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Article

Taste Neophobia, Latent Inhibition of Taste Aversion and Object Recognition Memory in Adolescent Rats

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ABSTRACT

Background: Adolescence in mammals is a period marked by increased novelty-seeking and enhanced responsiveness to the stressful properties of novel stimuli. Despite the need to taste potentially toxic novel foods during the adolescent growth spurt, there has been little study of taste neophobia and its attenuation. **Method:** Four experiments were carried out to compare taste neophobia and related memory processes in male and female adolescent (PND28) and adult (PND70) Wistar rats. Experiments 1 and 2 evaluated attenuation of taste neophobia to cider vinegar (3%) and sodium saccharin (0.1%) solutions were evaluated. Additionally, to test the role of memory in neophobia during adolescence, latent inhibition of taste aversion and object recognition memory were assessed in Experiment 3 and Experiment 4, respectively. **Results:** Adolescent and adult rats exhibited taste neophobia to the saccharin solution but adolescent rats required more exposure trials than adults to recognize the vinegar solution as safe. Both groups exhibited similar latent inhibition of taste aversion and object recognition memory. No sex effect was significant. **Conclusions:** Contrary to the accepted view associating adolescence with reduced neophobia, adolescent rats exhibited taste neophobia which even increased when sour tastes were encountered.

Neofobia Gustativa, Inhibición Latente de la Aversión Gustativa y Memoria de Reconocimiento de Objetos en Ratas Adolescentes

Palabras clave:
Adolescencia
Atenuación de la neofobia
Sabor
Memoria de reconocimiento
Rata

RESUMEN

Antecedentes: La adolescencia está marcada por búsqueda de la novedad y acentuada sensibilidad a las propiedades estresantes de los estímulos novedosos. A pesar de la necesidad de probar nuevos alimentos potencialmente tóxicos durante el periodo de crecimiento adolescente, la neofobia gustativa y su atenuación durante este periodo apenas ha sido estudiada. **Método:** Se evaluaron la neofobia gustativa y los procesos de memoria relacionados en ratas Wistar macho y hembra adolescentes (PND28) y adultas (PND70). En los Experimentos 1 y 2 Se exploró la atenuación de la neofobia gustativa a soluciones de vinagre de sidra (3%) y sacarina sódica (0,1%), respectivamente. En los experimentos 3 y 4, se evaluó también la inhibición latente de aversiones gustativas y la memoria de reconocimiento de objetos. **Resultados:** Adolescentes y adultos mostraron neofobia gustativa a la sacarina, pero las ratas adolescentes requirieron más exposiciones a la solución de vinagre para reconocerla como segura. No hubo diferencias entre los grupos en los Experimentos 3 and 4. No se hallaron efectos significativos del sexo. **Conclusiones:** A pesar de la ampliamente aceptada asociación entre adolescencia y reducida neofobia, las ratas adolescentes muestran neofobia al sabor que resulta incluso incrementada cuando se trata de sabores ácidos.

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Adolescence in mammals is a developmental period defined as the transition from infantile dependence on parental protection to adult independence. Reaching adult independence requires exposure to novel peers, environments and foods. Accordingly, perhaps the most prominent feature of adolescent behavior is increased novelty-seeking and risk-taking (Kelley, 2004). Adolescent novelty-seeking is highly conserved across species, with rodents being one of the most studied models (Spear, 2000; Varlinskaya & Spear, 2006). It is widely accepted that novelty seeking during adolescence has adaptive value, favoring the acquisition of skills and resources required for independent survival. However, novelty-seeking seems to be modulated by increased sensitivity to the emotional value of stimuli, which might protect survival in novelty-induced stressful situations. Hence, novelty-seeking in various domains should be assessed since they can lead to different outcomes.

Regarding the physical environment, adolescent rats exhibit increased exploration of novel objects (Douglas et al., 2003; Philpot & Wecker, 2008; Ramsaran et al., 2016; Stansfield & Kirstein, 2006) and novel environments (Lundberg et al., 2019; Lynn & Brown, 2010; Philpot & Wecker, 2008; Stansfield & Kirstein, 2006) in comparison to adults. However, a more precise evaluation of adolescent performance in the open field test and the elevated plus maze evidences avoidance of the risky central area (Lynn & Brown, 2010), increased velocity crossing the center and lower exploration of the open arms (Lynn & Brown, 2010) in comparison to adults (Lundberg et al., 2019). This might be attributed to the high anxiety levels reported during adolescence using various behavioral and physiological indices (Doremus-Fitzwater et al., 2009; Lundberg et al., 2019). The peculiar combination of increased novelty-seeking and higher anxiety in adolescents aids the acquisition of adult abilities and favor survival in risky novel environments.

