Loneliness depends on salivary estradiol levels in adolescent females

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Abstract

OBJECTIVE: Loneliness is one of the psychological characteristics in adolescence, during which sex hormones are elevated. The elevation of sex steroid hormones is known to sculpture and remodel neuronal circuits, which cause behavioral characteristics in adolescence. The aim of the present study is to investigate the relationship between loneliness and sex steroid hormones, testosterone (T) and 17β-estradiol (E2).
METHODS: Fifty-eight adolescents (28 boys and 30 girls) participated in this study. The salivary levels of T and E2 were measured by Enzyme-Linked Immunosorbent Assay (ELISA). Loneliness was assessed by the UCLA loneliness scale, which is widely used as a self-administered questionnaire.
RESULTS: The results showed that Salivary E2 levels had positive relevance to loneliness in females, whereas there was no relationship in males. Salivary T level was not shown to be relevant with loneliness in either sex group.
CONCLUSIONS: These findings suggest that E2 has gender specific effects on loneliness in adolescent females.

ORIGINAL ARTICLE

INTRODUCTION

Adolescence is a period of physical, psychological and social transition between childhood and adulthood (Spear 2000). One of the behavioral characteristics during adolescence is a high level of social interactions. Adolescents spend more time with peers (Csikszentmihalyi & Larson 1984), place greater value on peers' approval, advice and opinions (Brown 1990) and are more concerned about maintaining peer relationships (Parkhurst & Hopmeyer 1988), which enable them to be independent from their parents. As peer relationships become more important during adolescence, the potential consequences of rejection by peers, including loneliness, also becomes more severe. A review of related research has shown that the prevalence of loneliness peaks during adolescence (Heinrich & Gullone 2006).

Maturation of the reproductive system during adolescence results in elevated levels of gonadal steroid hormones. In addition to secondary sexual characteristics influencing the physical body appearance, gonadal hormones contribute to the development of neural function by binding to androgen or estrogen receptors in the brain (Casey *et al.* 2010). The elevation of these hormones during adolescence acts on the brain to

affect behavior in two distinctive ways (Phoenix *et al.* 1959; Peper *et al.* 2011): (i) activation effects; that is, hormonal stimulation functionally acts on neural pathways to activate certain behaviors, which are reversible, and (ii) organization effects; that is, steroids act on the central nervous system to structurally reorganize neural pathways, which are irreversible.

With regard to activation effects, many researchers have shown hormonal effects on socio-emotional behavior, such as aggression, mood and emotion recognition (Archer 2006; Lokuge *et al.* 2010; Derntl *et al.* 2008; Guapo *et al.* 2009). Additionally, a recent study has reported that adolescence, during which hormones are drastically increased, is positively associated with elevated physiological reactivity to emotional cues (Silk *et al.* 2009) and showed a positive association between testosterone levels and anticipation of reward with positive affect (Forbes *et al.* 2010). These findings suggest that the elevated level of gonadal hormones plays a prominent role in socio-emotional behavior during adolescence.

In regard to the organizational effects during adolescence, several studies using structural imaging have shown that the volume of structures in sub-cortical regions, such as the amygdala and hippocampus, increases (Giedd *et al.* 1996), whereas gray matter in the prefrontal regions thins between childhood and adulthood (Giedd *et al.* 1999). Similarly, a recent study has suggested that the development of top-down prefrontal regions during adolescence is immature relative to the development of bottom-up striatal regions involved in detecting salient cues in the environment (Somerville & Casey 2010). Therefore, these gaps of developmental progress in those brain areas may underlie characteristic adolescent behaviors, such as vulnerability to social rejection or isolation, which can, in turn, lead to loneliness.

