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Marker-assisted dissection of genetic influences on motor and neuroendocrine sensitization to cocaine in rats

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This study investigated genetic influences on behavioral and neuroendocrine responses to cocaine sensitization. We used male and female rats of the inbred strains Lewis (LEW) and spontaneously hypertensive rats (SHR), which display genetic differences in stress-related responses. The influence of two quantitative trait loci (QTL; *Ofil1* and *Ofil2* on chromosomes 4 and 7), which modulate stress reactivity in rats, on the effects of cocaine was also investigated through the use of recombinant lines (derived from a LEW × SHR intercross) selected by their genotype at *Ofil1* and *Ofil2*. Animals were given repeated cocaine or saline injections and tested for locomotion (induction of sensitization). Two weeks later, all animals were challenged with cocaine, and locomotion and corticosterone levels were measured (expression of sensitization). Results indicated that male SHR rats showed more behavioral sensitization than LEW rats, whereas no strain differences in sensitization were seen among females. When challenged with cocaine, LEW and SHR rats of both sexes pretreated with cocaine showed behavioral sensitization compared with saline pretreated animals; however, only LEW rats displayed an increase in the corticosterone levels. *Ofil1* was found to influence the induction of sensitization in males and *Ofil2* modulated the locomotor effect of cocaine in females. This study provides evidence of a genotype-dependent relationship between the induction and expression of cocaine sensitization, and between the behavioral and neuroendocrine responses induced by cocaine. Moreover, the *Ofil1* and *Ofil2* loci may contain one or more genes that control the behavioral effects of cocaine in rats.

Keywords: Anxiety, behavioral genetics, drug addiction, hypothalamic–pituitary–adrenal axis, marker-assisted selection, plasma corticosterone, sensitization, stress

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Repeated exposure to psychostimulants can induce behavioral sensitization, a progressive increase in the behavioral effects of the drug that may be involved in the development of drug addiction (Robinson & Berridge 2001). One of the biological mediators of the acute effects of psychostimulants and the development of sensitization is the activation of the stressor-responsive hypothalamic–pituitary–adrenocortical (HPA) axis (Cador *et al.* 1993; Przegalinski *et al.* 2000; Rivet *et al.* 1989). Furthermore, stress and the HPA axis have long been proposed to be implicated in the development of other psychiatric disorders, notably anxiety and depression (Arborelius *et al.* 1999). Thus, differences in the HPA axis system may explain in part the co-occurrence of drug addiction with other psychiatric illnesses.

Genetic factors are known to influence both the behavioral effects of cocaine and the HPA axis function (Mormede *et al.* 2002; Shuster *et al.* 1977), but the specific genes that modulate these phenotypes remain unknown. Quantitative trait loci (QTL) analysis is a powerful approach to identify chromosomal regions associated with a given quantitative phenotype and for the identification of specific genes. Multiple QTL associated with the locomotor effect of cocaine have been identified in mice (Boyle & Gill 2001; Janowsky *et al.* 2001; Jones *et al.* 1999; Miner & Marley 1995; Phillips *et al.* 1998; Tolliver *et al.* 1994); yet, to our knowledge, there are no reports of QTL influencing the behavioral effects of cocaine in rats.

To test the role of reactivity to stress in cocaine-induced sensitization, we used two inbred rat strains Lewis (LEW) and spontaneously hypertensive rats (SHR) that display genetic differences in stress-related responses. LEW and SHR rats show high and low indices of experimental anxiety, respectively (Ramos *et al.* 2002). Moreover, LEW rats submitted to some stressful situations show more severe and longer lasting stress responses than SHR rats (Berton *et al.* 1998; Vendruscolo *et al.* 2004). Differences in the stress-induced HPA axis activation between LEW and SHR rats have also been reported (Duclos *et al.* 2001; Gomez *et al.* 1998).

To investigate the molecular basis of the anxiety-related differences between LEW and SHR rats, a genome-wide QTL

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search was performed (Ramos *et al.* 1999). This study showed two QTL for open-field inner locomotion, referred to thereafter as *Ofil1* and *Ofil2* on chromosomes 4 and 7, respectively. Subsequent studies showed that these loci not only affect anxiety but also alcohol consumption, HPA axis function and stress reactivity (Potenza *et al.* 2004; Terenina-Rigaldie *et al.* 2003; Vendruscolo *et al.* 2006a,c). It was therefore of interest to determine whether these genomic regions influence other behavioral and neuroendocrine responses elicited by drugs of abuse.

This study compared LEW and SHR rats in terms of their sensitivity to cocaine sensitization, through the measurement of behavioral and neuroendocrine variables. The possible roles of the loci *Ofil1* and *Ofil2* on these responses were considered. Animals of both sexes were included because there is considerable evidence for quantitative and qualitative differences according to sex in the sensitivity toward drugs of abuse.

