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Acute anxiolytic effects of cocaine: The role of test latency and activity phase

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Abstract

The emotional effects of the initial consumption of cocaine can have a strong influence on the subsequent use of this drug. Several studies in rodents using long test latencies have reported anxiogenic effects of cocaine. Anxiogenesis, however, would seem to contradict cocaine's well known rewarding effects and its abuse liability. The present study was set up to shed light on conditions that influence cocaine's consequences, namely the time after administration and active vs. resting test phase. The aim of the first experiment was to investigate the effects of cocaine (0, 5, 10, 15 mg/kg, i.p.) on anxiety-related behavior in rats in their active phase with a short test latency of 10 min. In the open field test cocaine had an anxiolytic-like effect, which was confirmed in the elevated plus-maze test. A second experiment investigated the effects of cocaine after a latency of 30 min in animals in their active vs. resting phase. After 30 min significant anxiolytic-like effects were no longer observed in either of the paradigms, irrespective of the activity phase. This and other studies suggest that after first exposure cocaine has acute anxiolytic effects, which rapidly decline, and, may eventually reverse to a longer lasting anxiogenic state.

Keywords: Cocaine; Anxiety; Open field; Elevated plus-maze; Rats

1. Introduction

The initial experience with psychoactive drugs can have a profound influence on the future consumption pattern of such drugs. The initial effects of a psychoactive drug, however, depend on several parameters, such that the same drug can have very different effects in different individuals (Resnick et al., 1977; De Souza Silva et al., 2006) or in distinct settings (Volkow et al., 2006). Since the occurrence of pleasant or aversive initial effects is important for subsequent drug consumption, it is imperative to know what the determinants of these initial effects are.

A well known abused drug is cocaine (Nutt et al., 2007). During the first exposure of a low to medium dose, cocaine was reported to induce locomotor activation and arousal in humans and animals, and euphoria in humans (Gawin and Ellinwood, 1988; Johanson and Fischman, 1989). However, a considerable proportion of human users do not only report euphoria, but also agitation and anxiety (Post et al., 1974; Resnick et al., 1977). At higher doses, cocaine may not only cause schizophrenia-like symptoms (Siegel, 1978; Rich and Singer, 1991), but may also trigger panic reactions and long lasting anxiety disorders (Geracioti and Post, 1991). Nonetheless, if anxiety were a predominant acute effect, cocaine would probably not become a drug of abuse in a considerable proportion of the experimental consumers and in animal models of cocaine addiction (Gawin, 1991; Deroche-Gamonet et al., 2004).

Several animal models have investigated the initial effects of cocaine on anxiety-related behaviors in rodents and non-human primates. These studies reported either anxiogenic-like effects

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(Costall et al., 1989; Fontana and Commissaris, 1989; Rogerio and Takahashi, 1992a,b; Yang et al., 1992; Blanchard et al., 1999; Paine et al., 2002; De Souza Silva et al., 2006), no effects (Yang et al., 1992; Rogerio and Takahashi, 1992a; Sarnyai et al., 1995), or anxiolytic-like effects (De Souza Silva et al., 2006; Erhardt et al., 2006). Notably, the anxiogenic-like effects of cocaine in rodents were almost exclusively reported with long test latencies and when animals were tested in their resting phase, i.e. in the light phase. Anxiolytic-like effects tended to appear with short test latencies. Testing in the active phase, i.e. in the dark phase, has rarely been performed in rodent studies on anxiety-like behavior with cocaine. Nevertheless, important behavioral effects of cocaine, such as sensitization and conditioned place preference, appear to depend on circadian rhythm, i.e. the time of testing (e.g. Abarca et al., 2002; Uz et al., 2003).

The present experiments aimed to investigate the effects of cocaine on anxiety-related behavior in two different paradigms in rats, namely, the open field (Prut and Belzung, 2003) and the elevated plus-maze (EPM; Hogg, 1996), after a short latency of 10 min with animals in their active phase. Since the first experiment showed dose-dependent anxiolytic-like effects of cocaine, a second experiment was performed to investigate whether the reported anxiolytic-like effects are still persistent after longer test latencies, i.e. 30 min, and, if they possibly depend on the day-time of testing.

2. Material and methods

All experiments were conducted in conformity with the Animal Protection Law of the Federal Republic of Germany and the European Communities Council Directive (86/609/EEC).

2.1. Animals

Male Wistar rats (Tierversuchsanlage, University of Düsseldorf, Germany), weighing 347±4 g (mean±SEM) in experiment I and 323±4 g in experiment II were used. They were housed 5–6 animals per cage under standard laboratory conditions with food and water provided ad libitum. In experiment I all animals were kept with a reversed light–dark rhythm (light on from 19:00 to 7:00). In experiment II half of the animals were kept under a normal light–dark-rhythm (light on from 7:00–19:00), and half with a reversed light–dark rhythm (light on from 19:00 to 7:00). All animals were allowed to acclimatize to the respective rhythm for 14 days before testing commenced. They were handled daily for 5 days before experimentation.

2.2. Substances

Cocaine (0, 5, 10, 15, 20 mg/kg; Merck, Germany) was dissolved in phosphate buffered saline and injected in a volume of 1.0 ml/kg.