Concerning edibles, the rapid growth spurt during adolescence requires increased food intake but the reluctance to ingest novel tastes with unknown consequences might promote survival. This phenomenon is termed taste neophobia and exhibits adaptive changes throughout the life cycle (Gallo, 2018). Thus, if consumption is followed by visceral distress, a conditioned taste aversion (CTA) will take place and if there are no aversive consequences the attenuation of taste neophobia (AN) will increase consumption as the taste becomes familiar and safe (Reilly & Bornovalova, 2005). Studies that have systematically assessed taste neophobia and AN in adolescent rats using non-caloric solutions are lacking. While Dannenhoffer and Spear (2016) reported similar neophobia to a 3% sucrose + 0,125% saccharin solution in adult and adolescent rats, adolescent lower neophobia to a 6% sucrose solution was found by Vaidya et al. (2004). In both cases, the taste solutions had rewarding caloric properties and AN was not evaluated. In fact, adolescent rats have been reported to increase consumption of sweet caloric solutions (Friemel et al., 2010) that induce increased orofacial positive responses, thus indicating higher palatability (Wilmouth & Spear, 2009). This has been interpreted as increased sensitivity of adolescents to the appetitive properties of natural rewards.

A first experiment was aimed to evaluate taste neophobia and AN to a sour vinegar solution using the standard procedures applied in our laboratory (Exposito et al., 2020; Gómez-Chacón et al., 2012, 2016; Grau-Perales et al., 2019b, 2020, 2021; Grau-Perales & Gallo, 2020; Grau-Perales et al., 2019a; Morillas et al., 2017). If adolescents exhibit novelty seeking, then absence of taste neophobia can be expected. On the contrary, if adolescents are more sensitive than adults to the aversive properties of the taste, they would show higher taste neophobia than adults. The results indicated slower AN in adolescent than adult rats. This could be interpreted as increased taste neophobia to the non-palatable vinegar solution as more exposures were required to be attenuated, thus supporting the second hypothesis. Alternatively, however, a maturational deficit of the memory mechanisms delaying recognition of a familiar taste could not be discarded. Thus, in Experiment 2 we took advantage of the latent inhibition (LI) phenomenon for assessing taste memory using a palatable saccharin solution. LI is a well know learning phenomenon relying on the memory of previous experiences (Vicente & De la Casa, 2021). Previous exposures not followed by relevant consequences retard later conditioning to the same stimulus. This effect has been described in different learning tasks, including conditioned taste aversion (CTA). Latent inhibition of CTA requires a two-stage behavioral procedure. In the first stage, a taste is pre-exposed and in the second, the same taste is followed by aversive consequences. Attenuation or disruption of the learned aversion by previous exposures is assessed in a later one-bottle test. Hence, Experiment 2 included both a first saccharin exposure phase identical to that applied in Experiment 1 with the vinegar solution and a CTA second CTA phase. Taste neophobia and AN could be tested in the first phase. If the adolescent slower vinegar AN in Experiment 1 was due to increased sensitivity to aversive tastes, then it cannot be expected using the sweet palatable solution. Alternatively, if the results of Experiment 1 were due to taste memory deficits, adolescent rats would exhibit LI impairment as the phenomenon relays on taste memory. Experiment 3 was designed in order to assess more extensively the adolescent memory abilities in the spontaneous novel object recognition (NOR) task. This task is based on the innate preference of the rodents to explore novel objects compared with previously encountered ones. The standard procedure includes a sample phase in which two identical objects are available and a test phase in which one of the objects is substituted by a novel one. Higher exploration of the novel object indicates recognition as the animal remembers the familiar one. If adolescent rats exhibit a NOR performance similar to adult rats, a general memory impairment contributing to the delayed AN found in Experiment 1 could be discarded.

Given that there is some evidence of higher novel object exploration in adolescent males than females (Douglas et al., 2003) the effect of sex was also examined throughout the entire series of experiments.

Method

Participants

Male and female adolescent rat aged 28 days (PND28) at the beginning of the procedure and young adult Wistar rats (PND70) participated in these experiments. Body weight ranged from 114.61 ± 3.09 g (males) and 104.47 ± 3.64 g (females) in the adolescent groups and between 568.5 ± 25 g (males) and

295.38 ± 12.59 g (females) in the adult groups. In Experiment 1, a total number of 64 male and female Wistar rats were randomly assigned to the following groups according to each experimental condition defined by age and taste: adolescent vinegar 3% group (N = 16; 11 males, 5 females), adolescent water (N = 13; 5 males, 8 females), adult vinegar 3% (N = 22; 11 males, 11 females) and adult water group (N = 13; 10 males, 3 females). In Experiment 2, a total of 61 male and female Wistar rat were randomly assigned to the following groups according to each experimental condition defined by age and taste preexposure: adolescent saccharin pre-exposed group (N = 16; 8 males, 8 females), adolescent non pre-exposed group (N = 17; 9 males, 8 females), adult pre-exposed group (N = 14; 7 males, 7 females) and adult non pre-exposed group (N = 14; 7 males, 7 females). Finally, Experiment 3 evaluated object recognition memory in 28 Wistar rats according to each experimental condition defined by age: adolescent (N = 14; 7 males, 7 females) and adult (N = 14; 7 males, 7 females).