Materials and methods

Animals

Adult (10 weeks old), male and female LEW and SHR rats from our own colonies were used. Additionally, adult male and female recombinant F4 rats selected based on their genotype at *Ofil1* and *Ofil2* (marker-assisted selection) were used. A detailed description of the recombinant strains used in this study has been published previously (Vendruscolo *et al.* 2006a,b,c). Briefly, animals that inherited the homozygous genotypes LEW/LEW (L) or SHR/SHR (S) at each locus (4 = *Ofil1* and 7 = *Ofil2*) were selected, thus allowing the production of four recombinant rat lines (L4/L7, L4/S7, S4/L7 and S4/S7) with a known genotype at these loci only. The rest of the genome consisted of a random assortment of alleles from one or the other parental strains. The phenotypic differences between these groups were therefore the result of genetic variations within these chromosomal loci. All animals were kept in collective plastic cages (two to four rats per cage) with food and water available *ad libitum* under a 12-h light/dark cycle (lights on at 0700 h) at 21 ± 2°C. This study was conducted in conformity with the European Community Council Directive of 24 November 1986 (86/609/EEC).

Drugs

Cocaine was obtained from Coopérative Pharmaceutique Française (Bordeaux, France). Sterile saline (0.9% NaCl) was both the solvent and control vehicle.

Activity measurement

Animals were placed individually for 30 min (habituation period) in wire-mesh cages with transparent plastic sides (length 38.5 × width 23.5 × height 23 cm) located inside a rack equipped with infrared photo beams connected to a computer that recorded the number of cage crossings and rearings (Imetronic, Pessac, France). Each animal was then injected with cocaine (10 mg/kg) or saline (1 ml/kg) and left in the activity cages for an additional 60-min period. All animals received seven injections (one per day) at 2-day intervals. After this period of 'induction' of behavioral sensitization, the animals were kept undisturbed in their home cages for 15 days (drug-free period) and then submitted to a cocaine challenge session, when all animals (injected previously with cocaine or saline) were injected with cocaine 10 mg/kg to assess the 'expression' of behavioral sensitization. This session specifically consisted of 30 min of habituation in the activity cages followed by a 50-min (instead of 60 min) postinjection test. Immediately after the end of the behavioral test, the animals were transported to another room, decapitated and trunk blood was collected for corticosterone measurements. The behavioral tests were conducted under low-light conditions (<25 lx). Male and female rats were tested on alternate days between 0800 and 1300 h. Previous studies have shown that these experimental conditions are effective in achieving cocaine-induced sensitization (Cailhol & Mormede 1999).

Plasma corticosterone measurements

Blood was collected in chilled tubes coated with a 10% ethylenediaminetetraacetic acid solution and centrifuged (4500 ***g***, 15 min, 4°C). The plasma was then stored at −80°C for subsequent measurement of corticosterone. Plasma corticosterone concentrations were determined following extraction with absolute ethanol and using [^{3}H]-corticosterone as a radioligand and transcortin from rhesus monkey plasma as a binder, as previously described (Vendruscolo *et al.* 2006c).

Statistical analysis

Statistical analyses were performed separately for males and females. For comparisons between LEW and SHR, the induction of behavioral sensitization data were analyzed by a three-way analysis of variance [ANOVA; strain, treatment (cocaine vs. saline) and day] with the latter factor (1–7) being treated as repeated measures. The data for expression of behavioral sensitization and corticosterone levels were analyzed by a two-way ANOVA (strain and treatment). To analyze the influence of L or S alleles at each locus and their interaction, a four-way ANOVA with repeated measures (*Ofil1*, *Ofil2*, treatment and day) or a three-way ANOVA (*Ofil1*, *Ofil2* and treatment), was performed exclusively with the data of the four new rat lines. Fisher's LSD test was used for *post hoc* comparisons when appropriate. The accepted level of significance for all tests was $P < 0.05$.

Results

Induction of behavioral sensitization in SHR and LEW rats

Figure 1 illustrates the effect of the repeated cocaine or saline injections on the number of cage crossings and rearings shown by LEW and SHR rats of both sexes. For the number of cage crossings in males, the three-way ANOVA with repeated measures showed an overall effect of treatment ($F_{1,26} = 42.4$, $P < 0.0001$) and of day ($F_{6,156} = 9.6$, $P < 0.0001$). No difference was found for the strain factor ($F_{1,26} = 2.4$, $P = 0.1$). A significant treatment × day interaction ($F_{6,156} = 13.2$, $P < 0.0001$) showed that animals of both strains (LEW and SHR) progressively increased their locomotion with repeated cocaine injections. The *post hoc* comparisons indicated that the first cocaine injection did not induce hyperlocomotion in both LEW and SHR rats. However, cocaine-treated animals showed a significant increase in locomotion from the third to the seventh day when compared with the saline group as well as when compared with the first day (at least $P < 0.01$). For the number of rearings, the ANOVA showed an overall effect of treatment ($F_{1,26} = 20.4$, $P < 0.0001$) and of day ($F_{6,156} = 8.1$, $P < 0.0001$), but not of strain ($F_{1,26} = 2.4$, $P = 0.1$). A significant strain × treatment × day interaction was observed ($F_{6,156} = 2.2$, $P < 0.05$). The *post hoc* analyses indicated that cocaine-treated rats were not significantly different from saline-treated rats on the first day; however, cocaine-induced

