

Full Length Research Paper

Single and repeated exposure to methamphetamine induces altered sexual behavior in male sailfin molly (*Poecilia latipinna* Lesueur) (Pisces)

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In order to evaluate the effects of methamphetamine on sexual behavior of fish, saltwater-acclimatized male sailfin molly *Poecilia latipinna* L. (Pisces) adults were exposed to 0.1, 0.5, 1 mg/L methamphetamine (MA) concentrations and were observed for alterations in sexual behavior at 2nd, 5th or 7th exposure days. The overall changes displayed by the subjected fish included different acute and chronic responses.

Key words: Methamphetamine (N,α-dimethylphenethylamine), fish, sexual behavior, male.

INTRODUCTION

Methamphetamine (N,α-dimethylphenethylamine, C₁₀H₁₅N, "MA") is a prototypical basic (pK_a 9.9) drug (Logan, 2002). The drug was first synthesized in Japan (Ogata, 1919) and later licensed as the anorectic Methedrine® (Logan, 2002). It is a central nervous system (CNS) stimulant and its potent stimulating effects appear to result by promoting the release of biogenic amines from their stores in the nerve terminals, together with dopamine (DA) release from dopaminergic nerve terminals (Elshohly et al., 1992). These effects can result in arrhythmia, vasculature constriction and delayed ejaculation and enhanced intensity of orgasm in male human (Perez-Reyes et al., 1991; Logan, 2002; Ellinwood and Kilbey, 1980). The present study used a comprehensive approach to examine the effects of MA exposure on sexual behavior fish to examine if the fish can be used as appropriate model for studying behavioral effects of MA in human.

MATERIALS AND METHODS

Study species

Approximately, 300 adult sailfin mollies, *Poecilia latipinna* L., of both sexes (total weight = 20.5 ± 0.33 g (mean ± SD); total length = 85.73 ± 0.03 mm (mean ± SD)) were obtained from a local dealer and acclimated to 15 g/L saltwater to favor effective delivery of the agent from water to the animals in hyperosmotic condition (Nordlie et al., 1992). In order to select the animals with synchronous reproductive cycle, they were then maintained for another two weeks in a 1000 L community during which all of the females bred at the 15th day constituted experimental females (N = 89) and the males displaying least heterosexual mating attempts (< 2 attempts/h) at the same day were selected as experimental males (N = 56) and kept in sexually isolated tanks one week prior to experiment. They were fed daily with commercial dry pellets at 4% of body weight.

Drug

Water-soluble MA (hydrochloride salt) was purchased from local pharmaceuticals and verified for purity and enantiomeric ratio. The experimental concentrations of the drug were achieved by mixing different dosages of obtained MA (74.41% yield, 1.16 l/day) directly with phosphate-buffered saltwater (15 g/L) in approximately 15 min

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intervals before each trial.

Experimental design

During the experiment, a batch of 6 male fish were placed in 100 L fiberglass aquaria containing 0.1, 0.5 or 1 mg/L MA contaminated media and kept for 30 min (Logan, 2002). The fish were then immediately taken out, cleaned with a wet sponge, transferred to uncontaminated test tanks pre-stocked with 8 female fish and were observed for sexual behavior of each male at a 30 min (Schlupp and Plath, 2005) digitally video recorded test session after a 10 min acclimatization period (Heubela and Schlupp, 2008). The exposure schedule was conducted every day for seven days during which the observations were made at 2, 5 or 7th exposure day. The following parameters were considered: (1) heterosexual mating attempt (frequency of female-directed gonopodial thrusts) (Parzefall, 1969), (2) same sex mating attempt of male fish (frequency of male-directed gonopodial thrusts) (Cureton et al., 2010), (3) mean duration of full copulation time (Rodd and Sokolowski, 1995) and (4) threesome heterosexual mating attempts. For fine monitoring of behavioral changes, six fish were selected and observed as the pre-transfer group and also control groups receiving no drug were monitored throughout the test trials.

Data analysis

Data means were exposed to repeated-measures analyses of variance to test the effects of 'within subject' factor (exposure time) and 'between subjects' factor (dosage). Data were transformed to meet the assumptions of this analysis as follows: mean duration times of courtship were transformed using $\ln(\text{trait} + 1)$ (Zar, 1999) and other data were transformed using the square root of $(\text{trait} + 3/8)$ (Zar, 1999). The significant levels were defined at $P < 0.05$.

RESULTS

All fish exposed to the experimental concentrations of the MA survived the exposure period. The drug administered at 0.1, 0.5 or 1 mg/L concentrations resulted in significant alteration of all tested behavioral parameters. During the experiment, a significant ($p < 0.05$) time dependent, increased homosexual and heterosexual mating attempts and courtship time were recorded at 2nd and 5th exposure days (Figure 1). However, the aforementioned observed pattern was followed by a significant decrease in heterosexual mating attempts and mean duration of courtship during 7th exposure day (Figure 1). No constant dependence could be noted between threesome mating attempts and time (Figure 1).