The aim of the present study is to investigate the relationships between loneliness and gonadal steroid hormones, testosterone (T) and 17β-estradiol (E2), of which drastic elevations are characteristic features of adolescence. Several studies have examined sex differences in the behavioral (Kloep 1999) and neural (Eisenberger *et al.* 2009; Sebastian *et al.* 2010; Sebastian *et al.* 2011) responses to social isolation. However, the relationship between gonadal steroid hormone levels and loneliness in adolescence is still unclear. Therefore, we measured loneliness and salivary gonadal steroid levels by means of the UCLA loneliness scale and the Enzyme-Linked Immunosorbent Assay (ELISA) method, respectively, to investigate the relationships between these factors.

METHODS

Participants

A total of 58 healthy adolescents aged 15 to 17 years (28 boys and 30 girls, mean age 16.1±0.72 years) participated in the present study. None of the participants used hormonal contraceptives or medications that affect sex steroids. The participants were recruited from three high schools in different geographical areas of Nagasaki prefecture in Japan, which are representative of a range of socioeconomic status backgrounds. All participants provided written informed consent prior to the start of the experiment. The experimental protocol was conducted in accordance with the Declaration of Helsinki. The present study was approved by the Ethics Committee of the Nagasaki University Graduate School of Biomedical Sciences.

Measures of testosterone and 17β-estradiol levels in saliva

Saliva samples were collected from each participant between 12:00 and 13:00 on the day of the experiment (15–30 min before the noon meal), by having them spit through a straw into a small polypropylene tube. Saliva samples were frozen and stored at −80 °C in the laboratory. T and E2 were assayed in saliva duplicates using an ELISA technique (Salimetrics, State College, USA), with each sample being analyzed in duplicate. The average intra-assay coefficient of variation (CV) was 4.5% and 4.7%, respectively. In the present study, we did not control for menstrual cycle phase for each participant.

Measures of loneliness

In order to assess the loneliness of each participant, we used the UCLA loneliness scale (revised), a 20-item self-administered questionnaire measuring general feelings of social isolation, loneliness, and dissatisfaction with one's social interactions (Russell 1996). Participants were asked to rate how often they felt the way described by the items on a scale ranging from 1 (never) to 4 (often). The UCLA scale has been shown to have high reliability (alpha = 0.94) and is widely used. High scores reflect a high degree of loneliness. We used the Japanese version to assess subjective loneliness of all participants in the experiment (Moroi 1991).

RESULTS

The average score of each salivary gonadal steroid level (T and E2) and UCLA loneliness scale by sex are presented in Table 1 together with the standard deviations. Each averaged score by sex factor was tested by unpaired t-test. For each gonadal steroid level, it should be appreciated that the salivary T levels in males were significantly higher than those in females, and that salivary E2 levels in females were significantly higher than those in males. Moreover, the UCLA loneliness score in males were significantly higher than those in females. This result was in line with several previous findings which suggested that males are more lonely than females during adolescence (Avery 1982; Koenig *et al.* 1994).

Pearson's correlations were calculated to examine the relationships between each salivary gonadal steroid

level (T and E2) and the UCLA loneliness score, respectively. As shown in Table 2, there was no relationship between salivary T level and loneliness scores in either sex group. On the other hand, salivary E2 levels were significantly correlated with loneliness score in females (Figure 1), whereas there was no relationship in males. These results suggest that female adolescents with high E levels are more lonely than those with low E2 levels.

Tab. 1. Gonadal steroid levels and loneliness score by sex.

Variables	Male (n=28)		Female (n=30)		
	M	SD	M	SD	t
Testosterone (pg/ml)	159.06	49.07	73.07	21.05	8.57 ***
17β-estradiol (pg/ml)	1.47	0.81	2.37	1.09	3.54 ***
UCLA loneliness scale	38.82	8.74	32.87	7.09	2.84 **

**p<0.01; ***p<0.001

DISCUSSION

In the present study, we investigated the relationships between loneliness and salivary levels of gonadal steroid hormones, T and E2 during adolescence. We found that the level of salivary E2 had positive relevance to loneliness in adolescent females, whereas there was no relationship in adolescent males. On the other hand, salivary T levels did not affect loneliness in either sex group. These results suggest that loneliness in adolescent females is associated with salivary levels of E2 but not testosterone. On the other hand, loneliness in adolescent males is independent of the gonadal steroids levels.

