show that acute treatment with the dietary phenol resveratrol normalizes this sociability deficit. We further provide candidate molecular targets that change their activity in the prefrontal cortex following resveratrol treatment.

Resveratrol has been touted for a multitude of therapeutic applications from energy homeostasis, diabetes, neuronal degeneration, aging, heart disease, as well as cancer, to pain and inflammation (reviewed in Baur and Sinclair, 2006; Stuart and Robb, 2013). Previous behavioral studies reported its beneficial effects on depressive-like symptoms (Samardzic et al., 2013; Wang et al., 2013; Xu et al., 2010; Yu et al., 2012). Except when evaluating the effects of resveratrol on the consequences of prenatal stress (Sahu et al., 2013), studies showed no effects of resveratrol on anxiety-like behavior in light–dark box or open field locomotor data (Patisaul et al., 2009; Samardzic et al., 2013; Wang et al., 2013; Xu et al., 2010). This finding is in agreement with the absence of an effect on object exploration in our study, instead showing a selective effect on social investigation. No previous study examined the effects of resveratrol in sociability and, thus, a key contribution of the current study lies in extending the reported mood lifting effects

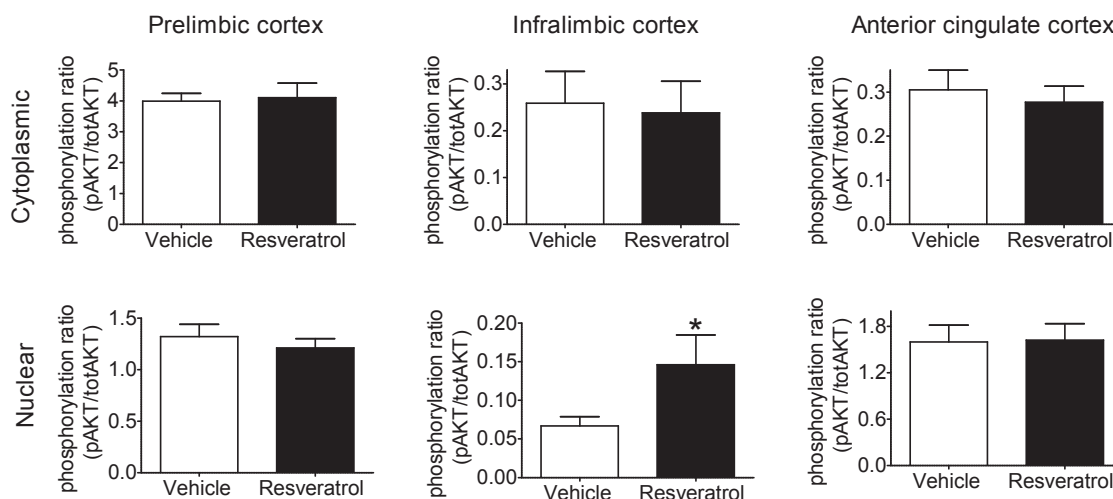




**Fig. 2.** Monoamine oxidase A enzymatic activity. The injection of resveratrol did not affect the enzymatic activity of monoamine oxidase A in equivalent amounts of mitochondrially-enriched protein. Mean values are presented, with error bars representing the standard error of the mean.



**Fig. 3.** Androgen receptor protein subcellular localization. In considering the subcellular localization of the androgen receptor, the injection of resveratrol was found to shift the balance to the nucleus over the cytoplasm in the prelimbic cortex, without affecting the ratio in the infralimbic or anterior cingulate cortices. Mean values are presented, with error bars representing the standard error of the mean. \*,  $p < 0.05$ .



**Fig. 4.** AKT protein activation. In considering the ratio of AKT<sup>Thr308</sup> phosphorylation to total AKT protein, the injection of resveratrol was found to increase relative activation selectively in the nucleus of the infralimbic cortex, and nowhere else. Mean values are presented, with error bars representing the standard error of the mean. \*,  $p < 0.05$ .

by showing that resveratrol is also effective to improve deficient social engagement.

