

## RESEARCH ARTICLE

# Social isolation in rats: Effects on animal welfare and molecular markers for neuroplasticity

Veronica Begni<sup>1</sup>✉, Alice Sanson<sup>1</sup>✉, Natascha Pfeiffer<sup>2</sup>, Christiane Brandwein<sup>2</sup>, Dragos Inta<sup>2,3</sup>, Steven R. Talbot<sup>4</sup>, Marco Andrea Riva<sup>1</sup>, Peter Gass<sup>2\*</sup>, Anne Stephanie Mallien<sup>1</sup> 

**1** Department of Pharmacological and Biomolecular Sciences, University of Milan, Milan, Italy, **2** Department of Psychiatry and Psychotherapy, Central Institute of Mental Health, Medical Faculty Mannheim of Heidelberg University, Mannheim, Germany, **3** Department of Psychiatry (UPK), University of Basel, Basel, Switzerland, **4** Institute for Laboratory Animal Science, Hannover Medical School, Hannover, Germany

✉ These authors contributed equally to this work.

\* [anne.mallien@zi-mannheim.de](mailto:anne.mallien@zi-mannheim.de)



## OPEN ACCESS

**Citation:** Begni V, Sanson A, Pfeiffer N, Brandwein C, Inta D, Talbot SR, et al. (2020) Social isolation in rats: Effects on animal welfare and molecular markers for neuroplasticity. PLoS ONE 15(10): e0240439. <https://doi.org/10.1371/journal.pone.0240439>

**Editor:** Judith Homberg, Radboud University Medical Centre, NETHERLANDS

**Received:** January 14, 2020

**Accepted:** September 27, 2020

**Published:** October 27, 2020

**Copyright:** © 2020 Begni et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Data Availability Statement:** All relevant data are within the manuscript and its Supporting Information files.

**Funding:** This work was supported by grants from the Deutsche Forschungsgemeinschaft (Forschergruppe 2591 'Severity assessment in animal based research', project P05) to P.G. and by grants from the Italian Ministry of University and Research (PRIN 2017AY8BP4 and PON "Ricerca e Innovazione" PerMedNet project ARS01\_01226) to M.A.R.

## Abstract

Early life stress compromises brain development and can contribute to the development of mental illnesses. A common animal model used to study different facets of psychiatric disorders is social isolation from early life on. In rats, this isolation can induce long-lasting alterations in molecular expression and in behavior. Since social isolation models severe psychiatric symptoms, it is to be expected that it affects the overall wellbeing of the animals. As also promoted by the 3Rs principle, though, it is pivotal to decrease the burden of laboratory animals by limiting the number of subjects (reduce, replace) and by improving the animals' wellbeing (refine). The aim of this study was therefore to test possible refinement strategies such as resocialization and mere adult social isolation. We examined whether the alternatives still triggered the necessary phenotype while minimizing the stress load on the animals. Interestingly, we did not find reduced wellbeing-associated burrowing performance in isolated rats. The hyperactive phenotype seen in socially isolated animals was observed for rats undergoing the adult-only isolation, but resocializing ameliorated the locomotor abnormality. Isolation strongly affected markers of neuroplasticity in the prefrontal cortex independent of timing: mRNA levels of *Arc*, *Bdnf* and the pool of *Bdnf* transcripts with the 3' long UTR were reduced in all groups. *Bdnf* splice variant IV expression was reduced in life-long-isolated animals. Some of these deficits normalized after resocialization; likewise, exon VI *Bdnf* mRNA levels were reduced only in animals persistently isolated. Conversely, social deprivation did not affect the expression of *Gad67* and *Pvb*, two GABAergic markers, whereas changes occurred in the expression of dopamine *d1* and *d2* receptors. As adult isolation was sufficient to trigger the hyperactive phenotype and impaired neuroplasticity in the prefrontal cortex, it could be a candidate for a refinement strategy for certain research questions. To fully grade the severity of post-weaning social isolation and the alternatives, adult isolation and resocialization, a more profound and multimodal assessment approach is necessary.

**Competing interests:** The authors have declared that no competing interests exist.

## Introduction

Chronic stress is a major environmental risk factor for the occurrence of psychiatric disorders. Especially early life stress, such as maternal separation or social isolation, compromises brain development, and can contribute to mental illness [1, 2]. Deficits in the social environment during neurodevelopment are relevant e.g. in autism spectrum disorder, attention-deficit/hyperactivity disorder, and schizophrenia [3, 4].

It has been proposed that social experiences and relationships may influence dopaminergic functionality [5, 6]. For instance, greater dopaminergic and cortisol responses to a psychological stressor were observed in young adults that reported low parental care when compared to controls [7], suggesting that early-life experiences may have a deep impact on systems implied in stress response. Corroborating this notion, exposure of children to abuse or unstable family situations is linked with elevated dopaminergic functionality in the striatum at adulthood [8], as well as with imbalance in excitatory/inhibitory neurotransmitters, which may underlie some cognitive dysfunctions [9]. Moreover, it is thought that childhood trauma might interplay with genetic factors, leading to several alterations—among which decreased levels of neurotrophins, aberrant DNA methylation, hypothalamic-pituitary-adrenal axis dysregulation—potentially culminating in psychotic episodes [10].

Nowadays, social isolation represents a significant health issue. Indeed, since children and adolescents are more sensitive to the detrimental effects of isolation, the COVID-19 containment measures (quarantine and forced isolation) might result in increased anxiety and depression rates among those fragile subjects [11], therefore this historical period demands deeper insights on the tangled consequences of social deprivation.

A common animal model used to study different facets of mental disorders is early-life social isolation in rodents [1]. In rats, this isolation can induce long-lasting alterations in functional connectivity, behavior, and molecular expression [1, 12–14]. The exposure to social deprivation is considered one of the most reliable preclinical models of schizophrenia, as it simulates the core features of mental disorders, e.g. cognitive deficits, alterations in social behavior, hyperactivity, and sensory gating deficits [1]. At the same time, social isolation supposedly impairs the wellbeing of the subjects, as the model aims to evoke psychiatric associated symptoms, which are generally considered disruptive [1]. Yet, it is unknown how much the animals are really burdened by these symptoms.

As in any experimental animal approach, the ethical dilemma between the wellbeing of the individual subject, and the opportunity to gain insight into a scientific question arises. The 3Rs principle states the importance of limiting the number of experiments (reduce, replace) and of improving the wellbeing of subjects (refine) as much as possible while maintaining efficacy. Since rats are social animals, it is advised to keep rats group-housed throughout life, but particularly early in life, when frequency and intensity of social contacts peak during adolescence [15, 16]. During adulthood, social interactions decline without ever diminishing completely [16]. Hence, the consequences of stress exposure are deeply influenced by the period of isolation and the timing thereof. Indeed, a relationship between brain responses to stress and its timing has been proposed. The early life period is very sensitive, characterized by intense brain development, during which external stimuli may influence or interfere with ongoing anatomical and functional changes [17, 18]. Therefore, it is highly likely that challenges that occur during this period may have long-term consequences on brain functions, potentially leading to the outbreak of pathological conditions later in life [19, 20]. During adulthood, by contrast, stress exposure determines more transitory changes in brain structures and functions. The mature brain possesses a heightened ability to respond to challenges with coping strategies

that may ultimately attenuate the consequences of such insults, even though chronic exposure may still be harmful to the overall health [17, 21, 22].

Therefore, one way of refining social isolation experiments in terms of animal welfare could be the isolation of subjects in the less sensitive adulthood phase.

Another idea is to resocialize the subjects during adulthood. This could also result in alleviation of some symptoms, while others may remain stable. Evidence from former resocialization studies is inconsistent in this respect [23]. Moreover, housing the rats in groups again after former isolation is often accompanied by aggressive behavior, which could inflict even more welfare concerns [24, 25]. In severe cases, it might become necessary to separate subjects from the group permanently. This, in turn, leads to the exclusion of the animal from the experiment as this repeated social isolation may confound the previous effects and abolishes the comparability of subjects and simultaneously acts against the idea of reducing animal numbers (3Rs).

The aim of this study was to assess the effect of resocialization and adult social isolation as possible refinement strategies for the social deprivation model. We intended to evaluate whether these strategies would still elicit isolation-associated hyperlocomotion and influence molecular parameters, while alleviating the burden on the rats.

The hyperlocomotion is an early onset and robust observation in isolation reared rats and is suggested as a criterion for the so-called isolation syndrome [1, 26]. It is detectable with simple means and is therefore frequently published and shows translational relevance since early-life deprived children have been associated with hyperlocomotion as well [27, 28]. To evaluate a wellbeing-associated parameter, we investigated the burrowing behavior. Burrowing is part of the natural behavioral repertoire of small rodents and supposedly has a self-rewarding component [29–31]. Reduction of this innate and spontaneous behavior can indicate welfare impairments in rodents [30, 32, 33]. It has been associated with many different aversive conditions including with hippocampal damage, epilepsy, stress-induced anhedonia, and/or lack of motivation [34–38].

At the molecular level, the exact mechanisms associated with the isolation-induced behavioral phenotypes are still unclear, although synaptic functions appear to be affected by social deprivation, coinciding with impairments of several neurotransmitters [1]. Impaired neurogenesis, long-term potentiation, and neuroplasticity were observed following isolation rearing conditions [1, 39–41]. Moreover, isolated animals showed hyperactivity of the dopamine system paralleled by reductions of serotonin functioning and reduction of parvalbumin positive gamma-amino-butyric acid (GABA) interneurons [1, 37, 38, 42, 43]. GABAergic transmission is thought to be implicated in cognitive activity and plays an important role in the brain response to stressors [44]. GABAergic dysfunctions are thought to be correlated with cognitive impairment, which is a core symptom of schizophrenia [45]. Similarly, dopamine is relevant for cognitive and emotional functions [46].

Therefore, we assessed the modulation of the expression of brain-derived neurotrophic factor (*Bdnf*), the immediate early gene (IEG) activity-regulated cytoskeleton-associated protein (*Arc*) and the postsynaptic density protein 95 (*Psd95*) involved in learning and memory processes, synaptic plasticity and brain connectivity. Additionally, two GABAergic markers, the glutamic acid decarboxylase 67 (*Gad67*) and parvalbumin (*Pvb*) were analyzed. Lastly, we measured the mRNA levels of the dopamine receptors D1 and D2. The analyses were conducted in the prefrontal cortex (PFC), a brain region highly relevant for the development of psychiatric disorders. Although the role of social behaviors for the development of PFC has been demonstrated [16], there are only a few data on the modulation in this area after a social isolation paradigm [47, 48].

## Materials and methods

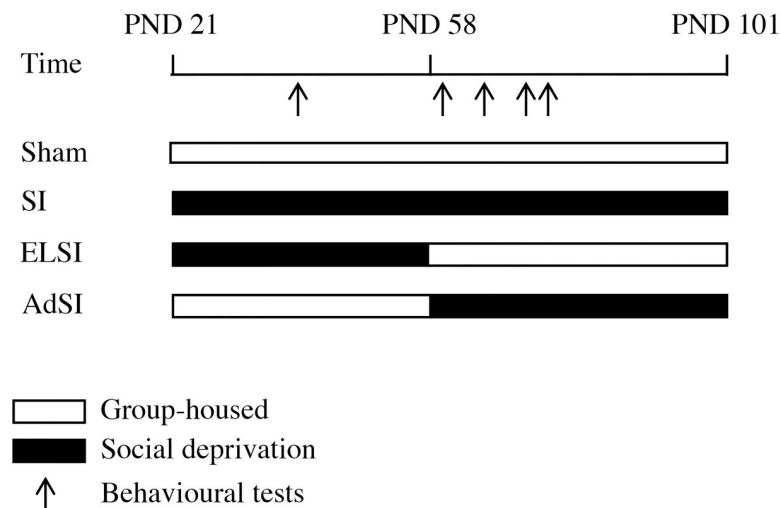
### Animals

The subjects of the study were wildtype male Lister Hooded rats, bred and maintained in the animal facility of the Central Institute of Mental Health, Mannheim, Germany. The dark/light cycle of the husbandry was 12h dark/light with lights on at 6 am. Room temperature and humidity were set to  $22\pm 1^\circ\text{C}$  and  $45\pm 5\%$ . Olfactory, visual, and auditory contact between the cages was not limited, while social interaction with the experimenter was limited to the handling during the weekly cage change. Water and food pellets (LasQCdiet Rod16-H, LasVendi GmbH, Soest, Germany) were available *ad libitum*. The type III (for single-housed rats) and type IV (for group-housed rats) macrolon cages were supplied with Abedd Espen MIDI (ABEDD, Vienna, Austria), but contained no environmental enrichment as suggested in previous publications [1]. All procedures were approved by the German animal welfare authorities (Regierungspräsidium Karlsruhe) and performed strictly according to the regulations of animal experimentation within the European Union (European Communities Council Directive 2010/63/EU).

**Social isolation paradigm.** As shown in Fig 1, the study comprised the analysis of four experimental groups with different exposure to isolation stress: *Social Isolation* (SI) rats were isolated from conspecifics throughout their lifespan ( $n = 12$ ), *Early Life Social Isolation* (ELSI) rats were only isolated during adolescence ( $n = 12$ ) and *Adult Social Isolation* (AdSI) rats during adulthood ( $n = 16$ ). Permanently group-housed rats (Sham) served as controls ( $n = 16$ ). All animals were weaned at  $\text{PND } 22 \pm 1$  and pseudo-randomly assigned to group- or solitary housing. The condition change in ELSI and AdSI was performed on  $\text{PND } 58 \pm 2$ . For group housing, four animals were kept per cage. ELSI were resocialized with three littermates. These social partners ( $n = 36$ ) were not included in the analysis.

