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Effects of suppressing gonadal hormones on response to novel objects in adolescent rats

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ABSTRACT

Human adolescents exhibit higher levels of novelty-seeking behaviour than younger or older individuals, and novelty-seeking is higher in males than females from adolescence onwards. Gonadal hormones, such as testosterone and estradiol, have been suggested to underlie age and sex difference in response to novelty; however, empirical evidence in support of this hypothesis is limited. Here, we investigated whether suppressing gonadal hormone levels during adolescence affects response to novelty in laboratory rats. Previously, we have shown that male adolescent Lister-hooded rats (postnatal day, *pnd*, 40) exhibit a stronger preference than same-aged females for a novel object compared to a familiar object. In the current study, 24 male and 24 female Lister-hooded rats were administered with Antide (a gonadotrophin-releasing hormone antagonist), or with a control vehicle solution, at pnd 28. Antide provided long-term suppression of gonadal hormone production, as confirmed by ELISA assays and measurement of internal organs. Response to novel objects was tested at pnd 40 in Antide-treated and control subjects using a 'novel object recognition' task with a short (2-minute) inter-trial interval. In support of previous findings, control males exhibited a stronger preference than control females for novelty when presented with a choice of objects. Antide-treated males exhibited a significantly lower preference for novel objects compared to control males, whilst Antide-treated females did not differ significantly from control females in their preference for novelty. Antide treatment did not affect total time spent interacting with objects. We discuss how gonadal hormones might influence sex differences in preference for novelty during adolescence.

Introduction

In human beings, adolescents generally exhibit higher levels of novelty-seeking behaviour than younger and older individuals (Arnett, 1992; Kelley et al., 2004), and males report engaging in more novelty-seeking behaviour than females from adolescence onwards (Zuckerman, 2006). Researchers have argued that these age and sex differences in behaviour could be adaptive; for instance, adolescents potentially gain important information about their environment by seeking out novel experiences at a time when they are becoming independent from their parents (Chambers et al., 2003), and sexual selection pressures can favour riskier behavioural strategies in males than females (Daly and Wilson, 1983; Spear, 2000). Thus, certain groups of individuals, particularly adolescent males, might be more strongly predisposed than other groups to prefer novel stimuli.

Understanding the mechanisms that might predispose adolescent males towards a preference for novelty is important, given that novelty-seeking has been closely linked to drug abuse (Bardo et al., 1996; Roberti, 2004) and adolescence is a period of significant vulnerability to addiction (Chambers et al., 2003; Crews et al., 2007; Spear, 2000; Witt, 2007). Gonadal hormones, such as testosterone and estradiol, have been suggested to play a role in the expression of novelty-seeking behaviour during adolescence and the difference in novelty-seeking tendencies between males and females (Doremus-Fitzwater et al., 2010; Ernst et al., 2009; Forbes and Dahl, 2010; Kuhn et al., 2010). However, experimental evidence in support of this hypothesis is currently limited. The aim of this study was to examine the effects of suppressing gonadal hormone production during adolescence on response to novelty in male and female laboratory rats (*Rattus norvegicus*).

Adolescence in rats encompasses the period from weaning (postnatal day, *pnd*, 21) to early adulthood (pnd 60), and this period can be further divided into early adolescence (pnd 21–33), mid-adolescence (pnd 34–46) and late adolescence (pnd 47–59) (based on Tirelli et al., 2003). During early adolescence, circulating ovarian hormone levels begin to rise in female rats and ovarian weight increases, and, similarly, testosterone levels rise and testicular weight increases in males (Gabriel et al., 1992; Pignatelli et al., 2006). In the wild, these young animals begin to explore the area immediately outside of the natal burrow (Calhoun, 1963). During mid-adolescence, females exhibit vaginal opening and irregular ovarian cycling, whilst testosterone levels continue to rise in males (Gabriel et al., 1992), and wild rats follow the

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mother on foraging trips away from the burrow at this age (Calhoun, 1963). By late adolescence, females exhibit regular ovarian cycles, and males are capable of producing fertile sperm (Gabriel et al., 1992; Tentler et al., 1997), and late adolescents often sleep in nest chambers away from the mother and littermates (Calhoun, 1963). After this age, young adult rats engage in sexual and aggressive interactions with other individuals and disperse from the natal area (Calhoun, 1963). As in most rodent species, male rats typically move further away from the natal burrow system than females (Calhoun, 1963; Krebs et al., 2007).

Previously, we have shown that mid-adolescent male rats (pnd 40) exhibit a stronger preference than same-aged females for a novel object compared to a familiar object in a laboratory setting (Cyrenne and Brown, 2011), and this sex difference is not observed in younger (pnd 28) or older (pnd 80) age groups (Cyrenne and Brown, 2011). To examine response to novelty, we have used a variant of the 'novel object recognition' (NOR) task (Berlyne, 1950; Ennaceur and Delacour, 1988), which forces rodents to *confront* novelty and also provides subjects with the opportunity to *choose* between a novel and a familiar stimulus. The procedure is to familiarise an animal to a novel arena, then place two objects into the arena and allow the animal to interact with the objects. During this first trial, Trial 1, the subject is confronted with novelty. One of the objects is then replaced with a new item and, in Trial 2, the animal has the choice of interacting with the novel or the familiar object. Previous studies have shown that rodents generally spend more time interacting with the novel than the familiar object in Trial 2 (Dere et al., 2007; Ennaceur and Delacour, 1988). The NOR task has been used extensively in rodent memory research, and increasing the delay between the first and second trials to several hours reduces the difference in response to the novel and familiar objects (e.g. 4 h or 24 h: Ennaceur and Delacour, 1988; Şik et al., 2003). However, the NOR task also allows researchers to investigate the mechanisms involved in novelty preference (Besheer et al., 2001), and we used a short interval between the two trials (i.e. 2 min) to reduce the probability that differences in response to the objects between groups would result from differences in memory ability.

