Available online at www.sciencedirect.com

SCIENCE DIRECT

Pharmacology, Biochemistry and Behavior 81 (2005) 530 – 534

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Naloxone increases ketamine-induced hyperactivity in the open field in female rats

Christopher Wilson*, Kelly Cone, Melanie Kercher, Jessica Hibbitts, Jennifer Fischer, Amber Van Lake, Jean Sumner

Department of Psychology, Sam Houston State University, Huntsville, TX 77341-2447, United States

Received 8 November 2004; received in revised form 9 March 2005; accepted 24 March 2005
Available online 3 June 2005

Abstract

In considering possible effects of ketamine, and its interactions with drugs that might be administered following ketamine abuse, researchers investigated the effects of administering combinations of saline, naloxone, and/or ketamine on open-field activity levels and frequencies of turning, reverse locomotion, head weaving, and rearing in 50-day-old rats. Female subjects having received ketamine combined with saline showed significant increases in open-field activity as compared with male subjects and subjects having received combinations of saline and/or naloxone. When combined with ketamine, naloxone caused an increase in the aforementioned ketamine-induced hyperactivity in female rats. In addition, ketamine caused increases in turning in female rats and increases in reverse locomotion and head weaving in male and female rats. Decreases in rearing were reported in both males and females with administration of ketamine. In general, naloxone had little, if any, effect on these behaviors. Thus, the primary effect of administration of naloxone reported in this study is an exacerbation of ketamine-induced open-field hyperactivity. The authors discuss the results with respect to indirect effects of ketamine on endogenous opiate and dopamine systems on the aforementioned behaviors. The researchers also consider sex differences and possible precautions when dealing with individuals having ingested psychedelic anesthetics.
© 2005 Elsevier Inc. All rights reserved.

Keywords: Ketamine; Naloxone; Hyperactivity; Rats

1. Introduction

Administration of subanesthetic doses of dissociative anesthetics such as ketamine and phencyclidine can result in behavioral abnormalities including psychotic episodes, mood alterations, cognitive disturbances, and hallucinations in humans (Jentsch and Roth, 1999; Vollenweider et al., 2000). These agents can produce hyperactivity, stereotypy, and ataxia in many mammalian models (Giuliani and Farrari, 1997).

Even though the primary mechanism of ketamine is to block *N*-methyl-D-aspartate (NMDA) receptors (Feldman et al., 1997), many of the behaviors, including hyperactivity, reported with low doses of this drug appear to depend on changes in dopamine (DA) activity (French and Ceci, 1990; White and Ryan, 1996; Yamamoto et al., 1997). In addition, there may be interactions between NMDA receptor blockers and endogenous opiate systems. Winters et al. (1998) reported the development of cross-tolerance and potentiation between ketamine and morphine with respect to tailflick latencies in rats. Administration of naloxone can suppress the antinociceptive effects of ketamine (Baumeister and Advokat, 1991). Forman (1999) also reported increases in reaction time in rats in the hot-plate test after administration of the NMDA antagonist MK-801, an effect that was partially reversed with administration of the opiate antagonist naloxone. Given these observations, it is logical that the interactive

* Corresponding author. Tel.: +1 936 294 3052; fax: +1 936 294 3798.
E-mail address: Wilson@shsu.edu (C. Wilson).

0091-3057/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2005.03.018

effect of morphine and ketamine is due to the latter drug's ability to induce the release of endogenous opiates, thus reducing the need for exogenous opiates.

Just as with administering subanesthetic doses of ketamine can lead to hyperactivity, so too can blocking opiate receptors. Gold and Pottash (1981) reported that administration of naloxone, causing acute opiate withdrawal in rats, resulted in increments in activity, an effect occurring only in animals that are, at the time, under the influence of opiates (Grant et al., 1988) and perhaps dependent upon noradrenergic (NA) systems (Gold, 1993; Roth et al., 1982). Additionally, naloxone administration can facilitate spinal reflexes in humans (Boureau et al., 1978) and can result in agitation and combativeness in individuals addicted to opiates (Stiegler, 2003). Interestingly, hospital protocols for dealing with drug overdose of "unknown etiology" often are to administer naloxone (Clinical Pathway for "Unconscious—Etiology Unknown"; Miller, 2004). If it is the case that opiate withdrawal leads to hyperreactivity and the analgesic effects of ketamine are a function of endogenous opiates, then administering naloxone, in combination with ketamine, may result in an additive effect on activity, with naloxone administration producing increments in behavior above that of ketamine administration alone. Based upon this rationale, it was hypothesized that combining ketamine and naloxone would result in greater increments in activity in rats than would administering either drug alone.

