This is the postprint (accepted) version of the final paper, published afterwards, here:
https://doi.org/10.1016/j.euroneuro.2019.12.119
We thank Elsevier for the opportunity.

# Antidepressant-like effects of pharmacological inhibition of FAAH activity in socially isolated female rats

**Luca Carnevali[a], Rosario Statello[a], Federica Vacondio[b], Francesca Ferlenghi[b], Gilberto Spadoni[c], Silvia Rivara[b], Marco Mor[b], Andrea Sgoifo[a],***

a *Stress Physiology Lab, Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parco Area delle Scienze 11/A, 43124 Parma, Italy*

b *Department of Food and Drug, University of Parma, Parma, Italy*

c *Department of Biomolecular Sciences, University of Urbino "Carlo Bo", Urbino, Italy*

∗ Corresponding author. *E-mail addresses:* luca.carnevali@unipr.it (L. Carnevali), andrea.sgoifo@unipr.it (A. Sgoifo).

**KEYWORDS** BDNF; Depression; Endocannabinoid; Females; Stress

**Abstract**

Pharmacological inhibition of the enzyme fatty acid amide hydrolase (FAAH), which terminates signaling of the endocannabinoid N-arachidonoylethanolamine (or anandamide, AEA), exerts favourable effects in rodent models of stress-related depression. Yet although depression seems to be more common among women than men and in spite of some evidence of sex differences in treatment efficacy, preclinical development of FAAH inhibitors for the pharmacotherapy of stress-related depression has been predominantly conducted in male animals. Here, adult female rats were exposed to six weeks of social isolation and, starting from the second week, treated with the FAAH inhibitor URB694 (0.3 mg/kg/day, i.p.) or vehicle. Compared to pair- housed females, socially isolated female rats treated with vehicle developed behavioral (mild anhedonia, passive stress coping) and physiological (reduced body weight gain, elevated plasma corticosterone levels) alterations. Moreover, prolonged social isolation provoked a reduction in brain-derived neurotrophic factor (BDNF) and AEA levels within the hippocampus. Together, these changes are indicative of an increased risk of developing a depressive-like state. Conversely, pharmacological inhibition of FAAH activity with URB694 restored both AEA and BDNF levels within the hippocampus of socially isolated rats and prevented the development of behavioral and physiological alterations. These results suggest a potential interplay between AEA- mediated signaling and hippocampal BDNF in the pathogenesis of depression-relevant behaviors and physiological alterations and antidepressant action of FAAH inhibition in socially isolated female rats.

## 1. Introduction

Prolonged or repeated exposure to stressors of psychosocial nature can act as a precipitating factor for the on- set of depression (Cohen et al., 2007; Dinan, 2005). One of the most susceptible brain regions to the effects of psychosocial stress is the hippocampus, a component of the limbic system that regulates emotional and cognitive processes related to psychiatric disorders (Belleau et al., 2019; Sheline et al., 2019). The hippocampus is also a major regulator of the hypothalamic-pituitary-adrenal (HPA) axis (Jacobson and Sapolsky, 1991), the neuroendocrine system responsible for the release of glucocorticoid stress hormones (i.e., cortisol in humans, corticosterone in rodents). In patients with depression, hippocampal volume is decreased (Sapolsky, 2000; Sheline, 1996) and the HPA axis is dysregulated (Stetler and Miller, 2011). Depletion of hippocampal neurogenesis has been implicated as one of the substrates that may explain the hippocampal volume loss seen in depression (Duman and Monteggia, 2006; Levone et al., 2015). Specifically, the neurotrophic hypothesis of depression proposes that stress-induced reductions in the expression of brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family regulating synaptic plasticity (Leal et al., 2017; Lu et al., 2014), occur in key limbic structures, including the hippocampus, to con- tribute to the pathogenesis of depression (Castren et al., 2007; Duman and Monteggia, 2006). Moreover, several lines of clinical and preclinical evidence indicate that conventional antidepressants (e.g., tricyclics, selective serotonin reuptake inhibitors and norepinephrine reuptake inhibitors) may in part exert their effects through BDNF upregulation (Hayley and Anisman, 2013; Pittenger and Duman, 2008; Tardito et al., 2006).

The past two decades have witnessed a driven focus on the identification of novel therapeutic targets for depression, in an attempt to overcome the notable limitations of conventional antidepressant treatments, poor efficacy being perhaps the most critical (Connolly and Thase, 2012). For example, substantial evidence

has ac- cumulated implicating a deficit in endocannabinoid (eCB) neurotransmission in the etiology of depression (for a comprehensive review see Gorzalka and Hill, 2011). At the preclinical level, a deficiency in the signaling mediated by the eCB N-arachidonoylethanolamine (or anandamide, AEA) has been noted in the hippocampus, hypothalamus, ventral striatum, and prefrontal cortex of rats exposed to several stressors (i.e., chronic unpredictable stress and social defeat stress) and presenting a "depressive-like" phenotype (reviewed in Carnevali et al., 2017b). These findings have triggered significant interest in the development of eCB-interacting drugs, including direct-acting receptor ligands and catabolism inhibitors for the pharmacotherapy of de- pression (Micale et al., 2013). Specifically, within preclinical models, facilitation of AEA signaling through pharmaco- logical inhibition of its degrading enzyme (i.e., fatty acid amide hydrolase (FAAH)) can enhance monoaminergic transmission, increase cellular plasticity and neurotrophin expression within the hippocampus, dampen HPA axis activity, and evoke antidepressant-like behavioral effects (reviewed in Carnevali et al., 2017b). However, while the literature has been unequivocal in showing that women experience depression at twice the rate of men (e.g., Grigoriadis and Robinson, 2007), very few preclinical studies have been conducted on female experimental animals (Beery, 2018; Kokras and Dalla, 2014). Moreover, despite the existence of sex differences in response to antidepressant treatment (Sloan and Kornstein, 2003), preclinical research on the antidepressant action of FAAH inhibitors has been predominantly conducted in male rodents (Carnevali et al., 2017b; Fowler, 2015). Therefore, there is a clear need to use female animals in preclinical models of stress to either confirm and generalize to females the previously obtained male animal-based findings or underscore potential sex differences in the etiology of depression and/or in the efficacy of new treatments.

Based on this background, the purpose of the cur- rent study was two-fold. First, we aimed at documenting the development of behavioral (passive stress coping, anhedonia) and biological (reduced hippocampal BDNF levels, HPA axis hyperactivity, body weight loss) alterations in adult female rats exposed to prolonged social isolation, a mild chronic social stressor that has been widely used to model symptoms that are often associated with an increased risk of developing a depressive- like state in rodents (Carnevali et al., 2017a). Second, we tested the hypothesis that pharmacological inhibition of FAAH activity would correct the alterations associated with prolonged social isolation. To this aim, we employed the FAAH inhibitor URB694 (6–hydroxy–[1,1'-biphenyl]−3-yl-cyclohexylcarbamate) which was shown to exhibit higher selectivity and more prolonged and profound access to the brain than the standard inhibitor URB597 (Clapper et al., 2009).

## 2. Experimental procedures

### 2.1. Animals and housing conditions

Four-month-old female wild-type Groningen rats were used in this study. This rat population, originally derived from the University of Groningen (the Netherlands) and currently bred in our laboratory under standard conditions, shows considerable individual differences in trait-like patterns of behavioral and physiological responses to environmental challenges (Carnevali et al., 2014; de Boer et al., 2017). After weaning, female animals were housed in same-sex sibling pairs and kept in rooms with controlled temperature (22 ± 2 °C) and humidity (50 ± 10%), under a reversed light-dark cycle (light on from 19:00 to 7:00 h), with food and water ad libitum except when required for the sucrose preference test (see below). A total of 40 pairs were included in the study, but only one female rat from each pair was submitted to the experimental procedures described below. Experiments were performed in accordance with the European Community Council Directive 2010/63/UE and approved by the Italian legislation on animal experimentation (D.L. 04/04/2014, n. 26, authorization n. 449/2017-PR). All efforts were made to reduce sample size and minimize animal suffering.

