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断乳后隔离对 BALB/c 小鼠的焦虑水平、社会行为及血清应激激素影响的性别差异

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摘 要: 社会隔离(社会剥夺)对行为和神经内分泌的效应已在社会性水平较高的动物被广泛研究, 但社会性水平较低的动物是否具有类似效应, 且这种效应是否具有性别差异还不清楚。BALB/c 品系小鼠具有较低的社会性, 为了探讨上述问题, 将断乳后的 BALB/c 小鼠单独或群居饲养 6 周至成体, 用旷场实验和同性社会互作实验分别检测雄性和雌性的焦虑水平、运动性及社会行为, 并用酶联免疫方法检测血清皮质酮(corticosterone, CORT)、去甲肾上腺素(norepinephrine, NE)和催产素(oxytocin, OT)的浓度。结果发现, 与群居饲养相比, 断乳后单独饲养增加了两性的社会探究行为, 同时增加了雌性的焦虑水平及雄性的运动性和攻击行为, 减少了雄性的身体接触。此外, 单独饲养增加了雌性和雄性血清 CORT 水平及雌性 NE 和 OT 水平。这些结果表明断乳后隔离也能引起低社会性水平动物的行为和应激内分泌改变, 且具有性别差异, 这种差异可能与 NE 和 OT 两种激素释放的差异有关。

关键词: 隔离; 应激; 焦虑; 社会性; 激素; BALB/c 小鼠
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Sex Differences in Post-weaning Isolation Induced Anxiety Levels, Social Behaviors and Serum Stress Hormones in BALB/c Mice

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Abstract: The effects of social isolation (social deprivation) on behavioral and neuroendocrine responses have been widely studied in prosocial animals. However, little is known about whether post-weaning isolation exerts similar effects in asocial animals, and whether there are sex-specific differences in these effects. BALB/c mice are generally less sociable. In order to investigate aforementioned question, weanling male and female BALB/c mice were singly or group housed for six weeks, then anxiety levels, locomotion and social behaviors were examined using an open field test and the same-sex social interaction test, and the concentrations of serum corticosterone (CORT), norepinephrine (NE) and oxytocin (OT) were measured using ELISA. The results showed that single rearing resulted in an increase in the social investigation behavior in both sexes compared to group rearing. Furthermore, the females experiencal single rearing enhanced the anxiety levels, while the males experienced single rearing increased the locomotion and the aggressive behavior, and decreased the contact behavior. Along with these changes, single rearing aggravated the levels of serum CORT in both sexes, while serum NE and OT were increased only in females. Taken together, these results indicate

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that post-weaning isolation results in sex-specific changes in behaviors and stress endocrine in asocial animals, which might be associated with the release differences of NE and OT.

Key words: isolation; stress; anxiety; sociability; hormone; BALB/c mice

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Psychosocial stress, specifically social isolation, results in profound behavioral and neurochemical abnormalities[1~3]. For instance, rats subjected to social isolation from weaning to early adulthood show a strong increase in stress related behaviors, such as increased anxiety-related behaviors[4, 5]. Similar to rats, mice also show increased anxiety, aggression and social interaction[6, 7]. In addition to behavioral changes, social isolation also results in an endocrinological response[5, 8]. For instance, social isolation leads to a significant increase in circulating adrenocorticotrophic hormone (ACTH), corticosterone (CORT) levels and corticotropin releasing hormone (CRH) receptor mRNA expression in prairie voles (*Microtus ochrogaster*)[9, 10]. CORT (cortisol, in human) is a stress hormone produced by the hypothalamic-pituitary-adrenal (HPA) axis, and its level is associated with a number of important behavioral disor ders, such as anxiety and depression[11]. Norepinephrine (NE) is released when a host of physiological changes are activated by a stressful event, and it has been reported that social isolation alters the metabolism and function of NE and increases its responses in the nucleus accumbens[12, 13]. Oxytocin (OT) is synthesized primarily in the supraoptic and paraventricular nuclei (PVN) of the hypothalamus and there are oxytocinergic projections to the posterior pituitary gland where it is released into the blood stream[14]. Increased levels of OT in the central and peripheral nervous systems have been associated with the experience of stressful events in humans and rodents[15~17]. For instance, during an acute shaker stress paradigm in male rats, OT was released specifically in the hypothalamic PVN and the plasma[16]. In addition, OT is able to interact with stress hormones in the HPA axis to regulate social behavior and emotion[18~20], which serves as a buffer against both physical and emotional stressors[19, 21, 22]. For instance, intracerebroventricular injections of OT could produce a comparable decrease in serum CORT levels[23].

The response to social isolation is not entirely consistent across rodents. Highly social species are more susceptible to social isolation[24~26], and permanent effects of post-weaning isolation are only observed in species that demonstrate high levels of social play during one stage of development[27]. To date, prosocial animals have been the primary subjects of studying how isolation influences behaviors[3, 28~30]. However, it is not well known whether social isolation exerts similar effects on asocial animals at the behavioral and endocrine levels. What's more, it is also unclear whether there are sexual differences in isolation-related response in asocial animals. BALB/c mice belong to inbred strain that shows low level sociability[31~33]. Thus, they can serve as a model for studying the effects of social isolation on asocial animals. The effects of isolation rearing may depend on the subjects' developmental stage during which isolation manipulations are conducted[7]. Early social isolation from weaning to adulthood is a procedure where animals are deprived of social contact with conspecifics during development. The present study aimed to explore the effects of post-weaning isolation on BALB/c mice and possible sexual differences in isolation-related behaviors and stress hormones.