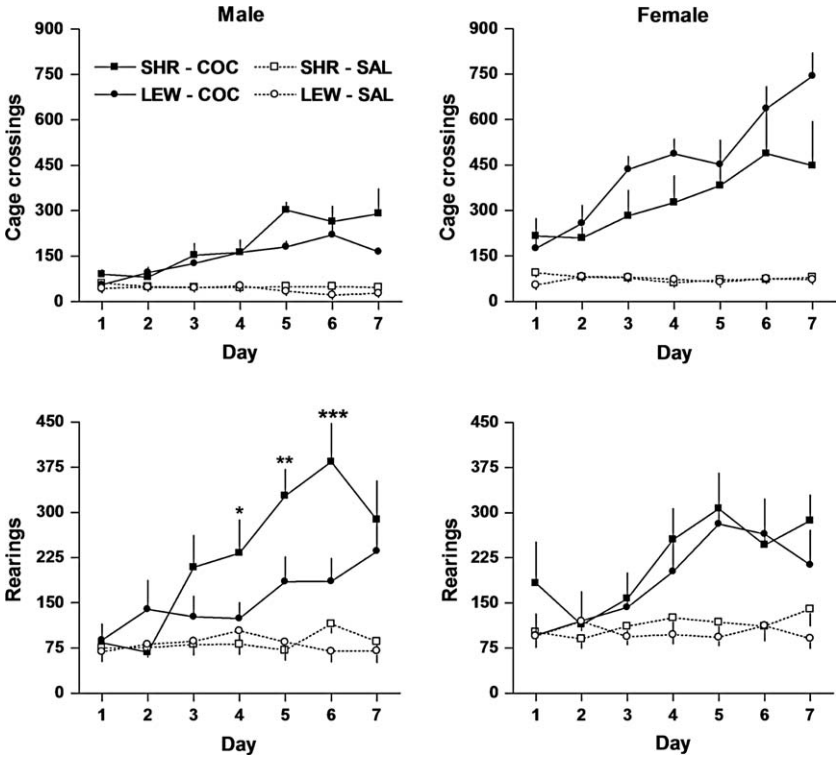

Figure 1: Induction of cocaine sensitization in LEW and SHR rats. Curves and vertical lines represent the mean and SEM of the number of cage crossings (upper panels) and rearings (lower panels) for 60 min, displayed by LEW and SHR rats of both sexes. $n = 7$–8 per group. For each sex, repeated cocaine injections induced behavioral sensitization (ANOVA, $P < 0.05$; see *Results* for further statistical details). *, ** and ***Significant differences between cocaine-treated SHR rats and cocaine-treated LEW rats on the same day of injection (ANOVA followed by Fisher's LSD test; $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively).

sensitization was significant from the third to the seventh day (at least $P < 0.05$). More importantly, cocaine-treated SHR rats showed a greater extent of behavioral sensitization, from the fourth to the sixth day (at least $P < 0.05$), than cocaine-treated LEW rats.

For females, the ANOVA showed an overall effect of treatment (cage crossings: $F_{1,28} = 50.6$, $P < 0.0001$; rearings: $F_{1,28} = 12.7$, $P < 0.001$) and of day (cage crossings: $F_{6,168} = 10.1$, $P < 0.0001$; rearings: $F_{6,168} = 5.6$, $P < 0.0001$), but not of strain (cage crossings: $F_{1,28} = 1.6$, $P = 0.2$; rearings: $F_{1,28} = 0.7$, $P = 0.4$) for motor activity. A significant treatment × day interaction was observed for the number of cage crossings ($F_{6,168} = 10.5$, $P < 0.0001$) and rearings ($F_{6,168} = 4.5$, $P < 0.001$). The *post hoc* comparisons indicated that cocaine-treated rats were not significantly different from saline-treated rats on the first day. However, cocaine-treated LEW and SHR female rats showed increased locomotion from the third to the seventh day, and made more rearings, from the fourth to the seventh day, compared with saline-treated rats, as well as compared with the first day (at least $P < 0.05$).

Expression of behavioral sensitization and corticosterone levels in SHR and LEW rats

Table 1 summarizes the results of a cocaine challenge on the number of cage crossings, the number of rearings and corticosterone levels in LEW and SHR rats of both sexes. For males, the two-way ANOVA showed a significant overall treatment effect for the number of cage crossings ($F_{1,26} = 4.3$, $P < 0.05$) and rearings ($F_{1,26} = 5.6$, $P < 0.05$). These data indicate that the animals previously treated with cocaine displayed greater locomotion and made more rearings than the animals previously treated with saline. No strain differences were found for the number of cage crossings ($F_{1,26} = 1.2$, $P = 0.3$) and rearings ($F_{1,26} = 0.1$, $P = 0.8$). Regarding corticosterone levels, the ANOVA did not show any significant effect of strain ($F_{1,26} = 0.1$, $P = 0.9$) and of treatment ($F_{1,26} = 3.6$, $P = 0.1$), but a significant strain × treatment interaction ($F_{1,26} = 4.3$; $P < 0.05$). *Post hoc* comparisons indicated that LEW rats, but not SHR rats, previously treated with cocaine showed higher levels of corticosterone than their control group, that is LEW rats previously treated with saline ($P < 0.01$).

