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Quasi-asymptotic development of conditioned hyperactivity induced by intermittent injections of cocaine in C57BL/6J mice

Christian Brabant, Sophie Tambour, Ezio Tirelli*

Behavioural Neuroscience and Psychopharmacology Laboratory, University of Liège, Boulevard du Rectorat 5/B-32, B-4000 Liège, Belgium

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Abstract

The emergence of a conditioned cocaine-induced hyperlocomotion was examined in C57BL/6J mice using a procedure that has not been used previously. Two days after a session of preexposure to the test chambers under saline, a first group of mice (cocaine-cued) received five once-daily injections of 10-mg/kg sc cocaine every other day (on the odd days of the chronic treatment period) and a saline injection on the 5 days following each cocaine injection day (on the even days of the treatment period), in all cases before being placed in the test chamber. Another group of mice (saline-cued) received 10 injections of saline on both the even and the odd days in the same context, and a third group of mice (cocaine-uncued) received five injections of saline on the even days in the test context and five injections of cocaine on the odd days in an alternative context. On the odd days sessions, the cocaine-cued group showed significant repeated increases in locomotion without behavioural sensitisation being induced, whereas the saline-cued levels of locomotion remained on baseline levels. On the first even session, the three groups did not differ from each other and showed lower levels of locomotion than on the preexposure session. During the two following even sessions, the cocaine-cued group showed an increase in locomotion that levelled off on the two remaining sessions, whereas the saline-cued and the cocaine-uncued groups (which presented comparable values) exhibited significantly lower levels of locomotion. That pattern of successive placebo responses resembles the typical S-shaped development of a Pavlovian conditioned response, albeit the increase described here was quite rapid. The protocol used here may provide an additional method for the experimental analysis of stimulant-induced conditioned placebo activity.

Keywords: Cocaine; Conditioned drug effects; C57BL/6J mice; Excitatory conditioning theory of sensitisation; Placebo response

1. Introduction

Many studies in rats and mice have shown that the presentation of contextual cues that had been repeatedly associated with drug administration such as cocaine or amphetamine can elicit the stimulant effects approximating those produced by the drug. This phenomenon has been most commonly proposed to result from classical conditioning (Ader, 1997; Stewart and Eikelboom, 1987; Cunningham, 1993; Stewart and Badiani, 1993). According to that view, the drug is thought to serve as the unconditioned stimulus (US), the initial drug effect as the unconditioned response (UR) and the test environment as the conditioned stimulus (CS). With the repeated injections of the drug, the test environment comes to evoke a conditioned drug-like effect on its own, namely the conditioned response (CR). Amongst the several procedures used to generate such a response, the most popular is probably the one based on the ''discriminative design'', which comprised three main experimental conditions (Beninger and Hahn, 1983; Cunningham, 1993; Crombag et al., 2000; Damianopoulos and Carey, 1992; Drew and Glick, 1988; Mattingly et al., 2000; Tilson and Rech, 1973; Tirelli and Terry, 1998). One group of animals receives the drug just prior to being placed in the test context and saline a certain time after completion of the session, prior to being returned to his home cage (or being placed in an alternative environment, the ''third world'', and then returned to the animal room). A second group of animals received the converse treatment: saline before testing and the drug in the animal room a certain time after completion of the test session. A third (control) group receives vehicle injections in both pre- and posttesting environments. The conditioned effects are evidenced on a

* Corresponding author. Tel.: +32-4-366-2018; fax: +32-4-366-2859.
E-mail address: ezio.tirelli@ulg.ac.be (E. Tirelli).