As altricial animals mature, the behavioral sensitivity to catecholaminergic (CA) agents apparently changes. For example, Spear and Brake (1983) suggested that periadolescent rats are hyposensitive to CA agonists, compared to either younger or older animals. In addition, several authors have reported a subsensitivity to the behavioral effects of cocaine (Laviola et al., 1995), amphetamine (Bolanos et al., 1998), and quinpirole (Frantz and Van Hartesveldt, 1999) in periadolescent rats when compared to rats at other ages. Given that cocaine functions primarily to block NA reuptake (Carmichael and Israel, 1973), it may well be that an age-related differential sensitivity to NA-agonists, along with DA, exists in rats. Interestingly, both Laviola et al. (1995) and Frantz and Van Hartesveldt (1999) reported gender differences with respect to sensitivity to DA agonists, with female rats showing greater responsiveness than males to these agents. These reports led the authors to hypothesize further that administration of ketamine, with or without naloxone, would result in more pronounced changes in behaviors in female rats than in male rats.

To test these hypotheses, 50-day-old animals were administered combinations of concentrations of naloxone and ketamine and placed in an open-field apparatus. In addition to activity levels, frequencies of rearing, turning, reverse locomotion, and head weaving were recorded. The authors chose to use 50-day-old rats, animals toward the end of periadolescence, because animals at this age might better reflect the age range of humans who have an increased probability of ketamine abuse and possible drug overdose.

2. Materials and method

2.1. Subjects

Subjects in this experiment were 80 (10 litters) Sprague–Dawley albino rats, 50 days of age at the time of testing. Subjects were derived from an established breeding colony at Sam Houston State University and were housed in Plexiglas breeding cages on a 12 L:12 D schedule with lights on at 07:00 h. Food and water were available ad lib. All testing occurred between 10:00 h and 14:00 h (light phase) under normal ambient light conditions.

2.2. Materials

Ketamine was purchased from NLS Animal Health, Baltimore, MD, naloxone was purchased from Sigma Chemical Co., St. Louis, MO, with doses based upon prior research (Dafny and Rigor, 1978; Wilson et al., 1984). The open-field apparatus used in this experiment was an Open-Field "Prime" system, Model 80093, purchased from Lafayette Instrument Co., Lafayette, IN. The Plexiglas floor measured 40×44 cm with photo beams positioned 3.5 cm above the surface.

2.3. Procedure

The procedures used in the present study were in compliance with the guidelines set forth by the National Institutes of Health Guide for Care and Use of Laboratory Animals.

Prior to parturition, pregnant female rats were placed in breeding cages containing wood chips as bedding. Cages were checked daily at 08:00 h and 16:00 h for the presence of newborn litters and the day a litter was first detected was considered postnatal day (PND) 0. On PND 1, litters were culled to 8–10 pups, and on PND 21, subjects were weaned by removing the mothers, but continued to be housed in groups in the breeding cages. To avoid the possibility of overcrowding and pregnancy, litters were separated into groups of males and females at PND 30. On the day of testing, PND 50, subjects were removed from their home cages, placed in cages containing fresh bedding, weighed, marked with a felt-tipped pen, and randomly assigned to various sex-drug dosage groups ($n = 10$). Five minutes prior to testing, each subject was sexed and was given an intraperitoneal (IP) administration of either saline or naloxone (10 mg/kg/ml). Immediately prior to testing, each subject was given an IP administration of either saline or ketamine (10 mg/kg/ml),

thus yielding eight groups: male-saline-saline, male-naloxone-saline, male-saline-ketamine, male-naloxone-ketamine, female-saline-saline, female-naloxone-saline, female-saline-ketamine, and female-naloxone-ketamine. The reason that rats in this experiment were pretreated with naloxone 5 min prior to testing was to ensure that the drug was biologically active, being within the range of drug onset and cessation of activity (see Gilman et al., 1980).

