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European Journal of Pharmacology 507 (2005) 57–67

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Methamphetamine sensitization in nociceptin receptor knockout mice: locomotor and c-*fos* expression

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Received 21 July 2004; received in revised form 12 November 2004; accepted 16 November 2004
Available online 29 December 2004

Abstract

The role of endogenous nociceptin in the development and expression of sensitization to repeated methamphetamine administration in a novel environment was studied in nociceptin receptor knockout mice. No differences in acute or sensitized locomotor responses were found in nociceptin receptor knockout mice. However, analysis of c-*fos* expression revealed significant interactions between chronic methamphetamine treatment and genotype in the nucleus accumbens and lateral septum. This was due to increased c-*fos* expression in chronically methamphetamine-treated nociceptin receptor knockout mice contrasted with reduced c-*fos* expression in chronically vehicle-treated nociceptin receptor knockout mice. Two further regions (nucleus accumbens core and ventromedial caudate putamen) showed significant interactions between genotype, chronic, and acute methamphetamine treatment due to accentuated c-*fos* expression in nociceptin receptor knockout mice sensitized and challenged with methamphetamine. These findings suggest endogenous nociceptin modulates the response of the central nervous system to repeated psychostimulant administration, although this is little reflected in locomotion.

Keywords: Nociceptin; Orphanin FQ; c-*fos*; Behavioral sensitization; Nociceptin receptor; Orphanin FQ receptor

1. Introduction

Nociceptin (known also as orphanin FQ) is the most recently discovered member of the opioid peptide family (see Mogil and Pasternak, 2001 for review). Although nociceptin shows strong homology with other endogenous opioid peptides, particularly dynorphin, nociceptin selectively binds and activates a distinct receptor (known variably as NOP, ORL1, or OP_4). Activation of the nociceptin receptor has wide-ranging effects including control of learning, anxiety and pain responses, and of particular interest to this laboratory, determining behavioral and neurochemical responses to rewarding drugs (see Koizumi et al., 2004 for references).

Repeated intermittent administration of psychostimulants (i.e., drugs with inherent locomotor activating properties) induces a phenomenon of increasing locomotor response known as behavioral sensitization (Robinson and Becker, 1986). This phenomenon is heavily context-dependent and occurs synchronous to sensitization of the mesolimbic dopaminergic tract (see Robinson and Berridge, 2003 for discussion), giving rise to the suggestion that behavioral sensitization may model some of the processes underlying the increased incentive value of drugs observed in human addicts during repeated consumption (De Vries et al., 1998; Kalivas et al., 1998; Robinson and Berridge, 2003).

At higher doses, nociceptin suppresses locomotion (see Mogil and Pasternak, 2001) by an action that is mediated in part through the mesolimbic tract (Narayanan et al., 2004). Several studies have assessed the effect of exogenous nociceptin administration on the development of behavioral sensitization to the psychostimulant cocaine in rats. These show that a single administration of nociceptin can sensitize locomotor responses to cocaine (Narayanan et al., 2002; Narayanan and Maidment, 1999), whereas when repeatedly coadministered at increasing doses, nociceptin suppresses

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doi:10.1016/j.ejphar.2004.11.034

the development of behavioral sensitization to cocaine (Lutfy and Maidment, 2002). Furthermore, repeated coadministration of nociceptin at increasing doses blocks sensitization of the stereotypic behaviors observed following repeated amphetamine administration (Kotlinska et al., 2003). These studies tentatively suggest that agonists of the nociceptin receptor may be beneficial in the treatment of addictive disorders, particularly as activation of the nociceptin receptor is not particularly aversive in rodents (Ciccocioppo et al., 2000; Devine et al., 1996; Kuzmin et al., 2003; Le Pen et al., 2002; Sakoori and Murphy, 2004).

The purpose of this study was to determine if *endogenous* nociceptin plays a role in determining chronic and acute responses to the psychostimulant methamphetamine, particularly the development and expression of behavioral sensitization. To this end, locomotor responses to repeated methamphetamine administration in a novel environment were compared between wild-type mice and mice heterozygous and homozygous for the nociceptin receptor, i.e., nociceptin receptor knockout mice. Furthermore, the effect of null mutation of the nociceptin receptor on the induction of c-*fos* expression (as a marker of neural activation, Herrera and Robertson, 1996) in response to chronic and acute methamphetamine administration was mapped throughout areas of the brain considered important in mediating the motivating and rewarding properties of abused drugs.

2. Materials and methods

2.1. Subjects

Male wild-type, heterozygous, and nociceptin receptor knockout mice were generated as previously described (Nishi et al., 1997) by mating of heterozygous pairs in a colony maintained in the RIKEN Brain Science Institute animal facility. Mice were housed in cages separated by sex, but amongst same generation siblings of mixed genotypes two to five per cage and maintained in a temperature- and humidity-controlled environment on a 12-h light/dark cycle (lights on 8:00 a.m.) with standard lab chow and water available ad libitum. A total of 84 mice, aged 61 to 96 days on the day of first drug treatment, were used. All experimental protocols used in the study were approved by the RIKEN Brain Science Institute review committee and were in accord with NIH ethics guidelines.

2.2. Drug and reagents

Methamphetamine hydrogen chloride (Dainippon Pharmaceutical, Tokyo) was freshly dissolved (corrected for salt and water content) in 0.9% NaCl vehicle and administered subcutaneously in a volume of 10 ml/kg. Unless otherwise stated, reagents were purchased from Wako (Osaka, Japan), Nacalai Tesque (Kyoto, Japan), or Sigma (Tokyo, Japan).

