Neuropsychopharmacology (2006) 31, 1431–1443

www.neuropsychopharmacology.org

σ_1 Receptor Ligands and Related Neuroactive Steroids Interfere with the Cocaine-Induced State of Memory

Pascal Romieu[1,2], Morgan Lucas[1,3] and Tangui Maurice*[,1,4]

[1]*Behavioral Neuropharmacology Group, INSERM U. 336, Institut de Biologie, Montpellier, France*

The present series of experiments examined the involvement of the σ_1 receptor and related neuroactive steroids in the memory state induced by a very low dose of cocaine. Using a modified passive avoidance procedure in mice, we examined whether cocaine induces state-dependent (StD) learning. Animals trained and tested with saline or the same dose of cocaine (0.1 or 0.3 mg/kg) showed correct retention, measured using two independent parameters: the retention latency and a ratio between the retention latency and the last training latency. Animals trained with cocaine (0.1 mg/kg) and tested with saline or cocaine (0.03, 0.3 mg/kg), or trained with saline and tested with cocaine, showed altered retention parameters, demonstrating that StD occurred. Therefore, cocaine administered before training produced a chemical state used as an endogenous cue to insure optimal retention. Since σ_1 receptor activation is an important event during the acquisition of cocaine reward, we tested several σ_1 ligands and related neurosteroids. The σ_1 agonist igmesine or antagonist BD1047 failed to produce StD, but modified the cocaine state. Among neuroactive steroids, pregnanolone and allopregnanolone, positive modulators of the γ-aminobutyric acid type A ($GABA_A$) receptor, produced StD. However, steroids also acting as σ_1 agonists, dehydroepiandrosterone (3β-hydroxy-5α-androsten-17-one (DHEA)), pregnenolone, or antagonist, progesterone, failed to induce StD but modified the cocaine state. Furthermore, optimal retention was observed with mice trained with (igmesine or DHEA) + cocaine and tested with a higher dose of cocaine, or with mice trained with (BD1047 or progesterone) + cocaine and tested with vehicle. This study demonstrated that: (i) low doses of cocaine induce a chemical state/memory trace sustaining StD; (ii) modulation of the σ_1 receptor activation, although insufficient to provoke StD, modulates the cocaine state; (iii) neuroactive steroids exert a unique role in state-dependent *vs* state-independent learning, via $GABA_A$ or σ_1 receptor modulation, and are able to affect the cocaine-induced mnesic trace at low brain concentrations.

Neuropsychopharmacology (2006) **31,** 1431–1443. doi:10.1038/sj.npp.1300885; published online 31 August 2005

Keywords: state dependence; cocaine; σ1 receptor; neuroactive steroids; learning; memory

INTRODUCTION

An information or behavioral response acquired in a particular affective, endocrine- or drug-induced, state will be optimally recalled when the organism is in the same state. In other words, recall in a different state will result in a lower efficacy or complete blockade of the retrieval of the response (Overton, 1978; Weingartner, 1978). This phenomenon, identified for many years (Girden and Culler, 1937), is called state dependence (StD) and experimental conditions have been defined allowing its reproducible examination in memory processes. Procedures have been defined using, for instance, classical operant conditioning (Colpaert, 1990; Jackson, 1995; Colpaert and Koek, 1996) or passive avoidance learning (Zarrindast *et al*, 2004; Zarrindast and Rezayof, 2004). Drug-induced StD has been observed when animals are administered with the same drug before acquisition and/or retention session of the operant or passive avoidance task. Indeed, animals receiving a repeated treatment (vehicle solution/vehicle solution or drug/drug) before acquisition and retention showed optimal retention capacities, whereas animals receiving crossed treatments (vehicle/drug or drug/vehicle) showed significant deficits in retention parameters. Several drugs acting on specific neurotransmitter systems could elicit StD, including naloxone (Oberling *et al*, 1993), morphine (Bruins Slot and Colpaert, 1999a, b; Zarrindast and Rezayof, 2004), pentobarbital (Overton, 1966), muscimol, diazepam (Nakagawa *et al*, 1993; Nakagawa and Iwasaki, 1996), chlordiazepoxide (Colpaert, 1990), or dizocilpine (Jackson *et al*,

*Correspondence: Dr T Maurice, INSERM U. 710, University of Montpellier II, CC 105, Place Eugène Bataillon, 34095 Montpellier Cedex 5, France, Tel: + 33 4 67 14 36 23, Fax: + 33 4 67 14 33 86, E-mail: maurice@univ-montp2.fr

[2]Current address: INSERM U. 575, Centre de Neurochimie, Strasbourg, France

[3]Current address: CNRS UMR 5541, Interactions neuronales et comportement, Université Victor Segalen Bordeaux 2, Bordeaux, France

[4]Current address: INSERM U. 710, University of Montpellier II, Montpellier, France

Received 9 September 2004; revised 5 July 2005; accepted 7 July 2005

Online publication: 3 August 2005 at http://www.acnp.org/citations/Npp080305040413/default.pdf

1992; Harrod *et al*, 2001). Endogenous agents may also sustain StD, such as the 3α-hydroxy-5-reduced metabolite of progesterone, pregnanolone (Bruins Slot *et al*, 1999), which acts as a positive modulator of the γ-aminobutyric acid type A ($GABA_A$) receptor (Paul and Purdy, 1992). Moreover, two pharmacologically related drugs may allow transfer of the memory trace and substitution may lead to optimal retention, as recently observed between pregnanolone and chlordiazepoxide (Bruins Slot *et al*, 1999). StD is now considered as an efficient neurophysiological mechanism allowing constraint of memory processes, in response not only to exogenous drug-induced perturbations, but also to various physiological conditions.

Addictive drugs, due to their reinforcing potential, are expected to induce, even at low dosage, a strong chemical state in the brain that may lead to effective StD. Indeed, StD was observed for ethanol in several behavioral paradigms (Swerdlow *et al*, 1983; Colpaert and Koek, 1995; Nakagawa and Iwasaki, 1996). Heroin-induced conditioned place preference was found to be significantly larger when animals conditioned with heroin were tested in a drug-treated state as compared to vehicle-treated ones (Bozarth, 1987), suggesting that the drug-induced state was used as an endogenous cue participating in an optimal retention performance.

StD has however not yet been described for cocaine, although the drug acts as a potent reinforcer in laboratory animals (Pickens and Thompson, 1968; Koob, 1992; Stolerman, 1992). While cocaine inhibits the reuptake of all monoamines, it is widely accepted that the addictive and reinforcing actions of cocaine are dependent on the drug ability to block the dopamine transporter, thereby increasing dopamine neurotransmission in both nigrostriatal and mesocorticolimbic pathways (Ritz *et al*, 1987; Kuhar, 1992; Woolverton, 1992). Cocaine self-administration and relapse are also mediated by glutamate neurotransmission in the nucleus accumbens (Cornish *et al*, 1999; Park *et al*, 2002). In addition, cocaine interacts with the σ_1 receptor at a similar dose range as observed for the dopamine transporter (Sharkey *et al*, 1988), and the σ_1 receptor is implicated in several of cocaine's effects such as locomotor stimulation, sensitization, acquisition and reactivation of conditioned place preference, convulsions, and lethality (Reith *et al*, 1986; Menkel *et al*, 1991; Ujike *et al*, 1992; Ritz and George, 1993; Romieu *et al*, 2000, 2002, 2003, 2004; for a review, see Maurice *et al*, 2002). This intracellular protein sharing some characteristics of neuromodulatory receptors is also a target for several neuroactive steroids, including pregnenolone, dehydroepiandrosterone (3β-hydroxy-5α-androsten-17-one (DHEA)), their sulfate esters, or progesterone, but not pregnanolone or allopregnanolone (Su *et al*, 1988; Monnet *et al*, 1995; Bergeron *et al*, 1996; Maurice *et al*, 1999).

The present study had a multiple purpose. First, after determining the memory effect of a wide dose range of cocaine (0.03–1 mg/kg) using a step-down-type passive avoidance memory test, we examined whether cocaine, at a dose ineffective directly on memory processes, could induce StD using a modified procedure of the test. Second, we examined whether the σ_1 receptor and its endogenous ligands, that is, neuroactive steroids, exhibited StD and/or are involved in the cocaine-induced StD. We report that cocaine, at low dosage, induces StD and that the selective σ_1 agonist igmesine or antagonist BD1047 failed to induce StD alone but could, respectively, amplify or mask the cocaine-induced chemical state. This effect is shared by σ_1 receptor-interacting neuroactive steroids, but not by steroids acting selectively as $GABA_A$ receptor modulators, which produced StD by themselves.

METHODS

Subjects

Male Swiss OF1 mice (Breeding Center of the Faculty of Pharmacy, Montpellier, France), aged 5–6 weeks and weighing 30 ± 2 g, were housed in groups. They had free access to laboratory food and water, except during behavioral experiments, and were kept in a regulated environment ($23 \pm 1^{\circ}C$, 40–60% humidity) under a 12-h light–dark cycle (light on at 0700 h). Experiments were carried out between 1000 and 1700 h, in a sound-attenuated and air-regulated experimental room, to which mice were habituated at least 30 min before each experiment. All animal procedures were conducted in strict adherence with the European Community Council Directive of 24 November 1986 (86-609/EEC).