As with males, the two-way ANOVA showed an overall effect of treatment ($F_{1,28} = 7.3$, $P < 0.05$), but not of strain ($F_{1,28} = 1.2$, $P = 0.3$) for the number of cage crossings, indicating that females previously treated with cocaine showed greater levels of locomotion than females previously treated with saline. For the number of rearings, the effects of treatment ($F_{1,28} = 4.4$, $P < 0.05$) and of strain ($F_{1,28} = 9.3$, $P < 0.01$) were significant, showing that rats previously treated with cocaine made more rearings than rats previously treated with saline, and SHR females made more rearings than LEW females. Regarding corticosterone levels, the ANOVA showed an overall effect of strain ($F_{1,28} = 8.1$, $P < 0.01$), but not of treatment ($F_{1,28} = 2.5$, $P = 0.1$). Moreover, a treatment × strain interaction was observed ($F_{1,28} = 4.4$, $P < 0.05$). The *post hoc* comparisons indicated that female LEW rats, but not female SHR rats, previously treated with cocaine showed increased levels of corticosterone compared with their control group (LEW rats previously treated with

Table 1: Expression of cocaine sensitization in LEW and SHR rats

Treatment	*n*	Sex	Strain	Body weight (g)	Number of cage crossings	Number of rearings	Corticosterone (ng/ml)
Prior SAL	7	M	LEW	303.4 ± 6.0	55.6 ± 12.3	83.4 ± 20.2	61.4 ± 14.6
Prior COC	8	M	LEW	306.0 ± 8.1	131.1 ± 24.7*	292.4 ± 82.2*	136.9 ± 28.9‡
Prior SAL	7	M	SHR	242.0 ± 3.0	85.1 ± 35.7	153.4 ± 50.4	97.6 ± 10.7
Prior COC	8	M	SHR	231.4 ± 2.9	205.5 ± 78.0*	250.3 ± 74.4*	94.4 ± 13.4
Prior SAL	8	F	LEW	187.9 ± 2.9	168.9 ± 28.4	103.5 ± 19.6	326.8 ± 91.0
Prior COC	8	F	LEW	188.7 ± 4.8	473.5 ± 83.9*	233.5 ± 65.8*	552.4 ± 65.9†
Prior SAL	8	F	SHR	152.4 ± 3.6	171.9 ± 73.8	281.1 ± 54.8§	280.8 ± 40.4
Prior COC	8	F	SHR	159.0 ± 4.4	296.6 ± 109.9*	358.6 ± 46.0*§	249.5 ± 29.0

Number of cage crossings and number of rearings during 50 min of test and corticosterone levels at the end of the test (mean ± SEM) shown by male (M) and female (F) rats previously injected with either saline (Prior SAL) or cocaine (Prior COC) and challenged with a dose of cocaine (10 mg/kg).

*Overall treatment effect (Prior SAL vs. Prior COC; ANOVA, $P < 0.05$) for each sex.

† and ‡Significant difference between Prior COC and Prior SAL in LEW rats (ANOVA followed by Fisher's LSD test; $P < 0.05$ and $P < 0.01$, respectively).

§Overall strain effect (LEW vs. SHR; ANOVA, $P < 0.01$) for females.

saline, $P < 0.05$) as well as compared with SHR previously treated with cocaine ($P < 0.01$).

Induction of behavioral sensitization in recombinant (F4) rats

The effect of repeated cocaine or saline injections on the number of cage crossings and rearings shown by male and female F4 rats (according to line) are illustrated in Fig. 2. For males, the four-way ANOVA with repeated measures showed a significant effect of treatment ($F_{1,44} = 19.1$, $P < 0.0001$) and of day ($F_{6,264} = 9.0$, $P < 0.0001$), but not of *Ofil1* ($F_{1,44} = 0.7$, $P = 0.4$) or *Ofil2* ($F_{1,44} = 0.5$, $P = 0.5$) for the number of cage crossings. A significant treatment x day interaction ($F_{6,264} = 10.3$, $P < 0.0001$) indicated that repeated administration of cocaine-induced sensitization of the response. Cocaine-treated rats were not significantly different from saline-treated rats at the first day, but the response to cocaine