1 Materials and methods

1.1 Experimental animals

Adult male and female BALB/c mice (Ningxia Medical University Laboratory Animal Center, Yinchuan, China) were housed in standard transparent Makrolon cages (l×w×h, 32×21.5×17 cm), and raised under a 12∶12 light-dark cycle (lights on 20: 00 h) at 23±2 ℃ with food and water avail-

able *ad libitum*. Virgin females and males were paired, and 20 days after pairing, females were checked daily for signs of labor. The day of birth was denoted as postnatal day zero. Pups were separated from parents after weaning at the age of 23 days. Male and female pups were separated, and divided into two groups: 1) reared in groups with four pups per cage (group rearing, cage size 38×27×16 cm); 2) reared singly in a cage for six weeks before testing (isolation rearing, cage size 38×27×16 cm). Subjects were therefore assigned to one of four treatment groups: group housed males (MG, n=10), isolated males (MI, n=10), group housed females (FG, n=10), and isolated females (FI, n=10). All animals were treated humanely according to guidelines approved by the Animal Care and Use Committee of Beifang University of Nationalities.

1.2 Open-field test

Following a six-week isolation, spontaneous motor activity and anxiety-like behavior were assessed in an open field chamber. The chamber was a brightly and evenly illuminated square arena (l×w×h, 50×50×25 cm) made of white glacial polyvinyl chloride and illuminated by four 60 W lamps mounted 1.5 m above the box (400 lx in the centre of arena). The area was divided into 16 quadrants (4 central and 12 peripheral quadrants)[34]. The tested mice were placed individually into the centre of the open field, left to explore for 5 min and videotaped using a video-recorder mounted 70 cm above the arena. After the test trial was completed, the chamber was thoroughly cleaned with 70% ethanol solution. The time each mouse spent in the central and the number of crossings between quadrants were recorded using Jwatcher 1.0.

1.3 Same-sex social interaction test

After the open-field test, the social interaction test was conducted. Tested mice had an almost equal body weight compared to stimulus mice. Stimulus mice were unfamiliar, sexually naive individuals of the same sex. Testing was conducted in a neutral plastic cage (46×31.5×20 cm), with approximately 2 cm of wood shavings on the floor and a removable clapboard in the middle. Prior to testing, stimulus and focal animals were placed on the opposite side of the cage and allowed to acclimate for 5 min while separated by the clapboard. Once the clapboard was removed, the dyadic social interaction was monitored for 15 min. Each individual's behavior was classified as follows: social investigation (sniffing the face, body or anogenital area of an individual), contact behavior (contact with another individual, including staying together or amicable grooming), aggressive behavior (pouncing (jumps or lunges), fighting (tumbling and biting) and chasing), rearing behavior (rising on the hind legs and sniffing into the air or the wall of the box), and self-grooming (cephalocaudal progression that begins with rhythmic movements of the paws around the mouth, face and ears, descending to the ventrum, flank, anogenital area and tail).

1.4 Serum CORT, NE and OT assays

Following behavioral testing, mice (males, n=6; females, n=6) were deeply anesthetized. Blood was collected from the retro-orbital sinus in the after noon (16: 00-17: 00). The collected blood samples were immediately centrifuged and separated, then serum was stored at -20 ℃. The concentrations of CORT, NE and OT were measured using a mouse-specific enzyme-linked immunosorbent assay kit (Suzhou Kaerwen Biotechnology, Suzhou, China) following the proprietary protocol. First, the prepared sample and the standard were incubated for 30 min at room temperature. Next, the prepared sample and the standard were placed in separate plate wells, and horseradish peroxidase-conjugate reagent was added, then samples/standards were incubated for 60 min at 37 ℃. Lastly, after the plate was washed four times, we added chromogen solutions A and B. After 15 min of incubation at 37 ℃, the reaction was terminated using the stop solution. The optimal dilution of serum (1 ∶ 5) was determined via dilution curves (1 ∶ 1, 1 ∶ 5 and 1 ∶ 10). The optical density of the sample was determined at 450 nm using a Bio-Rad iMark, with the blank well set as zero. The variation between duplicate measurements was less than 5%.

1.5 Statistical analysis

Statistical analyses were conducted using SPSS

13.0 (SPSS, Chicago, IL, USA). The data were analyzed using two-way analysis of variance (ANOVA). The variables were housing condition (group or isolation) and the sex (male or female). Post hoc tests were conducted using Fisher's least-significant difference (LSD) if a significant difference was found. All values were presented as mean±standard error and the alpha was set at 0.05.

2 Results

2.1 The behaviors of isolated or group-housed mice in open field

A two-way ANOVA revealed significant effects of sex and housing condition on the time spent in the central area (sex, $F_{1,36}$=11.290, P=0.002; housing condition, $F_{1,36}$=5.165, P=0.029) and on total transition (sex, $F_{1,36}$=12.191, P=0.001; housing condition, $F_{1,36}$=11.856, P=0.001). We also observed an interaction between sex and housing condition in the time spent in the central area ($F_{1,36}$=4.143, P=0.049) and transition ($F_{1,36}$=4.132, P=0.05). Group housed females spent significantly more time in the central area than group housed males (*mean difference*=29.547, P=0.001). In comparison to group housed mice, social isolation reduced the time spent in the central area in females (*mean difference*=-23.592, P=0.004) (Fig.1A) and increased transition in males (*mean difference*=33, P<0.001; Fig.1B).

2.2 Social interaction of isolated or group-housed mice

The significant effects of sex on aggression ($F_{1,36}$=72.822, P<0.001), contact behavior ($F_{1,36}$=11.905, P=0.001) and self-grooming behavior ($F_{1,36}$=8.346, P=0.007) were found. Housing condition resulted in significant differences in social investigation ($F_{1,36}$=30.631, P<0.001), aggression ($F_{1,36}$=72.133, P<0.001) and rearing behavior ($F_{1,36}$=8.024, P<0.001). For aggressive behaviors, we observed a significant interaction between sex and housing condition ($F_{1,36}$=72.133, P<0.001). Compared to the group-housed mice, the socially isolated mice significantly increased social investigation in both females (*mean difference*=16.287, P<0.001) and males (*mean difference*=43.697, P=0.011) (Fig.2A). Interestingly, isolated males significantly increased aggression (*mean difference*=79.867, P<0.001), and decreased contact behavior (*mean difference*=-94.376, P=0.026) (Fig.2B), while isolated females engaged in less rearing behavior (*mean difference*=-19.517, P=0.024) (Fig.2C). Moreover, group-housed males showed less self-grooming behavior than the group-housed females (*mean difference*=-39.182, P=0.037) (Fig.2D). It should be noted that aggressive behaviors during social interaction were observed only in isolated males and rarely observed in other three groups (data not shown).