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saline challenge test session in the drug-paired context. However, that procedure is not without disadvantages and pitfalls. For instance, it has been demonstrated that the familiarity with (or the degree of novelty of) the home cage in which the animal is usually placed after drug injection, markedly attenuates the amplitude of the stimulant effect of amphetamine or cocaine in rats, an effect that is susceptible to influence the ulterior behavioural responsiveness to saline (Robinson et al., 1998). That responsiveness also depends upon the physical and social characteristics of postinjection contexts used during repeated administration of the stimulant (Battisti et al., 2000; Willner et al., 1992; Kuribara, 1997). Perhaps more importantly, the most used conditioned druglike effect procedures provides no useful information on the curve of acquisition of the CR, a representative feature of Pavlovian conditioning (Kehoe and Macrae, 2002). In fact, the minimal number of trials yielding a postdrug significant CR is usually unknown. A few studies have attempted to generate an acquisition curve using a protocol involving conditioning (saline challenge) tests inserted between successive blocks of several once-daily injections of stimulant (Pickens and Crowder, 1967; Pickens and Dougherty, 1971; Ahmed et al., 1995; 1996). Unfortunately, the size of the CR induced after each block of injections remained more or less unchanged over the intermittent trials, with no asymptotic-like acquisition. It is probable that the number of injections within the first block and the stimulant dose were too high for initial CRs to be moderately induced, thereby impeding or masking any ulterior graded increment (sort of ceiling effect).

In this study, we obtained an increment of the successive placebo responses (interpreted as CRs) presenting some of the features of a typical asymptotic learning curve. That acquisition curve was generated in C57BL/6L mice that were challenged with saline 24 h after each of five successive once-daily injections of cocaine (cocaine was given on the odd days and saline on the even days in a period of 10 once-daily test sessions). It is noteworthy that C57BL/6J mice were used because these respond to cocaine with a relatively little individual variability and usually show unambiguous conditioned locomotion (in comparison with the out-bred strains examined in our laboratory).

2. Materials and methods

2.1. Subjects

Twenty-four experimentally naïve male C57BL/6J mice (Charles River Laboratories, Brussels, Belgium), aged 7–8 weeks at the beginning of the experiments, were housed in groups of four in white polyethylene cages [26 × 40.5 (surface) × 20 (height) cm] with pine sawdust bedding and provided with free access to tap water and food (standard pellets, Carfil Quality Bvda, Oud-Turnhout, Belgium). The housing room was maintained on a 12:12-h dark–light cycle (lights on at 07:30 h) and an ambient temperature of 19–22 °C. Animal maintenance and treatments were conducted conformably to the standards of animal welfare adopted by the European Communities Council (Directive No. 86/609 of 24 November 1986).

2.2. Pharmacological treatment

(−)-Cocaine hydrochloride (Belgopia, Louvain-La-Neuve, Belgium), dissolved in an isotonic saline solution (0.9% NaCl) at a volume of 0.01 ml/g of body weight, was injected into the nape of the neck at a dose of 10 mg/kg. Saline injections were given on the basis of the same volume.

2.3. Apparatus

Mice were individually tested in 10 test chambers, each one essentially consisting of a square enclosure made of 0.5-cm clear Plexiglas tablets, without base [internal dimensions 20.5 × 20.5 (surface) × 28 (height) cm]. An enclosure was placed on a square plate of 0.5-cm grey Forex that served as a floor, and a perforated clear Plexiglas plate served as a removable lid. Ambulatory activity was detected and measured by a pair of infrared light-beam sensors located on each side of the enclosure, at a height of 2 cm. Sensors were spaced 6.5-cm from each end of the side, so that the light-beams formed a matrix of 3 × 3 squares over the surface. A mouse had to traverse the full distance (at least 6.5 cm) between the beams for each activity count. Breakings of a single beam were not taken into consideration in the data analysis; breakings of the intersection between perpendicularly positioned beams were not recorded. Each apparatus was encased in a sound-attenuating shell [approximately 100 × 90 (surface) × 150 (height) cm], artificially ventilated, illuminated by an ‘‘energy saver’’ nonheating 60-W white light (625 lumen), and maintained in an ambient temperature of 21–23 °C. A one-way window on each shell door allowed direct visual surveillance.

