Molecular Neurobiology (2019) 56:611–620
https://doi.org/10.1007/s12035-018-1053-5

Behavioral and Noradrenergic Sensitizations in Vulnerable Traumatized Rats Suggest Common Bases with Substance Use Disorders

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Received: 21 July 2017 / Accepted: 29 March 2018 / Published online: 12 May 2018

Abstract

The aim of the present study was to strengthen our hypothesis of a common physiological basis for post-traumatic stress disorder (PTSD) and substance use disorders. This paper investigates the possibility that rats exposed to a PTSD model exhibit noradrenergic and behavioral sensitization, as observed following repeated drugs of abuse injections. First, rats received a single prolonged stress (SPS), combining three consecutive stressors. They were then tested, 2 weeks after the trauma for PTSD-like symptoms to discriminate between vulnerable and resilient rats. When microdialysis was performed in the prelimbic cortex (Experiment 1), larger increases of noradrenaline (NA) release in response to amphetamine were observed in vulnerable rats when compared to control and resilient animals. Experiment 2 showed that trauma-vulnerable rats exhibited increases in locomotor activity relative to controls, in response to an exposure to trauma-associated cues. These data demonstrate that a single trauma exposure induces in vulnerable animals both, a noradrenergic sensitization evidenced within the prelimbic cortex and behavioral sensitization obtained after a physiologic activation of the noradrenergic system. However, Experiment 3 showed that when NA system was activated by amphetamine (1 mg/kg), a decrease in behavioral sensitization was obtained in vulnerable rats. We proposed that this decreased locomotor activity results from an additional stress-induced increased reactivity of mesocortical dopaminergic neurons, known to counteract the consequences of cortical noradrenergic release in rats. These results support our hypothesis that noradrenergic sensitization represents a common physiological basis, involved both in PTSD and drug addiction and suggest new common therapeutic approaches for these pathologies.

Keywords Post-traumatic stress disorder (PTSD) · Single prolonged stress (SPS) · Prefrontal cortex · Microdialysis · Monoaminergic uncoupling

Introduction

Post-traumatic stress disorder (PTSD) and substance use disorders (SUDs) are two pathologies due to exposure to extreme negative and positive outcomes that have a 30–50% comorbidity [1]. They have extensive common ground [2] and in particular their high sensitivity to environmental cues leading either to re-experiencing (PTSD) or craving (SUD); both known to be responsible for the high rates of relapse in these pathologies. Such a hyper reactivity to reminder cues can be considered as responsible for the development of associated symptoms like hyper-arousal, sleep disorders, aggressiveness, and avoidance of associated stimuli. We proposed that exposure to outstanding events, such as trauma or drugs of abuse, induces memory reactivation disorders, sustained by common physiological processes [3, 4].

Using an animal model of addiction, one of us showed that repeated exposures to psychostimulants and other drugs of abuse (amphetamine, cocaine, MDMA, alcohol, morphine, nicotine) in mice induced behavioral and neurochemical sensitization [5–7]. More precisely, Tassin and collaborators showed that these mice exhibited large increases of noradrenaline (NA) and serotonin (5-HT) releases within the prelimbic

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12035-018-1053-5) contains supplementary material, which is available to authorized users.

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part (PL) of the medial prefrontal cortex (mPFC), as well as large increases of the locomotor behavior, in response to a drug activating these systems [7, 8]. To account for these results, they proposed that after repeated drug injections, the reciprocal control exerted by noradrenergic and serotonergic systems was disrupted, leading to an uncoupling of these monoaminergic systems, accounting for their release increases. This neurochemical sensitization has been shown to be responsible, through projections to the ventral tegmental area (VTA) and the nucleus accumbens (NAc), for the locomotor sensitization also observed in these mice [9, 10].

The aim of the present paper was to investigate whether a traumatic stress can induce similar sensitizations (behavioral and neurochemical), in response to a psychostimulant injection. In fact, cross-sensitization between stress and drugs has been widely illustrated in the literature (for a review see [11, 12]), and this phenomenon give support to our hypothesis of common physiological processes between SUDs and PTSD. For instance, repeated psychostimulant injections have been shown to increase the locomotor activity, and to activate HPA axis and DA receptor expression in mPFC, in response to a stressor [13–15]. Conversely, chronic and acute stress (as social defeat or restraint stress) can induce increases in locomotor activity in response to a drug injection (amphetamine, cocaine, or alcohol) and dopamine neurotransmission, as well as c-fos activation in the amygdala and the nucleus accumbens [16–19]. However, the long-term consequences of a single severe stress on locomotor activity and on prefrontal noradrenaline releases have never been explored.

