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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.

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-Rodriguez 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 μl; 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 × 27.5 × 31 cm; 200–250 lux). Elevated platforms were 95 cm above the ground (12 cm^2), 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 × 35 × 35 cm; adjacent target investigation areas, 30 × 35 × 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)-β-cyclodextrin (Sigma–Aldrich) with 10% ethanol