2.3. Sensitization procedure

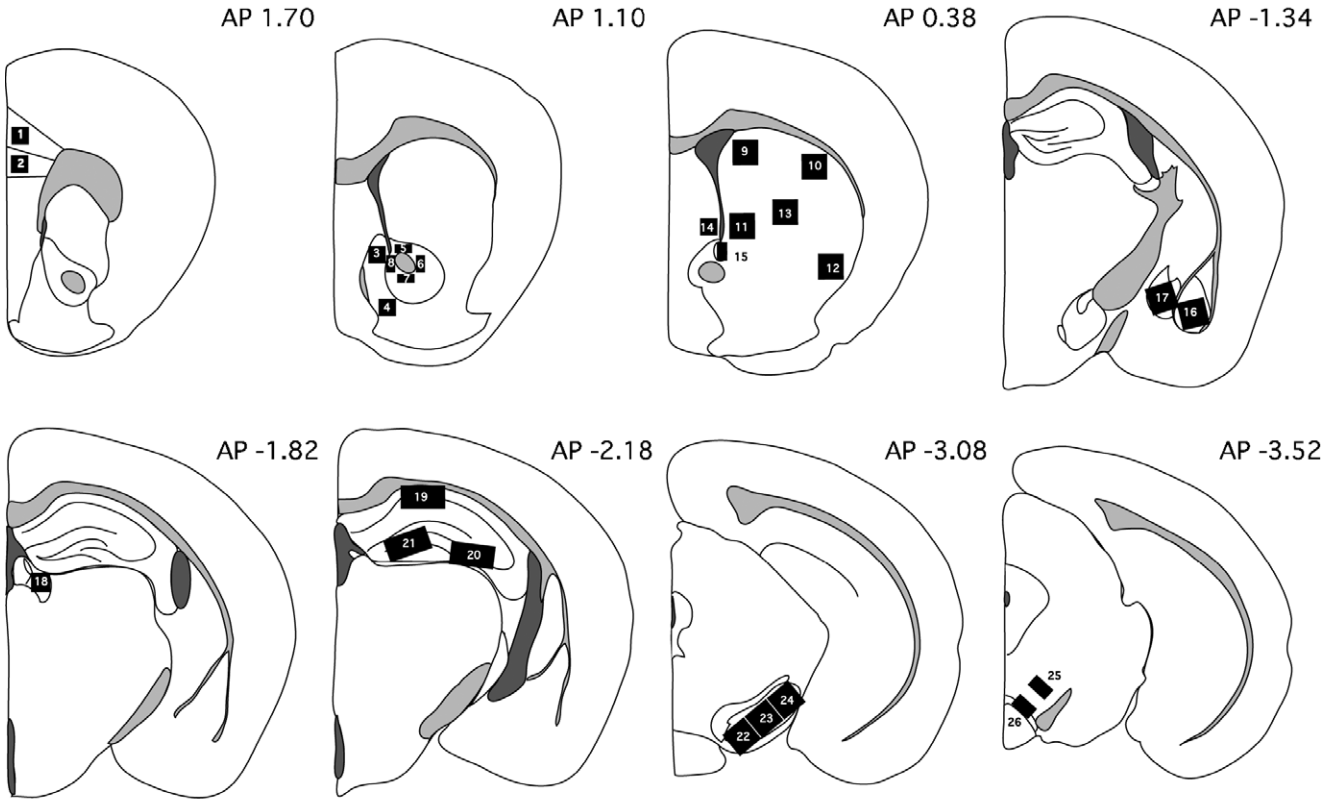
Animals were randomly assigned to four groups of seven or eight subjects. The sensitization protocol consisted of three "treatment" sessions on alternate days (day1, 3, and 5), followed by a drug "challenge" (day18) 13 days after the final treatment session. Animals remained in their home cages between locomotor monitoring sessions in their original groupings. Time of day and location of the test chamber were randomized within and between treatment groups. Prior to all locomotor monitoring sessions, animals were removed from their home cages, weighed, and then placed in one of six clear Plexiglas cages (18-cm diameter×21-cm height) containing wood chip bedding (Beta-Chips, Northeastern Products Caspian, MI). Each cage was located in a locomotor monitor (Coulbourn Instruments, Allentown, PA) consisting of an evenly spaced 16×16 array of infrared beams (covering an area of 25×25-cm) that automatically recorded horizontal locomotion to a personal computer. Activity monitors were enclosed in semitransparent (smoked gray) sound attenuating covered plastic chambers (25×25×38.5-cm). Following a 30-min period for determination of basal locomotor activity, each animal received subcutaneous drug injections of vehicle or methamphetamine (1 mg/kg), and monitored for a further 90 min. On the challenge day, each group was split into two further groups that received either a vehicle administration or methamphetamine (0.5 mg/kg) challenge. Following the 120-min locomotor monitoring period of the challenge day, i.e., 90 min after drug administration, animals were immediately sacrificed for simultaneous immunohistochemical analysis of Fos and tyrosine hydroxylase-immunoreactivity as previously described (Okabe and Murphy, 2004).

2.4. Quantification of c-fos expression and photography

Twenty-six regions of the brain (Fig. 1) were selected for quantification of c-*fos* expression by manual counting of Fos-immunoreactive profiles using a grid superimposed over the region of interest in both hemispheres by an observer blind to the experimental treatment. Counting was performed between 10× and 40× magnification (with 10× eyepieces) depending on the region of interest. Values from each hemisphere were averaged before being averaged for treatment/genotype group. Photographs were taken using a 3.3 megapixel digital camera (Olympus Optical, Tokyo) and were manipulated in no way prior to presentation.

2.5. Statistical analysis

Locomotor activity data prior to splitting groups at the time of drug administration on the challenge day was pooled for statistical analysis. Intersession basal locomotor activity (i.e., 30 min predrug administration period) was analyzed by univariate repeated measures analysis of variance (ANOVA, main factors: treatment and genotype) of recordings made



			mm²				mm²
1	Cingulate cortex area 1	Cg1	0.040	14	Lateral septal nucleus ventral	LSV	0.040
2	Prelimbic cortex	PrL	0.040	15	Bed nucleus of stria terminalis lateral dorsal	BSTLD	0.025
3	Nucleus accumbens dorsal shell	AcbDS	0.040	16	Central amygdaloid nucleus	CeA	0.090
4	Nucleus accumbens ventral shell	AcbVS	0.040	17	Basolateral amygdaloid nucleus	BLA	0.090
5	Nucleus accumbens dorsal core	AcbDC	0.020	18	Lateral habenular nucleus	LHb	0.040
6	Nucleus accumbens lateral core	AcbLC	0.020	19	Hippocampus CA1	CA1	0.150
7	Nucleus accumbens ventral core	AcbVC	0.020	20	Hippocampus CA3	CA3	0.150
8	Nucleus accumbens medial core	AcbMC	0.020	21	Dentate gyrus	DG	0.150
9	Caudate putamen dorsomedial	CPuDM	0.090	22	Substantia nigra reticular part medial	SNRM	0.090
10	Caudate putamen dorsolateral	CPuDL	0.090	23	Substantia nigra reticular part central	SNRCe	0.090
11	Caudate putamen ventromedial	CPuVM	0.090	24	Substantia nigra reticular part lateral	SNRL	0.090
12	Caudate putamen ventrolateral	CPuVL	0.090	25	Ventral tegmental area dorsal	VTAD	0.031
13	Caudate putamen central	CPuCe	0.090	26	Ventral tegmental area paranigral nucleus	VTAPN	0.031

