Social stress contagion in rats: Behavioural, autonomic and neuroendocrine correlates

Luca Carnevali\textsuperscript{a}, Nicola Montano\textsuperscript{b}, Rosario Statello\textsuperscript{a}, Gino Coudè\textsuperscript{c}, Federica Vacondio\textsuperscript{d}, Silvia Rivara\textsuperscript{d}, Pier Francesco Ferrari\textsuperscript{c}, Andrea Sgoifo\textsuperscript{a,}\textsuperscript{e}

\textsuperscript{a} Department of Chemistry, Life Sciences and Environmental Sustainability, Stress Physiology Lab, University of Parma, Italy
\textsuperscript{b} Department of Internal Medicine, Fondazione IRCCS Ca’ Granda, Ospedale Maggiore Policlinico, University of Milan, Italy
\textsuperscript{c} Institut des Sciences Cognitives Marc Jeannerod UMR 5229, CNRS—Université de Lyon, Bron Cedex, France
\textsuperscript{d} Department of Food and Drug, University of Parma, Italy

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A B S T R A C T

The negative emotional consequences associated with life stress exposure in an individual can affect the emotional state of social partners. In this study, we describe an experimental rat model of social stress contagion and its effects on social behaviour and cardiac autonomic and neuroendocrine functions. Adult male Wistar rats were pair-housed and one animal (designated as “demonstrator” (DEM)) was submitted to either social defeat stress (STR) by an aggressive male Wild-type rat in a separate room or just exposed to an unfamiliar empty cage (control condition, CTR), once a day for 4 consecutive days. We evaluated the influence of cohabitation with a STR DEM on behavioural, cardiac autonomic and neuroendocrine outcomes in the cagemate (defined “observer” (OBS)). After repeated social stress, STR DEM rats showed clear signs of social avoidance when tested in a new social context compared to CTR DEM rats. Interestingly, also their cagemate STR OBSs showed higher levels of social avoidance compared to CTR OBSs. Moreover, STR OBS rats exhibited a higher heart rate and a larger shift of cardiac autonomic balance toward sympathetic prevalence (as indexed by heart rate variability analysis) immediately after the first reunification with their STR DEMs, compared to the control condition. This heightened cardiac autonomic responsiveness habituated over time. Finally, STR OBSs showed elevated plasma corticosterone levels at the end of the experimental protocol compared to CTR OBSs. These findings demonstrate that cohabitation with a DEM rat, which has experienced repeated social defeat stress, substantially disrupts social behaviour and induces short-lasting cardiac autonomic activation and hypothalamic-pituitary-adrenal axis hyperactivity in the OBS rat, thus suggesting emotional state-matching between the OBS and the DEM rats. We conclude that this rodent model may be further exploited for investigating the neurobiological bases of negative affective sharing between social partners under chronic social stress conditions.

1. Introduction

Stress is increasingly present in everyday life in our fast-paced society and strongly influences our mental and physical well-being. It is well established that exposure to chronic stressful life events can favour the onset and progression of both psychological (e.g., depression, anxiety) and physical (e.g., cardiovascular) disorders in vulnerable individuals (Bjorkqvist, 2001; Rozanski et al., 2005; Slavich, 2016; Strike and Steptoe, 2004). In this context, the question arises as to what extent the adverse emotional consequences associated with stress exposure in family or friends have the potential to negatively impact our life, independently from whether or not we are directly exposed to stressful life events. Indeed, humans are highly sensitive to the emotional state of their social partners and may unconsciously adopt it through social interactions. In social neuroscience, the transmission of affect from one person to another is defined as affect contagion and has been suggested to function, in part, to facilitate social connection and coordination (Butler, 2011; Hatfield et al., 1994). The subjective state resulting from affective contagion is referred to as affective (or emotional) empathy (Bernhardt and Singer, 2012; Christov-Moore et al., 2014; de Waal, 2008). For example, the contagion of depressive symptoms is relatively well documented, as depression in family or friends might cumulatively increase the likelihood that a person will exhibit depressive behaviours (Bastiampillai et al., 2013; Joiner, 1994).

\textsuperscript{e} Corresponding author at: Department of Chemistry, Life Sciences and Environmental Sustainability, Stress Physiology Lab, University of Parma, Via Parco Area delle Scienze 11/a, 43124 Parma, Italy.
E-mail address: andrea.sgoifo@unipr.it (A. Sgoifo).

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Moreover, observing or interacting with an individual experiencing acute stress can activate the physiological stress response in the observer, as evidenced by increases in sympathetic nervous system activity and activation of the hypothalamic-pituitary-adrenal axis (Buchanan et al., 2012; Engert et al., 2014; Soto and Levenson, 2009).