### Behavioral analysis

All experiments were conducted approximately one hour after the beginning of the light phase and in separate experimental rooms. Acclimatization to the experimental rooms was only



**Fig 1. Summary of experimental design.** Timeline of stress exposure and behavioral testing in Lister Hooded male rats. Sham = lifelong group-housed animals; SI = lifelong isolated animals; ELSI = animals exposed to social isolation during adolescence; AdSI = animals exposed to social isolation at adulthood.

<https://doi.org/10.1371/journal.pone.0240439.g001>

necessary for the Open Field (OF) test (15 min). In a training phase during adolescence (PND 37–39) the subjects were familiarized with the burrowing procedure. The burrowing performance was monitored in the course of the project (PND 40, 59, 66, and 72) (Fig 2A) and locomotion was assessed in the OF test on PND 75.

**Burrowing.** Monitoring of burrowing behavior was assessed as previously described in [49]. Briefly, we placed gravel-filled (2.5 kg) plastic burrowing tubes (32 cm long × 10 cm Ø, opening 60 mm above the floor) in Macrolon Type IV cages and determined the latency for the animal to start burrowing and the burrowed material left in the tube after 60 min. Rats were familiarized with the experimental procedure in a preceding training phase. On the first

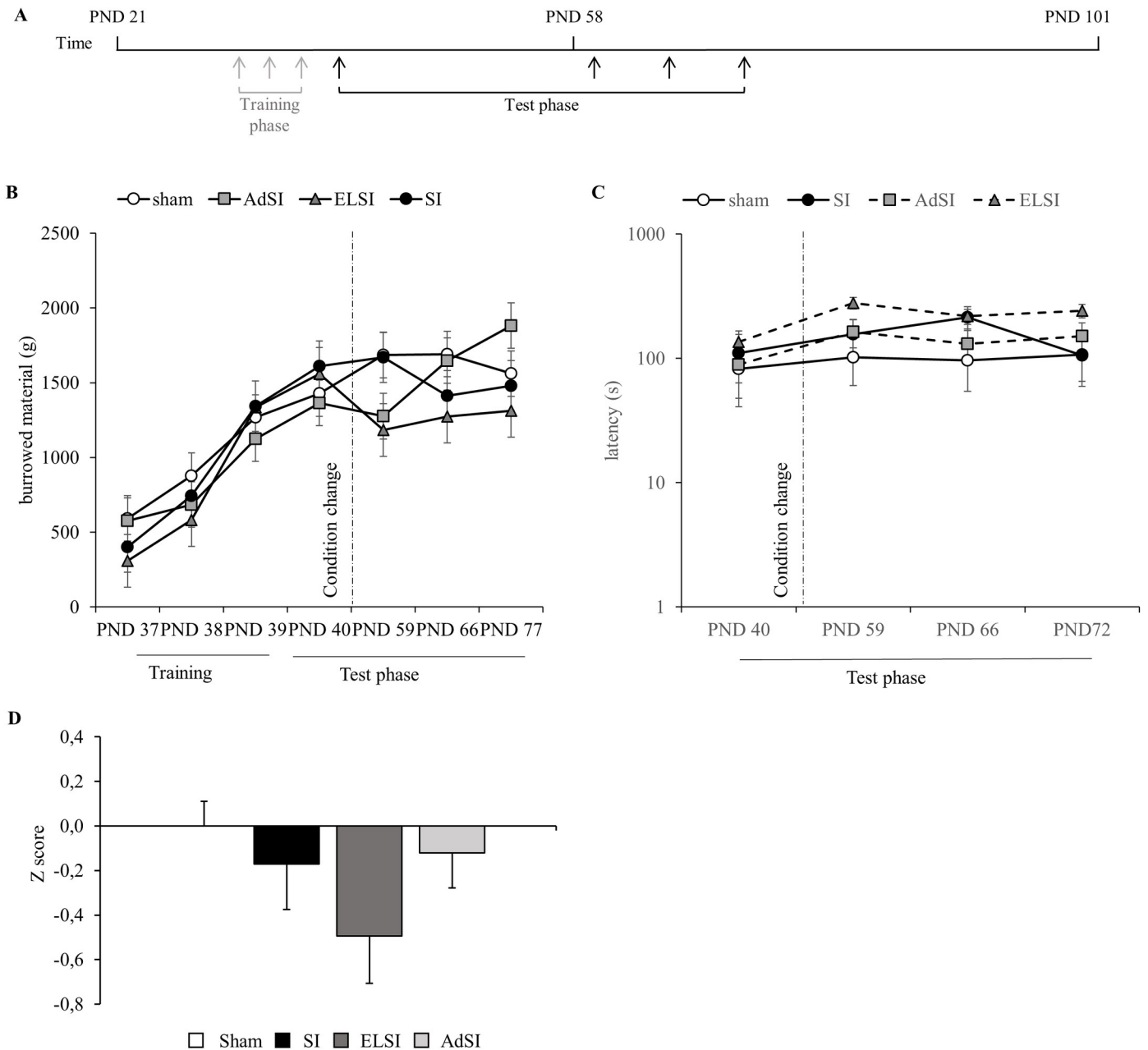


Fig 2.

<https://doi.org/10.1371/journal.pone.0240439.g002>

training day, each rat was free to explore an empty cage for 60 min before they had access to an empty tube for an additional 60 min. The procedure on the three subsequent training days already reflects the test condition: after a 60 min habituation to the empty cage, a gravel-filled tube was introduced into each cage. After another 60 mins, the gravel remaining in the tube was weighed and the amount of removed gravel determined. During the training phase, the latency to start burrowing was not recorded.

**Open Field test.** In the OF test the individual rat was placed into an unfamiliar arena (50 x 50 cm) illuminated from above with 15 lux for 60 min and their activity was recorded with a camera suspended above the arena. For analysis, this period was divided into 10 min time bins and the parameters *movement (time spent moving within the maze)*, *total distance moved*, *velocity*, *time spent in the center of the arena (the area more than 10 cm from the walls)* were analyzed using Ethovision XT, Noldus as described before [50, 51]. The tracking software, which calculates the center of the rat. We manually counted the *number of rearings* (vertical movement in which posture was upright and both front paws were lifted from the ground) and voided feces during the test. The test was performed on PND 75.

### Brain tissue collection, RNA isolation, and real-time PCR analyses

On PND 101±2, the animals were sacrificed. The brains were promptly extracted from the skull and the region of interest immediately dissected on ice. Dissected brains were stored at -80°C before RNA extraction. Total RNA was isolated from the prefrontal cortex by single-step guanidinium isothiocyanate/phenol extraction using PureZol RNA isolation reagent (Bio-Rad Laboratories, Italy) following the manufacturer's instructions, as previously described [22]. Total RNA was analyzed by TaqMan quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) to determine the levels of mRNA. Samples were run in triplicate as multiplexed reactions using the iScript™ one-step RT-PCR kit for probes (Bio-Rad Laboratories). Data were analyzed following the  $\Delta\Delta C_t$  method with a normalizing internal control, namely  $\beta$ -actin. All primers and probes information is listed in S1 Table.

### Statistical analyses

Data were tested for normal distribution using the Shapiro-Wilk test. Burrowing data were not normally distributed. However, while the residuals of the burrowed material were normally distributed after fitting a linear mixed model with the lme4 [52] package in R (burrowing ~ time\*condition + (1|ID)), the residuals of the latency data were not normally distributed. Therefore, the latency data were log-transformed and the model was calculated with the nlme package [53], also in R [54] (burrowing ~ time\*condition, random = ~1|ID) to obtain suitable residuals. In both calculations ID was considered a random effect. Factors were treated as simple treatment contrasts with the default level in the intercept. Post-hoc analyses were Tukey corrected.

Repeated measures ANOVA was used to analyze the Open Field parameters. Here, post hoc analyses were Bonferroni corrected. We chose single rats as the experimental unit. No data were excluded from the behavioral dataset.

Normalization of data using the z-score method was performed according to [55]. Basically, it indicates how many standard deviations an observation is above or below the mean of a control group. Applying this mathematical tool, we aimed to define an “emotionality” score enhancing the accuracy of the behavioral phenotyping. Accordingly, at a preclinical level, this approach has been documented as supportive in facilitating the behavioral assessment that is based on a set of converging traits rather than by a single one [55]. We applied z-normalization across multiple and complementary behavioral parameters measuring wellbeing, locomotor

activity, and anxiety-like traits. Z-scores in the burrowing task were calculated for each animal using the normalization of *burrowed material* values of each training and testing day. In the Open Field, for each animal the z-score of locomotion activity was calculated using the normalization of *velocity*, *total distance moved*, and *total movement* values. Similarly, z-scores of anxious behavior in the OF were calculated for each animal using the normalized *number of feces*, *number of rearings*, *time spent in the center*, and “distance to walls” values. As an example, z-scores assessing locomotor activity were calculated for each animal using the normalization of velocity, total distance moved, and total movement values.

$$Z \text{ locomotor activity} = \frac{\left(\frac{X-\mu}{\sigma}\right) \text{velocity} + \left(\frac{X-\mu}{\sigma}\right) \text{total distance moved} + \left(\frac{X-\mu}{\sigma}\right) \text{total movement}}{\text{number of parameters}}$$

X represents the individual value, whereas  $\mu$  and  $\sigma$  represent the mean and the standard deviation of sham animals.

Molecular changes produced by isolation were analyzed using univariate ANOVA (one-way ANOVA) followed by Bonferroni post-hoc comparisons, when appropriate. In addition, to evaluate the association between the hyperactive phenotype and the altered gene expression, Pearson product-moment correlation coefficients ( $r$ ) were calculated between the total distance moved (40–60 min) and the mRNA levels of *Bdnf*, *Arc*, *Psd95*, *Gad67*, *Pvb*, *Drd1* and *Drd2*.

A probability level of  $p < 0.05$  was considered significant in every test. All analyzes were done with SPSS Statistics software package, Version 25.

## Results

### Social isolation does not induce reduced wellbeing associated burrowing behavior

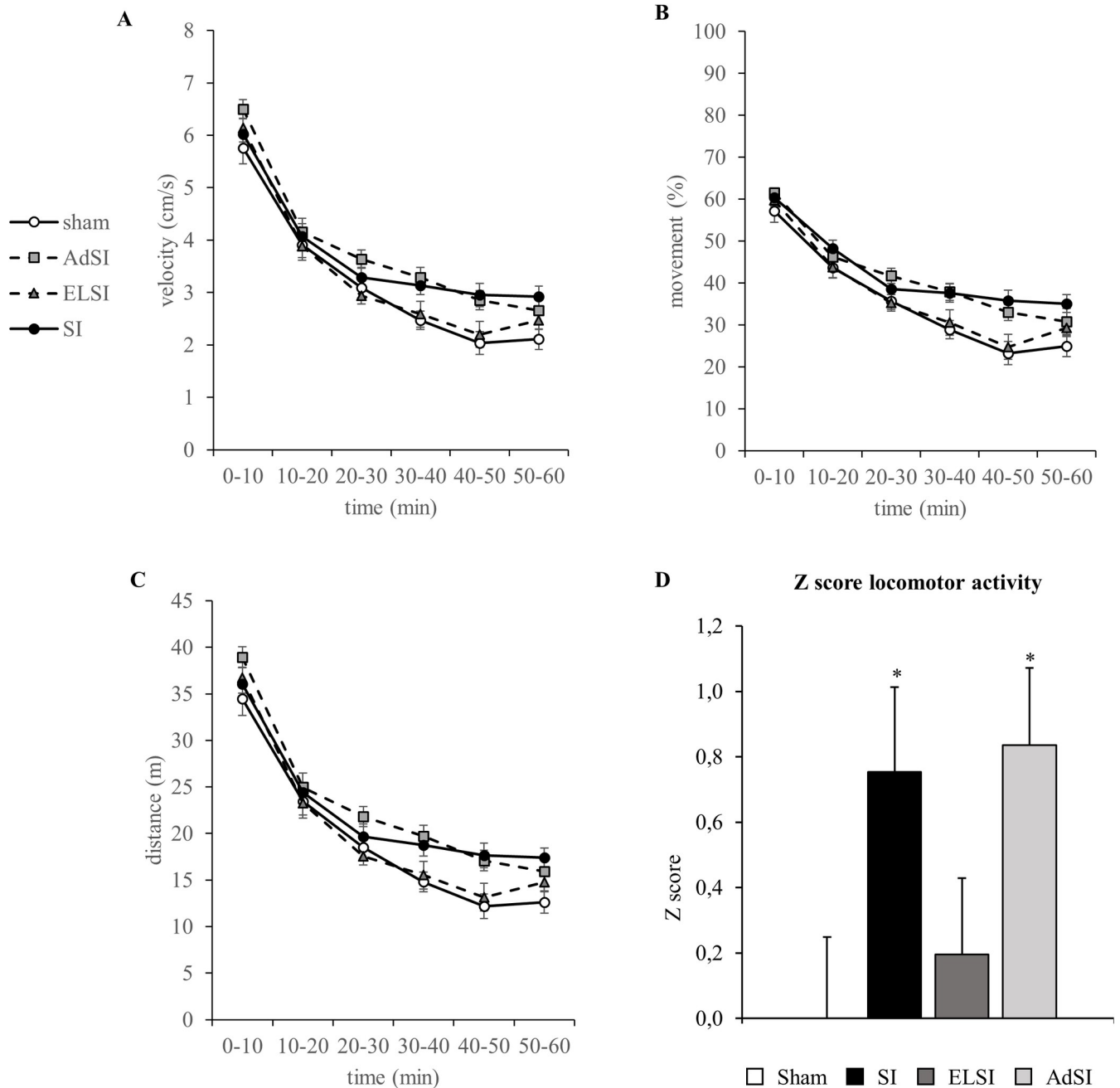
We used burrowing as a parameter for wellbeing associated behavior. We compared the amount of burrowed material in the preceding training phase and during the tests and found no significant differences between the treatment groups (Fig 2A).