2.4. Experimental procedure

Mice were first injected with saline and preexposed to the test chamber during a 60-min session. On the following day; mice were randomly assigned to three groups: the cocaine-cued group, the saline-cued group and the cocaine uncued group ($n = 8$). In the cocaine-cued group, before being individually placed in the test chambers mice received five once-daily injections of 10-mg/kg cocaine every other day, on the odd days of a 10-day period, and saline injections on the 5 days following each cocaine injection day on the even days of the treatment period. In the saline-cued group, mice received 10 injections of saline on both the even and the odd days before being placed in

the test context. In the cocaine-uncued group, mice received five injections of saline on the even days prior to placement in the test chamber and five injections of 10-mg/kg cocaine on the odd days prior to being individually placed in an alternative context. This context consisted of a clear polycarbonate colony cage [internal dimensions 16×32 (surface) $\times$ 17 (height) cm] provided with pine sawdust fresh bedding (but with no water or food) left for the duration of the session in one of the colony rooms (in which the animals from this experiment were not kept). After completion of a session, mice were returned to their home cage.

2.5. Data analyses

To evaluate habituation to the test context after the preexposure and the first saline sessions (animals were drug-free), ambulatory scores from these sessions were compared by means of a mixed-model 2×2 ANOVA, the test sessions (preexposure vs. first session) being considered as a within-subject factor and the ulterior psychopharmacological treatments (cocaine vs. saline) as a between-group variable. Ambulatory scores from the odd and even sessions were separately analysed with a mixed-model 3×5 ANOVA, the psychopharmacological treatments (Psychopharmacological treatments, three levels: cocaine-cued, saline-cued or cocaine-uncued) and the every-other-day test sessions (Sessions, five levels) being defined as between-group and within-subjects variables, respectively. Logarithmic (in base 10) transformations normalized raw data prior to ANOVA, more nearly meeting the assumption of homogeneity of variances (following a significant Levene's test, Glaser, 1983); for the sake of clarity means on the raw values are presented in the graphs. Relevant differences between means within and between sessions were assessed using the Student–Neuman–Keuls procedure derived from the appropriate error-term mean square (Winer et al., 1991). Otherwise specified, it is these analyses that are mentioned in the text. Statistical significance was set at a P level of .05.

3. Results

As shown in Fig. 1, across the drug treatment sessions (odd days), mice from the cocaine-cued group showed increased levels of ambulatory activity that were markedly greater than those exhibited by mice from the saline-cued group at each of the five odd-day sessions, without substantial changes between session being induced. That picture of effects was supported by a robustly significant main effect of Psychopharmacological treatment [$F(1,14) = 52.87$, $P < .0001$] and no interaction between the Psychopharmacological treatment and Session [$F(4,56) = 1.03$, $P > .40$]. The absence of a clear-cut increment of cocaine effect over the odd sessions indicated that no behavioural sensitisation

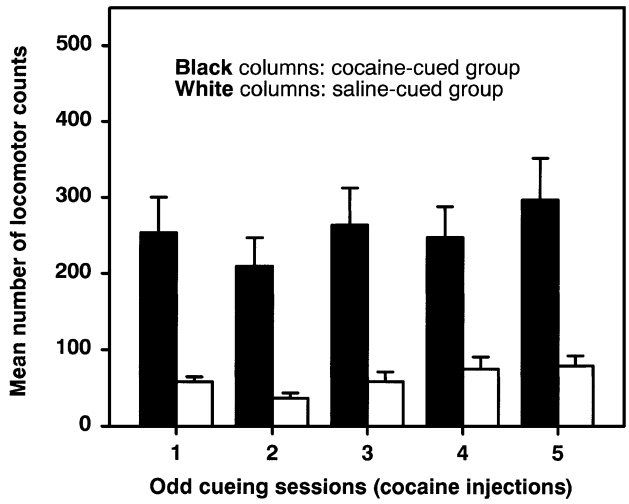

Fig. 1. Locomotion-activating effect of 10 mg/kg sc cocaine given every other day over five daily odd sessions. In the cocaine-cued group, mice received cocaine on odd days and saline on even days in the drug-paired context (effects presented in Fig. 2). In the saline-cued group, mice received saline on odd sessions and saline again on even sessions in the drug-paired context. ANOVA detected a significant main effect of the psychopharmacological treatment but no interaction between that factor and the session (details in text). Vertical brackets represent the SEM.

occurred, which was also confirmed by a less conservative (as compared to the ANOVA) Student t test comparing the value of the first session with that of the fifth session ($P > .45$). Note that the activity of the saline-cued group tended to increase over the sessions.