Since the seminal study by Church (Church, 1959), many studies, performed mostly in the contextual fear conditioning paradigm, have demonstrated that also rodents can be attuned to the affective state of a social partner (reviewed in Meyza et al., 2016; Panksepp and Lahvis, 2011). For example, after a painful (e.g., acetic acid) or stressful (e.g., foot shock) stimulus is delivered to a rodent (the demonstrator) in the presence of an observer rodent, the observer may mimic the demonstrator’s behavior as if it was directly experiencing pain or stress (Bredy and Barad, 2009; Langford et al., 2006). The social transfer of fear between rats can also occur through social interaction in the absence of direct observation. For example, during a social interaction with a recently fear-conditioned partner, naïve cage-mate observers showed increased freezing behaviour and neuronal activation in the amygdala and prefrontal cortex (Knapska et al., 2006; Mikosz et al., 2015). Importantly, emotional contagion in rodents appears to be dependent on familiarity (Gonzalez-Liencres et al., 2014; Jeon et al., 2010), similarly to humans (Engert et al., 2014; Martin et al., 2015). So far only a few rodent studies have investigated the effects of chronic stress in demonstrators on behavioural and physiological parameters in observers (e.g.: Gilmore et al., 2008; Boyko et al., 2015; Carrillo et al., 2015). For example, demonstrator mice were submitted to daily restraint stress for 15 days in close proximity to, but not in view of cagemate observers (Gilmore et al., 2008). When demonstrator mice returned to the cage, their observer partners showed higher increases in heart rate and core body temperature compared to observers of non-stressed animals, although this response habituated over time (Gilmore et al., 2008).

In another study, naïve rats were each housed with two rats that showed depressive-like behaviours after 5 weeks of chronic unpredictable stress (Boyko et al., 2015). Interestingly, naïve rats exhibited depressive-like behaviours (i.e., anhedonia and passive coping strategy during a forced swim test) after 5 weeks of cohabitation in the same cage with depressed rats (Boyko et al., 2015). These findings suggest that in rodents, similarly to humans, behavioural and physiological responses of an individual may be influenced by the affective state of its social partners.

Given that the most relevant stress factors associated with human psychopathology are thought to originate from an adverse social environment (Bjorkqvist, 2001; Rozanski et al., 2005; Slavich, 2016; Strike and Steptoe, 2004), in this study we attempted to set up an experimental rat model of social stress contagion by using a stress paradigm (social defeat) with high translational and naturalistic relevance for the human condition (Sgoifo et al., 2014; Carnevali et al., 2017). In particular, we tested the hypothesis that cohabitation with a demonstrator rat, which has been repeatedly submitted to social defeat stress in a separate room, would elicit behavioural, cardiac autonomic and neuroendocrine changes in the social partner.
2. Material and methods

2.1. Ethics statement and animals

All experiments were conducted in accordance with the guidelines for care and use of experimental animals of the European Communities Council (2010/63/UEL 276 20/10/2010) and with the Italian law (DL: 04.03.2014, N° 26) and approved by the Ethical Committee of the University of Parma. All efforts were made to minimize suffering and the numbers of animals used. In this study, 5-month-old male Wistar rats (350–450 g) were used as observers (OBSs) and 3-month-old male Wistar rats (300–350 g) were used as demonstrators (DEMs). The rats were provided by the animal facility of the University of Parma, and were housed in unisex groups of four individuals from weaning until the onset of experiments (day -19), when each OBS rat was pair-housed with an unfamiliar DEM rat in a plastic cage (60 × 40 × 40 cm) (Fig. 1). A wire mesh partition dividing the cage in two equal zones was used to separate each OBS rat from its cagemate DEM (Fig. 1). Thus, the pair was in sensory but not physical contact. OBS and DEM rats belonged to different groups of animals for each of the three experiments of this study. Additional 10-month-old male Wild-type Groningen rats weighing 500–600 g were housed in a separate room with an oviduct-ligated female partner and used as residents in the resident-intruder paradigm for all the experiments (see below). Finally, 5-month-old male Wistar rats were used as stimulus unfamiliar rats in the social approach/avoidance test (see below). All animals were kept in rooms with a constant temperature (22 ± 2 °C) and a reversed light-dark cycle (lights off from 9:00 until 21:00). Food and water were available ad libitum.