2.3. Apparatus

In order to test cocaine effects on anxiety-like behaviors, two behavioral paradigms were used, the open field test and the elevated plus-maze (EPM). The open field (60×60×39 cm) was made of grey PVC and located in a sound-attenuating chamber. Illumination was provided indirectly by four red-light bulbs (each 25 W) on the floor of the chamber. The illumination was 0.5 lux in the center of the maze and 0.3 lx in the corners. The EPM was also located in a sound-attenuating chamber. It was made of black perspex 'Plexiglas' and consisted of an open square in the center (10×10 cm) and 2 open (50×10 cm) and 2 enclosed (50×10×40 cm) arms with an open roof, arranged in a way that the 2 arms of each type were opposite to each other. The maze was elevated to a height of 50 cm. The floor of the maze was covered with a black rubber mat to prevent animals from falling off the open arms. Illumination was provided by white LED bulbs suspended 150 cm above the maze, providing illumination of 48 lx on the open arms, 0.3 lx on the closed arms, and 42 lx in the center. For online and post-hoc analysis, the behavior of the animals was recorded by a video camera located above the center of the mazes, connected to an EthoVision system (Noldus, The Netherlands).

2.4. Behavioral analysis

All behaviors were either analyzed automatically or post-hoc by experimenters blind to the treatment of the animals.

2.4.1. Open field

In the open field general behavioral effects of the treatments and effects on anxiety-related behaviors were scored. As general activity was measured: locomotor activity, as distance moved, velocity, as moved distance per time unit, rearing, as the number of times the animal lifted both forepaws from the ground, grooming, as the time that the animal groomed with its mouth and/or paws. To measure anxiety-related behavior in the open field the arena was virtually subdivided in a 30×30 cm center and a periphery. Center-entries were measured as the number of times the animal entered the center. Center-time was the time that the animal spent in the center. Center-locomotion, velocity, and rearing were measured as the respective behaviors in the center.

2.4.2. Elevated plus-maze

As general activity measures in the EPM locomotor activity was scored. For that purpose open and closed arms were subdivided into a proximal and a distal part of equal size. Locomotor activity was measured as entries form one compartment to another with all four paws. In addition, rearing behavior was scored. Since animals rarely show grooming in the EPM, this behavior was not analysed. As particular anxiety-related measures in the EPM we determined the number of entries into the open and closed arms, and into the center. An entry was counted when the animal entered the respective area with all four paws. We measured the time that the animal spent in the open and closed arms, and in the center. Entries and sojourn times were also determined separately for proximal and distal arm compartments.

2.5. Test procedure

The testing took place from 10:00–18:00. Each animal was tested twice with the same drug treatment, once in the open field

and 5–6 days later in the EPM. Animals were habituated to the red-light illuminated (2 × 25 W) holding-room 30 min prior to the injection. For the injection, each animal was removed from the group cage and transported in a dark container to the quiet dimly lit injection room. There it received the intraperitoneal (i.p.) injection and was returned to the transport container. After the respective latency, the animal was transported in the container to the sound-attenuating chamber in the experimenting room which contained the open field or EPM. For open field testing the animal was placed into the middle of the open field, facing away from the experimenter, for EPM testing, the animal was placed into the center of the maze facing one of the open arms. Immediately after the animal was placed into the maze, the experimenter closed the chamber for the testing time. After each animal, the mazes were cleaned with a 10% alcohol solution and dried completely.

2.5.1. Experiment I

In this experiment the effects of cocaine were investigated in animals during their activity phase, i.e. in the dark phase, 10 min after treatment injection. All animals were randomly assigned to one of four treatment groups that received an i.p. injection of 0, 5, 10 or 15 mg/kg cocaine (n = 16–18/group). After a latency of 10 min animals were tested in the open field for 10 min and in the EPM for 5 min and then returned to the home cage.

2.5.2. Experiment II

In experiment II the effects of cocaine were compared between animals in their active and resting phase, i.e. between dark and light phase, after a latency of 30 min. All animals were randomly assigned to one of six treatment groups (n = 12/group). Three groups were held with a normal light–dark rhythm, and three with a reversed light–dark rhythm. Each animal received an i.p. injection of 0, 10 or 20 mg/kg cocaine. After a latency of 30 min, they were tested in the open field for 20 min and in the EPM for 5 min, and then returned to the home cage.

2.6. Statistical analysis

Behavioral data are presented as mean ± SEM. For statistical analysis in experiment I, a one-way-ANOVA was used with the

