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Deletion of Melanin-Concentrating Hormone Receptor-1 gene accentuates D-amphetamine-induced psychomotor activation but neither the subsequent development of sensitization nor the expression of conditioned activity in mice

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Abstract

The present study aimed to test the hypothesis that mice lacking the MCHR1 receptor (Melanin-Concentrating Hormone Receptor-1) present an elevated vulnerability towards the neurobehavioural effects of D-amphetamine, presumably due to previously established up-regulations of dopamine D1 receptors in these mice. We examined the psychomotor effects of five once-daily injections of 1.5 and 3 mg/kg D-amphetamine (i.p.) or ten once-daily injections of 2.25 mg/kg D-amphetamine in knockout (KO) mice lacking the MCHR1 receptor. The first injection of D-amphetamine induced a greater psychomotor response amongst the KO mice at 2.25 and 3.0 mg/kg. On all subsequent D-amphetamine injections, KO mice still showed greater levels of psychomotor activity than the WT mice, but with no between-genotype difference in the rate of development of sensitization (similar slopes of the curves). Furthermore, 24 h after the last injection of 2.25 mg/kg D-amphetamine both genotypes exhibited a significant post-sensitization conditioned activity. Thus, MCHR1 receptors are likely not deeply involved in the mechanisms of induction of sensitization and related conditioned activity induced by D-amphetamine, albeit our results confirm a contribution of these receptors to the mechanisms of the acute effects of that drug, possibly via an inhibitory action on the dopaminergic mesolimbic system. Our results do not support the hypothesis of a functional contribution of MCHR1 receptors to the addictive effects of D-amphetamine.

Keywords: MCHR1 receptor; D-amphetamine; Knockout mice; Psychomotor activity; Behavioural sensitization; Conditioned drug effect

1. Introduction

Melanin-Concentrating Hormone (MCH) and its receptor MCHR1 are involved in the modulating action that the lateral hypothalamus (LH) naturally exerts on food intake and energy homeostasis (Saper et al., 2002). For instance, hypothalamic administrations of MCH or its over-expression in transgenic mice stimulate feeding behavior and increase body weight. Consistently, prepro-hormone ppMCH and MCHR1 knockout mice are lean and display relatively high metabolic rates, in spite of showing signs of mild hyperphagia (for a review see Xu et al., 2004). Anatomically, MCH neurons send projections to many brain areas such as the cortex, the brainstem, the hippocampal formation and limbic structures (Bittencourt et al., 1992). The distribution of MCHR1 transcripts correlates well with MCH projections. In particular, high expression of MCHR1 mRNA has been identified in the shell of the nucleus accumbens (NAC) and to a lesser extent in the ventral tegmental area (VTA), two major components of the reward dopaminergic mesolimbic system (Saito et al., 2001). The fact

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that the shell of the NAC is critically involved in mediating food reward (Di Chiara, 2002) is suggestive of an involvement of MCH and MCHR1 receptors in the mediation of the rewarding effect of food, which would take place from the LH towards the NAC region (Georgescu et al., 2005; Saper et al., 2002). Likewise, the fact that dopaminergic projections from the VTA to the NAC are critical in the mediation of the neurobehavioral and rewarding effects of addictive substances like cocaine or the amphetamines (Di Chiara, 2002; Vanderschuren and Kalivas, 2000) raises the possibility of a participation of the MCHR1 receptors to that mediation (Di Leone et al., 2003). Amongst the few studies having explored that possibility, Smith et al. (2005) have recently reported that MCHR1-deficient mice, which were hyperactive in a novel context and hypersensitive to a single injection of 2 and especially 3 mg/kg D-amphetamine, exhibited up-regulation of dopamine D1 receptors in the VTA and in both the NAC shell and core and norepinephrine (NE) transporters in the NAC shell and the globus pallidus. Consistently, a marked psychomotor reactivity to the D1 agonist SKF38393 was also found in MCHR1–KO mice. As most theories of behavioral sensitization to psychomotor stimulants postulate that sensitization processes involves possibly the norepinephrine system (albeit this is still controversial) and especially the dopamine D1 receptors (i.e., Juhila et al., 2005; Vezina, 1996; Vanderschuren and Kalivas, 2000), these results provide the empirical grounds for the hypothesis that MCHR1 receptors exert an inhibiting action on mesolimbic monoamine activity, and are thereby involved in the neural mechanisms of addictive effects (Smith et al., 2005). An obvious prediction of that theoretical contention is that mice lacking MCHR1 receptors should exhibit a greater vulnerability to chronic exposure to substances of abuse, in particular D-amphetamine. For example, as compared to intact mice MCHR1-deficient mice should exhibit a greater propensity to develop amphetamine-induced sensitization (greater rates of sensitization), a core feature of psychomotor stimulants dependence (Robinson and Berridge, 2003). Since the post-drug conditioned psychomotor response (to the test context) is inherent to the neurobehavioral effects of intermittently given D-amphetamine, we also investigated that response. In other words, the present study was aimed at verifying that prediction, using D-amphetamine doses comparable to those used in the single-injection study by Smith et al. (2005). The initial test session provided information on between-genotype differences in acute psychomotor activation and the following sessions on the development or induction of sensitization. The rates of sensitization, assessed via simple linear regression analysis, were then compared between genotypes, a high rate of sensitization testifying of a great susceptibility (or vulnerability) to D-amphetamine. In a first experiment, mice were tested according to a simple design involving five once-daily drug injections of 1.5 or 3.0 mg/kg D-amphetamine and a behavioral measurement after each injection. Based on the results of the first experiment, the design of the second experiment involved ten once-daily injections of 2.25 mg/kg D-amphetamine with the aim to maximize the likelihood of inducing sensitization at an intermediate and slower rate and to facilitate the manifestation of potential genotype-related differences. The expression of conditioned psychomotor activity was assessed under a saline challenge 24 h after the last sensitizing injection.

2. Methods

2.1. Animals and drugs

The inactivation of the mchr1 allele, the generation of knockout animals and the genotyping method have been described previously (Adamantidis et al., 2005). Heterozygous founders were maintained in a hybrid 129X1/SvJ×C57BL/6J background for 3 generations. Knockout (KO) and wild-type (WT) littermates were derived from the intercrossing of a population of heterozygous mice.

Forty F4 and F5-derived male mice, aged 7–9 weeks and experimentally naïve at the start of testing, were housed in groups of two in transparent polycarbonate tubs (11×30-cm surface, 13-cm height) and provided with pine sawdust bedding and free access to tap water and food (standard pellets, CARFIL QUALITY, Oud-Turnhout, Belgium). The housing room was maintained on a 12:12 h dark–light cycle (lights on at 08:00 h) and at an ambient temperature of 20–24 °C. All experimental treatments and animal maintenance were carried out according to the guidelines of animal welfare laid down by the European Community (EEC Council Directive No. 86/609 of the 24 November 1986).