Fig. 1. Schematic representations of coronal sections illustrating areas analyzed (shown as solid boxes) for quantification of Fos-immunoreactivity and abbreviations used in this study. Anterior–posterior (AP) coordinates (according to the atlas of Franklin and Paxinos, 1997) in millimeters are shown upper right to each section. Notes: (1) Global nucleus accumbens core score was calculated by summing areas 5, 6, 7, and 8. (2) Fos-immunoreactivity in CA1 and CA3 hippocampal areas was quantified in the pyramidal cell layer only. Fos-immunoreactivity in the dentate gyrus was quantified in the granule cell layer only. (3) Fos-immunoreactivity in the ventral tegmental area and substantia nigra compacta represents that colocalized in cells also immunoreactive for tyrosine hydroxylase.

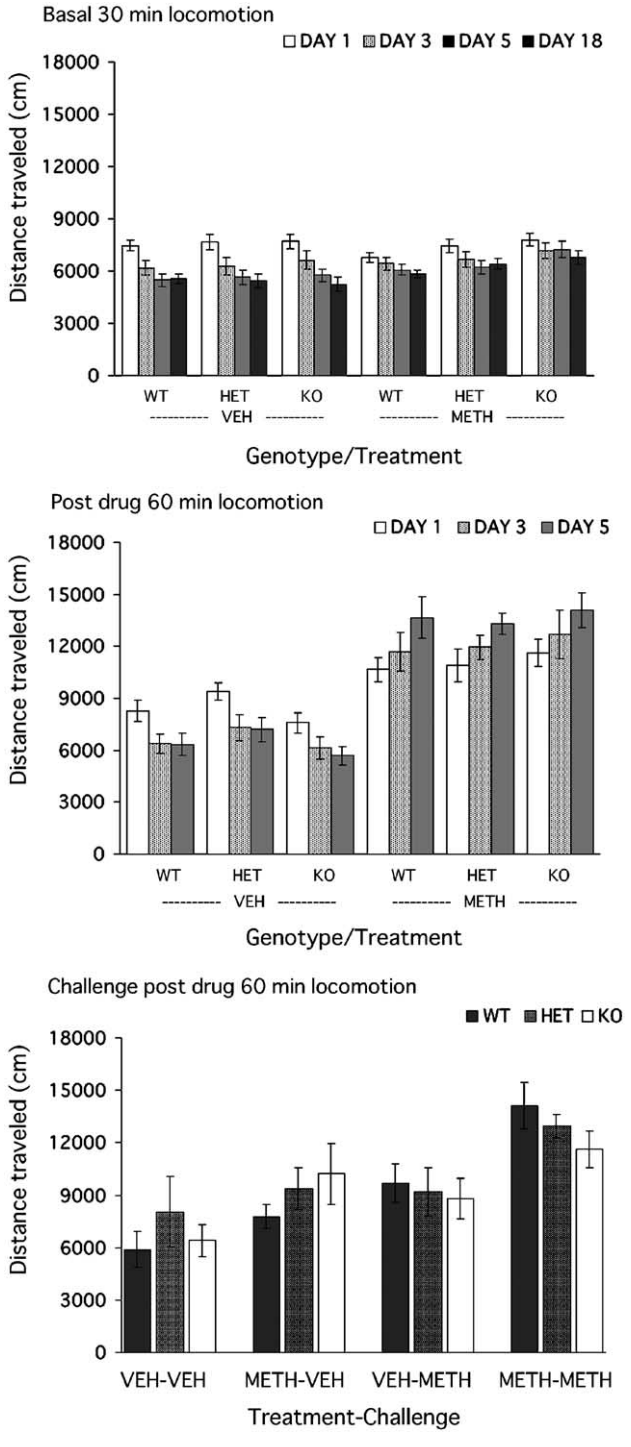
on days 1, 3, 5, and 18. The 60-min postdrug administration period was selected for analysis as it was during this period that most changes in locomotor activity were observed. Intersession drug-stimulated locomotor activity (i.e., the 60-min postdrug administration period) was analyzed by univariate repeated measures ANOVA (main factors: treatment and genotype) of recordings made on days 1, 3, and 5. Within-session drug-stimulated locomotor activity (i.e., 60 min postdrug period, day 18) and c-*fos* expression on the challenge day was analyzed by three-way ANOVA (main factors: treatment, challenge, and genotype). All results are expressed as the mean ± standard error of the mean (S.E.M.). ANOVA was performed using SuperANOVA software (Abacus Concepts, Berkeley, CA). Statistical significance was taken at P values less than 0.05.

3. Results

3.1. Effect of null mutation of the nociceptin receptor gene on the locomotor response to single and repeated methamphetamine administration

Locomotor activity data from four mice were lost due to a computer malfunction, leaving group sizes of six to eight mice. Comparing basal locomotion over the four locomotor

recording sessions showed no significant main effect of genotype or interaction of genotype with time. There was no significant main effect of methamphetamine treatment, although a significant effect of time ($F3,234=54.814$, $p<0.0001$) and interaction of time and methamphetamine treatment ($F3,234=10.533$, $p<0.0001$) were found reflecting a generalized decrease in total basal locomotion (due to habituation) as mice were repeatedly tested, and an ability of methamphetamine treatment to counteract the decrease (Fig. 2, upper panel).



