Progress in Neuropsychopharmacology & Biological Psychiatry xxx (xxxx) xxx

Contents lists available at ScienceDirect

Progress in Neuropsychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

Altered hedonic, novelty-, stress- and D-amphetamine-induced response due to social isolation in peripuberty

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ARTICLE INFO

Keywords:
Elevated plus maze
Sucrose preference
Corticosterone
C-FOS
EGR1
Adolescence

ABSTRACT

Reduction in direct social contact with peers during early adolescence is thought to be a risk factor for an increase in depressive symptoms, but there is still no clear evidence to suggest early behavioral manifestations and their association with the later outcome of social distancing during this period. To address this question, we used social isolation paradigm in peripubertal rats as the rodent model of adolescence. The litter was an experimental unit. On postnatal day 29, each litter gave group-housed and single-housed males, which were reared and tested one week and two weeks thereafter. Psychomotor/emotional response to novelty in exploration-based tasks, behavioral and neuronal responses to the drug reward (D-amphetamine), motivation/hedonic behavior, physiological and response to physiological stress were examined. Social isolation in peripubertal rats manifested through: hyper-reactivity/agitation and the state anxiety/risk-taking at an early stage; reduced behavioral response to D-amphetamine and altered neural processing of this stimulus, at a later stage; consummatory hypohedonia that deepened over time without changing the motivation to eat; unchanged body weight gain and resting blood corticosterone, cortisol and glucose levels over time; altered blood biochemistry (silenced corticosterone and increased glucose) due to overnight fasting only at an early stage. Our results highlight that the outcome of reduced direct social contact with peers during peripuberty is dynamic, with the cluster of atypical early symptoms that evolve into the syndrome that is delicate for assessment through routinely measurable behavior and biomarkers of stress, but with progressive consummatory hypohedonia and unaffected motivation to eat as stable marks.

1. Introduction

There is a view that neural architecture of social beings evolved under the conditions of interpersonal attachments, including non-visual and non-auditory contacts (Baumeister and Leary, 1995), and that a decrease in or removal of a system's key inputs may risk destabilizing of the system (Badcock et al., 2017). The reduction in direct social contact with peers during early adolescence has been suspected to be a risk factor for an increase in depressive symptoms (Primack et al., 2017; Twenge et al., 2019; Witvliet et al., 2010), the appearance of which sharply rises from late childhood through early adolescence (Merikangas et al., 2010; Weinberger et al., 2018). There is still no clear experimental evidence to suggest early behavioral manifestations and their association with the later outcome of social distancing during this critical period. Importantly, reinforcement of social interactions, defined as "social reward" (Insel, 2003) is found not only in human adolescents (Schriber and Guyer, 2016), but also in peripubertal rats (Trezza et al., 2011) that, as social species, provide good subjects for studying the influence of social contacts on health. Experimental findings indicate that the endogenous opioid system is a critical component of the neural circuit that mediates the positive affective states associated with social interactions (Trezza et al., 2011).

Depressed mood/irritability or anhedonia must be present for a depression diagnosis but depressive disorder in adolescents can manifest differently than it does in adults (Thapar et al., 2012). High anxiety–depression co-morbidity has been documented (Cummings et al., 2014) with still unresolved questions on the order in which individual symptoms appear, as each increases the risk for the emergence and/or exacerbation of the other (Liu et al., 2018). Importantly, recent findings have highlighted the significance of anhedonia, but not irritability, as a hallmark of adolescent depression, without reference to age, gender, body mass index and ethnicity (Gabbay et al., 2015). Anhedonia is

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https://doi.org/10.1016/j.pnpbp.2020.110186
Received 23 June 2020; Received in revised form 10 November 2020; Accepted 18 November 2020
Available online 22 November 2020

conceptualized and assessed as a unitary construct, but is actually a multifaceted construct that reflects changes in motivational (wanting, mediated by mesolimbic dopamine) and consummatory (liking, mediated by opiate system) capacity (Treadway and Zald, 2011). Although it is not fully known how these two sub-components of anhedonia contribute to the development of depression, motivational anhedonia has been addressed better due to the effects of dopamine manipulation in animals and to the findings about deficient mesolimbic dopamine in some forms of depression, such as that with marked psychomotor retardation (Dichter et al., 2010). Such patients show a strong positive correlation between degree of depression and rewarding effects of D-amphetamine (D-AMPH, a dopamine releaser and dopamine reuptake inhibitor), reflecting sensitization of the dopamine system in the disease (Tremblay et al., 2002). However, there are also patients with poor response to D-AMPH who also have a limited responsiveness to tricyclic therapy (Fawcett and Siomopoulos, 1971) – the first recognized and the strongest determinant of atypical depression (Lojko and Rybakowski, 2017). Less depression severity, mood reactivity, increased appetite, higher comorbidity with anxiety, low or normal rather than elevated basal levels of cortisol, are some of the features of atypical depression in adults (Davidson et al., 1982; Lojko and Rybakowski, 2017). Abnormalities in neural responses to rewarding stimulus (i.e. regular activation of ventral striatum but increased recruitment of cortical midline structures/dorsal anterior cingulate cortex) have also been revealed in depression (Knutson et al., 2008).

Despite advances in treatments, the usage of antidepressants in young people up to the age of 24 has no clear therapeutic benefit (McCain, 2009; Vitiello and Ordonez, 2016) and preventing depression is another aspect to which much importance is attached by the World Health Organization (WHO, 2017). However, early recognition of even sub-thresholds and atypical symptoms associated with future mental health problems remains a challenge.

To get a better insight into fundamental biological principles related to social determinants of health in adolescence, we examined early and advanced manifestations of a decrease in direct social contacts with peers in peripubertal male rats as a rodent model of adolescence. Considering all the above-mentioned, we have addressed: 1) psychomotor/emotional response to novelty in free-exploration paradigms, using tests of unconditioned anxiety – motor activity in rectangular arena, the novel object exploration test, elevated plus maze test, with assessment of reactivity and adaptability over time to the conditions given in each of the tests; the first and the third test indicate state anxiety and the second one indicates trait anxiety (van Gaalen and Steckler, 2000), 2) behavioral and neural responses to the drug reward (D-AMPH) – in rodents, locomotor activation induced by psychostimulants, such is D-AMPH, models the unconditioned response to the rewarding nature of drugs (Wise and Bozarth, 1987) and is followed by the synthesis of immediate early proteins, biochemical indicators of neuronal activation in the brain (Morgan and Curran, 1991; Sheng and Greenberg, 1990), 3) consummatory anhedonia and motivation for goal-directed behavior – sucrose preference test along with food consumption monitoring (Der-Avakian and Markou, 2012; Palmiter, 2008; Pavkovic et al., 2020), 4) physiological response and 5) response to physiological stress – overnight fasting (Djordjevic et al., 2008). We have hypothesized that targeted decline in direct social contacts with peers, with preserved motivation, would primarily interrupt liking component of reward processing, thus affecting behavioral response to environmental/drug stimuli in the way different from that observed in principally motivational deficit.