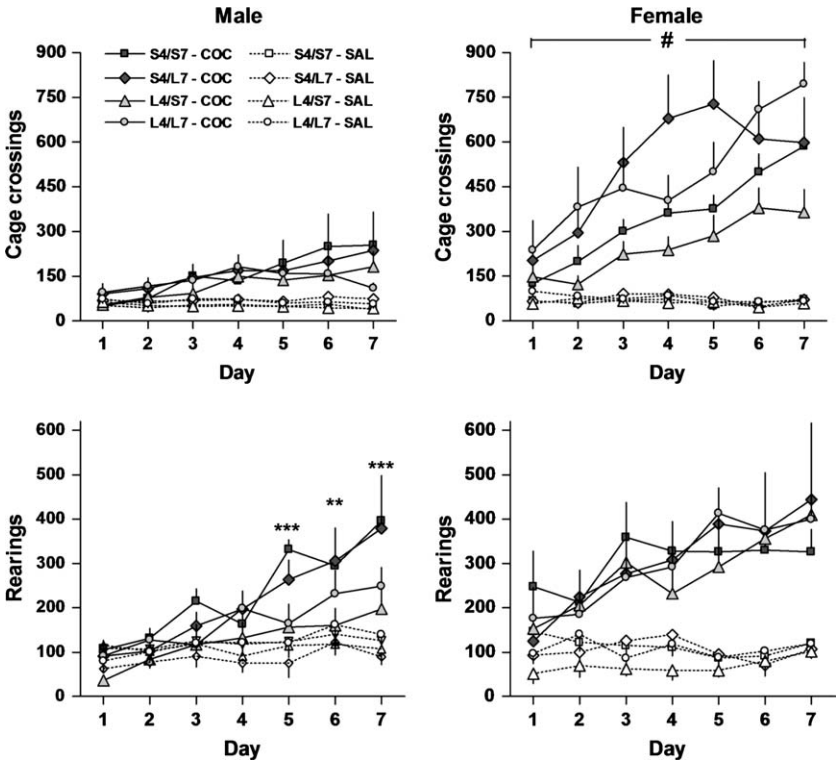

Figure 2: Induction of cocaine sensitization in recombinant F4 rats. Curves and vertical lines represent the mean and SEM of the number of cage crossings (upper panels) and rearings (lower panels) for 60 min displayed by F4 rats of both sexes. $n = 5$–8 per group. For each sex, repeated cocaine injections induced behavioral sensitization (ANOVA, $P < 0.05$; see *Results* for further statistical details). ** and ***Significant effect of the *Ofil1* locus, on chromosome 4 (ANOVA followed by Fisher's LSD test; $P < 0.01$ and $P < 0.001$, respectively). #Significant effect of the *Ofil2* locus on chromosome 7 (ANOVA, $P < 0.05$).

was significantly increased from the third to the seventh day in relation to the saline group as well as in relation to the first day (at least $P < 0.01$). For the number of rearings, an overall effect of treatment ($F_{1,44} = 19.3$, $P < 0.0001$) of *Ofil1* ($F_{1,44} = 6.5$, $P < 0.05$) and of day ($F_{6,264} = 22.0$, $P < 0.0001$) but not of *Ofil2* ($F_{1,44} = 1.0$, $P = 0.3$) were significant, as well as the treatment × *Ofil1* × day interaction ($F_{6,264} = 3.2$, $P < 0.01$). The *post hoc* comparisons indicated that cocaine-treated rats were not significantly different from saline-treated rats on the first day; however, cocaine-induced sensitization was significant from the fourth to the seventh day compared with saline (at least $P < 0.05$) and from the third to the seventh day compared with the first day ($P < 0.0001$). More importantly, cocaine-treated animals carrying SHR alleles in *Ofil1* (S4/S7 and S4/L7) made more rearings, from the fifth to the seventh day, than cocaine-treated animals carrying LEW alleles in *Ofil1* (L4/L7 and L4/S7) (at least $P < 0.01$).

For females, the ANOVA showed an overall effect of treatment ($F_{1,46} = 77.8$, $P < 0.0001$) of *Ofil2* ($F_{1,46} = 8.3$, $P < 0.01$) and of day ($F_{6,276} = 16.3$, $P < 0.0001$) but not of *Ofil1* ($F_{1,46} = 0.6$, $P = 0.4$) for the number of cage crossings. A significant treatment × day interaction ($F_{6,276} = 18.2$, $P < 0.0001$) indicated that repeated administration of cocaine elicited sensitization of locomotion. The *post hoc* comparisons indicated that cocaine produced a significant increase in locomotor activity from the first (acute effect of cocaine) to the seventh day (at least $P < 0.05$) compared with the saline group. Moreover, locomotion after the first day was lower than that observed after all other days (at least $P < 0.05$). Furthermore, a significant *Ofil2* × treatment interaction ($F_{1,46} = 6.65$, $P < 0.05$) indicated that the cocaine-treated animals with LEW alleles at *Ofil2* (L4/L7 and S4/L7) showed overall higher locomotion than the animals with SHR alleles at this locus (L4/S7 and S4/S7).

For the number of rearings, the ANOVA showed a significant effect of treatment ($F_{1,46} = 25.6$, $P < 0.0001$) and of day ($F_{6,276} = 4.9$, $P < 0.0001$) but not of *Ofil1* ($F_{1,46} = 0.2$, $P = 0.6$) and *Ofil2* ($F_{1,46} = 0.1$, $P = 0.7$). A significant treatment × day interaction ($F_{6,276} = 5.52$, $P < 0.0001$) indicated that cocaine elicited an increased number of rearings with repeated injections. Cocaine-treated rats were not significantly different from saline-treated rats at the first day, but cocaine-induced sensitization was significant from the third to the seventh day compared with the saline group as well as compared with the first day (at least $P < 0.001$).