D-amphetamine sulfate (BELGOPIA, Louvain-La-Neuve, Belgium) was dissolved in an isotonic saline solution (0.9% NaCl) and was injected via the peritoneal route (i.p.) at 1.5, 2.25 or 3.0 mg/kg in a volume of 0.01 ml/g body weight. The control treatment (saline solution) was administered in the same volume and manner.

2.2. Behavioural apparatus

Mice were tested individually with eight custom-made activity-meters. Each activity-meter consisted of a wooden base placed under a removable clear polycarbonate tub (12×22 cm surface×12 cm height), a transparent acrylic-glass tablet serving as a lid. Locomotion (psychomotor activity) was measured by two pairs of infrared light-beam sensors (2-cm height) mounted on the base and spaced 6 cm from each other on both long sides of the tub. A mouse had to traverse the full distance between the beams for each activity count. Interruptions of a single beam were not taken into account in the data analyses. Activity counts were recorded by a personal computer to which all activity-meters were connected. Each apparatus was encased in a white-paint sound-attenuating shell (65×80 cm surface×80 cm height) that was artificially ventilated, illuminated by a non-heating energy-saver white light and maintained at an ambient temperature of 20–24 °C. A one-way window in each shell door allowed direct visual surveillance during testing.

2.3. Experimental procedure

Prior to experimentation, mice were handled daily for a dozen of min over eight days without being exposed to the behavioral apparatus. All procedures were conducted between 9:00 and 12:00 h, mice being weighed and tail-marked with a felt-tipped pen before every test session. This study comprised two experiments. In Experiment I (Exp.I), mice from both genotypes received five once-daily injections of saline, 1.5 mg/kg or 3.0 mg/kg D-amphetamine and their psychomotor activity was measured on every sessions. In Experiment II (Exp.II), whose parameters were established on the basis of the results derived from Exp.I, saline or 2.25 mg/kg D-amphetamine were given once-daily over ten sessions and psychomotor activity measured on each test session. Additionally, 24 h following the tenth drug-treatment session, mice were tested for post-drug conditioned hyperactivity under saline in the same contextual conditions as those used for the D-amphetamine pretreatment. The occurrence of the conditioned response was declared when the value derived from the drug-pretreated mice was significantly greater than those of the saline-pretreated mice recorded on the saline-challenge test session as well as on the initial session of the pretreatment phase (Cunningham, 1993; Damianopoulos and Carey, 1992; Tirelli and Terry, 1998). In Exp.I, the design comprised six experimental conditions resulting from the factorial combination of the three possible D-amphetamine pretreatments (saline, 1.5 or 3.0 mg/kg) with the genotypes (WT or KO). The groups corresponding to these conditions were labeled as follows: WT/Sal (wild-type mice receiving saline injections), WT/1.5 (wild-type mice receiving injections of 1.5 mg/kg D-amphetamine), WT/3.0 (wild-type mice receiving injections of 3.0 mg/kg D-amphetamine, KO/Sal (mutants receiving saline injections), KO/1.5 (mutants receiving injections of 1.5 mg/kg D-amphetamine), KO/3.0 (mutants receiving injections of 3.0 mg/kg D-amphetamine). In Exp. II, the design comprised four experimental conditions resulting from the factorial combination of the two possible D-amphetamine pretreatments (saline or 2.25 mg/kg) with the genotypes (WT or KO). The groups corresponding to these four conditions were labeled as follows: WT/Sal (wild-type mice receiving saline injections), WT/2.25 (wild-type mice receiving injections of 2.25 mg/kg D-amphetamine), KO/Sal (mutants receiving saline injections), and KO/2.25 (mutants receiving injections of 2.25 mg/kg D-amphetamine). Within each genotype, mice were randomly allocated to the possible experimental groups seven days prior to testing ($n=10$ in Exp. I and $n=14$ in Exp. II). In each session from both experiments, mice from all groups were first placed into the test chamber for 15 min, injected with their respective treatment and then replaced in the test chamber for a period of 70 min, the pre- and post-injection periods being separated by a 5-min interval (during which the injection was performed). Locomotor activity measurements were broken down into intervals of 5 min for all sessions. Mice were returned to their home-cages within 10 min after testing.

2.4. Data analyses

The acute amphetamine-induced psychomotor effects derived from the 1st session from both experiments were analyzed with 3-way mixed-model ANOVAs that included the genotype (Genotype, 2 levels) and D-amphetamine doses (Drug, 3 levels in Exp.I; 2 levels in Exp.II) as between-group variables and the post-injection 5-min intervals as a within-subjects variable (Interval: 14 levels).

Data pertaining to the development of sensitization over the five (Exp.I) or the ten (Exp.II) once-daily test sessions were analyzed using 3-way mixed-model ANOVAs incorporating the genotype (Genotype, 2 levels) and D-amphetamine dose (Drug, 3 levels in Exp. I; 2 levels in Exp. II) as between-group factors, and the successive once-daily test sessions (total post-injection activity counts over 70 min) as a within-subject factor (Session, 5 levels in Exp. I; 10 levels in Exp. II). The individual rate of sensitization over the successive (five or ten) sessions was measured using a simple linear regression analysis, a relatively high slope (or regression coefficient) signifying a high rate of sensitization. A delta score was also computed to quantify the final amplitude achieved by sensitization (the difference between the total behavioral counts on the last and the 1st session), a relatively large delta score indicating an ample final sensitized effect. Potential genotype- and dose-related differential rates and amplitudes of sensitization werethen assessed using 2-way fixed-model ANOVAs incorporating the genotype (Genotype, 2 levels) and D-amphetamine doses (Drug, 2 or 3 levels) as fixed factors. Since sensitization-related increases in D-amphetamine-induced hyperactivity were expected, confirmatory F-based planned comparisons were conducted to test the reliability of the related between-mean differences (last-session value against the first-session one; Keppel and Wickens, 2004).

Data pertaining to the conditioning test (Exp. II) were analyzed with a $2\times2\times14$ mixed-model ANOVA incorporating the genotype (Genotype, 2 levels) and previously-given D-amphetamine intermittent treatment (Drug pretreatment, 2 levels) as between-group factors, and the post-injection 5-min intervals as a within-subject factor (Interval, 14 levels). Additionally, a 2-way fixed-model ANOVA was conducted on the total number of counts recorded on the entire session (70 min, without taking into account the interval); it incorporated D-amphetamine pretreatment (Drug pretreatment, 2 levels) and the genotype (Genotype, 2 levels) as between-group factors. In order to ascertain that the post-injection saline-induced activity constituted a real conditioned drug effect, and not a sort of lack-of-habituation effect, values from the conditioning test day (session on day 11) were compared with those of the first session, a significantly greater value on the saline-challenge test session reflecting the occurrence of such an effect (Damianopoulos and Carey, 1992; Tirelli and Terry 1998). This was performed with a 2-way fixed-model ANOVA treating the genotype (Genotype, 2 levels) as a