The prominent decrease on PND 59 of the ELSI group was not sufficient to cause statistical differences. An overall time effect ( $F(6,309) = 9.249$ ,  $p < 0.0001$ ) was detected, but there was no interaction between time and treatment group (Fig 2B). The intercept ( $F(1,309) = 15.117$ ,  $p = 0.0001$ ) of the default model was 592.2 g (SE 152.3 g).

The intercept ( $F(1,157) = 245.787$ ,  $p < 0.001$ ) for the latency to burrow was 53.6 s (SE 1.3 s). We found no differences in the latency to burrow between the conditions, over time or interaction. In comparison of the fixed effects we found that ELSI rats at PND 59 showed a significant increase ( $p = 0.0304$ ) with an alteration of 2.6 s (SE 1.6 s). No significant differences in z-scores were detected (Fig 2D).

### Isolation-induced hyperactivity is age-independent and can be ameliorated by resocialization

Social isolation is known to induce hyperactivity in rats [1]. We detected the locomotor behavior in the Open Field test and observed the progress over time in different parameters (Fig 3A–3C). We found the typical decrease of activity over time for velocity (time  $F(5,260) = 370.430$ ,  $p = 5.02E-116$ ), movement (time  $F(5,260) = 246.846$ ,  $p = 1.59E-96$ ) and distance moved ( $F(5,260) = 370.192$ ,  $p = 5.40E-116$ ). Additionally, we found interactions with the housing condition (distance moved: time\*housing  $F(15,260) = 1.685$ ,  $p = 0.054$ ; velocity:



**Fig 3. Effects of social isolation on locomotor activity investigated in the Open Field test to assess schizophrenic-like phenotypes.** Analyses of velocity in cm/s (A), movement in % (B) and distance moved in m (C) in the Open Field test for all four groups. Normalization of data using the z-score method was performed for *velocity*, *total distance moved*, and *total movement* values using the Sham group as the baseline (D). Each value represents the mean ± SEM. \* p<0.05, \*\* p<0.01 vs Sham animals post-hoc.

<https://doi.org/10.1371/journal.pone.0240439.g003>

time\*housing F(15,260) = 1.730, p = 0.046; movement: time\*housing F(15,260) = 246.846, p = 0.010).

Interestingly, while rats from SI and AdSI showed higher activity, the behavioral outcome from ELSI rats rather resembled the sham controls. We discovered a strong tendency for housing-specific effects (distance moved: housing F(3,52) = 2.716, p = 0.054; velocity: housing F



(3,52) = 2.718,  $p = 0.054$ ), but was only significant for the movement parameter (housing:  $F(3,52) = 3.372$ ,  $p = 0.025$ ). Post-hoc analysis revealed increased activity of SI rats, reflected in a statistical trend ( $p = 0.074$ , Bonferroni corrected).

The z-score analysis (Fig 3D) further confirmed these results, showing a significant main effect of housing condition ( $F(3,55) = 2.936$ ,  $p = 0.042$ ).

The number of rearings in the Open Field test decreased significantly over time (time:  $F(5,260) = 331.429$ ,  $p = 5.84E-76$ ), but was not influenced by housing (Fig 4A). The time spent in the center was neither influenced by time nor housing (Fig 4B). All groups voided a comparable number of feces (Fig 4C). There was no significant interaction between time and housing. These data were confirmed by using the z scores approach (Fig 4D), as no effect of housing condition has been observed ( $F(3,55) = 1.370$ ,  $p = 0.262$ ).

All rats showed a gain in body weight (time:  $F(7,371) = 6875.075$ ,  $p = 2.45E-166$ ), but with different intercepts (time\*housing:  $F(21,371) = 2.568$ ,  $p = 2.16E-4$ ), which did not result in differences between the housing groups (Fig 4E). While all animals gained weight over time, SI rats showed the highest increase, resulting in a slightly, but insignificantly higher weight.

### Deprivation of social interactions strongly affects neuroplasticity regardless of the timing of stress exposure

**Analysis of *Bdnf* mRNA expression.** To assess the differential impact of social isolation throughout life on neuroplasticity, total *Bdnf* mRNA levels, the pool of *Bdnf* transcripts with the long 3'-UTR that are usually targeted to dendrites, and of two major *Bdnf* isoforms, namely transcript IV and VI, were analyzed in the prefrontal cortex.

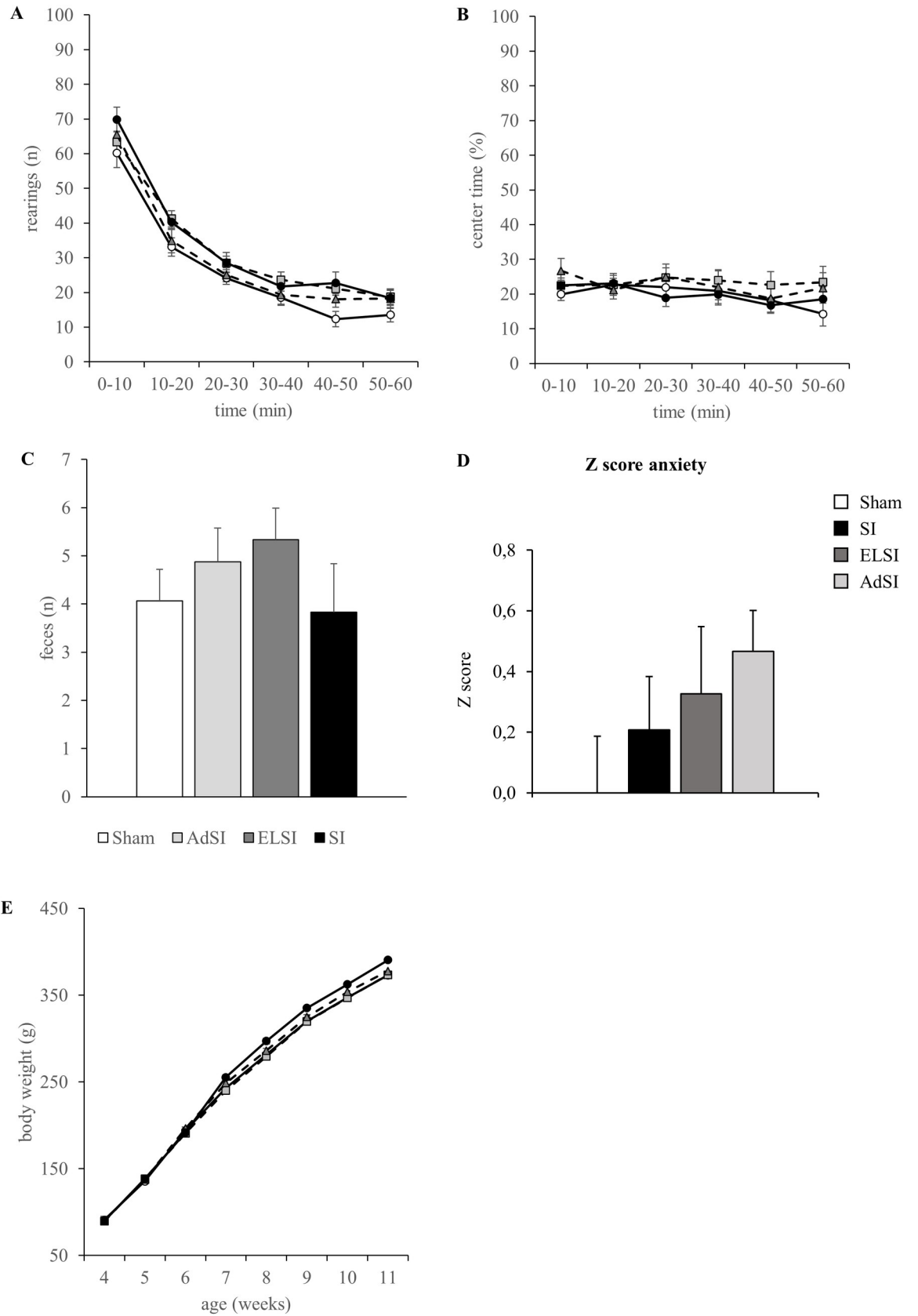
We found that housing conditions determined a significant main effect on the expression of total *Bdnf* mRNA levels within the prefrontal cortex ( $F(3, 49) = 8.776$ ,  $p = 0.0001$ , see Fig 5A). Indeed, animals with lifelong isolation as well as animals which were isolated exclusively during adulthood showed a significant down-regulation of total *Bdnf* mRNA levels, as compared to animals always housed in group (post-hoc:  $p < 0.01$  and  $p < 0.001$ , respectively). Contrastingly, this reduction did not reach statistical significance in animals isolated only during adolescence ( $p = 0.075$ ).

Similarly, a significant main effect of the rearing condition on the expression of *Bdnf* long 3'-UTR mRNA levels was found ( $F(3, 52) = 6.750$ ,  $p = 0.00067$ , see Fig 5B), with a significant decrease in the expression of *Bdnf* long 3'-UTR within the prefrontal cortex of animals isolated only during adolescence or adulthood (post-hoc:  $p < 0.05$  for ELSI and  $p < 0.001$  for AdSI), whereas this reduction was barely not statistically significant in lifelong isolated animals (post-hoc:  $p = 0.0559$ ).

The expression of the two major *Bdnf* isoforms IV and VI were partially divergent from what observed for total *Bdnf* and *Bdnf* long 3'-UTR. As for the expression of *Bdnf* transcripts containing exon IV, we observed a significant main effect of the housing condition ( $F(3, 54) = 3.302$ ,  $p = 0.027$ , see Fig 5C). In detail, animals with a lifelong social deprivation showed decreased exon IV containing-*Bdnf* mRNA levels, as compared to controls (post-hoc:  $p < 0.05$ ), whereas animals isolated exclusively during adolescence or adulthood did not.

In a similar way, data obtained from the analysis of the expression of *Bdnf* transcripts containing exon VI highlighted a significant main effect of housing condition ( $F(3, 51) = 3.007$ ,  $p = 0.039$ , see Fig 5D), with a significant down-regulation of gene expression only in animals lifelong socially deprived as compared to control grouped animals (post-hoc:  $p < 0.05$ ).

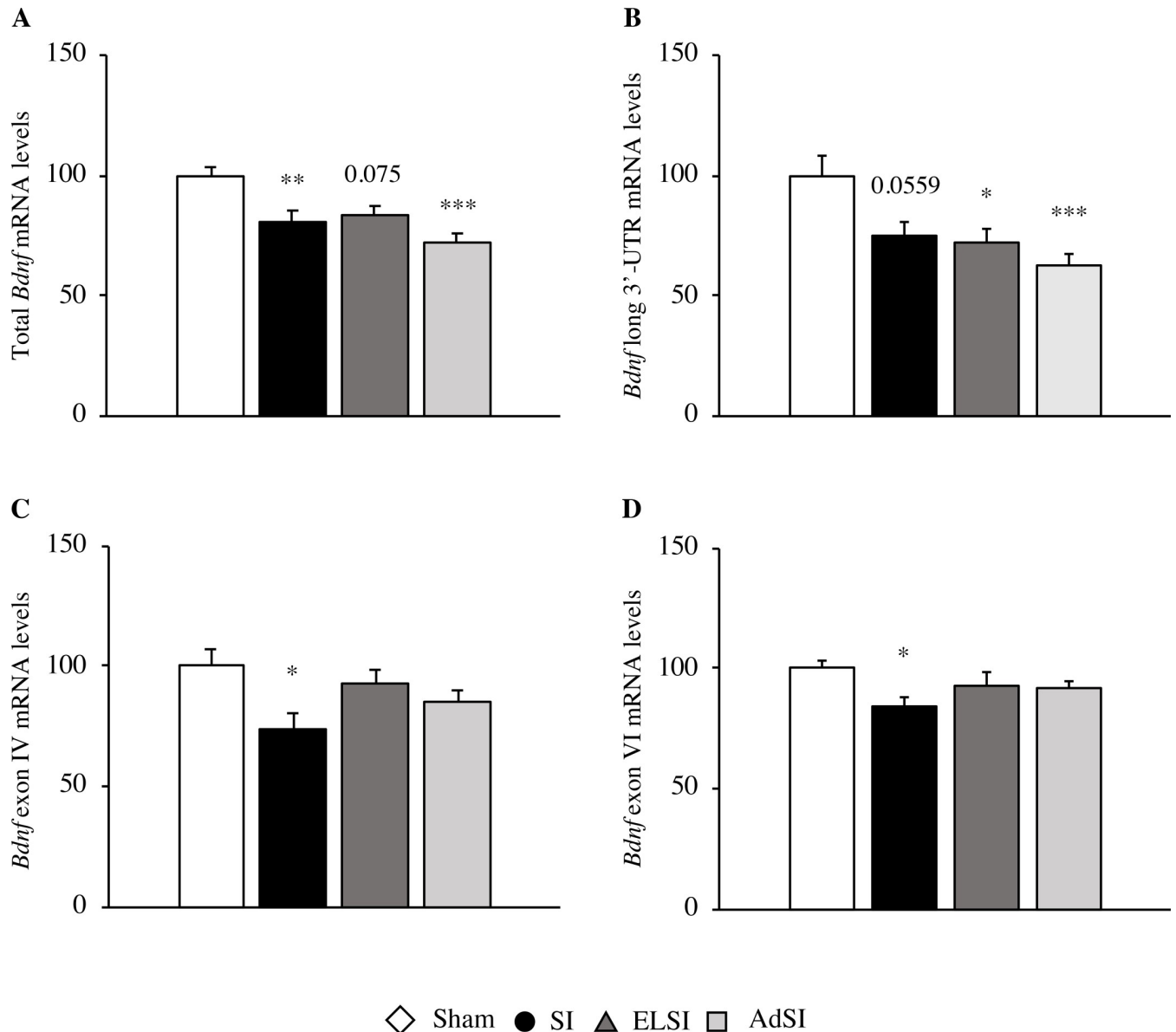
In light of these findings, to further investigate if impairments in *Bdnf* expression were associated with the hyperlocomotion observed in the OF test, total *Bdnf*, *Bdnf* long 3'-UTR, *Bdnf* IV transcripts and *Bdnf* VI transcripts mRNA levels were then plotted against the distance



**Fig 4. Effects of social isolation on exploration and anxiety in the Open Field.** Analysis of the total number of rearings (A), time spent in the center zone (B), and the number of voided feces (C) in the Open Field test. Normalization of data using the z-score method was performed for the *number of feces*, the *number of rearings*, *time spent in the center*, and “*distance to walls*” values using the Sham group as the baseline (D). Each value represents the mean  $\pm$  SEM. Body weight development in g over time (E).