2.2. Surgery and radiotelemetry

Radiotelemetric transmitters (TA11CTA-F40; Data Sciences International, St. Paul, MN, USA) for recording ECG signals were implanted in OBS rats on day -12 (Fig. 1). Animals were anesthetized with tiletamine hydrochloride plus zolazepam hydrochloride (Zoletil; 20 mg/kg, s.c.) and the transmitters were implanted according to a surgical procedure that guarantees high-quality ECG recordings. The transmitter body was placed in the abdominal cavity; one electrode was fixed to the dorsal surface of the xyphoid process, and another electrode was placed in the anterior mediastinum close to the right atrium (Costoli et al., 2004). After surgery, rats were injected for 2 days with gentamicin sulfate (Aagent, Fatro, 0.2 ml/kg, s.c.) and allowed at least 10 days of recovery before the start of the experiments (Fig. 1). Recovery from surgery was eventful. ECG waves (sampling frequency 1000 Hz) were picked up by a radiotelemetry receiver (RPC-1) and recorded via ART-Gold 4.2 data acquisition system (Data Sciences Int., St. Paul, MN, USA).

2.3. Experiment 1

This experiment was designed to investigate whether cohabitation with a socially stressed DEM rat would alter behavioural and physiological responses of the cagemate OBS in a new social context (social approach/avoidance test). Specific experimental procedures are described below.

2.3.1. Repeated social defeat stress/control procedure

Initially, OBS rats were pair-housed with DEM rats (n = 18 pairs) (Fig. 1). Each pair was randomly assigned to one of two experimental conditions: a) a repeated social defeat stress (STR, n = 9), or b) a control (CTR, n = 9) procedure for 4 consecutive days (Fig. 1). The social defeat stress was based on a classical “resident-intruder” paradigm (Miczek, 1979). On the experimental day, STR DEM rats (intruders) were removed from their cages, transferred to an acoustically isolated room (room 2) and put for 15 min in the cage of an aggressive male Wild-type rat (resident) (Fig. 1), whose female partner had previously been removed from it (Carnevali et al., 2013b, 2015). During this phase, STR DEM rats were attacked and defeated by the resident (i.e., when the intruder rat assumed a supine posture that was held for at least 5 s). On the contrary, CTR DEM rats were put for 15 min in an empty cage with clean bedding in room 1. During social defeat/control procedure, OBS rats were otherwise left undisturbed in their home cages and were not able to hear the vocalization of STR DEM rats from room 2. At the end of this 15-min period, STR and CTR DEM rats were reunited with their respective cagemate OBSs. This procedure was repeated for 4 consecutive days (days 1–4, Fig. 1) at the onset of the dark phase of the light/dark cycle. To avoid large individual differences in the intensity of received aggression, every time we exposed STR DEM rats to a different opponent in a rotational design (average number of received attacks: 4.4 ± 1.0). Upon every session of social defeat, all STR DEM rats were quickly examined for wounds (though none were ever observed).

2.3.2. Social approach/avoidance test

On day 5 (Fig. 1), STR DEMs were not subjected to any social stress procedure. All OBS and DEM rats were instead submitted to a social approach/avoidance test, which is considered a reliable procedure to measure experimental anxiety in a social context (Haller and Bakos, 2002; Haller et al., 2003; Toth and Neumann, 2013), after the onset of the dark phase (i.e., when STR DEMs were expecting to be moved to the resident’s cage). The apparatus consisted of a large cage (60 × 40 × 40 cm) divided by a black plastic wall in a social and a non-social (40 × 40 × 40 cm and 20 × 40 × 40 cm respectively) compartment, which were connected by a sliding door (10 × 10 cm). The social compartment contained a sub-chamber (15 × 40 × 40 cm) delimited by a wire mesh partition in which a stimulus unfamiliar male Wistar rat was confined, as previously described (Carnevali et al., 2014; Nicolas and Prinssen, 2006). On the day of the test, all DEM and OBS rats were individually introduced into the non-social compartment for a 3-min habituation period. After this period, the sliding door was opened and the experimental rat was allowed to move freely between the non-social and social compartments for 10 min. The test apparatus did not permit physical contact between the experimental and stimulus animals. The behaviour of OBS and DEM rats was recorded using a video camera positioned above the apparatus. Continuous ECG recordings were performed in STR OBS and CTR OBS rats during the 10-min test period. At the end of the test, both the experimental and the stimulus rat were removed and the apparatus was carefully cleaned. Stimulus rats were used maximally twice.

2.4. Experiment 2

This experiment was designed to investigate potential cardiac autonomic and neuroendocrine changes in STR OBS rats. Differently from experiment 1, in this experiment animals received an auditory cue, which signalled the removal of the DEM from its cage and the beginning of the social stress/control procedure. The auditory cue therefore became the conditioned stimulus and was randomly and unpredictably presented during the dark phase of the light-dark cycle to better investigate the specific changes of autonomic activity independently from the light-dark transitions. Specific experimental procedures are described below.