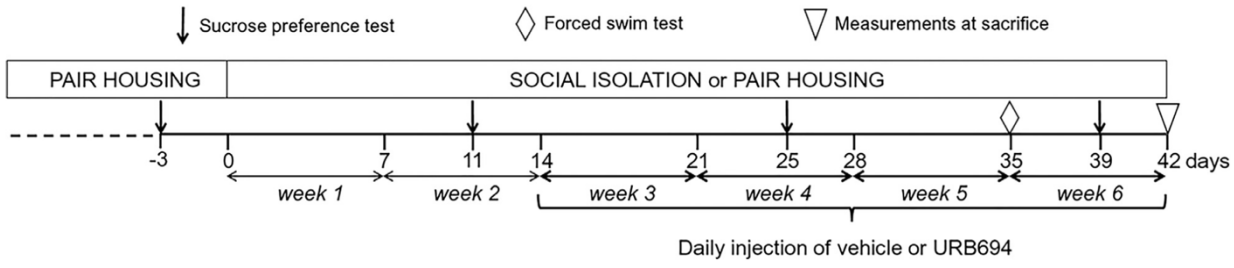

**Fig. 1** Timeline of experimental procedures.

**2.2. Experimental design**

The experimental timeline is depicted in Fig. 1. Specific procedures and data analysis are described in the following sections. On day 0, animals were randomly divided in socially isolated (SI) and paired- housed (PH) groups. Female rats from the SI group were separated from their respective sibling and individually housed in a sound- proof room for 6 weeks to avoid any sensory (visual, olfactory, and acoustic) contact with their conspecifics. On the contrary, female rats from the PH group were continually housed with their respective sibling and kept in the same room with other pairs. Handling and cage cleaning were matched between the two groups. Starting from the beginning of the third week of the social isolation/pair- housing condition, animals received daily i.p. injection of either the FAAH inhibitor URB694 or vehicle (VEH). Thus, four experimental subgroups emerged: (i) SI + VEH ($n$ = 10), (ii) SI + URB694 ($n$ = 10), (iii) PH + VEH ($n$ = 10), and (iv) PH + URB694 ($n$ = 10). Experiments were conducted on separate cohorts of 8 experimental animals each ($n$ = 4 SI and $n$ = 4 PH rats), starting with the VEH-treated animals. Experimental animals were tested four times in the sucrose preference test and once in the forced swim test during the dark phase of the daily cycle between 10.00 and 12.00 h. At sacrifice (day 42), trunk blood, adrenal glands, and hippocampus were harvested. Body weight was measured weekly throughout the study. Moreover, the estrous cycle phase of female rats was determined immediately after each behavioral test and before sacrifice using vaginal smear cytology. Vaginal smears were collected by gently introducing a moistened (0.9% NaCl) cotton swab in the rat's vagina. The sample was transferred to a glass slide and examined microscopically following Giemsa staining. The phase of the cycle (metaestrous, diestrous, pro-estrous or estrous) was deter- mined based upon the presence of leukocytes, nucleated epithelial or cornfield epithelial cells (Marcondes et al., 2002).

**2.3. Drug treatment**

URB694 is a carbamate FAAH inhibitor that irreversibly carbamoylates the nucleophile catalytic serine in FAAH active site (Tarzia et al., 2006). URB694 is a second generation inhibitor with improved metabolic stability and selectivity for FAAH (Clapper et al., 2009). URB694 was freshly dissolved in VEH containing 5% PEG, 5% Tween 80, and 90% saline. VEH (vol:1 ml/kg) or URB694 (0.3 mg/kg, i.p.) were injected i.p. between 11.00 and 13.00 h and, on the days of the sucrose solution and forced swim tests, at least 1 h after the completion of the test. URB694 dose was chosen based on our previous studies (Carnevali et al., 2015a, 2015b), and a pilot study showing that FAAH activity in the brain of female wild-type Groningen rats was substantially inhibited 24 h after administration of this drug dose (Supplemental Fig. S1).

**2.4. Sucrose preference test**

*Ad libitum* 2% sucrose solution was available for 5 days before the beginning of the experimental procedures to allow adaptation to its taste. Food and water were removed from the cage for 16 h before each sucrose preference test; moreover, one hour before the test, all experimental animals (paired and isolated) were moved into individual cages to ensure accurate fluid intake measurements of paired animals. Water and 2% sucrose solution were placed in premeasured bottles in the individual cage, and fluid intake was monitored for 1 h. Animals were returned to their respective home cages immediately after the test (Grippo et al., 2007). Sucrose preference tests were conducted in baseline conditions (day−3) and after 11, 25, and 39 days of social isolation (Fig. 1). Sucrose solution intake was expressed as the relative percentage of the total liquid intake and was taken as an operational index of anhedonia, defined as reduced sucrose preference relative to control animals and baseline values (Grippo et al., 2007).

**2.5. Forced swim test**

An adapted version of the forced swim test originally described by Porsolt (Porsolt et al., 1977) was used. On day 35 (Fig. 1), female rats were forced to swim individually for 5 min in a Plexiglas cylinder (height: 40 cm, diameter: 30 cm) filled with water (temperature: 24 ± 2 °C; depth: 30 cm). During the test, rats' behavior was video-taped. The overall time spent in immobility (floating and making only those movements necessary to keep the head above water) was scored by a trained experimenter blind to animals' condition and treatment. Immobility during the single session of the forced swim test was used as an index of passive stress coping (Commons et al., 2017).

### 2.6. Measurements at sacrifice

Twenty-four hours after the last administration of URB694 or VEH (i.e., at 11.00 h; day 42, Fig. 1), female rats were euthanized by decapitation under isoflurane anesthesia (2% in 100% oxygen). Trunk blood was collected in EDTA-coated tubes (Sarsted AG, Numbrecht, Germany) and plasma was separated by centrifugation (2600 g, 4 °C, 10 min). Brains were immediately removed and the hippocampus rapidly dissected and snap-frozen in nitrogen. All samples were stored at −80 °C until further analysis, as described below. Adrenal glands were also removed and weighed.

#### 2.6.1. Plasma corticosterone levels

Plasma was deproteinized by addition of two volumes of organic sol- vent (ice-cold acetonitrile), containing the internal standard dexamethasone (structural analog of corticosterone, 75 nmol/L). After centrifugation (14,000 g, 4 °C, 10 min), the supernatant was directly injected in the liquid chromatography/tandem mass spectrometry system (HPLC/MS/MS) for quantification of corticosterone levels, in accordance with previously published analytical methods (Plenis et al., 2011). A detailed description of the HPLC/MS/MS analytical method and related MS instrumentation is reported in the Supplemental Material.

#### 2.6.2. BDNF hippocampal content

BDNF content in the hippocampus was measured using a commercially available sandwich enzyme-linked immune sorbent assay (ELISA) kit (Quantikine ®ELISA-Total BDNF, R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. A de- tailed description of the experimental procedure is reported in the Supplemental Material. BDNF tissue content was expressed as a per- centage of the control group (PH+VEH rats).

#### 2.6.3. AEA hippocampal levels

AEA was extracted from 10% w/v hippocampal tissue homogenates employing two volumes of ice-cold acetonitrile containing the deuterated internal standard AEA-d4 and quantified by HPLC/MS/MS as previously reported (Carnevali et al., 2015a). The analytical standards AEA and AEA-d4 were purchased from Cayman Chemical (Ann Arbor, MI, USA) as stock solutions in ethanol. AEA levels were expressed as pmol/g wet weight of tissue. A detailed description of the HPLC/MS/MS analytical method and related MS instrumentation is reported in the Supplemental Material.