Using the Single Prolonged Stress (SPS; [20]), combining 2-h restraint stress, followed by a 20-min forced-swim and a loss of consciousness produced by CO_2 to model a traumatic stress, we showed earlier that some traumatized rats developed PTSD-like symptoms, as well as noradrenergic-dependent long-term behavioral sensitization, supporting our hypothesis [3]. Since then, we have developed a procedure that is able to reliably differentiate trauma resilient and vulnerable rats [21]. The aim of the present experiments was to test our hypothesis by investigating the possibility of obtaining in vulnerable rats exposed to a single prolonged stress (trauma):

(1) increases of NA release, using in vivo microdialysis within the prelimbic area (neurochemical sensitization) (Experiment 1).
(2) increases in locomotor activity (behavioral sensitization) following an exposure to trauma-related cues known to induce in human a noradrenergic activation as well as re-experiencing the trauma ([22, 23]; Experiment 2) and following an amphetamine injection used to activate the noradrenergic system (Experiment 3).

In all these experiments, rats were first behaviorally characterized before being exposed to the trauma, and then classified as vulnerable or resilient on the basis of PTSD-like symptom tests, 2 weeks after the SPS.

Material and Methods

Animals

The subjects were 81 male Sprague Dawley rats weighing 250–275 g upon arrival, from Harlan Laboratories, France. Rats were housed in pairs under a 12-h light/dark cycle (lights on at 07:30), with food and water available ad libitum. They were habituated to the colony room at least 14 days prior to the start of the experiment, then handled, numbered, and weighed. All efforts were made to minimize the number of animals used and their discomfort. All experiments were approved by the ethic committee CEE59 (Project number 2015-07) and in accordance with the European Communities Council Directive [2010/63/EU, 22 September 2010].

Single Prolonged Stress (SPS)

The trauma procedure was already detailed elsewhere (see SI and [21, 24]). Briefly, rats were restrained for 2 h, and then placed for 20 min in a water tank. Finally and after a 15-min rest, rats were enclosed in a small chamber saturated in CO_2, until loss of consciousness. Control rats (CTRL) were exposed to the same situation but without any stress (see SI for more information). In Experiment 2, an inhibitory avoidance with a weak electrical foot shock was delivered just before the SPS in order to provide a context predicting the occurrence of the trauma (see SI and [24]).

Behavioral Tests

At the beginning of each experiment, rats performed an elevated plus maze (EPM) to evaluate the level of anxiety and a corridor test to quantify the amount of locomotor activity.

After the SPS procedure, an EPM and a light-dark test was performed to evaluate the level of anxiety, as well as an acoustic startle response (ASR) test to evaluate the level of arousal (for more details see SI and [21, 24]).

Behavioral Characterization: Selection of Resilient and Vulnerable Rats

In Experiments 2 and 3, traumatized rats were separated into resilient and vulnerable on the basis of the results obtained on three main symptom tests: two exploring anxiety (EPM and light-dark), and one the arousal (acoustic startle response ASR). A rat was considered to demonstrate a PTSD-like symptom [25] when its performance differed by one standard deviation (SD) from the mean level of the performance

obtained by the CTRL group for the light-dark test and ½ SD for EPM and ASR. Rats exhibiting two or three symptoms were considered as *Vulnerable* (SPS-V), while the remaining rats were considered as *Resilient* (SPS-R). In Experiment 1, to reduce potential interference of the behavioral tests on NA release, the trauma vulnerability of rats was assessed on the basis of an EPM test performed before the microdialysis and confirmed by other tests delivered after (see SI for more details).

Experimental Procedures

In Vivo Microdialysis (Experiment 1)

The aim of Experiment 1 was to determine whether trauma may induce a neurochemical sensitization. In vivo microdialysis was used to quantify the noradrenergic release within the prefrontal cortex (see SI for more details). One week after the SPS, a permanent unilateral cannula was placed at the edge of the prelimbic part of the prefrontal cortex (Fig. S1). The microdialysis was performed on awake and freely moving animals, 20 days after the trauma. After the probe insertion, perfusate samples (20 μl) were collected every 20 min. The first five samples (100 min) were used to determine the basal extracellular NA level. Then, amphetamine (1 mg/kg, i.p.) was injected and samples were collected for 220 min. Dialysate samples were analyzed by a high-performance liquid chromatography (HPLC), performed with a reverse-phase column. Data from microdialysis were expressed as a percentage of the respective mean basal value.

Cue-Test (Experiment 2)

Experiment 2 investigated the possibility of observing behavioral sensitization after a re-exposure to trauma-associated cues thought to model re-experiencing in humans [22–24]. To that end, rats were exposed to a trauma reminder cue, a procedure thought to activate naturally the noradrenergic system [23, 26]. The locomotor activity was recorded for 40 min, during the basal phase, then each rat was transported to the SPS room and placed, for a 1-min period, in the safe white box of the inhibitory avoidance a cue known as a powerful trauma reminder. The locomotor activity was then recorded in the corridor for an additional 20-min period.

Sensitization Test (Experiment 3)

Experiment 3 explored the possibility to get behavioral sensitization in vulnerable rats when the noradrenergic system was activated by an amphetamine injection. Rats were first placed in a circular corridor (see SI for details) during 1 h (habituation phase). Rats then received a saline injection and were recorded during another 1-h period (basal phase). Finally, they were injected with a d-amphetamine solution (1 mg/kg, i.p.) before being recorded for a third hour (amphetamine phase). Their levels of locomotor activity were recorded every 10 min during each phases.