2.4.1. Repeated social defeat stress/control procedure

Initially, OBS rats were pair-housed with DEM rats (n = 20 pairs) (Fig. 1). Each pair was randomly designated to either a repeated social defeat stress (STR, n = 10) or a control (CTR, n = 10) procedure for 4 consecutive days (Fig. 1). Differently from experiment 1, the beginning of the social defeat stress/control procedure (i.e., the removal of the DEM rats from their cages) was preceded every day by a tone (intensity: 70 dB; frequency: 2000–2200 Hz), which was played for 5 s at a
randomized time between 10:00 and 13:00 and was used as a conditioned stimulus. On day 1 and 4, continuous ECG recordings were performed in STR OBS and CTR OBS rats for 15 min (i) before the conditioned stimulus, (ii) during social defeat/control procedure in DEM rats, and (iii) after the return of the DEMs to their home cages. The behaviour of OBS and DEM rats was recorded using a video camera positioned in front of the cages.

On day 5, the conditioned stimulus was played for 5 s at 10:00, but the animals were then left otherwise undisturbed in their cages. Continuous ECG recordings were performed in STR OBS and CTR OBS rats for 30 min before and 30 min after the conditioned stimulus. During these phases, the behaviour of OBS and DEM rats was recorded using a video camera positioned in front of the cages.

2.4.2. Plasma collection

Tail vein blood (average vol: 0.3 ml) was collected in EDTA tubes in baseline condition (day -2) and on day 5 (i.e., 30 min after the presentation of the auditory cue) (Fig. 1) at the same time of the day (10:30), in order to avoid effects of circadian cycle on corticosterone levels. We used five different cohorts of animals for this experiment, each composed of n = 2 STR and n = 2 CTR pairs. This allowed the collection of blood from n = 2 STR OBS and n = 2 CTR OBS rats within a 10-min period, in a random order. Blood was centrifuged (2600 rpm; 4 °C; 10 min), and the plasma was collected and stored at −20 °C until analysis for the determination of plasma corticosterone levels.

2.5. Experiment 3

This experiment was designed to demonstrate that olfactory stimuli originating from the cage of an aggressive, dominant rat are not sufficient to induce autonomic and neuroendocrine changes in OBS rats. Specific experimental procedures are described below.

2.5.1. Exposure to olfactory stimuli

Initially, OBS rats were pair-housed with DEM rats (n = 6 pairs) (Fig. 1). On days 1–4, DEM rats were removed from their cages immediately after a tone (i.e., the conditioned stimulus) had been played for 5 s at a random time between 10:00 and 13:00, identically to experiment 2. The DEM rats were then put in an empty cage with clean bedding in the same room, while in another room a male Wistar rat was played for 5 s at a random time between 10:00 and 13:00, identically to the approach/avoidance test, the social compartment was virtually divided into two zones of equal dimensions: proximal to and distal from the stimulus rat, as previously described (Carnevali et al., 2014; Nicolas and Prinsen, 2006). We calculated the time spent by the animals (i) in the non-social compartment, and (ii) in the proximal and distal areas of the social compartment (expressed as% of total time). For the behauioural analyses on day 1, day 4 and day 5, the home cage compartments belonging to DEM and OBS rats were virtually divided into two zones of equal dimensions (15 × 40 × 40 cm): proximal to and distal from the wire mesh partition that separated each pair. We calculated the time spent by the animals in the proximal and distal zones (expressed as% of total time), as well as the time during which the animals were engaged in the following three behavioural categories (expressed as% of total time): (i) freezing (head up, no body movements excluding those necessary for breathing), (ii) resting (small body movements including those associated with grooming or sniffing, or head down and no body movements excluding those necessary for breathing), and (iii) motion (moving or turning around).

2.6. Data analysis

2.6.1. ECG analysis

Initially, each raw ECG signal was visually inspected to ensure that all R-waves were correctly detected. Those parts of ECG recordings which were non-stationary and/or exhibited recording artefacts were excluded from the analysis. Heart rate (HR; reported in beats per minute: bpm) and frequency-domain parameters of heart rate variability (HRV) were then quantified using ChartPro 5.0 software (ADInstruments, Sydney, Australia). A fast Fourier transform-based method (Welch’s periodogram: 256 points, 50% overlap, and Hamming window) was applied to extract the power of the high frequency band (HF, 0.75–2.5 Hz), which reflects parasympathetic modulation and includes oscillations of HR linked to respiration (Ramaekers et al., 2002; Reyes del Paso et al., 2013). The power measure was then transformed to its natural logarithm to normalize the distribution of the estimates to limit the impact of large differences (i.e., outlying values). The ratio between the low frequency band (LF, 0.2–0.75 Hz) and the HF band was also calculated as a measure of sympathovagal balance (Carnevali et al., 2013a; Ramaekers et al., 2002).