Immediately following the second administration, i.e., ketamine/saline, each subject was placed singly into the open-field apparatus. The numbers of both central and perimeter photo beams broken were automatically recorded for a total testing time of 10 min. Also, during the testing phase, two independent observers, seated approximately 2.0 m from the apparatus, recorded the number of times the rat made a 360° turn, the number of times it reared, the number of bouts of reverse locomotion, and the number of head weaves. For these measures, the means of the two observers were used for statistical analyses. The study was run blind with the observers having no prior knowledge as to which drug condition any particular animal had been assigned.

2.4. Data analysis

Because of a violation of the assumption of homogeneity of variance with respect to the open-field activity data, Gupta's (1988) non-parametric procedures were used to analyze these data. Alpha was set at 0.05.

Due to the violation of the assumption of homogeneity of variance and the inordinately high number of tied ranks with respect to data for the individual behaviors, Kruskal–Wallis non-parametric procedures were used to determine overall differences among groups in this study. Differences between individual pairs of groups on these behaviors were assessed using Ryan's Procedure (Linton and Gallo, 1975).

3. Results

3.1. Open-field activity

Although the data were analyzed using non-parametric procedures, for ease of comparison with results from other experiments, the data for open-field activity levels are presented, in Fig. 1, using conventional parametric statistics. Analysis of these data yielded a significant sex effect, $H(1)=30.17$, $P<0.05$, and a significant sex × ketamine interaction effect, $H(1)=13.94$, $P<0.05$. In addition, the ketamine × naloxone × sex triple interaction effect was statistically significant, $H(1)=66.15$, $P<0.05$. No other effects were statistically significant. The data show that ketamine increased open-field activity in females but not males and the combination of ketamine and naloxone produced an increment in activity in females over that produced by ketamine alone.

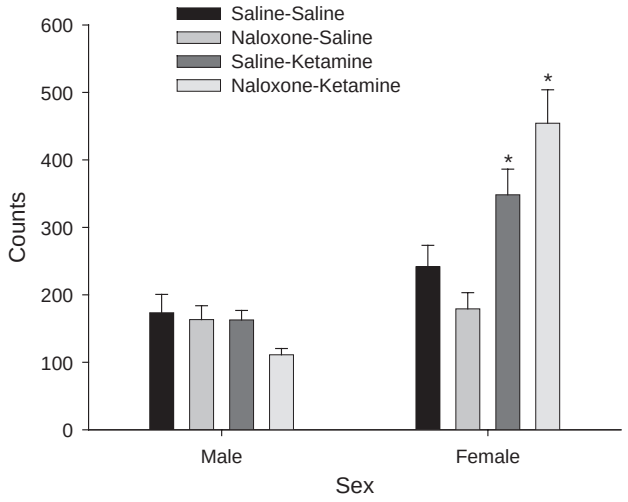

Fig. 1. Mean counts over a 10-min open-field test session for male and female rats having received combinations of ketamine, naloxone, and saline (*denotes significantly different from all other groups).

3.2. Turning, reverse locomotion, head weaving, rearing

Data for the four specific behaviors measured in this experiment are presented in Table 1. Again, for ease of comparison with other studies, data are presented using conventional parametric statistics.

A Kruskal–Wallis test of turning behavior yielded a significant effect, $\text{Chi2}(7)=51.52$, $P<0.05$. Post hoc analyses showed that females under the influence of ketamine combined with saline or naloxone had more bouts of turning than subjects in other groups.

An analysis of the data for reverse locomotion yielded a significant effect, $\text{Chi2}(7)=60.73$, $P<0.05$. Post hoc analyses showed that females that had received ketamine, in combination with saline or naloxone, had a significantly greater number of bouts of reverse locomotion than subjects in all other groups. Males having received ketamine combined with saline demonstrated an intermediate level of reverse locomotion, showing significantly lower levels than females with ketamine, with or without naloxone, but significantly higher levels than all other groups.

The analysis of the data for head weaving yielded a significant effect, $\text{Chi2}(7)=62.47$, $P<0.05$. Females having received ketamine, with or without naloxone, had greater numbers of bouts of head weaving than subjects in all other groups. As with reverse locomotion, males having received ketamine in combination with saline showed lower levels of head weaving than females with ketamine, but higher levels than all other groups.