Statistical Analyses

Statistical analyses were performed with standard analyses of variance (ANOVAs), using VAR3 software, allowing planned comparisons [27]. Two-way ANOVAs were used with group as a main factor (CTRL/SPS-R/SPS-V) and time (duration of the test) as a within group factor (repeated measures) for microdialysis data (NA level; Experiment 1) and for locomotor activity experiments (number of ¼ turns; Experiments 2 and 3). One-way ANOVA was used with group as a main factor during the cue exposure test (percent of freezing; Exp 2). Pearson correlation coefficients were calculated between percent of freezing and locomotor activity (Experiment 2) using R software. Significant differences were set at $p < 0.05$.

Results

The aim of this study was to investigate whether rats exposed to SPS, an animal model of PTSD can demonstrate (1) a neurochemical sensitization (Experiment 1) and (2) behavioral sensitization after an exposure to a trauma-associated cue mimicking re-experiencing (Experiment 2) and after an amphetamine injection (Experiment 3).

1. Experiment 1: Neurochemical sensitization to amphetamine (Fig. 1)

In the first experiment, among the 14 rats exposed to the SPS, seven were identified as vulnerable (50%; see Supplemental Information, Results 1). Experiment 1 included three different groups: a control group (CTRL; $n = 14$), a SPS-resilient group (SPS-R; $n = 7$ but $n = 5$ after histological analysis) and a SPS-vulnerable group (SPS-V; $n = 7$).

At D20, the microdialysis probe was inserted within the medial prefrontal cortex, at the level of the prelimbic cortex. Samples were collected every 20 min, over a 320-min period, beginning 140 min after the probe insertion. Typical chromatograms of the HPLC analyses are shown on Fig. S2.

During the determination of the basal level of NA release, no between groups difference can be noted ($F < 1$). As illustrated in Fig. 1, the acute *d*-amphetamine (1 mg/kg) injection increased the cortical extracellular NA level in every group. Noradrenaline level increases began to be significant relative to the basal levels, 20–40 min after the amphetamine injection ($F(1–23) = 11.29$, $p = 0.002$). This effect was obtained for the three groups of rats (CTRL: $F(1–13) = 6.55$, $p = 0.02$; SPS-R:

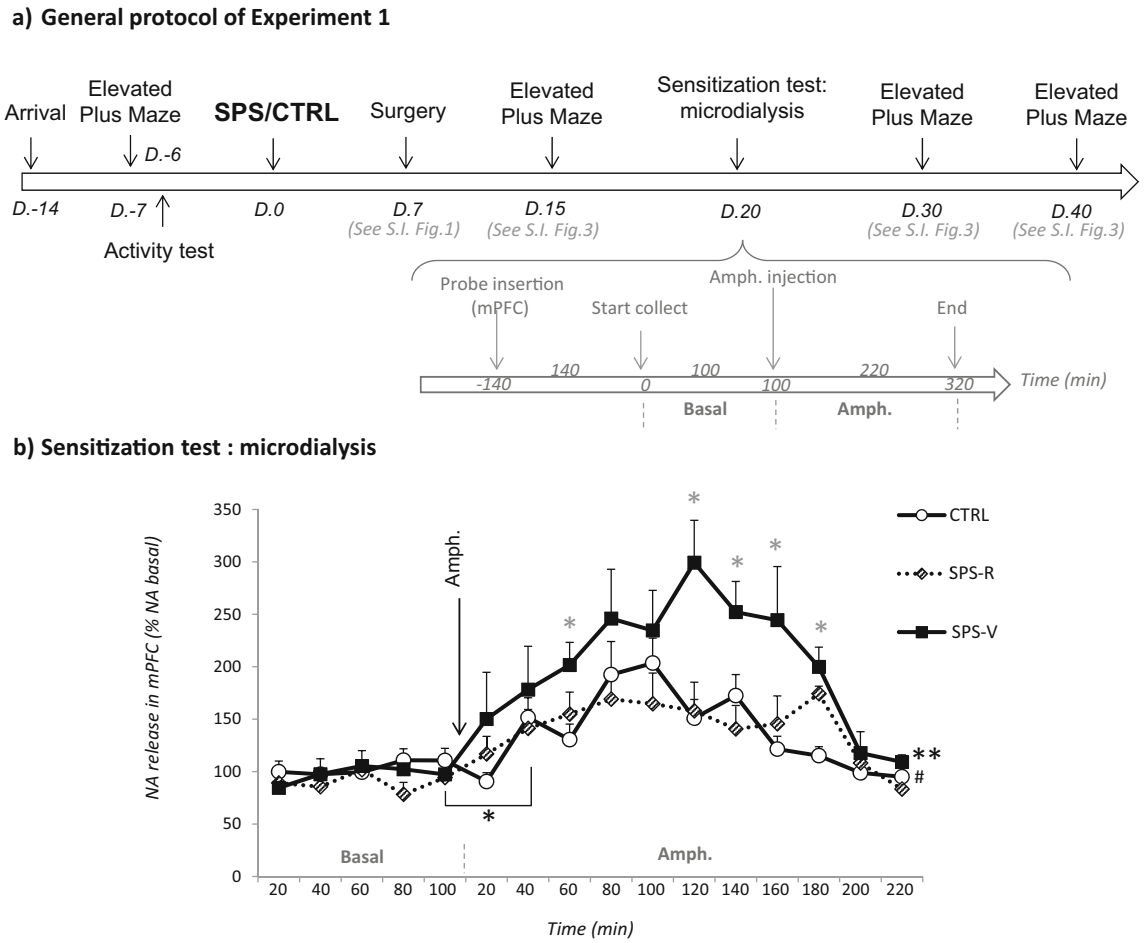

Fig. 1 Experiment 1. **a** Timeline of general protocol: Rats were behaviorally characterized 1 week before the trauma with an elevated plus maze test and with an activity test. At D0, rats received either the SPS or the CTRL procedure. One week later, a microdialysis cannula was inserted in the mPFC area (D7). Rats were determined as resilient or vulnerable (-R or -V) on the basis of an EPM test delivered at D15. During the sensitization test, which took place at D20, rats were placed in the microdialysis device, the probe was inserted and the sample began to be collected (after a 140-min period) every 20 min for 100 min before the amphetamine injection (1 mg/kg) and 220 min thereafter. **b** Noradrenergic release during the amphetamine sensitization test: Percent of NA basal level obtained in mPFC for CTRL, trauma-resilient (SPS-R), and vulnerable rats (SPS-V), during 100 min before and 220 min after the amphetamine injection. Data are expressed as mean ± standard error of the mean (SEM). *.05 > p > .01; **.01 > p > .001: SPS-V compared to CTRL; #.05 > p > .01: SPS-V compared to SPS-R. Gray stars correspond to a group effect obtained for a specific sample

F(1–4) = 8.80, $p = 0.04$; SPS-V: F(1–6) = 12.79, $p = 0.01$). The ANOVA analysis performed during the 220-min period taking placed after the amphetamine injection indicated a group effect, (F(2–23) = 4.21, $p = 0.02$), a time effect (F(8–184) = 6.35, $p < .001$, as well as a significant interaction between time and group (F(16–184) = 1.72, $p = 0.04$). Complementary analyses indicated that vulnerable rats exhibited higher increases in cortical extracellular NA level than CTRL (F(1–19) = 6.81, $p = 0.016$), and SPS-R rats (F(1–10) = 5.36, $p = 0.04$), with no difference between the last two groups (F < 1).

SPS-vulnerable rats demonstrated a neurochemical sensitization in response to an amphetamine injection.

2. Experiment 2: Behavioral sensitization to a trauma-associated cue

Experiment 2 investigated whether behavioral sensitization can be obtained in SPS-vulnerable rats after a trauma memory reactivation induced by a short exposure (1 min) to a SPS-associated cue, delivered 15 days after the exposure to the prolonged stress. In the present experiment, involving 21 rats, PTSD-like symptoms tests revealed that 6 among 11 traumatized rats were classified as vulnerable (55%). Three different groups were considered: a control group (CTRL; $n = 10$), a SPS-resilient group (SPS-R; $n = 5$), and a SPS-vulnerable group (SPS-V; $n = 6$).

During the one-min exposure to the predictive cue (i.e., the safe compartment of the inhibitory avoidance apparatus), a group effect was obtained on the percent of freezing (F(2–18) = 18.56, $p < .001$), with both SPS groups showing significantly more freezing than the CTRL group (SPS-V: F(1–14) = 49.56, $p < .001$ and SPS-R: F(1–13) = 10.31, $p = 0.006$,

respectively). SPS-V group further showed more freezing than SPS-R group, although the difference felt just short of significance ($F(1–9) = 4.05$, $p = 0.07$).

In order to determine behavioral sensitization, rats were placed in the corridor test, before and after the exposure to the SPS predictive cue (Fig. 2). During the 40-min preceding the cue exposure, the level of activity significantly decreased ($F(5–90) = 113$, $p < .001$), with no group effect, even during the 10-min period following the saline injection. Exposure to the predictive cue resulted in a significant increase in the locomotor activity relative to the pre-exposure period ($F(1–18) = 17.27$, $p < .001$). This effect was significant for the SPS-V rats ($F(1–5) = 9.07$, $p = 0.04$) but did not reach a significance level for the other two groups. The analysis of the activity scores obtained during the first 10 min following the cue exposure indicated a group effect ($F(2–18) = 3.75$, $p = 0.042$). SPS-V group showed higher activity scores than the two other groups, but the difference was significant only with the CTRL rats ($F(1–13) = 9.05$, $p = 0.009$), and not with SPS-R rats ($F(1–9) = 2.37$, $p = 0.15$). Moreover, SPS-V rats exhibited a strong correlation between the level of activity after the cue exposure and the level of freezing during the cue exposure (Fig. 2b, $r = 0.78$, $p = 0.05$), a result not obtained for the other two groups (CTRL: $r = 0.16$, ns; SPS-R: $r = -0.5$, ns, see supplemental information), indicating that the relationship between these two indexes was restricted to vulnerable rats.