All animals were born in the breeding colony of the University of Granada. On PND2 the litters were culled to 12 pups balancing sex as much as possible. After weaning on PND17, the animals were individually housed in 40 × 20 × 24 cm Plexiglas cages with wood shaving and environmental enrichment. Water and food were available ad libitum. The humidity was kept at 55% and the temperature at 20-24º. The animals were maintained on a 12-hour light-dark cycle (lights on at 08:00) and the experimental procedures were carried out in the light phase. The procedures were approved by the University of Granada Ethics Committee for Animal Research and by the Regional Ministry of Agriculture, Fisheries and Rural Development of Andalusia (17-02-15-195).

Instruments

The flavors used were 3% cider vinegar (Experiment 1) and 0,1% sodium saccharin (Experiments 2 and 3) solutions. In Experiment 3 we used lithium chloride (LiCl, 0.15 M; 2% b.w.) to induce CTA. Object recognition memory (Experiment 4) was assessed in an open box made of black painted wood (52 x 52 x 40 cm). Two pairs of objects consisting of stacked simple figures of decreasing size (cubes tower and spheres tower of 6 cm wide and 12 cm high) were used as novel or familiar objects. Velcro was attached to each object to be secured to the floor. Overhead lighting illuminated the testing area reducing room context information. Sessions were recorded with an overhead video camera and the SMART 3.0 software (Panlab SL) was used for analyzing the exploration behavior.

Procedure

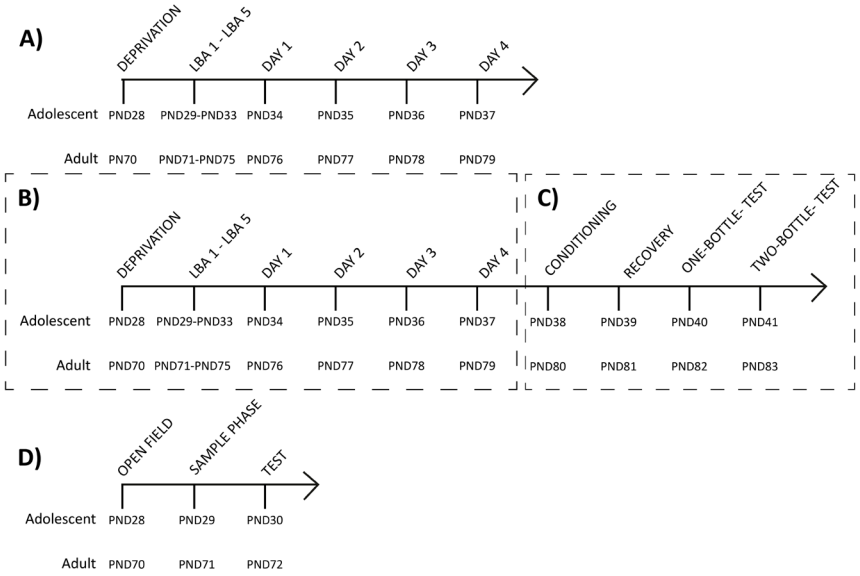
An unequivocal demonstration of neophobia requires at least two (but ideally multiple) exposures to the taste solution. In order to assess neophobia and AN (Experiments 1 and phase 1 of Experiment 2), the procedure consisted in daily 15-minute morning drinking sessions in which consumption was recorded and additional 20-minute rehydration sessions in the afternoon at 10:00 am and 16:00 pm respectively. Adolescent rats were deprived of water in their PND28 and finished both procedures 9 days later, at PND37 (Figure 1).

Along the 5 day-baseline period, water intake during the morning drinking session was recorded. Once the water intake baseline (BL) was stabilized the experimental sessions begun. Depending on the group, rats had access along the 4 following days to the flavored solution or water during the daily morning drinking sessions and consumption (ml) was recorded after each session. In order to allow comparisons between different age groups, an intake index was calculated by obtaining the percentage of ingested solution during the experimental drinking sessions in comparison with that of the last baseline day (Daygr x 100)/LBgr). Thus, a low index indicated high taste neophobia.

Assessment of latent inhibition of CTA (Experiment 2, phase 2) was performed after the saccharin preexposure phase by inducing a CTA in the same animals. Thus, on the first day of Experiment 2, phase 2, and throughout the experimental procedure, at 10:00 am, all the animals had 15 min access to 0.1% sodium saccharin solution and the amount drank was recorded. Non-pre-exposed control groups drank the saccharin solution for the first time while the pre-exposed groups drank the familiar saccharin solution for the fifth time. Fifteen minutes after the end of the saccharin drinking session, all the animals received an i.p. injection of lithium chloride (LiCl, 0.15 M; 2% b.w.) and returned to the home cage receiving a rehydration session at 16:00 pm. The next day they were allowed to recover from the LiCl-induced visceral distress with access to water during both drinking periods. On Day 3, only the saccharin solution was available to all groups. Intake was recorded and a rate was calculated with respect to LB to be able to compare adolescent and adult rats. Adolescent rats received aversive conditioning at PND38 and completed the procedure at PND41 (Figure 1).