Adolescence is characteristic of dramatic elevation both in the level of gonadal steroid hormones and loneliness (Heinrich & Gullone 2006). These two adolescence characteristics are well known, but the relationships have not been investigated directly. Our current data suggest that loneliness is modulated by E2 levels in adolescent females, but not males. To the best of our knowledge, the present study is the first to clarify the association between E2 levels and loneliness in women.

Although the neuronal mechanism underlying the positive relationship between E2 levels and the loneliness could not be clarified in the present study, one possible explanation is that E2 may affect brain regions which modulate the sensitivity to social distress. The most broadly accepted definition of loneliness is the distress that results from discrepancies between desired and perceived social relationships (Perlman & Peplau 1981), indicating that loneliness can be considered as the most typical form of social distress. In a brain imaging study using a virtual ball-tossing game task, which creates a social distress (peer rejection) artificially, it was found that the activation of amygdala (Masten *et al.* 2009) and dorsal anterior cingulate cortex (dACC) (Eisenberger *et al.* 2003; Way *et al.* 2009) have a positive correlation with the distress induced by social rejection. Several other findings have suggested that ERs are abundant in the amygdala and cingulate cortex compared with other brain regions (Goldstein *et al.* 2001; Ostlund *et al.* 2003; Merchenthaler *et al.* 2004; Shughrue *et al.* 1997; Butler *et al.* 1999; Gerlach *et al.* 1983). It is, therefore, plausible that E2 modulates the activity of the amygdala and/or dACC, which are involved in the vulnerability to social distress in human relationships.

Another possible neural mechanism is that E2 may modulate the sensitivity to threats in a social context.

Tab. 2. Correlation with gonadal steroid levels and loneliness score.

	Male (n=28)	Female (n=30)
Testosterone (pg/ml)	–0.078	0.073
17β-estradiol (pg/ml)	–0.014	0.442 *

*p<0.05

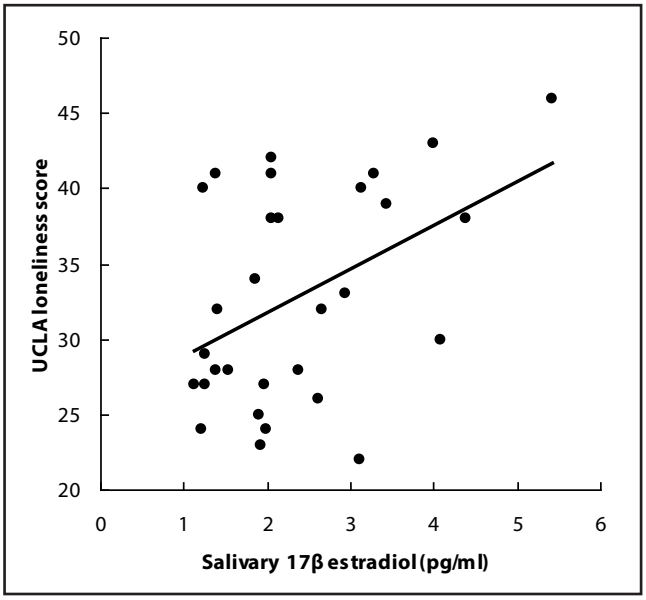

Fig. 1. Scatterplot of the salivary 17β-estradiol (E2) levels against the UCLA loneliness score in females.