Drugs

Cocaine was obtained from Cooper (Marseille, France). (+)-*N*-cyclopropylmethyl-*N*-methyl-1,4-diphenyl-1-ethyl-but-3-en-1-ylamine (igmesine) was provided by Dr François J Roman (Pfizer GRD, Fresnes, France), *N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(dimethylamino)ethylamine (BD1047) by Dr Wayne D Bowen (NIDDK/NIH, Bethesda, MD). Pregnanolone (3α-hydroxy-5β-pregnan-20-one), allopregnanolone (3α-hydroxy-5α-pregnan-20-one), pregnenolone (3β-hydroxy-5α-pregnen-20-one), DHEA, and progesterone (4-pregnene-3,20-dione) were from Sigma Aldrich (Saint-Quentin Fallavier, France). Cocaine was dissolved in physiological saline, σ_1 ligands were dissolved in distilled water and steroids suspended in pure sesame oil. Cocaine and σ_1 ligands were injected intraperitoneally (i.p.) and steroids subcutaneously (s.c.), in a volume of 100 μl per 20 g of body weight.

Passive Avoidance Apparatus

The apparatus consisted of a transparent acrylic cage ($30 \times 30 \times 40\,cm^3$ high) with a grid-floor, inserted in an outer box ($35 \times 35 \times 90\,cm^3$ high). A 15 W lamp lit the cage during the experimental period. A wooden platform ($4 \times 4 \times 4\,cm^3$) was fixed at the center of the grid-floor. Electric shocks (1 Hz, 500 ms, 35 or 43 V DC) could be delivered to the grid-floor using an isolated pulse stimulator (Model 2100, AM Systems, Everett, WA, USA).

Two-Trial Acquisition (Classical Step-Down Passive Avoidance Procedure)

The classical step-down type passive avoidance procedure consisted in two training sessions, at 90-min time interval, and a retention session, carried out 24 h after the first training (Hiramatsu *et al*, 1992; Maurice *et al*, 1994, 1998).

Cocaine was administered i.p. either 15 min before or immediately after the first training session, or 30 min before the retention session. During training sessions, each mouse was placed on the platform. When it stepped down and placed its four paws on the grid-floor, moderate intensity shocks (35 V) were delivered for 15 s. The step-down latency and number of vocalizations and flinching reactions were measured. Shock sensitivity was evaluated by adding the number of vocalizations and flinching reactions. Animals that did not step down within 60 s during the second session were considered as remembering the task and taken off, without receiving further electric shocks. The retention session was performed in a similar manner as training, except that shocks were not applied to the grid-floor. Each mouse was placed again on the platform and the latency recorded, with an upper cut-off time of 300 s. Two parametric measures were analyzed during the retention session: (i) the step-down latency, expressed as median and interquartile (25–75%) range (IR), since an upper cutoff time was set, and (ii) the number of animals reaching an avoidance criterion, expressed as percentage. The retention criterion was considered as reached if the latency measured during the retention session was greater than three-fold the latency showed by the animal during the second training session and, at least, greater than 60 s. Untreated animals trained under this two-trial acquisition procedure showed a median retention latency of 75 (16–216) s and a number of animals to a criterion of 44% ($n = 18$). This procedure allows the observation of significant deterioration or improvement of retention parameters.

Same-State Multiple-Trial Acquisition (State-Dependence Procedure)

StD was measured in animals trained by repetitive training sessions that led to a similar, high level of acquisition. Animals received injections 15 min before the first training. Then, they were trained repeatedly, with an intertrial time interval of 5 min, until they remained on the platform for 60 s. The number of session-to-criterion was determined and considered as an index of the quality of memory acquisition. Animals that did not reach the criterion after 10 sessions were discontinued. The retention session was performed after 24 h in a similar manner as training, except that shocks were not applied to the grid-floor. Each mouse was again injected 15 min before being placed on the platform. The step-down latency was recorded, with an upper cutoff time of 300 s. Two parametric measures of retention were analyzed: (i) the step-down latency, expressed as median and interquartile (25–75%) range, and (ii) the latency ratio, calculated as: (latency measured during the retention session/latency measured during the last trial before reaching the criterion). The ratio value was log-transformed in order to normalize the data and allow parametric analysis of variance. Untreated animals trained under the multiple-trial acquisition procedure showed a median retention latency of 300 (300–300) s and a mean latency ratio of 20.5 ± 1.9, leading to a log-transformed mean of 1.31 ± 0.03 ($n = 61$). This procedure allows to determine moderate but significant diminution of learning quality, in terms of diminutions of latency and/or latency ratio.

Experimental Design

Examination of the direct effect of cocaine on memory abilities. In order to determine the putative memory effects of low doses of cocaine, a first series of experiments examined the effect of cocaine (0, 0.03, 0.1, 0.3, 1 mg/kg i.p.) in mice submitted to the normal two-trial acquisition passive avoidance procedure. Cocaine was administered either 15 min before the first training, or immediately after, or 30 min before the retention session. These different administration schedules allowed to determine whether the drug affected the acquisition, consolidation, or retention phase, respectively.

Determination of the cocaine-induced state-dependent learning. The StD induced by cocaine was examined using the modified, same-state multiple-trial acquisition, passive avoidance procedure. Animals were administered with either saline or cocaine (0.1, 0.3 mg/kg i.p.) before the first training session and/or before the retention session in repeated or crossed treatments, and the quality of retention was examined.

Examination of the ability of σ_1 ligands to induce state-dependent learning. The doses of each compound used in this study were selected according to a series of preliminary experiments or previous results from our team. In order to examine specifically StD, through the impact of the drug-induced chemical state and without interference from any memory effects, the highest dose subactive in the classical two-trial acquisition passive avoidance procedure was selected. The σ_1 receptor agonist igmesine (Roman *et al*, 1990) exerted significant antiamnesic effects in several models of amnesia at the dose of 1 mg/kg i.p. (Maurice *et al*, 1996). BD1047 is a selective σ_1 antagonist (Matsumoto *et al*, 1995) blocking several σ_1 agonist-mediated behaviors at doses of 3 and 10 mg/kg i.p. (Romieu *et al*, 2000, 2002; Urani *et al*, 2001). Therefore, the doses of 0.5 and 1 mg/kg were selected for igmesine and BD1047, respectively. Animals were administered with either saline or σ_1 ligands before the first training session and/or before the retention session in repeated or crossed treatments, and the quality of retention was examined. The effect of igmesine and BD1047 on the cocaine-induced StD was then examined. Animals were simultaneously injected i.p. with igmesine and cocaine or BD1047, and cocaine either before the first training session and/or before the retention session.

Examination of the ability of neuroactive steroids to sustain state-dependent learning. Pregnanolone and allopregnanolone are two steroids acting as positive modulators of the $GABA_A$ receptor complex and devoid of affinity for the σ_1 receptor. Pregnenolone, DHEA, and progesterone have been shown to interact with the σ_1 receptor, in parallel to the direct effects on $GABA_A$ or NMDA receptors (for reviews, see Maurice *et al*, 1999, 2001). Pregnanolone was tested at the dose of 10 mg/kg, previously used to show StD in an operant procedure in rats (Bruins Slot *et al*, 1999). In preliminary experiments, we observed that allopregnanolone and progesterone impaired significantly learning at the dose of 10 mg/kg s.c., but not 5 mg/kg (data not shown). Pregnenolone or DHEA showed antiamnesic effects at the

dose of 20 mg/kg s.c. (Urani *et al*, 1998; Maurice *et al*, 1997). Therefore, the following doses were used: 5 mg/kg for allopregnanolone and progesterone, and 10 mg/kg for pregnenolone and DHEA. The effects of the steroids interacting with the σ_1 receptor on the cocaine-induced StD were also examined. Animals were simultaneously injected with DHEA + cocaine or progesterone + cocaine, either before the first training session and/or before the retention session.

Determination of the agonist/antagonist effect. We finally examined the nature of the chemical state induced by coadministration of the σ_1 ligands or steroids with cocaine. First, we compared the learning quality following coadministration of BD1047 + cocaine or progesterone + cocaine with administration of the vehicle solutions. Second, we compared the learning quality following coadministration of igmesine + cocaine or DHEA + cocaine with the administration of a higher dose, 0.3 mg/kg, of cocaine.

Data Analysis

The number of session-to-criterion was analyzed using the Student's *t*-test. Latencies measured during the passive avoidance test retention did not show a normal distribution, since cutoff times were set. They were thus analyzed using the Kruskal–Wallis nonparametric analysis of variance (KW values), group comparisons being made with Dunn's nonparametric multiple comparisons test. Log-transformed latency ratios were analyzed using one-way ANOVA (F values). *Post hoc* comparisons were performed using the Newmann–Keuls' test for multiple comparisons. Statistical significance was defined as $P<0.05$.