Our data indicated that SPS-vulnerable rats demonstrated an increase of locomotor activity relative to control rats after being shortly exposed to trauma-related cues, known to activate the noradrenergic system and thought to produce re-experiencing in human. This result was not observed after an exposure to a short stressor not related to the trauma, such as the one induced by the saline injection.

3. Experiment 3: Behavioral sensitization to amphetamine

In this experiment, behavioral sensitization was expected in SPS-vulnerable rats in response to the activation of the noradrenergic system by an amphetamine injection. Among the 32 rats of this experiment, 11 over the 22 rats exposed to the prolonged stress were identified as vulnerable after the three PTSD-like symptoms tests. Three different groups were thus considered: a control group (CTRL; $n = 10$), a SPS-resilient group (SPS-R; $n = 11$) and a SPS-vulnerable group (SPS-V; $n = 11$).

As depicted in Fig. 3, behavioral sensitization was investigated 20 days after the SPS. An ANOVA performed on the locomotor activity during the basal phase indicated no groups effects ($F(2–29) = 1.44$, ns), a significant effect of Time due to the progressive habituation ($F(5–145) = 24.13$, $p < .001$), and no interaction between these two factors ($F < 1$). However, after the amphetamine injection, the ANOVA performed on the number of ¼ turns indicated a significant group effect ($F(2–29) = 3.08$, $p = 0.05$), as well as a significant time effect ($F(5–145) = 23.02$, $p < .001$), with no interaction between these two factors ($F < 1$). Planned comparisons during the amphetamine phase showed that SPS-V rats demonstrated locomotor activity significantly lower than CTRL ($F(1–19) = 5.83$, $p = 0.024$), and SPS-R rats ($F(1–20) = 4.26$, $p = 0.049$), with no difference between these latter two groups ($F < 1$).

Contrary to what was expected, SPS-vulnerable rats demonstrated a behavioral sensitization significantly lower (and not higher) that the one observed in the other two groups, an effect that we termed “decrease in behavioral sensitization”.

Discussion

We have proposed that the high susceptibility to environmental cues characterizing PTSD and SUD might be viewed as memory reactivation disorders resulting from the exposure to outstanding events thought to induce an “uncoupling” of monoaminergic systems [2]. Supporting our hypothesis, the present results showed that neurochemical sensitization, such as already demonstrated within cortical neurons located in the prelimbic area obtained after repeated exposures to a psychostimulant [5, 7, 8], can also be observed after a single prolonged stress exposure, used to model a traumatic stress. More precisely, we showed a dramatic increase in prelimbic cortical extracellular noradrenergic level, restricted to SPS-vulnerable rats, in response to the activation of the catecholaminergic systems by a d-amphetamine injection, examined 2 weeks after the trauma. For Tassin and his collaborators, the hyper-reactivity of the noradrenergic neurons is associated with a simultaneous and persistent desensitization of somatodendritic alpha2 A-adrenergic auto-receptor function thought to be responsible for the massive NA releases in the mPFC [5–8, 28]. Here we show for the first time that a similar and durable sensitization of noradrenergic neurons can be reproduced at least in some rats exposed to a single prolonged stress exposure. Moreover, since NA cortical releases in resilient rats were undistinguishable from those observed in control rats, the mechanisms underlying this sensitization must be linked to the vulnerability to trauma.

After repeated drug exposures, Tassin and collaborators [5] reported increases of the locomotor activity and behavioral sensitization, in response to amphetamine. As this behavioral sensitization was correlated with the NA release in mice [8], and disappeared when α1b-adrenergic receptors are blocked [29], behavioral sensitization has been considered as mainly resulting from the activation of the NA system, even though the involvement of the dopamine, serotonin, and glutamate systems cannot be entirely excluded. In the present paper, we also investigated the possibility that a single prolonged stress exposure induced long-term behavioral sensitization, similar to repeated drug exposures, as was already shown after