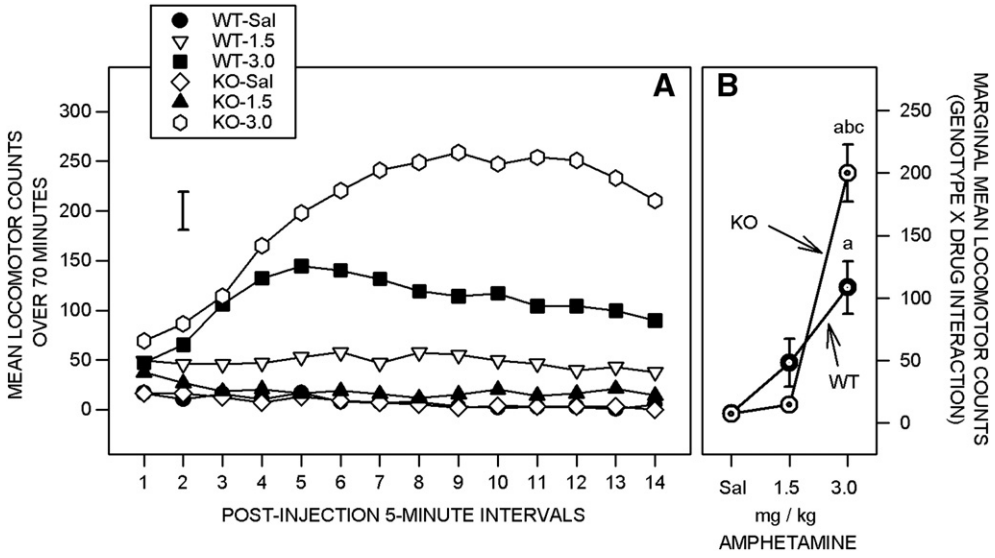

Fig. 1. Psychomotor activation induced by 1.5 or 3.0 mg/kg D-amphetamine on the first session in MCHR1–KO and MCHR1–WT mice (Exp.I). (A): Expression of the effects as a function of the 14 post-injection intervals; (B): Marginal means of the first-order Genotype-by-Drug interaction. (a): value significantly different from the respective saline-injected group within each genotype, (b): value significantly different from the respective group treated with 1.5 mg/kg D-amphetamine within each genotype; (c): significant between-genotype difference at 3.0 mg/kg D-amphetamine, as supported by Tukey–HSD tests taken at least at $P<0.025$. The vertical brackets are the pooled SEM×2 in the panel A and the individual SEMs×2 in the panel B.

between-group factor and the relevant test sessions (Day, 2 levels; values from the D-amphetamine-pretreated group on day 11 and values from the saline-pretreated group on day 1) as a within-subject factor; confirmatory *F*-based planned comparisons were conducted to ascertain that the conditioning-related differences were reliable (Keppel and Wickens, 2004). Other relevant between-mean differences were assessed using a posteriori Tukey–HSD tests derived from the appropriate error-terms (Keppel and Wickens, 2004). Prior to performing the ANOVAs, data were submitted to a square-root transformation in order to more nearly meet the assumption of homoscedasticity (for the sake of clarity, raw values are presented in the graphs). The differences were conventionally declared significant at the *P*-level of 0.05.

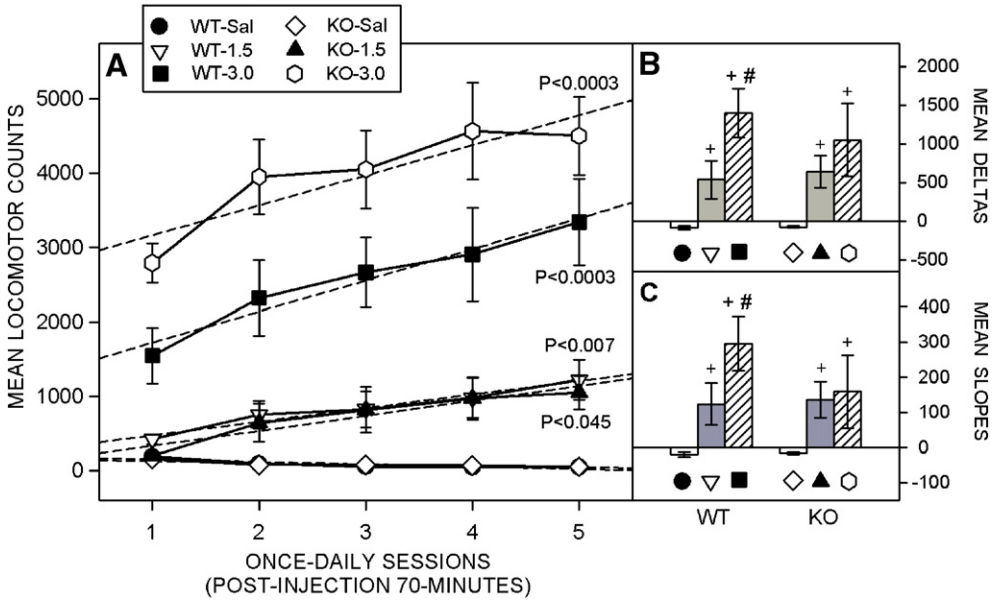

Fig. 2. Development of psychomotor sensitization over five once-daily injections of 1.5 or 3.0 mg/kg D-amphetamine in MCHR1–KO and MCHR1–WT mice (Exp.I). (A): Emergence of the sensitized effects over the five 70-min test sessions; (B): Mean relative increase in psychomotor activity for each of the six experimental groups (delta scores); (C): Mean rates of sensitization for the same groups (slopes). Each *P*-level indicated in the graph of panel A refers to a significant difference between the mean value of the last session and that of the first one (*F*-based planned comparisons). (+): value significantly different from the respective saline-injected group within each genotype; (#): value significantly different from the respective group having received 1.5 mg/kg D-amphetamine within each genotype, as yielded by *F*-based planned tests taken at least at $P<0.025$ (slopes). Note that the regression lines in the panel A are derived from the mean values plotted in the graph for iconographic reasons and do not represent the mean slopes shown in panel C. The vertical brackets are the SEM×2.

3. Results

3.1. Experiment I

Fig. 1, presents the within-session time-courses of the (acute) effects as recorded in the first session of the intermittent treatment. ANOVA on these data brought about a robustly significant Genotype-by-Drug-by-Interval interaction ($F[26,546]=6.31$, $P<0.0001$), whose values are shown in panel A, and a significant Genotype-by-Drug interaction ($F[2,42]=6.99$, $P<0.0025$), whose specific values are depicted in panel B (marginal means). As shown in panel A, 3.0 mg/kg D-amphetamine induced levels of psychomotor activation that were much greater in KO mice than in their WT counterparts, these effects being significantly higher than those induced by saline and 1.5 mg/kg D-amphetamine in both genotypes (Tukey–HSD tests taken at least at $P<0.025$). At 1.5 mg/kg, D-amphetamine induced non-significant (WT mice) or no (KO mice) psychomotor hyperactivity.