RESULTS

Effect of Cocaine on Normal Acquisition of Passive Avoidance

A first series of experiments examined the direct effect of cocaine, in a large dose range, on the learning capacities in the classical two-trial acquisition passive avoidance procedure. Cocaine, administered at 0, 0.03, 0.1, 0.3, and 1 mg/kg to Swiss mice, failed to affect the passive avoidance response when it was administered 15 min before the first training session (latencies: KW = 2.49, $P>0.05$, $n=15$–18, data not shown) or immediately after the first training session (latencies: KW = 2.92, $P>0.05$, $n=12$ each group, data not shown). Therefore, the drug did not affect the acquisition and consolidation phases of the memory process. Noteworthy, the pre-training administration of cocaine failed to affect the shock sensitivity, whatever the dose ($F_{(4,81)}=0.77$, $P>0.05$). When cocaine was administered 30 min before the retention session, examined 24 h after training, the drug significantly improved the quality of retention, with a significant effect in latency (KW = 9.15, $P<0.05$; Figure 1a) and percentage of animals to criterion (Figure 1b). The dose of 0.3 mg/kg led to significant differences as compared with the vehicle-treated group. This observation indicated that a low dose of cocaine ameliorated memory retrieval in Swiss mice.

Cocaine-Induced State-Dependent Learning

The StD was examined using the modified passive avoidance procedure. A narrow dose range was intentionally selected, 0.1–0.3 mg/kg, in order to limit the interference with direct drug effects on memory. The cocaine treatments failed to affect acquisition, since the number of session-to-criterion was not significantly different among treatment groups (Table 1a). However, significant differences were observed in retention parameters, both in terms of latency

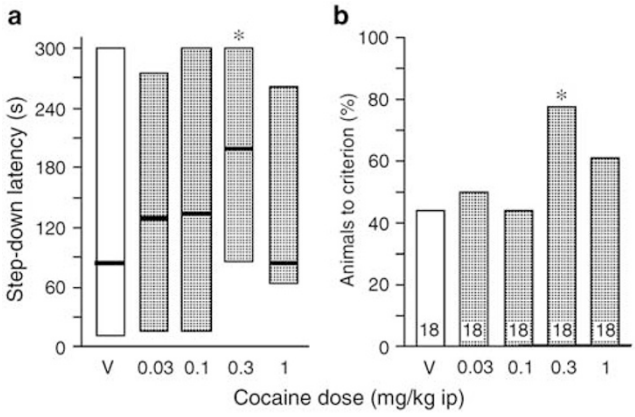

Figure 1 Cocaine-induced memory-enhancing properties in the two-trial passive avoidance acquisition procedure: (a) median step-down latency and (b) percentages of animals to criterion during the retention session. Animals were trained twice at 90-min interval and retention was examined after 24 h. Cocaine (0.03–1 mg/kg) or vehicle solution (V, 0.9% saline) was administered 30 min before retention. The drug is ineffective when administered before or after training (data not shown). The number of animals per group is indicated within columns in (b). *$P<0.05$; Dunn's test in (a) or χ^2 test in (b).

Table 1 Effects of the Different Treatments on Acquisition in the Same-State Multiple-Trial Acquisition Passive Avoidance Procedure, Measured in Terms of Numbers of Session-to-Criterion

Treatment (mg/kg)	Session-to-criterion	*n*	ANOVA or *t*-test
(a) Saline solution	3.5 ± 0.2	64	
Cocaine (0.1)	4.0 ± 0.2	46	
Cocaine (0.3)	4.2 ± 0.4	38	$F_{(2,147)}=1.75$, $P>0.05$
(b) Saline solution	3.3 ± 0.2	39	
Igmesine (0.5)	3.8 ± 0.3	36	$t=1.62$, $P>0.05$
(c) Saline solution	3.1 ± 0.3	34	
BD1047 (1)	2.7 ± 0.2	36	$t=1.56$, $P>0.05$
(d) Vehicle solution	4.3 ± 0.4	33	
Pregnanolone (10)	3.9 ± 0.2	37	$t=0.80$, $P>0.05$
Vehicle solution	3.6 ± 0.2	36	
Allopregnanolone (5)	3.7 ± 0.2	36	$t=0.18$, $P>0.05$
(e) Vehicle solution	3.0 ± 0.2	30	
Pregnenolone (10)	3.2 ± 0.2	29	$t=1.06$, $P>0.05$
Vehicle solution	2.9 ± 0.1	30	
DHEA (10)	3.1 ± 0.2	30	$t=0.77$, $P>0.05$
Vehicle solution	2.7 ± 0.2	30	
Progesterone (5)	2.7 ± 0.2	30	$t=0.14$, $P>0.05$

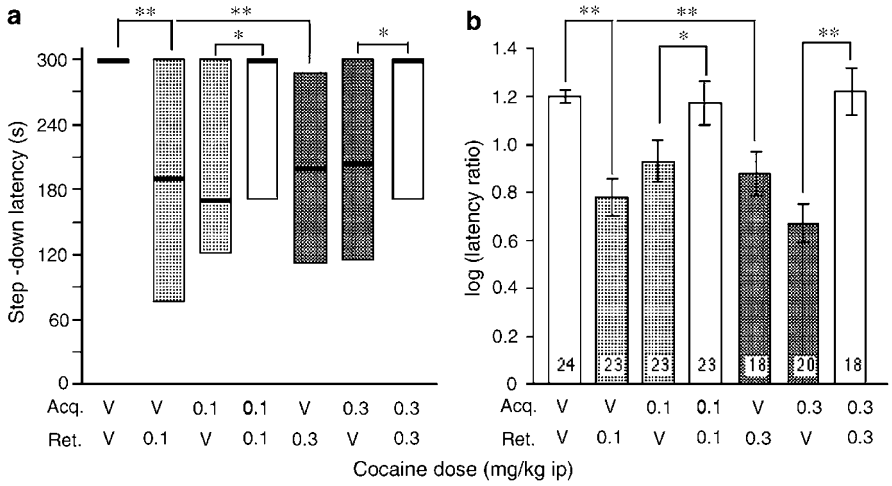

Figure 2 Cocaine-induced state-dependent learning in the multiple trial passive avoidance procedure: (a) step-down latency and (b) log-transformed latency ratio. Animals were injected with either cocaine (0.1 or 0.3 mg/kg) or saline (V), 15 min before the first passive avoidance training session (Acq.), and then trained every 5 min until they reach the learning criterion. After 24 h, animals were injected with either cocaine (0.1 or 0.3 mg/kg) or saline (V) in repeated or crossed treatments, and retention was examined. The latency was expressed as median and IR. The latency ratio is the ratio of the latency found in the test session to the latency found in the last training session before reaching the criterion, expressed as log-transformed. The number of animals per group is indicated within columns in (b). $^{*}P<0.05$, $^{**}P<0.01$; Dunn's test in (a) or Newmann–Keuls' test in (b).

Table 2 Cocaine-Induced State-Dependent Learning is Dose Dependent

Acquisition treatment	Retention treatment	*n*	Latency median (IR) (s)	Latency ratio (log-transformed)
Cocaine (0.1)	Cocaine (0.03)	30	259 (159–300)	1.05 ± 0.07
Cocaine (0.1)	Cocaine (0.1)	36	300 (171–300)	1.19 ± 0.07
Cocaine (0.1)	Cocaine (0.3)	36	145 (139–300)	0.94 ± 0.08*

Animals were injected with 0.1 mg/kg cocaine 15 min before the first passive avoidance training session and then trained every 5 min until they reached the learning criterion. After 24 h, animals were injected with 0.03, 0.1, or 0.3 mg/kg cocaine and retention was examined. The latency was expressed as median and IR. The latency ratio was caluclated as the ratio of the latency measured during the retention session to the latency measured during the last training session before reaching the criterion, expressed as log-transformed. ANOVA analyses: KW = 4.04, $P>0.05$ for latencies; $F_{(2,101)}=3.31$, $P<0.05$ for latency ratios. $^{*}P<0.05$ *vs* the cocaine (0.1 mg/kg) repeated treatment; Newmann–Keuls' test.

(KW = 35.56, $P<0.0001$; Figure 2a) and latency ratio ($F_{(6,148)}=7.36$, $P<0.0001$; Figure 2b). Animals receiving crossed treatments, that is, trained with cocaine and tested with vehicle or *vice-versa*, showed significantly lower retention parameters than animals treated with the repeated, vehicle/vehicle or cocaine/cocaine, treatments. This observation denoted a lower quality of learning (Figure 2a and b). Therefore, cocaine, at 0.1 and 0.3 mg/kg, induced state-dependent learning that could be demonstrated using the modified passive avoidance procedure in mice.

Moreover, the cocaine-induced StD appeared dose-dependent. Animals trained with 0.1 mg/kg cocaine were tested with 0.03, 0.1 or 0.3 mg/kg cocaine. Latency measures failed to show a significant difference (Table 2). However, latency ratio calculations significantly differed among groups, and particularly animals trained with 0.1 mg/kg cocaine and tested with 0.3 mg/kg cocaine showed a significant diminution of the latency ratio (Table 2).

Effects of σ_1 Receptor Ligands on Cocaine-Induced StD

The igmesine (0.5 mg/kg) treatment did not affect acquisition, since the number of session-to-criterion was similar in vehicle- or igmesine-treated groups (Table 1b). Moreover, the drug did not induce state-dependent learning, since the repeated (vehicle/vehicle or igmesine/igmesine) or crossed (vehicle/igmesine or igmesine/vehicle) treatments led to optimal retention performances with no significant variation of latency or latency ratio (Table 3a). Similarly, the BD1047 treatment did not affect acquisition, since the number of session-to-criterion was similar in BD1047- or vehicle-treated animals (Table 1c). The repeated or crossed treatment with BD1047 showed that the drug did also not induce state-dependent learning. Optimal retention was observed whatever the pre-acquisition or pre-retention treatments, both in terms of latency or latency ratio (Table 3b). These observations indicated that neither the σ_1 agonist nor the σ_1 antagonist induced a chemical state within the brain sufficient to sustain StD.