2.6.2. Plasma analysis

Plasma was deproteinized by addition of two volumes of organic solvent (ice-cold acetonitrile), containing the internal standard dexamethasone (structural analogue of cortisol, 75 nmol/L). After centrifugation (14,000g, 4 °C, 10 min), the supernatant was directly injected in the liquid chromatography/tandem mass spectrometry system for quantification of corticosterone levels, as previously reported (Plenis et al., 2011; Carnevali et al., 2015). Mass spectrometric analyses were done in positive ion mode. A Thermo Accela UHPLC gradient system coupled to a Thermo TSQ Quantum Max triple quadrupole mass spectrometer (Thermo Italia, Milan, Italy) equipped with an heated electrospray ionization (H-ESI) ion source was employed. Corticosterone calibration curves were prepared in the 10–1000 pmol/ml range by spiking 2 μl of a DMSO stock solution containing the analyte mixture in 198 μl of charcoal treated rat plasma and processing the calibration standards following the same experimental procedure described for unknown samples. The limit of quantification was 10 pmol/ml for corticosterone. Calibration curves showed good linearity with the coefficients of correlation (r2) > 0.99 for all curves.

2.6.3. Behavioural analysis

Animals’ behaviour was analyzed using the Ethovision 6.0 software (Noldus, The Netherlands) by a trained experimenter blind to the experimental condition. For the behavioural analysis during the social approach/avoidance test, the social compartment was virtually divided into two zones of equal dimensions: proximal to and distal from the stimulus rat, as previously described (Carnevali et al., 2014; Nicolas and Prinsen, 2006). We calculated the time spent by the animals (i) in the non-social compartment, and (ii) in the proximal and distal areas of the social compartment (expressed as% of total time). For the behauioural analyses on day 1, day 4 and day 5, the home cage compartments belonging to DEM and OBS rats were virtually divided into two zones of equal dimensions (15 × 40 × 40 cm): proximal to and distal from the wire mesh partition that separated each pair. We calculated the time spent by the animals in the proximal and distal zones (expressed as% of total time), as well as the time during which the animals were engaged in the following three behavioural categories (expressed as% of total time): (i) freezing (head up, no body movements excluding those necessary for breathing), (ii) resting (small body movements including those associated with grooming or sniffing, or head down and no body movements excluding those necessary for breathing), and (iii) motion (moving or turning around).

2.6.4. Statistical analysis

Repeated measures data (ECG parameters on day 1 and 4, corticosterone levels) were analyzed with a two-way ANOVA, with “time” as a within-subject factor (11 and 2 levels, respectively) and “group” as a between-subject factor (stress and control). The time spent by the animals in the different zones of the social approach/avoidance apparatus (experiment 1) and their home cages (day 1, 4 and 5; experiment 2) was analyzed with group (OBS or DEM) × condition (STR or CTR) × zone factorial design ANOVAs. The behaviour of the animals on day 1, 4 and 5 (experiment 2) was analyzed with group (OBS or DEM) × condition (STR or CTR) × behaviour (freezing or resting or motion) factorial design ANOVAs. Follow-up analyses were
conducted using Student’s t-tests, with a Bonferroni correction for multiple comparisons for each outcome variable separately. A priori pair-wise comparisons were conducted using Student’s t-tests, after checking the equality of variance with a Levene’s test. Significance was set at p < 0.05.

3. Results

3.1. Experiment 1

3.1.1. Social approach/avoidance test

The behaviour of OBS and DEM rats during the social approach/avoidance test is depicted in Fig. 2. The ANOVA yielded significant group × zone (F(2,78) = 58.3, p < 0.01), condition × zone (F(2,78) = 6.1, p < 0.01), and group × condition × zone (F(2,78) = 4.0, p < 0.05) interactions. Specifically, follow up analyses revealed that STR DEM rats spent more time in the non-social compartment (t(16) = 5.5, p < 0.01) and less time in the zone proximal to the stimulus rat in the social compartment (t(16) = −8.1, p < 0.01) compared to CTR DEM counterparts (Fig. 2). Interestingly, also STR OBS rats spent more time in the non-social compartment (t(16) = 4.1, p < 0.01) and less time in the proximal zone of the social compartment (t(16) = −4.0, p < 0.01) compared to CTR OBS rats (Fig. 2). We found no differences in HR and HRV parameters between STR OBS and CTR OBS rats during the test (HR: CTR OBS = 430 ± 11 bpm vs. STR OBS = 439 ± 11 bpm; LF/HF: CTR OBS = 0.9 ± 0.4 ln(ms²) vs. STR OBS = 0.5 ± 0.3 ln(ms²); LF/HF = CTR OBS = 1.1 ± 0.1 vs. STR OBS = 1.3 ± 0.2).