The standard behavioral procedure to assess novel object recognition memory (NOR) consists of three phases. On day 1, habituation to the testing box took place. Each animal was allowed to freely explore the empty open-field arena for 5 minutes after previous handling and room acclimatization. On day 2 the sample phase consisted in a 5 min session in which the arena contained two identical objects either spheres or cube towers. The animals were placed into the cage with its head oriented to the opposite direction of the object's location and they were allowed to explore the pair of objects. On day 3, twenty-four hours after the sample phase, object recognition was tested. The rats were placed for 5 minutes in the box and one copy of the object from the sample phase was substituted by a novel object. The exploration time (ET) of each object was recorded in seconds. Object ET was defined as having more than 1 second of the rat's head directed to the object with the nose within 2cm of the object and vibrissae moving. Climbing onto the object (unless the rat sniffs the object it has climbed on) or chewing the object is not considered exploration. Both the sample phase and tests were video recorded and the total time spent exploring each of the two objects was scored by the experimenter with two stop watches. Objects and their relative positions were counterbalanced. The arena and objects were cleaned after each session with 70% ethanol to avoid odor cues. For experiment 3 all animals were naïve, so the adolescent rats started the procedure at their PND28 and finished at their PND30 (Figure 1).

Figure 1
Timeline of the Experimental Procedures



Note. A) Experiment 1: Attenuation of taste neophobia to a cider vinegar 3% solution. B) Experiment 2, phase 1: Attenuation of taste neophobia to a socium saccharim 0.1% solution. C) Experiment 2, phase 2: Latent inhibition of conditioned taste aversión. D) Experiment 3: Novel object recognition task.

Data Analysis

All statistical analyses were performed with the statistical program SPSS. We applied global mixed ANOVAs with between-subjects factors (Age, Sex, Preexposure) and within-subjects repeated measures factor (Days, Novelty) according to the particular experiment. Analyses of simple effects and interactions with a α-level of 0.05 were conducted. Bonferroni post-hoc comparisons were performed and Greenhouse & Geisser (1959) were used when the assumption of sphericity was not met.

Results

Mean (± SEM) direct consumption (ml) of the exposure periods in Experiment 1, Experiment 2, phase 1 and Experiment 2, phase 2 are shown in Table 1.

In the Experiments 1 and 2, there were no differences between the groups in water intake on the last BL day, except for the fact that males exhibited higher intake than females. There were no differences either between the adolescent groups in the body weight gain throughout the experimental procedure.

Fig. 2 shows mean vinegar intake indices of the different adolescent (Fig. 2a) and adult (Fig. 2b) groups along the experimental days in the Experiment 1.

A global mixed 2 (Age) x 2 (Sex) x 2 (Group) x 4 (Day) ANOVA yielded a significant effect of Day [$F(3,170) = 13.42$; $p < .001$, $\eta 2 = .19$], interaction Age x Day [$F(3,170) = 8.11$; $p < .001$, $\eta^2 = .13$], Group x Day [$F(3,170) = 11.86$; $p < .001$, $\eta^2 = .17$] and a triple interaction Age x Group x Day [$F(3,170) = 2.64$; $p = .05$, $\eta^2 = .04$] but no main effect or interaction of the Sex factor. Analysis of the triple interaction showed an effect of Day [$F(3,70) = 4.03$; $p = .014$, $\eta^2 = .13$] and an interaction Group x Day [$F(3,70) = 4.92$; $p = .006$, $\eta^2 = .15$] in the adolescent group, and also an effect of Day [$F(4, 136) = 28.82$; $p < .001$, $\eta^2 = .46$] and interaction Group x Day [$F(4,136) = 14.07$; $p < .001$, $\eta^2 = .29$] in the adult group. Analysis of the interaction in the adolescent group revealed a significant main effect of Day in vinegar group [$F(2,25) = 9.16$; $p = .002$, $\eta^2 = .38$] but not in water group. Post-hoc Bonferroni tests showed a higher intake index of the vinegar solution in the third exposure day than the second exposure day in the adolescent vinegar group ($p = .001$) but no differences in the intake index of vinegar solution between the first and second day ($p = 1$). Similarly, in the case of adult group, analysis of the interaction revealed a main effect of Day in the vinegar group [$F(3,60) = 64.58$; $p < .001$, $\eta^2 = .75$], but not in the water group. However, adults rats showed higher intake index in the second exposure day compared to first exposure day ($p < .001$), according to Bonferroni post-hoc test, suggesting a delayed AN in the adolescent group compared to the adults.

Fig. 3 shows mean saccharin intake indices of the adolescent (Fig. 3a) and adult (Fig. 3b) groups during the first exposure phase of Experiment 2.