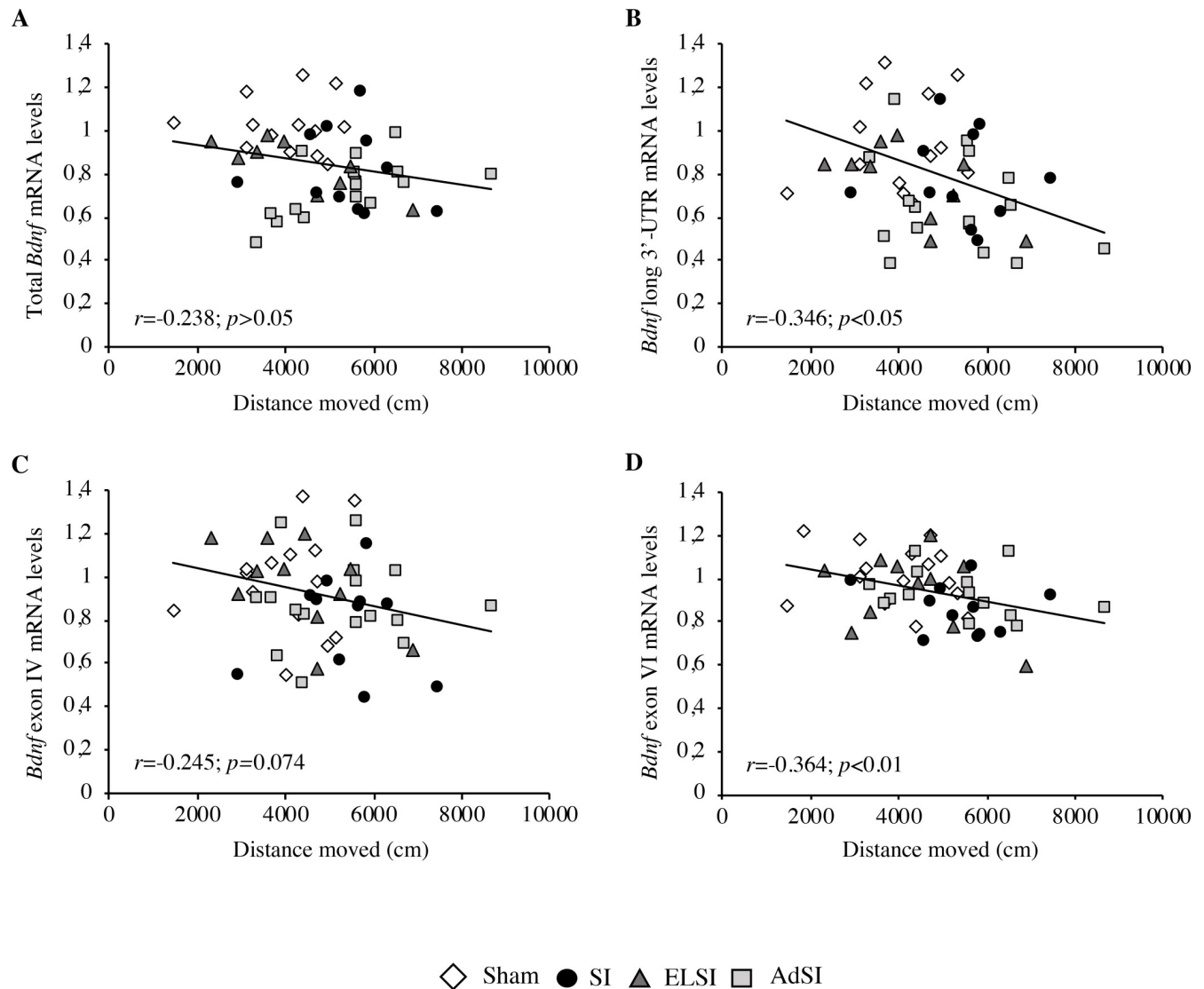
<https://doi.org/10.1371/journal.pone.0240439.g004>

moved in the second part of the behavioral test (40–60 min), where the differences appeared most prominent (Fig 6). While no correlation between the distance moved and the expression of total *Bdnf* was found ( $r = -0.238$ ,  $p > 0.05$ , Fig 6A), the statistical analysis showed a significant correlation between hyperlocomotion and *Bdnf* long 3'-UTR down-regulation ( $r = -0.346$ ,



**Fig 5. Effects of social isolation on *Bdnf* mRNA expression in the prefrontal cortex of adult Lister Hooded male rats.** The mRNA levels of total *Bdnf* (A), *Bdnf* long 3'-UTR (B), *Bdnf* exon IV (C) and *Bdnf* exon VI (D) were analyzed in the prefrontal cortex of male rats that were lifelong group-housed (Sham), lifelong isolated (SI), exposed to social isolation during adolescence (ELSI) or exposed to social isolation during adulthood (AdSI). The data expressed as the % vs. Sham animals set at 100%, are the mean  $\pm$  SEM. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  vs Sham animals post-hoc.

<https://doi.org/10.1371/journal.pone.0240439.g005>



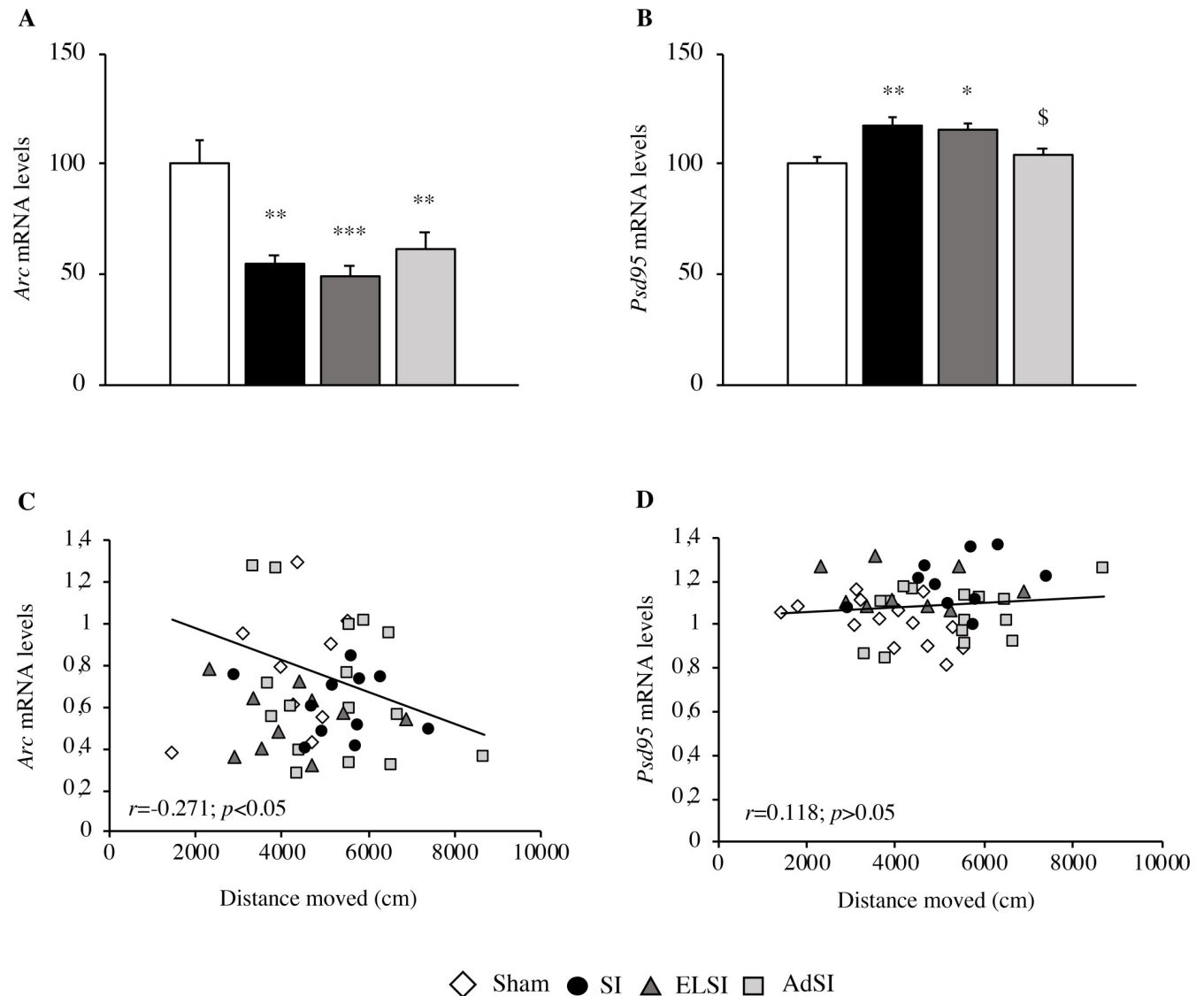
**Fig 6. Correlation analyses between the locomotor activity assessed in the OF test and the expression of *Bdnf* within the prefrontal cortex of adult Lister Hooded male rats exposed to social isolation.** The correlations between the distance moved in the second part of the OF test (40–60 min) and the mRNA levels of total *Bdnf* (A), *Bdnf* long 3'-UTR (B), *Bdnf* exon IV (C) and *Bdnf* exon VI (D) in the prefrontal cortex of male rats that were lifelong group-housed (Sham), lifelong isolated (SI), exposed to social isolation during adolescence (ELSI) or exposed to social isolation during adulthood (AdSI) were analyzed by Pearson product-moment correlation ( $r$ ).

<https://doi.org/10.1371/journal.pone.0240439.g006>

$p < 0.05$ , Fig 6B) as well as between hyperlocomotion and *Bdnf* VI transcripts down-regulation ( $r = -0.364$ ,  $p < 0.01$ , Fig 6D), with a considerable trend toward significance also for the correlation with *Bdnf* IV transcripts expression ( $r = -0.245$ ,  $p = 0.074$ , Fig 6C). Indeed, lower levels of *Bdnf* were associated with higher moved distances in the OF test.

**Analysis of *Arc* and *Psd95* mRNA expression.** Next, we evaluated the levels of the immediate early gene (IEG) Activity Regulated Cytoskeleton Associated Protein (*Arc*) and Post-Synaptic Density Protein 95 (*Psd95*).

We found that housing environment significantly affected *Arc* expression ( $F(3, 53) = 8.860$ ,  $p = 0.00008$ , Fig 7A), resulting in a significant decrease of mRNA levels in the prefrontal cortex of each stressed group, as compared to controls (post-hoc:  $p < 0.01$  for SI and AdSI,  $p < 0.001$



**Fig 7. Effects of social isolation on the expression of genes involved in neuroplasticity in the prefrontal cortex of adult Lister Hooded male rats.** The mRNA levels of *Arc* (A) and *Psd95* (B) were analyzed in the prefrontal cortex of male rats that were lifelong group-housed (Sham), lifelong isolated (SI), exposed to social isolation during adolescence (ELSI) or exposed to social isolation during adulthood (AdSI). The data expressed as the % vs. Sham animals set at 100%, are the mean  $\pm$  SEM of at least 9 animals per group. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\*\* $p < 0.001$  vs Sham animals; \$ $p < 0.05$  vs SI animals post-hoc. The correlations between the distance moved in the second part of the OF test (40–60 min) and the mRNA levels of *Arc* (C) and of *Psd95* (D) in the prefrontal cortex of male rats that were lifelong group-housed (Sham), lifelong isolated (SI), exposed to social isolation during adolescence (ELSI) or exposed to social isolation during adulthood (AdSI) were analyzed by Pearson product-moment correlation ( $r$ ).

<https://doi.org/10.1371/journal.pone.0240439.g007>

for ELSI). Furthermore, we observed that the molecular changes in *Arc* expression correlated with the locomotor activity assessed in the OF test. Indeed, there was a negative correlation between the two variables ( $r = -0.271$ ,  $p < 0.05$ , Fig 7C), indicating that animals expressing lower *Arc* levels moved more during the second part of the locomotor assessment (40–60 min).