A Kruskal–Wallis test on rearing data yielded a significant difference among groups, $\text{Chi2}(7)=27.12$, $P<0.05$. Post hoc analyses showed that subjects having

Table 1
Mean frequencies (+S.E.M.) over a 10-min test session for turning, reverse locomotion, head weaving and rearing for male and female rats having received combinations of ketamine, naloxone, and saline

		Sal-Sal	Nal-Sal	Sal-Ket	Nal-Ket
Turning	Male	0.0 (0.00)	0.0 (0.00)	0.3 (0.30)	0.0 (0.00)
	Female	0.0 (0.00)	0.0 (0.00)	2.9 (1.49)*	6.7 (1.59)*
RevLoc	Male	0.0 (0.00)	0.0 (0.00)	1.0 (0.33)*,**	0.4 (0.22)
	Female	0.0 (0.00)	0.0 (0.00)	9.1 (1.91)*	11.8 (2.68)*
Hweave	Male	0.2 (0.20)	0.0 (0.00)	4.3 (1.60)*	1.1 (0.55)
	Female	0.1 (0.10)	0.0 (0.00)	29.4 (6.20)*,**	35.6 (4.31)*,**
Rearing	Male	10.2 (2.41)	9.6 (1.67)	4.3 (0.75)*	5.3 (1.15)*
	Female	17.2 (3.80)	12.7 (2.74)	7.4 (3.09)*	1.4 (0.45)*

*indicates significantly different from other groups but not from each other; **indicates significantly different from male-saline-ketamine group on this behavior.

received ketamine, with or without naloxone, had fewer rears than subjects in all other groups.

4. Discussion

We investigated the effects of naloxone on ketamine-induced behaviors in rats. With respect to open-field activity, ketamine, when given in combination with saline resulted in hyperactivity in female, but not male, rats. Administration of naloxone exacerbated this ketamine-induced increase in behavior; however naloxone, when given in combination with saline, did not affect open-field activity in either male or female animals.

Administration of ketamine along with saline caused increments in the frequency of turning in female, but not male, rats. Administration of naloxone with ketamine, though, did not result in any further increase in the behavior. Ketamine also increased reverse locomotion in female rats with no added effect of naloxone, and increased, albeit, in a much lower fashion, this behavior in male rats. Again, naloxone had no effect on the females and slightly lowered the response in males. Frequencies of head weaving also increased in males and females following ketamine administration, with naloxone having no incremental effect in female rats and actually decreasing the frequency in male rats. Finally, administration of ketamine decreased rearing, regardless of sex, with no effect of administration of naloxone.

With respect to the hypothesized interaction between naloxone and ketamine, it appears that the primary interaction was in open-field activity in female animals. The initial effect of ketamine, with saline, may be due to an increase in DA activity, as ketamine has been shown to stimulate this system (French and Ceci, 1990; White and Ryan, 1996; Yamamoto et al., 1997). The added effect of combining naloxone with ketamine may be due to an increase in NA activity (Gold, 1993; Roth et al., 1982). Female rats may be more sensitive to DA agonists than male rats (Frantz and Van Hartesveldt, 1999; Laviola et al., 1995) which may account for the sex difference with ketamine alone. The combination of ketamine and naloxone was ineffective in changing levels of open-field activity in male rats, perhaps because of a relative hyposensitivity to CA agents in male animals (Spear and Brake, 1983). Regardless, females were much more sensitive to the manipulations employed in this study than were the males, at least with respect to open-field activity.

The presence, or absence, of female hormones might also have contributed to the sex difference in open-field activity in this study. Frantz and Van Hartesveldt (1999) studied the locomotor effects of quinpirole, a DA agonist, on age and gender in rats and reported that females generally are more responsive than males to that drug; i.e., females were more prone to locomotor activation by a mid-range dose of quinpirole than males of the same age. The females were "also less susceptible to locomotor depression by a low dose of quinpirole" than were the males (Frantz and Van Hartesveldt, 1999, p. 824). These authors proposed that the differences may be related to drug metabolism and that the higher variability in activity shown by female rats may be caused by variations in the estrous cycle.