Expression of behavioral sensitization and corticosterone levels in recombinant (F4) rats

Table 2 summarizes the effect of a cocaine challenge on the number of cage crossings, the number of rearings and corticosterone levels shown by F4 (according to lines) rats of both sexes. For males, the three-way ANOVA showed a significant overall treatment effect for the number of cage crossings ($F_{1,44} = 12.2$, $P < 0.01$) and rearings ($F_{1,44} = 5.4$, $P < 0.05$). These data indicate that the animals previously treated with cocaine showed higher levels of locomotion and made more rearings than the animals previously exposed to saline, regardless of genotype. No significant effects were found for *Ofil1* (cage crossings: $F_{1,44} = 1.1$, $P = 0.3$; rearings: $F_{1,44} = 4.0$, $P = 0.1$) and *Ofil2* (cage crossings: $F_{1,44} = 0.1$, $P = 0.9$; rearings: $F_{1,44} = 1.5$, $P = 0.2$). Regarding corticosterone levels, no significant differences were detected by ANOVA for treatment ($F_{1,44} = 1.6$, $P = 0.2$), *Ofil1* ($F_{1,44} = 2.0$, $P = 0.2$) and *Ofil2* ($F_{1,44} = 0.7$, $P = 0.4$). However, a significant *Ofil1* × *Ofil2* interaction was found ($F_{1,43} = 6.39$, $P < 0.05$). The *post hoc* comparisons indicated that the animals

Table 2: Expression of cocaine sensitization in recombinant F4 rats

Treatment	*n*	Sex	F4 line	Body weight (g)	Number of cage crossings	Number of rearings	Corticosterone (ng/ml)
Prior SAL	7	M	L4/L7	284.3 ± 18.0	96.1 ± 20.6	247.0 ± 44.9	84.5 ± 22.1
Prior COC	8	M	L4/L7	289.5 ± 17.7	219.9 ± 49.4**	410.5 ± 80.8*	81.2 ± 16.8
Prior SAL	5	M	L4/S7	234.8 ± 20.7	62.4 ± 16.3	156.6 ± 62.6	49.3 ± 12.5
Prior COC	5	M	L4/S7	243.0 ± 24.8	209.2 ± 64.7**	199.8 ± 44.2*	64.4 ± 18.8
Prior SAL	8	M	S4/L7	342.1 ± 20.2	118.4 ± 41.1	247.8 ± 77.0	58.4 ± 9.6
Prior COC	8	M	S4/L7	332.0 ± 19.6	212.4 ± 43.7**	478.9 ± 92.9*	76.9 ± 15.9
Prior SAL	6	M	S4/S7	306.7 ± 27.2	147.8 ± 53.1	324.5 ± 86.0	93.2 ± 21.7
Prior COC	5	M	S4/S7	306.0 ± 21.6	248.4 ± 62.7**	424.4 ± 105.7*	162.0 ± 81.6
Prior SAL	6	F	L4/L7	173.5 ± 10.6	222.8 ± 55.4	177.3 ± 24.4	270.5 ± 42.8
Prior COC	6	F	L4/L7	166.2 ± 3.1	551.5 ± 111.7***	428.5 ± 101.3*	386.3 ± 64.2**
Prior SAL	5	F	L4/S7	151.6 ± 7.6	179.6 ± 34.8	100.8 ± 9.8	201.6 ± 60.3
Prior COC	8	F	L4/S7	179.2 ± 11.9	254.4 ± 49.3***	440.7 ± 149.0*	359.1 ± 99.3*
Prior SAL	8	F	S4/L7	189.1 ± 7.1	226.6 ± 59.2	197.0 ± 83.0	280.5 ± 62.9
Prior COC	8	F	S4/L7	195.2 ± 9.4	541.3 ± 105.9***	393.7 ± 109.8*	331.9 ± 28.3*
Prior SAL	5	F	S4/S7	191.2 ± 8.5	237.8 ± 87.1	319.6 ± 99.8	236.0 ± 67.8
Prior COC	8	F	S4/S7	199.0 ± 9.1	484.4 ± 74.9***	282.6 ± 54.3*	370.6 ± 72.4*

Number of cage crossings and number of rearings during 50 min of test and corticosterone levels at the end of the test (mean ± SEM) shown by male (M) and female (F) F4 rats (according to line) previously injected with either saline (Prior SAL) or cocaine (Prior COC) and challenged with a dose of cocaine (10 mg/kg). For each sex *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ indicate an overall treatment effect (Prior SAL vs. Prior COC; ANOVA).

with SHR alleles at both loci (i.e. S4/S7) showed higher levels of corticosterone than the S4/L7 and L4/S7 rats ($P < 0.05$).