2. Material and methods

2.1. Animals

Male Wistar Han rats, aged 29 postnatal days (P29; the onset of peripubertal age in the rat (Tzanoulinou et al., 2014), maintained under standard housing conditions on a 12 h light-dark cycle (lights on at 7:00 am) were used in the experiments. The efforts were made to minimize the suffering of the animals and the number of rats used. All animal procedures were in compliance with EU Directive 2010/63/EU and were approved by the Ethical Committee of the Institute (01-01/19) and by the National Ethic Research Committee (323-07-05339/2020-05).

2.2. Drugs

D-amphetamine sulfate (D-AMPH; Sigma-ALDRICH Chemie, Germany) was used in the dose of 1.5 mg/kg, dissolved in saline (0.9% NaCl) as 1.5 mg/mL (applied as 1 mL/kg). In rats, this dose produces dopamine-mediated psychomotor activation with the accent on locomotor hyperactivity, which has been observed in peripubertal rats as well (Pavkovic et al., 2017).

2.3. Experimental procedure

The litter was a biological replicate, with a total of 43 litters used in the experiment. At P29 male rats from the same litter (weaned at P21) were subjected to separation in the way that 3 rats were randomly chosen for further group housing and 1 for single housing. The cages were placed side by side and rats could smell, see and hear each other, but interact socially only in the group cages. Four experiments were performed. Schematic presentation of the experimental design is given in Fig. 1.

Experiment 1 ($N = 8$ litters) was performed to examine:

1) Spontaneous activity in exploration-based tasks – in rectangular arena and thereafter to the novel object in a familiar arena as well as in the elevated plus maze (EPM); combining results obtained in the open arena and the EPM can provide useful information as in both tests the behavior is the result of conflict between the motivation to explore and the motivation to avoid potentially dangerous situations, but different environment could produce different behavioral profiles (Ennaceur and Chazot, 2016; Rodgers and Dalvi, 1997).

2) Psychomotor response to D-AMPH in animals habituated to the experimental arena, followed by decapitation, brain tissue sampling (the medial prefrontal cortex (mPFC) and the striatum) and Western blot analysis to gain the expression of proteins encoded by the immediate early genes, i.e.c-FOS and EGR1; in response to a stimulus the synthesis of immediate early proteins requires a period of up to 90 min (Morgan and Curran, 1991; Sheng and Greenberg, 1990). Experimental protocols followed previously described procedures (Pavkovic et al., 2018; Pavkovic et al., 2020; Pavkovic et al., 2017). In group housed animals one animal per group/cage passed all tests predicted by the Experiment 1.

Experiment 2 ($N = 7$ litters) was performed to assess consummatory anhedonia and motivation for goal-directed behavior at the end of the 1st and the 2nd week of defined housing, using sucrose preference in two-bottle choice paradigm along with food consumption monitoring, as previously described (Pavkovic et al., 2020). In group housed animals group preference was measured, as all the animals per cage were from the same litter. Sucrose consumption and preference for palatable solutions displays "liking" (consummatory) capacity of hedonic response (Der-Avakian and Markou, 2012; Willner, 2017). We introduced measurement of food consumption along with sucrose consumption/preference (Pavkovic et al., 2020) considering that i) feeding is goal-directed behavior, ii) general motivation to eat is dependent on striatal dopamine (Palmiter, 2008), iii) consumption of sucrose solution increases extracellular dopamine levels within the striatum at concentration-dependent manner (Hajnal et al., 2004), and iv) animals that are not motivated to engage in goal-directed behaviors still have a preference for sucrose (Palmiter, 2008).

Experiments 3 and 4 were performed to evaluate physiological response and response to overnight fasting at the end of the 1st and the 2nd week of defined housing (N = 7 litters per experiment per time