Fig. 2 depicts the activating effects of the sensitizing repeated injections of D-amphetamine over the five daily sessions. ANOVA on these data yielded highly significant Session and Drug main effects ($F[4,216]=12.28$, $P<0.0001$ and $F[4,54]=4.31$, $P<0.020$, respectively), a significant Genotype-by-Drug interaction ($F[4,216]=12.28$, $P<0.0001$) and no Genotype-by-Drug-by-Session interaction ($P>0.91$). For each D-amphetamine-injected group shown in panel A, *F*-based planned comparisons confirmed that the value from the last session was significantly greater than that of the first session, indicating that sensitization did occur (*P*-levels between 0.045 and 0.0003). At 3.0 mg/kg D-amphetamine,

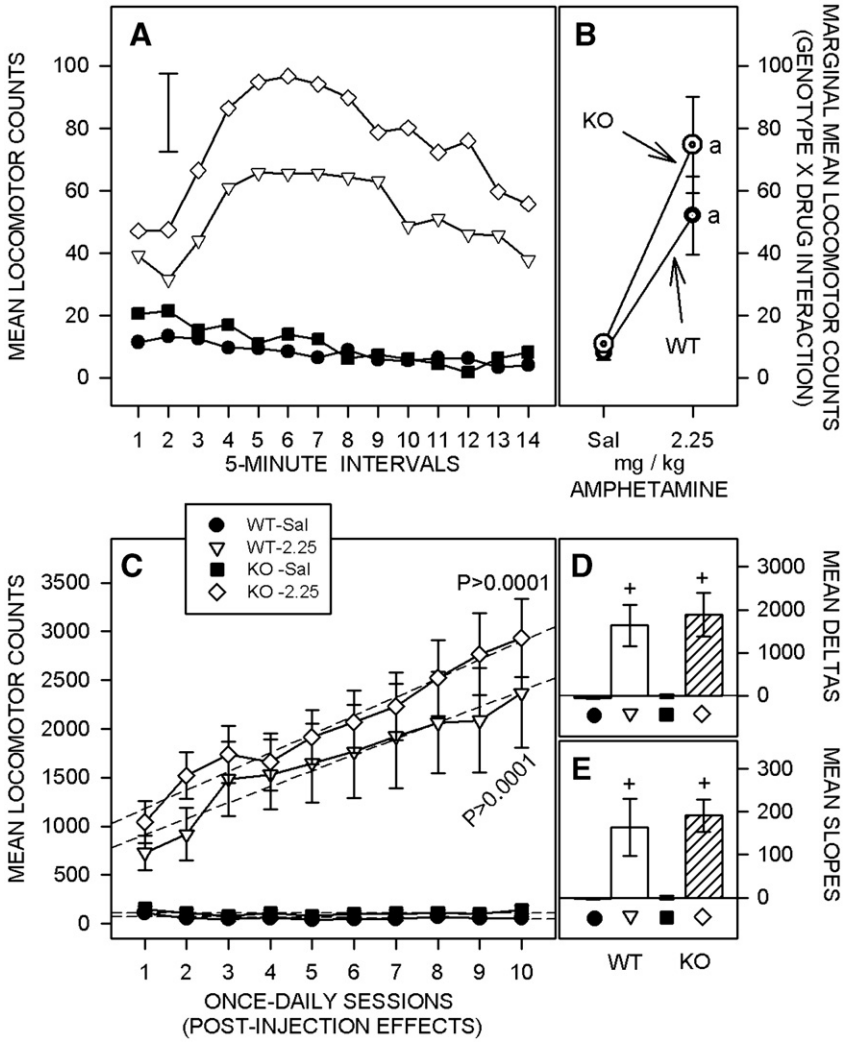

Fig. 3. Psychomotor activation and sensitization induced by 2.25 mg/kg D-amphetamine in MCHR1–KO and MCHR1–WT mice (Exp.II). (A): Expression of D-amphetamine-induced psychomotor activation as a function of the 14 post-injection intervals of the first session; (B): Marginal means of the first-order Genotype-by-Drug interaction derived from the first session data analysis. (C): Development of psychomotor sensitization over the five 70-min test sessions; (D): Mean relative increase in psychomotor activity for each of the six experimental groups (delta score); (E): Mean rates of sensitization for each of the six groups (slopes). (a): value significantly different from the respective saline-injected group within each genotype, as yielded by Tukey–HSD tests taken at least at $P<0.010$ (any measure). Note that the regression lines in the panel C are derived from the mean values plotted in the graph for iconographic reasons and do not represent the mean slopes shown in panel E. The vertical brackets are as in Figs. 1 and 2.

KO mice exhibited levels of psychomotor activation significantly higher than those displayed by the WT mice from the first to the fourth sessions, the incremental trajectories of these sensitizing effects being grossly parallel. The slight attenuation that occurred on the 5th session among the KO mice might have resulted from the few yet unambiguous stereotypical movements (via behavioral competition) observed in some mice through the door shell. At 1.5 mg/kg D-amphetamine, the sensitization curves did not differ at all between genotypes. While the rates and amplitudes of 1.5 and 3.0 mg/kg D-amphetamine sensitization were significantly greater than those derived from the saline-treated groups in both genotypes, these measures did not differ substantially between KO and WT mice, as can be seen in panels B and C. Consistently, there were no significant Genotype-by-Drug interaction (deltas: $P>0.90$; slopes: $P>0.74$) while a robustly significant main effect of Drug was found in both measures (deltas: $F[2,42]=27.25$, $P<0.0001$; slopes: $F[2,42]=13.67$, $P<0.0001$). F-based planned tests within each genotype indicated that both the rates and the amplitudes of sensitization were dose-dependently increased in the WT but not in the KO mice, in which there were no between-dose differences (mean deltas: at last at $P<0.010$, mean slopes: at least at $P<0.025$). That small between-genotype qualitative difference reflected the above-mentioned slight blunting of the sensitized effect of 3.0 mg/kg D-amphetamine on the last session in the KO mice (occurrence of a few stereotypic movements).