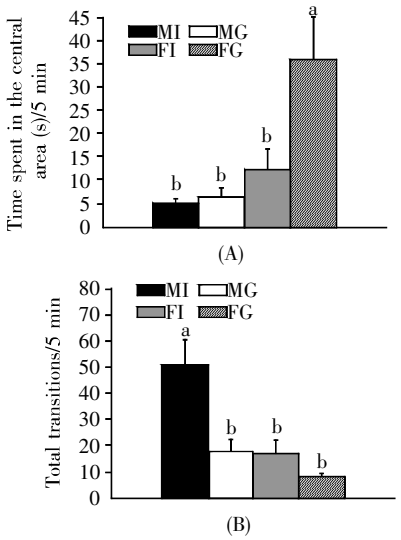

Fig.1 Behaviors of isolated or group-housed BALB/c mice in an open field test

(A) Time spent in the central area; (B) Transitions in the open field. Groups not sharing same letters are significantly different (P≤0.05). MI, isolated males; MG, group housed males; FI, isolated females; FG, group housed females.

2.3 The levels of serum CORT, NE and OT in isolated or group-housed mice

A two-way ANOVA revealed significant effects of sex and housing condition on the levels of serum CORT (sex, $F_{1,20}$=5.889, P=0.025; housing condition, $F_{1,20}$=63.966, P<0.001) and OT (sex, $F_{1,20}$=39.354, P<0.001; housing condition, $F_{1,20}$=5.025, P=0.036), while only sex had an effect on NE levels ($F_{1,20}$=62.611, P<0.001). There was an interaction between sex and housing condition in serum CORT ($F_{1,20}$=9.183, P=0.007), NE ($F_{1,20}$=18.488, P<0.001) and OT levels ($F_{1,20}$=16.326, P=0.001). The post-hoc test revealed that serum CORT levels were significantly higher in the isolated males and females compared

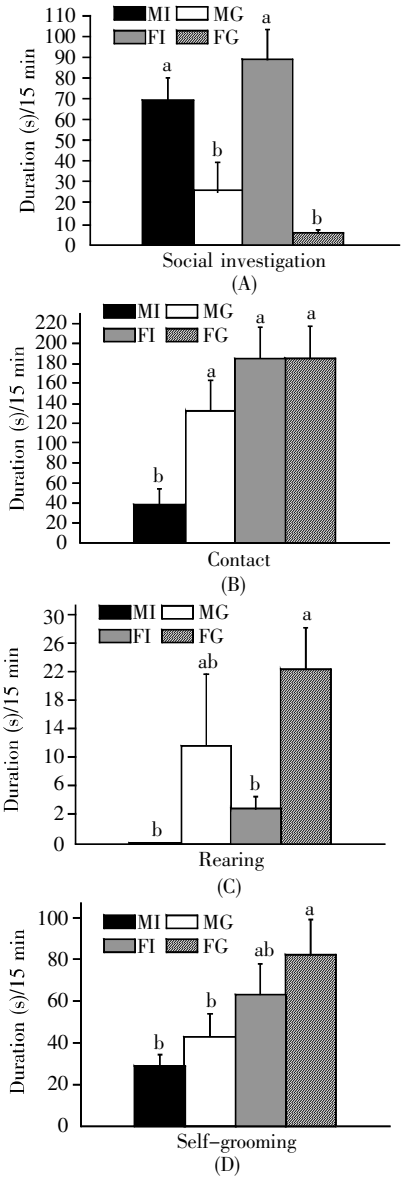

Fig.2　The same-sex social interaction in isolated or group-housed BALB/c mice

(A) Social investigation; (B) Contact behavior; (C) Rearing behavior; (D) Self-grooming behavior. Groups not sharing same letters are significantly different ($P \leqslant 0.05$). MI, isolated males; MG, group housed males; FI, isolated females; FG, group housed females.

to the group housed males and females (males, *mean difference* =12.94, P=0.013; females, *mean difference*=28.71, P<0.001) (Fig.3A). The difference in serum CORT levels was not found between group housed males and females (*mean difference*=1.57, P=1) (Fig.3A). Isolated females had significantly higher levels of NE than the group housed females (*mean difference*=0.89, P=0.002), while no significant difference was observed in the males (*mean difference* = −0.34, P=0.63). We observed a significant difference in serum NE levels between group housed males and females (*mean difference*=−0.53, P<0.001) (Fig.3B). In addition, isolation significantly elevated the serum OT levels in females (*mean difference* = 2.317, P<0.001), but no differences were found in males (*mean difference*=−0.663, P=0.218) (Fig.3C).

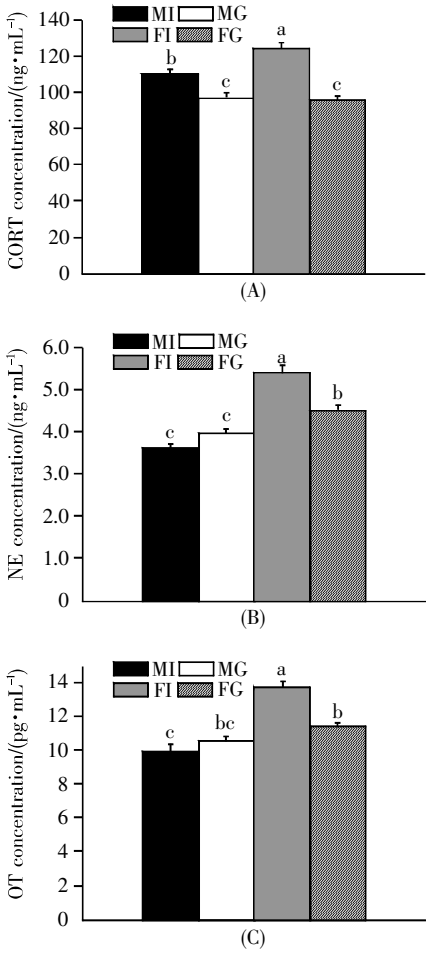

Fig.3　The concentrations of serum CORT (A), NE (B) and OT (C) in isolated or group-housed BALB/c mice

Groups not sharing same letters are significantly different ($P \leqslant 0.05$). MI, isolated males; MG, group housed males; FI, isolated females; FG, group housed females.