The effect of each drug on the cocaine-induced StD was then examined. Animals received co-treatments of igmesine (0.5 mg/kg) plus cocaine (0.1 mg/kg) or BD1047 (1 mg/kg) plus cocaine (0.1 mg/kg), either before the first training session or before the retention session. The retention parameters were compared with animals receiving a repeated cocaine (0.1 mg/kg) treatment. As shown in Figure 3, both igmesine and BD1047 interfered with the cocaine-induced chemical state. Administration of igmesine + cocaine resulted

Table 3 Selective σ_1 Receptor Ligands Failed to Induce State-Dependent Learning

Acquisition treatment	Retention treatment	*n*	Latency median (IR) (s)	Latency ratio (log-transformed)
(a) σ_1 receptor agonist igmesine				
Vehicle	Vehicle	19	247 (150–300)	1.19±0.10
Vehicle	Igmesine (0.5)	20	262 (60–300)	1.14±0.13
Igmesine (0.5)	Vehicle	16	300 (146–300)	1.04±0.10
Igmesine (0.5)	Igmesine (0.5)	20	293 (87–300)	1.05±0.10
(b) σ_1 receptor antagonist BD1047				
Vehicle	Vehicle	18	300 (300–300)	1.18±0.11
Vehicle	BD1047 (1)	16	300 (220–300)	1.23±0.14
BD1047 (1)	Vehicle	18	300 (250–300)	1.22±0.10
BD1047 (1)	BD1047 (1)	18	300 (277–300)	1.32±0.11

Drug injections were performed 15 min before the acquisition or test session (see legend of Table 2 for details). ANOVA analyses: (a) KW = 0.87, $P > 0.05$, for latencies and $F_{(3,74)} = 0.43$, $P > 0.05$ for latency ratios; (b) KW = 2.01, $P > 0.05$ for latencies and $F_{(3,72)} = 0.26$, $P > 0.05$ for latency ratios.

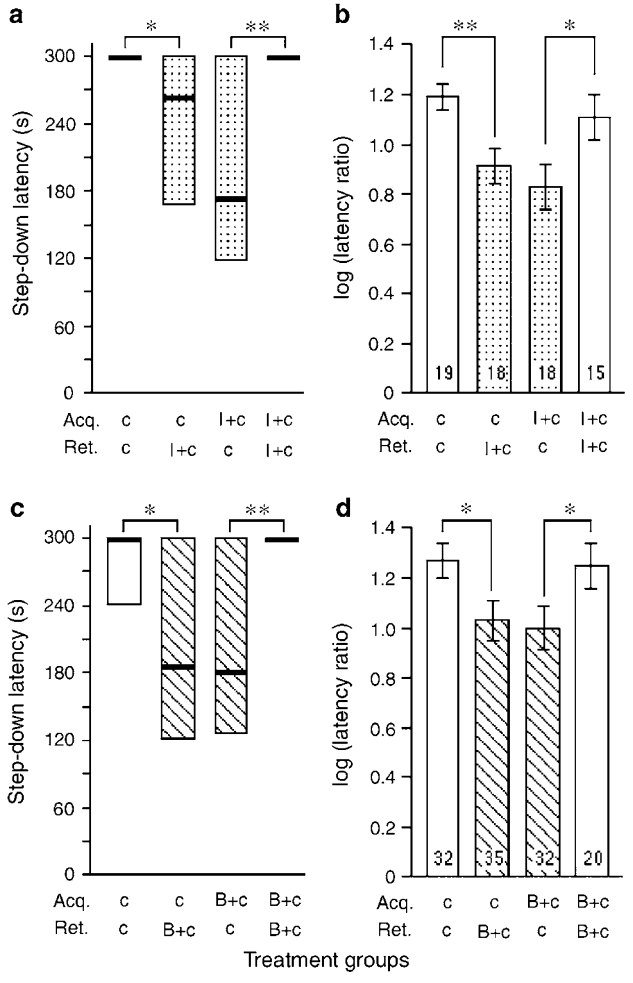

Figure 3 σ_1 Receptor ligands affected the cocaine-induced state-dependent learning. Pretreatment with the σ_1 receptor ligands affected the cocaine-induced state-dependent learning. Animals received: (a, b) igmesine (I, 0.5 mg/kg) or vehicle solution or (c, d) BD1047 (B, 1 mg/kg) or vehicle solution, simultaneously with cocaine (c, 0.1 mg/kg) in repeated or crossed treatments before the acquisition (Acq.) and retention (Ret.) sessions. The number of animals per group is indicated in (b, d). *$P < 0.05$, **$P < 0.01$, Dunn's test in (a, c) and Newmann–Keuls' test in (b, d).

in significant diminutions of the retention parameters, observed for latency (KW = 15.18, $P < 0.01$; Figure 2a) or latency ratio ($F_{(3,69)} = 5.43$, $P < 0.01$; Figure 2b). Similarly, the modification of the cocaine-induced chemical state by the BD1047 pretreatment altered the quality of retention, for both parameters (KW = 15.91, $P < 0.01$; Figure 2c; $F_{(3,118)} = 2.72$, $P < 0.05$; Figure 2d).

Effects of Neuroactive Steroids

Some neuroactive steroids have been reported to interact with the σ_1 receptor (Maurice *et al*, 1999, 2001) and to sustain StD (Bruins Slot *et al*, 1999). Therefore, their ability to induce state-dependent learning in the modified passive avoidance procedure was examined.

Animals were treated with pregnanolone (10 mg/kg) or allopregnanolone (5 mg/kg) and vehicle before the first training session and/or before the retention session in repeated and crossed treatments. The treatments with each steroid failed to affect acquisition, since the number of session-to-criterion was similar between steroid- and vehicle-treated groups (Table 1d). Each steroid, however, induced a marked StD. For pregnanolone, significant differences were observed in retention parameters, both in terms of latency and latency ratio (Table 4a). For allopregnanolone, significant differences were also observed for latency, when the steroid was substituted for vehicle before retention and latency ratio, when vehicle was substituted for allopregnanolone before retention (Table 4b). These results confirmed that $GABA_A$ receptor-interacting steroids could provoke a significant StD.

The treatments with pregnenolone, DHEA, or progesterone failed to affect acquisition, since the number of session-to-criterion was similar between steroid- and vehicle-treated groups (Table 1e). The repeated or crossed treatment with each steroid led to similar memory parameters, as shown in Table 5a for pregnenolone, Table 5b for DHEA, and Table 5c for progesterone. These observations indicated that the steroids failed to induce a chemical state in the brain sustaining state-dependent learning.

Table 4 StD Induced by Pregnanolone or Allopregnanolone, Two Reduced Metabolites of Progesterone

Acquisition treatment	Retention treatment	*n*	Latency median (IR) (s)	Latency ratio (log-transformed)
(a) Pregnanolone				
Vehicle	Vehicle	19	300 (220–300)	1.10±0.09
Vehicle	Pregnanolone (10)	18	135 (130–212)**	0.84±0.08*
Pregnanolone (10)	Vehicle	15	159 (90–300)$^{\#}$	0.78±0.08$^{\#}$
Pregnanolone (10)	Pregnanolone (10)	18	300 (190–300)	1.00±0.05
(b) Allopregnanolone				
Vehicle	Vehicle	16	300 (300–300)	1.14±0.10
Vehicle	Allo (5)	20	232 (100–300)*	0.92±0.07
Allo (5)	Vehicle	20	176 (122–300)	0.86±0.07$^{\#}$
Allo (5)	Allo (5)	16	294 (186–300)	1.16±0.10

Drug injections were performed 15 min before the acquisition or test session (see legend of Table 2 for details). ANOVA analyses: (a) KW = 11.17, $P<0.05$, for latencies and $F_{(3,69)} = 3.40$, $P<0.005$ for latency ratios; (b) KW = 13.58, $P<0.01$ for latencies and $F_{(3,71)} = 2.95$, $P<0.05$ for latency ratios.
*$P<0.05$, **$P<0.01$ *vs* the vehicle repeated treatment; $^{\#}P<0.05$ *vs* the steroid repeated treatment; Dunn's test for latency and Newmann–Keuls' test for ratio.