Comparing drug-stimulated locomotion over the three treatment sessions revealed a significant main effect of methamphetamine treatment ($F1,78=78.918$, $p<0.0001$), time ($F2,156=3.971$, p=0.0208), and interaction between methamphetamine treatment and time ($F2,156=46.373$, $p<0.0001$) reflecting a generalized ability of methamphetamine to induce increases in locomotor activity that strengthened over repeated administration, whereas animals treated with vehicle showed declining levels of locomotion during repeated treatment (Fig. 2, middle panel). This was confirmed by post hoc contrast analysis, which revealed significantly ($F=57.857$, $p<0.0001$) higher locomotion during the third methamphetamine treatment session compared to the first, whereas significantly ($F=34.755$, $p<0.0001$) lower locomotion was observed in the postinjection period of mice treated with vehicle during the third treatment session compared with the same mice during the first treatment session. Thus, locomotor sensitization to methamphetamine occurred during the treatment sessions. However, no main effect of genotype or interaction of genotype with any other factor on postdrug locomotion was observed during the drug treatment sessions, indicating that null mutation of the nociceptin receptor gene did not influence the development of behavioral sensitization to methamphetamine.

Multifactorial analysis of postdrug locomotion on the challenge day continued to show no significant main effect of genotype, or significant interaction of genotype with either methamphetamine treatment or methamphetamine challenge (Fig. 2, lower panel). Significant main effects of methamphetamine treatment ($F1,72=20.162$, $p<0.0001$) and methamphetamine challenge ($F1,72=21.681$, $p<0.0001$) were evident due in both cases to increased levels of locomotion in mice that had been either chronically treated or challenged with methamphetamine. No significant interaction between methamphetamine treatment and methamphetamine challenge was observed due to a generally additive effect of the effects of treatment and challenge on locomotor activity in the postdrug period. Nonetheless, post hoc contrast analysis

Fig. 2. Comparison of the development and expression of behavioral sensitization to methamphetamine in a novel environment in wild-type (WT), heterozygous (HET), and nociceptin receptor knockout (KO) mice. Mice were repeatedly treated on alternate days with injections of vehicle (VEH) or methamphetamine (METH, 1 mg/kg) for 5 days (days 1, 3, and 5), during which behavioral sensitization was observed in mice of all three genotypes. On the 18th day, mice were subdivided into 12 new groups for challenge with either vehicle or methamphetamine (0.5 mg/kg). Graphs show total basal horizontal locomotor activity recorded during either the 30-min predrug injection period (upper panel), the 60-min postdrug injection period during treatment sessions (middle panel), and the 60-min postdrug injection period during the challenge session (lower panel). Although statistical analysis found no significant differences between wild-type and nociceptin receptor knockout mice on either acute or sensitized locomotor responses to methamphetamine, it is notable that nociceptin receptor knockout mice tended towards stronger conditioned locomotor activity (i.e., METH-VEH group), and weaker sensitized locomotor activity (METH-METH group) responses compared to wild-type mice on the challenge day. All data are expressed as mean±S.E.M. (upper and middle panels: $n=13$ to 15/group; lower panel: $n=6$ to 8/group).

Table 1
Quantification of Fos-immunoreactive profiles in various regions of the brain 90 min after methamphetamine (METH) challenge in methamphetamine-naïve and methamphetamine-experienced wild-type (WT), heterozygous (HET), and nociceptin receptor knockout (KO) mice