3.2. Experiment II

Fig. 3 presents the time-courses of the acute (first session) psychomotor effects of 2.25 mg/kg D-amphetamine as recorded on the first test session of Exp.II (panels A and B), and the development of psychomotor sensitization induced by 2.25 mg/kg D-amphetamine given over ten once-daily test sessions (panels C, D and E). Three-way ANOVA on data from the first session brought about a robustly significant main effect of Drug ($F[1,52]=28.65$; $P<0.0001$), but neither a significant Genotype-by-Drug-by-Interval interaction ($P>0.49$), whose values are depicted in panel A, nor a significant Genotype-by-Drug interaction ($P>0.34$), whose values are depicted in panel B. In fact, the first injection of D-amphetamine induced an overall significant psychomotor activation without clear-cut differentiation of the genotypes, in spite of a trend for the KO mice to achieve greater levels than their WT counterparts (Tukey–HSD tests on the relevant individual marginal means taken at least at $P<0.010$). ANOVA on the 10-session data (panel C) yielded highly significant main effects of Drug and Session ($F[1,52]=68.87$; $P<0.0001$ and $F[4,216]=12.28$; $P<0.0001$, respectively), a robustly significant Drug-by-Session interaction ($F[9,468]=9.13$; $P<0.0001$), but no interaction involving the genotype and no Genotype main effect (all terms at least at $P>0.95$). In other words, drug-induced psychomotor activation augmented near-monotonously over the successive test sessions in both KO and WT mice, without substantial differences between the trajectories

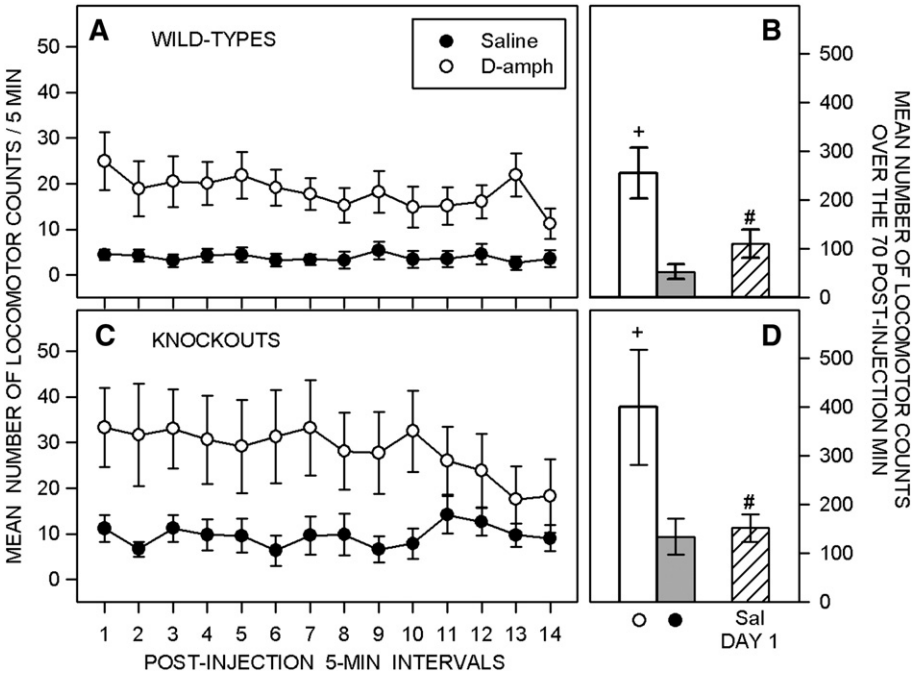

Fig. 4. Expression of the conditioned psychomotor activation generated under a saline challenge 24 h following the 10-injection treatment of 2.25 mg/kg D-amphetamine, i.e. on day 11 of Exp. II (see Fig. 4). (A) and (C): Conditioned activity as a function of the 14 post-injection intervals in MCHR1–WT and MCHR1–KO mice, respectively; (B) and (D): Conditioned activity expressed in terms of locomotor counts summed over the entire 70-min session in MCHR1–WT and MCHR1–KO mice, respectively. The hatched columns represent the values derived from the first session of the saline-pretreated groups. (+): value significantly greater than that of the respective saline-pretreated group, at $P<0.003$ (WT) or $P<0.030$ (KO); (#): value significantly smaller than that derived from the D-amphetamine-pretreated group at $P<0.007$ (WT) or $P<0.025$ (KO), as yielded by F-based planned comparisons. The vertical brackets are the SEM×2 in all graphs.

of two sensitization curves being induced. In fact, within the two D-amphetamine-treated groups, the values of the last (10th) test session achieved levels that were almost three-fold significantly higher than those recorded on the respective first sessions, as supported by *F*-based planned comparisons taken at $P<0.0001$. As shown in panels D and E, these effects were confirmed by the measures of the amplitude and the rate of sensitization, since the levels of these measures in the cocaine-treated group were significantly greater than those of the saline-treated animals in both genotypes, a profile of effects supported by strong main effects of Drug (deltas: $F[1,52]=26.02$, $P<0.0001$; rates: $F[1,52]=21.98$, $P<0.0001$), the absence of significant Genotype-by-Drug interaction (deltas: $P>0.77$; rates: $P>0.74$), and *F*-based planned comparisons between the relevant values (at least at $P<0.010$ for both measures). Note that, in this experiment, there was little evidence for habituation in the saline-treated groups in both genotypes since mean deltas and mean slopes were close to zero.

Fig. 4 presents the psychomotor response to a saline challenge in D-amphetamine- and saline-pretreated mice recorded 24 h following the 10th test session of the D-amphetamine intermittent pretreatment (counts per interval in panels A and C, whole-session counts in panels B and D). The three-way ANOVA on the time-courses data yielded no Genotype-by-Drug-by-Interval and Genotype-by-Drug pretreatment interactions ($P>0.125$ and $P>0.650$, respectively), no main effect of Genotype ($P>0.110$), but a robustly significant main effect of Drug pretreatment ($F[1,52]=11.72$, $P<0.002$). This suggests that an average conditioned activity was actually generated in both genotypes, between which there were no substantial differences. Also, conditioned activity was more or less constantly expressed over the 14 intervals, albeit less clearly during the four last intervals in the KO mice, as supported by a significant Drug-by-Session interaction ($F[13,676]=2.39$, $P<0.004$). As depicted in panels B and D, mice having received D-amphetamine exhibited a significantly greater overall response (summed counts on entire session) than the saline-pretreated mice within each genotype, the absence of substantial between-genotype difference being confirmed. That profile of effects was corroborated by significant main effects of Drug pretreatment and Genotype ($F[1,52]=23.12$, $P<0.0001$ and $F[1,52]=4.72$, $P<0.035$, respectively) and in particular by significant *F*-based planned tests at least at $P<0.030$. It can also be seen in panels B and D that D-amphetamine-pretreated mice from each genotype expressed levels of total psychomotor activity on the saline-challenge session that were greater than those, induced by novelty, derived from the respective saline-treated mice tested on the first session. This profile of effects was supported by a robustly significant Drug pretreatment main effect ($F[1,52]=12.26$, $P<0.001$) and especially significant *F*-based planned comparisons at least at $P<0.025$.

4. Discussion

The present study yielded the following findings. (1) A single injection of 3.0 mg/kg D-amphetamine induced a greater psychomotor activation in the MCHR1-deficient KO mice than in their WT counterparts, the lower dose of 1.5 mg/kg D-amphetamine exerting little effect in both genotypes. (2) On the subsequent once-daily sessions, 3.0 mg/kg D-amphetamine induced an unequivocal psychomotor sensitization that develops at significantly higher levels in the KO than in the WT mice. (3) More importantly, psychomotor sensitization developed at comparable rates and amplitudes in both genotypes at all doses of D-amphetamine. (4) Both genotypes exhibited an unambiguous post-sensitization conditioned psychomotor activity (under saline).