#### 2.6.4. FAAH activity in the hippocampus

For ex vivo determination of FAAH activity, frozen hippocampi were thawed and homogenized in ice-cold Tris buffer (10 vol, 50 mM, pH 7.5) containing 0.32 M sucrose. The homogenates were centrifuged (1000 g, 10 min, 4 °C) and total protein content was quantified in the supernatant by the bicinchoninic acid (BCA) protein kit (Pierce Biotechnology, Rockford, IL, USA). FAAH activity was measured at 37 °C for 30 min in 0.5 mL Tris buffer (50 mM, pH 7.5) containing fatty acid-free bovine serum albumin (BSA) (0.05%, w/v), 50 μg of protein from brain homogenates, 10 μM AEA and [3H]- AEA (10,000 disintegrations per minute) as previously described (Clapper et al., 2009). Briefly, the reactions were stopped with 1 mL chloroform:methanol (1:1). After centrifugation (2000 g, 10 min, 4 °C), [3H]-ethanolamine was measured in the aqueous phase by liquid scintillation counting. [3H]-AEA (specific activity: 60 Ci/mmol), employed as a substrate for ex vivo FAAH assay, was purchased from American Radiolabeled Chemicals (St. Louis, MI, USA).

### 2.7. Statistical analysis

All statistical analyses were performed using SPSS v. 25 (IBM soft- ware package). Data are presented as mean ± standard error of the mean (SEM). The influence of the estrous cycle phase on behavioral and biochemical measurements was controlled in all statistical analyses. A three-way ANOVA for repeated measures with "condition" (2 levels: isolation, pair-housing) and "treatment" (2 levels: VEH, URB694) as the between subject factors, and "time" as the within subject factor (3 levels: days 11, 25, and 39) was ap- plied on delta changes in sucrose solution preference with respect to baseline. All other data were analyzed with 2 (factor "condition": isolation or pair-housing) x 2 (factor "treatment": URB694 or VEH) factorial design ANOVAs. Follow-up analyses were conducted using Student's "t" tests, with a Bonferroni correction for multiple

comparisons. Pearson's *r* correlations were performed to assess the correlation between plasma corticosterone levels, BDNF hippocampal content and AEA hippocampal levels. Statistical significance was set at $p < .05$.

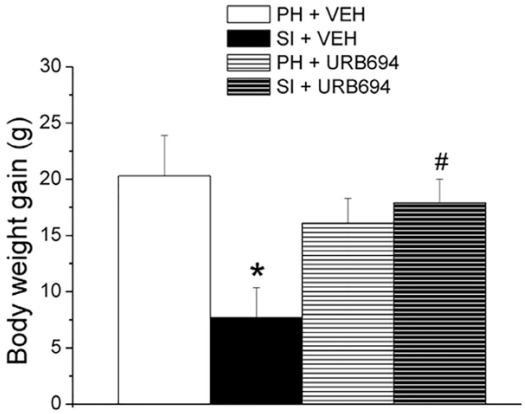


**Fig. 2** Body weight gain of paired-housed (PH) and socially isolated (SI) female rats treated with vehicle (VEH) or URB694, calculated as the difference between weight at the end (im- mediately before animals were euthanized) and at the start (when animals were assigned to the different housing conditions) of the experiment ($n = 10$ per group). Data are ex- pressed mean±SEM. ∗ = significantly different from corresponding PH + VEH group; # = significantly different from corresponding SI + VEH group (*p* values are reported in the text).

## 3.Results

### 3.1. Body weight

There were no significant differences in body weight among groups at the start of the experiment (i.e., when animals were assigned to the different housing conditions) (PH + VEH = 230 ± 2 g; IS + VEH = 237 ± 5 g; PH + URB694 = 231 ± 4 g; IS + URB694 = 226 ± 8 g). However, a significant time x condition interaction emerged on body weight gain calculated as the difference between weight at the end (i.e., immediately before animals were euthanized) and at the start of the experiment ($F = 7.1$, $p = .012$). As shown in Fig. 2, socially isolated female rats treated with VEH gained significantly less weight compared with their respective pair-housed counterparts ($p = .002$). This effect of social isolation was prevented by URB694 treatment (SI + URB694 vs SI + VEH, $p = .012$).

### 3.2. Sucrose preference test

Total fluid intake did not differ among groups at each assessment point (Supplemental Table S1). Also, there were no significant differences among groups in their base- line preference for the consumption of the sucrose solution (PH + VEH = 85 ± 2%; IS + VEH = 88 ± 2%; PH + URB694 = 83 ± 3%; IS + URB694 = 82 ± 3%). Of note, the estrous cycle phase had no effect on baseline sucrose solution preference ($F = 0.3$, $p = .543$). However, facto- rial ANOVA yielded a significant time x condition interaction ($F = 5.1$, $p = .028$) on preference changes during the social isolation period (calculated as the difference between each assessment point and the baseline), with no significant effects of the estrous cycle phase ($F = 0.4$, $p = .497$). Specifically, as shown in Fig. 3, no group differences were observed on day 11. However, on day 25, socially-isolated female rats treated with VEH showed a significantly larger reduction in the preference for sucrose solution consumption compared with their respective pair-housed counter- parts (p = .025). This effect was prevented by URB694 treatment (SI + URB694 vs. SI + VEH, p = .003). A similar trend was observed on day 39, although differences did not reach full statistical significance (SI + VEH vs PH + VEH, p = .056; SI + VEH vs. SI + URB694, p = .067).

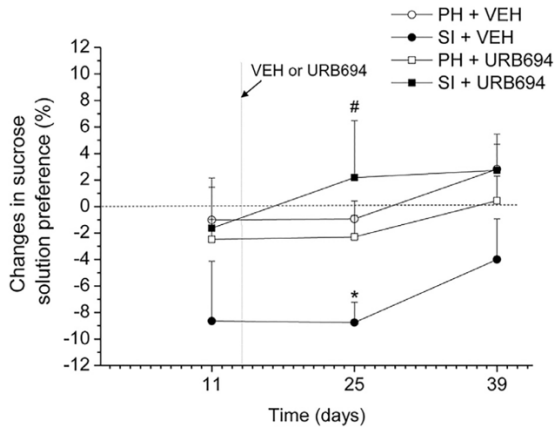


**Fig. 3** Changes in sucrose solution preference in paired- housed (PH) and socially isolated (SI) female rats treated with vehicle (VEH) or URB694, calculated as the difference between each assessment point during the social isolation period and the baseline ($n$ = 10 per group). Data are expressed mean±SEM. ∗ = significantly different from corresponding PH + VEH group; # = significantly different from corresponding SI + VEH group ($p$ values are reported in the text).

### 3.3. Forced swim test

Behavior during the forced swim test is illustrated in Fig. 4. Factorial ANOVA yielded a significant effect of treatment ($F$ = 4.9, $p$ = .033) and a strong trend for condition x treatment interaction ($F$ = 3.5, $p$ = .071) on immobility time, with no significant effects of estrous cycle phase ($F$ = 0.2, $p$ = .632). Specifically, socially isolated female rats treated with VEH spent significantly more time in immobility compared with their respective pair-housed counterparts ($p$ = .024). This behavioral effect of social isolation was significantly corrected by URB694 treatment (SI + URB694 vs. SI + VEH, $p$ = .007).