Using a face recognition task, many studies have shown that fearful faces are threat-related stimuli (Bishop *et al.* 2007). In women, it was reported that the sensitivity to fearful faces altered depending on the phase of the menstrual period, and the recognition rate for fearful faces was enhanced in the period of high E2 levels during the menstrual cycle (Pearson & Lewis 2005). On the other hand, fearful faces have been proved to activate the amygdala, which is prominent in size during adolescence compared with other developmental stages (Adolph 2008; Guyer *et al.* 2008). Moreover, Hariri and colleagues found that people with below-average levels of social support (therefore, probably considered

as being lonely) exhibit more activity in the amygdala for fearful faces than people with above-average levels (Hyde *et al.* 2011). Taking these findings together, there is the possibility that the amygdala activation by E2 modulates the sensitivity to threats in a social context. Accordingly, if loneliness can be considered as a consequence of threats in a social context, a similar mechanism may be involved.

In the present study, we found no association between E2 levels and loneliness in adolescent males. Although we could not determine how E2 levels influence loneliness exclusively in adolescent females, two possible explanations can be made. First, sex differences in the expression of ERs in the brain areas which are involved in loneliness may account for the sex specific effect of E2 on loneliness. Indeed, individual differences in sensitivity for social distress may be attributed to the responsiveness of the amygdala, which includes ERs abundantly (Goldstein *et al.* 2001; Ostlund *et al.* 2003; Merchenthaler *et al.* 2004; Shughrue *et al.* 1997; Butler *et al.* 1999; Gerlach *et al.* 1983). Furthermore, animal studies showed that there was an obvious difference of ERβ expression between males and females, and that the expression levels of females in the amygdala were higher than those of males (Zhang *et al.* 2002). These sex differences in ER expression in the amygdala, which is a neural correlate of social distress, suggest the sex differences in E2 have effects on loneliness.

The second explanation concerns the sex difference in psychological factors underlying loneliness. It has been revealed that high social anxiety is a significant predictor of loneliness for adolescent females whereas low self-esteem was significant for adolescent males (Inderbitzen-Pisaruk *et al.* 1992). Therefore, if E2 levels have an influence on the activation of the amygdala, which is a social anxiety-related brain region (Etkin & Wager 2007), but not on the activation in medial PFC (BA10), which is a self-esteem-related brain region (Eisenberger *et al.* 2011), E2 levels in adolescent males would not affect loneliness. As described above, the amygdala contains relatively high levels of ERs compared with other brain regions whereas medial PFC (BA10) contains relatively low levels of ERs (Goldstein *et al.* 2001). Additionally, the expression levels of ERβ in the amygdala in females were higher than those in males (Zhang *et al.* 2002). These findings support the hypothesis that the sex differences of brain functioning caused by E2 underlies the sex difference of loneliness.

The present study has also investigated the relationship between loneliness and T levels, both of which show drastic elevation during adolescence (Heinrich & Gullone 2006). However, there was no relationship between them in either sex group. It has been reported that T levels have a positive association with the traits required in competitive situations, such as success and dominance (Archer 2006), aggression (Christiansen & Knussmann 1987; Archer 2006), impulsivity (Bjork *et al.* 2001; Fujisawa et al.), and distrust (Bos *et al.* 2010). In contrast, many previous findings have showed that oxytocin has a positive correlation with the traits required in cooperative situations, such as interpersonal trust (Kosfeld *et al.* 2005) and empathy (Domes *et al.* 2007), sensitivity for non-verbal information in communication (Hollander *et al.* 2007). On the other hand, E2 increases the number of oxytocin receptor and the production of oxytocin (Uvnas-Moberg *et al.* 2003). These findings suggest the possibility that E2 serves as an affiliation motivation with others (Schacter 1959) via oxytocinergic pathways, whereas T serves as an achievement motivation by competing with others (McClelland 1965). Therefore, T levels might not affect loneliness in either adolescent males or females.

In conclusion, the present results showed that E2 levels have positive relevance to loneliness in females but not in males during adolescence, whereas there was no relationship between loneliness and T levels in either sex group. These findings suggest that E2 modulates loneliness levels by enhancing the sensitivity to social distress in human relationships among adolescent females. However, we could not clarify the neural mechanism underlying this effect. The measurement of brain activity using imaging techniques will be fruitful in further elucidating the mechanisms underlying loneliness during adolescence.

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