A global mixed 2 (Age) x 2 (Sex) x 2 (Group) x 4 (Day) repeated measures ANOVA yielded significant effects of Day [$F(3,157) = 35.43$; $p < .001$, $\eta^2 = .40$], Group x Day [$F(3,157) = 8.90$; $p < .001$, $\eta^2 = .14$], Age x Day [$F(3,157) = 5.44$; $p = .001$, $\eta^2 = .09$] and a triple interaction Group x Age x Day [$F(3,157) = 5.58$; $p = .001$, $\eta^2 = .09$], but not main effect or interaction of the factor Sex. The analysis of the triple interaction indicated an effect of Day [$F(3,86) = 21.71$; $p < .001$, $\eta^2 = .41$] and an interaction Group x Day [$F(3,86) = 14.24$; $p < .001$, $\eta^2 = .31$] in the adolescent group as well as an effect of Day [$F(3,71) = 16.95$; $p < .001$, $\eta^2 = .39$] and interaction Group x Day [$F(3, 71) = 6.98$; $p = .001$, $\eta^2 = .21$] in the adult group. The analysis of the Group x Day interaction in adolescent group revealed a main effect of Day factor in the saccharin group [$F(3,44) = 26.01$; $p > .001$, $\eta 2 = .63$] and water group [$F(2,34) = 5.92$; $p = .005$, $\eta^2 = .27$]. Bonferroni post-hoc test showed higher saccharin solution

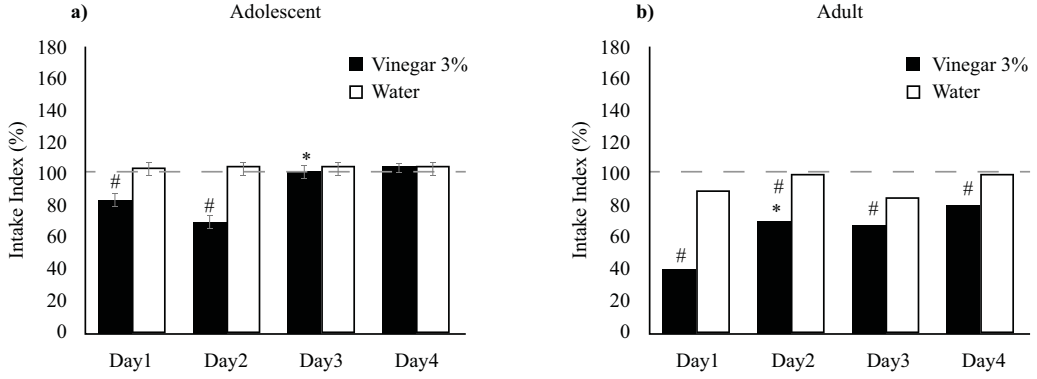
intake index on the second exposure day than on the first exposure day in adolescent saccharin group (p =.038). Likewise, analysis of the Group x Day interaction in adult group revealed a main effect of Day in the saccharin group [$F(4,35) = 18.09$; p >.001, η^2 = .58] but not in the water group. Bonferroni post-hoc comparisons revealed a higher saccharin solution intake index on the second exposure day than on the first exposure day (p =.002). This can be interpreted as similar saccharin AN on the second exposure day both in the adolescent and the adult group.

With respect to the second phase of Experiment 2, saccharin intake indices of the adolescent and adult groups during the conditioning and one-bottle test sessions are shown in Figure 4. A mixed 2 (Age) x 2 (Sex) x 2 (Pre-exposure) x 2 (Session) ANOVA yielded significant effects of the main factor Session [$F(1,53) = 160.43$; p <.001, η^2 = .75] and the interaction Session x Pre-exposure [$F(1,53) = 18.33$; p <.001, η^2 = .26], but no effect or interactions of the factors Age and Sex. The analysis of the Session x Pre-exposure interaction showed a significant effect of session in both pre-exposed [$F(1,29) = 24.27$; p > .001, η^2 = .46] and non-pre-exposed [$F(1,30) = 170.17$; p >.001, η^2 = .85]. Post-hoc Bonferroni tests showed a decrease in the one-bottle test intake index compared to the conditioning day in both, pre-exposed (p < .001) and non-pre-exposed groups (p < .001), thus indicating CTA in both groups. The non-pre-exposed group, however, showed lower saccharin intake than the pre-exposed group in both, conditioning (p < .001). These results demonstrated an intact latent inhibition phenomenon, as the previous taste exposure retarded the later conditioned aversion. No age effect was found.

Regarding NOR assessment in Experiment 3, there were no differences among the groups in ET during the sample phase as the rats explored both copies of the objects equally. A 2 (Age) x 2 (Sex) x 2 (Novelty) ANOVA during the test phase showed a main effect of Novelty [$F(1, 24) = 38.43$; p = .005, η^2 = .62] and an interaction Novelty x Age [$F(1, 24) = 4.05$; p = .05, η^2 = .14], but no interaction Novelty x Sex [$F(1, 24) = 0.07$; p =.79, η^2 = .00], nor triple interaction Novelty x Sex x Age [$F(1, 24) = 0.11$; p =.74, η^2 = .00].