3.2. Experiment 2

3.2.1. Social defeat/control procedure

The time course of HR and HRV changes in OBS rats during the social defeat/control procedure on day 1 and day 4 is presented in Fig. 3. Two-way ANOVAs for repeated measures yielded (i) significant effects of time (F(10,180) = 9.2, p < 0.01) and group (F(1,18) = 4.2, p < 0.05) for HR values, and (ii) a significant time x group interaction for HF values (F(10,180) = 5.6, p < 0.05) on day 1. Follow-up analyses revealed that, before the removal of DEM rats from their cages, mean HR and HRV parameters were similar between STR OBS and CTR OBS rats (Fig. 3A, C, E). Similarly, no group differences were observed in the HR and HRV responses to the removal of the DEMs (Fig. 3A, C, E). However, after the return of the DEMs following social defeat/control procedure, the magnitude of the HR response (evaluated as the area under the curve (AUC) with respect to the pre-return value) was significantly larger in STR OBS than CTR OBS rats (AUC STR OBS = 679 ± 118 bpm vs. AUC CTR OBS = 342 ± 98 bpm, t(18) = 2.2, p < 0.05). Specifically, absolute mean HR values were significantly higher in STR OBSs during the first 9 min after the reunification with their respective DEMs compared to CTR OBSs (min 18: t(18) = 3.3, p < 0.01; min 21: t(18) = 2.7, p < 0.05; min 24: t(18) = 2.2, p < 0.05) (Fig. 3A). Moreover, after the return of the DEMs, STR OBS rats showed significantly lower HF values (min 18: t(18) = −2.2, p < 0.05; min 21: t(18) = −3.2, p < 0.01) (Fig. 3C) and a significantly higher LF to HF ratio (min 18: t(18) = 2.2, p < 0.05) (Fig. 3E) compared to CTR OBSs. On day 4, STR OBS and CTR OBS rats showed similar values of HR and HRV parameters during the social defeat/control procedure (Fig. 3B, D, F).

The behaviour of OBS and DEM rats during the first 3 min after the return of the DEMs following social defeat/control procedure is depicted in Fig. 4. The ANOVA yielded a significant group × condition × zone interaction (day 1: F(1,72) = 6.6, p < 0.05; day 4: F(1,72) = 13.2, p < 0.01). In particular, STR DEM rats spent significantly less time in the zone proximal to the STR OBS’s compartment upon returning to the home cage compared to CTR OBS rats, both on day 1 (t(18) = −2.1, p < 0.05) and day 4 (t(18) = −2.2, p < 0.05), while STR OBS and CTR OBS rats spent a similar amount of time in the proximal and distal zones (Fig. 4A, B). No differences were observed in the amount of freezing behaviour, resting behaviour and motion between STR DEM and CTR DEM rats and between STR OBS and CTR OBS rats, both on day 1 and day 4 (Fig. 4A, B).

3.2.2. Presentation of the acoustic cue (day 5)

Behavioural responses of DEM and OBS rats to the presentation of the acoustic cue on day 5 are depicted in Fig. 5A. The ANOVA revealed that, before the acoustic cue was presented, STR OBS rats spent significantly less time in the zone proximal to the STR OBS’s
compartment compared to their respective CTR DEM counterparts ($t_{(18)} = -2.6, p < 0.05$). Moreover, freezing behaviour was significantly greater in STR DEM than CTR DEM rats ($t_{(18)} = 2.6, p < 0.05$).

There were no significant behavioural differences between STR OBS and CTR OBS rats during the first 3 min that followed the conditioned stimulus. Cardiac autonomic responses of OBS rats to the presentation of the acoustic cue on day 5 are represented in Fig. 5B. During pre-stimulus conditions HR and HRV parameters were similar between STR OBS and CTR OBS rats (HR: STR OBS = 351 ± 11 bpm vs. CTR OBS = 335 ± 6 bpm; HF: STR OBS = 1.3 ± 1.1 ln(ms$^2$) vs. CTR OBS = 1.6 ± 0.3 ln(ms$^2$); LF/HF: STR OBS = 0.8 ± 0.1 vs. CTR OBS = 0.7 ± 0.1). Similarly, there were no significant group differences in HR and HRV parameters during the first 3 min that followed the conditioned stimulus (Fig. 5B).