DISCUSSION

Psychostimulant drugs such as MA, which facilitate catecholamine release and/or block their uptake, are commonly used for psychostimulation (Martins et al., 2008). The MA dosages investigated in the current study induced different patterns of sexual behavior alterations

due to exposure time. Our results show that acute exposure to MA can result in sexual arousal in male fish. D1 and D2, DA receptors are clearly implicated in the induction of sexual arousal (Da prada et al., 1973) and it has been suggested that presynaptic activation of DA transmission is a key pharmacologic property mediating the sex-promoting effects of stimulants (Paglietti et al., 1978). One region where DA may act to facilitate male sexual behavior is the medial preoptic area (MPOA), a region at the rostral end of the hypothalamus (Dominguez and Hull, 2005) probably by removing tonic inhibition, thereby enhancing sensory-motor integration (Chevalier and Deniau, 1990; Hull et al., 2002). On the other hand, chronic exposure to the agent resulted in induction of a lassitude phase for all tested parameters accordingly except that of homosexual behavior in our study. Its now well demonstrated that, long-term administrations of the MA may lead to persistent DAergic deficits (Wilson et al., 1996). Under normal circumstances, DAergic signaling involves a delicate balance between dopamine (DA) release and re-uptake by the presynaptic nerve terminal by coordinated function of DA transporter (DAT) and the vesicular monoamine transporter-2 (VMAT-2) (Riddle et al., 2006). MA-induced decrease in DAT and VMAT-2 activity may contribute to the persistent DAergic deficits caused by MA (Riddle et al., 2006). Interestingly, MA exposure in the current experiment resulted in progressive increase in homosexual behavior of subjected fish at even highest dosage which may be a direct result of the disrupting effects of MA on pheromonal communication (Logan, 2002). In this case lack of heterosexual discrimination based on chemical cues among male mollies may results in "mistaken identity" phenomenon (Ambrogio and Pechenic, 2008).

In summary, it can be speculated that the extant of the responses to MA is preserved between fish and human and sailfin molly may constitute a useful model system in the study of the biological mechanisms of MA effects considering ethical concerns associated with administration of MA to humans.

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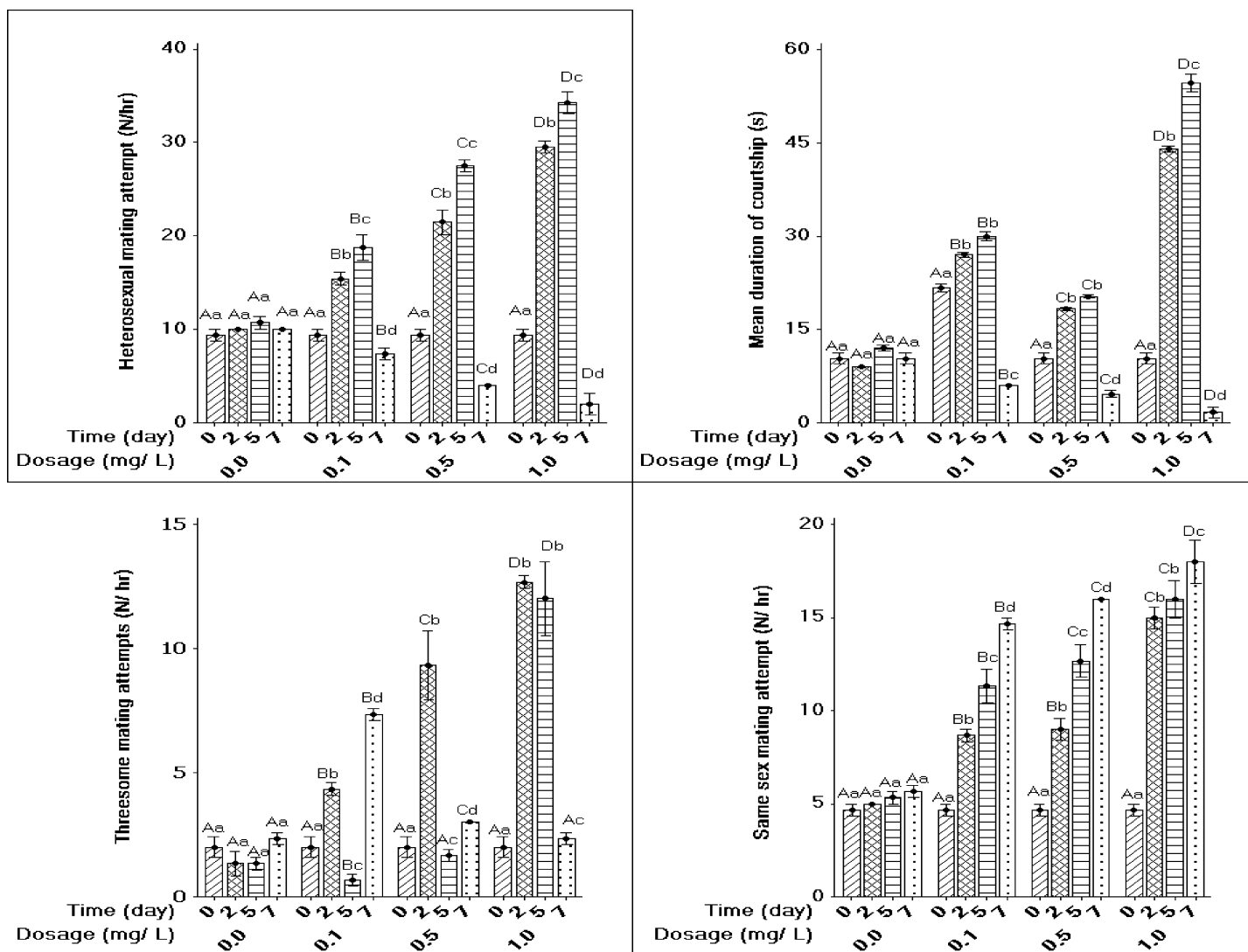


Figure 1. Time course of sexual behavior variations in sailfin molly (*Poecilia latipinna* Les.) exposed to different methamphetamine concentrations. Values having different upper case superscript letter are significantly different ($P < 0.05$) among different dosages of the drug; values having different lower case superscript letter are significantly different ($P < 0.05$) among different time points.

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