Fig. 2 presents the levels of locomotion displayed during the first exposure to the test chamber (preexposure session under saline) and the incremental development of ambulatory activity across the repeated administration of saline on the five even sessions. Mice from the three groups (cocaine-cued, saline-cued and cocaine-uncued) showed on the first session comparable levels of locomotion that were clearly lower than those obtained for the same animals on the preexposure session 48 h earlier. That difference was supported by an ANOVA that brought about a robustly significant main effect of Session [preexposure vs. first session, $F(1,21) = 35.94$, $P < .0001$] with no significant interaction between that factor and Psychopharmacological treatment [$F(8,84) = 2.97$, $P < .005$]. As regards the subsequent even sessions, the difference between the value of the cocaine-cued group and those of the saline-cued and cocaine-uncued groups emerged from the second session (after the preexposure session) onwards, whereas these values did not differ from each other on the first session. That profile of effects was supported by robust significant main effects of Psychopharmacological treatment [$F(2,21) = 7.06$, $P < .005$] and Session [$F(4,84) = 4.04$, $P < .005$], and especially by a significant interaction between these factors [$F(8,84) = 2.97$, $P < .006$]. Subsequent between-mean comparisons tests specified that the value of

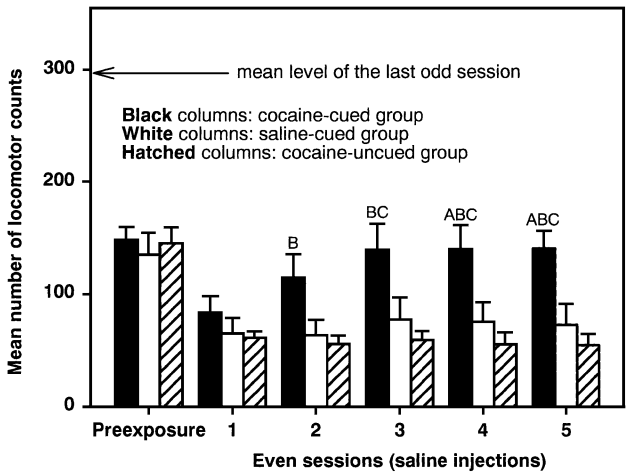

Fig. 2. Locomotor activity exhibited by cocaine-cued, saline-cued and cocaine-uncued mice after saline injections given on even sessions in the cocaine-cued context. The preexposure session shows that all animals were habituated to the drug-paired context on the first even session (ANOVA-yielded significant main effect of the session; see text for details). On odd days sessions; mice from the cocaine-cued group received cocaine in the drug-paired context, mice from the saline-cued group received saline in the drug-paired context, mice from the cocaine-uncued group were left undisturbed in a novel colony home cage after a cocaine injection. (A) indicates that the mean score was significantly greater than the corresponding value of the cocaine-cued group on the first session; (B) the mean score was significantly greater than that of the saline-cued group within a given session; (C) the mean score was significantly greater than that of the cocaine-uncued group within a given session; as yielded by Student–Neuman–Keuls between-mean comparisons tests taken at least at $P < .05$. Vertical brackets represent the SEM.

the cocaine-cued group was significantly greater than those of the saline-cued and cocaine-uncued groups on the second, third, fourth and fifth even sessions (Student–Neuman–Keuls tests using the between-subject mean square denominator for F, taken at $P < .05$). Additionally, the values of the cocaine-cued group on the third, fourth and fifth sessions were significantly different from the corresponding value derived from the first session, but not from the corresponding value of the second session (Student–Neuman–Keuls tests using the interaction mean square denominator for F, taken at $P < .05$). The effect exhibited in the cocaine-cued group on the second session was smaller than that of the following session and greater than that of the preceding one, albeit nonsignificantly so (Student–Neuman–Keuls tests), contributing to the asymptotic-like curve of acquisition of the placebo response.