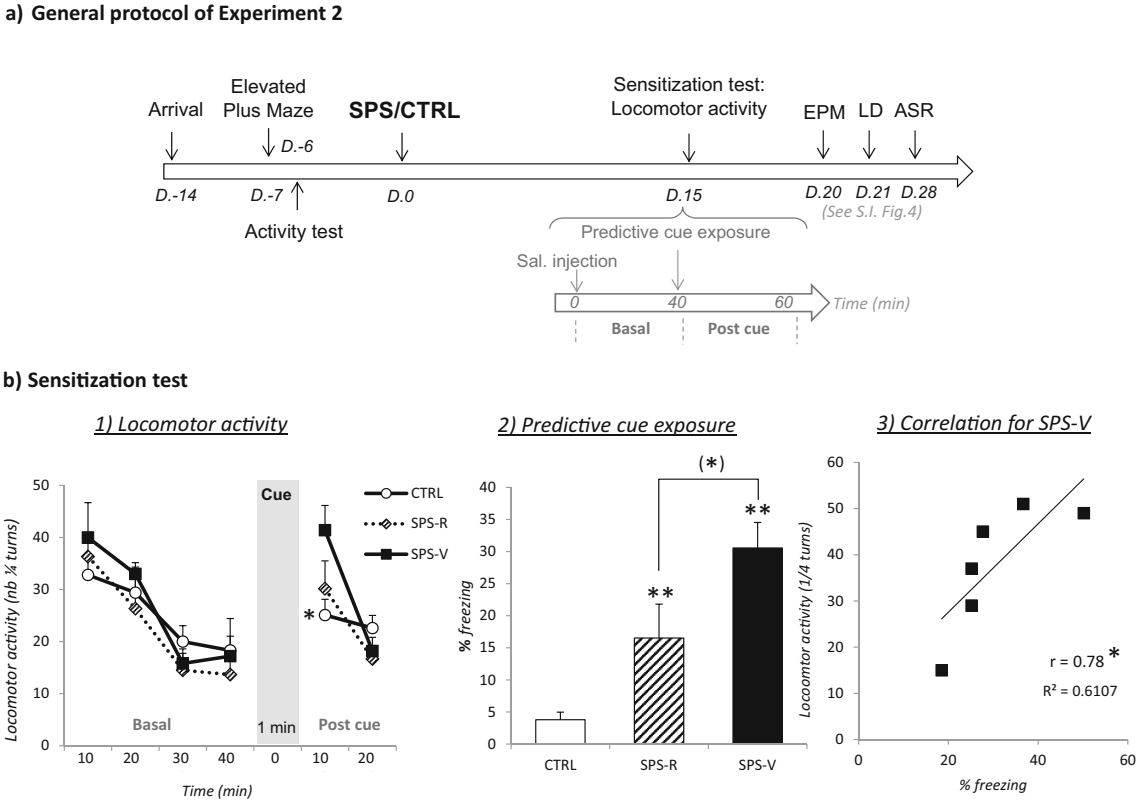

Fig. 2 Experiment 2. **a** Timeline of general protocol: Rats that were behaviorally characterized received the trauma or the control procedure at D0. The behavioral tests performed between D20 and 28 were used to determine the resilience to the trauma. The cue sensitization test took place at D15. Rats received a saline injection before being placed in the corridor for 40 min (basal). They were then placed in the predictive box for 1 min before being placed back in the corridor for an additional 20-min period. Their locomotor activities were recorded every 10 min. **b** Locomotor activity during behavioral sensitization test with exposure to a trauma-cue: (1) *Locomotor activity* (number of ¼ turns) obtained before and after the cue exposure (10-min blocks) for CTRL and SPS-R and SPS-V. (2) *Exposure to the predictive cue:* percentage of freezing obtained during the 1-min exposure to the predictive cue. (3) *Correlation* between the level of freezing obtained during the cue exposure and locomotor activity developed after the cue exposure in SPS-V rats. Data are expressed as mean ± standard error of the mean (SEM). *.05 > p > .01; **.01 > p > .001: SPS-V compared to CTRL

chronic stress [16–18]. Our results indeed indicate that 2 weeks after the trauma, SPS-vulnerable rats demonstrated behavioral sensitization, when the noradrenergic system was stimulated by a short exposure to SPS-associated cues used as a model for re-experiencing in humans. It is noteworthy that we already reported similar results in some rats exposed to repeated and massed amphetamine injections, in response to an exposure to reminder cues [30]. Hence, exposure to drug and SPS reminders are both able to sustainably induce the expression of behavioral sensitization suggesting that they both stimulate the noradrenergic system. This finding is further supported by previous studies demonstrating that increases of retention performance resulting from a pre-test exposure to reminder cues can be mimicked by increases of NA release [31]. It has also previously been shown that blocking NA release during amphetamine and trauma exposures prevent the sensitization developments [3, 32]. Taken together, these findings reinforce our hypothesis postulating similarities between drug and trauma consequences.