### 3.4. Measurements at sacrifice

#### 3.4.1. Plasma corticosterone levels and adrenal weight

Factorial ANOVA yielded a significant condition x treatment interaction ($F$ = 7.1, $p$ = .012) on plasma corticosterone levels at the end of the experimental protocol, with no significant effects of the estrous cycle phase ($F$ = 0.6, $p$ = .430). As depicted in Fig. 5, socially isolated female rats treated with VEH had significantly higher plasma corticosterone levels than their respective pair-housed counterparts (p = .016). URB694 treatment prevented the effect of social isolation on plasma corticosterone levels (SI + URB694 vs. SI + VEH, p = .003). There were no significant effects of condition and/or treatment on adrenal weight corrected for body weight at the end of the experiment (PH + VEH = 0.021 ± 0.002 mg/g; IS + VEH = 0.027 ± 0.003 mg/g; PH + URB694 = 0.027 ± 0.002 mg/g; IS + URB694 = 0.026 ± 0.002 mg/g).

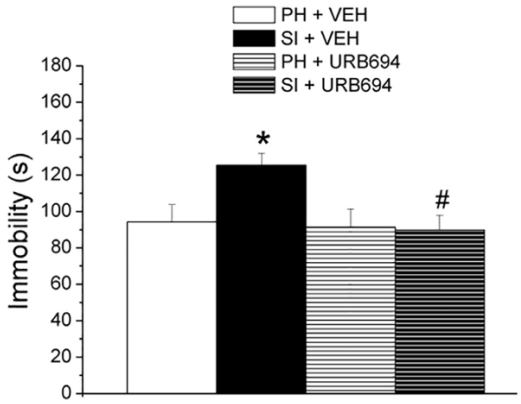


**Fig. 4** Time spent in immobility during the forced swim test by paired-housed (PH) and socially isolated (SI) female rats treated with vehicle (VEH) or URB694 ($n$ = 10 per group). Data are expressed mean±SEM. ∗ = significantly different from corresponding PH + VEH group; # = significantly different from corresponding SI + VEH group ($p$ values are reported in the text).

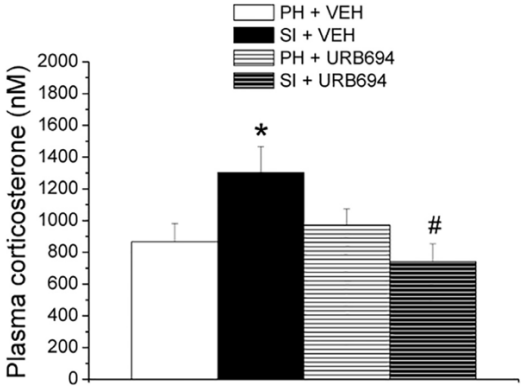


**Fig. 5** Plasma corticosterone levels at the end of the experimental protocol in paired-housed (PH) and socially isolated (SI) female rats treated with vehicle (VEH) or URB694 (*n* = 10 per group). Data are expressed mean±SEM. ∗ = significantly different from corresponding PH + VEH group; # = significantly different from corresponding SI + VEH group (*p* values are re- ported in the text).

### 3.4.2. BDNF hippocampal content

Factorial ANOVA yielded a significant effect of treatment ($F$ = 7.3, $p$ = .012) and a significant condition x treatment interaction ($F$ = 6.9, $p$ = .014) on BDNF content in the hippocampus at the end of the experimental protocol. As illustrated in Fig. 6A, socially isolated female rats treated with VEH showed a significantly lower BDNF hippocampal content compared with their respective pair-housed counterparts (p = .023). This effect of social isolation was prevented by URB694 treatment (SI + URB694 vs SI + VEH, p = .001). Moreover, we found a negative, although not significant, correlation between plasma corticosterone levels and BDNF hippocampal content (Table 1).

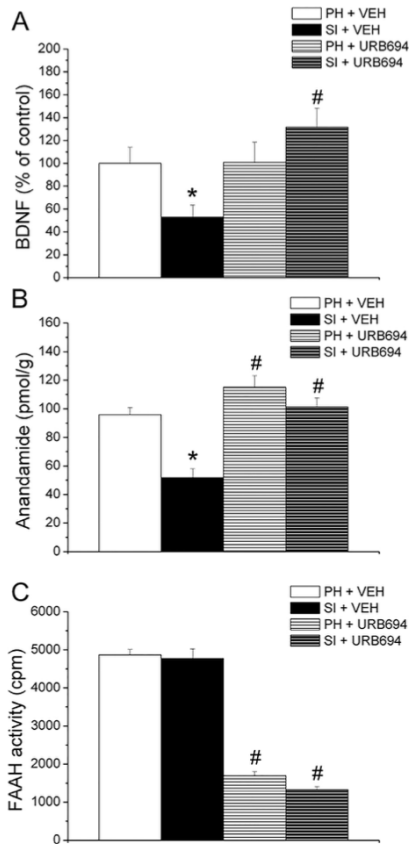


**Fig. 6** Brain-derived neurotrophic factor (BDNF; panel A) and anandamide (panel B) levels, and fatty acid amide hydrolase (FAAH) activity (panel C) in the hippocampus of paired-housed (PH) and socially isolated (SI) female rats treated with vehicle (VEH) or URB694 (*n* = 10 per group). Data are expressed mean±SEM. BDNF

values are expressed as a percentage of the control group (PH+VEH rats). ∗ = significantly different from corresponding PH + VEH group; # = significantly different from corresponding VEH group (*p* values are reported in the text).

**Table 1** Correlation matrix between plasma corticosterone levels, brain-derived neurotrophic factor (BDNF) hippocampal content, and anandamide (AEA) hippocampal levels at the end of the experimental protocol.

| | | Corticosterone | BDNF | AEA |
|---|---|---|---|---|
| Corticosterone | r | - | | |
| | p | | | |
| BDNF | r | −0.32 | - | |
| | p | .082 | | |
| AEA | r | −0.31 | .44 | - |
| | p | .068 | .015 | |

### 3.4.3. AEA hippocampal levels

Factorial ANOVA yielded significant effects of condition ($F = 19.7$, $p < .001$) and treatment ($F = 27.6$, $p < .001$), and a significant condition x treatment interaction ($F = 5.3$, $p = .028$) on AEA hippocampal levels at the end of the experimental protocol. As shown in Fig. 6B, socially isolated female rats treated with VEH showed significantly lower AEA hippocampal levels compared with their respective pair housed counterpart ($p < .001$). As expected, URB694- treated groups showed significantly greater AEA levels than corresponding VEH-treated groups, both in the social isolation ($p < .001$) and pair-housing ($p = .040$) condition. Moreover, we found a significant positive correlation be- tween AEA levels and BDNF content within the hippocampus (Table 1), as well as a strong trend for a negative correlation between AEA hippocampal levels and plasma corticosterone levels (Table 1).

### 3.4.4. FAAH activity

Factorial ANOVA yielded a significant effect of treatment ($F = 456.0$, $p < .001$) on FAAH activity in the hippocampus, being, as expected, significantly lower in URB694-treated than VEH-treated rats in both the social isolation ($p < .001$) and pair-housing ($p < .001$) condition (Fig. 6C).

## 4. Discussion

The major findings of the current investigation are the following. Compared to pair-housed females, socially isolated female rats developed behavioral (mild anhedonic state, passive stress coping) and physiological (reduced body weight gain, elevated plasma corticosterone levels) changes, and showed a reduction in BDNF and AEA levels within the hippocampus. Together, these changes are indicative of an increased risk of developing a depressive-like state. Notably, pharmacological inhibition of FAAH activity with URB694 restored AEA and BDNF hippocampal levels, and prevented the development of behavioral and physio- logical alterations following prolonged social isolation.