Changes in the estrous cycle have been linked with variations in responsiveness to amine-related agents. Ovariectomized female rats with estrogen implants show much greater levels of cocaine- and MDMA-induced hyperactivity than do ovariectomized rats without implants (Zhou et al., 2003). Sell et al. (2000) reported similar results but also noted increments in cocaine-induced activity in normally cycling rats during proestrus and estrus, compared to diestrus. Thus, levels of estrogen may play a key role in CA-related hyperactivity. A related possibility is that levels of other gonadal hormones, e.g., follicle-stimulating hormone or luteinizing hormone, which vary during the estral cycle, may be critical to the aforementioned effects. If the mechanisms involved in ketamine-induced hyperactivity are similar to those implicated in cocaine- and MDMA-induced hyperactivity, then perhaps endogenous hormone levels are key to the changes in activity reported in the current study. This possibly could explain why females were affected by the ketamine and males were not.

The addition of naloxone to ketamine was remarkable in its ineffectiveness in changing the four behavioral measures recorded in this experiment. With the exception of turning in males, ketamine did increase three of the four behaviors, turning, reverse locomotion, and head weaving, measured and decreased one, rearing. Naloxone, basically, did nothing to these frequencies in the females, and in fact, lowered the frequencies of two of the behaviors, reverse locomotion and head weaving, in the males. The ketamine-induced increases in males, in general, was not as great as those in females, typically producing intermediate levels between those of the females and the control animals. A possible mechanism for these results is the relative insensitivity of males to DA input, or the relative hypersensitivity of females to DA input. Regardless, looking at interactions, naloxone basically did very little, perhaps

indicating that these behaviors are primarily under control of DA systems, with little input from systems evoked by blocking endogenous opiates.

The reductions in rearing with ketamine may be a function of ataxia induced by the drug (Giuliani and Farrari, 1997). Thus, this effect could be the result of a lack of balance or coordination and not the direct effect of the drug itself. Consistent with this assumption, Silveri and Spear (2002) reported decreases in the onset of righting response and increases in the time to regain this reflex in 26-day-old rats given MK-801 in combination with ethanol as opposed to a combination of saline and ethanol.

In summary, ketamine is effective in altering the activity levels of 50-day-old female, but not male, rats and administration of naloxone, in combination with ketamine, can intensify this effect. The mechanism underlying the increments in open-field activity with the combination of naloxone and ketamine may be a function of an additive effect of both DA and NA systems and that induction of DA systems may be a necessary component for the additional increment in activity seen with naloxone. As for the particular behaviors measured in this experiment, in general, the interaction between naloxone and ketamine was, at best, suspect and the authors consider differences in those behaviors with combinations of those drugs as less than compelling.

As a final note, the protocol in many emergency rooms in dealing with drug overdose of "unknown etiology" is to administer naloxone (Clinical Pathway for "Unconscious—Etiology Unknown"; Miller, 2004). In this light, although it is highly speculative, if the results reported in this experiment can be generalized to humans, emergency room physicians may inadvertently and unexpectedly induce hyperactivity in individuals, especially females, who have ingested ketamine by administering naloxone. Given this possibility, it might be judicious to check for horizontal and vertical gaze nystagmus, symptoms indicative of psychedelic anesthetic abuse, but not indicative of opiate abuse, prior to administering naloxone.

References

Baumeister A, Advokat C. Evidence for a supraspinal mechanism in the opioid-mediated antinociceptive effect of ketamine. Brain Res 1991;566:351–3.

Bolanos CA, Glatt SJ, Jackson D. Subsensitivity to dopaminergic drugs in periadolescent rats: a behavioral and neurochemical analysis. Dev Brain Res 1998;111:25–33.

Boureau F, Willer JC, Dauthier C. Study of naloxone in normal awake man: effects on spinal reflexes. Neuropharmacology 1978;17:565–8.

Carmichael FJ, Israel Y. In vitro inhibitory effects of narcotic analgesics and other psychotrophic drugs on the active uptake of norepinephrine in mouse brain tissue. J Pharmacol Exp Ther 1973;186:253–60.

Clinical Pathway for "Unconscious—Etiology Unknown". Conroe Regional Medical Center Emergency Room. 504 Medical Center Blvd, Conroe, TX.

Dafny N, Rigor BM. Dose effects of ketamine on photic and acoustic field potentials. Neuropharmacology 1978;17:851–62.

Feldman RS, Meyer JS, Quenzer LF. Principles of neuropsychopharmacology. Sunderland, MA: Sinauer Associates; 1997.