Considering *Psd95* levels, a significant main effect of the housing condition was found ( $F(3, 48) = 6.889$ ,  $p = 0.0006$ , Fig 7B). Conversely to what we observed so far, animals that underwent isolation during adolescence, as well as animals that were always housed in isolation, showed significantly up-regulated mRNA levels in the prefrontal cortex, as compared to

controls ( $p < 0.01$  for SI and  $p < 0.05$  for ELSI). Moreover, animals reared in constant isolation showed a significant increase of *Psd95* also as compared to animals exposed to isolation rearing only during adulthood ( $p < 0.05$ ). A Pearson product-moment correlation coefficient was computed to assess the relationship between *Psd95* levels and distance moved in the second part of the OF test (40–60 min). However, the hyperactivity state did not seem to correlate with the specific modulation of *Psd95* mRNA levels ( $r = 0.118$ ,  $p > 0.05$ , Fig 7D).

**Analysis of *Gad67* and *Pvb* mRNA expression.** We next evaluated the expression of two  $\gamma$ -aminobutyric acid (GABA) related markers, glutamic acid decarboxylase 67 (*Gad67*), and *Parvalbumin* (*Pvb*).

With regard to *Gad67* mRNA expression, we observed no significant main effect of the housing condition (Fig 8A). The lack of a correlation between mRNA levels and locomotor activity assessed during the second part of the OF test (40–60 min) ( $r = -0.083$ ,  $p > 0.05$ , Fig 8C) further confirms this.

Although the expression of *Pvb* was not impacted by isolation rearing conditions (Fig 8B), the correlation analysis between the molecular and the behavioral data showed an inverse relationship close to significance ( $r = -0.276$ ,  $p = 0.052$ , Fig 8D). This result indicates that hyperactive animals expressed lower *Pvb* within the prefrontal cortex.

**Analysis of *Drd1* and *Drd2* mRNA expression.** Lastly, we measured the expression of *Drd1* and *Drd2*, genes encoding for dopaminergic receptors subunits D1 and D2, respectively.

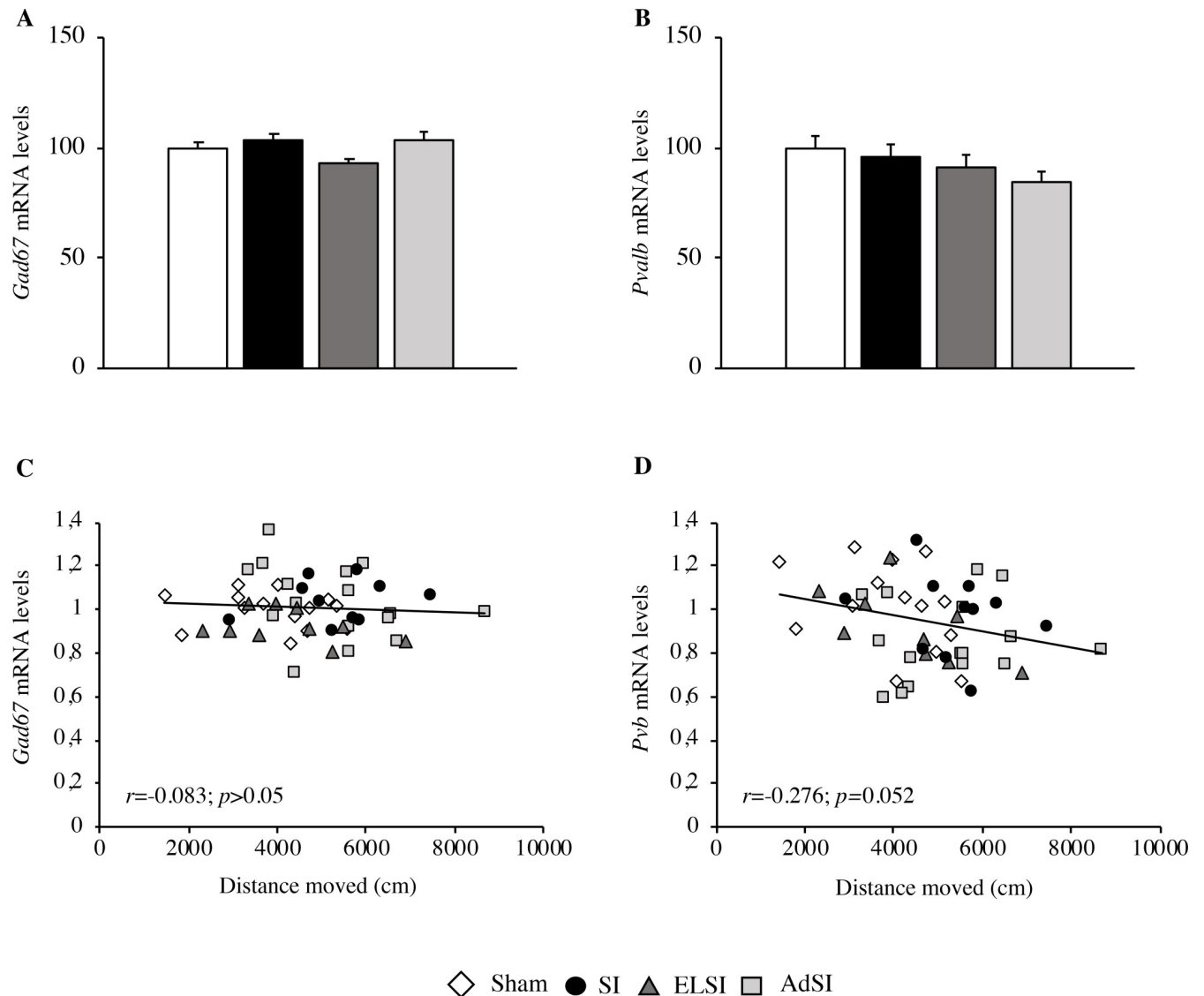
Considering *Drd1*, we found a substantial trend toward the significance of housing condition ( $F(3, 49) = 2.653$ ,  $p = 0.0596$ , Fig 9A). With respect to *Drd2* mRNA levels, we observed a significant main effect of the housing environment ( $F(3, 45) = 3.773$ ,  $p = 0.017$ , Fig 9B). Indeed, animals isolated only during adulthood show an elevation of *Drd2* expression, which was statistically significant when compared to lifelong isolated animals (post-hoc:  $p < 0.05$ ).

Despite these effects, we did not find any correlation between *Drd1* and *Drd2* gene expression and locomotor activity assessed during the second part of the OF test (40–60 min, data not shown).

## Discussion

In this study, we aimed to investigate the effect of social isolation at different developmental stages of male rats, namely during adolescence, young adulthood, or both phases and the effects of resocialization. One reason to investigate this issue was to find potential refinement strategies, which may inflict as little suffering in the subjects as possible, while still being able to investigate the effects produced by social deprivation. To ensure this, we tested the behavioral outcome by assessment of hyperlocomotion, a core behavioral domain for this manipulation. Additionally, we used burrowing behavior as a readout for animal welfare and eventually, we correlated the results with selected molecular changes.

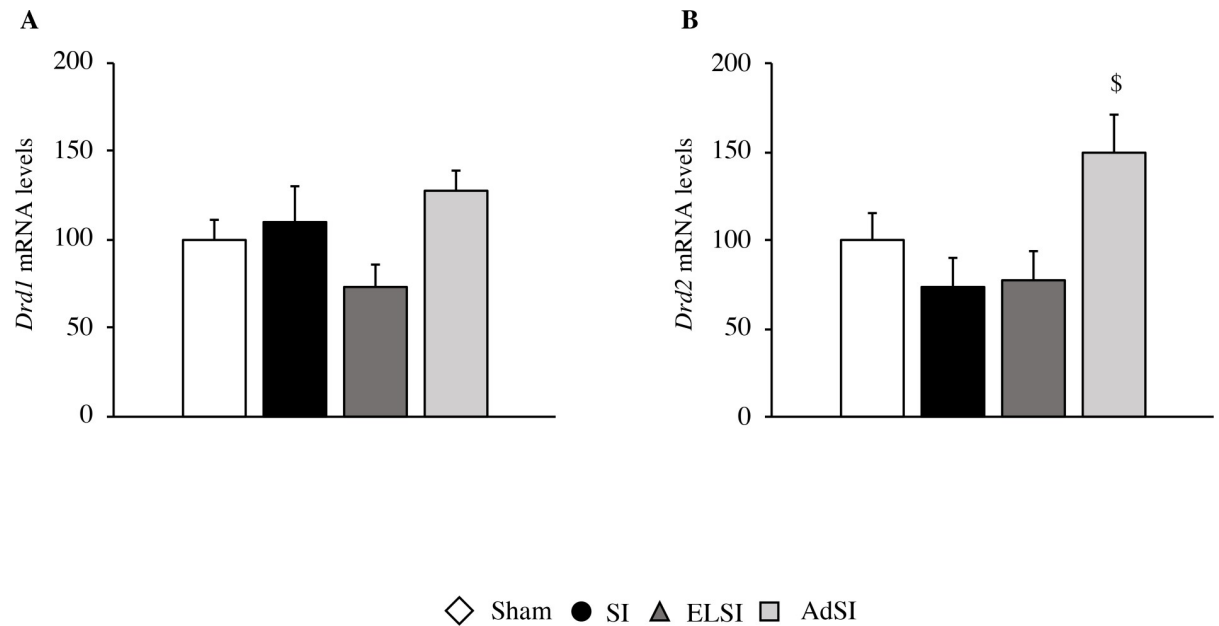
The hyperactivity in a novel environment observed in the Open Field test is typical for the utilized Lister Hooded strain [56–58]. We found that animals, which were socially isolated for their entire life, showed increased locomotor activity, mainly after 40 min within the start of the behavioral task. Similarly, animals isolated only during adulthood displayed a hyperactive phenotype. Contrary, returning to a social context during adolescence normalized the behavioral phenotype. Interestingly, subjects that were isolated at the time of testing showed the typical hyperactivity, independent from the separation status during adolescence. This implies that social isolation acts as an age-independent stressor and that social isolation of rats should be avoided throughout life, whenever possible; not only to minimize confounding factors in scientific readouts but also to warrant animal welfare and to comply with the 3Rs principle of animal research. Similar to previous findings, we found that the resocialized ELSI group did not



**Fig 8. Effects of social isolation on the expression of GABAergic markers in the prefrontal cortex of adult Lister Hooded male rats.** The mRNA levels of *Gad67* (A) and *Parvalbumin* (*Pvalb*) (B) were analyzed in the prefrontal cortex of male rats that were lifelong group-housed (Sham), lifelong isolated (SI), exposed to social isolation during adolescence (ELSI) or exposed to social isolation during adulthood (AdSI). The data expressed as the % vs. Sham animals set at 100%, are the mean  $\pm$  SEM of at least 9 animals per group. The correlations between the distance moved in the second part of the OF test (40–60 min) and the mRNA levels of *Gad67* (C) and *Parvalbumin* (*Pvalb*) (D) in the prefrontal cortex of male rats that were lifelong group-housed (Sham), lifelong isolated (SI), exposed to social isolation during adolescence (ELSI) or exposed to social isolation during adulthood (AdSI) were analyzed by Pearson product-moment correlation ( $r$ ).

<https://doi.org/10.1371/journal.pone.0240439.g008>

display hyperactive behavior [56]. However, some behavioral responses cannot be attenuated by social contact after the critical period for social development, e.g. social anxiety, sexual or aggressive interaction [59–62]. Others can be reversed, including approach behavior upon pro-social calls or abnormal habituation to novel environments [63]. The findings on resocialization are inconsistent and appear dependent on different rat strains, sex, or the timing and duration period of social isolation [23]. Typically, rats are isolated after weaning for 2 to 6 weeks and are then resocialized for some time before the experimental onset. This time of resocialization varies highly, from hours to weeks—which of course limits the comparability of the



**Fig 9. Effects of social isolation on the expression of dopaminergic markers in the prefrontal cortex of adult Lister Hooded male rats.** The mRNA levels of *Drd1* (A) and *Drd2* (B) were analyzed in the prefrontal cortex of male rats that were lifelong group-housed (Sham), lifelong isolated (SI), exposed to social isolation during adolescence (ELSI) or exposed to social isolation during adulthood (AdSI). The data expressed as the % vs. Sham animals set at 100%, are the mean  $\pm$  SEM of at least 9 animals per group. <sup>\$</sup> $p < 0.05$  vs SI animals.

<https://doi.org/10.1371/journal.pone.0240439.g009>

outcomes. However, some studies indicate a critical developmental phase in which for example anxiety, aggression and reduced social interaction and defense behavior remained impaired and could not be recovered by resocialization ([24, 25, 59, 61, 64]; for review read [23]). Other studies show beneficial effects of resocialization on anxiety- and depression-like behavior, as well as aggression and social interaction and response to social ultrasonic calls [63, 65–67]. A direct comparison between adolescent isolation and adult isolation was hardly ever described. Seffer *et al.* Seffer, Rippberger [63] did compare those isolation forms with respect to social approach behavior and found no impairment in isolated adults, but a deficit in resocialized rats [63]. Hence, it is advisable to be aware of whether the isolation-induced effects of interest are distinct after the isolation at different developmental stages.

We also investigated whether the timing of social isolation inflicted a different intensity of severity on the animals' wellbeing. In principle, social isolation did not derogate the execution of burrowing. Especially during the training and early test phase, no constraints became apparent indicating that the wellbeing of rats was affected by early-onset social isolation.

The AdSI group did not show an increase in burrowing directly after the condition change, but continuously improved afterwards. In contrast, joining a group without previous socialization during adolescence tended to diminish the wellbeing-associated burrowing and increased the latency to start burrowing. The neglect of such "luxury behavior" could be explained by the urge to satisfy more vital needs. The time in the burrowing test may serve as an undisturbed opportunity to sleep, since the rats are alone within the experimental cage. We observed this in several cases. Other than that, we did not detect signs for impaired welfare. Body weight was similar in all treatment groups and we observed no aggressive behavior within the groups, although early life social deprivation has been reported to lead to long-lasting aggressive behaviors [25]. Our data indicate that remaining in social isolation was less stressful than resocialization for adult male rats. Hence, resocialization might not be suitable for refining



experiments, although the effects were only tendencies, mild and limited to a short period. It might be advisable to keep this in mind, when performing social alterations in any behavioral experiment.