However, contrary to what was expected, an opposite effect was obtained in response to a d-amphetamine injection, since SPS-vulnerable rats exhibited a large decrease in behavioral sensitization. Opposed results of that kind, i.e., a behavioral sensitization after exposure to reminder cues and a decrease in behavioral sensitization in response to a d-amphetamine injection [30] has already been shown in rats that were exposed to repeated and massed amphetamine injections. It should be noted that after a reminder exposure, the noradrenergic activation seems to be relatively weak since the locomotor activity was increased over a relatively short period of time (the first 10 min). This effect contrasts with that obtained in response to a d-amphetamine injection, for which increases over more than a 1-h time period (present results and [30]) were reported, suggesting that the noradrenergic system was more strongly activated by the psychostimulant than by an exposure to trauma reminders. Decreases in behavioral sensitization have already been reported in SPS rats in response to cocaine [33], as well as in rats exposed to repeated amphetamine

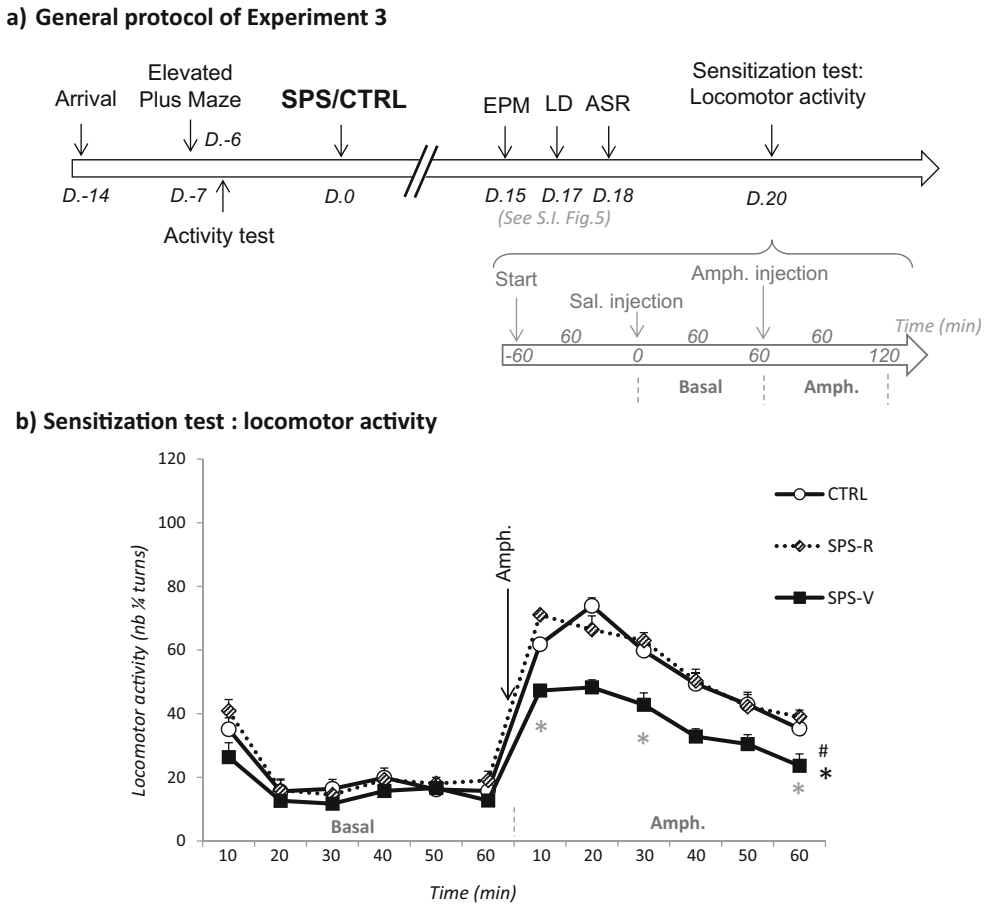

Fig. 3 Experiment 3. **a** Timeline of general protocol: Rats were first behaviorally characterized with an elevated plus maze test and with an activity test. The SPS or the control procedure was performed at D0. Three behavioral tests were performed between D15 and D18 to identify resilient and vulnerable rats (-R or -V). During the sensitization test which took place at D20, rats were placed in the corridor test for 1 h. They then received a saline injection and were replaced in the test for 1 h and finally received an amphetamine injection (1 mg/kg) before being recorded for an additional 1-h period. **b** Locomotor activity during behavioral sensitization test with amphetamine: Number of ¼ turns obtained by blocks of 10 min for SPS-R, SPS-V, and CTRL rats, for 1 h after a saline injection (basal) and for 1 h after the amphetamine injection (1 mg/kg, i.p.). Data are expressed as mean ± standard error of the mean (SEM). *.05 > p > .01: SPS-V compared to CTRL; $^{\#}$.05 > p > .01: SPS-V compared to SPS-R. Gray stars correspond to a group effect obtained for a specific block of time

treatments [34, 35]. These latter results strengthen the similarity of the effects induced by drugs of abuse and trauma, and suggest common processes in both situations. Obviously, decreases in locomotor activities during the amphetamine test could result from the occurrence of stereotyped behaviors, but, this seems unlikely for several reasons. First, we did not notice any stereotypies in our rats. Similarly, no more stereotyped responses have been reported in SPS rats, after a single methamphetamine challenge using a stronger dose than ours (2.5 mg/kg; [36]). Second, in a previous study [3], we showed that SPS rats demonstrating less locomotor activity than control rats, in response to a single amphetamine injection, showed progressive increases in their motor activity with additional amphetamine injections. This finding challenges the stereotypy hypothesis since stereotypies are known to increase with additional drug injections. Finally, none of the previously cited studies reporting decrease in locomotor activity after a psychostimulant administration occurring after SPS or repeated amphetamine exposures have considered stereotypies as a possible explanation, without, however, providing a clear explanation.