4. Discussion

4.1. Progressive development of the saline-challenges responses

In the present study, 48 h after a session of preexposure to the test context the mice receiving cocaine on the odd days and saline on the even days, of a period of 10 days, in the same experimental context (cocaine-cued group) showed repeated increases of locomotor activity without behavioural sensitisation being induced (over Days 1, 3, 5, 7 and 9). Over the even days of the same period of time (Days 2, 4, 6, 8 and 10), mice from the cocaine-cued group showed a progressive increase of locomotion, whereas mice from the saline-cued group (that received saline on all sessions) and the cocaine-uncued group (that received saline on the even sessions and cocaine outside of the testing context on the odd sessions) exhibited lower, baseline, levels of locomotor activity throughout the testing period. Note that the greatest of the saline-challenges effects was approximately twofold smaller than the last hyperlocomotor effect induced by cocaine (fifth odd session).

The increasing effects of the repeated saline challenges in the cocaine-cued group can be reasonably interpreted in terms of Pavlovian conditioning, the context paired with cocaine on the odd days becoming a CS that elicits cocaine stimulant effects over the even sessions. Closer inspection of the data indicates that the development of these saline responses did not totally reproduce the typical acquisition S-shaped curve of many nonpharmacological Pavlovian conditioning preparations (Domjan, 1998; Kehoe and Macrae, 2002). In such studies, the magnitude or the strength of the CR (like salivation in dogs, eye-blinking in rabbits or freezing in rats) appears gradually with repetition of the US–CS association, with a little increase on the initial trials, then a larger increase, until some asymptotic level is achieved at which the rate of increase levels off (plateau). In other words, the ascendant slope often involves several (at least two or three) trials that induce significantly different CRs prior to reaching the plateau, which did not occur in the present study. To obtain such a pattern of effects, the value derived from the second even session should have been significantly different from the values of both the preceding and the successive even sessions (see Fig. 2). And the picture would have been even more convincing if at least another placebo effect significantly different from the others occurred in the ascendant slope. Nevertheless, the significant differences between the saline challenge values on the third, fourth and fifth sessions and that of the first session is still suggestive of some gradual emergence of a conditioned placebo effect. It is noteworthy to remember that the all-or-none emergence of the CR has been theorised in the field of learning psychology by Estes (1955). According to that theory, learning occurs in an all-or-none manner for each individual at a given trial, the number of individuals showing the CR increasing over the series of acquisition trials, until all individuals show the response when the asymptote is reached; that profile of successive appearances of the individual CRs would be masked by the S-shaped curve of acquisition. However, the individual scores that our mice from the cocaine-cues group exhibited under saline on the even-day sessions are not consistent with such a conception since the eight mice tended to exhibit a parallel

pattern of acquisition of the CR, all mice having unambiguously acquired the CR (assumed when the value at a given session was merely greater than that of the first session) by the third session (Table 1).

To some extent the procedure used in the present study resembles that used in the few previous studies having examined the development of the drug-like placebo response (induced by d-amphetamine or bromocriptine) with the number of cumulated trials within the same animals (Pickens and Crowder, 1967; Pickens and Dougherty, 1971; Mazurski and Beninger, 1991; Hoffman and Wise, 1992). These authors conducted multiple tests for placebo activity interspersed between a series of blocks of drug-context pairings (three to seven blocks of three to six intermittent injections given at least 24 h apart), which can be considered analogous to the successive blocks of acquisition trials in a typical Pavlovian preparation. Unfortunately, the magnitude of the successive saline-challenge responses remained more or less constant over the trials. Other studies, using *independent groups* of animals undergoing a different number (two to eight) of amphetamine-context pairings have also failed to obtain a positive function between the number of such pairings and the size of the druglike placebo effect (Ahmed et al., 1995, 1996).

In these later studies, it was suggested that the stimulant-like placebo responses would be generated according to an all-or-none, possibly nonassociative process. However, it is likely that the number of injections (US−CS pairings) given within each blocks was too high for an increase or a quasi-increase of the placebo effect to be revealed in these rat studies. Without using such blocks of trials, we have recently obtained in C57BL/6J mice a positive relationship between the druglike placebo effect and the number of once-daily contextual cocaine injections, mice undergoing 12 trials showing the greatest CR and those receiving only 3 trials the smallest one (Michel et al., 2003). Note that these results confirmed and complement the work of Pihl and Altman (1971) who reported that the greatest saline-challenge locomotor response was produced by the largest number of previous drug-context trials (15) in amphetamine-treated rats, although the smallest number of trials (three) did not yield a significant CR and at least nine trials were necessary for a CR to be induced, the CR produced by 15 trials barely accentuating that CR. Additionally, given that in pharmacological placebo experiments the dose plays the role of the magnitude of the US, which is positively related with the magnitude or the strength of the CR in Pavlovian conditioning theory, it is also plausible that *the dose* of amphetamine used in the abovementioned inconclusive studies was excessive (Michel and Tirelli, 2002a,b).