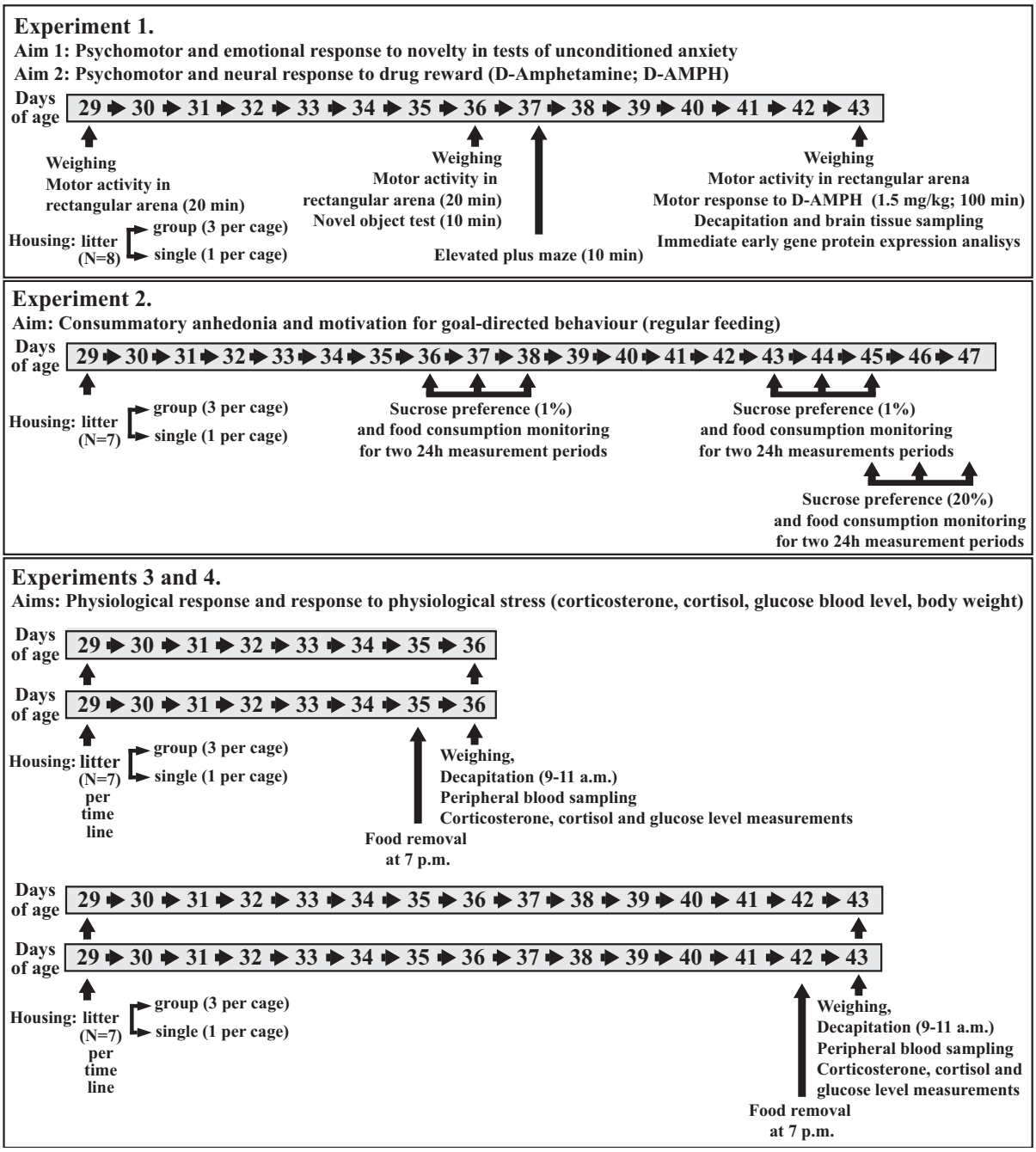

Fig. 1. Schematic presentation of the experimental design.

point) respectively (parameters measured: corticosterone, cortisol and glucose blood level, body weight gain). In group housed animals, one animal per group/cage was examined. Overnight fasting was used as physiological stress that has the advantage of producing uniformly low baseline blood glucose level (Djordjevic et al., 2008).

All behavioral procedures are given in detail as supplementary material (Appendices 1–6) along with the list of antibodies and methods/devices used for blood analysis.

2.4. Statistical analysis

The data were presented as means ± standard deviation (SD), with individual data plots along the column bars, and statistically analyzed using Statistica 6.0 software (StatSoft Inc.). Normality of data sets was estimated by Shapiro-Wilk's test. The accepted level of significance was $p < 0.05$.

The results obtained in behavioral tests were analyzed using nonparametric statistics (Wilcoxon's test for repeated measures to assess adaptive behavior over time, Mann-Whitney *U* test for pairwise comparisons) as some data did not have normal distribution even after transformation. The Western blot data were analyzed using *t*-test for independent samples.

Blood parameters and body weight gain data were transformed to reach normal distribution and analyzed using two-way ANOVA with the housing condition and the overnight fasting as factors for data obtained at the end of the 1st and the 2nd week of defined housing, followed by post hoc Tukey test, if appropriate (statistics given as Supplementary Table 1).

3. Results

3.1. Early changes in psychomotor and emotional response to novelty in peripubertal male rats due to social isolation

To test early changes in psychomotor/emotional response to novelty in peripubertal rats due to social isolation, we examined animals' spontaneous activity in exploration-based tasks, i.e. in rectangular arena (Fig. 2A–C), in response to novel object in familiar arena (Fig. 2D–F) and in the EPM (Fig. 2G–N) at the end of the 1st week of defined housing. The results obtained in the rectangular arena before animals were housed in groups or as singles are given as supplementary Fig. 3, as a confirmation that there were no differences in basal motor activity of the animals. Statistical comparisons and exact p values are given as supplementary material, Table S1.

In the rectangular arena, single-housed animals showed hyperactive behavior compared to group-housed controls, i.e. increased locomotor, stereotypy-like and vertical activities during the first 10 min of testing (Fig. 2A–C, * $p < 0.05$, U test), reaching control-like level during the next 10 min of testing. There was a decrease across time in both groups regarding all three activities (Fig. 2A–C, # $p < 0.05$, Wilcoxon test). No significant changes between groups were detected regarding the number of entries and time spent in the central (aversive) zone of the arena (supplementary Fig. 4).

In the novel object test, there were no significant differences between experimental groups in all examined parameters: latency to approach novel object, the number of approaches to it and the time spent in the object exploration (Fig. 2D–F).