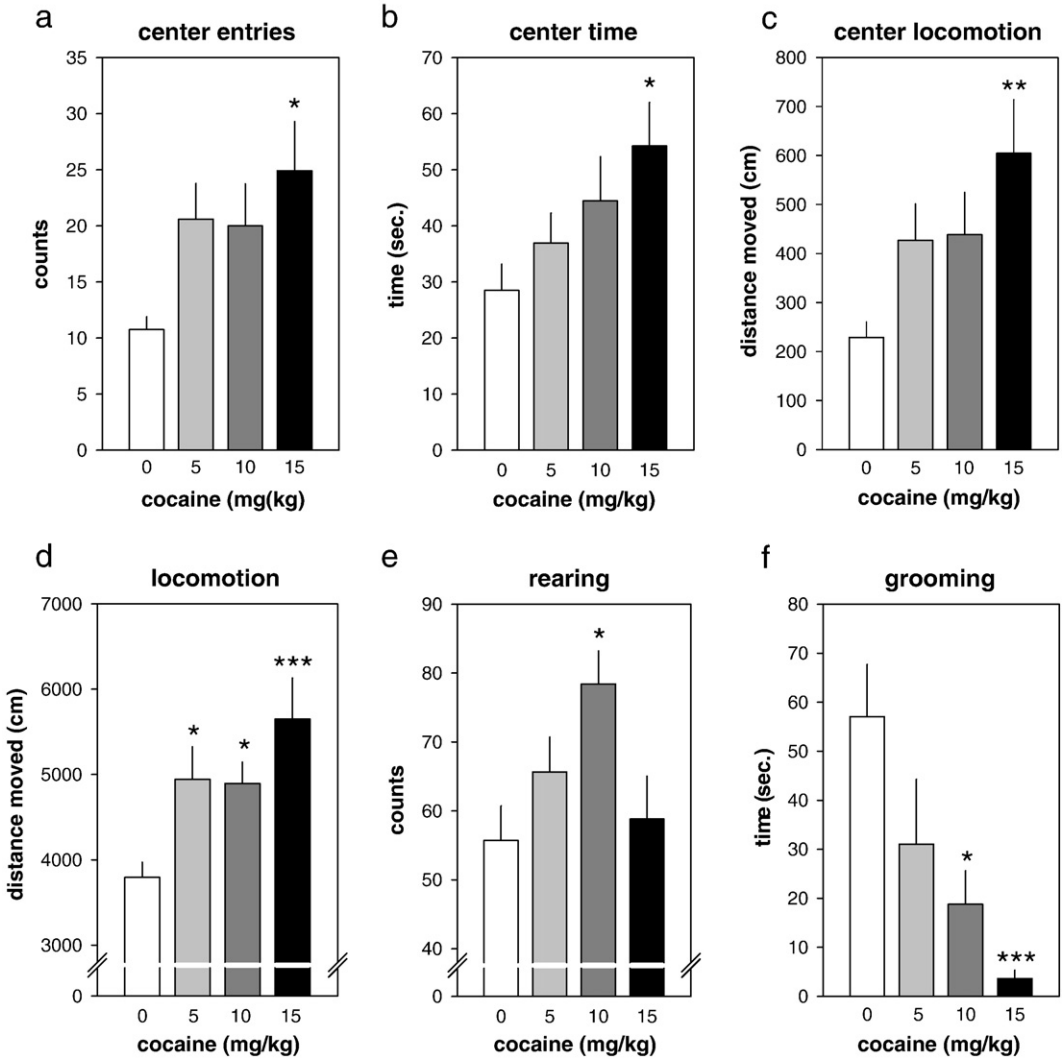

Fig. 1. The effects of cocaine on anxiety-related behavior and general behavioral activity in the open field test (mean ± S.E.M.). Animals were tested in their active phase, i.e. dark phase, 10–20 min after receiving an i.p. injection of cocaine (*p < 0.05, **p < 0.01, pre-planned Bonferroni-corrected Fisher's LSD test vs. saline).

factor treatment. In order to determine group differences, pre-planned Bonferroni-corrected Fisher's LSD-tests vs. saline control were used (Ramsey, 1993). In experiment II, a two-way ANOVA with activity phase and treatment as factors was used. In order to determine group differences, pre-planned Bonferroni-corrected Fisher's LSD-tests vs. the respective saline control were applied. A p-value of <0.05 was used for statistical significance. The software Statistica 4.0 was used for analysis.

3. Results

3.1. Experiment I

The number of center entries was increased 10–20 min after the cocaine treatment ($F_{3,33}=3.00$, $p=0.044$; Fig. 1a). Pre-planned comparisons showed a significantly increased number of center entries after 15 mg/kg cocaine ($p=0.019$), but not after 5 or 10 mg/kg ($p>0.05$). Cocaine increased the time spent in the center of the open field (Fig. 1b), although, analysis of variance only found a tendency ($F_{3,33}=2.76$, $p=0.058$). Pre-planned comparisons, however, showed a significantly increased time in the center after 15 mg/kg cocaine ($p=0.027$), but not after 5 or 10 mg/kg ($p>0.05$). Also, the locomotion in the center of the open field was increased by cocaine ($F_{3,33}=3.65$, $p=0.022$; Fig. 1c). Pre-planned comparisons revealed a significantly increased distance moved in the center after 15 mg/kg cocaine ($p=0.0069$), but not after 5 or 10 mg/kg ($p>0.05$). Interestingly, neither the velocity in the center nor rearing in the center were increased by cocaine ($p>0.05$; data not shown).

When the behavior in the whole maze was considered, cocaine significantly increased locomotor activity ($F_{3,33}=6.20$, $p=0.002$; Fig. 1d). Pre-planned comparisons showed a significant increase after 5 mg/kg ($p=0.039$), 10 mg/kg ($p=0.048$), and 15 mg/kg ($p=0.00048$). Also, overall velocity was significantly increased after cocaine ($F_{3,33}=6.11$, $p=0.002$). Pre-planned comparisons showed a significant increase after 5 mg/kg ($p=0.039$), 10 mg/kg ($p=0.051$), and 15 mg/kg ($p=0.00053$) cocaine (data not shown). Rearing behavior was