	WT	HET	KO	WT	HET	KO	WT	HET	KO	WT	HET	KO
Pretreat METH	−	−	−	+	+	+	−	−	−	+	+	+
Challenge METH	−	−	−	−	−	−	+	+	+	+	+	+
Cg1	23.2±6.1	26.1±5.5	19.5±5.0	25.0±4.6	28.1±5.2	31.6±4.0	30.6±4.7	28.8±6.0	29.1±8.3	21.9±5.9	27.3±7.5	35.7±10.3
PrL	29.1±6.1	34.4±5.4	29.3±5.2	29.5±2.7	37.1±5.7	34.9±4.5	35.5±3.3	36.2±6.2	24.7±4.3	25.4±4.8	27.7±5.2	39.4±6.6
AcbDsh	32.5±3.3	30.6±2.8	28.4±4.3	31.8±3.4	34.4±3.0	36.2±2.8	43.8±2.0	35.8±4.0	34.6±3.2	25.1±4.3	40.1±5.4	35.8±4.5
AcbVsh	5.1±2.1	5.5±1.7	3.2±1.0	7.1±1.5	6.8±1.4	5.4±1.3	6.8±1.6	5.7±1.6	5.5±1.0	4.9±1.0	6.6±1.5	6.1±1.5
AcbDC	3.6±0.9	4.4±1.1	2.3±0.5	6.9±1.4	5.2±1.1	6.6±1.2	12.4±1.1	9.8±1.4	7.5±1.8	7.7±2.0	12.9±1.8	12.3±2.7
AcbLC	1.6±0.6	2.8±1.3	0.8±0.2	4.1±1.0	3.9±1.1	3.3±0.8	8.1±1.1	6.7±1.0	6.1±1.1	4.9±1.5	6.5±1.6	7.9±1.0
AcbVC	2.8±0.9	3.8±1.4	3.4±1.2	6.8±1.1	6.7±1.4	5.7±1.3	7.6±0.9	7.4±1.6	6.8±1.7	5.9±1.3	11.9±2.5	6.6±1.7
AcbMC	6.4±1.6	6.1±1.1	4.5±1.1	5.9±0.8	7.4±1.2	6.1±1.4	9.9±2.3	9.1±1.7	5.3±1.1	6.6±2.1	10.0±1.7	9.2±2.1
Global core	3.6±0.9	4.3±1.1	2.7±0.7	5.9±0.9	5.8±1.0	5.4±1.0	9.5±0.9	8.3±1.2	6.4±1.2	6.3±2.6	10.3±4.2	9.0±3.7
CPuDM	13.4±2.4	27.4±8.7	14.6±4.0	15.7±2.3	24.3±6.3	20.2±3.4	33.8±6.2	16.8±3.9	25.7±4.6	20.6±6.4	31.5±6.0	32.6±5.7
CPuDL	2.9±1.5	9.6±6.5	7.3±4.0	6.8±2.5	11.1±6.7	7.7±2.8	18.0±6.7	10.4±3.0	12.1±3.9	8.7±3.8	17.4±4.2	16.1±5.9
CPuVM	10.4±3.8	14.2±4.0	11.4±4.8	21.9±5.5	19.8±6.1	17.6±4.1	44.8±8.8	26.0±7.2	23.5±4.3	28.1±8.2	47.1±7.9	33.8±10.5
CPuVL	1.6±0.7	1.6±1.0	1.0±0.4	1.4±0.7	0.9±0.5	1.5±0.7	3.9±0.8	5.6±2.7	2.2±0.7	3.2±1.2	7.3±2.2	6.2±1.3
CPuCe	2.4±0.9	10.1±5.1	2.5±1.1	5.6±1.8	8.1±4.1	4.6±1.7	22.6±4.9	18.9±5.0	10.9±3.6	11.0±4.2	17.5±4.9	21.1±8.1
LSV	49.1±4.4	46.9±5.5	45.4±4.2	39.4±4.8	44.8±5.3	59.8±2.2	54.6±4.8	45.6±4.3	51.7±4.9	34.6±7.2	41.4±4.7	47.9±5.1
BSTLD	7.4±1.6	8.4±2.0	4.6±1.5	4.1±1.3	3.2±1.0	4.9±1.7	50.6±4.9	52.4±7.6	42.9±6.1	8.4±2.7	19.9±3.7	14.2±2.1
CeA	8.5±1.8	10.4±2.8	6.5±1.9	7.9±1.4	6.3±1.2	9.1±2.6	84.0±10.2	85.2±7.9	66.6±6.7	27.6±4.4	38.9±4.6	28.6±5.9
BLA	27.6±3.9	28.4±4.4	26.2±4.6	30.4±1.7	29.6±4.2	34.7±2.4	36.6±4.1	32.9±4.5	29.5±3.2	24.5±4.6	39.3±3.7	35.4±5.0
LHb	6.7±1.9	9.6±2.1	12.2±3.8	9.4±3.5	11.9±3.0	9.0±3.1	9.9±2.5	11.8±3.1	8.5±1.4	10.2±4.1	14.6±3.5	19.4±7.3
CA1	9.7±4.0	11.3±4.6	6.0±2.0	10.9±3.3	12.4±3.2	12.4±2.2	14.9±4.1	11.3±2.5	7.4±2.2	10.0±3.7	19.4±4.2	15.4±5.3
CA3	8.1±1.5	8.4±2.0	5.9±1.4	9.8±1.2	10.3±1.9	12.9±1.4	8.6±1.0	9.9±2.2	8.2±1.6	11.7±2.5	12.6±1.4	12.5±3.1
DG	16.8±3.4	16.6±3.8	14.9±3.5	17.8±2.6	18.7±2.2	21.5±2.1	16.1±1.7	20.1±3.6	14.4±2.3	17.6±3.9	24.4±3.0	25.9±5.0
SNRM	21.9±5.6	18.4±8.6	16.0±5.2	20.3±4.4	21.9±6.2	19.9±4.1	27.9±4.8	20.2±4.6	20.4±4.6	18.1±3.7	23.1±6.1	21.8±5.2
SNRCe	3.9±1.7	3.4±2.0	3.1±1.3	4.3±1.5	7.5±3.6	4.4±1.5	5.7±1.4	5.1±1.0	3.3±0.5	6.6±2.0	7.0±2.4	10.2±5.1
SNRL	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.1	0.3±0.3	0.0±0.0	0.1±0.1	0.1±0.1	0.1±0.1	0.1±0.2	0.5±0.3
VTAD	1.1±0.3	1.9±0.5	1.1±0.5	1.5±0.5	2.3±0.8	1.9±0.4	1.1±0.5	0.2±0.1	1.2±0.3	0.9±0.3	2.5±0.6	2.1±0.6
VTAPN	8.4±3.8	8.2±3.6	5.4±1.8	5.9±2.2	10.2±3.4	10.8±2.2	7.8±2.1	4.0±1.1	6.6±1.5	5.2±1.6	7.1±1.7	8.1±4.0

Quantification of two further regions that showed a statistically significant interaction between all three main factors is shown graphically in Fig. 4. All data are expressed as mean±S.E.M. (n=7 to 8/group). See Fig. 1 for abbreviation definitions.

showed that animals treated with methamphetamine over the three treatment sessions showed significantly (F=15.961, p=0.0002) higher levels of locomotion following methamphetamine challenge than those that had received vehicle administrations over the first three locomotor test sessions (Fig. 2, lower panel). No significant genotype-dependent differences in locomotion were found on the challenge day, although it is notable that nociceptin receptor knockout mice tended towards stronger methamphetamine conditioned locomotor activity on the challenge day, and weaker methamphetamine-sensitized locomotor activity compared to wild-type mice (Fig. 2, lower panel).

3.2. Effect of null mutation of the nociceptin receptor gene on c-fos expression after single and repeated methamphetamine administration

The results of analysis of c-*fos* expression throughout the brain regions studied are shown in Table 1. A significant main effect of methamphetamine treatment on c-*fos* expression was found in approximately a third of brain regions quantified (Table 2). This effect was observed as a mild up-regulation of c-*fos* expression in most regions, with the exception of the lateral division of the bed nucleus stria terminalis and central nucleus of the amygdala where decreases in c-*fos* expression were observed in mice that had received methamphetamine treatment during the first three locomotor monitoring sessions. Acute challenge with methamphetamine induced significant changes in c-*fos* expression in almost half of the brain regions studied that was in almost all cases observed as increased c-*fos* expression. Amongst the regions of the brain quantified, subcortical areas of the forebrain (putamen and nucleus accumbens) and elements of the extended amygdala showed the largest increases in c-*fos* expression. No significant main effect of genotype on c-*fos* expression was found in any of the 27 regions (including global nucleus accumbens score) studied.