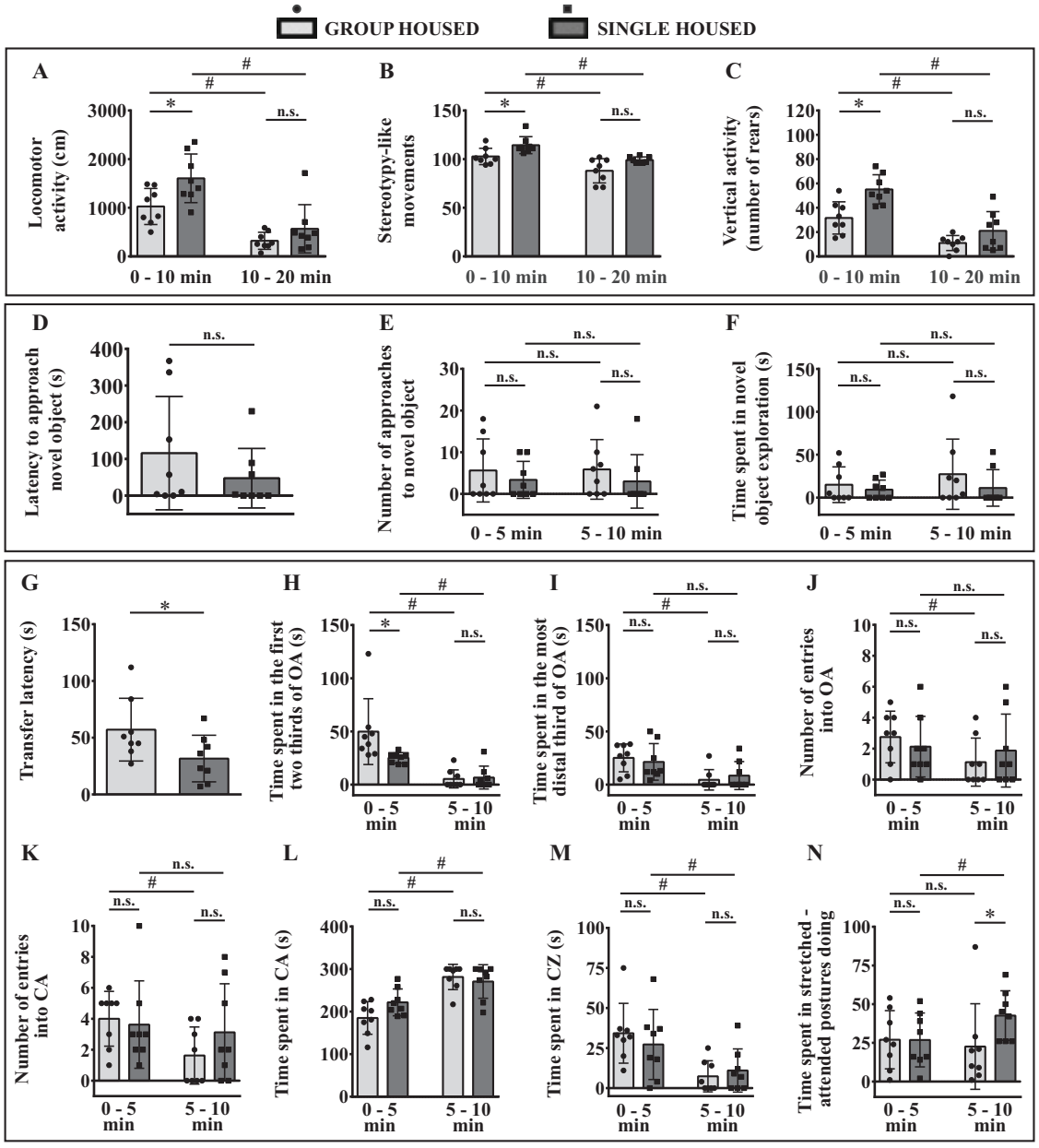

Fig. 2. Psychomotor and emotional response of peripubertal rats to novelty, at the end of the 1st week of defined housing, which began on the 29th postnatal day. Behavior of the animals examined in the open field (A–C), novel object (D–F) and elevated plus maze test (G–N). The data are represented as mean ± SD, with individual data plots along the column bars ($n = 8$ animals per group). Comparisons between groups and within a group across time were performed as indicated by lines above the graph bars. * $p < 0.05$, U test; # $p < 0.05$, Wilcoxon test. OA – open arms, CA – closed arms, CZ – central zone. Statistical comparisons and exact p values are given as supplementary material, Table S1.

In the EPM, single-housed animals had shorter transfer latency and spent significantly less time in the first two thirds of the open arms during the first 5 min of testing than group-housed animals (Fig. 2G and H, respectively, * $p < 0.05$, *U* test). No significant differences between groups were detected regarding time spent in the most distal third of the open arms (Fig. 2I), the number of open arms entries (Fig. 2J) and the number of closed arms entries (Fig. 2K); however, while group-housed animals showed a decrease in all three parameters across time (# $p < 0.05$, Wilcoxon test) this was not confirmed in single-housed animals. Time spent in closed arms and in central zone of the EPM was similar in both groups (Fig. 2L and M, respectively). Single-housed animals spent more time in stretched-attended postures across the testing session (Fig. 2N, # $p < 0.05$) and were more engaged than group-housed animals in doing this during the second 5 min of testing (Fig. 2N, * $p < 0.05$).

3.2. Motor and neural response to D-AMPH in peripubertal male rats due to social isolation

Single- and group-housed animals showed highly similar motor/exploratory activity in rectangular open field arena after 2 weeks of defined housing (Fig. 3A–C; the number of entries and time spent in the central (aversive) zone of the arena are given as supplementary Fig. 5). Importantly, single-housed animals showed a weaker locomotor and stereotypy-like response to subsequent D-AMPH treatment (1.5 mg/kg) than group-housed animals did during the initial 50 min of monitoring (Fig. 3D and E, * $p < 0.05$, *U* test). In group-housed animals there was a decrease in both activities across time (Fig. 3D and E, # $p < 0.05$, Wilcoxon test), while in single-housed animals a decrease was observed regarding stereotypy-like activity only (Fig. 3E, # $p < 0.05$). The vertical activity was similar in both groups (Fig. 3F), but it did not decrease significantly across time in single-housed animals as it did in group-housed animals (Fig. 3F, # $p < 0.05$, Wilcoxon test).

A weaker motor response to D-AMPH in single-housed animals was accompanied by an increased expression of c-FOS and EGR1, which were used as molecular markers of neuronal activation, in the mPFC of these animals (Fig. 3G–H, * $p < 0.05$, *t*-test), without significant changes in the striatum (Figs. 3I–J).

Statistical comparisons and exact *p* values are given as supplementary material, Table S2.

3.3. Consummatory hypohedonia and food consumption in peripubertal male rats due to social isolation

The preference for 1% sucrose solution (the average for two consecutive days of measurement) was lower in isolated than in group-housed animals after 1 week of defined housing (Fig. 4A, * $p < 0.05$, *U* test). This was evident after 2 weeks as well (Fig. 4B, * $p < 0.05$), with the outcome being more pronounced than that seen after 1 week (# $p < 0.05$, Wilcoxon test). There was no change in food intake between single- and group-housed animals, but there was a decrease across the examined period regardless of housing conditions (Fig. 4C and D, # $p < 0.05$, Wilcoxon test). No differences in the examined parameters were found in any of the experimental groups in the second in relation to the first day of measurement (Fig. 4A–D).

The preference for 20% sucrose solution (the average for two consecutive days of measurement) was less in isolated than in control animals (Fig. 4E, * $p < 0.05$) without significant changes in the average amount of food consumed (Fig. 4F). However, there was an important difference: in group-housed animals no change in preference for 20% sucrose was accompanied by an increase in food consumption across the days of testing (Fig. 4F, # $p < 0.05$, Wilcoxon test), while in isolated animals there was an increase in preference for 20% sucrose (Fig. 4E, # $p < 0.05$) without the changes in food consumption (Fig. 4F).