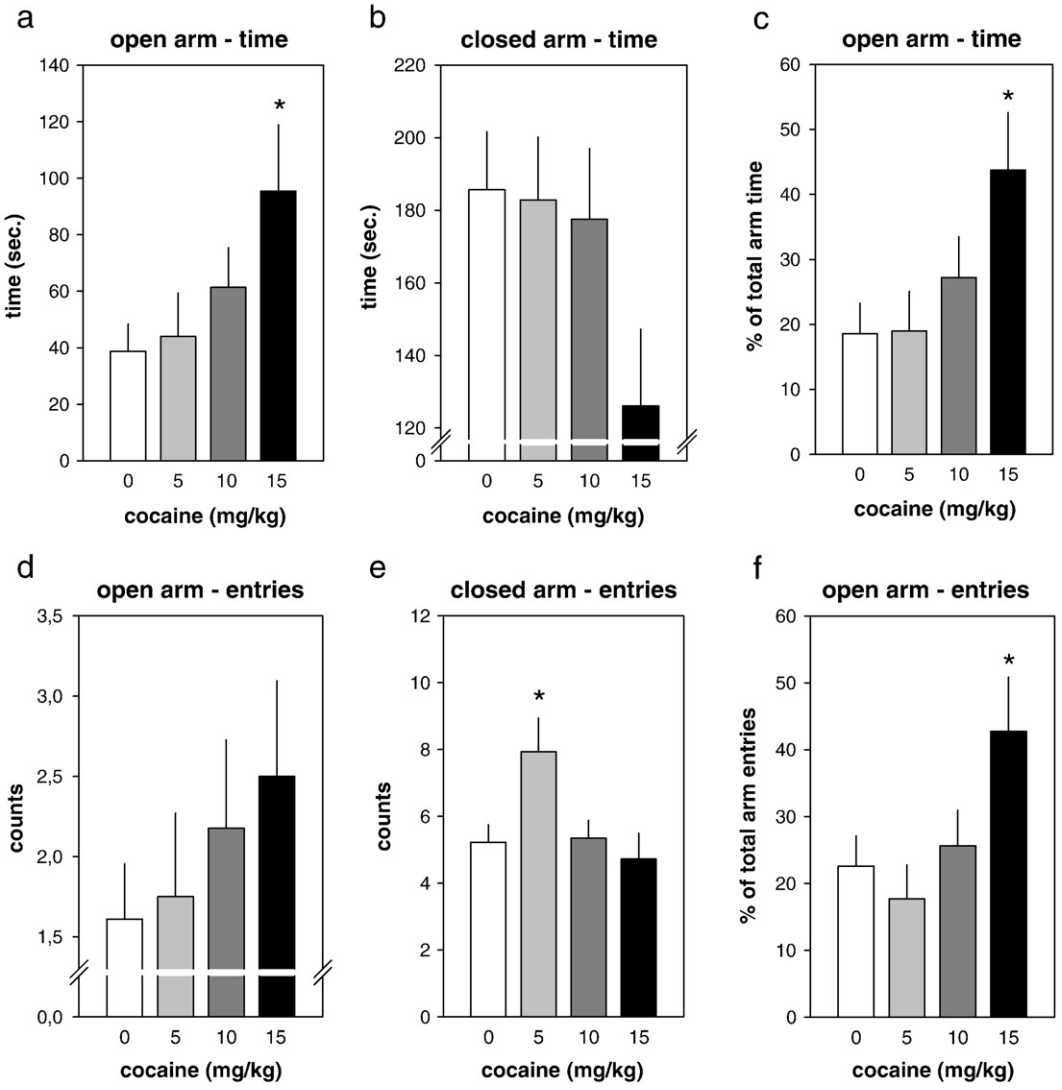

Fig. 2. The effects of cocaine on anxiety-related behavior in the elevated plus-maze (mean±S.E.M.). Animals were tested in their active phase, i.e. dark phase, 10–15 min after receiving an i.p. injection of cocaine (*$p<0.05$, pre-planned Bonferroni-corrected Fisher's LSD test vs. saline).