Table 5 Neuroactive Steroids Pregnenolone, DHEA, and Progesterone Failed to Induced State-Dependent Learning

Acquisition treatment	Retention treatment	*n*	Latency median (IR) (s)	Latency ratio (log-transformed)
(a) Pregnenolone				
Vehicle	Vehicle	14	259 (126–300)	1.19±0.10
Vehicle	Pregnenolone (10)	15	300 (131–300)	1.00±0.11
Pregnenolone (10)	Vehicle	15	300 (188–300)	1.21±0.13
Pregnenolone (10)	Pregnenolone (10)	15	300 (190–300)	1.22±0.13
(b) DHEA				
Vehicle	Vehicle	20	300 (212–300)	1.28±0.12
Vehicle	DHEA (10)	20	300 (114–300)	1.09±0.12
DHEA (10)	Vehicle	20	300 (180–300)	1.16±0.08
DHEA (10)	DHEA (10)	20	300 (164–300)	1.13±0.09
(c) Progesterone				
Vehicle	Vehicle	15	300 (225–300)	1.27±0.12
Vehicle	Progesterone (5)	15	300 (240–300)	1.10±0.11
Progesterone (5)	Vehicle	15	300 (171–300)	1.02±0.12
Progesterone (5)	Progesterone (5)	15	300 (175–300)	1.12±0.08

Drug injections were performed 15 min before the acquisition or test session (see legend of Table 2 for details). ANOVA analyses: (a) KW = 1.95, $P>0.05$, for latencies and $F_{(3,58)} = 0.77$, $P>0.05$ for latency ratios; (b) KW = 0.58, $P>0.05$ for latencies and $F_{(3,79)} = 0.60$, $P>0.05$ for latency ratios; (c) KW = 0.28, $P>0.05$ for latencies and $F_{(3,59)} = 0.90$, $P>0.05$ for latency ratios.

Administration of DHEA before cocaine led, however, to a modification of the chemical state induced by cocaine alone. Significant diminutions of the retention parameters were measured for latency (KW = 19.11, $P<0.001$; Figure 4a) or latency ratio ($F_{(3,77)} = 5.37$, $P<0.01$; Figure 4b). Progesterone also affected the cocaine-induced chemical state. When the steroid was administered before cocaine during retention, animals showed a correct transfer to cocaine, suggesting that the cocaine-induced state was not modified. However, when progesterone was administered before cocaine during training, significant diminutions of the retention parameters were observed (KW = 32.82, $P<0.0001$; Figure 4c; $F_{(3,77)} = 9.35$, $P<0.0001$; Figure 4d).

Nature of the σ_1 Receptor Ligand-Induced Modification of the Cocaine-Induced Chemical State

As shown in Table 6a, co-treatment of cocaine with the σ_1 receptor antagonist BD1047 or progesterone, administered

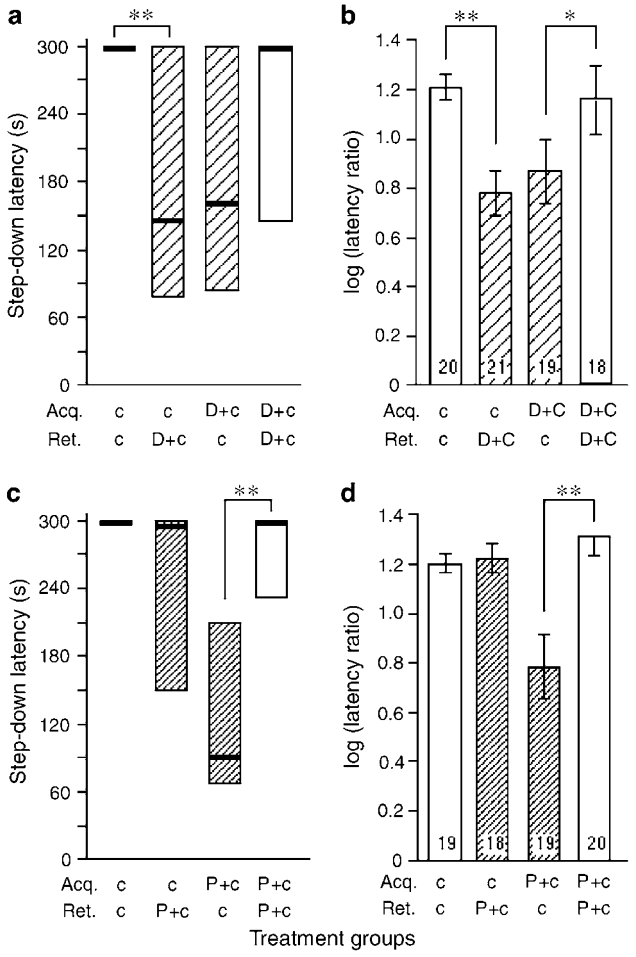

Figure 4 σ_1 Receptor-interacting steroids affected the cocaine-induced state-dependent learning. Pretreatment with DHEA or progesterone affected the cocaine-induced state-dependent learning. Animals received: (a, b) DHEA (D, 10 mg/kg) or (c, d) progesterone (P, 5 mg/kg) simultaneously with cocaine (c, 0.1 mg/kg) or only cocaine (0.1 mg/kg) in repeated or crossed treatments before the acquisition (Acq.) and retention (Ret.) sessions. Retention was analyzed in terms of median step-down latency (a, c) and log-transformed latency ratios (b, d). The number of animals per group is indicated within columns in (b, d). $^{*}P<0.05$, $^{**}P<0.01$, Dunn's test in (a, c) and Newmann–Keuls' test in (b, d).

before training or retention, showed transfer to vehicle solutions, since similar retention parameters were measured in all groups (KW = 10.07, $P>0.05$ for latency; $F_{(6,135)} = 0.96$, $P>0.05$ for latency ratio; Table 6a).

Co-administration of the σ_1 receptor agonist igmesine or DHEA before 0.1 mg/kg cocaine, administered before training or retention, showed transfer to 0.3 mg/kg cocaine, since similar retention parameters were measured in all treatment groups (KW = 7.03, $P>0.05$ for latency; $F_{(6,122)} = 0.39$, $P>0.05$ for latency ratio; Table 6b). Thus, the co-treatment with BD1047 or progesterone allowed a complete 'masking' of the cocaine-induced chemical state, since transfer was observed with the vehicle solution, whereas a co-treatment with igmesine or DHEA allowed to 'amplify' the chemical state induced by cocaine, since transfer was observed with a higher dose of the drug.

DISCUSSION

Cocaine-Induced StD

The preliminary observation described herein showed that a low dose of cocaine ameliorated memory retrieval in Swiss mice submitted to a classical passive avoidance procedure. The effect appeared bell-shaped and limited to a narrow dose range. No effect was observed on the acquisition or consolidation phase. This observation confirmed previous reports showing memory-enhancing effects for cocaine (Introini-Collison and McGaugh, 1989; Janak *et al*, 1992; Weinberger *et al*, 1992; White *et al*, 1995). Noteworthy, at a higher dosage, 10–40 mg/kg i.p., cocaine was reported to produce learning deficits (Branch and Dearing, 1982; Hudzik and Wenger, 1993; Wenger and Wright, 1990; Baron *et al*, 1998; Quirk *et al*, 2001). The observation that 0.3 mg/kg of cocaine allowed to improve a memory parameter, the retention quality, led us to select 0.1 mg/kg for the further examination of the ability of cocaine to produce state-dependent learning.

The present study demonstrated for the first time that cocaine produces StD, since cocaine/saline or saline/cocaine crossed treatment between acquisition and retention sessions of a passive avoidance response resulted in significant diminutions of the memory quality. On the contrary, when animals were trained and tested under the same cocaine state, they showed optimal retention parameters, similar to animals treated repeatedly with saline, that is, presumably in normophysiological conditions. This cocaine-induced StD, resulting from the modification of the brain chemical state, occurred at low doses, in the 0.03–0.3 mg/kg range, whereas hyperlocomotion, learning impairments, and reward properties are observed in the 10–40 mg/kg dose range, depending on mouse strains (Romieu *et al*, 2000, 2002, 2004; Quirk *et al*, 2001; Phan *et al*, 2002). This StD was dose-dependent and animals trained with 0.1 mg/kg cocaine showed an adequate transfer when tested at the same dose and not at 0.03 or 0.3 mg/kg. Such narrow bell-shaped dose range for StD was already observed for morphine-induced StD in rats (Bruins Slot and Colpaert, 1999a, b). It was proposed that the morphine-induced memory trace is specific to a particular magnitude of opiate receptor activation (Bruins Slot and Colpaert, 1999a, b). Similarly, cocaine, at the doses used, may induce only a partial blockade of the dopamine transporter, and conceivably discretely modify the rate of dopamine reuptake, resulting in a particular physiological state for both the pre- and post-synaptic neurons in monoaminergic target structures, such as the nucleus accumbens, striatum, amygdala, or frontal cortex. The observation of a clear StD for cocaine confirmed that acute administration of the drug provoked rapid and intense pharmacological effects in the brain. It is thus conceivable that the abused drug provoked at higher doses, even after acute administration, an intense adaptative plasticity, as already described electrophysiologically, in terms of modification of the NMDA/AMPA receptor long-term potentiation responses in the ventral tegmental area (Ungless *et al*, 2001). The cocaine ability to change the chemical state after acute injection may also contribute to its potent and long-term reinforcing effect. For instance, self-administration induced by a single exposure to cocaine in food-trained rats could

Table 6 Modification of the Cocaine-Induced Chemical State by σ_1 Ligands or σ_1 Receptor-Interacting Steroids