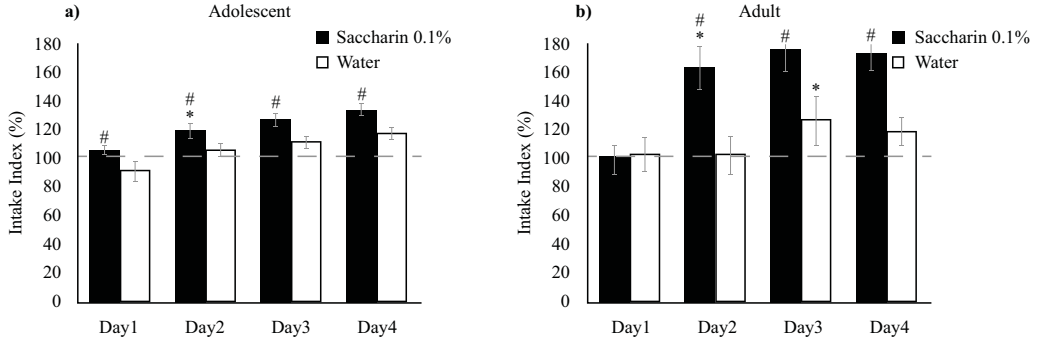
The analysis of the Novelty x Age interaction indicated a main effect of novelty in adolescent [$F(1, 13) = 32.10$; p >.001, η^2 = .71] and adult [$F(1, 13) = 12.84$; p =.003, η^2 = .50] rats. Post-hoc Bonferroni comparisons revealed that adolescent rats spend more time than adult rats exploring the novel object (p > .001) and the familiar object (p = .04) (Fig. 5). These indicated that although both age groups remembered equally the previously presented object, the adolescents showed higher exploratory activity.

Figure 2
Experiment 1



Note: (a) Mean ± SEM intake indices of the Adolescent groups receiving either cider-vinegar solution (Vin 3%) or Water exposures; (b) Mean ± SEM intake indices of the Adult groups receiving either cider-vinegar solution (Vin 3%) or Water exposures.* versus previous day (p < .05), # versus Water group.

Figure 3
Experiment 2, Phase 1



Note: (a) Mean ± SEM intake indices of the Adolescent groups receiving either saccharin solution (Saccharin 0,1%) or Water exposures; (b) Mean ± SEM intake indices of the Adult groups receiving either saccharin solution (Saccharin 0,1%) or Water exposures.* versus previous day (p < .05), # versus Water group.

Table 1
Mean (± SEM) Direct Consumption (ml) of the Exposure Periods in Experiment 1, Experiment 2, Phase 1 and Experiment 2, Phase 2

Experiment 1. Attenuation of taste neophobia to a cider vinegar 3% solution.

Age	Sex	Experimental group	WBL	Day 1	Day 2	Day 3	Day 4
Adolescent	Male	Vin 3%	6.10 (± .40)	4.29 (± .47)	4.04 (± .38)	5.52 (± .32)	5.69 (± .29)
		Water	6.50 (± .50)	6.60 (± .64)	7.06 (± .69)	7.26 (± .66)	5.66 (± 1.19)
	Female	Vin 3%	4.98 (± .19)	4.66 (± .91)	3.42 (± .38)	5.36 (± .34)	5.64 (± .33)
		Water	6.02 (± .33)	6.33 (± .32)	6.10 (± .47)	5.77 (± .41)	6.78 (± .49)
Adult	Male	Vin 3%	9.68 (± 0.52)	3.5 (± .20)	6.05 (± .32)	6.21 (± 0.38)	7.82 (± .37)
		Water	10.01 (± .48)	9.46 (± .60)	9.95 (± .42)	8.88 (± .34)	10.80 (± .60)
	Female	Vin 3%	7.30 (± .39)	3.24 (± .22)	5.52 (± .37)	5.12 (± .38)	5.7 (± .42)
		Water	7.06 (± .29)	5.4 (± 1.40)	8.93 (± .76)	7.06 (± .64)	6.83 (± 1.57)

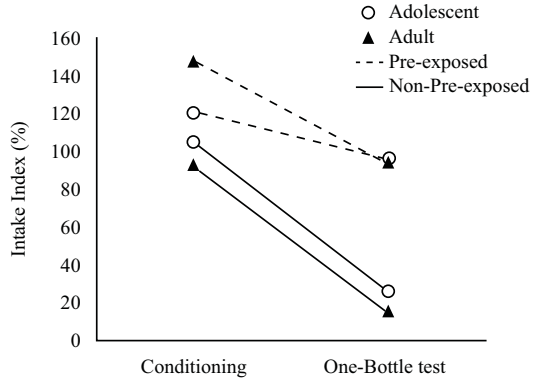
Experiment 2, phase 1. Attenuation of taste neophobia to a sodium Saccharin 0.1% solution.