### 4.1. Depressive-like changes in socially isolated female rats

Psychiatric disorders in humans have been linked prevalently with social stress and/or reduced social interaction (Bjorkqvist, 2001; Heinrich and Gullone, 2006). Within pre-clinical models, the social defeat paradigm has been shown to have a substantial impact on depression-relevant behavioral and physiological parameters in adult male rats, while solitary housing is particularly effective in precipitating depressive-like symptoms in previously group-housed female rats (Beery and Kaufer, 2015; Carnevali et al., 2017a). Of note, the social isolation protocol adopted in this study included both solitary housing and long-term deprivation of sensory stimuli originating from the surrounding social environment. Therefore, it is likely that the described effects are due to a combination of both. Specifically, female rats showed a reduction in body weight gain, signs of a mild anhedonic-like state (i.e., reduced preference for the consumption of a sucrose solution), passive coping (i.e., in- creased immobility in the forced swim test), and elevated plasma corticosterone levels. Deficits in body weight gain in isolated rats may be explained by reduced food intake, as previously demonstrated in individually housed mice and rats (Izadi et al., 2018; Sun et al., 2014), particularly around light-dark phase transitions (Sun et al., 2014). Interestingly, reductions in heat production

and in the respiratory ex- change ratio were also found during light-dark transitions in individually housed mice (Sun et al., 2014), suggesting that metabolic functions may have been affected also in our socially isolated rats. Moreover, the mild reduction in the preference for the consumption of a palatable solution observed only after 25 days of social isolation resembles the time course of changes reported in female Wistar rats exposed to chronic mild stress (Grippo et al., 2005) and in socially isolated female prairie voles (Grippo et al., 2007). However, we acknowledge that the interpretation of this result is limited by the difference, albeit not statistically significant, between the two stressed groups on day 11 (i.e., before the start of the pharmacological treatment). Notably, the estrous cycle phase did not seem to have any effect on any of the behavioral and biological variables assessed in the cur- rent study, although our analysis is limited by the small sample size given that four different stages were considered. Nevertheless, this is in line with empirical research across multiple rodent species demonstrating that estrous cyclicity is not a major source of variability in females or, at least, is not greater than intrinsic variability in males (Beery, 2018; Finnell et al., 2018; Kokras et al., 2015).

Animal and human studies have provided support for the role of stress in the pathogenesis of depression via alterations in BDNF-mediated signaling (Hashimoto, 2010; Stepanichev et al., 2014), a neurotrophin that primarily regulates synaptic plasticity (Leal et al., 2017; Lu et al., 2014). In line with these findings, we found that BDNF con- tent was reduced in the hippocampus of socially isolated female rats with depressive-like symptoms. Remarkably, such downregulation of hippocampal BDNF was paralleled by a decrease in AEA hippocampal levels. Converging lines of evidence support the possibility that AEA signaling at the cannabinoid receptor 1 (CB1R) may be an important mediator of neuroplastic phenomena within the hippocampus (Aguado et al., 2005; Hashimotodani et al., 2007; Hill et al., 2010; Scarante et al., 2017; Burstein et al., 2018). Particularly relevant for the current results are findings of de- creased BDNF levels in the hippocampus of CB1R knockout mice (Aso et al., 2008). Thus, we hypothesize that a deficiency in AEA-mediated signaling at the CB1R might be im- plicated in the downregulation of BDNF hippocampal con- tent observed in socially isolated female rats. Moreover, the positive correlation found here between AEA levels and BDNF content further supports a role for the eCB system in adult hippocampal neurogenesis (Scarante et al., 2017). Notably, while one study reported a similar decrease in AEA levels in the hippocampus of chronically stressed male rats (Hill et al., 2008), other studies showed no changes in AEA hippocampal levels upon chronic stress exposure (Bortolato et al., 2007; Carnevali et al., 2015a; Hill et al., 2005). Of note, our data suggest that reduced AEA levels in the hippocampus of socially isolated rats were not due to an up- regulation of FAAH enzymatic activity. This is in line with previous studies showing that FAAH activity is not affected by chronic stress exposure in rats (Bortolato et al., 2007; Hill et al., 2008), suggesting that the stress-induced de- cline in the hippocampal pool of AEA might be due to di- minished biosynthetic mechanisms. Empirical evidence indicates the eCB system may be a biochemical effector of glucocorticoids in the brain (Hill and McEwen, 2010). Notably, the hippocampus itself is particularly sensitive to the action of glucocorticoid stress hormones due the rich con- centration of receptor sites for glucocorticoids (De Kloet et al., 1998). The negative, although only marginally significant, correlation found between plasma corticosterone levels and AEA hippocampal levels prompts further investigation into the specific mechanisms underlying the effects of stress exposure on AEA metabolism and their causal relationship with BDNF hippocampal downregulation. Interestingly, sex-specific mechanisms of eCB-mediated synaptic modulation within the hippocampus have been proposed to partly explain sex disparities in prevalence of depression (Huang and Woolley, 2012; Tabatadze et al., 2015). De- creased levels of BDNF may contribute to the atrophy of the hippocampus that has been observed in patients with depression (Sheline, 1996; Sheline et al., 2019). Recently, Belleau et al. (2019) proposed a model according to which chronic life stress can trigger the initial development of hippocampal volume reduction. However, this reduction would be neither necessary nor sufficient to produce a major de- pressive episode (Belleau et al., 2019). On the other hand, stress also initiates a set of neurotoxic processes (HPA axis dysregulation, inflammation, and neurotransmitter disturbances) that interact and may drive the development of a more chronic type of depression marked by further hippocampal volume reduction (Belleau et al., 2019). Although hippocampal volume was not assessed in the current study, we speculate that AEA–BDNF interactions might be implicated in the development of depressive symptoms and hippocampal volume decline under chronic life stress. Future longitudinal studies in rodent models of social stress may be informative in this regard.

### 4.2. Antidepressant-like effects of the FAAH inhibitor URB694

In an attempt to replicate findings of our previous study demonstrating antidepressant-like effects of the FAAH inhibitor URB694 in chronically stressed male rats (Carnevali et al., 2015a), pharmacological treatment with URB694 started after two weeks of social isolation (i.e., we anticipated that depressive-like behaviors would al- ready have begun to manifest by then). However, contrary to our expectations, we failed to conclusively demonstrate the onset of an anhedonic-like state before the start of the treatment. Thus, the fact that URB694-treated females did not show depressive-like behavioral and biological symptoms after a prolonged period of social isolation suggests, more cautiously, that inhibition of FAAH activity represents an effective preventive measure in this animal model. These results are in line with a growing body of evidence demonstrating that pharmacological inhibition of FAAH activity produces an antidepressant response in chronically stressed male rodents (Carnevali et al., 2017b). Interestingly, FAAH inhibitors have been shown to increase hippocampal neurogenesis in adult rats (Goncalves et al., 2008; Hill et al., 2006; Marchalant et al., 2009) and prevent stress- induced BDNF downregulation in the brain (Burstein et al., 2018), supposedly via facilitation of CB1R-mediated activation of the extracellular signal-regulated kinase signaling pathway (Derkinderen et al., 2003; Rubino et al., 2005). Therefore, given that CB1Rs are highly abundant in the rodent (and human) hippocampus (Mackie, 2005), we hypothesize that the antidepressant-like action of the FAAH inhibitor URB694 in socially isolated female rats may be partly mediated by a preservation of hippocampal BDNF content via enhancement of AEA signaling at the CB1R. However, the antidepressant-like effects of URB694 may also be interpreted in light of experimental evidence showing that AEA-signaling enhancement at the CB1R facilitates adaptive stress coping behaviors (Haller et al., 2013) and attenuates the neuroendocrine response to psychological stressors (Gorzalka et al., 2008). Moreover, given that FAAH inhibitors also increase the levels of other fatty acid amines with activity at peroxisome proliferator activated receptor-α (N-oleoylethanolamine (OEA) and N-palmitoylethanolamine (PEA)), the possibility of other non-cannabinoid receptor- mediated mechanisms cannot be completely ruled out. For example, a growing body of preclinical evidence suggests that PEA could have antidepressant-like activity (De Gregorio et al., 2019). On the other hand, increases in the endogenous levels of OEA may reduce food intake by regulating systems that control hunger and satiety in the brain (Romano et al., 2015). However, these compounds might also prolong and enhance AEA biological activity by competing with AEA for FAAH-mediated degradation (Petrosino et al., 2009). Of note, the current drug regimen had no effects on control animals, suggesting that the FAAH inhibitor did not affect normal biological processes and behavioral responses.