Statistical comparisons and exact *p* values are given as supplementary material, Table S3.

3.4. Physiological and response to physiological stress in peripubertal male rats due to social isolation

Blood parameters and body weight gain were analyzed using two-way ANOVA with the housing condition and the stress presence (overnight fasting) as factors at the end of the 1st and the 2nd week of defined housing, followed by post hoc Tukey test, if appropriate. For all data sets (Fig. 5A–D, left and right panels) *F* statistic is given as supplementary material, Table S4.

The morning blood level of corticosterone, cortisol and glucose, as well as weight gain, were highly similar in group-housed and isolated non-fasted animals in both time points examined (Fig. 5A–D). Significant difference between fasted group- and single-housed rats was detected regarding corticosterone (decreased) and glucose (increased), at the end of the 1st week of defined housing (Fig. 5A and C, * $p < 0.05$, Tukey test).

Compared to non-fasted group-housed rats, fasted ones showed an increase in stress hormones and a decrease in glucose at the end of the 1st and the 2nd week of defined housing (Fig. 5A–C, # $p < 0.05$, Tukey test). Compared to non-fasted single-housed rats, fasted ones showed an increase in corticosterone only, specifically in the later time point (Fig. 5A, # $p < 0.05$), while glucose, like in group-housed animals, was decreased in both time points (Fig. 5C, # $p < 0.05$). Less weight gain than age-matched non-fasted counterparts was observed only in younger group of examined animals (i.e. at the end of the 1st week of defined housing) regardless of housing conditions (Fig. 5D; body weights of the animals are given as supplementary Fig. 6).

4. Discussion

Our study brings comprehensive findings on the direct neurobehavioral and physiological outcome of reduced direct social contacts with peers in peripubertal male rats as a rodent model of adolescence, revealing: 1) hyper-reactivity/agitation and the state anxiety at an early stage, 2) impaired behavioral response to D-AMPH and altered neural processing of this stimulus at a later stage, 3) consummatory hypohedonia that deepened over time without changes in motivation to eat, 4) unchanged body weight gain and resting blood corticosterone, cortisol and glucose levels over time, and 5) altered blood biochemistry (silenced corticosterone and increased glucose) due to overnight fasting only at an early stage. Obtained results highlight that the outcome of reduced direct social contact with peers during peripuberty is dynamic, with the cluster of atypical early symptoms that progress into the syndrome delicate for assessment through routinely measurable behavior and biomarkers of stress, but with consummatory hypohedonia and unaffected motivation to eat as stable marks. These findings confirm our hypothesis that the restriction of direct social contacts with peers in adolescence, without initially reduced motivation to socialize, would primarily manifest through the affected liking component of reward processing and behavioral response to environmental/drug stimuli in the way different from that observed in principally motivational deficit.

Immediate consequences of social isolation in peripuberty have been sparsely examined experimentally (Leussis and Andersen, 2008) while stability of the outcomes during the isolation period has not been addressed at all. Using the method of extended observation, our study showed that excessive motor activity, which has been described as the most consistent result in animal models of prolonged social isolation-induced depression-like behavior (Del Arco et al., 2004; Robbins et al., 1996), in peripubertal stage should be expected as an early and transient behavioral manifestation. In fact, it should rather be termed hyper-reactivity as it was observed only during the initial phase of testing, along with intense stereotypy-like activities as a sign of agitation (Mason, 1991). This hyper-reactivity did not influence either adaptability of the animals to the testing cage or their activity in the central (aversive) zone of the cage. Hyper-reactivity was not evident in the novel object exploration, supporting the view that an object and open