On the initial sessions, the saline-treated mice exhibited no between-genotype difference in basal psychomotor activity, neither before nor after the injection (compare values of the WT–Sal and KO–Sal groups in Figs. 1 and 3). Only some hint of a lack of habituation in saline-treated KO mice can be detected in Exp.II, where their activity on the 11th session (conditioning test) was still comparable to that of the first session. That pattern of observations concords with several studies reporting no genotypic difference in locomotor activity measured during the daytime (Adamantidis et al., 2005; Astrand et al., 2004; Marsh et al., 2002; Roy et al., 2006; Zhou et al., 2005). In some of these studies, MCHR1-deficient mice exhibited a significant hyperactivity at night, their behavior being monitored through the entire light–dark cycle was used (Astrand et al., 2004; Marsh et al., 2002; Zhou et al., 2005). However, the present results on locomotion disagree with some other previous studies, including some of ours, where relatively short 15–60-min psychomotor activity tests were performed in a novel environment during the light phase (unpublished data from this laboratory obtained with mutants derived from a 129X1/SvJ × 129S2/SvPas genomic background; Lalonde and Qian, 2007; Smith et al., 2005; Tyhon et al., 2006). Taken together, these results strongly suggest that the relative hyperactivity of the MCHR1–KO mice is sensitive to between- and within-laboratory methodological dissimilarities (sample sizes, use of a pre-injection monitored period, sensitivity of the behavioral measure) and strongly depends on the circadian moment of the behavioral test. As regards our own conflicting results, any potential effect of the backcross status (F2–F3 and F3–F4 in the previous studies, F4–F5 in the present one) should have been minimized by the maintenance of a very large number of mating couples coming from the chimeras in all of our studies. Note also that the experimenter and animal husbandry can be ruled out since these factors did not change in our laboratory.

The psychomotor-activating effects of acute (first session) D-amphetamine in our MCHR1-deficient mice substantially confirm those reported by Smith et al. (2005), even if their mutant mice showed three-fold increases at 2 mg/kg D-amphetamine whereas ours did not significantly differ from their WT counterparts at comparable doses (1.5 and 2.25 mg/kg D-amphetamine). Along with the usual and countless laboratory-specific procedural factors, it is likely that this between-laboratory qualitative difference were due to the genomic background from which the KO mice were generated. In Smith et al. (2005), MCHR1–KO mice were derived from the mixed

129SvEv×C57BL/6J background (or 129S6/SvEvTac×C57BL/6J) while our mice were generated from the mixed 129X1/SvJ×C57BL/6J background (WT). Mice from the 129SvEv strain have been reported to respond relatively weakly to acute and even intermittent D-amphetamine or cocaine, whereas 129X1/SvJ mice respond much more intensively (Gould et al., 2007; Miner 1997; Ralph et al., 2001; MacKerchar et al., 2006; Walters and Blendy, 2001). Therefore, it is plausible that the attenuated between-genotype differences in acute D-amphetamine sensitivity of our mice were due to the influence of the parental 129X1/SvJ strain. On the other hand, the relatively moderate stimulating effects of D-amphetamine expressed by the mice from the mixed 129S6/SvEvTac×C57BL/6J background (WT) was likely driven by the parental 129S6/SvEvTac strain, facilitating the establishment of the relatively large amphetamine-induced increases in psychomotor activity amongst the mutants in Smith et al.'s study. Since these MCHR1–KO mice present pronounced up-regulations of the D1 receptors in representative regions of the mesolimbic system (Smith et al., 2005), one can also hypothesize that our genomic background presents lesser such up-regulations (to be verified).

By contrast, we found no between-genotype differences at all in the rate or amplitude of sensitization development, a finding that unambiguously disconfirm the prediction of Smith et al.'s hypothesis (2005) that repeated injections of D-amphetamine should lead to a magnified psychomotor sensitization and related conditioned activity in mice lacking the MCHR1 receptor. That hypothesis stipulates that MCHR1 receptors in intact animals exert a natural inhibitory control on the monoamine activity in the mesoaccumbens axis, thereby potentially participating in the mechanisms of drug addiction (see also Di Leone et al., 2003). It is well known that the rate and amplitude of psychomotor sensitization to amphetamines, and even the likelihood to induce it, is tightly dependent upon procedural factors such as the behavioral measure, the test context and especially the route and regime of drug administration; marked between-injection intervals often lead to clear-cut sensitization whereas more continuous drug administration can produce tolerance in mice and rats (Chaudry et al., 1987; Kuribara, 1996; Segal and Kuczenski, 1994). This suggests that the use of another schedule of D-amphetamine administration, given in presumably more appropriate conditions, could have facilitated in our mice the manifestation of between-genotype differences in sensitization. However, this is unlikely since the fact that we utilized a representative sensitization protocol, and that we confirmed the magnified psychomotor sensitivity to acute amphetamine of the MCHR1–KO mice, strengthens the validity of the present results. Furthermore, if the above-mentioned possibility was true, the generality of the involvement of the MCH–MCHR1 system in mesolimbic mechanisms would be restricted to some conditions of testing, anyway weakening the tested hypothesis.

Dopaminergic and glutamatergic transmissions in the NAC, the VTA, the basolateral amygdala and the prefrontal cortex, notably, are well established to participate in the mechanisms of induction of amphetamine sensitization (see reviews by Di Chiara, 2002; Vanderschuren and Kalivas, 2000). As regards the VTA, a predominant body of evidence indicates that it constitutes the main structure responsible for the induction of amphetamine sensitization, to a much larger extent than the NAC, whereas both structures seem equally indispensable for cocaine sensitization to be induced (Vanderschuren and Kalivas, 2000). MCHR1 receptors and MCH are both unambiguously present in the shell and the core of the NAC and in the VTA (Bittencourt et al., 1992; Saito et al., 2001). However, because of several failures to demonstrate a direct interaction between the mesoaccumbens dopaminergic transmission and the MCH–MCHR1 system, one must admit that the functional significance of that anatomical localization remains puzzling. For instance, infusion of MCH failed to facilitate the firing rate of rat VTA neurons in experiments using single-unit extra-cellular and whole-cell patch-clamp recordings (Korotkova et al., 2003). Consistently, intra-VTA infusion of MCH in rats has no effect on steady state levels of dopamine release and metabolism neither in the caudate nucleus not in the NAC (Sanchez et al., 2001). Note also that MCHR1 KO and WT mice do not differ in basal or acute amphetamine-evoked tissue levels of monoamines or metabolites within the NAC (Smith et al., 2005). Because of such a deep involvement of the VTA in the induction of amphetamine sensitization, these neurobiological results are consistent with the absence of differences in the rate and amplitude of sensitization between our WT and KO mice.

