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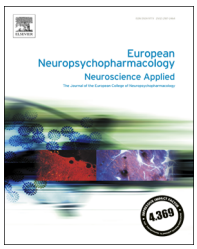

Repeated amphetamine injections alter behavior and induce a delayed behavioral sensitization modulated by reactivity to novelty: Similarities and differences with trauma consequences

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Abstract
Supporting our hypothesis of common biological bases for post-traumatic stress disorder (PTSD) and addiction, we recently reported that rats exposed to a single prolonged stress (SPS), a PTSD model, develop a delayed behavioral sensitization of the noradrenergic system, similar to that observed in mice after four repeated drug administrations. However, sensitization after trauma was modulated by reactivity to novelty, and this aspect that had not been explored in the addiction model. The first aim of the paper was thus to investigate the influence of reactivity to novelty on delayed behavioral sensitization in rats after four repeated amphetamine injections. Injections were either distributed over 4 days, as conducted in mouse models of addiction, or massed during a single session, reproducing SPS conditions. The second aim was to investigate whether repeated amphetamine injections have similar behavioral consequences to those induced by PTSD.
Our results showed that massed amphetamine injections induced more anxiety than distributed injections, and led to avoidance of drug-associated cues avoidance, while distributed injections somewhat reduced the startle response, such as is seen in SPS. In addition, massed amphetamine injections induced a delayed behavioral sensitization clearly affected by the reactivity to novelty, reproducing results observed following exposure to traumatic events. Finally, all rats receiving repeated amphetamine injections exhibited a behavioral sensitization in response to exposure to drug-associated cues. Taken together, these data strengthen the position that drug addiction and PTSD share some common mechanisms that we tried to clarify in this paper.

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1. Introduction

Dependence on drugs of abuse and posttraumatic stress disorder (PTSD) both result from exposure to extreme stimuli. Both are associated with vivid re-experiences generally triggered by environmental cues associated with the trauma or drug taking. In both cases, the revival of these episodes maintains the pathologies and may precipitate relapses even after long periods of remission. These elements, combined with a high frequency of comorbidity between PTSD and drug addiction, suggest common mechanisms possibly underlying the dramatic increase in susceptibility to reminder cues in these two pathologies (Gisquet-Verrier, 2009). In a model of drug abuse using repeated injections of a psychostimulant (Steketee and Kalivas, 2011), noradrenergic dependant behavioral sensitization (increases in locomotor activity) and neurochemical sensitization (increases in noradrenaline and serotonin release) were demonstrated a month later, in mice tested with an amphetamine injection (Lanteri et al., 2008; Salomon et al., 2006). It has been proposed that both outcomes may result from the loss of control that noradrenergic and serotonergic systems may exert on each other (also termed uncoupling; See Tassin, 2008; Doucet et al., 2013). We proposed that this uncoupling could be the common mechanism responsible for the increased sensitivity to retrieval cues (Toledano et al., 2013). Support for our hypothesis was obtained with the single prolonged stress (SPS), a model of PTSD (Liberzon et al., 1997). Here, rats showed numerous behavioral alterations reproducing PTSD symptoms, as well as a noradrenergic dependent behavioral sensitization demonstrated two weeks later in response to a single amphetamine injection (Toledano et al., 2013). One principle difference in these two models is that following repeated drug injections, behavioral sensitization was observed in all mice, while behavioral sensitization following trauma was only observed in rats showing a weak response to a novel environment, (low responders or LR). In contrast rats that showed high responses (HR) to a novel environment showed behavioral desensitization, with lower motor activity than their respective controls.

Individual differences between high and low responders is known to modulate the sensitivity to drugs of abuse (Piazza et al., 1989). However, although experiments have studied the involvement of high and low responders during initiation (1st injection) and development (successive injections) of behavioral sensitization (Hooks et al., 1991), to date there are no studies that have investigated the role of this individual difference in the long term expression of behavioral sensitization induced by repeated drug exposures, which is critical for addiction models. Obtaining this information was the first aim of the present study.

The second aim was to determine whether repeated drug injections induced behavioral alterations similar to those induced by trauma. As the main difference between drug and trauma studies rely on the way drugs are administered (distributed exposure for the drug versus massed exposure for the trauma), the consequences of four repeated amphetamine injections when delivered either in a single day (massed) or over four consecutive days (distributed) were studied. If, according to our hypothesis, the uncoupling of the monoaminergic systems induces hypersensitivity to drug-related environmental cues, then exposure to these cues should induce monoamine release and thus affect the subsequent motor activity. This possibility was explored in a final experiment.

2. Experimental procedures

2.1. Animals

Eighty male Sprague Dawley rats (Charles River Laboratories, France) weighing 250–275 g upon arrival were used in these experiments. They were housed in pairs under a 12-h light/dark cycle (lights on at 07:30), with food and water freely available. They were habituated to the colony room at least 6 days prior to the start of the experiments, during which time they were handled, numbered and weighed. All efforts were made to minimize the number of animals used and their discomfort. All experiments were performed in accordance with the European Communities Council Directive [2010/63/EU, 22 September 2010].

2.2. Drugs

D-amphetamine sulfate (Sigma-Aldrich, Saint Quentin Fallavier, France) was dissolved in saline (.9%) before use. Amphetamine was injected intraperitoneal (i.p., 1 mg/kg/ml) with the drug expressed as the weight of the salt.

2.3. Behavioral protocols

2.3.1. Video tracking system

An ANY-maze™ (Stoelting Co, Wood Dale, USA) flexible video tracking system was used for automated scoring in the elevated plus maze, Light/Dark exploration and avoidance of a drug related odor. A video camera was placed above the apparatus and information was relayed to a monitor in an adjoining room to score behavior in real time.

2.3.2. Elevated Plus Maze test

The Elevated Plus Maze (EPM) test was used to determine the level of anxiety (Walf and Frye, 2007). The apparatus, constructed of black PVC, was 60 cm above the floor and placed in a dimly lit room. Four arms (50 cm long and 11 cm wide, two open and two closed with 50 cm high walls) formed a cross with two arms of the same type facing each other. At the start of the experiment the animal was placed in the center of the maze facing a closed arm. When 80% of the animal's full body entered each arm, it was considered an entry, while an exit was defined as 70% outside the area. At the end of a 5 min period, rats were returned to their home cages and the plus maze was cleaned with a 70% ethanol solution. The relative percentage of time spent in the open arms [time in the open arms/(time open arms+closed arms)*100] was used as an index of anxiety. For each cohort, the median score of the index of anxiety was determined, based on the scores for each rat.

2.3.3. Locomotor activity tests

Eight concentric circular corridors (each 14 cm) were constructed of black Plexiglas sidewalls and a stainless steel running platform (Imetronic, Pessac, France). Four infrared beams were placed 5 cm above the floor, every 90°, and locomotor activity was counted when animals interrupted two successive beams; thus traveling a quarter of the circular corridor. The number of quarter turns, recorded via a computer fitted with appropriate software, was used as an index of the locomotor activity.

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