3　Discussion

In this study, social isolation of BALB/c mice from weaning to adulthood resulted in increased social investigation behavior. Isolated males showed an increase in locomotion accompanied by higher aggression, while isolated females showed increased anxiety levels accompanied by less rearing behavior. In addition, we observed effects of social isolation and sex on the concentrations of serum CORT,

NE and OT, which may underlie the behavioral changes.

3.1 Effects of post-weaning isolation on locomotion and anxiety-like behavior

Compared to group-housed controls, isolated females showed increased anxiety levels, whilst isolated males did not show changes in anxiety levels (Fig.1A). Studies in rats have also observed similar results where isolation-rearing increasd anxiety-like behavior only in females[35~38]. However, in gerbils (*Meiiones Unguiculataus*), which are social animals that live in family groups in the wild, singly housed males showed increased anxiety but the females didn't[29]. Since the social structure of a species has a strong effect on social isolation, the inconsistent findings between these studies may be a result of species-specific response to social isolation[24]. In addition, the range of test situations can also influence the behavioral changes that result from isolation in males and females[29]. We observed that post-weaning isolation significantly increased locomotive activity in male BALB/c mice (Fig.1B), which was consistent with some previous studies where isolated rats increased spontaneous locomotor activity[2, 39, 40]. However, we did not observe any differences in locomotion in females. Locomotor activity in a novel environment is a complex behavior that may be influenced by many factors, including anxiety levels, responsiveness to stress and novelty[41]. Here, isolated females showed increased anxiety, whilst there were no specific effects in males; isolated males increased locomotion, whilst there were no specific effects in females. These results suggest that the levels of locomotion do not represent anxiety levels in BALB/c mice, and emotionality of females is more susceptible to early isolation than that of males. In addition, we found that group-housed female BALB/c mice spent more time in the central area in open field than males, suggesting that males have higher levels of anxiety than females (Fig.1A). Meanwhile, similar to a previous study[33], we did not observe sex differences in locomotion in group housed BALB/c mice (Fig.1B).

3.2 Effects of post-weaning isolation on social behaviors

Isolated male and female BALB/c mice engaged in more social investigation compared to the group-housed animals (Fig.2A). These findings concured with some reports showing that early social isolation enhanced social investigation[42~44], but differed from other studies suggesting that isolation decreased social interaction[29, 45]. In addition, we found that isolated male mice showed decreased contact behavior (Fig.2B) and increased aggression, which concured with previous reports from rats and prairie voles where post-weaning isolation resulted in increased aggression levels[3, 42, 44, 46]. In our experiments, isolated females showed less rearing behavior when compared to the group-housed females (Fig.2C). The differences in rearing suggest they may be different in emotional processes when engaging in female-female interaction[47]. Male BALB/c mice are known to be less social than females[33], and our findings support the proposal that low level of social behavior does not correlate with low level of locomotion[30, 48]. Group-housed female BALB/c mice exhibited more rearing behaviors when interacting with same-sex individuals compared to males (Fig.2C). It has been found that ICR mice show similar behavioral differences[49]. Female BALB/c mice exhibited more self-grooming behaviors than males (Fig.2D), and this difference suggests that there may be sex difference in emotional state during social interaction[47, 50].

3.3 Effects of post-weaning isolation on stress hormones

Although peripheral hormone levels may not fully capture central hormone levels[51], the peripheral measures are likely to reflect responses to a range of stimuli, including social stimuli[52]. Compared to group-housed controls, we found a significant increase in the concentration of serum CORT in isolated males and females (Fig.3A). Social isolation is stressful, and the physiological changes elicited by stress comprise a cascade of neuroendocrine events mediated by stress systems, such as the HPA axis. The increase in CORT levels suggests that HPA axis activity is enhanced in the socially isolated in-

dividuals. When animals are subjected to stress, CRH is secreted from the hypothalamus, which results in the secretion of CORT from the adrenal cortex to guard against stress disorders. However, our results were not consistent with a previous study that found no change in CORT levels of BALB/cJ mice isolated for 3 weeks[53]. This discrepancy may be due to the difference in the length of isolation in the experiments. For example, it has been shown that plasma CORT levels are higher in mice isolated for 5 weeks than in mice isolated for 2 weeks[54].

The locus coeruleus and postganglionic neurons of the sympathetic nervous system, adrenal medulla release NE into the blood. We observed higher NE levels in group-housed females compared to group-housed males. It has been reported that female ICR mice exhibit higher responses to stress and higher levels of serum CORT under both basal and stressed conditions compared to the males[55, 56]. These findings demonstrated the possible sex differences in the HPA axis and adrenergic system activity. What's more, isolated females showed an increase in serum NE levels, whilst there were no specific effects in the isolated males (Fig.3B). Therefore it appears that isolation induces release of NE mainly in females, likely contributing to the increased anxiety-behavior that was observed in females.