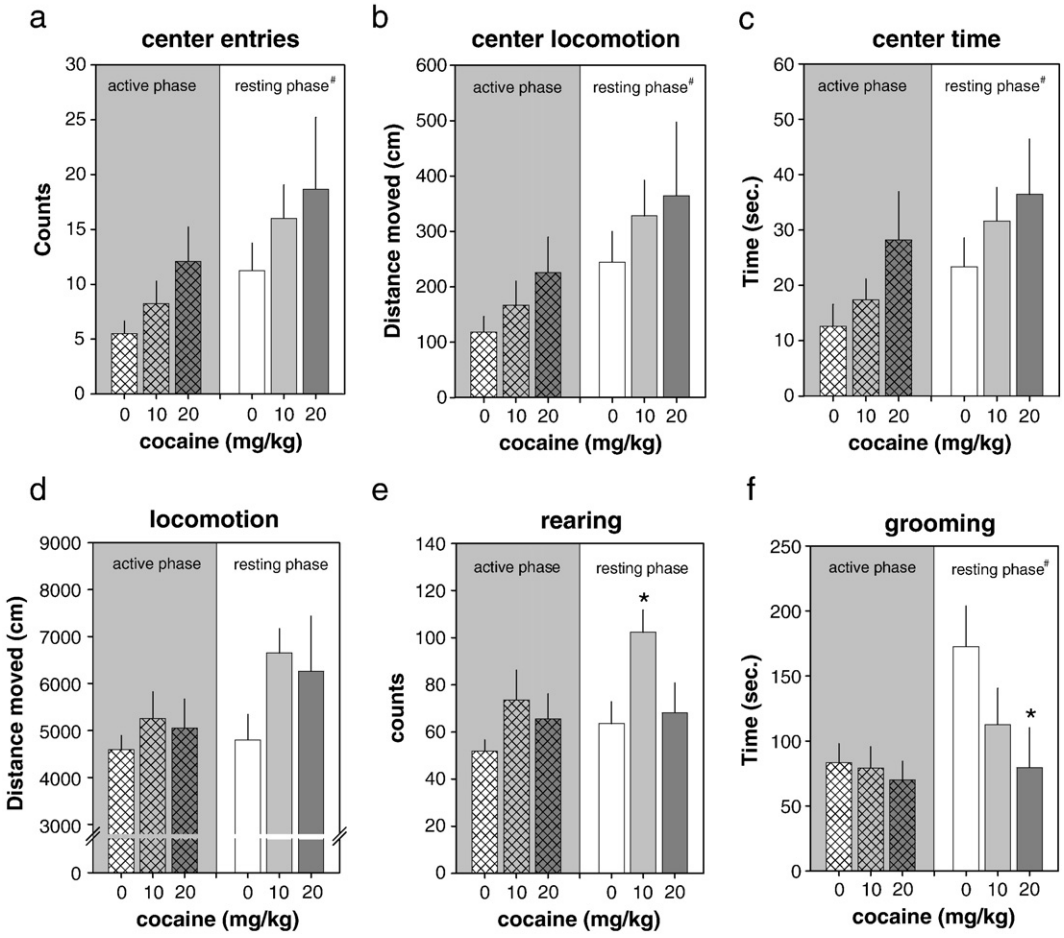

Fig. 3. The effects of cocaine on anxiety-related behavior and general behavioral activity in the open field test (mean±S.E.M.). Half of the animals were tested in their active phase, i.e. dark phase (grey), and half in their resting phase, i.e. light phase (white). Animals were tested 30–50 min after receiving an i.p. injection of cocaine ($^{\#}p<0.05$, two-way ANOVA, factor: activity phase; $*p<0.05$, pre-planned Bonferroni-corrected Fisher's LSD test vs. saline; for further statistical details: see text).

also enhanced after cocaine treatment ($F_{3,33}=3.23$, $p=0.035$; Fig. 1e). This effect was significant for the 10 mg/kg ($p=0.021$) dose, but not for 5 or 15 mg/kg dose (p>0.05). Grooming behavior decreased after cocaine treatment ($F_{3,33}=6.57$, $p=0.0013$). Pre-planned comparisons showed significant effects after 10 mg/kg ($p=0.012$) and 15 mg/kg ($p=0.00045$) cocaine, but not after 5 mg/kg ($p>0.05$).

The time spent in the open arms of the EPM was increased 10–15 min after the cocaine treatment, although an ANOVA failed to reach significance ($F_{3,65}=2.46$, $p=0.07$; Fig. 2a). Pre-planned comparisons, however, showed a significant increase in the time spent in the open arms after 15 mg/kg cocaine ($p=0.049$), but not after 5 or 10 mg/kg ($p>0.05$). Considering the time in the open arms as percent of the total time spent in the arms of the EPM (Fig. 2c), cocaine had a significant effect ($F_{3,65}=3.18$, $p=0.03$). Pre-planned comparisons showed a significant increase after 15 mg/kg ($p=0.024$), but not after 5 or 10 mg/kg ($p>0.05$). Visual inspection revealed that 15 mg/kg cocaine tended to reduce the time spent in the closed arms of the EPM (Fig. 2b), although neither ANOVA ($F_{3,65}=2.35$, $p=0.08$), nor pre-planned comparisons revealed statistically significant effects ($p>0.05$). There was also no cocaine effect on the time spent in the center of the EPM ($p>0.05$, data not shown). Visual inspection showed that cocaine increased the number of entries into the open arms of the EPM (Fig. 2d), although neither an ANOVA nor pre-planned comparisons revealed statistically significant effects ($p>0.05$). Considering the number of open arm entries as percent of the total arm entries (Fig. 2f), cocaine had a significant effect ($F_{3,65}=3.37$, $p=0.023$). Pre-planned comparisons showed a significant increase after 15 mg/kg ($p=0.049$) cocaine, but not after 5 or 10 mg/kg ($p>0.05$). The number of entries into the closed arms of the EPM was increased after the cocaine treatment ($F_{3,65}=3.83$, $p=0.014$; Fig. 2e). Pre-planned comparisons showed a significantly increased number of entries after 5 mg/kg cocaine ($p=0.03$), but not after 10 or 15 mg/kg ($p>0.05$). There was no cocaine effect on the number of entries into the center of the EPM ($p>0.05$, data not shown).

3.2. Experiment II

Testing the animals in their resting phase 30–50 min after the injection resulted in more center entries than in the active phase in all treatment groups (Fig. 3a). A two-way-ANOVA yielded a

significant effect for the factor test phase ($F_{1,66}=5.44$, $p=0.023$), but no significant treatment effect or test phase × treatment interaction ($p>0.05$). Locomotor activity in the center (Fig. 3b) was generally higher in the resting vs. active phase animals after all treatments ($F_{1,66}=5.74$, $p=0.019$). The effect of the treatment and the test phase × treatment interaction failed to reach statistical significance ($p>0.05$). Also the time spent in the center of the open field was generally higher in animals tested in their resting phase compared to the active phase animals ($F_{1,66}=4.06$, $p=0.048$). However, no significant treatment effect or test phase × treatment interaction was observed ($p>0.05$). The velocity and rearing behavior in the center of the maze were neither affected by the test phase nor by the treatment ($p>0.05$). There were also no significant phase × treatment interactions ($p>0.05$; data not shown).