In considering the present promising results for their applicability to humans via the preferred oral route, a concern could be that the intraperitoneal route used here is only subjected to partial first-pass metabolism effects, as unlike the oral route it bypasses the gastrointestinal tract and the gut wall, themselves also implicated in resveratrol processing (Amri et al., 2012; Wenzel and Somoza, 2005). It is worth noting that pre-clinical studies using the same resveratrol concentration as ours have previously demonstrated mood lifting effects, not just following intraperitoneal administration (acute or chronic regimen, respectively; Samardzic et al., 2013; Wang et al., 2013), but also after intra-gastric administration (acute or chronic regimen, respectively; Xu et al.,

2010; Yu et al., 2012), thereby alleviating some of the concerns surrounding first-pass effects. In contrast to these studies, we used both (ethanol and 2-Hydroxypropyl)- $\beta$ -cyclodextrin to increase the aqueous solubility and stability of resveratrol, an approach that, while not likely to be effective at enhancing the extent of the oral bioavailability, can nonetheless enhance its rate (Amri et al., 2012; Das et al., 2008). Future investigations may address issues of optimization of oral bioavailability by considering alternative formulations (Amri et al., 2012), the use of resveratrol derivatives with higher oral availability, such as pterostilbene (e.g. Yeo et al., 2013), and since only relatively little resveratrol appears to find its way to the brain (Liang et al., 2013; Sale et al., 2004), the role of the parent compound relative to its metabolites, also known to be active (cf. Amri et al., 2012).

In addition to the pharmacological normalization of the peripubertal stress-induced social deficit, the present study identified alterations in certain prefrontal cortex subregions following resveratrol treatment. Prefrontal cortex engagement has been shown to be critically involved in social behaviors in humans (e.g. Dichter et al., 2009; Ho et al., 2012), and in animal models (Avale et al., 2011; Covington et al., 2010; Stack et al., 2010; but see Kumar et al., 2013). In the latter, prefrontal cortex damage, whether widespread (Gonzalez et al., 2000; Shah and Treit, 2003) or localized to the prelimbic cortex (Avale et al., 2011), has been reported to increase social exploration. In contrast, anterior cingulate lesions have yielded a reduction in social interest, rather unaffected by orbitofrontal lesions (Rudebeck et al., 2007). Our data shows an engagement of prelimbic and infralimbic cortices following acute resveratrol treatment.

The resveratrol dose used in our study was not effective to detect MAOA activation differences in prefrontal cortex subregions at the time point evaluated, although effects in other molecular targets were observed. A previous study of the acute effects of resveratrol showed that while the same low dose of resveratrol (20 mg/kg) and time-delay from treatment to testing (30 min) as in our study produced behavioral changes and reduced MAOA activity in whole-brain homogenates, monoamines and their metabolites were only statistically significantly affected at higher doses, whether in the frontal cortex, hippocampus or hypothalamus (Xu et al., 2010). Furthermore, while chronic administration of this low dose of resveratrol significantly reduced MAOA activity in the frontal cortex, monoamines and their metabolites again were only affected at higher doses (Yu et al., 2012). While our subregional analysis of prefrontal cortex did not reveal MAOA alterations, it nonetheless revealed prelimbic and infralimbic cortex alterations in other molecules previously associated with resveratrol effects, the androgen receptor and the AKT protein kinase, discussed next.

Androgen receptor function in men has long been associated with affect, notably aggressive behavior, but also social extraversion (Lukaszewski and Roney, 2011) and depression (Colangelo et al., 2007; Seidman et al., 2001). The androgen receptor exhibits interacting slow (30 min or more) and fast (seconds to minutes) mechanisms of action, respectively genomic and non-genomic (Bennett et al., 2010; Michels and Hoppe, 2008). This receptor is typically translocated from the cytoplasm to the nucleus when bound by androgen ligands, testosterone or its metabolite, 5 $\alpha$ -dihydrotestosterone. Androgens – notably testosterone – are known to play a key role in social behaviors, both in animals (Bluthé et al., 1993, 1990) and humans (Dabbs et al., 2001), as well as with prefrontal cortex metabolism in a social context (Stanton et al., 2009; Volman et al., 2011). We thus investigated potential changes in the subcellular localization of the androgen receptor, finding an increase in the nuclear localization of the androgen receptor in the prelimbic cortex following resveratrol treatment.