Age	Sex	Experimental group	WBL	Day 1	Day 2	Day 3	Day 4
Adolescent	Male	Sac 0.1%	8.97 (± .54)	9.65 (± .60)	10.50 (± .50)	11.22 (± .51)	12.12 (± .74)
		Water	9.17 (± .68)	9.05 (± .54)	9.54 (± .78)	10.07 (± .86)	10,87 (± 1.03)
	Female	Sac 0.1%	7.36 (± .35)	7.36 (± .32)	8.42 (± .31)	9.01 (± .33)	9.42 (± .31)
		Water	7.70 (± .37)	6.00 (± .85)	7.93 (± .46)	8.32 (± .33)	8.97 (± .29)
Adult	Male	Sac 0.1%	9.42 (± .61)	9.17 (± .87)	15.94 (± 1.87)	14.94 (± 1.68)	16.74 (± 1.96)
		Water	9.71 (± 1.36)	10.51 (± .74)	10.04 (± .81)	13.28 (± .51)	11.14 (± .63)
	Female	Sac 0.1%	6.29 (± .31)	6.12 (± 1.15)	9.50 (± 1.09)	11.71 (± .1.11)	10.70 (± .77)
		Water	7.30 (± .44)	6.02 (± .43)	5.97 (± 0.65)	6.80 (± .62)	8.04 (± .82)

Experiment 2, phase 2. Latent Inhibition of CTA.

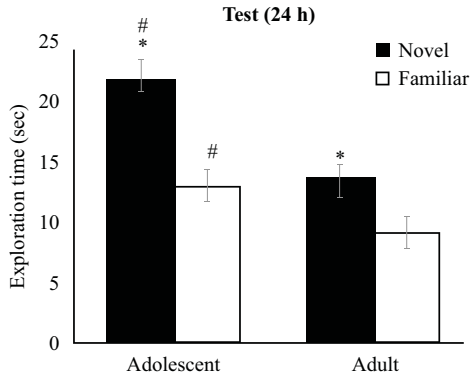
Age	Sex	Experimental group	Conditioning	One Bottle Test
Adolescent	Male	Pre-exposed	11.213 (± .55)	9.13 (± .47)
		Non pre-expo	10.22 (± .91)	2.2 (± .58)
	Female	Pre-exposed	8.2 (± 1.09)	6.55 (± .54)
		Non pre-expo	7.53 (± .61)	2.38 (± .92)
Adult	Male	Pre-exposed	13.07 (± 1.63)	7.02 (± 1.69)
		Non pre-expo	9.58 (± 1.09)	1.10 (± .11)
	Female	Pre-exposed	9.54 (± .69)	7.17 (± .66)
		Non pre-expo	5.71 (± .91)	1.32 (± .22)

Figure 4
Experiment 2, Phase 2



Note: Mean ± SEM intake indices during the Conditioning session and One-Bottle test (Saccharin 0.1%) of the pre-exposed and non pre-exposed adolescent and adult groups. *versus Pre-exposed (p <.05) group, #versus Conditioning session.

Figure 5
Experiment 3



Note: Novel object recognition (NOR) test performance. Mean (+SEM) novel and familiar objects' exploration time of adolescent and adult groups. * versus Familiar (p < .05), # versus Adult.

Discussion

The main finding reported in the present series of experiments is that unlike what has been reported with object and physical environments when it comes to tastes, adolescent rats do not exhibit more novelty-seeking than adults but a similar or even enhanced taste neophobia.

The results of Experiment 1 and the first phase of Experiment 2 evidenced vinegar and saccharin neophobia respectively in adolescent rats. Even though in Experiment 2 saccharin intake indices were similar to water intake indices during the first saccharin exposure, neophobia was evident as consumption increased in later exposures. This demonstrates that although similar to water, saccharin intake was reduced on the first encounter. Moreover, adolescent vinegar neophobia, but not saccharin neophobia, required two exposure trials to be attenuated in contrast to the adult groups that attenuated neophobia to both tastes after one exposure. The delayed AN to a novel vinegar solution in adolescents does not seem related with immature taste memory. Safe taste memory was not impaired in the adolescent groups as demonstrated by an intact latent inhibition effect induced by previous taste exposures on CTA (Experiment 23). Also, adolescent rats showed recognition of a familiar object after a 24h delay in a NOR task similarly to adult rats (Experiment 34). This delay is the same applied between flavor exposures during the AN procedure. Thus, a potential explanation of the slower attenuation of vinegar neophobia by global memory deficits is not supported by our results.

Therefore, the delayed AN to the sour vinegar solution could be the outcome of a greater vinegar than saccharin neophobia in adolescents. This could be due to a higher sensitivity to the aversive properties of the taste in comparison to adults. Accordingly, it has been reported hypersensitivity in young humans to aversive salty solutions (Galvan & McGlennen, 2013). These results challenge also the claim based on data obtained with other natural rewarding stimuli that adolescents are more responsive to appetitive rewards but less sensitive to negative outcomes (Doremus-Fitzwater et al., 2010). They found decreased aversive orofacial responses to quinine solution in adolescent rats. Our results, however, support increased responsiveness to the novel sour vinegar solution. It should be noted that enhancing the cautious neophobic response to potentially dangerous tastes in adolescents might be adaptive when confronted with poisons thus favoring survival and it is consistent with the evidence of higher responsiveness to stressful stimuli during novel environment exploration. Noteworthy, adolescent rats are more cautious than adult rats exploring the risky central areas in the open-field arena (Lundberg et al., 2019; Lynn & Brown, 2010) and the open arms in the elevated plus maze (Lynn & Brown, 2010). However, this might not be adaptive in the case of the sweet taste since it predicts nutrients sources of great value during the adolescent growth spurt. In fact, sweet taste preferences during adolescence have been reported not only in animals but in humans.