Acquisition treatment	Retention treatment	*n*	Latency median (IR) (s)	Latency ratio (log-transformed)
(a) Transfer between σ_1 antagonists+cocaine and vehicle solution				
Vehicle	Vehicle	20	300 (233–300)	1.08±0.09
Vehicle	BD1047+cocaine	20	300 (240–300)	1.25±0.12
BD1047+cocaine	Vehicle	20	208 (146–300)	1.16±0.13
BD1047+cocaine	BD1047+cocaine	20	300 (300–300)	1.26±0.12
Vehicle	Progesterone+cocaine	18	300 (218–300)	1.21±0.10
Progesterone+cocaine	Vehicle	18	300 (219–300)	1.17±0.14
Progesterone+cocaine	Progesterone+cocaine	20	300 (229–300)	1.31±0.11
(b) Transfer between σ_1 agonists+cocaine (0.1 mg/kg) and cocaine (0.3 mg/kg)				
Cocaine (0.3)	Cocaine (0.3)	18	300 (200–300)	1.22±0.14
Cocaine (0.3)	Igmesine+cocaine (0.1)	18	300 (206–300)	1.16±0.16
Igmesine+cocaine (0.1)	Cocaine (0.3)	18	300 (225–300)	1.06±0.06
Igmesine+cocaine (0.1)	Igmesine+cocaine (0.1)	15	300 (300–300)	1.11±0.13
Cocaine (0.3)	DHEA+cocaine (0.1)	16	300 (219–300)	1.17±0.21
DHEA+cocaine (0.1)	Cocaine (0.3)	20	300 (252–300)	1.22±0.14
DHEA+cocaine (0.1)	DHEA+cocaine (0.1)	18	300 (145–300)	1.16±0.18

(a) Animals were injected with BD1047 (1 mg/kg) or progesterone (5 mg/kg) or vehicle solution immediately before cocaine (0.1 mg/kg), 15 min before the first training session, and/or before the retention session in repeated or crossed treatments. (b) Animals were injected with igmesine (0.5 mg/kg) or DHEA (10 mg/kg) immediately before cocaine (0.1 mg/kg) or with cocaine (0.3 mg/kg), 15 min before the first training session, and/or before the retention session in repeated or crossed treatments. No statistically significant difference was observed.

be reactivated after several months, as recently reported (Ciccocioppo *et al*, 2004). However, it must be noted that, if addictive effects of cocaine are largely believed to be mediated by dopamine transporter blockade, StD observed in our results can also ensue from cocaine's actions on other targets, with comparable or higher affinity such as other monoamine transporters or σ_1 receptor.

The σ_1 Receptor and Cocaine-Induced StD

The σ_1 receptor is presently considered to be an essential component of the intracellular regulation and trafficking mechanisms (Su and Hayashi, 2001). The receptor regulates several neuronal functions and particularly intracellular Ca^{2+} mobilization, Ca^{2+} channel activity, neurotransmitter release, and neuronal firing (Hayashi *et al*, 2000; Maurice *et al*, 2002). However, even at large, physiologically relevant dosages, acute or chronic administration of σ_1 ligands, agonists, as well as antagonists failed to induce notable effects in control animals. At the behavioral level, as well as in numerous physiological tests, σ_1 receptors act as pure neuromodulatory receptors, devoid of effect in control conditions, but highly effective when a pharmacological or pathological unbalanced state is induced. Here, we extended these observations to the notion of state-dependent learning. Selective σ_1 receptor ligands failed to induce a particular memory trace by their own, at a dose subactive in terms of direct effects on memory abilities. Larger dose remains to be tested. However, compounds, at the dose tested, induced a partial activation or antagonism of the σ_1 receptor, sufficient to modify the cocaine-induced StD, thus clearly involving the σ_1 receptor in the acquisition of the drug-induced state.

The σ_1 receptor plays an important role in several pharmacological effects induced by cocaine. The drug presents a low micromolar affinity for the σ_1 receptor (Sharkey *et al*, 1988), but whether its interaction could be direct or indirect is still under controversy (Maurice *et al*, 2002). However, its involvement in the drug-induced acute locomotor effect and stereotypies, in the sensitization of these behavioral responses, and toxic effects after high doses of the drug were already reported (for a review, see Maurice *et al*, 2002). The σ_1 receptor activation is also involved in the acquisition, expression, and reactivation of cocaine-induced conditioned place preference (Romieu *et al*, 2000, 2002, 2004), suggesting that the rewarding effects of the drug and relapse to drug craving is highly influenced by this intraneuronal modulatory system. The present study showed that if modulation of the σ_1 receptor alone, using the agonist igmesine or antagonist BD1047, did not result in a particular chemical state, its activation was involved in the memory state induced by cocaine administration. As said above, cocaine-induced StD is likely to involve the rate of occupancy of monoamine transporters and therefore to result from an increase in monoaminergic neurotransmission. However, the co-treatment with the σ_1 agonist igmesine + cocaine resulted in an amplification of the cocaine-induced memory state, since transfer of the retention quality was observed to the higher, 0.3 mg/kg, dose of cocaine. On the contrary, the co-treatment with the σ_1 antagonist BD1047 + cocaine resulted in a complete blockade of the cocaine-induced memory state, since transfer to the vehicle solution was observed. Therefore,

the fact that both the agonist and antagonist modified the cocaine-induced memory state was not the reflection of a similar behavioral effects, but an amplification for the agonist *vs* a masking effect for the antagonist. A similar involvement of the σ_1 receptor was previously observed for the acquisition of cocaine-induced appetitive properties (Romieu *et al*, 2000, 2002). Conceivably, the abused drug seems to misappropriate a natural neuromodulatory system to acquire and amplify its effects, from the subtlest ones, the memory trace (this study), to the most drastic, including hyperlocomotion, convulsions, and lethality (Reith *et al*, 1986; Menkel *et al*, 1991; Ujike *et al*, 1992; Ritz and George, 1993). As such, the receptor appeared as a very important component mediating the neuroplastic changes induced by cocaine.

Influence of Neuro(active) Steroids on Cocaine-Induced StD

The σ_1 receptor represents one of the particular targets by which some neuroactive steroids act to exert their rapid nongenomic effects in the brain (Su *et al*, 1988; Maurice *et al*, 1999). These steroids are synthesized in the periphery or centrally in the brain by glial cells and neurons, and modulate the brain activity through both genomic and nongenomic rapid neuromodulatory actions affecting several neurotransmitters and second messenger systems (Baulieu, 1981; Paul and Purdy, 1992). *In vitro* and *in vivo* pharmacological tests, as well as behavioral studies, demonstrated that pregnenolone, DHEA, and their sulfate esters act as σ_1 receptor agonists, whereas progesterone acts as a potent antagonist (Monnet *et al*, 1995; Bergeron *et al*, 1996; Maurice *et al*, 1997; Maurice and Privat, 1997). These steroids also interact with excitatory and inhibitory neurotransmitter receptors. The reduced metabolites of progesterone, including pregnanolone and allopregnanolone, are devoid of effect on the σ_1 receptor, but are efficient positive modulators of the $GABA_A$ receptor (Schumacher and McEwen, 1989).

In the present study, we observed that pregnanolone and allopregnanolone produced StD in mice, while pregnenolone, DHEA, and progesterone failed to provoke a reliable memory state sustaining StD. $GABA_A$ receptor modulators, including benzodiazepines, like diazepam or chlordiazepoxide, have been shown to produce strong memory traces, resulting in StD in rodents and allowing transfer of retention quality to pregnanolone (Colpaert, 1990; Colpaert and Koek, 1996; Bruins Slot and Colpaert, 1999a, b). This notion has even been proposed as a unique concept for the psychophysiological effects of exogenous benzodiazepines or GABA receptor-modulating endogenous neuroactive steroids (Colpaert, 1990; Bruins Slot *et al*, 1999b). The latter steroids, like pregnanolone or allopregnanolone, may thus produce StD through their $GABA_A$ receptor interaction. They, interestingly, showed plasma and brain levels varying considerably with exogenous conditions. In particular, stressful events lead to marked increases in steroid levels that in turn are able to constraint the memory for particular environmental conditions (Healy and Drugan, 1996; Bruins Slot and Colpaert, 1999b). Such a role for these GABAergic steroids in the constraint of memory must be taken into account and deserves more extensive studies.

Moreover, in the present study, we observed that other precursor steroids, like pregnenolone, DHEA, or even progesterone, failed to induce an observable memory trace in the brain, at the doses tested. If these steroids interacted mainly with $GABA_A$ receptors, or even NMDA receptors, blockade of which is also known to sustain StD (Jackson *et al*, 1992; Harrod *et al*, 2001), they would be expected to modify the brain chemical state in a way allowing the observation of StD. Their interaction with the σ_1 receptor, known to occur at the dosage tested in the present study (Maurice *et al*, 2001), may explain the lack of memory trace, further suggesting that acting through the σ_1 receptor could also allow to modulate the chemical states induced by GABAergic or glutamatergic drugs. Indeed, DHEA behaved as igmesine. It failed to induce a memory state by itself, but amplified the cocaine (0.1 mg/kg)-induced memory state and produced transfer to the higher dose of drug. Progesterone behaved also similarly as BD1047, by producing a clear masking effect of the cocaine-induced memory state. These observations bring a physiological substratum for the involvement of the σ_1 receptor activation in the cocaine StD and suggest, together with previous conditioned place preference results (Romieu *et al*, 2003, 2004), that the endogenous neurosteroid tonus may influence cocaine vulnerability.