Interestingly, we observed higher serum OT in isolated females (Fig.3C). It is surprising, as high peripheral levels of OT and arginine vasopressin have been associated with improved social functioning in children with autism spectrum disorders[57, 58]. However, it was consistent with previous reports that showed increased plasma OT levels in female prairie voles isolated for 4 weeks[59] and greater OT content in the PVN in the singly housed female prairie voles[60]. Furthermore, the plasma OT increment following stress in rats is sexually dimorphic, with females exhibiting greater responses than males[61]. Churchland *et al.* (2012)[62] proposed that plasma OT reflected coordinated release by magnocellular cells in the PVN, and that serum levels of OT correlated with OT expression in the PVN. Since OT can be anxiolytic[63], and isolation results in anxiety behavior in females, the elevation of OT levels in isolated females may serve to protect females from the negative consequences of isolation[59]. Another idea is that anxiolytic effects of OT are male-specific[64]. Higher OT in women correlates with greater attachment anxiety[64], and OT may serve as an indicator of interpersonal stress, especially in women[65]. Nevertheless, our findings provide further evidence to support the sexual dimorphic role of OT in early isolation. Furthermore, the elevation of serum OT levels induced by isolation co-occurred with changes of NE levels in females, indicating a possible association between the two hormones.

In conclusion, isolation from weaning to early adulthood altered behaviors and stress hormones release in BALB/c mice, particularly in females. Our results demonstrate the importance of group life to develop behavioral flexibility even in asocial animals. The different effects of isolation on stress hormones help explain sexual differences in isolation-induced behavioral and emotional changes.

References:

[1] FONE K C, PORKESS M V. Behavioural and neurochemical effects of post-weaning social isolation in rodents-relevance to developmental neuropsychiatric disorders[J]. Neuroscience and Biobehavioral Reviews, 2008, 32(6): 1087-1102.

[2] HEIDBREDER C A, WEISS I C, DOMENEY A M, *et al*. Behavioral, neurochemical and endocrinological characterization of the early social isolation syndrome[J]. Neuroscience, 2000, 100(4): 749-768.

[3] SCOTTI M A, CARLTON E D, DEMAS G E, *et al*. Social isolation disrupts innate immune responses in both male and female prairie voles and enhances agonistic behavior in female prairie voles (*Microtus ochrogaster*)[J]. Hormones and Behavior, 2015, 70: 7-13.

[4] LENG A, FELDON J, FERGER B. Long-term social isolation and medial prefrontal cortex: dopaminergic and cholinergic neurotransmission[J]. Pharmacology Biochemistry and Behavior, 2004, 77(2): 371-379.

[5] WEISS I C, PRYCE C R, JONGEN-RELO A L, *et al*. Effect of social isolation on stress-related behavioural and neuroendocrine state in the rat[J]. Behavioural Brain Research, 2004, 152(2): 279-295.

[6] GUIDOTTI A, DONG E, MATSUMOTO K, *et al*. The socially-isolated mouse: a model to study the putative role of allopregnanolone and 5α-dihydroprogesterone in psychiatric disorders[J]. Brain Research Reviews, 2001, 37(1-3): 110-115.

[7] FERDMAN N, MURMU R P, BOCK J, *et al*. Weaning age, social isolation, and gender, interact to determine adult explorative and social behavior, and dendritic and spine morphology in prefrontal cortex of rats[J]. Behavioural Brain Research, 2007, 180(2): 174-182.

[8] PAN Y L, LIU Y, YOUNG K A, *et al*. Post-weaning social isolation alters anxiety-related behavior and neurochemical gene expression in the brain of male prairie voles[J]. Neuroscience Letters, 2009, 454(1): 67-71.

[9] GRIPPO A J, GERENA D, HUANG J, *et al*. Social isolation induces behavioral and neuroendocrine disturbances relevant