To our knowledge this is the first assessment of taste neophobia in adolescent rats using the standard procedures applied in adult rats. Previous incidental reports of adolescent neophobia to sweet caloric solutions in experiments not focused on this issue have yielded controversial results. While Dannenhoffer and Spear (2016) reported similar neophobia during 30 min access to a 3% sucrose + 0,125% saccharin solution in adult and adolescent rats, Vaidya et al., (2004) found increased consumption in comparison to adults of a novel 6% sucrose solution in a 4 h drinking session. The authors interpreted this result as reduced neophobia but it cannot be excluded that the increased consumption could be due to the sucrose rewarding caloric properties that can be detected along a 4h drinking session. This would be in accordance with reports of increased adolescent preference for sweet caloric solutions (Friemel et al., 2010; Vaidya et al., 2004; Wilmouth & Spear, 2009). In spite of the fact that recordings of the orofacial responses indicate that the palatability of the sucrose sweet taste might be enhanced in adolescent rats (Wilmouth & Spear, 2009), we have not found differences in taste neophobia compared to adults using the non-caloric saccharin solution in the Experiment 2. Moreover, the adult group exhibited even a higher increase than the adolescent group in the amount of saccharin drank along the AN sessions. Therefore, enhanced sweet solutions consumption can be interpreted as an increased sensitivity of adolescents to the appetitive properties of natural rewards while we found no evidence of reduced taste neophobia measuring consumption on a non-rewarding sweet taste solution.

Overall, our results indicate that adolescent rats are able to reduce the ingestion of novel tastes and that taste neophobia is even more affected than in adults by aversive tastes. This is the case of the vinegar solution intake which requires more exposures to be recovered as it becomes familiar. Unexpectedly, the reduced ingestion of novel tastes seems to be contrary to novelty-seeking that characterizes adolescent behavior. Nonetheless, it should be taken into account that novelty-seeking has been defined in terms of exploratory behavior of the external environmental cues which involves immediate consequences. Hence, adolescent rats exhibit increased cautious exploration of novel places (Lundberg et al., 2019; Lynn & Brown, 2010; Philpot & Wecker, 2008; Stansfield & Kirstein, 2006) and objects (Douglas et al., 2003; Philpot & Wecker, 2008; Ramsaran et al., 2016; Stansfield & Kirstein, 2006) accompanied by increased stress responsiveness (Doremus-Fitzwater et al., 2010; Lundberg et al., 2019; Lynn & Brown, 2010). However, the exploration of chemical cues such as tastes with internal consequences requires monitoring the visceral interoceptive effects of ingestion. Thus, maintaining or increasing the neophobic responses along several trials might reflect increased cautious exploration of tastes by adolescent rats. Taking into account such definition, our results are in accordance with increased novelty-seeking during adolescence. Moreover, they point to the need of considering the peculiarities of the interoceptive environment exploration when assessing developmental behavioral changes. Moreover, our results demonstrate intact LI and object recognition memory in adolescent rats. This indicates that the brain mechanisms required for long term taste and visual memory are mature at this age.

The age range of the adolescent groups used in the present study covers the entire adolescent period in rats according to the most conservative criteria that establish adolescence from PND28 to PND42 (Spear, 2000). This classification was based on behavioral transitions which included play, exploratory behavior and peer interaction in wild rats as well as on developmental brain events. The age ranges used to define adolescence in rodents might vary from strict definitions covering PND28 to PND 34 (Philpot & Wecker, 2008) to broader definitions extending the

adolescence to PND60 (Arenas et al., 2016; Lynn & Brown, 2010). However, in the second case the authors often distinguish several stages. The age range covered in our experiments corresponds to the middle adolescence and it is considered as prototypic adolescence (Varlinskaya & Spear, 2006). We have chosen this conservative range of age in order to assure that assessments have been performed during the adolescence age range.

With respect to the effect of sex our results do not indicate differences between male and female adolescent rats in any of the behavioral tasks applied. Although there are some indications of greater impact of adolescence on males regarding novel objects exploratory behavior (Douglas et al., 2003), response to stress induced by the open arms in the elevated plus maze (Lynn & Brown, 2010) and alcohol consumption (Vetter-O'Hagen et al., 2009), the evidence is scarce and there are authors who do not find sex-dependent differences (Doremus-Fitzwater et al., 2009; Hammerslag & Gulley, 2014).

In all, our results question a simple view of novelty-seeking associated to risk-taking during adolescence as the result of the immaturity of the cognitive system. On the contrary, our findings evidence a careful risk assessment based on an increased sensitivity to aversive taste stimuli which contributes to an altered decision making regulating the ingestive behavior to protect survival. This prompts an approach to study the adolescent behavior as aimed to cover the specific needs of this developmental period rather than considering it as underdeveloped adult behavior. Similar approaches are emerging in the field of addictive adolescent behavior (Bernheim et al., 2013).

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