We looked at *Bdnf*, a marker for psychiatric disorders, which is used as an indicator for wellbeing as well [68]. *Bdnf* is an important player for neuronal development and function, neuroprotection and neuronal plasticity [68, 69]. The latter activity is crucial for adaptation to adverse environmental conditions, which is also associated with the development of neuropsychiatric disorders [70–72]. We found that *Bdnf* expression was markedly reduced in the prefrontal cortex of isolated animals, regardless of the timing of isolation exposure. The *Bdnf* gene is very complex and consists of several splice variants that are localized in different neuronal compartments, mediating synaptic plasticity within each area [73, 74]. Similar to total *Bdnf* expression, we observed that also the levels of the pool of *Bdnf* transcripts with the long 3'-UTR are downregulated within the PFC of isolated animals, without any difference due to the timing of stress exposure. The pool of *Bdnf* transcripts comprising the long 3'-UTR are preferentially targeted to dendrites, contributing to activity-dependent *Bdnf* translation [73]. This may suggest a widespread impairment of the neurotrophic functionality, both in the cell body and in distal dendrites, regardless of the timing of stress exposure. Accordingly, a late deprivation of social contacts, as well as resocialization, seems to be a suitable refinement alternative, leading to a deficiency similar to SI animals. However, it has been suggested that also the specific 5'UTR sequence must be taken into account for dendritic targeting [74]. Accordingly, it has been proposed that *Bdnf* transcripts containing 5'UTR exon I or IV are localized to the cell body and proximal dendrites, while *Bdnf* transcripts containing 5'UTR exon II or VI are found in distal dendrites [74]. Interestingly, we found that exon IV- and exon VI-containing *Bdnf* transcripts were significantly reduced only in lifelong isolated animals. However, *Bdnf* expression patterns differ based on the cell type (neurons or astrocytes) and other minor *Bdnf* transcripts could also play a pivotal role [75, 76]. The adult cortex expresses more exon IV-containing *Bdnf* transcripts than exon VI-transcripts [76]. Stratifying the results at the cellular level, this ratio is confirmed only in neurons, while astrocytes present more exon VI-containing *Bdnf* transcripts in the cortex [75].

A few studies investigating isolation-induced effects on *Bdnf* expression within the PFC found no changes immediately after stress exposure during adolescence [77], while returning to social housing after adolescent social isolation seemed to increase cortical *Bdnf* expression [78]. Similarly, exposure to social deprivation at adulthood induced an up-regulation of *Bdnf* expression in the PFC [79]. Even though our results are in contrast with these findings, they confirm the post-mortem studies in schizophrenia and depressed patients, which showed a consistent decrease of cortical *Bdnf* expression [80]. Furthermore, we found that the isolated animals with reduced *Bdnf* mRNA levels within the PFC were more active in the OF testing. This evidence suggests that the behavioral dysfunction in locomotor activity may be associated with an impairment of neuroplasticity. As both parameters are well-established hallmarks of mental diseases, this correlation strengthens our findings.

The detrimental effects of social deprivation exposure on neuroplasticity were further confirmed by the investigation of *Arc* expression. Similar to BDNF, *Arc* encodes for a multifunctional protein that is fundamental in several processes, comprising synaptic plasticity and structural dendritic spine remodeling [81, 82]. We observed a decrease in *Arc* mRNA expression within the prefrontal cortex of each stressed group, in line with previous studies that found a link between post-weaning isolation and reduced *Arc* levels [83]. A sharp negative correlation between *Arc* levels and locomotor activity further supported that deprivation of social interaction at any time of life can induce a hyperactivity phenotype coupled with impaired neuroplasticity. This finding confirms previously reported data showing how AdSI hyperactive

mice also display reduced *Arc* mRNA levels [84]. Nevertheless, further research should investigate isolation at different life-stages to better understand if a link between *Arc* expression and a hyperactivity phenotype is consistent.

Taken together, our data suggest that deprivation of social contacts at any time of life can lead to an overall distress condition of the animals. Hereby, at least as far as *Bdnf* and *Arc* mRNA expression are concerned, we could speculate that the application of social deprivation only during adulthood might be considered as an alternative to long-lasting isolation, inducing the onset of an acute hyperactive behavior as well as a deficit of neuroplasticity-related markers. On the contrary, shifting to a situation of social housing during adulthood normalizes hyperactive phenotypes while still showing molecular impairments, in association with a reduction of wellbeing.

Last, considering the key role of glutamate and GABA in mental illnesses, we investigated two prototypes markers of these systems. We investigated the Postsynaptic Density Protein 95 (PSD95), one of the most abundant proteins of the postsynaptic densities and a key regulator of synaptic transmission and plasticity [85, 86]. Indeed, PSD95 is involved in synaptic maturation by modulating N-methyl-d-aspartic acid receptors (NMDARs) and  $\alpha$ -amino-3-hydroxy-5-methyl-4-isox-azolepropionic acid receptors (AMPA). Accordingly, a reduction in PSD95 levels has been found in the brain of schizophrenic patients [87], supporting its postulated role in the outbreak of the disease [88]. However, our data were in contrast with these findings, as we found a significant increase in *Psd95* mRNA levels both in animals that experienced isolation only during adolescence or lifelong isolation. Animals isolated only during adulthood did not show any modulation. In contrast, reduced PSD95 expression has been found in the PFC of female rats exposed to social isolation rearing condition [89].

Furthermore, dysfunctions in the GABAergic transmission have been observed following exposure to chronic stress as well as in association with major depression disorder (MDD) and schizophrenia [44, 90]. The synthesis of GABA mainly relies on the enzyme *Gad67*, whose level is reduced in schizophrenic and MDD patients along with chronically stressed animals [90, 91]. Such a decrease is predominant in neurons expressing the calcium-binding protein parvalbumin (PVB), which is accordingly decreased in such pathological outcomes [45]. However, we did not find any modulation of *Gad67* and *Pvb* expression following our stress procedure, in contrast with a previous study showing a significant down-regulation of *Pvb* after three weeks of social deprivation at adulthood [92]. Although the expression of *Pvb* was not impacted by isolation rearing conditions, we found that hyperactive animals expressed lower *Pvb*, confirming the fact that impairments in the GABAergic system are coupled with a hyperactive schizophrenic-like phenotype.

Considering the role of dopamine in schizophrenia, we found limited changes in D1 and D2 receptors following SI during adolescence, with a trend toward a decreased expression, whereas we observed a consistent elevation of D2 mRNA levels after social isolation at adulthood. These changes are in line with a previous report showing that chronic stress exposure may increase *Drd2* levels [93, 94] and suggest a complex regulation of this system based on the timing of the adverse experience.

Both behavioral and molecular data indicate that AdSI might be a suitable candidate for refining social isolation experiments. AdSI showed comparable hyperactivity, no long-lasting impact on the wellbeing parameter, and most molecular features of SI. However, we would like to emphasize that this observation might be strain- or sex-specific. Since male rats are generally more susceptible to SI outcomes [1] and due to technical limitations in the lab, we focused on male rats in this study. Furthermore, we concentrated the analysis for psychiatry-related parameters to the novelty induced exploration, since this a robust marker for the isolation syndrome. We decided to limit our analysis on locomotor hyperactivity as an indicator to

prevent frequent handling, as this could confound the outcome [28]. For other research questions different parameters, e.g. sensory gating might be relevant as well.

In terms of welfare, there is growing evidence that multimodal analysis is a more valuable approach towards evidence-based assessment of severity in laboratory animals [95–97]. In this study, we focused on burrowing and could not find differences between the groups besides the short-term decrease directly after resocialization. More studies on other relevant parameters like nesting, corticosterone release, saccharine preference, grimace scale, or heart rate variability are necessary to fully comprehend the potential benefit of AdSI over SI.

In summary, our study demonstrates that the deprivation of social contacts, at any time of life in laboratory rats, can firmly impair neuroplasticity processes within the prefrontal cortex. These findings have strong translational value, suggesting that an isolation housing condition could be detrimental not only if experienced during the first stages of life but also later on. Moreover, we established that some deficits induced by adolescent stress are long-lasting since they were not attenuated after a period of social housing. Surprisingly, we could not find altered wellbeing due to the isolation housing per se. We observed only a short-term effect after resocialization. Although some evidence suggests that resocialization might normalize isolation-induced parameters, the regrouping acts as a temporary stressor. This should be taken into consideration when changing social groups during experimental procedures. The shift of the isolation period from the more susceptible adolescence phase to the adult phase could be an adequate refinement measure for research questions. It is important to approach the refinement of animal experiments whenever possible and consider potential improvements for the subjects.

## Supporting information

### **S1 Data. Raw data burrowing long format.**

(XLSX)

### **S2 Data. Raw data burrowing.**

(XLSX)

### **S3 Data. Raw data mRNA.**

(XLSX)

### **S4 Data. Raw data open field.**

(XLSX)

### **S5 Data. Raw Data Z scores.**

(XLSX)

### **S1 Table. Primers and probes assays.**

(DOCX)

### **S1 File. The ARRIVE essential 10: Author checklist.**

(PDF)

## Author Contributions

**Conceptualization:** Natascha Pfeiffer, Christiane Brandwein, Dragos Inta, Steven R. Talbot, Marco Andrea Riva, Peter Gass, Anne Stephanie Mallien.

**Data curation:** Veronica Begni, Alice Sanson.

**Formal analysis:** Veronica Begni, Steven R. Talbot, Anne Stephanie Mallien.

**Funding acquisition:** Marco Andrea Riva, Peter Gass.

**Investigation:** Alice Sanson, Natascha Pfeiffer, Christiane Brandwein.

**Project administration:** Anne Stephanie Mallien.

**Resources:** Peter Gass.

**Supervision:** Marco Andrea Riva.

**Visualization:** Veronica Begni, Alice Sanson, Steven R. Talbot, Anne Stephanie Mallien.

**Writing – original draft:** Veronica Begni, Alice Sanson, Anne Stephanie Mallien.

**Writing – review & editing:** Veronica Begni, Alice Sanson, Natascha Pfeiffer, Christiane Brandwein, Dragos Inta, Steven R. Talbot, Marco Andrea Riva, Peter Gass, Anne Stephanie Mallien.

## References

1. Fone KC, Porkess MV. Behavioural and neurochemical effects of post-weaning social isolation in rodents—relevance to developmental neuropsychiatric disorders. *Neurosci Biobehav Rev.* 2008; 32(6):1087–102. <https://doi.org/10.1016/j.neubiorev.2008.03.003> PMID: 18423591
2. Varese F, Smeets F, Drukker M, Lieverse R, Lataster T, Viechtbauer W, et al. Childhood adversities increase the risk of psychosis: a meta-analysis of patient-control, prospective- and cross-sectional cohort studies. *Schizophr Bull.* 2012; 38(4):661–71. <https://doi.org/10.1093/schbul/sbs050> PMID: 22461484
3. Meyer-Lindenberg A, Tost H. Neural mechanisms of social risk for psychiatric disorders. *Nat Neurosci.* 2012; 15(5):663–8. <https://doi.org/10.1038/nn.3083> PMID: 22504349
4. Meyer-Lindenberg A. Social neuroscience and mechanisms of risk for mental disorders. *World Psychiatry.* 2014; 13(2):143–4. <https://doi.org/10.1002/wps.20121> PMID: 24890061
5. Selten JP, Booij J, Buwalda B, Meyer-Lindenberg A. Biological Mechanisms Whereby Social Exclusion May Contribute to the Etiology of Psychosis: A Narrative Review. *Schizophr Bull.* 2017; 43(2):287–92. <https://doi.org/10.1093/schbul/sbw180> PMID: 28053019
6. Howes OD, McCutcheon R, Owen MJ, Murray RM. The Role of Genes, Stress, and Dopamine in the Development of Schizophrenia. *Biol Psychiatry.* 2017; 81(1):9–20. <https://doi.org/10.1016/j.biopsych.2016.07.014> PMID: 27720198
7. Pruessner JC, Champagne F, Meaney MJ, Dagher A. Dopamine release in response to a psychological stress in humans and its relationship to early life maternal care: a positron emission tomography study using [<sup>11</sup>C]raclopride. *J Neurosci.* 2004; 24(11):2825–31. <https://doi.org/10.1523/JNEUROSCI.3422-03.2004> PMID: 15028776
8. Egerton A, Valmaggia LR, Howes OD, Day F, Chaddock CA, Allen P, et al. Adversity in childhood linked to elevated striatal dopamine function in adulthood. *Schizophrenia research.* 2016; 176(2–3):171–6. <https://doi.org/10.1016/j.schres.2016.06.005> PMID: 27344984
9. Allen P, Sommer IE, Jardri R, Eysenck MW, Hugdahl K. Extrinsic and default mode networks in psychiatric conditions: Relationship to excitatory-inhibitory transmitter balance and early trauma. *Neurosci Biobehav Rev.* 2019; 99:90–100. <https://doi.org/10.1016/j.neubiorev.2019.02.004> PMID: 30769024
10. Misiak B, Krefft M, Bielawski T, Moustafa AA, Sasiadek MM, Frydecka D. Toward a unified theory of childhood trauma and psychosis: A comprehensive review of epidemiological, clinical, neuropsychological and biological findings. *Neurosci Biobehav Rev.* 2017; 75:393–406. <https://doi.org/10.1016/j.neubiorev.2017.02.015> PMID: 28216171
11. Loades ME, Chatburn E, Higson-Sweeney N, Reynolds S, Shafran R, Brigden A, et al. Rapid Systematic Review: The Impact of Social Isolation and Loneliness on the Mental Health of Children and Adolescents in the Context of COVID-19. *J Am Acad Child Adolesc Psychiatry.* 2020.
12. Reinwald JR, Becker R, Mallien AS, Falfan-Melgoza C, Sack M, Clemm von Hohenberg C, et al. Neural Mechanisms of Early-Life Social Stress as a Developmental Risk Factor for Severe Psychiatric Disorders. *Biol Psychiatry.* 2018; 84(2):116–28. <https://doi.org/10.1016/j.biopsych.2017.12.010> PMID: 29397900
13. Levine JB, Youngs RM, MacDonald ML, Chu M, Leeder AD, Berthiaume F, et al. Isolation rearing and hyperlocomotion are associated with reduced immediate early gene expression levels in the medial