Although neuropharmacological studies confer a pivotal role to the D1 receptors within the VTA in the induction of sensitization to amphetamine, other studies yielding opposite conclusions suggest that the evidence linking D1 receptors and sensitization is in fact highly complex (see Vanderschuren and Kalivas, 2000; McDougall et al., 2005). On one hand, the blockade of D1 receptors in the VTA can prevent the induction of amphetamine psychomotor sensitization and related dopamine-dependent neuroadaptations (Vanderschuren and Kalivas, 2000; Vezina, 1996). On the other hand, psychomotor sensitization to amphetamine has been convincingly generated in D1-deficient mice, sometimes at greater levels than in the WT controls (Xu et al., 2000; Corvol et al., 2007; McDougall et al. 2005). Consistent with these latter results, cross-sensitization between amphetamine (prior history) and representative D1-agonists has not been successfully obtained, cross-sensitization to D2 agonists occurring readily (Ujike et al., 1990; Vandeshuren et al., 1999). Thus, it is openly plausible that the D1 receptors were not involved in the development of sensitization in our MCHR1-deficient mice, in spite of a probable up-regulation of these sites in that genotype. To some extent, our results can even be taken as indirectly disconfirming such an involvement.

The D-amphetamine-induced conditioned responses readily expressed in both genotypes, with some more inter-individual variability in MCHR1-deficient mice, concord with comparable results in previous reports having used representative inbred and outbred strains of mice (Cabib 1993; Hayashi et al., 1980; McDougall et al., 2005; Mead and Stephens 1998; Steckler and Holboer 2001; Tirelli and Terry, 1998). According to the "lack-of-habituation" hypothesis, some degree of novelty (that produces hyperactivity) is preserved by the drug intermittent

drug treatment, via an attenuation or suppression of habituation to the test context (especially during the establishment of a sensitization). The drug-free hyperactivity shown on the conditioning test by the previously drugged animals has thus been proposed to represent a novelty-induced effect (Damianopoulos and Carey, 1992; Tirelli and Terry, 1998). This cannot be the case in our study because the conditioned response was much greater than the novelty-induced hyperactivity, a fact that would even disconfirm the "lack-of-habituation" hypothesis (along with several previous studies, for example Ahmed et al., 1996; Tirelli and Heidbreder, 1999; Tirelli and Terry, 1998). From our results, it can obviously be concluded that there are likely no convincing between-genotype dissimilarities in the neural mechanisms underlying D-amphetamine-induced conditioned psychomotor activity. Note that part of these mechanisms relies on a differential involvement of D1, D2 and D3 receptors in the NAC core and the basolateral amygdala in that activity or in sensitization and non-sensitized psychomotor activation produced by D-amphetamine (Aujla and Beninger, 2004; Martin-Iverson and McManus, 1990; Mazurski and Beninger, 1991; Sellings and Clarke, 2006).

In conclusion, the present results confirm that MCHR1-deficient mice are certainly hypersensitive to the acute administration of D-amphetamine, suggesting that MCHR1 receptors may contribute to the brain mechanisms of the psychomotor effects of that drug, perhaps by exerting a direct inhibitory action on the processes mediating motor and behavioral activation in the mesocorticolimbic system (given the anatomic localization of MCHR1 receptors). However, we found no strong evidence for an involvement of the MCHR1 receptor in the mechanisms of induction of psychomotor sensitization and related conditioned hyperactivity. Therefore, the present results, together with those we have previously obtained with cocaine (Tyhon et al., 2006), disprove the hypothesis that MCHR1 receptors directly or indirectly exert an inhibiting action on mesolimbic monoamine activity, which subserves the chronic psychomotor and addictive effects of stimulant drugs (Smith et al., 2005).

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References

Adamantidis A, Thomas E, Tyhon A, Seutin V, Tirelli E, Grisar T. Disrupting the melanin-concentrating hormone receptor 1 in mice leads to cognitive deficits and alterations of NMDA receptor function. Eur J Neurosci 2005;21:2837–44.

Ahmed SH, Oberling P, Di Scala G, Sandner G. Amphetamine-induced conditioned activity does not result from a failure of rats to habituate to novelty. Psychopharmacology 1996;123:325–32.

Astrand A, Bohlooly M, Larsdotter S, Mahlapuu M, Andersen H, Tornell J, et al. Mice lacking melanin-concentrating hormone receptor-1 demonstrate increased heart rat associated with altered autonomic activity. Am J Physiol, Regul Integr Comp Physiol 2004;287:R749–58.

Aujla H, Beninger RJ. Intra-BLA or intra-NAC infusions of the dopamine D3 receptor partial agonist, BP897, block intra-NAC amphetamine conditioned activity. Behav Neurosci 2004;118:1324–30.

Bittencourt JC, Presse F, Arias C, Peto C, Vaughan J, Nahon JL, et al. The melanin-concentrating hormone system of the rat brain: an immuno- and hybridization histochemical characterization. J Comp Neurol 1992;319:218–45.

Cabib S. Strain-dependent behavioural sensitization to amphetamine: role of environmental influences. Behav Pharmacol 1993;4:367–74.

Chaudry IA, Turkanis SA, Karler R. Characteristics of reverse tolerance to amphetamine-induced locomotor stimulation in mice. Neuropharmacology 1987;27:777–85.

Corvol JC, Valjent E, Pascoli V, Robin A, Stipanovich A, Luedtke RR, et al. Quantitative changes in G alpha olf protein levels, but not D1 receptors, after specifically acute response to psychostmulants. Neuropsychopharmacology 2007;32:1109–21.

Cunnigham CL. Pavlovian drug conditioning. In: van Haaren F, editor. Methods in behavioural pharmacology. Amsterdam: Elsevier; 1993. p. 349–81.

Damianopoulos EN, Carey RJ. Pavlovian conditioning of CNS drug effects: a critical review and new experimental designs. Rev Neurosci 1992;3:65–77.

Di Chiara G. Nucleus accumbens shell and core dopamine: differential role in behavior and addiction. Behav Brain Res 2002;137:75–114.

Di Leone JR, Georgescu D, Nestler EJ. Lateral hypothalamic neuropeptides in reward and drug addiction. Life Sci 2003;73:759–68.

Georgescu D, Sears RM, Hommel JD, Barrot M, Bolanos CA, Marsh DJ, et al. The hypothamic neuropeptide melanin-concentrating hormone acts in the nucleus accumbens to modulate feeding behaviour and forced-swim performance. J Neurosci 2005;25:2933–40.

Gould TD, O'Donnell KC, Picchini AM, Manji HK. Strain differences in lithium attenuation of D-amphetamine-induced hyperlocomotion: a mouse model for the genetics of clinical response to lithium. Neuropsychopharmacology 2007;32:1321–33.

Hayashi T, Ohashi K, Tadokoro S. Conditioned drug effects to D-amphetamine- and morphine-induced motor acceleration in mice: experiential approach for placebo effect. Jpn J Pharmacol 1980;30:93–100.