### 3.2.3. Corticosterone levels

Plasma corticosterone levels in OBS rats in baseline conditions (day-2) and on day 5 are represented in Fig. 6. A two-way ANOVA for repeated measures yielded a significant time x group interaction ($F_{(1,18)} = 4.7, p < 0.05$). Follow-up analysis revealed that baseline plasma corticosterone levels were similar between STR OBS and CTR OBS rats. On day 5, STR OBSs showed significantly higher plasma corticosterone levels compared to CTR OBSs ($t_{(18)} = 3.9, p < 0.05$) and the respective baseline (day -2) level ($t_{(18)} = 4.1, p < 0.05$) (Fig. 6).

### 3.3. Experiment 3

#### 3.3.1. Olfactory stimulus

HR responses of OBS rats to the presentation of the olfactory stimulus on day 1 and 4 are depicted in Fig. 7. There were no significant changes in mean HR values after presentation of the olfactory stimulus compared to the pre-stimulus phase, neither on day 1 nor on day 4 (Fig. 7). Similarly, no changes were observed in HRV parameters (data not shown).
3.3.2. Corticosterone levels

In OBS rats, plasma corticosterone levels on day 5 were similar to the baseline (day -2) levels (day -2 = 320 ± 28 nM vs. day 5 = 256 ± 37 nM).

4. Discussion

In this study we attempted to set up an experimental rat model of social stress contagion between one animal (designated as “demonstrator” (DEM)) and its cagemate social partner (defined “observer” (OBS)). Our major and novel findings are that cohabitation with a socially stressed DEM induced (i) social avoidance in a new social context, (ii) cardiac autonomic activation, and (iii) hypothalamic-pituitary-adrenal axis hyperactivity in the OBS.

In our experiments, OBS and DEM rats had been paired in the same cage for 20 days to achieve familiarity before the beginning of the repeated social defeat stress procedure. Our first objective was to investigate whether behavioural and cardiac autonomic responses of OBS rats would be influenced by previous sensory exposure to a socially stressed familiar DEM (experiment 1). As expected, following repeated episodes of social defeat, STR DEM rats exhibited higher levels of social fear and social avoidance directed toward an unknown conspeciﬁc rat (i.e., not the dominant Wild-type rat that had defeated them) compared to CTR DEMs. This result is an essential conﬁrmation that the social defeat procedure adopted here induced behavioural signs of social stress in STR DEMs. Strikingly, the behaviour of STR OBSs paralleled that of STR DEMs, with STR OBS animals clearly showing higher levels of social avoidance and social fear in a new social context compared to CTR OBSs. This is indicative of the fact that the social interaction with a stressed cagemate may provide valuable information about potential threats in the environment. In the last few years, studies in mice and rats have shown that the direct observation of a companion in pain or distress (i.e., animals received foot shock or an injection of a drug causing pain) induces similar behavioural changes (freezing or writhing behaviour, respectively) in the observer (Jeon et al., 2010; Langford et al., 2006). In addition to this, the observers are not only passively perceiving the distress of others but are capable of displaying prosocial...
beaviours apparently aimed at ameliorating the stressful condition of the cagemate. For example, rodents can intentionally free a cagemate trapped in a restrainer (Ben-Ami Bartal et al., 2011) or greatly increase grooming behaviour directed toward familiar conspecifics (but not strangers) that have experienced a stressor (Burkott et al., 2016). Importantly, in our study STR OBS rats did not directly witness the DEM being socially defeated and subordinated by an aggressive rat, but interacted with it in a familiar environment before and after each episode of social defeat. This indicates that emotional-state matching between observer and demonstrator rats can occur through social interaction in the absence of direct observation of the source of stress. Despite clear signs of social avoidance behaviour in STR OBS rats, we found that cardiac autonomic responses to the social approach/avoidance test (evaluated as means of the 10-min test) were relatively close proximity to the stimulus rat compared to CTR OBSs). This might avoidance test (evaluated as means of the 10-min test) were relatively close proximity to the stimulus rat compared to CTR OBSs). This might avoidance test (evaluated as means of the 10-min test) were relatively close proximity to the stimulus rat compared to CTR OBSs). This might have limited the revelation of potentially different patterns of autonomic activity between the two groups.

Motivated by these encouraging findings, we then explored cardiac autonomic and neuroendocrine correlates of emotional state-matching between STR OBSs and STR DEMs using a conditioned stimulus that was unpredictable (experiment 3). We found that cardiac autonomic responses to the social approach/avoidance test (evaluated as means of the 10-min test) were relatively unaffected by previous cohabitation with a stressed partner. Unfortunately, due to technical reasons we could not reliably assess HR and HRV parameters during the exploration of the different zones of the apparatus (i.e., HR might have been higher when STR OBSs were in close proximity to the stimulus rat compared to CTR OBSs). This might have limited the revelation of potentially different patterns of autonomic activity between the two groups.