- prefrontal cortex. *Neuroscience*. 2007; 145(1):42–55. <https://doi.org/10.1016/j.neuroscience.2006.11.063> PMID: 17239545
14. Cattaneo A, Riva MA. Stress-induced mechanisms in mental illness: A role for glucocorticoid signalling. *The Journal of steroid biochemistry and molecular biology*. 2015.
  15. Vanderschuren LJ, Achterberg EJ, Trezza V. The neurobiology of social play and its rewarding value in rats. *Neurosci Biobehav Rev*. 2016; 70:86–105. <https://doi.org/10.1016/j.neubiorev.2016.07.025> PMID: 27587003
  16. Vanderschuren LJ, Trezza V. What the laboratory rat has taught us about social play behavior: role in behavioral development and neural mechanisms. *Current topics in behavioral neurosciences*. 2014; 16:189–212. [https://doi.org/10.1007/7854\\_2013\\_268](https://doi.org/10.1007/7854_2013_268) PMID: 24338663
  17. Andersen SL. Trajectories of brain development: point of vulnerability or window of opportunity? *Neurosci Biobehav Rev*. 2003; 27(1–2):3–18. [https://doi.org/10.1016/s0149-7634\(03\)00005-8](https://doi.org/10.1016/s0149-7634(03)00005-8) PMID: 12732219
  18. Andersen SL. Exposure to early adversity: Points of cross-species translation that can lead to improved understanding of depression. *Dev Psychopathol*. 2015; 27(2):477–91. <https://doi.org/10.1017/S0954579415000103> PMID: 25997766
  19. Inta D, Vogt MA, Elkin H, Weber T, Lima-Ojeda JM, Schneider M, et al. Phenotype of mice with inducible ablation of GluA1 AMPA receptors during late adolescence: relevance for mental disorders. *Hippocampus*. 2014; 24(4):424–35. <https://doi.org/10.1002/hipo.22236> PMID: 24339333
  20. Giovanoli S, Engler H, Engler A, Richetto J, Voget M, Willi R, et al. Stress in puberty unmasks latent neuropathological consequences of prenatal immune activation in mice. *Science*. 2013; 339(6123):1095–9. <https://doi.org/10.1126/science.1228261> PMID: 23449593
  21. Calabrese F, Brivio P, Gruca P, Lason-Tyburkiewicz M, Papp M, Riva MA. Chronic Mild Stress-Induced Alterations of Local Protein Synthesis: A Role for Cognitive Impairment. *ACS Chem Neurosci*. 2017; 8(4):817–25. <https://doi.org/10.1021/acscchemneuro.6b00392> PMID: 28094500
  22. Luoni A, Macchi F, Papp M, Molteni R, Riva MA. Lurasidone exerts antidepressant properties in the chronic mild stress model through the regulation of synaptic and neuroplastic mechanisms in the rat prefrontal cortex. *Int J Neuropsychopharmacol*. 2014; 18(4).
  23. Lukkes JL, Watt MJ, Lowry CA, Forster GL. Consequences of post-weaning social isolation on anxiety behavior and related neural circuits in rodents. *Frontiers in behavioral neuroscience*. 2009; 3:18. <https://doi.org/10.3389/neuro.08.018.2009> PMID: 19738931
  24. Potegal M, Einon D. Aggressive behaviors in adult rats deprived of playfighting experience as juveniles. *Developmental psychobiology*. 1989; 22(2):159–72. <https://doi.org/10.1002/dev.420220206> PMID: 2925003
  25. Tulogdi A, Toth M, Barsvari B, Biro L, Mikics E, Haller J. Effects of resocialization on post-weaning social isolation-induced abnormal aggression and social deficits in rats. *Developmental psychobiology*. 2014; 56(1):49–57. <https://doi.org/10.1002/dev.21090> PMID: 23168609
  26. Heidbreder CA, Weiss IC, Domeney AM, Pryce C, Homberg J, Hedou G, et al. Behavioral, neurochemical and endocrinological characterization of the early social isolation syndrome. *Neuroscience*. 2000; 100(4):749–68. [https://doi.org/10.1016/s0306-4522\(00\)00336-5](https://doi.org/10.1016/s0306-4522(00)00336-5) PMID: 11036209
  27. Wongwitdecha N, Marsden CA. Social isolation increases aggressive behaviour and alters the effects of diazepam in the rat social interaction test. *Behav Brain Res*. 1996; 75(1–2):27–32. [https://doi.org/10.1016/0166-4328\(96\)00181-7](https://doi.org/10.1016/0166-4328(96)00181-7) PMID: 8800657
  28. Holson RR, Scallet AC, Ali SF, Turner BB. "Isolation stress" revisited: isolation-rearing effects depend on animal care methods. *Physiol Behav*. 1991; 49(6):1107–18. [https://doi.org/10.1016/0031-9384\(91\)90338-o](https://doi.org/10.1016/0031-9384(91)90338-o) PMID: 1896492
  29. Deacon RM, Raley JM, Perry VH, Rawlins JN. Burrowing into prion disease. *Neuroreport*. 2001; 12(9):2053–7. <https://doi.org/10.1097/00001756-200107030-00052> PMID: 11435945
  30. Jirkof P. Burrowing and nest building behavior as indicators of well-being in mice. *J Neurosci Methods*. 2014; 234:139–46. <https://doi.org/10.1016/j.jneumeth.2014.02.001> PMID: 24525328
  31. Teeling JL, Felton LM, Deacon RM, Cunningham C, Rawlins JN, Perry VH. Sub-pyrogenic systemic inflammation impacts on brain and behavior, independent of cytokines. *Brain Behav Immun*. 2007; 21(6):836–50. <https://doi.org/10.1016/j.bbi.2007.01.012> PMID: 17367989
  32. Deacon RM. Burrowing in rodents: a sensitive method for detecting behavioral dysfunction. *Nat Protoc*. 2006; 1(1):118–21. <https://doi.org/10.1038/nprot.2006.19> PMID: 17406222
  33. Deacon RM. Burrowing: a sensitive behavioural assay, tested in five species of laboratory rodents. *Behav Brain Res*. 2009; 200(1):128–33. <https://doi.org/10.1016/j.bbr.2009.01.007> PMID: 19373978
  34. Strekalova T, Steinbusch HW. Measuring behavior in mice with chronic stress depression paradigm. *Progress in neuro-psychopharmacology & biological psychiatry*. 2010; 34(2):348–61.

35. Andrews N, Legg E, Lisak D, Issop Y, Richardson D, Harper S, et al. Spontaneous burrowing behaviour in the rat is reduced by peripheral nerve injury or inflammation associated pain. *Eur J Pain*. 2012; 16(4):485–95. <https://doi.org/10.1016/j.ejpain.2011.07.012> PMID: 22396078
36. Koska I, van Dijk RM, Seiffert I, Di Liberto V, Moller C, Palme R, et al. Toward evidence-based severity assessment in rat models with repeated seizures: II. Chemical post-status epilepticus model. *Epilepsia*. 2019; 60(10):2114–27. <https://doi.org/10.1111/epi.16330> PMID: 31471910
37. King MV, Seeman P, Marsden CA, Fone KC. Increased dopamine D2High receptors in rats reared in social isolation. *Synapse*. 2009; 63(6):476–83. <https://doi.org/10.1002/syn.20624> PMID: 19217027
38. Harte MK, Powell SB, Swerdlow NR, Geyer MA, Reynolds GP. Deficits in parvalbumin and calbindin immunoreactive cells in the hippocampus of isolation reared rats. *J Neural Transm (Vienna)*. 2007; 114(7):893–8. <https://doi.org/10.1007/s00702-007-0627-6> PMID: 17594127
39. Silva-Gomez AB, Rojas D, Juarez I, Flores G. Decreased dendritic spine density on prefrontal cortical and hippocampal pyramidal neurons in postweaning social isolation rats. *Brain Res*. 2003; 983(1–2):128–36. [https://doi.org/10.1016/s0006-8993\(03\)03042-7](https://doi.org/10.1016/s0006-8993(03)03042-7) PMID: 12914973
40. Lu L, Bao G, Chen H, Xia P, Fan X, Zhang J, et al. Modification of hippocampal neurogenesis and neuroplasticity by social environments. *Exp Neurol*. 2003; 183(2):600–9. [https://doi.org/10.1016/s0014-4886\(03\)00248-6](https://doi.org/10.1016/s0014-4886(03)00248-6) PMID: 14552901
41. Roberts L, Greene JR. Post-weaning social isolation of rats leads to a diminution of LTP in the CA1 to subiculum pathway. *Brain Res*. 2003; 991(1–2):271–3. <https://doi.org/10.1016/j.brainres.2003.08.022> PMID: 14575904
42. Preece MA, Dalley JW, Theobald DE, Robbins TW, Reynolds GP. Region specific changes in forebrain 5-hydroxytryptamine1A and 5-hydroxytryptamine2A receptors in isolation-reared rats: an in vitro autoradiography study. *Neuroscience*. 2004; 123(3):725–32. <https://doi.org/10.1016/j.neuroscience.2003.10.008> PMID: 14706784
43. Filipovic D, Stanisavljevic A, Jasnica N, Bernardi RE, Inta D, Peric I, et al. Chronic Treatment with Fluoxetine or Clozapine of Socially Isolated Rats Prevents Subsector-Specific Reduction of Parvalbumin Immunoreactive Cells in the Hippocampus. *Neuroscience*. 2018; 371:384–94. <https://doi.org/10.1016/j.neuroscience.2017.12.020> PMID: 29275206
44. Mora F, Segovia G, Del Arco A, de Blas M, Garrido P. Stress, neurotransmitters, corticosterone and body-brain integration. *Brain Res*. 2012; 1476:71–85. <https://doi.org/10.1016/j.brainres.2011.12.049> PMID: 22285436
45. Lewis DA, Hashimoto T, Volk DW. Cortical inhibitory neurons and schizophrenia. *Nat Rev Neurosci*. 2005; 6(4):312–24. <https://doi.org/10.1038/nrn1648> PMID: 15803162
46. Shinohara R, Taniguchi M, Ehrlich AT, Yokogawa K, Deguchi Y, Cherasse Y, et al. Dopamine D1 receptor subtype mediates acute stress-induced dendritic growth in excitatory neurons of the medial prefrontal cortex and contributes to suppression of stress susceptibility in mice. *Mol Psychiatry*. 2018; 23(8):1717–30. <https://doi.org/10.1038/mp.2017.177> PMID: 28924188
47. Murinova J, Hlavacova N, Chmelova M, Riecanaky I. The Evidence for Altered BDNF Expression in the Brain of Rats Reared or Housed in Social Isolation: A Systematic Review. *Frontiers in behavioral neuroscience*. 2017; 11:101. <https://doi.org/10.3389/fnbeh.2017.00101> PMID: 28620285
48. Filipovic D, Todorovic N, Bernardi RE, Gass P. Oxidative and nitrosative stress pathways in the brain of socially isolated adult male rats demonstrating depressive- and anxiety-like symptoms. *Brain structure & function*. 2017; 222(1):1–20.
49. Di Liberto V, van Dijk RM, Brendel M, Waldron AM, Moller C, Koska I, et al. Imaging correlates of behavioral impairments: An experimental PET study in the rat pilocarpine epilepsy model. *Neurobiol Dis*. 2018; 118:9–21. <https://doi.org/10.1016/j.nbd.2018.06.010> PMID: 29933054
50. Zueger M, Urani A, Chourbaji S, Zacher C, Roche M, Harkin A, et al. Olfactory bulbectomy in mice induces alterations in exploratory behavior. *Neurosci Lett*. 2005; 374(2):142–6. <https://doi.org/10.1016/j.neulet.2004.10.040> PMID: 15644281
51. Richter SH, Garner JP, Zipser B, Lewejohann L, Sachser N, Touma C, et al. Effect of population heterogenization on the reproducibility of mouse behavior: a multi-laboratory study. *PLoS One*. 2011; 6(1):e16461. <https://doi.org/10.1371/journal.pone.0016461> PMID: 21305027
52. Bates DM, Martin; Bolker, Ben; Walker, Steve. Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*. 2015; 67(1):1–48.
53. Pinheiro J BD, DebRoy S, Sarkar D, R Core Team \_nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1–147 ed2020.
54. R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2019.