Table 2
Multifactorial analysis of Fos-immunoreactivity in brain regions quantified 90 min after vehicle (VEH) or methamphetamine (METH) challenge to wild-type (WT), heterozygous (HET), or nociceptin receptor knockout (KO) mice either methamphetamine-naïve or methamphetamine-experienced

	Main factors			Two-factor interaction			Three-factor interaction
	Treatment (VEH vs. METH)	Challenge (VEH vs. METH)	Genotype (WT vs. HET vs. KO)	Treatment× challenge	Treatment× genotype	Challenge× genotype	Treatment×challenge× genotype
	F value	F value	F value	F value	F value	F value	F value
Cg1	0.37	0.95	0.43	0.91	1.28	0.29	0.09
PrL	0.09	0.11	0.71	0.59	3.00	0.29	1.50
AcbDsh	0.04	3.18	0.34	4.03[a]	5.46[b]	0.24	1.84
AcbVsh	1.17	0.30	0.72	1.60	0.29	0.50	0.44
AcbDC	5.78[a]	47.86[c]	0.43	1.16	3.46[a]	0.38	3.83[a]
AcbLC	1.53	49.17[c]	0.34	4.96[a]	1.61	0.70	2.22
AcbVC	6.06[a]	12.84[c]	2.09	1.93	1.22	0.94	1.78
AcbMC	0.69	7.51[b]	1.78	0.03	2.67	0.10	0.81
Global core	4.80[a]	35.91[c]	1.48	1.96	2.25	0.26	2.50
CPuDM	0.62	7.21[b]	0.72	0.05	1.82	2.71	2.88
CPuDL	0.25	6.30[a]	0.49	0.07	0.68	0.33	1.43
CPuVM	3.26	26.41[c]	0.91	0.17	1.78	0.31	3.44[a]
CPuVL	1.49	27.40[c]	1.56	1.85	1.48	2.01	0.89
CPuCe	0.00	26.07[c]	1.15	0.20	1.94	0.27	2.27
LSV	2.57	0.36	2.85	3.72	4.93[b]	0.14	0.78
BSTLD	91.51[c]	178.36[c]	1.81	66.29[c]	1.64	1.27	0.85
CeA	79.30[c]	309.76[c]	2.62	74.86[c]	1.45	2.29	0.82
BLA	0.97	2.80	0.56	0.95	2.77	0.71	1.90
LHb	1.94	1.86	1.17	1.18	0.13	0.05	1.79
CA1	3.05	1.94	1.04	0.05	2.02	0.05	1.01
CA3	12.28[c]	1.84	0.17	0.00	1.24	0.10	0.44
DG	6.91[a]	1.44	1.00	0.55	1.86	0.73	0.14
SNRM	0.00	0.57	0.26	0.42	0.98	0.03	0.15
SNRCe	4.37[a]	2.41	0.11	0.29	0.68	0.33	0.88
SNRL	3.54	1.57	2.59	0.01	1.55	0.27	0.20
VTAD	8.22[b]	1.32	1.76	0.57	1.92	0.96	2.14
VTAPN	0.70	1.44	0.14	0.10	1.83	0.49	0.30

Data are shown analyzed by three-way ANOVA. Neuroanatomical regions showing significant interactions between genotype and other main effects are summarized graphically in Figs. 3 and 4. All data are expressed as mean±S.E.M. (n=7 to 8/group). See Fig. 1 for abbreviation definitions.

[a] P<0.05.

[b] P<0.01.

[c] P<0.001.

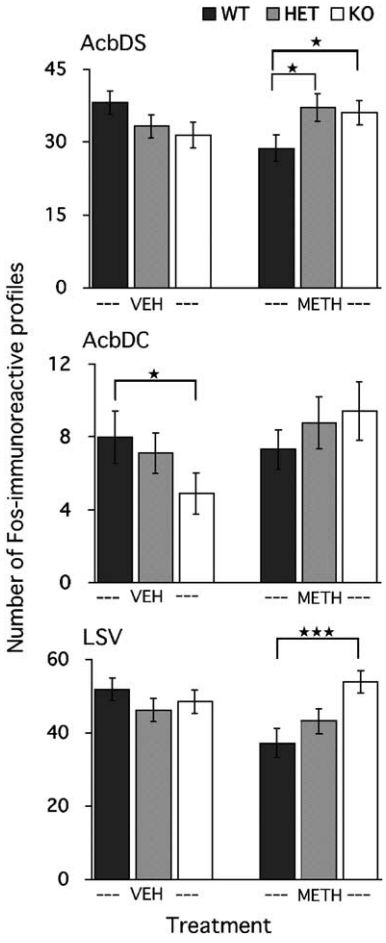


Fig. 3. Graphical summary of Fos-immunoreactivity in neuroanatomical regions showing statistically significant interactions between chronic methamphetamine (METH) treatment and genotype. All data are expressed as mean±S.E.M. (n=14 to 16/group). (★) $p< 0.05$, (★★★) $P< 0.001$. VEH, vehicle; WT, wild-type; HET, heterozygous; KO, nociceptin receptor knockout. See Fig. 1 for additional abbreviation definitions.

Significant interactions between methamphetamine treatment and methamphetamine challenge on c-*fos* expression were found in four of the areas of the brain quantified (Tables 1 and 2), these being most striking in the bed nucleus stria terminalis and central nucleus of the amygdala, where mice treated with methamphetamine during the first three locomotor monitoring sessions showed severely reduced responses to acute methamphetamine challenge compared to those that had received only vehicle during the first three locomotor monitoring sessions. A similar effect, although much milder, was also found in the dorsal region of the nucleus accumbens shell and the lateral area of the nucleus accumbens core, where blunted responses to acute methamphetamine challenge were observed in mice treated with methamphetamine during the first three locomotor monitoring sessions.

A significant interaction between methamphetamine treatment and genotype was found in three regions: the dorsal areas of the nucleus accumbens core and shell and the lateral septum. This was due to an enhanced c-*fos* expression in nociceptin receptor knockout mice treated with methamphetamine during the first three locomotor monitoring sessions contrasted against a reduced c-*fos* expression in nociceptin receptor knockout mice repeatedly treated with vehicle during the same period (Fig. 3). Although a significant interaction was found only in the above three regions, a similar trend was observed in the prelimbic cortex, basolateral amygdala, and dentate gyrus of the hippocampus.