### 4.3. Conclusion

The results of this study suggest a potential interplay between AEA-mediated signaling and BDNF at the level of the hippocampus in the development of depression- relevant behaviors and physiological changes in female rats exposed to prolonged social isolation. Moreover, the current results document the ability of the FAAH inhibitor URB694 to correct the alterations associated with prolonged social isolation. One should note, however, that the current results are merely suggestive, and their translational implications for depression should be interpreted within the context of their limitations. First, the behavioral and biological changes described here after social isolation are often associated with an increased risk of developing major depression, but are not clinical symptoms of major depression per se. Second, we adopted a rodent model of prolonged social isolation, which should not be intended as a diagnostic model, but rather as a model of risk and vulnerability factor of stress-related depression. Moreover, we must acknowledge that, at present, clinical research on FAAH inhibitors has been slowed down by the serious adverse effects caused by the FAAH inhibitor BIA 10–2474 for the treatment of pain (von Schaper, 2016), which displayed both intrinsic toxic effects at high doses and off-targets effects (van Esbroeck et al., 2017). Investigations conducted by a Temporary Specialist Scientific Committee concluded that the toxicity of BIA 10-2474 is unlikely due to FAAH inhibition (Temporary Specialist Scientific Committee, 2016). A communication from the U.S. Food and Drug Administration also reported that the unique toxicity of this drug does not extend to other FAAH inhibitors (Food and Drug Administration, 2016), which are well tolerated by patients enrolled in clinical trials, and remarkably lack of the common adverse events elicited by exogenous cannabinoid-like compounds, including impairment in cognition, motor coordination, and psychoses (Mallet et al., 2016). The disorders for which these agents are being tested are mostly neuropsychiatric, such as pain conditions, depression, anxiety disorders, and phobias (Mallet et al., 2016). Nevertheless, the current results in female rats and previous

research in male rodents using the carbamate FAAH inhibitors URB597 (e.g. Bortolato et al., 2007) and URB694 (Carnevali et al., 2015a) warrant more translational studies to examine the mood-modulating properties of this class of FAAH inhibitors (Gururajan et al., 2019). Recently, sex differences in hippocampal response to pharmacological inhibition of FAAH activity have been reported in rats after acute intense stress (Zer-Aviv and Akirav, 2016). This suggests that preclinical development of FAAH inhibitors for the pharmacotherapy of stress- related depression should aim at comparing the underlying neurobiological mechanisms between males and females.

**Role of funding source**
The funding sources had no role in study design; in the col- lection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

**Contributors**
Authors LC and RS performed the experiments and analysed the data. Authors FV and FF analyzed the data. Authors LC, RS and AS designed the study. Author GS synthesized URB694. Author LC wrote the first draft of the manuscript. Authors RS, FV, SR, MM and AS revised the article critically for important intellectual content. All authors interpreted the results and contributed to and have approved the final manuscript.

**Conflict of Interest**
All authors declare that they have no conflicts of interest.

**Acknowledgements**
This research was financially supported by the Italian Ministry for University and Research (MIUR, PRIN 2017, 20175SA5JJ project) and the by the Programme “FIL-Quota Incentivante” of University of Parma and co-sponsored by Fondazione Cariparma.

**References**
- Aguado, T., Monory, K., Palazuelos, J., Stella, N., Cravatt, B., Lutz, B., Marsicano, G., Kokaia, Z., Guzman, M., Galve-Rop- erh, I., 2005. The endocannabinoid system drives neural progenitor proliferation. Faseb J. 19, 1704–1706.
- Aso, E., Ozaita, A., Valdizan, E.M., Ledent, C., Pazos, A., Maldonado, R., Valverde, O., 2008. BDNF impairment in the hippocampus is related to enhanced despair behavior in CB1 knockout mice. J. Neurochem. 105, 565–572.
- Beery, A.K., 2018. Inclusion of females does not increase variability in rodent research studies. Curr. Opin. Behav. Sci. 23, 143–149.
- Beery, A.K., Kaufer, D., 2015. Stress, social behavior, and resilience: insights from rodents. Neurobiol. Stress 1, 116–127.
- Belleau, E.L., Treadway, M.T., Pizzagalli, D.A., 2019. The impact of stress and major depressive disorder on hippocampal and medial prefrontal cortex morphology. Biol. Psychiatry 85, 443–453.
- Bjorkqvist, K., 2001. Social defeat as a stressor in humans. Physiol. Behav. 73, 435–442.
- Bortolato, M., Mangieri, R.A., Fu, J., Kim, J.H., Arguello, O., Du- ranti, A., Tontini, A., Mor, M., Tarzia, G., Piomelli, D., 2007. Antidepressant-like activity of the fatty acid amide hydrolase inhibitor URB597 in a rat model of chronic mild stress. Biol. Psychiatry 62, 1103–1110.
- Burstein, O., Shoshan, N., Doron, R., Akirav, I., 2018. Cannabinoids prevent depressive-like symptoms and alterations in BDNF ex- pression in a rat model of PTSD. Prog. Neuropsychopharmacol. Biol. Psychiatry 84, 129–139.
- Carnevali, L., Montano, N., Statello, R., Sgoifo, A., 2017a. Rodent models of depression-cardiovascular comorbidity: bridging the known to the new. Neurosci. Biobehav. Rev. 76, 144–153.
- Carnevali, L., Nalivaiko, E., Sgoifo, A., 2014. Respiratory patterns reflect different levels of aggressiveness and emotionality in Wild-type Groningen rats. Respir. Physiol. Neurobiol. 204, 28–35.
- Carnevali, L., Rivara, S., Nalivaiko, E., Thayer, J.F., Vacondio, F., Mor, M., Sgoifo, A., 2017b. Pharmacological inhibition of FAAH activity in rodents: a promising pharmacological approach for psychological-cardiac comorbidity? Neurosci. Biobehav. Rev. 74, 444–452.