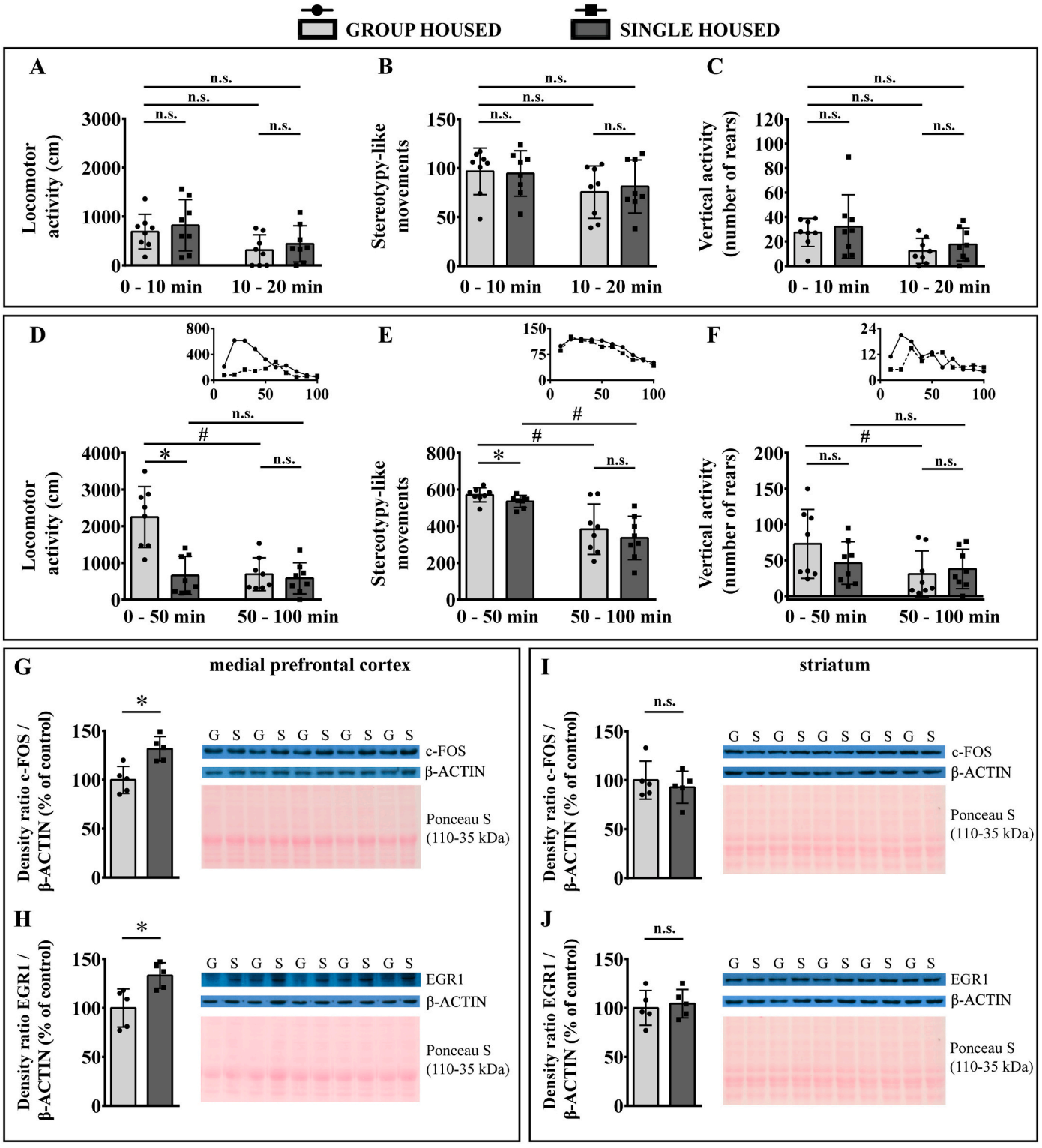

Fig. 3. Motor and neural response to D-AMPH in peripubertal rats at the end of the 2nd week of defined housing, which began on the 29th postnatal day. After habituation to the experimental arena (A–C) motor response to D-AMPH challenge (1.5 mg/kg, i.p.) was monitored (D–F). After that, brain tissue was taken for the analysis of drug-induced expression of biochemical indicators of neuronal activation, c-FOS and EGR1, in the medial prefrontal cortex (G, H) and the striatum (I, J). The data are represented as mean ± SD, with individual data plots along the column bars (n = 8 animals per group for behavioral analysis, 5 of which are randomly chosen for further biochemical analysis). Panels G, H, I and J contain representative immunoblots and Ponceau S staining (G – group housed, S – single housed), * $p < 0.05$, *t*-test for independent samples. For data related to motor response, comparisons between groups and within a group across time were performed as indicated by lines above the graph bars; * $p < 0.05$, *U* test; # $p < 0.05$, Wilcoxon test. Inserted line graphs illustrating the exact dynamics of changes in the examined parameters over time after D-AMPH injection (D-locomotor activity, *E*-stereotype, F-vertical activity) were not subjected to detailed statistical analysis, as this was done for summary values derived from these profiles of activities. Statistical comparisons and exact *p* values are given as supplementary material, Table S2.