to depression in female and male prairie voles[J]. Psychoneuroendocrinology, 2007, 32(8–10): 966–980.
[10] POURNAJAFI–NAZARLOO H, PARTOO L, YEE J, *et al*. Effects of social isolation on mRNA expression for corticotrophin–releasing hormone receptors in prairie voles[J]. Psychoneuroendocrinology, 2011, 36(6): 780–789
[11] COWEN P J. Not fade away: the HPA axis and depression[J]. Psychological Medicine, 2010, 40(4): 1–4.
[12] STOLK J M, CONNOR R L, BARCHAS J D. Social environment and brain biogenic amine metabolism in rats[J]. Journal of Comparative and Physiological Psychology, 1974, 87(2): 203–207.
[13] KARKHANIS A N, LOCKE J L, MCCOOL B A, *et al*. Social isolation rearing increases nucleus accumbens dopamine and norepinephrine responses to acute ethanol in adulthood[J]. Alcoholism–Clinical and Experimental Research, 2014, 38(11): 2770–2779.
[14] NELSON E, PANKSEPP J. Oxytocin mediates acquisition of maternally associated odor preferences in preweanling rat pups[J]. Behavioral Neuroscience, 1996, 110(3): 583–592.
[15] NISHIOKA T, ANSELMO–FRANCI J A, *et al*. Stress increases oxytocin release within the hypothalamic paraventricular nucleus[J]. Brain Research, 1998, 781(1–2): 57–61.
[16] GRIPPO A J, BELTZ T G, WEISS R M, *et al*. The effects of chronic fluoxetine treatment on chronic mild stress–induced cardiovascular changes and anhedonia[J]. Biological Psychiatry, 2006, 59(4): 309–316.
[17] TAYLOR S E, GONZAGA G C, KLEIN L C, *et al*. Relation of oxytocin to psychological stress responses and hypothalamic–pituitary–adrenocortical axis activity in older women[J]. Psychosomatic Medicine, 2006, 68(2): 238–245.
[18] TURNER R A, ALTEMUS M, ENOS T, *et al*. Preliminary research on plasma oxytocin in normal cycling women: investigating emotion and interpersonal distress[J]. Psychiatry, 1999, 62(2): 97–113.
[19] RAZZOLI M, CUSHING B S, CARTER C S, *et al*. Hormonal regulation of agonistic and affiliative behavior in female Mongolian gerbils (*Meriones unguiculatus*)[J]. Hormones and Behavior, 2003, 43(5): 549–553.
[20] LIM M M, YOUNG L J. Neuropeptidergic regulation of affiliative behavior and social bonding in animals[J]. Hormones and Behavior, 2006, 50(4): 506–517.
[21] NEUMANN I D, KRÖMER S A, TOSCHI N, *et al*. Brain oxytocin inhibits the (re)activity of the hypothalamo–pituitary–adrenal axis in male rats: involvement of hypothalamic and limbic brain regions[J]. Regulatory Peptides, 2000, 96(1–2): 31–38.
[22] CARTER C S. Developmental consequences of oxytocin[J]. Physiology & Behavior, 2003, 79(3): 383–397.
[23] DEVRIES A C, CHO M M, CARDILLO S, *et al*. Oxytocin can suppress the HPA axis in prairie voles[Z]. Society for Neuroscience Abstract, 1997.
[24] SHIMOZURU M, KIKUSUI T, TAKEUCHI Y, *et al*. Effects of isolation–rearing on the development of social behaviors in male Mongolian gerbils (*Meriones unguiculatus*)[J]. Physiology & Behavior, 2008, 94(3): 491–500.
[25] SHAPIRO L E, INSEL T R. Infant's response to social separation reflects adult differences in affiliative behavior: a comparative developmental study in prairie and montane voles[J]. Developmental Psychobiology, 1990, 23(5): 375–393.
[26] STOWE J R, LIU Y, CURTIS J T, *et al*. Species differences in anxiety–related responses in male prairie and meadow voles: the effects of social isolation[J]. Physiology & Behavior, 2005, 86(3): 369–378.
[27] EINON D F, HUMPHREYS A P, CHIVERS S M, *et al*. Isolation has permanent effects upon the behavior of the rat, but not the mouse, gerbil or guinea pig[J]. Developmental Psychobiology, 1981, 14(4): 343–355.
[28] HOL T, VAN DEN BERG C L, VAN REE J M, *et al*. Isolation during the play period in infancy decreases adult social interactions in rats[J]. Behavioural Brain Research, 1999, 100(1–2): 91–97.
[29] STARKEY N J, NORMINGTON G, BRIDGES N J. The effects of individual housing on 'anxious' behaviour in male and female gerbils[J]. Physiology & Behavior, 2007, 90(4): 545–552.
[30] WANG J L, ZHANG L X, ZHANG P, *et al*. Cocaine–induced rewarding properties, behavioural sensitization and alteration in social behaviours in group–housed and postpuberty isolated female mandarin voles[J]. Behavioral Pharmacology, 2012, 23(7): 693–702.
[31] BRODKIN E S. BALB/c mice: low sociability and other phenotypes that may be relevant to autism[J]. Behavioural Brain Research, 2007, 176(1): 53–56.
[32] JACOME L F, BURKET J A, HERNDON A L, *et al*. Genetically inbred Balb/c mice differ from outbred Swiss Webster mice on discrete measures of sociability: relevance to a genetic mouse model of autism spectrum disorders[J]. Autism Research, 2011, 4(6): 393–400.
[33] AN X L, ZOU J X, WU R Y, *et al*. Strain and sex differences in anxiety–like and social behaviors in C57BL/6J and BALB/cJ mice[J]. Experimental Animals, 2011, 60(2): 111–123.
[34] FIORE L, RATTI G. Remote laboratory and animal behaviour: an interactive open field system[J]. Computers & Education, 2007, 49(4): 1299–1307.
[35] ARAKAWA H. Ontogeny of sex differences in defensive burying behavior in rats: effect of social isolation[J]. Aggressive Behavior, 2007, 33(1): 38–47.
[36] LEUSSIS M P, ANDERSEN S L. Is adolescence a sensitive period for depression? Behavioral and neuroanatomical findings from a social stress model[J]. Synapse, 2008, 62(1): 22–30.
[37] LUKKES J L, ENGELMAN G H, ZELIN N S, *et al*. Post–weaning social isolation of female rats, anxiety–related behavior, and serotonergic systems[J]. Brain Research, 2012, 1443: 1–17.
[38] HERMES G, LI N, DUMAN C, *et al*. Post–weaning chronic social isolation produces profound behavioral dysregulation with decreases in prefrontal cortex synaptic–associated protein expression in female rats[J]. Physiology & Behavior, 2011, 104(2): 354–359.
[39] WEISS I C, DI IORIO L, FELDON J, *et al*. Strain differences in the isolation–induced effects on prepulse inhibition of the acoustic startle response and on locomotor activity[J]. Behavioral Neuroscience, 2000, 114(2): 364–373.
[40] WEISS I C, FELDON J, DOMENEY A M. Isolation rearing–induced disruption of prepulse inhibition: further evidence for fragility of the response[J]. Behavioral Pharmacology, 1999, 10(2): 139–149.
[41] LEE M, CHEN K, SHIH J C, *et al*. MAO–B knockout mice exhibit deficient habituation of locomotor activity but normal nicotine intake[J]. Genes, Brain and Behavior, 2004, 3(4): 216–227.
[42] WONGWITDECHA N, MARSDEN C A. Social isolation increases aggressive behaviour and alters the effects of diazepam in the rat social interaction test[J]. Behavioural Brain Research, 1996, 75(1–2): 27–32.
[43] ESTELLES J, RODRÍGUEZ–ARIAS M, AGUILAR M A, *et al*. Social behavioural profile of cocaine in isolated and grouped male mice[J]. Drug and Alcohol Dependence, 2004, 76(2): 115–123.
[44] MENG Q X, LI N X, HAN X, *et al*. Peri–adolescence isolation rearing alters social behavior and nociception in rats[J]. Neuroscience Letters, 2010, 480(1): 25–29.
[45] HOLLOWAY K S, SUTER R B. Play deprivation without social, isolation: housing controls[J]. Developmental Psychobiology, 2004, 44(1): 58–67.
[46] HAN X, WANG W W, SHAO F, *et al*. Isolation rearing alters social behaviors and monoamine neurotransmission in the medial prefrontal cortex and nucleus accumbens of adult rats[J]. Brain Research, 2011, 1385: 175–181.
[47] TO C T, BAGDY G. Anxiogenic effect of central CCK administration is attenuated by chronic fluoxetine or ipsapirone treatment[J]. Neuropharmacology, 1999, 38(2): 279–282.
[48] BOLIVAR V J, CALDARONE B J, REILLY A A, *et al*. Habituation of activity in an open field: a survey of inbred strains and F1 hybrids[J]. Behavior Genetics, 2000, 30(4): 285–293.
[49] ZHAN B, MA H Y, WANG J L, *et al*. Sex differences in morphine–induced behavioral sensitization and social behaviors in ICR mice[J]. Zoological Research, 2015, 36(2): 103–108.
[50] CAREY R J, DEPALMA G, DAMIANOPOULOS E. Evidence for Pavlovian conditioning of cocaine–induced responses linked to emotional behavioral effects[J]. Pharmacology Biochemistry and Behavior, 2005, 80(1): 123–134.
[51] KAGERBAUER S M, MARTIN J, SCHUSTER T, *et al*. Plasma oxytocin and vasopressin do not predict neuropeptide concentrations in human cerebrospinal fluid[J]. Journal of Neuroendocrinology, 2013, 25(7): 668–673.
[52] KENKEL W M, SUBOC G, SUE CARTER C. Autonomic, behavioral and neuroendocrine correlates of paternal behavior in male prairie voles[J]. Physiology & Behavior, 2014, 128: 252–259.
[53] LIU X, WU R Y, TAI F D, *et al*. Effects of group housing on stress induced emotional and neuroendocrine alterations[J]. Brain Research, 2013, 1502: 71–80.
[54] FRANCÈS H, GRAULET A, DEBRAY M, *et al*. Morphine–induced sensitization of locomotor activity in mice: effect of social isolation on plasma corticosterone levels[J]. Brain Research, 2000, 860(1–2): 136–140.