For females, a significant main effect of treatment was observed for the number of cage crossings ($F_{1,46} = 18.1$, $P < 0.0001$), the number of rearings ($F_{1,46} = 7.0$, $P < 0.05$) and corticosterone levels ($F_{1,46} = 5.4$, $P < 0.05$). These findings indicate that cocaine challenge elicited an increase of all these parameters in females previously treated with cocaine as compared with females previously treated with saline. No significant differences were detected for *Ofil1* (cage crossings: $F_{1,46} = 1.5$, $P = 0.2$; rearings: $F_{1,46} = 0.03$, $P = 0.9$; corticosterone levels: $F_{1,46} = 0.01$, $P = 1.0$) and *Ofil2* (cage crossings: $F_{1,46} = 2.9$, $P = 0.1$; rearings: $F_{1,46} = 0.03$, $P = 0.8$; corticosterone levels: $F_{1,46} = 0.3$, $P = 0.6$).

Discussion

The present results substantiate previous studies showing that repeated cocaine injections produce psychomotor sensitization in rats of both sexes. This effect, however, was influenced by genotype. In this study, the induction of behavioral sensitization was higher in male SHR than LEW rats, with no strain differences being observed in females. The behavioral sensitization induced by cocaine was long-lasting as animals preexposed to cocaine showed higher locomotion and made more rearings than animals preexposed to saline when challenged with cocaine after a 2-week drug-free period. Interestingly, LEW rats but not SHR rats of both sexes pretreated with cocaine showed increased levels of corticosterone compared with their respective saline pretreated controls after cocaine challenge. The *Ofil1* locus on chromosome 4 was found to modulate cocaine sensitization in males, whereas the *Ofil2* locus on chromosome 7 modulated the locomotor effect of cocaine in females.

In agreement with our previous studies (Vendruscolo *et al.* 2006a), no strain (LEW × SHR) or line (recombinant rats) differences were observed in this study for baseline levels of locomotion (saline-treated animals). Acute cocaine treatment did not elicit a hyperlocomotor effect in any experimental group except in recombinant F4 females, which showed a modest increase in locomotion (but not rearing) as compared with the control group. This finding indicates that the dose of cocaine (10 mg/kg) used in this study was moderate. However, cocaine sensitization was observed in all groups through a progressive enhancement in locomotion and rearings with repeated cocaine injections.

SHR male rats were more sensitive to cocaine sensitization, as revealed by the number of rearings, but not locomotion, than LEW male rats. This result is in agreement with previous studies reporting that SHR rats are more sensitive to psychostimulants than other rat strains (Pamplona *et al.* 2007; Tsai & Lin 1988). In studies with cocaine, LEW rats are frequently compared with Fischer 344 rats, with LEW rats of both sexes showing higher sensitization (Kosten *et al.* 1994; Sircar & Kim 1999). Sircar and Kim (1999) reported that LEW and Sprague–Dawley rats showed cocaine sensitization, whereas Fischer 344 rats failed to develop sensitization with repeated cocaine injections. These latter findings, together with the present results, suggest that Fischer 344 rats are hyposensitive to the behavioral effects of cocaine, as opposed to LEW rats being more sensitive. Similar strain differences were observed in the development of food restriction-induced hyperactivity (Duclos *et al.* 2005).

Behavioral activation during the expression of cocaine sensitization was similar between LEW and SHR strains. Only one behavioral difference was detected in this test, female SHR rats (preexposed to either saline or cocaine) made more rearings than female LEW rats. This effect, which was not observed after the first cocaine injection (acute effect), suggests that female SHR rats become more sensitive to the behavioral effects of cocaine after the experimental procedure. A more interesting finding, however, was the genotype-specific effect of cocaine sensitization on the levels of corticosterone. Male and female LEW rats, but not SHR rats, preexposed to cocaine (sensitized rats) showed higher levels of corticosterone after a cocaine challenge than rats preexposed to saline. This finding indicates that the relationship between neuroendocrine and behavioral responses induced by cocaine depends on the genotype. Increased levels of corticosterone after exposure of rats to psychostimulants have been observed in some (Barr *et al.* 2002; Schmidt *et al.* 1995; Vanderschuren *et al.* 1999) but not in other studies (Borowsky & Kuhn 1991; Levy *et al.* 1992; Torres & Rivier 1992). Similar to the results with LEW and SHR obtained here, it has been reported that DBA/2 mice displayed behavioral sensitization and increased corticosterone levels after repeated cocaine exposure, whereas only behavioral sensitization was observed for C57BL/6 mice. Furthermore, adrenalectomy prevented behavioral sensitization and induced significant changes on brain dopamine system in the DBA/2 strain but had only a marginal effect in the C57BL/6 mice (de Jong *et al.* 2007, 2008). In humans, the HPA axis and corticosterone have long been implicated in the development of drug addiction (Mello & Mendelson 1997) and other psychiatric illnesses such as anxiety and depression (Arborelius *et al.* 1999). Thus, it could be hypothesized that increased corticosterone levels by repeated cocaine exposure in vulnerable individuals contribute to the transition from controlled drug use to compulsive drug abuse and, perhaps, to the high rates of psychiatric comorbidity observed in these population groups (Merikangas *et al.* 1998). In this regard, we predict that LEW and SHR rats might have a differential susceptibility to develop addiction-like and anxiety/depression-like behaviors induced by repeated cocaine exposure. This possibility remains to be tested.