- Carnevali, L., Vacondio, F., Rossi, S., Callegari, S., Macchi, E., Spadoni, G., Bedini, A., Rivara, S., Mor, M., Sgoifo, A., 2015a. Antidepressant-like activity and cardioprotective effects of fatty acid amide hydrolase inhibitor URB694 in socially stressed Wistar Kyoto rats. Eur. Neuropsychopharmacol. 25, 2157–2169.
- Carnevali, L., Vacondio, F., Rossi, S., Macchi, E., Spadoni, G., Bedini, A., Neumann, I.D., Rivara, S., Mor, M., Sgoifo, A., 2015b. Cardioprotective effects of fatty acid amide hydrolase inhibitor URB694, in a rodent model of trait anxiety. Sci. Rep. 5, 18218.
- Castren, E., Voikar, V., Rantamaki, T., 2007. Role of neurotrophic factors in depression. Curr. Opin. Pharmacol. 7, 18–21.
- Clapper, J.R., Vacondio, F., King, A.R., Duranti, A., Tontini, A., Silva, C., Sanchini, S., Tarzia, G., Mor, M., Piomelli, D., 2009. A second generation of carbamate-based fatty acid amide hydro- lase inhibitors with improved activity in vivo. ChemMedChem 4, 1505–1513.
- Cohen, S., Janicki-Deverts, D., Miller, G.E., 2007. Psychological stress and disease. JAMA 298, 1685–1687.
- Commons, K.G., Cholanians, A.B., Babb, J.A., Ehlinger, D.G., 2017. The rodent forced swim test measures stress-coping strategy, not depression-like behavior. ACS Chem. Neurosci. 8, 955–960.
- Connolly, K.R., Thase, M.E., 2012. Emerging drugs for major depressive disorder. Expert. Opin. Emerg. Drugs 17, 105–126.
- de Boer, S.F., Buwalda, B., Koolhaas, J.M., 2017. Untangling the neurobiology of coping styles in rodents: towards neural mechanisms underlying individual differences in disease susceptibility. Neurosci. Biobehav. Rev. 74 (Pt B), 401–422.
- De Gregorio, D., Manchia, M., Carpiniello, B., Valtorta, F., Nobile, M., Gobbi, G., Comai, S., 2019. Role of palmitoylethanolamide (PEA) in depression: translational evidence: special section on "Translational and neuroscience studies in affective disorders". Section editor, Maria nobile MD, Ph.D. This section of JAD focuses on the relevance of translational and neuroscience studies in providing a better understanding of the neu- ral basis of affective disorders. The main aim is to briefly summaries relevant research findings in clinical neuroscience with particular regards to specific innovative topics in mood and anxiety disorders. J. Affect. Disord. 255, 195–200.
- De Kloet, E.R., Vreugdenhil, E., Oitzl, M.S., Joels, M., 1998. Brain corticosteroid receptor balance in health and disease. Endocr. Rev. 19, 269–301.
- Derkinderen, P., Valjent, E., Toutant, M., Corvol, J.C., Enslen, H., Ledent, C., Trzaskos, J., Caboche, J., Girault, J.A., 2003. Regulation of extracellular signal-regulated kinase by cannabinoids in hippocampus. J. Neurosci. 23, 2371–2382.
- Dinan, T.G., 2005. Stress: the shared common component in major mental illnesses. Eur. Psychiatry 20 (3), S326–S328 Suppl.
- Duman, R.S., Monteggia, L.M., 2006. A neurotrophic model for stress-related mood disorders. Biol. Psychiatry 59, 1116–1127.
- Finnell, J.E., Muniz, B.L., Padi, A.R., Lombard, C.M., Mof- fitt, C.M., Wood, C.S., Wilson, L.B., Reagan, L.P., Wilson, M.A., Wood, S.K., 2018. Essential role of ovarian hormones in susceptibility to the consequences of witnessing social defeat in female rats. Biol. Psychiatry 84, 372–382.
- Food and Drug Administration, Drug Safety and Availability Report, 2016. FDA finds drugs under investigation in the U.S. related to French BIA 10-2474 drug do not pose similar safety risks. https://www.fda.gov/drugs/drug-safety-and-availability/fda-finds-drugs-under-investigation-us-related-french-bia-10-2474-drug-do-not-pose-similar-safety.
- Fowler, C.J., 2015. The potential of inhibitors of endocannabinoid metabolism as anxiolytic and antidepressive drugs–a practical view. Eur. Neuropsychopharmacol. 25, 749–762.
- Goncalves, M.B., Suetterlin, P., Yip, P., Molina-Holgado, F., Walker, D.J., Oudin, M.J., Zentar, M.P., Pollard, S., Yanez– Munoz, R.J., Williams, G., Walsh, F.S., Pangalos, M.N., Do- herty, P., 2008. A diacylglycerol lipase-CB2 cannabinoid pathway regulates adult subventricular zone neurogenesis in an age-dependent manner. Mol. Cell. Neurosci. 38, 526–536.
- Gorzalka, B.B., Hill, M.N., 2011. Putative role of endocannabinoid signaling in the etiology of depression and actions of antidepressants. Prog. Neuropsychopharmacol. Biol. Psychiatry 35, 1575–1585.

- Gorzalka, B.B., Hill, M.N., Hillard, C.J., 2008. Regulation of endo- cannabinoid signaling by stress: implications for stress-related affective disorders. Neurosci. Biobehav. Rev. 32, 1152–1160.
- Grigoriadis, S., Robinson, G.E., 2007. Gender issues in depression. Ann. Clin. Psychiatry 19, 247–255.
- Grippo, A.J., Cushing, B.S., Carter, C.S., 2007. Depression-like behavior and stressor-induced neuroendocrine activation in female prairie voles exposed to chronic social isolation. Psychosom. Med. 69, 149–157.
- Grippo, A.J., Sullivan, N.R., Damjanoska, K.J., Crane, J.W., Car- rasco, G.A., Shi, J., Chen, Z., Garcia, F., Muma, N.A., Van de Kar, L.D., 2005. Chronic mild stress induces behavioral and phys- iological changes, and may alter serotonin 1A receptor function, in male and cycling female rats. Psychopharmacology (Berl) 179, 769–780.
- Gururajan, A., Reif, A., Cryan, J.F., Slattery, D.A., 2019. The future of rodent models in depression research. Nat. Rev. Neurosci. 20, 686–701.
- Haller, J., Goldberg, S.R., Pelczer, K.G., Aliczki, M., Panlilio, L.V., 2013. The effects of anandamide signaling enhanced by the FAAH inhibitor URB597 on coping styles in rats. Psychopharmacology (Berl) 230, 353–362.
- Hashimoto, K., 2010. Brain-derived neurotrophic factor as a biomarker for mood disorders: an historical overview and future directions. Psychiatry Clin. Neurosci. 64, 341–357.
- Hashimotodani, Y., Ohno-Shosaku, T., Kano, M., 2007. Endocannabinoids and synaptic function in the CNS. Neuroscientist 13, 127–137.
- Hayley, S., Anisman, H., 2013. Neurotrophic paths in the treatment of depression. J. Psychiatry Neurosci. 38, 291–293.
- Heinrich, L.M., Gullone, E., 2006. The clinical significance of loneliness: a literature review. Clin. Psychol. Rev. 26, 695–718.
- Hill, M.N., Carrier, E.J., McLaughlin, R.J., Morrish, A.C., Meier, S.E., Hillard, C.J., Gorzalka, B.B., 2008. Regional alterations in the endocannabinoid system in an animal model of depression: effects of concurrent antidepressant treatment. J. Neurochem. 106, 2322–2336.
- Hill, M.N., Kambo, J.S., Sun, J.C., Gorzalka, B.B., Galea, L.A., 2006. Endocannabinoids modulate stress-induced suppression of hippocampal cell proliferation and activation of defensive behaviours. Eur. J. Neurosci. 24, 1845–1849.
- Hill, M.N., McEwen, B.S., 2010. Involvement of the endocannabinoid system in the neurobehavioural effects of stress and glucocorticoids. Prog. Neuropsychopharmacol. Biol. Psychiatry 34, 791–797.
- Hill, M.N., Patel, S., Carrier, E.J., Rademacher, D.J., Ormerod, B.K., Hillard, C.J., Gorzalka, B.B., 2005. Down- regulation of endocannabinoid signaling in the hippocampus following chronic unpredictable stress. Neuropsychopharmacology 30, 508–515.
- Hill, M.N., Titterness, A.K., Morrish, A.C., Carrier, E.J., Lee, T.T., Gil-Mohapel, J., Gorzalka, B.B., Hillard, C.J., Christie, B.R., 2010. Endogenous cannabinoid signaling is required for voluntary exercise-induced enhancement of progenitor cell proliferation in the hippocampus. Hippocampus 20, 513–523.
- Huang, G.Z., Woolley, C.S., 2012. Estradiol acutely suppresses inhibition in the hippocampus through a sex-specific endocannabinoid and mGluR-dependent mechanism. Neuron 74, 801–808.
- Izadi, M.S., Radahmadi, M., Ghasemi, M., Rayatpour, A., 2018. Effects of isolation and social subchronic stresses on food intake and levels of leptin, ghrelin, and glucose in male rats. Adv. Biomed. Res. 7, 118.
- Jacobson, L., Sapolsky, R., 1991. The role of the hippocampus in feedback regulation of the hypothalamic-pituitary-adrenocortical axis. Endocr. Rev. 12, 118–134.
- Kokras, N., Antoniou, K., Mikail, H.G., Kafetzopoulos, V., Papadopoulou-Daifoti, Z., Dalla, C., 2015. Forced swim test: what about females? Neuropharmacology 99, 408–421.
- Kokras, N., Dalla, C., 2014. Sex differences in animal models of psychiatric disorders. Br. J. Pharmacol. 171, 4595–4619.
- Leal, G., Bramham, C.R., Duarte, C.B., 2017. BDNF and hippocampal synaptic plasticity. Vitam. Horm. 104, 153–195.
- Levone, B.R., Cryan, J.F., O'Leary, O.F., 2015. Role of adult hippocampal neurogenesis in stress resilience. Neurobiol. Stress 1, 147–155.