Forman LJ. NMDA receptor antagonism produces antinociception which is partially mediated by brain opioids and dopamine. Life Sci 1999; 64:1877–87.

Frantz KJ, Van Hartesveldt C. The locomotor effects of quinpirole in rats depend on age and gender. Pharmacol Biochem Behav 1999;64:821–6.

French ED, Ceci A. Non-competitive *N*-methyl-D-aspartate antagonists are potent activators of ventral tegmental A10 dopamine neurons. Neurosci Lett 1990;119:159–62.

Gilman AG, Goodman LS, Gilman A. Goodman and Gilman's the pharmacological basis of therapeutics. New York: Macmillan; 1980.

Giuliani D, Farrari F. Involvement of dopamine receptors in the antipsychotic profile of (−)eticlopride. Physiol Behav 1997;61:563–7.

Gold MS. Opiate addiction and the locus coeruleus: the clinical utility of clonidine, naltrexone, methadone, and buprenorphine. Psychiatr Clin North Am 1993;16:61–73.

Gold MS, Pottash AL. Endorphins, locus coeruleus, clonidine, and lofexidine: a mechanism for opiate withdrawal and new nonopiate treatments. Adv Alcohol Subst Abuse 1981;1:33–52.

Grant SJ, Huang YH, Redmond DE. Behavior of monkeys during opiate withdrawal and locus coeruleus stimulation. Pharmacol Biochem Behav 1988;30:13–9.

Gupta SP. Nonparametric two way ANOVA by ranks. Indian J Psychom Educ 1988;19:57–64.

Jentsch JD, Roth RH. The neuropsychopharmacology of phencyclidine: from NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. Neuropsychopharmacology 1999;20:201–25.

Laviola G, Wood RD, Kuhn C. Cocaine sensitization in periadolescent and adult rats. J Pharmacol Exp Ther 1995;275:345–57.

Linton M, Gallo Jr PS. The practical statistician: simplified handbook of statistics. Monterey (CA): Brooks/Cole; 1975.

Miller RK. Clear Lake emergency medical corps medical protocols, Clear Lake, TX; 2004.

Roth RH, Elsworth JD, Redmond DE. Clonidine suppression of noradrenergic hyperactivity during morphine withdrawal by clonidine: biochemical studies in rodents and primates. J Clin Psychiatry 1982; 43:42–6.

Sell SL, Scalzitti JM, Thomas ML. Influence of ovarian hormones and estrous cycle on the behavioral response to cocaine in female rats. J Pharmacol Exp Ther 2000;293:879–86.

Silveri MM, Spear LP. The effects of NMDA and GABA-sub(A) pharmacological manipulations on ethanol sensitivity in immature and mature animals. Alcohol Clin Exp Res 2002;26:449–56.

Spear LP, Brake SC. Periadolescence: age-dependent behavior and psychopharmacological responsivity in rats. Dev Psychobiol 1983; 16:83–109.

Stiegler P. EMT-Basic/EMT Basic IV technician protocols. Dane County Emergency Medical Services; 2003.

Vollenweider FX, Vontobel P, Oye I. Effects of (S) ketamine on striatal dopamine: a [-sup-1-sup-1C] raclopride PET study of a model psychosis in humans. J Psychiatr Res 2000;34:35–43.

White JM, Ryan CF. Pharmacological properties of ketamine. Drug Alcohol Rev 1996;15:145–55.

Wilson C, Cullen E, Sendell K. A pharmacologic investigation of the "transport response" in the white rat. Int J Dev Neurosci 1984;2:323–9.

Winters WD, Hance AJ, Cadd GG. Ketamine- and morphine-induced analgesia and catalepsy: I. Tolerance, cross-tolerance, potentiation, residual morphine levels and naloxone action in the rat. J Pharmacol Exp Ther 1998;244:51–7.

Yamamoto M, Mizuki Y, Suetsugi M. Effects of dopamine antagonists on changes in spontaneous EEG and locomotor activity in ketamine-treated rats. Pharmacol Biochem Behav 1997;57:361–5.

Zhou W, Cunningham KA, Thomas ML. Estrogen effects on the hyperactivity induced by (+)-MDMA and cocaine in female rats. Behav Neurosci 2003;117:84–94.