No significant interactions between genotype and acute methamphetamine challenge on c-*fos* expression were observed in any of the brain regions quantified. A significant interaction between methamphetamine treatment, methamphetamine challenge, and genotype on c-*fos* expression was found in two regions: the dorsal area of the nucleus accumbens core and the ventromedial area of the caudate putamen (Figs. 4 and 5). In both of these regions, post hoc contrast analysis showed significantly lower c-*fos* expression in methamphetamine-naïve nociceptin receptor knockout mice challenged with methamphetamine compared to wild-type mice treated in the same manner. This was contrasted against an enhanced c-*fos* expression in methamphetamine-experienced methamphetamine-challenged

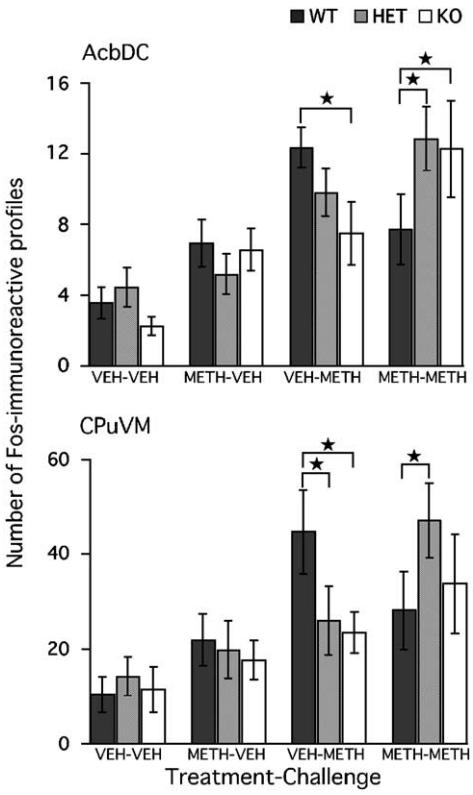


Fig. 4. Graphical summary of Fos-immunoreactivity in neuroanatomical regions showing statistically significant interactions between chronic methamphetamine (METH) treatment, acute methamphetamine challenge, and genotype. All data are expressed as mean±S.E.M. (n=7 to 8/group). (★) $P<0.05$. VEH, vehicle; WT, wild-type; HET, heterozygous; KO, nociceptin receptor knockout. See Fig. 1 for additional abbreviation definitions.

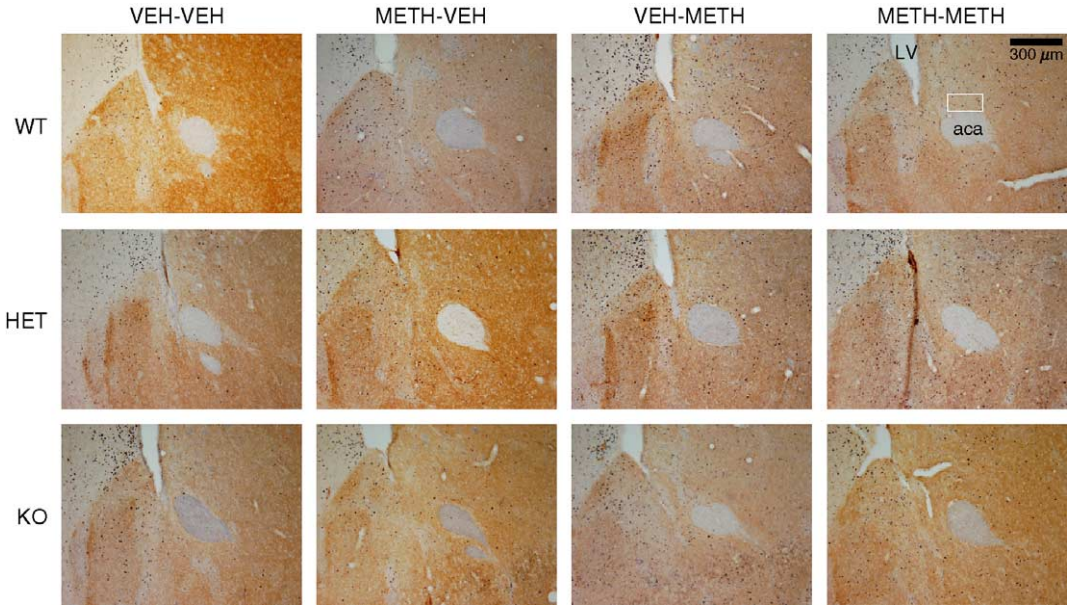


Fig. 5. Representative examples of differential c-*fos* expression dependent on chronic methamphetamine (METH) treatment, acute methamphetamine challenge, and genotype. Representative photomicrographs show Fos (black) and tyrosine hydroxylase (brown) immunoreactivity in the dorsal core region of the nucleus accumbens (photomicrographs correspond to data presented graphically in the upper panel of Fig. 4) following vehicle (VEH) or methamphetamine challenge on the final day (day 18) of the experiment. Outline indicates area used for quantification of Fos-immunoreactivity. LV, lateral ventricle; aca, anterior commissure; WT, wild-type; HET, heterozygous; KO, nociceptin receptor knockout.

nociceptin receptor knockout mice that compared to wild-type mice, showed significantly greater c-*fos* expression in the dorsal region of the nucleus accumbens core.

4. Discussion

Central administration of nociceptin blocks several of the behavioral and neurobiological responses to rewarding drugs. For example, nociceptin blocks both the acquisition and expression of conditioned place preferences to morphine, amphetamine, cocaine, and alcohol (Ciccocioppo et al., 2004; Kotlinska et al., 2003; Kotlinska et al., 2002; Murphy et al., 1999; Sakoori and Murphy, 2004; Zhao et al., 2003) and attenuates responding for ethanol in a self-administration paradigm (Ciccocioppo et al., 2004; Ciccocioppo et al., 1999). These actions may be partially due to a suppressive effect of nociceptin on the activity of the mesolimbic dopamine system (Di Giannuario and Pieretti, 2000; Di Giannuario et al., 1999; Koizumi et al., 2004; Lutfy et al., 2001). Of particular relevance to this study, administration of increasing doses of nociceptin attenuates the development of behavioral sensitization to cocaine (Lutfy et al., 2002), suggesting that nociceptin may influence changes in the incentive value of rewarding drugs observed during repeated administration.