Role of Neuro(active) Steroids in Physiological StD

Noteworthy, the observation that neuroactive steroids differentially sustained StD by themselves, besides their putative involvement in cocaine-induced memory trace, suggests a particular physiological role for this neuromodulatory system involved in controlling the excitatory/inhibitory balance in the brain. Depending on physiological conditions and neuroendocrine responses, neurosteroids may contribute to the constraint of memory according to StD concept or not. For instance, in stressful situations, steroid biosyntheses led to very high levels in reduced metabolites of progesterone as compared to precursor steroids (Paul and Purdy, 1992; Barbaccia *et al*, 1996; Urani *et al*, 2001), and thus to state-dependent memory processes, whereas, in normophysiological conditions, state-independent processes may prevail. In addition, basal levels in neuroactive steroids and life-long variations in biosyntheses levels vary considerably among individuals. Their involvement in the memory trace induced by cocaine, as well as in the acquisition of appetitive properties as recently reported (Romieu *et al*, 2003), confirmed their importance as a factor of vulnerability to develop drug addiction. Indeed, it has been demonstrated that pre-administration of DHEA or pregnenolone during the conditioning phase of place preference protocol enhanced cocaine-rewarding properties. In contrast, administration of progesterone or treatment with finasteride, a 5α-reductase inhibitor which accumulates endogenous progesterone by blocking the formation of reduced progesterone metabolites, led to abolition of cocaine place preference (Romieu *et al*, 2003). It could then be suggested that some neuro(active) steroids could interfere with cocaine subjective effects. DHEA is able to reinstate cocaine-seeking behavior in a place preference reactivation procedure (Romieu *et al*, 2004). The memory state induced by cocaine, and its physiological effects, may

therefore largely depend on the endogenous neurosteroid tonus. It could be proposed that neurosteroids, that is, glucocorticoids, sexual hormones, or reduced progesterone metabolites, may be involved in the stress-induced regulation of drug intake, in a state-dependant recall of consumption behavior. In other less stressful situations, cocaine use could be maintained by state-independent memory processes, where σ_1 receptor-related neurosteroids would be more effective in altering the subjective effects of the drug.

In conclusion, the present study showed that cocaine induced in the brain, at very low doses, a memory trace sufficient to sustain StD, in a passive avoidance response. This state of memory was dependent upon σ_1 receptor activation, although a selective agonist administered alone was not sufficient to provoke any particular chemical state. This observation confirmed the major involvement of the σ_1 receptor as a neuromodulatory system for the drug of abuse and for the resulting adaptative plastic changes in the brain structures. Neuroactive steroids played a particular role in the involvement of StD in learning and memory processes. On the one hand, the $GABA_A$ receptor-related steroids pregnanolone or allopregnanolone sustained StD. On the other hand, the σ_1 receptor-interacting steroids pregnenolone, DHEA, or progesterone failed to sustain StD, although they also interacted with $GABA_A$ or NMDA receptors. Two major physiological consequences for these observations are proposed. First, variations among steroid levels, in normophysiological *vs* stressful conditions, for instance, may switch physiological memory processes, from state-independent to state-dependent learning, with major consequences on the intensity of the memory trace. Second, variations in steroid levels and particularly the DHEA/progesterone ratio, among individuals or neuroendocrine conditions, may markedly influence the intensity of the chemical state induced by an acute injection of cocaine. Therefore, neurosteroids may conceivably play a major influence on the differential propensity to develop addiction among individuals.

ACKNOWLEDGEMENTS

We heartily thank Dr FC Colpaert (Pierre-Fabre Labs., Castres, France) for his careful reading of the manuscript and helpful comments. We also thank Drs FJ Roman (Fresnes, France) and WD Bowen (Bethesda, MD) for their gift of drugs. This work was supported by the Institut de la Santé et de la Recherche Médicale (INSERM). No third party funding was received.

REFERENCES

Barbaccia ML, Roscetti G, Trabucchi M, Mostallino MC, Concas A, Purdy RH *et al* (1996). Time-dependent changes in rat brain neuroactive steroid concentrations and GABAA receptor function after acute stress. *Neuroendocrinology* **63**: 166–172.

Baron SP, Wright D, Wenger GR (1998). Effects of drugs of abuse and scopolamine on memory in rats: delayed spatial alternation and matching to position. *Psychopharmacology* **137**: 7–14.

Baulieu EE (1981). Steroid hormones in the brain: several mechanisms?. In: Fuxe K, Gustafson JA, Wettenberg L (eds). *Steroid Hormone Regulation of the Brain*. Pergamon Press: Oxford. pp 3–14.

Bergeron R, de Montigny C, Debonnel G (1996). Potentiation of neuronal NMDA response induced by dehydroepiandrosterone and its suppression by progesterone: effects mediated via sigma receptors. *J Neurosci* **16**: 1193–1202.

Bozarth MA (1987). Conditioned place preference: a parametric analysis using systemic heroin injections. In: Bozarth MA (ed). *Methods of Assessing the reinforcing Properties of Abused Drugs*. Springer Verlag: New York. pp 241–272.

Branch MN, Dearing ME (1982). Effects of acute and daily cocaine administration on performance under a delayed-matching-to-sample procedure. *Pharmacol Biochem Behav* **16**: 713–718.

Bruins Slot LA, Colpaert FC (1999a). Recall rendered dependent on an opiate state. *Behav Neurosci* **113**: 337–344.

Bruins Slot LA, Colpaert FC (1999b). Opiate states of memory: receptor mechanisms. *J Neurosci* **19**: 10520–10529.

Bruins Slot LA, Piazza PV, Colpaert FC (1999). Neuroactive steroids and the constraint of memory. *Eur J Neurosci* **11**: 4081–4088.

Ciccocioppo R, Martin-Fardon R, Weiss F (2004). Stimuli associated with a single cocaine experience elicit long-lasting cocaine-seeking. *Nat Neurosci* **7**: 495–496.

Colpaert FC (1990). A memory trace locked into the benzodiazepine state of memory. *Psychopharmacology* **102**: 28–36.

Colpaert FC, Koek W (1995). Empirical evidence that the state dependence and drug discrimination paradigms can generate different outcomes. *Psychopharmacology* **120**: 272–279.

Colpaert FC, Koek W (1996). Behavioral specificity of chlordiazepoxide-produced StD. *Behav Pharmacol* **7**: 49–55.

Cornish JL, Duffy P, Kalivas PW (1999). A role for nucleus accumbens glutamate transmission in the relapse to cocaine-seeking behavior. *Neuroscience* **93**: 1359–1367.

Girden E, Culler EA (1937). Conditioned responses in curarized striate muscle in dogs. *J Comp Psychol* **23**: 261–274.

Harrod SB, Flint RW, Riccio DC (2001). MK-801 induced retrieval, but not acquisition, deficits for passive avoidance conditioning. *Pharmacol Biochem Behav* **69**: 585–593.

Hayashi T, Maurice T, Su TP (2000). Ca^{2+} signaling via $sigma_1$-receptors: novel regulatory mechanism affecting intracellular Ca^{2+} concentration. *J Pharmacol Exp Ther* **293**: 788–798.

Healy DJ, Drugan RC (1996). Escapable stress modulates retention of spatial learning in rats: preliminary evidence for involvement of neurosteroids. *Psychobiology* **24**: 110–117.

Hiramatsu M, Koide T, Ishihara S, Shiotani T, Kameyama T, Nabeshima T (1992). Involvement of the cholinergic system in the effect of nefiracetam (DM-9384) on carbon monoxide (CO)-induced acute and delayed amnesia. *Eur J Pharmacol* **216**: 279–285.

Hudzik TJ, Wenger GR (1993). Effects of drugs of abuse and cholinergic agents on delayed matching-to-sample responding in the squirrel monkey. *J Pharmacol Exp Ther* **265**: 120–127.

Introini-Collison IB, McGaugh JL (1989). Cocaine enhances memory storage in mice. *Psychopharmacology* **99**: 537–541.

Jackson A (1995). State-dependent effects of atypical benzodiazepine-receptor agonists. *Psychopharmacology* **119**: 399–404.

Jackson A, Koek W, Colpaert FC (1992). NMDA antagonists make learning and recall state-dependent. *Behav Pharmacol* **3**: 415–421.

Janak PH, Keppel G, Martinez JL (1992). Cocaine enhances retention of avoidance conditioning in rats. *Psychopharmacology* **106**: 383–387.

Koob GF (1992). Drugs of abuse: anatomy, pharmacology and function of reward pathways. *Trends Pharmacol Sci* **13**: 177–184.

Kuhar MJ (1992). Molecular pharmacology of cocaine: a dopamine hypothesis and its implications. *Ciba Found Symp* **166**: 81–89.

Matsumoto RR, Bowen WD, Tom MA, Vo VN, Truong DD, De Costa BR (1995). Characterization of two novel sigma receptor ligands: antidystonic effects in rats suggest sigma receptor antagonism. *Eur J Pharmacol* **280**: 301–310.

Maurice T, Hiramatsu M, Itoh J, Kameyama T, Hasegawa T, Nabeshima T (1994). Behavioral evidence for a modulating role of σ ligands in memory processes. I. Attenuation of dizocilpine (MK-801)-induced amnesia. *Brain Res* **647**: 44–56.