When the behavior in the whole maze was considered, the resting phase tested animals showed more locomotor activity than the active phase tested animals (Fig. 3d), which, however, became only evident as a tendency ($F_{1,66}=2.89$, $p=0.09$). The effect of the treatment and the test phase × treatment interaction failed to reach statistical significance ($p>0.05$). Rearing behavior tended to differ between active and resting phase treated animals (Fig. 3e). A two-way ANOVA, however, yielded only a tendency for the factor phase ($F_{1,66}=2.89$, $p=0.09$), a significant effect for the factor treatment ($F_{2,66}=4.54$, $p=0.014$), but no significant phase × treatment interaction ($p>0.05$). Pre-planned comparisons showed a significant effect of the 10 mg/kg cocaine vs. saline in the animals tested in the resting phase ($p=0.039$). Animals in the resting phase spent significantly more time grooming (Fig. 3f) than animals in the active phase ($F_{1,66}=5.15$, $p=0.026$). A two-way ANOVA failed to find a significant treatment effect ($F_{2,66}=2.52$, $p=0.088$), or a significant interaction ($p>0.05$). Pre-planned comparisons showed a significant difference between the 20 mg/kg cocaine and saline-treated animals in the resting phase ($p=0.03$). In the resting phase there was a tendency for a higher velocity compared with the active phase ($F_{1,66}=2.89$, $p=0.09$). The effect of the treatment and the phase × treatment interaction failed to reach statistical significance ($p>0.05$; data not shown).

Neither the test phase, nor the cocaine treatment, had a significant effect on the entries and time spent in the open arms (Fig. 4a and d), closed arms (Fig. 4b and e), or center (data not shown) of the EPM when animals were tested 30–35 min after the injection ($p>0.05$). There was no significant test phase × treatment interaction for all these parameters ($p>0.05$). Also, the correction of open arm times and entries for the total of all arm

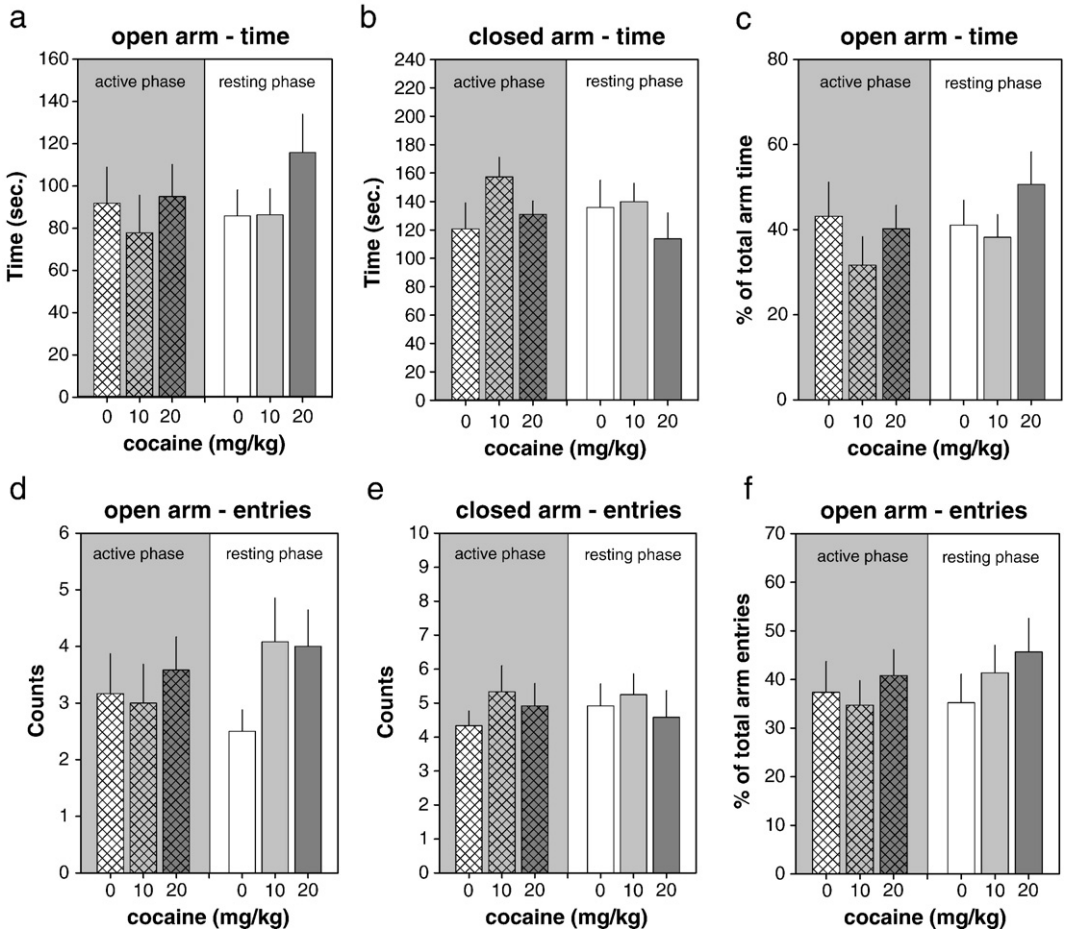

Fig. 4. The effects of cocaine on anxiety-related behavior in the elevated plus-maze (mean ± S.E.M.). Half of the animals were tested in their active phase, i.e. dark phase (grey), and half in their resting phase, i.e. light phase (white). Animals were tested 30–35 min after receiving an i.p. injection of cocaine.

times and entries (Fig. 4c and f) failed to show a significant effect in a two-way ANOVA ($p>0.05$). In a fine-analysis of EPM behavior, entries and times in the distal compartments of the open and closed arms were analyzed separately. However, two-way ANOVAs yielded only a tendency for a treatment effect for the time spent in the distal open arms ($F_{2,66}=2.69, p=0.075$). Neither the test phase, nor the cocaine treatment, had any effect on the entries or time spent in the distal closed arms of the EPM ($p>0.05$; data not shown).