The current study investigated whether the sex difference in performance on the NOR task during mid-adolescence is influenced by suppression of circulating gonadal hormone levels. To suppress gonadal hormone production from early adolescence onwards, male and female rats were administered with a long-acting gonadotrophin-releasing hormone (GnRH) antagonist, Antide. GnRH antagonists act at the pituitary gland, where they strongly bind with GnRH receptors and hence prevent endogenous GnRH from stimulating gonadotrophin production (reviewed by Herbst, 2003). As a consequence of the lack of gonadotrophins, gonadal hormone production is blocked and gonadal development is retarded. The effects of these drugs are rapid in onset, and, unlike traditional gonadectomy, surgery is not required, as antagonists are administered via subcutaneous or intra-peritoneal routes. In our study, Antide was administered subcutaneously at pnd 28, which marks the start of the rise in circulating gonadal hormone levels in both sexes (Gabriel et al., 1992; Pignatelli et al., 2006), and the chosen dose was predicted to suppress hormone production through to pnd 40 in both males and females (Habenicht et al., 1990; Takeyoshi et al., 2002). We used a GnRH antagonist, rather than gonadectomy, as the effects of GnRH antagonists wear off over time and future studies could potentially investigate the effects of 'delaying' puberty.

The response of Antide-treated and control subjects to novelty was tested in the NOR task at pnd 40 using a 2-minute inter-trial interval. In addition to measuring the strength preference for the novel object in Trial 2, time spent moving and total amount of time spent in contact with the objects during both trials was recorded, in order to examine whether the hormone manipulations also influenced these measures. We hypothesised that control males would exhibit a stronger preference for the novel object than control females at pnd 40 (Cyrenne and Brown, 2011), and that Antide treatment would influence response to novelty in one or both sexes. A small number of studies have suggested that preference for novel objects is reduced by removal of gonadal hormones in adult rodents (e.g. males: Aubele et al., 2008; Ceccarelli et al., 2001; females: Wallace et al., 2006). However, these studies used a NOR task with longer inter-trial intervals than the current study; thus, the effects of gonadectomy might have resulted from changes in memory performance rather than from changes in initial preference for novelty. Whether manipulating gonadal hormone levels influences preference for novelty at short inter-trial intervals in adolescent rats has not been examined previously.

Methods

Subjects and housing

The subjects were 24 male and 24 female Lister-hooded rats bred in-house from stock (Harlan, U.K.). All animals were housed in cages (measuring 25 cm × 45 cm × 15 cm) with ad libitum access to soy-free rodent pellets and water. Housing rooms were controlled for temperature (20 ± 1 °C) and humidity (55 ± 5%), and maintained on a 12-hour light:dark cycle (lights on 7 am). From pnd 17, pups were handled once per day and were weaned into same-sex sibling groups at pnd 21, then housed as same-sex pairs from pnd 28 onwards. The subjects were taken from 16 litters, with no more than one individual in each experimental group taken from a single litter. All appropriate guidelines and regulations were adhered to, as set out in the Principles of Laboratory Animal Care (NIH, Publication No. 85–23, revised 1985) and the UK Home Office Animals (Scientific Procedures) Act 1986.

Experimental design

On pnd 28, experimental animals (12 males, 12 females) were treated with a gonadotrophin-hormone releasing hormone (GnRH) antagonist, Antide (Bachem Distribution Services, Germany; dissolved in 1:1 mixture of propylene glycol:saline) via subcutaneous injection at a dose of 6 mg/kg (based on Habenicht et al., 1990; Takeyoshi et al., 2002). Control animals (12 males, 12 females: cage-mates of Antide-treated subjects) were administered with a subcutaneous injection of the vehicle solution at pnd 28. For all subjects, body weight and ano-genital distance were measured at pnd 21, 28, 35 and 40. Behavioural testing was conducted on pnd 40. Immediately after testing, subjects were euthanised, and testes and uteri were removed and weighed. Blood was also collected for hormonal analysis at this time, and the serum was stored at −80 °C prior to assay.

Hormone assays

Serum samples from male subjects were analysed using a testosterone ELISA assay kit (Assay Designs, Enzo Life Sciences, U.K.). Samples were diluted (1:10) and run in duplicate. This kit has a lower limit of detection of 5.67 pg/ml, an inter-assay coefficient of variation of 11.3% and an intra-assay coefficient of variation of 10.0%. Serum samples from female subjects were analysed using a progesterone ELISA assay kit (Assay Designs, Enzo Life Sciences, U.K.). Samples were diluted (1:100) and run in duplicate. This kit has a lower limit of detection of 8.57 pg/ml, an inter-assay coefficient of variation of 8.3% and an intra-assay coefficient of variation of 5.4%.

Apparatus

The apparatus for the NOR task was a wooden, light grey-painted square chamber (67 cm × 67 cm × 45 cm, l × w × h: similar in size to previous studies, e.g. Cain et al., 2005; Ennaceur and Delacour, 1988; Inagaki et al., 2010). Five objects (yellow rubber toy, glass jar filled with rocks, blue plastic bottle filled with sand, orange plastic toy