One of the main findings of this study is the influence of *Ofil1* and *Ofil2* on the behavioral effects of cocaine. *Ofil1* affected the induction of cocaine sensitization in males, whereas *Ofil2* modulated the hyperlocomotor effect of cocaine in females. In males, the profile of behavioral responses related to the *Ofil1* genotype was consistent with the parental strain, that is SHR alleles increasing sensitization relative to LEW alleles. This finding suggests that the *Ofil1* locus on chromosome 4 contributes to the behavioral differences related to cocaine sensitization observed between male LEW and SHR rats. In female cocaine-treated F4 rats, those carrying LEW alleles at the *Ofil2* locus on chromosome 7 showed an overall enhanced locomotion compared with those carrying SHR alleles, despite showing similar levels of

sensitization. The lack of behavior differences between SHR and LEW rats suggests that other loci yet to be identified modulates the cocaine effects in an opposite direction. Several putative QTL associated with the effects of cocaine on motor activity have been identified in mice (Boyle & Gill 2001; Janowsky *et al.* 2001; Jones *et al.* 1999; Miner & Marley 1995; Phillips *et al.* 1998; Tolliver *et al.* 1994). The present results are the first evidence of QTL affecting the behavioral effects of cocaine in rats. Interestingly, Phillips *et al.* (1998) have reported a significant QTL for cocaine sensitization in the mouse chromosome 6, a region that is syntenic to *Ofil1*. This finding strengthens the assumption that *Ofil1* contains one or more genes controlling cocaine sensitization.

During the expression of cocaine sensitization, *Ofil1* and *Ofil2* did not affect behavioral and neuroendocrine responses in either male or female F4 rats. The genetic effects on the induction, but not the expression, of cocaine sensitization that was observed in the present study is in agreement with the assumption that there is a dissociation between induction and expression of behavioral sensitization (Vanderschuren & Kalivas 2000), and suggests that these traits are controlled by different genetic mechanisms.

From the perspective of psychiatric comorbidity, an effort to understand the genetic mechanisms involved in the co-occurrence of psychiatric disorders is clearly needed, considering the difficulties involved in the diagnosis and treatment of these conditions. Together, the results of the present study and those of our previous studies suggest that *Ofil1* affects simultaneously anxiety-like behavior and alcohol consumption in females (Vendruscolo *et al.* 2006a). Also, in females, *Ofil2* was found to modulate prepulse inhibition, an endophenotype of schizophrenia and other neuropsychiatric disorders (Vendruscolo *et al.* 2006b), and the hyperlocomotor effect of cocaine (present study). The neural substrates involved in prepulse inhibition and the effects of psychostimulants partially overlap (Swerdlow *et al.* 2001). In males, *Ofil1* concomitantly affected stress-induced analgesia (Vendruscolo *et al.* 2006c) and cocaine sensitization (present study). Stress, analgesia and the effects of drugs of abuse also share common biological mechanisms (Franklin 1998). These findings suggest the following possibilities: the presence of two or more linked genes independently controlling different phenotypes or one single gene simultaneously affecting different phenotypes.

The influence of *Ofil1* and *Ofil2* on behavior, especially cocaine induced, is supposed to result from the modification (polymorphism) of genes located in these regions. Genes in *Ofil1* that are directly or indirectly associated with the cocaine effects include those encoding neuropeptide Y, corticotropin-releasing hormone receptor 2, α-synuclein, neurokinin 1 receptor, thyrotropin-releasing hormone, histamine receptor H1, synapsin II and genes related to the glutamate system (glutamate receptor, ionotropic, delta 2; glutamate receptor interacting protein 2; glutamate receptor, ionotropic, *N*-methyl D-aspartate 2B and glutamate receptor metabotropic 7). Genes encoding synapsin III, tryptophan hydroxylase 2, neurokinin 2 receptor and thyrotropin-releasing hormone receptor are located in *Ofil2* and are also potentially associated with the effects of cocaine (see, for additional genes and greater details, rat genome database at http://rgd.mcw.edu/). We consider all of these aforementioned genes as candidates for *Ofil1* and *Ofil2*. Given the key role of the *nucleus accumbens* on cocaine sensitization (Taylor *et al.* 2007), future studies on protein expression in this brain region may contribute to reduce the number of candidate genes in *Ofil1* and *Ofil2* and help us understand the gender and genetic mechanisms of cocaine sensitization.

In conclusion, this study provides evidence of a genotype-dependent relationship between the induction and expression of cocaine sensitization, and between the behavioral and neuroendocrine responses induced by cocaine. The *Ofil1* locus, on chromosome 4, and the *Ofil2* locus, on chromosome 7, may contain one or more genes controlling cocaine sensitization and the hyperlocomotor effect of cocaine in male and female rats, respectively. Moreover, the marker-assisted selection used in this study proved to be a valuable approach for detecting, confirming and fine-mapping behavioral QTL and searching for genetic correlations between different phenotypes. Congenic rats are currently being produced to study *Ofil1* in greater detail.

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