As yet, few studies have attempted to identify a role for endogenous nociceptin in the control of drug seeking and reward. We have recently shown that central administration of the nonpeptide nociceptin receptor antagonist UFP-101 induces a conditioned place preference (Sakoori et al., 2003) indicative of a rewarding experience, presumably by disinhibiting a suppressive effect of endogenous nociceptin on hedonic state. However, any influence of endogenous nociceptin on the maintenance of hedonic balance appears to occur independently of effects on the mesolimbic dopamine system. That is, neither administration of UFP-101, nor knockout of the nociceptin receptor influences mesolimbic activity (Koizumi et al., 2004; Murphy et al., 2002).

In this study, we employed a genetic approach to assess the role of endogenous nociceptin in the response to acute and repeated methamphetamine administration. We found that locomotor responses to methamphetamine were barely altered by knockout of the nociceptin receptor gene. However, c-*fos* expression in several brain regions (dorsal shell of the nucleus accumbens, dorsal core of the nucleus accumbens, lateral septal nuclei) was differentially affected by chronic methamphetamine administration dependent on absence or presence of the nociceptin receptor gene. Specifically, compared to wild-type mice, nociceptin receptor knockout mice tended towards increased c-*fos* expression following repeated methamphetamine administration, but showed decreased expression of c-*fos* following repeated vehicle administration. Two brain regions (dorsal core of the nucleus accumbens, ventromedial caudate putamen) showed a significant difference in acute and sensitized c-*fos* expression in response to methamphetamine dependent on the presence of the nociceptin receptor gene. That is, amongst methamphetamine-naïve mice, nociceptin receptor knockout mice showed reduced c-*fos* expression in response to acute methamphetamine challenge, whereas amongst methamphetamine-experienced (i.e., sensitized) mice, nociceptin receptor

knockout mice showed an enhanced c-*fos* expression in responses in acute methamphetamine challenge in one of these regions.

These findings suggest that endogenous nociceptin participates in determining the response of the central nervous system to chronic methamphetamine administration, but has no major role in determining responses to a single administration of methamphetamine. The general direction of the effects observed in this study agrees with previous studies showing that nociceptin (when repeatedly administered at increasing doses) can attenuate behavioral sensitization to the psychostimulant cocaine (Lutfy et al., 2002). Null mutation of the nociceptin receptor ought to remove the influence of endogenous nociceptin, which given the generally suppressive nature of nociceptin, would be anticipated to enhance c-*fos* expression in response to the stimulatory effects of methamphetamine. Indeed, this was the case in the current study, supporting a role for endogenous nociceptin in the response to chronic methamphetamine administration. However, this effect was surprisingly mild and restricted to a small number of brain regions, despite the wide distribution of nociceptin and the nociceptin receptor (Houtani et al., 2000; Neal et al., 2001; Neal et al., 1999). Furthermore, this effect was not reflected in behavioral responses. It is interesting to note that the strongest effect of null mutation of the nociceptin receptor gene on the response to methamphetamine was found outside of brain regions most commonly considered central in mediating the rewarding and conditioned effects of abused drugs (e.g., cortical and extended amygdaloid regions). That is, the lateral septum showed the most statistically significant alteration in genotype-dependent response to chronic methamphetamine treatment. Indeed, this region narrowly failed to show a statistically significant main effect of genotype alone. The lateral septal nuclei show some of the highest levels of nociceptin receptor expression in the brain, particularly when considering expression of reporter genes (Houtani et al., 2000). Our previous studies using the nociceptin receptor antagonist J-113397 (known also as Compound B) also suggest a strong influence of endogenous nociceptin in this region (Okabe and Murphy, 2004), which may underlie the altered anxiety profile of nociceptin receptor knockout mice that has been previously reported (Millan, 2003, see below).

Previous studies show that both exogenous and endogenous nociceptin are generally inhibitory towards memory processes (Higgins et al., 2002; Manabe et al., 1998; Redrobe et al., 2000; Sandin et al., 1997; Sandin et al., 2004). The strong context sensitivity of behavioral sensitization (see De Vries et al., 1998; Kalivas et al., 1998; Robinson and Berridge, 2003) implies the strengthening of stimulus–stimulus links that reflect the creation of associative memories (Anagnostaras et al., 2002). The generally up-regulated expression of c-*fos* observed in nociceptin receptor knockout mice repeatedly treated with methamphetamine in this study could reflect a form of enhanced mnemonic ability when mice are returned to the drug-associated environment. Exogenous and endogenous nociceptin have also been found to modulate anxiety-like responses, although it remains to be resolved if nociceptin is anxiogenic, anxiolytic, or both, as current evidence suggests all three possibilities (Blakley et al., 2004; Ciccocioppo et al., 2002; Fernandez et al., 2004; Griebel et al., 1999; Jenck et al., 1997; Jenck et al., 2000; Koster et al., 1999; Ouagazzal et al., 2003). An interaction between elimination of endogenous nociceptin tone and the expression of c-*fos* in the brains of mice repeatedly treated with vehicle was found in this study. That is, nociceptin receptor knockout mice repeatedly treated with vehicle showed reduced c-*fos* expression in several regions of the brain compared to wild-type mice. Further study will be necessary to determine if this difference in neural reactivity is due to differences in reactivity to repeated handling, or simply reflects a lower level of neural activity in nociceptin receptor knockout mice.

In considering the results of this study, it is important to bear in mind possible developmental adaptations that may occur in the absence of the nociceptin receptor gene. Such adaptations may mask the contribution of the endogenous nociceptin system to the response of the central nervous system to methamphetamine. Furthermore, despite an overlapping mechanism of action (i.e., their ability to stimulate central release of biogenic amines), methamphetamine and cocaine responses may differ in their regulation by exogenous or endogenous nociceptin (see above), and thus comparison with previous studies (Lutfy et al., 2002; Narayanan et al., 2002; Narayanan and Maidment, 1999) must be made with care. However, taken at face value, these data suggest that endogenous nociceptin plays only a mild and highly selective role in determining neural responses to methamphetamine administration.

In summary, this study suggests that the endogenous nociceptin system plays little role in determining locomotor responses to acute or repeated methamphetamine administration, but contributes to the response of select areas of the central nervous system.

Acknowledgments

The authors thank Ms. Naoko Midorikawa for technical assistance and Ms. Kiyoko Yamada for secretarial assistance.

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