Juhila J, Honkanen A, Sallinen J, Haapalinna A, Korpi ER, Schenin M. Alpha (2A)-adrenoceptors regulate d-amphetamine-induced hyperactivity and behavioural sensitization in mice. Eur J Pharmacol 2005;517:74–83.

Keppel G, Wickens TD. Design and analysis: a researcher's handbook. Fourth edition. Upper Saddle River, N.J.: Pearson Education; 2004.

Korotkova TM, Sergeeva OA, Eriksson KS, Haas HL, Brown RE. Excitation of ventral tegmental area dopaminergic and nondopaminergic neurons by orexins/hypocretins. J Neurosci 2003;23:7–11.

Kuribara H. Effects of interdose interval on ambulatory sensitization to methamphetamine, cocaine and morphine in mice. Eur J Pharmacol 1996;316:1–5.

Lalonde R, Qian S. Exploratory activity, motor coordination, and spatial learning in Mchr1 knockout mice. Behav Brain Res 2007;178:293–304.

McDougall SA, Reichel CM, Cyr MC, Karper PE, Nazarian A, Crawford CA. Importance of D1 receptors for associative components of amphetamine-induced behavioral sensitization and conditioned activity: a study using D1 receptor knockout mice. Psychopharmacology 2005;183:20–30.

MacKerchar TL, Zarcone TJ, Fowler SC. Use of a force-plate actometer for detecting and quantifying vertical leaping induced by amphetamine in BALB/cJ mice, but not in C57BL/6J, DBA/2J, 129X1/SvJ, C3H/HeJ, and CD-1 mice. J Neurosci Methods 2006;153:48–54.

Marsh DJ, Weingarth DT, Novi DE, Chen HY, Trumbauer ME, Chen AS, et al. Melanin-concentrating hormone 1 receptor-deficient mice are lean, hyperactive, and hyperphagic and have altered metabolism. Proc Natl Acad Sci U S A 2002;99:3240–5.

Martin-Iverson MT, McManus DJ. Stimulant-conditioned locomotion is not affected by blockade of D1 and/or D2 dopamine receptors during conditioning. Brain Res 1990;521:175–84.

Mazurski EJ, Beninger RJ. Effects of selective drugs for dopaminergic D1 and D2 receptors on conditioned locomotion. Psychopharmacology 1991;105:107–12.

Mead AN, Stephens DN. AMPA-receptors are involved in the expression of amphetamine-induced behavioural sensitization, but not in the expression of

amphetamine-induced conditioned activity in mice. Neuropharmacology 1998;37:1131–8.
Miner LL. Cocaine reward and locomotor activity in C57BL/6J and 129/SvJ inbred mice and their F1 cross. Pharmacol Biochem Behav 1997;58:25–30.
Ralph RJ, Paulus MP, Geyer MA. Strain-specific effects of amphetamine on prepulse inhibition and patterns of locomotor behavior in mice. J Pharmacol Exp Ther 2001;298:148–2001.
Robinson TE, Berridge KC. Addiction. Ann Rev Psychol 2003;54:25–53.
Roy M, David NK, Danao JV, Baribault H, Tian H, Giorgetti M. Genetic inactivation of melanin-concentrating hormone receptor subtype-1 (MCHR1) in mice exerts anxiolytic-like behavioral effects. Neuropsychopharmacology 2006;31:112–20.
Saito Y, Cheng M, Leslie FM, Civelli O. Expression of the melanin-concentrating hormone (MCH) receptor mRNA in the rat brain. J Comp Neurol 2001;435:26–40.
Sanchez MS, Barontini M, Armando I, Celis ME. Correlation of increased grooming behavior and motor activity with alterations in nigrostriatal and mesolimbic catecholamines after alpha-melanotropin and neuropeptide glutamine-isoleucine in the rat ventral tegmental area. Cell Mol Neurobiol 2001;21:523–33.
Saper CB, Chou TC, Elmquist JK. The need to feed: homeostatic and hedonic control of eating. Neuron 2002;36:199–211.
Segal DS, Kuczenski R. Behavioral pharmacology of amphetamine. In: Cho AK, Segal DS, editors. Amphetamine and its analogs: psychopharmacology, toxicology, and abuse. San Diego: Academic Press; 1994. p. 115–50.
Sellings LHL, Clarke PBS. 6-hydroxydopamine lesions of nucleus accumbens core abolish amphetamine-induced conditioned activity. Synapse 2006;59:374–7.
Smith DG, Tzavara ET, Shaw J, Luecke S, Wade M, Davis R, et al. Mesolimbic dopamine super-sensitivity in melanin-concentrating hormone-1 receptor deficient mice. J Neurosci 2005;25:914–22.
Steckler T, Holsboer F. Conditioned activity to amphetamine in transgenic mice expressing an antisense RNA against the glucocorticoid receptor. Behav Neurosci 2001;115:207–19.
Tirelli C, Heidbreder C. Conditioning of and contextual sensitization to apomorphine-induced climbing in mice: evidence against the habituation hypothesis. Behav Neurosci 1999;113:368–76.
Tirelli E, Terry P. Amphetamine-induced conditioned activity and sensitization: the role of habituation to the test context and the involvement of Pavlovian processes. Behav Pharmacol 1998;9:409–19.
Tyhon A, Amantidis A, Foidart A, Grisar T, Lakaye B, Tirelli E. Mice lacking the melanin-concentrating hormone receptor-1 exhibit an atypical psychomotor susceptibility to cocaine and no conditioned cocaine response. Behav Brain Res 2006;173:94–103.
Ujike H, Akiyama K, Otsuki S. D2 nut not D1 dopamine agonists produce augmented behavioral response in rats after sub-chronic treatment with methamphetamine or cocaine. Psychopharmacology 1990;102:459–64.
Vanderschuren LJMJ, Kalivas PW. Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. Psychopharmacology 2000;151:99–120.
Vandershuren LJ, Schoffenlmeer AN, Mulder AH, De Vries TJ. Dopaminergic mechanisms mediating the long term expression of locomotor sensitization following pre-exposure to morphine or amphetamine. Psychopharmacology 1999;143:244–53.
Vezina P. D1 dopamine receptor activation is necessary for the induction of sensitization to amphetamine in the ventral tegmental area. J Neurosci 1996;16:2411–20.
Walters CL, Blendy JA. Different requirements for CAMP response element binding protein in positive and negative reinforcing properties of drugs of abuse. J Neurosci 2001;21:9438–44.
Xu M, Guo Y, Vorhees CV, Zang J. Behavioral responses to cocaine and amphetamine administration in mice lacking the dopamine D1 receptor. Brain Res 2000;852:198–207.
Xu YL, Jackson VR, Civelli O. Orphan G protein-coupled receptors and obesity. Eur J Pharmacol 2004;500:243–53.
Zhou D, Shen Z, Strack AM, Marsh DJ, Shearman LP. Enhanced running wheel activity of both Mch1r- and Pmch-deficient mice. Regul Pept 2005;124:53–63.