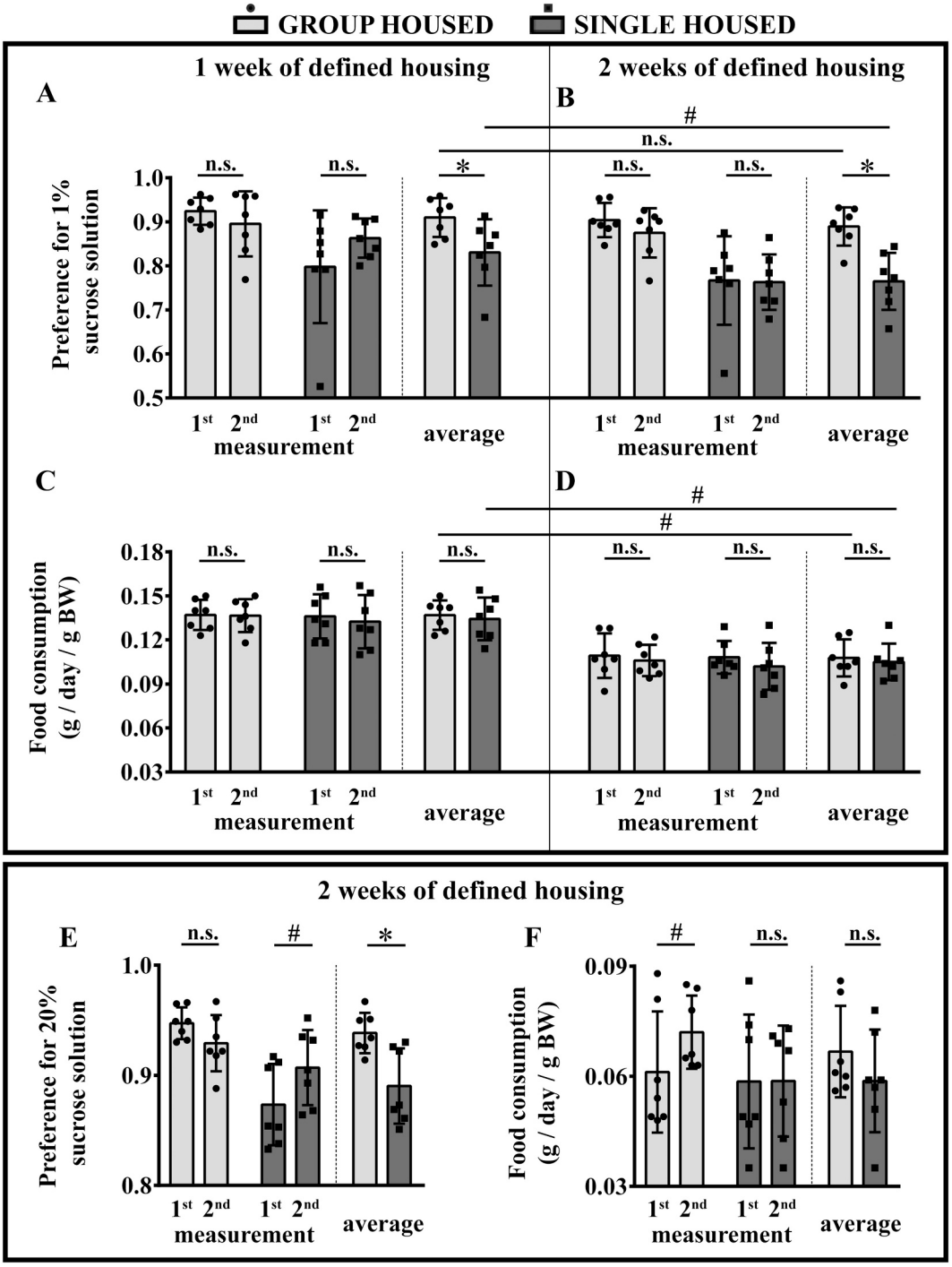

Fig. 4. Preference for sucrose solution and food consumption in peripubertal rats at the end of the 1st and the 2nd week of defined housing, which began on the 29th postnatal day. Measurements related to 1% sucrose solution were performed at the end of both the 1st (A, C) and the 2nd (B, D) week. Measurements related to 20% sucrose solution were performed immediately after 1% sucrose consumption monitoring at the end of the 2nd week only because high percentage sucrose solution should have rewarding effects that could influence upcoming measurements and final findings. For each time point of interest (the end of the 1st and the 2nd week of defined housing) measurements were given for two consecutive days of testing, as well as their average. The first measurement was performed 24 h after the placement of the bottles with sucrose and water, when the consumed liquids per cage were measured, as well as the food eaten, and the preference was calculated. The measurement process was performed again for the next 24 h. The data are represented as mean ± SD, with individual data plots along the column bars ($n = 7$ animals per group for single housed condition; n = 7 cages (3 animals per cage, all from the same litter) for group housed condition). Comparisons between groups and within a group across time were performed as indicated by lines above the graph bars. * $p < 0.05$, U test; # $p < 0.05$, Wilcoxon test. Statistical comparisons and exact p values are given as supplementary material, Table S3.

field exploratory behaviors are processed differently in the brain (Fuhrmann et al., 2015; Gangadharan et al., 2016). In animal models of depression-like behavior, an increase in locomotor response has been related to changes in the function of the dopaminergic mesolimbic system (Del Arco et al., 2004; Robbins et al., 1996), but hyperdopaminergic state is rather associated with a non-habituating hyperactivity that does not distinguish between environmental and object novelty (Zhuang et al., 2001). Even in the EPM, regardless of the clear

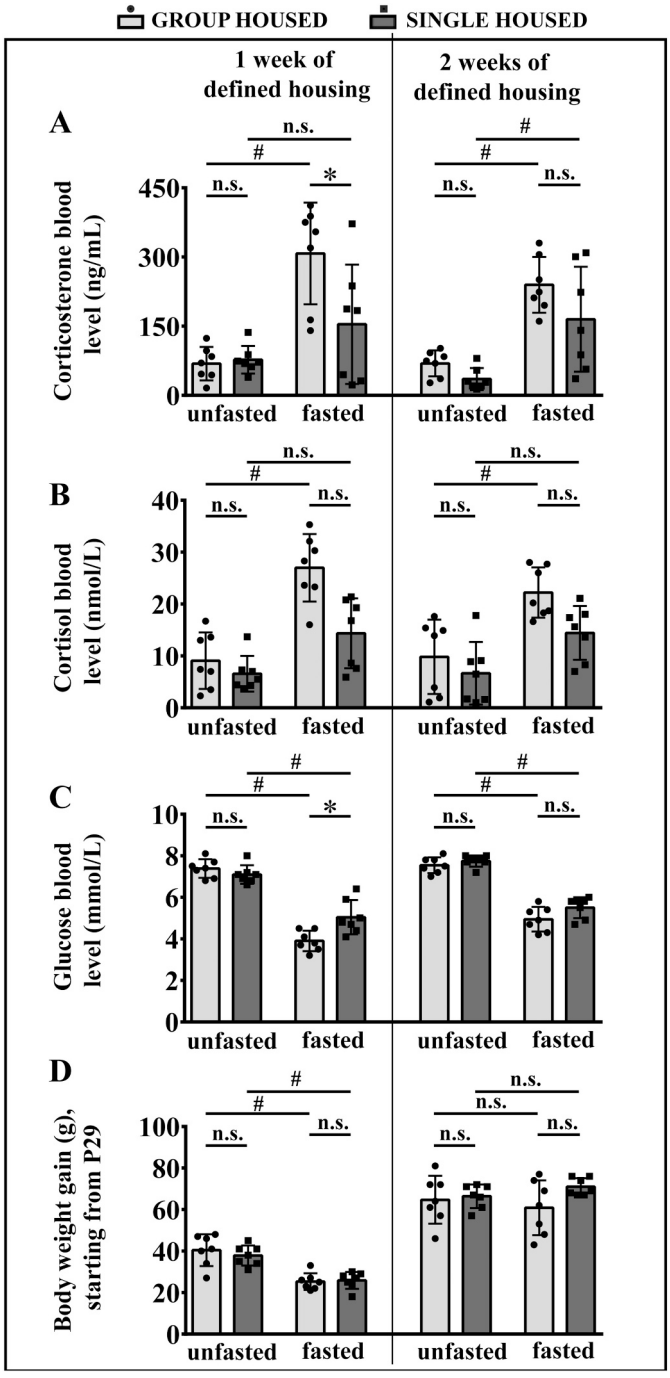

Fig. 5. Blood corticosterone (A), cortisol (B), glucose (C) and body weight gain (D) in peripubertal rats at the end of the 1st and the 2nd week of defined housing, which began on the 29th postnatal day. The data are represented as mean ± SD, with individual data plots along the column bars (n = 7 animals per group). Comparisons between groups were performed using Tukey test as a post-hoc for two-way ANOVA, as indicated by lines above the graph bars. * $p < 0.05$ within the same feeding regimen, # $p < 0.05$ within the same housing regimen. Statistical comparisons and exact p values are given as supplementary material, Table S4.

manifestation of anxiety-like behavior (a decrease in the amount of time spent in proximal two-thirds of open arms), isolation-reared animals showed signs of hyper-reactivity to this novel environment, i.e. did not decrease arms entries across testing session. The number of entries into closed arms has been considered an indicator of general locomotor activity (Rodgers et al., 1999). Moreover, they were engaged in stretch-attended postures doing and the most distal thirds of the open arms staying, which indicated a propensity for risk-assessment activity/sensation seeking – stretch-attended postures are the only parameter of the EPM that is positively correlated with the circulating level of corticosterone (Reis et al., 2012; Rodgers et al., 1999) that is responsible for appetitive properties of stress (Piazza et al., 1993). Overall, the first part of this study shows that hyper-reactivity and agitation in novel environment are early behavioral manifestations of reduced direct social contacts with peers in adolescence, being more delicately expressed in the surroundings with high anxiogenic potential where anxiety-like behavior and sensation seeking are present as well. This anxiety is state rather than trait, as it did not manifest as a stable characteristic of isolated animals (van Gaalen and Steckler, 2000). It should be emphasized that, in the context of early and atypical symptoms of depressive disorder onset, all the results of our study have a clear basis in the findings on humans: agitated behavior is a part of mood dysregulation syndrome that appears in childhood and predicts risk for later depressive disorders (Brotman et al., 2006; Thapar et al., 2012); a history of attention deficit and hyperactivity in adolescence is associated with an elevated risk of depressive disorder as well (Meinzer et al., 2012); people who are anxious in routine situations should be expected to seek sensation-producing experiences (Burkhart et al., 1978); even more, it has been suggested that adolescents found to be engaged in risk-taking behaviors should be assessed for depressive symptoms (Testa and Steinberg, 2010).