Maurice T, Junien JL, Privat A (1997). Dehydroepiandrosterone sulfate attenuates dizocilpine-induced learning impairment in mice via σ_1 receptors. *Behav Brain Res* **83**: 159–164.

Maurice T, Martin-Fardon R, Romieu P, Matsumoto RR (2002). Selective sigma$_1$ (σ_1) receptor antagonists as a new promising strategy to prevent cocaine-induced behaviors and toxicity. *Neurosci Biobehav Res* **26**: 499–527.

Maurice T, Phan VL, Urani A, Kamei H, Noda Y, Nabeshima T (1999). Neuroactive neurosteroids as endogenous effector for the sigma$_1$ (σ_1) receptor: pharmacological evidences and therapeutic opportunities. *Jpn J Pharmacol* **81**: 125–155.

Maurice T, Privat A (1997). SA4503, a novel cognitive enhancer with σ_1 receptor agonist properties, facilitates NMDA receptor-dependent learning in mice. *Eur J Pharmacol* **328**: 9–18.

Maurice T, Roman F, Su TP, Privat A (1996). Beneficial effects of σ agonists on the age-related learning impairment in senescence-accelerated mouse (SAM). *Brain Res* **733**: 219–230.

Maurice T, Su TP, Privat A (1998). Sigma$_1$ (σ_1) receptor agonists and neurosteroids attenuate β_{25-35}-amyloid peptide-induced amnesia in mice through a common mechanism. *Neuroscience* **83**: 413–428.

Maurice T, Urani A, Phan VL, Romieu P (2001). The interaction between neuroactive steroids and the sigma$_1$ (σ_1) receptor function: behavioral consequences and therapeutic opportunities. *Brain Res Rev* **37**: 116–132.

Menkel M, Terry M, Pontecorvo M, Katz JL, Witkin JM (1991). Selective σ ligands block stimulant effects of cocaine. *Eur J Pharmacol* **201**: 251–252.

Monnet FP, Mahé V, Robel P, Baulieu EE (1995). Neurosteroids, via sigma receptors, modulate the [^{3}H]norepinephrine release evoked by *N*-methyl-D-aspartate in the rat hippocampus. *Proc Natl Acad Sci USA* **92**: 3774–3778.

Nakagawa Y, Iwasaki T (1996). Ethanol-induced state-dependent learning is mediated by 5-hydroxytryptamine-3 receptors but not by *N*-methyl-D-aspartate receptor complex. *Brain Res* **706**: 227–232.

Nakagawa Y, Iwasaki T, Ishima T, Kimura K (1993). Interaction between benzodiazepine and GABA-A receptors in state-dependent learning. *Life Sci* **52**: 1935–1945.

Oberling P, Rocha B, Di Scala G, Sandner G (1993). Evidence for state-dependent retrieval in conditioned place aversion. *Behav Neural Biol* **60**: 27–32.

Overton DA (1966). State-dependent learning produced by depressant and atropine-like drugs. *Psychopharmacology* **10**: 6–31.

Overton DA (1978). Basic mechanisms of state-dependent learning. *Psychopharmacol Bull* **14**: 67–68.

Park WK, Bari AA, Jey AR, Anderson SM, Spealman RD, Rowlett JK *et al* (2002). Cocaine administered into the medial prefrontal cortex reinstates cocaine-seeking behavior by increasing AMPA receptor-mediated glutamate transmission in the nucleus accumbens. *J Neurosci* **22**: 2916–2925.

Paul SM, Purdy RH (1992). Neuroactive steroids. *FASEB J* **6**: 2311–2322.

Phan VL, Urani A, Romieu P, Maurice T (2002). Strain differences in σ_1 receptor-mediated behaviours are related to neurosteroid levels. *Eur J Neurosci* **15**: 1523–1534.

Pickens R, Thompson T (1968). Cocaine-reinforced behavior in rats: effects of reinforcement magnitude and fixed-ratio size. *J Pharmacol Exp Ther* **161**: 122–129.

Quirk PL, Richards RW, Avery DD (2001). Subchronic cocaine produces training paradigm-dependent learning deficits in laboratory rats. *Pharmacol Biochem Behav* **68**: 545–553.

Reith MEA, Meisler BE, Sershen H, Lajtha A (1986). Structural requirements for cocaine congeners to interact with dopamine and serotonin uptake sites in mouse brain and to induce stereotyped behavior. *Biochem Pharmacol* **35**: 1123–1129.

Ritz MC, George FR (1993). Cocaine-induced seizures and lethality appear to be associated with distinct central nervous system binding sites. *J Pharmacol Exp Ther* **264**: 1333–1343.

Ritz MC, Lamb RJ, Goldberg SR, Kuhar MJ (1987). Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science* **237**: 1219–1223.

Roman FJ, Pascaud X, Martin B, Vauché D, Junien JL (1990). JO 1784, a potent and selective ligand for rat and mouse brain sigma-sites. *J Pharm Pharmacol* **42**: 439–440.

Romieu P, Martin-Fardon R, Bowen WD, Maurice T (2003). Sigma$_1$ (σ_1) receptor-related neuroactive steroids modulate cocaine-induced reward. *J Neurosci* **23**: 3572–3576.

Romieu P, Martin-Fardon R, Maurice T (2000). Involvement of the σ_1 receptor in the cocaine-induced conditioned place preference. *Neuroreport* **11**: 2885–2888.

Romieu P, Meunier J, Garcia D, Zozime N, Martin-Fardon R, Bowen WD *et al* (2004). The sigma$_1$ (σ_1) receptor activation is a key step for the reactivation of cocaine conditioned place preference by drug priming. *Psychopharmacology* **175**: 154–162.

Romieu P, Phan VL, Martin-Fardon R, Maurice T (2002). The sigma$_1$ receptor involvement in cocaine-induced conditioned place preference is consequent upon dopamine uptake blockade. *Neuropsychopharmacology* **26**: 444–455.

Schumacher M, McEwen BS (1989). Steroid and barbiturate modulation of the GABA$_A$ receptor. *Mol Neurobiol* **3**: 275–280.

Sharkey J, Glen KA, Wolfe S, Kuhar MJ (1988). Cocaine binding at σ receptors. *Eur J Pharmacol* **149**: 171–174.

Stolerman I (1992). Drugs of abuse: behavioural principles, methods and terms. *Trends Pharmacol Sci* **13**: 170–176.

Su TP, Hayashi T (2001). Cocaine affects the dynamics of cytoskeletal proteins via sigma$_1$ receptors. *Trends Pharmacol Sci* **22**: 456–458.

Su TP, London ED, Jaffe JH (1988). Steroid binding at σ receptors suggests a link between endocrine, nervous, and immune systems. *Science* **240**: 219–221.

Swerdlow NR, van der Kooy D, Koob GF, Wenger JR (1983). Cholecystokinin produces conditioned place-aversions, not place-preferences, in food-deprived rats: evidence against involvement in satiety. *Life Sci* **32**: 2087–2093.

Ujike H, Tsuchida K, Akiyama K, Otsuki S (1992). Supersensitivity of σ receptors after repeated administration of cocaine. *Life Sci* **51**: PL31–PL36.

Ungless MA, Whistler JL, Malenka RC, Bonci A (2001). Single cocaine exposure *in vivo* induces long-term potentiation in dopamine neurons. *Nature* **411**: 583–587.

Urani A, Privat A, Maurice T (1998). The modulation by neurosteroids of the scopolamine-induced learning impairment in mice involves an interaction with sigma$_1$ (σ_1) receptors. *Brain Res* **799**: 64–77.

Urani A, Roman FJ, Phan VL, Su TP, Maurice T (2001). The antidepressant-like effect induced by sigma$_1$-receptor agonists and neuroactive steroids in mice submitted to the forced swimming test. *J Pharmacol Exp Ther* **298**: 1269–1279.

Weinberger SB, Riedel CA, Janak PH, Martinez JL (1992). Cocaine enhances one-way avoidance responding in mice. *Pharmacol Biochem Behav* **41**: 851–854.

Weingartner H (1978). Human state dependent learning. In: Ho BT, Richards III DW, Chute DL (eds). *Drug Discrimination*

and Stae Dependent Learning. Academic Press: New York. pp 363–383.

Wenger GR, Wright DW (1990). Disruption of performance under a titrating matching-to-sample schedule of reinforcement by drugs of abuse. *J Pharmacol Exp Ther* **254**: 258–269.

White IM, Christensen JR, Flory GS, Miller DW, Rebec GV (1995). Amphetamine, cocaine, and dizocilpine enhance performance on a lever-release, conditioned avoidance response task in rats. *Psychopharmacology* **118**: 324–331.

Woolverton WL (1992). Determinants of cocaine self-administration by laboratory animals. *Ciba Found Symp* **166**: 149–161.

Zarrindast MR, Jafari MR, Ahmadi S, Djahanguiri B (2004). Influence of central administration ATP-dependent K^+ channel on morphine state-dependent memory of passive avoidance. *Eur J Pharmacol* **487**: 143–148.

Zarrindast MR, Rezayof A (2004). Morphine-state dependent learning: sensitization and interactions with dopamine receptors. *Eur J Pharmacol* **497**: 197–204.