In several studies, mice or rats receiving an intermittent administration of a stimulating drug in a constant test context and being exposed to that context (drug-free or under saline) for a few minutes *before* each drug injection, came to exhibit an anticipatory responding that progressively grew during the preinjection period (Hayashi et al., 1980; Mucha et al., 1981; Tirelli and Terry, 1998; Szumlinski et al., 2000; Fraioli et al., 1999). In these studies, an asymptotic-like increase of hyperlocomotion induced by d-amphetamine, cocaine or morphine and heightened rearing induced by d-amphetamine occurred over the successive preinjection periods (for an all-or-none pattern of results, see Steckler and Holsboer, 2001). A notable dissimilarity between the protocol of these studies and ours is that the animals were exposed to the drug-paired environment prior to the drug injection, when the CS mainly comprised cues from the administration ritual and handling. In fact, the anticipatory conditioned activity was relatively limited in comparison to the postinjection conditioned activity as measured in the present study.

4.2. Implications for the excitatory conditioning explanation of contextual sensitisation

In the current study, the posttreatment conditioned hyperactivity was generated without any convincing sign of sensitisation to the stimulant effect of cocaine, a profile of effects that can also be found in numerous previous reports using cocaine or amphetamine (e.g., Adams et al., 2000; Ahmed et al., 1995, 1996; Carey and Gui, 1998; Devries and Pert, 1998; Gold and Koob, 1989; Kiyatkin, 1992; Martin-Iverson and Fawcett, 1996; Herz and Beninger, 1987). In our study, the lack of sensitisation to cocaine might be due to a contrasting influence of extinction-like processes taking place on the saline-challenges sessions, a phenomenon sometimes called in the learning theory literature "partial reinforcement effect" (in which the US is delivered after some but not all CS trials, attenuating the acquisition of the CR; Domjan, 1998). According to that hypothesis, mice that would pair the test context with cocaine on odd days without being exposed to the test chamber during the even days (and left undisturbed in their home cage) should exhibit convin-

Table 1
Individual scores on the even days derived for the mice having received cocaine on the odd days and saline on the even days in the drug-paired context (cocaine-cued group)

Animal	Session 1	Session 2	Session 3	Session 4	Session 5
Mouse 1	85	132	103	162	136
Mouse 2	76	138	153	171	111
Mouse 3	27	37	56	42	68
Mouse 4	134	184	237	143	166
Mouse 5	126	162	210	170	177
Mouse 6	45	40	100	134	164
Mouse 7	121	152	170	226	197
Mouse 8	58	77	91	75	110

One cannot state that the individual conditioned responses emerged according to an all-or-none fashion, successively at a different session for each individual.

cing evidence of sensitisation (as compared to the cocaine-cued mice used here). In fact, such an effect did occur in a still unpublished study of ours where the absence of sensitisation (repeated constant effect) found here was also replicated (manuscript in preparation). However, the CRs generated on a unique posttreatment saline-challenge test for the two main groups (exposed to the test context either every daily session or every other day) were practically identical in magnitude, contrarily to the prediction of the "partial reinforcement effect" account of contextual sensitisation, according to which the CR of the sensitised group should have been greater than that of the nonsensitised group. More generally these results and the obtaining of a robust CR together with the absence of sensitisation found in the present study are sharply incompatible with the excitatory conditioning theory of contextual sensitisation. That theory stipulates that a CR grows from one drug injection to the next (from trial to trial), extending an otherwise unchanging drug-induced UR, and thereby shaping the incremental component of the sensitising response. Thus, the CR would represent the difference between the nonsensitised unconditioned response and the final sensitised one (Silverman and Bonate, 1997; Post et al., 1992; Siegel, 1985). According to that hypothesis, our cocaine-cued mice should *not have exhibited* clear-cut CRs on saline challenges. In fact, in the literature, several lines of observations do not support a hypothetical functional involvement of the CR in the origin of contextual sensitisation. Doubts about the validity of that hypothesis have firstly arisen from the observation (common to most sensitisation studies) of a marked disparity in size between the CR and the final sensitised UR (and the difference between the sensitised and the initial responses). Strong contextually sensitised locomotor responses to the drug (apomorphine, quinpirole or cocaine in adult and suckling rats) have been generated even without any conditioned activity being expressed on the postdrug saline challenge (Mattingly and Gotsick, 1989; Szechtman et al., 1993; Wood et al., 1998).