Resveratrol was also previously shown to activate the AKT pathway (Brasnyó et al., 2011; Patel et al., 2011). Here, although AKT is involved in both genomic and non-genomic actions of the androgen receptor (reviewed in Bennett et al., 2010; Michels and Hoppe, 2008), no AKT alterations were concurrently observed in the subregion with apparent nuclearization of the androgen receptor, the prelimbic cortex. In contrast, AKT recruitment was seen in the infralimbic cortex, where no translocation was suggested for the androgen receptor, potentially hinting that genomic actions of this receptor were not favored in this area. AKT exhibits synapse-modulating properties, in particular an ability to effect post-synaptic receptor recruitment (Wang et al., 2003). Like resveratrol in the present study, high frequency stimulation can induce AKT<sup>Thr308</sup> phosphorylation, without affecting total protein levels

(Tsokas et al., 2007; though see Sui et al., 2008). AKT function has been associated with prefrontal cortex structural alterations in both humans and mice (Lai et al., 2006; Tan et al., 2008), together with alterations in social information processing (Lin et al., 2012) and mental health conditions associated with disordered mood, such as depression, autism, bipolar disorder and schizophrenia (De Lacy and King, 2013; Ebert and Greenberg, 2013; Emamian, 2012; Kitagishi et al., 2012; Marsden, 2013; Zheng et al., 2012). Resveratrol increased Thr308 phosphorylation of AKT, noteworthy as the brains of depressed (suicide and non-suicide) subjects exhibit prefrontal cortex reductions in AKT enzymatic activity, including Thr308 phosphorylation, and also in the absence of protein level changes (Dwivedi et al., 2010; Karège et al., 2011, 2007). Furthermore, in trying to understand the mechanisms at play, it is worth noting that, where present, resveratrol administration-induced increases in AKT activation were observed selectively in the nuclear fractions, potentially related to oxidative activity that can trigger such nuclear activation (Uranga et al., 2013). Thus, developments in the generation of nucleus-specific AKT modulators (Maiuri et al., 2010) may be a tantalizing prospect.

Development during the peripubertal period can program adolescent and adult behaviors. In contrast to adulthood, the experienced of the form of adversity sustained in the present paradigm during this sensitive peripubertal period has pervasive social consequences from the individual to others in their path, with effects even felt across generations (Cordero et al., 2013, 2012; Márquez et al., 2013; Poirier et al., 2013; Veenit et al., 2013). Organizational effects of androgens or glucocorticoids could influence the sensitivity of adult males to activational effects of either of these hormones (Romeo, 2003; Viau, 2002), affecting prefrontal cortex activation and network engagement. We propose that differences in neurochemistry arising as a result of the adverse peripubertal experience could account for the differential behavioral effect of the resveratrol, with an apparent lack of effect of this treatment on the social interest of the control subjects in the present study. Although independent mechanisms may be invoked by peripubertal stress and resveratrol treatment, it is also possible that the behavioral outcome is rather mediated by the targeting of a molecular alteration initially programmed by the peripubertal experience, and successfully reversed by resveratrol.

The results of the current study highlight the potential of resveratrol in the development of acute therapeutic interventions tackling deficient social relations. Targets of potential of interest include those nuclear molecular changes here identified to be engaged by resveratrol treatment, namely prefrontal cortex androgen receptor recruitment and infralimbic cortex AKT activation. Considering the pervasive ramifications of deficient social engagement, novel interventions could prove beneficial to both individuals and society.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jpsychires.2014.05.017>.

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The funders had no role in study design, data collection, analysis, or preparation of the manuscript.

## Contributors

GLP was involved in the conceptual input, planning of the studies, all data acquisition and analysis, and preparation of the first draft and final editing of the manuscript. NI and OZ were involved in the protein data acquisition. CS was involved in conceptual input and planning of the studies, as well as manuscript preparation and final editing.

## Conflicts of interest

All authors declare that they have no conflicts of interest.

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