(下转第 357 页)

[6] TAKAHASHI M, HIGUCHI M, MAKOKHA G N, *et al*. HTLV-1 Tax oncoprotein stimulates ROS production and apoptosis in T cells by interacting with USP10[J]. Blood, 2013, 122(5): 715-725.
[7] SONCINI C, BERDO I, DRAETTA G. Ras-GAP SH3 domain binding protein (G3BP) is a modulator of USP10, a novel human ubiquitin specific protease[J]. Oncogene, 2001, 20(29): 3869-3879.
[8] KOMANDER D, CLAGUE M J, URBE S. Breaking the chains: structure and function of the deubiquitinases[J]. Nature Reviews Molecular Cell Biology, 2009, 10(8): 550-563.
[9] BOMBERGER J M, BARNABY R L, STANTON B A. The deubiquitinating enzyme USP10 regulates the post-endocytic sorting of cystic fibrosis transmembrane conductance regulator in airway epithelial cells[J]. The Journal of Biological Chemistry, 2009, 284(28): 18778-18789.
[10] NIKOLAEV A Y, LI M, PUSKAS N, *et al*. Parc: a cytoplasmic anchor for p53[J]. Cell, 2003, 112(1): 29-40.
[11] YUAN J, LUO K T, ZHANG L Z, *et al*. USP10 regulates p53 localization and stability by deubiquitinating p53[J]. Cell, 2010, 140(3): 384-396.
[12] JOCHEMSEN A G, SHILOH Y. USP10: friend and foe[J]. Cell, 2010, 140(3): 308-310.
[13] MICHISHITA E, MCCORD R A, BERBER E, *et al*. SIRT6 is a histone H3 lysine 9 deacetylase that modulates telomeric chromatin[J]. Nature, 2008, 452(7186): 492-496.
[14] KAIDI A, WEINERT B T, CHOUDHARY C, *et al*. Human SIRT6 promotes DNA end resection through CtIP deacetylation[J]. Science, 2010, 329(5997): 1348-1353.
[15] SEBASTIAN C, ZWAANS B M M, SILBERMAN D M, *et al*. The histone deacetylase SIRT6 is a tumor suppressor that controls cancer metabolism[J]. Cell, 2012, 151(6): 1185-1199.
[16] CHAROLLAIS R H, BUQUET C, MEATER J. Butyrate blocks the accumulation of CDC2 mRNA in late G1 phase but inhibits both the early and late G1 progression in chemically transformed mouse fibroblasts BP-A31[J]. Journal of Cellular Physiology, 1990, 145(1): 46-52.
[17] CHAROLLAIS R H, MESTER J. Resumption of cell cycle in BALB/c-3T3 fibroblasts arrested by polyamine depletion: relation with "competence" gene expression[J]. Journal of Cellular Physiology, 1988, 137(3): 559-564.
[18] DALAL S N, SCHWEITZER C M, GAN J, *et al*. Cytoplasmic localization of human cdc25C during interphase requires an intact 14-3-3 binding site[J]. Molecular and Cellular Biology, 1999, 19(6): 4465-4479.
[19] ELY C M, LEFTWICH J A, CHENEVIX-TRENCH G, *et al*. Altered regulation of c-myc in an HL-60 differentiation resistant subclone, HL-60-1E3[J]. Cancer Research, 1987, 47(17): 4595-4600.
[20] FURUKAWA Y, PIWNICA-WORMS H, ERNST T J, *et al*. *cdc*2 gene expression at the G1 to S transition in human T lymphocytes[J]. Science, 1990, 250(4982): 805-808.
[21] GAZITT Y, REDOS G W, COHEN R J. Ultrastructural localization and fluctuation in the level of the proliferating cell nuclear antigen and myc oncoproteins in synchronized neuroblastoma cells[J]. Cancer Research, 1993, 53(8): 1899-1905.
[22] KELLY K, COCHRAN B H, STILES C D, *et al*. Cell-specific regulation of the *c-myc* gene by lymphocyte mitogens and platelet-derived growth factor[J]. Cell, 1983, 35(3): 603-610.
[23] LIN Z H, YANG H, TAN C, *et al*. USP10 antagonizes c-Myc transcriptional activation through SIRT6 stabilization to suppress tumor formation[J]. Cell Reports, 2013, 5(6): 1639-1649.
[24] KOBAYASHI S. Choose delicately and reuse adequately: the newly revealed process of autophagy[J]. Biological & Pharmaceutical Bulletin, 2015, 38(8): 1098-1103.
[25] ITAKURA E, KISHI C, INOUE K, *et al*. Beclin 1 forms two distinct phosphatidylinositol 3-kinase complexes with mammalian Atg14 and UVRAG[J]. Molecular Biology of the Cell, 2008, 19(12): 5360-5372.
[26] LIANG C Y, FENG P H, KU B, *et al*. Autophagic and tumour suppressor activity of a novel Beclin1-binding protein UVRAG[J]. Nature Cell Biology, 2006, 8(7): 688-699.
[27] MATSUNAGA K, SAITOH T, TABATA K, *et al*. Two Beclin 1-binding proteins, Atg14L and Rubicon, reciprocally regulate autophagy at different stages[J]. Nature Cell Biology, 2009, 11(4): 385-396.
[28] ZHONG Y, WANG Q J, LI X T, *et al*. Distinct regulation of autophagic activity by Atg14L and Rubicon associated with Beclin 1-phosphatidylinositol-3-kinase complex[J]. Nature Cell Biology, 2009, 11(4): 468-476.
[29] LIU J L, XIA H G, KIM M, *et al*. Beclin1 controls the levels of p53 by regulating the deubiquitination activity of USP10 and USP13[J]. Cell, 2011, 147(1): 223-234.
[30] MA J, SIEGEL R, JEMAL A. Pancreatic cancer death rates by race among US men and women, 1970-2009[J]. Journal of National Cancer Institute, 2013, 105(22): 1694-1700.
[31] LIU H, XU X F, ZHAO Y, *et al*. MicroRNA-191 promotes pancreatic cancer progression by targeting USP10[J]. Tumor Biology, 2014, 35(12): 12157-12163.
[32] SUN J M, LI Q, GU H Y, *et al*. *Interleukin* 10 rs1800872 T>G polymorphism was associated with an increased risk of esophageal cancer in a Chinese population[J]. Asian Pacific Journal of Cancer Prevention, 2013, 14(6): 3443-3447.
[33] PICKENS A, ORRINGER M B. Geographical distribution and racial disparity in esophageal cancer[J]. The Annals of Thoracic Surgery, 2003, 76(4): S1367-S1369.
[34] YANG Q Y, OU C, LIU M, *et al*. NRAGE promotes cell proliferation by stabilizing PCNA in a ubiquitin-proteasome pathway in esophageal carcinomas[J]. Carcinogenesis, 2014, 35(7): 1643-1651.
[35] DENG S S, ZHOU H Y, XIONG R H, *et al*. Over-expression of genes and proteins of ubiquitin specific peptidases (USPs) and proteasome subunits (PSs) in breast cancer tissue observed by the methods of RFDD-PCR and proteomics[J]. Breast Cancer Research and Treatment, 2007, 104(1): 21-30.
[36] GRUNDA J M, NABORS L B, PALMER C A, *et al*. Increased expression of thymidylate synthetase (TS), ubiquitin specific protease 10 (USP10) and survivin is associated with poor survival in glioblastoma multiforme (GBM)[J]. Journal of Neuro-Oncology, 2006, 80(3): 261-274.