- Lu, B., Nagappan, G., Lu, Y., 2014. BDNF and synaptic plasticity, cognitive function, and dysfunction. Handb. Exp. Pharmacol. 220, 223–250.
- Mackie, K., 2005. Distribution of cannabinoid receptors in the central and peripheral nervous system. Handb. Exp. Pharmacol. 299–325.
- Mallet, C., Dubray, C., Dualé, C., 2016. FAAH inhibitors in the limelight, but regrettably. Int. J. Clin. Pharmacol. Ther. 54, 498–501.
- Marchalant, Y., Brothers, H.M., Wenk, G.L., 2009. Cannabinoid agonist WIN-55,212-2 partially restores neurogenesis in the aged rat brain. Mol. Psychiatry 14, 1068–1069.
- Marcondes, F.K., Bianchi, F.J., Tanno, A.P., 2002. Determination of the estrous cycle phases of rats: some helpful considerations. Braz. J. Biol. 62, 609–614.
- Micale, V., Di Marzo, V., Sulcova, A., Wotjak, C.T., Drago, F., 2013. Endocannabinoid system and mood disorders: priming a target for new therapies. Pharmacol. Ther. 138, 18–37.
- Petrosino, S., Ligresti, A., Di Marzo, V., 2009. Endocannabinoid chemical biology: a tool for the development of novel therapies. Curr. Opin. Chem. Biol. 13, 309–320.
- Pittenger, C., Duman, R.S., 2008. Stress, depression, and neuro- plasticity: a convergence of mechanisms. Neuropsychopharmacology 33, 88–109.
- Plenis, A., Konieczna, L., Oledzka, I., Kowalski, P., Baczek, T., 2011. Simultaneous determination of urinary cortisol, cortisone and corticosterone in parachutists, depressed patients and healthy controls in view of biomedical and pharmacokinetic studies. Mol. Biosyst. 7, 1487–1500.
- Porsolt, R.D., Le Pichon, M., Jalfre, M., 1977. Depression: a new animal model sensitive to antidepressant treatments. Nature 266, 730–732.
- Romano, A., Tempesta, B., Provensi, G., Passani, M.B., Gaetani, S., 2015. Central mechanisms mediating the hypophagic effects of oleoylethanolamide and N-acylphosphatidylethanolamines: different lipid signals? Front. Pharmacol. 6, 137.
- Rubino, T., Forlani, G., Vigano, D., Zippel, R., Parolaro, D., 2005. Ras/ERK signalling in cannabinoid tolerance: from behaviour to cellular aspects. J. Neurochem. 93, 984–991.
- Sapolsky, R.M., 2000. The possibility of neurotoxicity in the hippocampus in major depression: a primer on neuron death. Biol. Psychiatry 48, 755–765.
- Scarante, F.F., Vila-Verde, C., Detoni, V.L., Ferreira-Junior, N.C., Guimaraes, F.S., Campos, A.C., 2017. Cannabinoid modulation of the stressed hippocampus. Front. Mol. Neurosci. 10, 411.
- Sheline, Y.I., 1996. Hippocampal atrophy in major depression: a result of depression-induced neurotoxicity? Mol. Psychiatry 1, 298–299.
- Sheline, Y.I., Liston, C., McEwen, B.S., 2019. Parsing the hippocampus in depression: chronic stress, hippocampal volume, and major depressive disorder. Biol. Psychiatry 85, 436–438.
- Sloan, D.M., Kornstein, S.G., 2003. Gender differences in depression and response to antidepressant treatment. Psychiatr. Clin. N. Am. 26, 581–594.
- Stepanichev, M., Dygalo, N.N., Grigoryan, G., Shishkina, G.T., Gulyaeva, N., 2014. Rodent models of depression: neurotrophic and neuroinflammatory biomarkers. Biomed. Res. Int. 2014, 932757.
- Stetler, C., Miller, G.E., 2011. Depression and hypothalamic-pituitary-adrenal activation: a quantitative summary of four decades of research. Psychosom. Med. 73, 114–126.
- Sun, M., Choi, E.Y., Magee, D.J., Stets, C.W., During, M.J., Lin, E.J., 2014. Metabolic effects of social isolation in adult C57BL/6 mice. Int. Sch. Res. Not. 2014, 690950.
- Tabatadze, N., Huang, G., May, R.M., Jain, A., Woolley, C.S., 2015. Sex differences in molecular signaling at inhibitory synapses in the hippocampus. J. Neurosci. 35, 11252–11265.
- Tardito, D., Perez, J., Tiraboschi, E., Musazzi, L., Racagni, G., Popoli, M., 2006. Signaling pathways regulating gene expression, neuroplasticity, and neurotrophic mechanisms in the ac- tion of antidepressants: a critical overview. Pharmacol. Rev. 58, 115–134.
- Tarzia, G., Duranti, A., Gatti, G., Piersanti, G., Tontini, A., Ri- vara, S., Lodola, A., Plazzi, P.V., Mor, M., Kathuria, S., Piomelli, D., 2006. Synthesis and structure-activity relationships of FAAH inhibitors: cyclohexylcarbamic acid biphenyl esters with chemical modulation at the proximal phenyl ring. ChemMedChem 1, 130–139.
- Temporary Specialist Scientific Committee, 2016. FAAH (Fatty acid amide hydrolase), on the causes of the accident during a phase 1 clinical trial in Rennes in January 2016.

https://ansm.sante.fr/var/ansm_site/storage/original/application/744c7c6daf96b141bc9509e2f85c227e.pdf

- van Esbroeck, A.C.M., Janssen, A.P.A., Cognetta, A.B., Oga- sawara, D., Shpak, G., van der Kroeg, M., Kantae, V., Bagge- laar, M.P., de Vrij, F.M.S., Deng, H., Allarà, M., Fezza, F., Lin, Z., van der Wel, T., Soethoudt, M., Mock, E.D., den Dulk, H., Baak, I.L., Florea, B.I., Hendriks, G., De Petrocellis, L., Overkleeft, H.S., Hankemeier, T., De Zeeuw, C.I., Di Marzo, V., Maccarrone, M., Cravatt, B.F., Kushner, S.A., van der Stelt, M., 2017. Activity-based protein profiling reveals off-tar- get proteins of the FAAH inhibitor BIA 10-2474. Science 356, 1084–1087.
- von Schaper, E., 2016. Bial incident raises FAAH suspicions. Nat. Biotechnol. 34, 223.
- Zer-Aviv, T.M., Akirav, I., 2016. Sex differences in hippocampal response to endocannabinoids after exposure to severe stress. Hippocampus 26, 947–957.