Extreme behavioral changes following exposure to the stressful stimuli in rodents were significantly more prevalent in animals with a blunted hypothalamic–pituitary–adrenocortical (HPA) axis response than in wild type rats with normal HPA axis functioning or in rats with an excessively responsive HPA system (Cohen et al., 2006). In our experiment, the blood level of basal corticosterone/cortisol was normal and it should be noted that low or normal cortisol was often observed in atypical depression (Davidson et al., 1982; Lojko and Rybakowski, 2017). Importantly, stress-induced level of circulating glucocorticoids was deficient in socially-isolated peripubertal rats at the end of the 1st week of isolation housing, i.e. at the time of above discussed behavioral examinations. Overnight fasting also revealed that a disturbed glucose metabolism (hepatic glucose production is known to happen in a fasting state, Sharabi et al., 2015) is important early mark of social isolation in peripuberty, as it becomes evident only at the end of the 1st week of isolation housing. It is known that hepatic glucose production is promoted by glucocorticoids (Sharabi et al., 2015), but their circulating level in given stress condition was lower in socially-isolated than in group-housed animals. Nevertheless, further studies, which would accentuate glucocorticoid receptor in target organs, are needed to fulfill this point. Stress-related metabolic specificities of younger examined group of rats is supported by reduced weight gain in these animals in response to overnight fasting, regardless of the housing conditions.

Novel clinical findings highlighted the significance of anhedonia, without changes in body mass index, as a hallmark of adolescent depression (Gabbay et al., 2015). Data obtained in the present study support this, showing that isolation-induced appearance of consummatory hypohedonia and its deepening over time are not related to affected motivation to eat. Considering that general motivation to eat is dependent on striatal dopamine (Palmiter, 2008), and that the consumption of high percentage sucrose solution increases extracellular dopamine within the striatum (Hajnal et al., 2004), consummatory behavior of experimental animals assessed with 20% sucrose solution additionally showed that functional continuum between consummatory hedonic drive and the dopamine flow within the striatum (i.e. opiate-dopamine interaction) in control animals was preserved. In distinction, it was disturbed in socially-isolated animals. A small number of studies have been conducted using the sweet taste test for the assessment of consummatory anhedonia in humans (Berlin et al., 1998; Dichter et al., 2010; McCabe et al., 2009) with one of them reporting decreased activation of the striatum in depressed patients compared to healthy

controls (McCabe et al., 2009).

At the end of the 2nd week, isolated rats did not show changes in psychomotor behavior in novel environment, but showed prominent reduction in psychomotor response to D-AMPH challenge. The mesolimbic dopamine system is mainly responsible for locomotor and rewarding effects of D-AMPH (Kelly and Iversen, 1976; Wise and Bozarth, 1987) but, as endogenous opioid system is involved as well (Jayaram-Lindstrom et al., 2004), the above-discussed interruption in the opiate-dopamine integrity in the limbic system of the isolated animals has additional grounds for further examination. Importantly, reduced psychomotor response to D-AMPH in the isolated animals was accompanied by an increased expression of the biochemical indicators of neuronal activation, c-FOS and EGR1, in the mPFC of socially-isolated compared to group-housed animals, without changes in the striatum (for the expression of c-FOS and EGR1 in the forebrain after the application of addictive drugs, including D-AMPH, please see the reference Harlan and Garcia, 1998). In normal conditions, in doses highly similar to that used in the current study, D-AMPH produces inhibitory responses in the mPFC of experimental animals and, at the same time, exaggerates salient value of a rewarding experience (Homayoun and Moghaddam, 2006). Importantly, increased recruitment of cortical midline structures in response to rewarding stimulus was revealed in depression (Knutson et al., 2008), and recent findings in rodents confirmed that mPFC overactivity suppresses reward-motivated behaviors (Ferenczi et al., 2016). Nevertheless, the issue of the correctness of neural response to D-AMPH in depression deserves further attention, as some reports accentuate a strong positive correlation between the degree of depression and rewarding effects of the drug (Tremblay et al., 2002) – considering all the above discussed, that phenomenon should not be related to the mPFC over-activity.

Although the study has notable strengths, its limitation is the absence of data in female rats. Parallel experiments on both sexes were not possible due to technical and spatial limitations. The absence of saline-injected controls in the D-AMPH-related neurobehavioral outcome can be considered a limitation as well. Nevertheless, observed results represent an important contribution to understanding social determinant of health in adolescence and providing directions for larger, in-depth studies.

In conclusion, using the rodent model, this study provides the first comprehensive analysis of emotional, physiological, neural and mood changes due to social isolation in peripuberty, emphasizing its profound and complex consequences. The findings indicate that the cluster of atypical early symptoms, including agitation, risk-taking, state anxiety and altered blood biochemistry in response to fasting-induced stress, along with consummatory hypohedonia and unaffected body mass index, have important predictive role regarding the social context of adolescent health. Overall, the importance of direct social contacts with peers in peripuberty/adolescence should be promoted as much as possible, because their lack has an undeniably strong impact on well-being, outweighing the benefits of all other ideal living conditions.

Author's contribution

Study conception and design: MP, ŽP, VP; Acquisition of data (experimental work): MP, ŽP, NLV; Analysis and interpretation of data: MP, VP, SK; Wrote the paper: MP, VP. All authors have read and approved the final version of this article.

Funding

This work was supported by the Ministry of Education, Science and Technological Development of Republic of Serbia (Contract Number 451-03-68/2020-14/200007).

Ethical Statement

All animal procedures were in compliance with EU Directive 2010/63/EU and were approved by the Ethical Committee of the Institute (01-01/19) and by National Ethic Research Committee (323-07-05339/2020-05).

Declaration of Competing Interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pnpbp.2020.110186.

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