In conclusion, our findings demonstrate that cohabitation with a socially stressed rat produces physiological and behavioural effects in the social partner. These results are consistent with previous findings suggesting that rodents are able to socially share a state of fear or distress experienced by others in order to appropriately respond to the threats of the environment (Atsak et al., 2011; Ben-Ami Bartal et al., 2011; Meyza et al., 2016; Panksepp and Lahvis, 2011). Such capacity is considered to be a basic form of empathy, in which the emotion directly experienced by an individual activates similar emotional states in the observer. Although this capacity has been long considered to be uniquely human, animal studies in nonhuman primates and more recently in rodents have shown that some forms of empathy, such as emotional contagion, are widespread among animals, including rodents. In interpreting our results, we must acknowledge strengths and limitations of this study. A strength is undoubtedly the use of a biologically relevant social stress paradigm, which represents a novel, intriguing approach in rodent empathy research. Secondly, while rodent studies in the field of affective contagion almost exclusively rely on behavioural parameters to assess social transmission of emotional states (Meyza et al., 2016; Panksepp and Lahvis, 2011), here the glucocorticoid synthesis blocker), might suggest that reduced freezing in observer rats does not necessarily indicate a disappearance in the affective response to the distress of the demonstrator (Carrillo et al., 2015).

The social transmission of emotional states could exploit different mechanisms and sensory channels. Social communication in mammals has evolved to facilitate reproductive behaviour and for protection against environmental threats and predation. Rodents may communicate their emotional state through ultrasonic vocalization (Jones and Monfils, 2016; Saito et al., 2016), visual (Kavaliers et al., 2001; Langford et al., 2006), and odour or pheromone cues (Bredy and Barad, 2009; Rottman and Snowdon, 1972), each with profound influences on empathic-like responses. For example, a recent study effectively demonstrated that the duration of negative affect-associated ultrasonic vocalizations (around 22 kHz), which were emitted by demonstrator rats during a fear conditioning paradigm, was positively correlated with the freezing behaviours displayed by observers the following day (Jones and Monfils, 2016). In addition, visual and olfactory cues are thought to play a primary role in mediating pain communication between paired animals (Langford et al., 2006; Smith et al., 2016). Our results ruled out a potential involvement of olfactory stimuli originating from the cage of dominant rats in triggering cardiac autonomic responses in OBS rats (experiment 3). However, we suppose that STR OBS rats acquired the stress state of their social partners also through the observation of distinctive patterns of behaviour. Indeed, we found that STR DEMs tended to avoid the zone proximal to the STR OBS’s compartment upon returning to their home cage following social defeat, a behavioural response that might indicate social anxiety. Moreover, when the conditioned stimulus was presented on day 5, it clearly elicited a fear response (i.e., freezing behaviour) in STR DEM rats, which once again tended to avoid the zone proximal to the STR OBS’s compartment. Here, we found no differences in cardiac autonomic and behavioural responses of STR OBS rats to the auditory cue, which further suggests an habituation-like effect following prolonged cohabitation with a stressed partner (Carrillo et al., 2015). On the other hand, STR OBS animals showed a strong elevation of plasma corticosterone levels after the presentation of the acoustic cue on day 5. Of note, this neuroendocrine response was not found in animals which had been simply exposed to olfactory stimuli originating from the cage of an aggressive, dominant rat (experiment 3). Our data do not clarify whether this increase in plasma corticosterone levels in STR OBS rats was triggered by social transmission of fear in response to the acoustic cue on day 5 or by prolonged cohabitation with a STR DEM, or a combination of both. Nevertheless, this finding suggests that the emotional state of the STR DEM somewhat permeated to the neuroendocrine stress response of the STR OBS rat.
investigation has been extended to cardiac autonomic and neuroendocrine readouts. Several questions, however, arise as a result of the findings reported here. Firstly, we did not precisely measure how the behaviour of STR DEM rats relates to the behaviour/physiology of STR OBS rats. Secondly, we did not accurately investigate the sensory modality(ies) through which emotional information is transferred from STR DEM to STR OBS rats. Thirdly, a major limitation is that we have no insight into the neurobiological mechanisms underlying the behavioural and physiological changes associated with the social interaction with a STR DEM. Finally, we did not examine whether behavioural and physiological correlates of social stress contagion in rats do differ according to familiarity, gender or age. In light of these considerations, we believe that future work with this rodent model may pave the way for a systematic investigation of these research questions.

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