4. Discussion

The present study investigated the effects of cocaine on anxiety-related behavior in two different tests in rats. The first experiment described cocaine's effects on anxiety-related behavior in the animal's active period, i.e. the dark phase, 10–20 min after cocaine injection. It was found that cocaine significantly increased the number of entries, the time spent, and the locomotion in the center of the open field. Velocity and rearing in the center was not influenced by cocaine. These effects were observed parallel to the well known increase in locomotor activity and the decrease in grooming behavior. In the EPM test, cocaine increased the time spent in the open arms and the percent of open arm entries in relation to total arm entries. Accordingly, the present data advocate an anxiolytic-like effect of cocaine 10–20 min after injection in animals tested in their active phase. In the second experiment, the effects of cocaine were investigated 30–50 min after injection in animals tested in the active phase, compared with animals tested in their resting phase. In the open field the animals tested in the resting phase showed significantly more entries and locomotion in the center of the maze, and spent more time in the center than animals tested in their active phase. This might suggest that the baseline level of anxiety is lower in the resting phase than in the active phase. However, this effect only occurred in the open field, but not in the EPM test. The cocaine treatment did not significantly increase the number of center entries and time spent in the center 30–50 min after injection, nor did it affect any other anxiety-related parameter. No interaction of the test phase with the cocaine treatment was observed with any of the behavioral parameters. This suggests that cocaine had no significant effects on anxiety in the open field 30–50 min after application, irrespective of whether animals were tested in their active or resting phase. In the EPM no effect of the test phase was observed. Neither was there an effect of cocaine treatment on entries into open arm, closed arm or center, nor on time spent in the respective compartments. Overall, there was no interaction of the test phase with the treatment in the EPM 30–35 min after cocaine application. This supports the findings in the open field test, suggesting no significant anxiolytic-like effect of cocaine 30–50 min after application irrespective of the test phase.

Our studies advocate an initial, but not late, anxiolytic-like effect of cocaine exposure in rats. The anxiolytic-like effect was only observed after the short 10 min test latency during the animal's active phase. Neither anxiolytic-like, nor anxiogenic-like, effects were observed 30–50 min after cocaine application in this study, irrespective of whether the animals were tested in their active or resting phase. It should be noted that the testing in our studies was performed after an extensive handling of the animals. This might have facilitated anxiolytic-like drug effects (Andrews and File, 1993). We found an effect of the test phase on anxiety-related behavior in the open field, which suggests that animals tested in their resting phase are less anxious and generally more active than animals in their active phase. However, this observation may have been an effect of the sudden darkness for the animals in the resting, i.e. light phase, during the testing procedure. Sudden darkness was shown to induce a high activity and low anxiety state in rats (Nasello et al., 1998).

Although we investigated cocaine effects on anxiety-like behaviors in two different paradigms, thus, taking different measures, it has to be noted that in both paradigms anxiety-like behaviors were not independent measures and were related to the overall locomotor activity. Several authors have pointed out that this might induce "false positive" effects, in particular when psychostimulant drugs are evaluated (Dawson and Tricklebank, 1995; Weiss et al., 1998). In respect of the present data, it might be argued that the increase in the distance moved in the center of the open field after an acute cocaine challenge merely resulted from a general increase of locomotor activity. Interestingly, in experiment I the velocity in the center of the open field was not affected by cocaine, however, the distance moved and time spent in the center significantly increased. Since overall locomotion significantly increased after an acute cocaine challenge, this effect was surely due to an increase of peripheral velocity. As such, the increase in center distance and time in the open field test were unlikely unspecific locomotor effects. This view is supported in the EPM data of experiment I, showing that the number of open arm entries was still significantly increased by cocaine when corrected for total arm entries, i.e. a measure of general locomotor activity (Fig. 2f).

Anxiolytic-like effects of initial cocaine exposure were also reported in CF1 mice 30 min after application in the EPM, but not 60 min thereafter in the open field test (Erhardt et al., 2006). While no effects of cocaine on anxiety-related measures have also been reported in some studies (Yang et al., 1992; Rogerio and Takahashi, 1992a; Sarnyai et al., 1995), the majority of studies have reported anxiogenic-like effects of cocaine in rodent models of anxiety. Acute cocaine treatment increased anxiety-related behaviors in mice in a black–white box test, when tested 40 min after injection. This acute effect was shown to attenuate with repeated treatment after only 3 days, and reversed to an anxiolytic-like response (Costall et al., 1989). Enhanced escape and flight responses were reported following cocaine treatment in mice confronted with an anaesthetized rat as threat stimulus, and in rats in a runway test (Blanchard et al., 1999). Anxiogenic-like effects of cocaine were found in rats 20 min (Yang et al., 1992) and 40 min (Paine et al., 2002) after application in the EPM, and after 30 min in a punished drinking conflict paradigm (Fontana and Commissaris, 1989). Rogerio and Takahashi (1992a) tested rats in the EPM with a shorter latency after injection. Interestingly, 15 min after cocaine application there was a clear tendency towards an anxiolytic effect. However, after a repeated pre-