(上接第 332 页)

[55] AOKI M, SHIMOZURU M, KIKUSUI T, *et al*. Sex differences in behavioral and corticosterone responses to mild stressors in ICR mice are altered by ovariectomy in peripubertal period[J]. Zoological Science, 2010, 27(10): 783-789.
[56] YAMAURA K, BI Y, ISHIWATARI M, *et al*. Sex differences in stress reactivity of hippocampal BDNF in mice are associated with the female preponderance of decreased locomotor activity in response to restraint stress[J]. Zoological Science, 2013, 30(12): 1019-1024.
[57] MODAHL C, GREEN L A, FEIN D, *et al*. Plasma oxytocin levels in autistic children[J]. Biological Psychiatry, 1998, 43(4): 270-277.
[58] GREEN L A, FEIN D, MODAHL C, *et al*. Oxytocin and autistic disorder: alterations in peptide forms[J]. Biological Psychiatry, 2001, 50(8): 609-613.
[59] POURNAJAFI-NAZARLOO H, KENKEL W, MOHSENPOUR S R, *et al*. Exposure to chronic isolation modulates receptors mRNAs for oxytocin and vasopressin in the hypothalamus and heart[J]. Peptides, 2013, 43: 20-26.
[60] AHERN T H, YOUNG L J. The impact of early life family structure on adult social attachment, alloparental behavior, and the neuropeptide systems regulating affiliative behaviors in the monogamous prairie vole (*Microtus ochrogaster*)[J]. Fronters in Behavioral Neuroscience, 2009, 3: 17.
[61] CARTER D A, SARIDAKI E, LIGHTMAN S L. Sexual differentiation of oxytocin stress responsiveness: effect of neonatal androgenization, castration and a luteinizing hormone-releasing hormone antagonist[J]. Acta Endocrinologica (Copenhagen), 1988, 117(4): 525-530.
[62] CHURCHLAND P S, WINKIELMAN P. Modulating social behavior with oxytocin: how does it work? What does it mean?[J]. Hormones and Behavior, 2012, 61(3): 392-399.
[63] YOSHIDA M, TAKAYANAGI Y, INOUE K, *et al*. Evidence that oxytocin exerts anxiolytic effects via oxytocin receptor expressed in serotonergic neurons in mice[J]. Journal of Neuroscience, 2009, 29(7): 2259-2271.
[64] WEISMAN O, ZAGOORY-SHARON O, SCHNEIDERMAN I, *et al*. Plasma oxytocin distributions in a large cohort of women and men and their gender-specific associations with anxiety[J]. Psychoneuroendocrinology, 2013, 38(5): 694-701.
[65] TSENG H C, CHI M H, LEE L T, *et al*. Sex-specific associations between plasma oxytocin levels and schizotypal personality features in healthy individuals[J]. Journal of Psychiatric Research, 2014, 51(1): 37-41.