55. Guilloux JP, Seney M, Edgar N, Sibille E. Integrated behavioral z-scoring increases the sensitivity and reliability of behavioral phenotyping in mice: relevance to emotionality and sex. *J Neurosci Methods*. 2011; 197(1):21–31. <https://doi.org/10.1016/j.jneumeth.2011.01.019> PMID: 21277897
56. Gentsch C, Lichtsteiner M, Frischknecht HR, Feer H, Siegfried B. Isolation-induced locomotor hyperactivity and hypoalgesia in rats are prevented by handling and reversed by resocialization. *Physiol Behav*. 1988; 43(1):13–6. [https://doi.org/10.1016/0031-9384\(88\)90091-1](https://doi.org/10.1016/0031-9384(88)90091-1) PMID: 3413245
57. Geyer MA, Wilkinson LS, Humby T, Robbins TW. Isolation rearing of rats produces a deficit in prepulse inhibition of acoustic startle similar to that in schizophrenia. *Biol Psychiatry*. 1993; 34(6):361–72. [https://doi.org/10.1016/0006-3223\(93\)90180-I](https://doi.org/10.1016/0006-3223(93)90180-I) PMID: 8218603
58. Weiss IC, Di Iorio L, Feldon J, Domeney AM. Strain differences in the isolation-induced effects on prepulse inhibition of the acoustic startle response and on locomotor activity. *Behav Neurosci*. 2000; 114(2):364–73. PMID: 10832797
59. van den Berg CL, Hol T, Van Ree JM, Spruijt BM, Everts H, Koolhaas JM. Play is indispensable for an adequate development of coping with social challenges in the rat. *Developmental psychobiology*. 1999; 34(2):129–38. PMID: 10086231
60. Lukkes J, Vuong S, Scholl J, Oliver H, Forster G. Corticotropin-releasing factor receptor antagonism within the dorsal raphe nucleus reduces social anxiety-like behavior after early-life social isolation. *J Neurosci*. 2009; 29(32):9955–60. <https://doi.org/10.1523/JNEUROSCI.0854-09.2009> PMID: 19675229
61. Lukkes JL, Mokin MV, Scholl JL, Forster GL. Adult rats exposed to early-life social isolation exhibit increased anxiety and conditioned fear behavior, and altered hormonal stress responses. *Horm Behav*. 2009; 55(1):248–56. <https://doi.org/10.1016/j.yhbeh.2008.10.014> PMID: 19027017
62. Mikics E, Guirado R, Umemori J, Toth M, Biro L, Miskolczi C, et al. Social Learning Requires Plasticity Enhanced by Fluoxetine Through Prefrontal Bdnf-TrkB Signaling to Limit Aggression Induced by Post-Weaning Social Isolation. *Neuropsychopharmacology*. 2018; 43(2):235–45. <https://doi.org/10.1038/npp.2017.142> PMID: 28685757
63. Seffer D, Rippberger H, Schwarting RK, Wöhr M. Pro-social 50-kHz ultrasonic communication in rats: post-weaning but not post-adolescent social isolation leads to social impairments-phenotypic rescue by resocialization. *Frontiers in behavioral neuroscience*. 2015; 9:102. <https://doi.org/10.3389/fnbeh.2015.00102> PMID: 25983681
64. Wright IK, Upton N, Marsden CA. Resocialisation of isolation-reared rats does not alter their anxiogenic profile on the elevated X-maze model of anxiety. *Physiol Behav*. 1991; 50(6):1129–32. [https://doi.org/10.1016/0031-9384\(91\)90572-6](https://doi.org/10.1016/0031-9384(91)90572-6) PMID: 1798767
65. Vale AL, Montgomery AM. Social interaction: responses to chlordiazepoxide and the loss of isolation-reared effects with paired-housing. *Psychopharmacology (Berl)*. 1997; 133(2):127–32. <https://doi.org/10.1007/s002130050382> PMID: 9342778
66. Kokare DM, Dandekar MP, Singru PS, Gupta GL, Subhedar NK. Involvement of alpha-MSH in the social isolation induced anxiety- and depression-like behaviors in rat. *Neuropharmacology*. 2010; 58(7):1009–18. <https://doi.org/10.1016/j.neuropharm.2010.01.006> PMID: 20080115
67. Meng Q, Li N, Han X, Shao F, Wang W. Peri-adolescence isolation rearing alters social behavior and nociception in rats. *Neurosci Lett*. 2010; 480(1):25–9. <https://doi.org/10.1016/j.neulet.2010.05.067> PMID: 20638959
68. Kowianski P, Lietzau G, Czuba E, Waskow M, Steliga A, Morys J. BDNF: A Key Factor with Multipotent Impact on Brain Signaling and Synaptic Plasticity. *Cell Mol Neurobiol*. 2018; 38(3):579–93. <https://doi.org/10.1007/s10571-017-0510-4> PMID: 28623429
69. Leal G, Comprido D, Duarte CB. BDNF-induced local protein synthesis and synaptic plasticity. *Neuropharmacology*. 2014; 76 Pt C:639–56.
70. Begni V, Riva MA, Cattaneo A. Cellular and molecular mechanisms of the brain-derived neurotrophic factor in physiological and pathological conditions. *Clin Sci (Lond)*. 2017; 131(2):123–38.
71. Bennett MR, Lagopoulos J. Stress and trauma: BDNF control of dendritic-spine formation and regression. *Prog Neurobiol*. 2014; 112:80–99. <https://doi.org/10.1016/j.pneurobio.2013.10.005> PMID: 24211850
72. Cattaneo A, Cattaneo N, Begni V, Pariante CM, Riva MA. The human BDNF gene: peripheral gene expression and protein levels as biomarkers for psychiatric disorders. *Translational psychiatry*. 2016; 6(11):e958. <https://doi.org/10.1038/tp.2016.214> PMID: 27874848
73. An JJ, Gharami K, Liao GY, Woo NH, Lau AG, Vanevski F, et al. Distinct role of long 3' UTR BDNF mRNA in spine morphology and synaptic plasticity in hippocampal neurons. *Cell*. 2008; 134(1):175–87. <https://doi.org/10.1016/j.cell.2008.05.045> PMID: 18614020

74. Baj G, Leone E, Chao MV, Tongiorgi E. Spatial segregation of BDNF transcripts enables BDNF to differentially shape distinct dendritic compartments. *Proc Natl Acad Sci U S A*. 2011; 108(40):16813–8. <https://doi.org/10.1073/pnas.1014168108> PMID: 21933955
75. Rousseaud A, Delepine C, Nectoux J, Billuart P, Bienvenu T. Differential Expression and Regulation of Brain-Derived Neurotrophic Factor (BDNF) mRNA Isoforms in Brain Cells from Mecp2(308/y) Mouse Model. *J Mol Neurosci*. 2015; 56(4):758–67. <https://doi.org/10.1007/s12031-014-0487-0> PMID: 25634725
76. Perovic M, Tesic V, Mladenovic Djordjevic A, Smiljanic K, Loncarevic-Vasiljkovic N, Ruzdijic S, et al. BDNF transcripts, proBDNF and proNGF, in the cortex and hippocampus throughout the life span of the rat. *Age (Dordr)*. 2013; 35(6):2057–70.
77. Wall VL, Fischer EK, Bland ST. Isolation rearing attenuates social interaction-induced expression of immediate early gene protein products in the medial prefrontal cortex of male and female rats. *Physiol Behav*. 2012; 107(3):440–50. <https://doi.org/10.1016/j.physbeh.2012.09.002> PMID: 22982514
78. Li M, Du W, Shao F, Wang W. Cognitive dysfunction and epigenetic alterations of the BDNF gene are induced by social isolation during early adolescence. *Behav Brain Res*. 2016; 313:177–83. <https://doi.org/10.1016/j.bbr.2016.07.025> PMID: 27435421
79. Adzic M, Djordjevic J, Djordjevic A, Niciforovic A, Demonacos C, Radojic M, et al. Acute or chronic stress induce cell compartment-specific phosphorylation of glucocorticoid receptor and alter its transcriptional activity in Wistar rat brain. *The Journal of endocrinology*. 2009; 202(1):87–97. <https://doi.org/10.1677/JOE-08-0509> PMID: 19406955
80. Reinhart V, Bove SE, Volfson D, Lewis DA, Kleiman RJ, Lanz TA. Evaluation of TrkB and BDNF transcripts in prefrontal cortex, hippocampus, and striatum from subjects with schizophrenia, bipolar disorder, and major depressive disorder. *Neurobiol Dis*. 2015; 77:220–7. <https://doi.org/10.1016/j.nbd.2015.03.011> PMID: 25796564
81. Steward O, Farris S, Pirbhoy PS, Darnell J, Driesche SJ. Localization and local translation of Arc/Arg3.1 mRNA at synapses: some observations and paradoxes. *Frontiers in molecular neuroscience*. 2014; 7:101. <https://doi.org/10.3389/fnmol.2014.00101> PMID: 25628532
82. Nikolaienko O, Patil S, Eriksen MS, Bramham CR. Arc protein: a flexible hub for synaptic plasticity and cognition. *Seminars in cell & developmental biology*. 2018; 77:33–42.
83. Pisu MG, Dore R, Mostallino MC, Loi M, Pibiri F, Mameli R, et al. Down-regulation of hippocampal BDNF and Arc associated with improvement in aversive spatial memory performance in socially isolated rats. *Behav Brain Res*. 2011; 222(1):73–80. <https://doi.org/10.1016/j.bbr.2011.03.021> PMID: 21420441
84. Ieraci A, Mallei A, Popoli M. Social Isolation Stress Induces Anxious-Depressive-Like Behavior and Alterations of Neuroplasticity-Related Genes in Adult Male Mice. *Neural Plast*. 2016; 2016:6212983. <https://doi.org/10.1155/2016/6212983> PMID: 26881124
85. Kim E, Sheng M. PDZ domain proteins of synapses. *Nat Rev Neurosci*. 2004; 5(10):771–81. <https://doi.org/10.1038/nrn1517> PMID: 15378037
86. Zeng M, Shang Y, Araki Y, Guo T, Haganir RL, Zhang M. Phase Transition in Postsynaptic Densities Underlies Formation of Synaptic Complexes and Synaptic Plasticity. *Cell*. 2016; 166(5):1163–75 e12. <https://doi.org/10.1016/j.cell.2016.07.008> PMID: 27565345
87. Ohnuma T, Kato H, Arai H, Faull RL, McKenna PJ, Emson PC. Gene expression of PSD95 in prefrontal cortex and hippocampus in schizophrenia. *Neuroreport*. 2000; 11(14):3133–7. <https://doi.org/10.1097/00001756-200009280-00019> PMID: 11043537
88. Coley AA, Gao WJ. PSD95: A synaptic protein implicated in schizophrenia or autism? *Progress in neuro-psychopharmacology & biological psychiatry*. 2018; 82:187–94.
89. Hermes G, Li N, Duman C, Duman R. Post-weaning chronic social isolation produces profound behavioral dysregulation with decreases in prefrontal cortex synaptic-associated protein expression in female rats. *Physiol Behav*. 2011; 104(2):354–9. <https://doi.org/10.1016/j.physbeh.2010.12.019> PMID: 21185848
90. Fogaca MV, Duman RS. Cortical GABAergic Dysfunction in Stress and Depression: New Insights for Therapeutic Interventions. *Frontiers in cellular neuroscience*. 2019; 13:87. <https://doi.org/10.3389/fncel.2019.00087> PMID: 30914923
91. Volk DW, Austin MC, Pierri JN, Sampson AR, Lewis DA. Decreased glutamic acid decarboxylase67 messenger RNA expression in a subset of prefrontal cortical gamma-aminobutyric acid neurons in subjects with schizophrenia. *Archives of general psychiatry*. 2000; 57(3):237–45. <https://doi.org/10.1001/archpsyc.57.3.237> PMID: 10711910
92. Filipovic D, Zlatkovic J, Gass P, Inta D. The differential effects of acute vs. chronic stress and their combination on hippocampal parvalbumin and inducible heat shock protein 70 expression. *Neuroscience*. 2013; 236:47–54. <https://doi.org/10.1016/j.neuroscience.2013.01.033> PMID: 23352936



93. Bagalkot TR, Jin HM, Prabhu VV, Muna SS, Cui Y, Yadav BK, et al. Chronic social defeat stress increases dopamine D2 receptor dimerization in the prefrontal cortex of adult mice. *Neuroscience*. 2015 Dec 17; 311:444–52. <https://doi.org/10.1016/j.neuroscience.2015.10.024> Epub 2015 Oct 17. PMID: [26484605](https://pubmed.ncbi.nlm.nih.gov/26484605/).
94. Tomas-Roig J, Havemann-Reinecke U. Gene expression signature in brain regions exposed to long-term psychosocial stress following acute challenge with cannabinoid drugs. *Psychoneuroendocrinology*. 2019 Apr; 102:1–8. <https://doi.org/10.1016/j.psyneuen.2018.11.023> Epub 2018 Nov 20. PMID: [30476795](https://pubmed.ncbi.nlm.nih.gov/30476795/).
95. Abdelrahman A, Kumstel S, Zhang X, Liebig M, Wendt EHU, Eichberg J, et al. A novel multi-parametric analysis of non-invasive methods to assess animal distress during chronic pancreatitis. *Sci Rep*. 2019; 9(1):14084. <https://doi.org/10.1038/s41598-019-50682-3> PMID: [31575986](https://pubmed.ncbi.nlm.nih.gov/31575986/)
96. Kumstel S, Wendt EHU, Eichberg J, Talbot SR, Hager C, Zhang X, et al. Grading animal distress and side effects of therapies. *Ann N Y Acad Sci*. 2020.
97. van Dijk RM, Koska I, Bleich A, Tolba R, Seiffert I, Moller C, et al. Design of composite measure schemes for comparative severity assessment in animal-based neuroscience research: A case study focussed on rat epilepsy models. *PLoS One*. 2020; 15(5):e0230141. <https://doi.org/10.1371/journal.pone.0230141> PMID: [32413036](https://pubmed.ncbi.nlm.nih.gov/32413036/)