treatment with saline, a cocaine challenge produced an anxiogenic-like effect. Another study in rats showed that only animals with low basal anxiety-related behavior in the EPM displayed an anxiogenic-like response to cocaine (Rogerio and Takahashi, 1992b). Notably, these anxiogenic-like effects in rodent studies were predominantly observed with long test latencies. In marmoset monkeys an increase of anxiety-related behavior was reported within 20 min after injection, but only in animals which did not show a hyperlocomotor response. Animals which responded with an increase in locomotor activity showed attenuated anxiety-related behaviors (De Souza Silva et al., 2006). Overall, acute anxiogenic-like effects of cocaine must be reconciled with cocaine's reinforcing properties and its abuse liability in animals and humans.

Nonetheless, anxiety-related behavior was reported after cocaine in various paradigms after repeated treatment (DeVries and Pert, 1998), such as an operant runway model of cocaine self-administration (Ettenberg and Geist, 1991, 1993; Geist and Ettenberg, 1997; Raven et al., 2000), discriminative stimulus tests (Shearman and Lal, 1981; Mantsch and Goeders, 1998), and cocaine withdrawal (e.g. Wood and Lal, 1987; Sarnyai et al., 1995; Basso et al., 1999; Paine et al., 2002). It has been argued that, particularly, cocaine's effects on serotonergic (5-HT) activity may be responsible for anxiogenic effects, since 5-HT appears to be closely linked to anxiety-related behavior (Handley et al., 1993; Graeff et al., 1996; Schwarting et al., 1998). Acute cocaine treatment increases extracellular 5-HT levels in various brain structures related to anxiety. During withdrawal, however, extracellular 5-HT levels fall below baseline (for a review see: Müller et al., 2007a). Our results and those of others might suggest that the acute effects of cocaine on anxiety-related behavior have a multi-phasic time course. Immediately after injection, when 5-HT responses in the brain are still rising (e.g. Parsons and Justice, 1993; Andrews and Lucki, 2001; Müller et al., 2002, 2007b; Pum et al., 2007), cocaine might have a predominantly anxiolytic-like effect. After a time of 30 min, this effect reverses and later in time an anxiogenic-like response dominates. This effect is hypothesized to coincide with the peak and decline of the acute 5-HT response. In addition, a long lasting drop in level of 5-HT below baseline might be associated with cocaine withdrawal-induced anxiety (Parsons et al., 1995; Sizemore et al., 2000). This view would be consistent with the failure of buspirone to reverse late cocaine-induced anxiety-related behavior and withdrawal-related anxiety, since the 5-HT_{1A}-receptor agonist buspirone is known to reduce extracellular 5-HT levels in terminal regions of the serotonergic projections (Müller et al., 2007a). The proposed time course of cocaine effects on anxiety-related behavior is also supported by reports in humans. Resnick et al. (1977) investigated the effects of cocaine in human volunteers. Significant subjective effects, such as "high" and "pleasure", were found at an intranasal dose of $\geq$25 mg. However, 4 out of 19 volunteers reported a biphasic effect after 25 and 100 mg cocaine, which was characterized by an initial euphoria followed after 20–30 or 45–60 min by dysphoria, anxiety and fatigue. The other 15 volunteers did not report the late phase anxiogenic effect (Resnick et al., 1977).

Concerning the acute cocaine action, it becomes evident that the emotional effects critically depend on various parameters, such as individual differences (Rogerio and Takahashi, 1992b; De Souza Silva et al., 2006) or test latency (Erhardt et al., 2006). This view is expanded by the present study, which suggests that cocaine can have anxiolytic-like effects after a short latency when applied in the active phase of the animals, and no anxiogenic-like effects after a longer latency. Additional factors, such as individual differences, test phase, or pre-test stress levels, might, therefore, shift the proposed time course of the cocaine effects on anxiety-related behavior and/or influence its amplitude. It may be argued that the neurochemical effects of cocaine, in particular on 5-HT activity (Müller et al., 2007a), during the increase in the transmitter concentration, might amplify the present emotional state of the organism. High stress levels, as they can be assumed during EPM testing of unhandled animals (e.g. Costall et al., 1989), or testing under aversive conditions (e.g. high lighting contrast between open and closed arms of the EPM) might, therefore, mask an early anxiolytic-like cocaine effect (e.g. Rogerio and Takahashi, 1992a; Yang et al., 1992).

Taken together, the present study provides evidence for an anxiolytic-like effect of cocaine in rats in their active, i.e. dark phase, in the open field and EPM 10–20 min after injection. At a time of 30–50 min after injection, no significant anxiolytic-like effect was observed, irrespective of the test phase, i.e. if animals were tested in their resting or active phase. These findings, together with other studies, might suggest that cocaine, after first exposure, can have temporary anxiolytic-like behavioral effects, which decline rapidly, and, may eventually reverse to an anxiogenic state. We propose a multi-phasic time course of the initial cocaine effects on anxiety that can be modulated in duration and amplitude by the conditions of exposure.

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