In studies explicitly designed to test the excitatory conditioning account of sensitisation, several parameters of contextual sensitisation to amphetamine and cocaine in rats and mice (e.g., slopes of the individual sensitisation curves) have been found to be unambiguously *uncorrelated* with the magnitude of postsensitisation conditioned activity, indicating that the latter is likely not a functional component of the former (Crombag et al., 2000; Michel and Tirelli, 2002a; Tirelli et al., 2003). *No correlation* at all has also been found when testing the hypothetical relationships of the UR with the CR (the latter supposedly mimicking the former), and of the UR with the difference between the finally sensitised UR and the initial UR, the sensitised UR being supposedly the summation of the CR onto the initial UR (Badiani et al., 1995; Michel and Tirelli, 2002a; Tirelli et al., 2003). Additional objections come from the manipulation of Pavlovian conditioning features and parameters, which do not systematically have repercussions on the expression of contextual sensitisation (and vice versa). For example, there have been several reports that context-specific sensitisation to d-amphetamine-induced rotational behaviour in 6-OHDA-lesioned rats or hyperactivity in intact rats and cocaine-induced hyperlocomotion in rats and mice persists without any decrease in amplitude after extinction of the CR, instead of being extinguished as well (Anastognoras and Robinson, 1996; Cabib, 1993; Carey and Gui, 1998; Drew and Glick, 1988; Stewart and Vezina, 1991). In the same vein, preexposure to the test context has been reported to attenuate the expression of the CR, conforming to the Pavlovian principle of latent inhibition, but without affecting the rate and the amplitude of contextual sensitisation in rats treated with d-amphetamine (Drew and Glick, 1988; Crombag et al., 2001).

The divergence between the contextually sensitised response and the CR has probably neurobiological basis since the two responses are differentially sensitive to pharmacological blockade. For example, haloperidol attenuates cocaine-induced locomotor sensitisation without affecting the CR, whereas nimodipine blocks the CR despite only limited effects on sensitisation (Reimer and Martin-Iverson, 1994). Conversely, it has been found that the D2-type dopamine receptor antagonist eticlopride completely blocked the development of sensitisation to the locomotor-activating effect of the D2/D3 agonist 7-OHDPAT without affecting the occurrence of a subsequent CR in rats (Mattingly et al., 1998).

In conclusion, (1) the design used here may provide a relatively simple method to examine the development (the acquisition) of the conditioned placebo responses induced by psychomotor stimulants in laboratory rodents, pending on the identification of doses, injection regimes and behavioural measures allowing a more graded increment (to a plateau) of the placebo response over the successive tests challenges than that reported here. (2) An important general implication of the finding of a postdrug conditioned placebo response without sensitisation (along with the above-commented experimental objections to the excitatory theory of contextual sensitisation) is that the repeated administration of a drug in the same context endows this context with the ability to accentuate the repeatedly induced responding to the drug in a context-specific manner *independently* of its ability to produce a CR, and therefore without any associative mechanisms being involved (Stewart and Badiani, 1993; Anastognoras and Robinson, 1996; Crombag et al., 2000; Tirelli et al., 2003). Therefore, contextual sensitisation might represent an autonomous form of neural plasticity that is not amenable to another one (e.g., conditioning) and that should be experimentally characterised per se.

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