It remains the case, however, that after a d-amphetamine injection, a lower locomotor activity was obtained in vulnerable rats, while this effect was not obtained in mice, after repeated drug injections. This is probably due to a particular effect of stress on monoamine cortical releases. As previously mentioned, repeated consumption in mice of a drug of abuse, like cocaine, amphetamine, and alcohol, has been associated with both cortical noradrenergic and behavioral sensitization. Moreover, it has been established that cortical noradrenergic releases resulting from repeated drug exposures induce secondary increases of dopamine release within the accumbens nucleus [37] known to be responsible for the locomotor hyperactivity [38]. However, such an explanation might be modified when repeated drug exposures are replaced by stress in rats. Indeed, stress is well known to induce noradrenergic

release in the prefrontal cortex [39–41]. Some studies, using chronic stress, reported not only a cortical noradrenergic release, but also an additional cortical dopamine release due to the activation of mesocortical dopaminergic (DA) neurons [42, 43]. Interestingly, mesocortical DA activations induced by electrical stimulation of the ventral tegmental area (VTA) are known to induce a marked inhibition of the spontaneous activity of prefronto-cortical cells [44]. At the opposite, deletion of cortical dopaminergic innervation was shown to be correlated with an increased locomotor activity suggesting that mesocortical DA could exert an inhibitory role on the locomotor behavior [45]. We propose that in our conditions, where rats are exposed to a prolonged trauma experience, the amphetamine injection in vulnerable rats not only induced a noradrenergic release, as assessed by the results obtained in Experiment 1, but also a dopaminergic cortical release [43]. This last one counteracts the NA activation, thus leading to a reduced activity of the nucleus accumbens cells [29] and accounting for the decreased locomotor activity observed in vulnerable rats when compared to resilient rats. This hypothesis may further account for the lack of agreement between the time course of sensitization and the decrease of locomotor activity that began after the increase of noradrenergic release. Such a view may also account for the decreases in locomotor activity obtained in rats after massed exposures to psychostimulant reported in a previous study, a condition known to be more stressful than distributed exposures ([30]; see also [34, 35]). Obviously, this hypothesis has, however, to be confirmed by complementary experiments before being adopted.

Conclusion, Limitations, and Perspectives

The present results provide several additional clues strengthening the similarity between PTSD and SUDs. We show that similarly as after repeated exposure to drugs of abuse, exposure to a single prolonged stress has long-term consequences on the noradrenergic system in vulnerable rats, increasing the reactivity of noradrenergic transmission and inducing a stable sensitization of noradrenergic neurons that is still present more than 2 weeks later.

We also show that exposure to SPS-related cues is able to reveal behavioral sensitization, similar to the exposure to drug-associated cues [30, 46]. All these findings constitute strong evidence supporting the idea that trauma and addiction rely on some common physiological processes: an uncoupling of noradrenergic and serotonergic systems. We must acknowledge that our experiments only concern the noradrenergic system and not the serotonergic one and that additional experiments are required to establish this point.

Obviously, drug addiction and PTSD are two different pathologies that cannot be considered as the same. However, these pathologies are characterized by striking similarities, namely their hyper-reactivity to drug and trauma-associated cues that may be accounted by some similar processes. We propose that in both cases, exposure to extreme events such as a trauma or a drug of abuse breaks the inhibitory control that the noradrenergic and the serotonergic systems exert on one another, inducing hyper-reactivity of both noradrenergic and serotonergic neurons. Such an uncoupling could account for the hyper-reactivity of vulnerable rats to drug and trauma-associated cues, inducing craving and re-experiencing, respectively, which are responsible for the high rates of relapse in these pathologies. In line with these data, we recently started to explore new therapeutic approaches. The first approach is an attempt to recouple the monoaminergic systems. Indeed, it has recently been shown that delivering a combination of alpha1b and 5HT2A receptor antagonists in alcohol-dependent mice blocked behavioral sensitization to amphetamine and reversed their alcohol preference [47]. The aim of the second strategy is to modify the emotional valence of the trauma/drug memory in order to reduce the consequences of drug/trauma reminders. Presently, such an emotional remodeling, combining a pharmacological treatment with memory reactivation, has been shown to be effective in traumatized rats [4], as well as in cocaine addicted humans [48].

Acknowledgments We are very grateful to Professor D. C. Riccio for the correction of the English text. We thank Aurelie Bonilla, Céline Dubois, and Fabien Lhéricel for the animal care.

Funding This work was supported by “les Gueules Cassées” foundation (grant number 45-2017). Claire Le Dorze was supported by a DGA-MRIS (Direction Générale de l’Armement) scholarship.

Compliance with Ethical Standards

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Conflict of Interest The authors declare that they have no conflict of interest.

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