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MATING BEHAVIOR OF MALE MICE AFTER MSG ADMINISTRATION DURING INTRA UTERINE TO MATURE PERIODS OF LIFE

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ABSTRACT

Monosodium glutamate (MSG), one of the most popular *flavor enhancer* in the world, with the average daily consumption of 0.55 – 3 g, is categorized as a safe food additive. However, several reports (from both field and laboratory studies) indicate that MSG could induce disorders in structure and function of several system organs, including reproductive system. This study was aimed to investigate the impacts of daily long term exposure of MSG, from intra uterine up to adult periods of life, on mating behavior of male mice. Pregnant parental (P) female mice were orally treated with aqueous solution of MSG (4 mg/kg bw daily), started from day 0 of pregnancy until the end of weaning periods. This step was aimed to indirectly expose male F1 mice with MSG by means of placenta during intra uterine and then during lactating periods of life. After weaning, F1 male litter were separated from their female counterparts and then given the same oral dose of MSG, daily for five consecutive weeks until they were reproductively mature for mating. During four days mating period, male mice were analysed for mating behavior, i.e. mounting attempt frequency, length of time for vaginal plug, mating succes and copulation rate. All mice were housed in fly-proof plastics cages, fed with commercially prepared food and water was provide *ad libitum*. Our results show that F1 male mice previously received MSG both indirectly during intra uterine and lactating periods and directly during postlactation period of life have a lower mounting frequency per 30 minutes (9.5 ± 6.7) than control group (12.7 ± 3.2). The MSG-treated males also needs a longer time (48.6+15.4 hours) to copulate female counterparts than the control group (32.8 ± 10.5). The administration of MSG decreases mating succes of the males (70% MSG vs 90% control). The results indicate that chronic administration of MSG during intrauterine development, lactation, post lactation (weaning) and adult periods of life can interfere with the male mating behavior.

Key words: *Monosodium glutamate (MSG), male mice, reproductive behavior, long term exposure*

INTRODUCTION

Monosodium glutamate (MSG) is a white crystalline powder of the sodium salt of a naturally occurring non-essential amino acid, glutamic acid, has been using by human population for a very long time as food flavour enhancer additive with global consumption average varied between 0.55 – 3 g/day/person (NAS, 1979; Rhodes *et al.*, 1991; Prawirohardjono *et al*., 2000). MSG is added in almost all industrial processed food products such as potato chips and the other angel foods, soup, vegetables, saosage, canned meat,

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frozen meat, instant noodle and in almost all restaurant fast foods (Geha *et al*, 2000; Hodgson, 2001; Loliger, 2010).

MSG is categorized by FDA, European Community Commission, and even by WHO as safe food additive without effect on health (Geha *et al*., 2000; Hodgson, 2001). However, several controversies remained from case or experimental studies need to be confirmed. These studies indicate that MSG seemly related to several healthy problems such as cancer, heart attacts, obesity, asthma, reproductive, nervous and the other system function disorders. Subcutan injection of MSG in infant rats can induce brain damage (Olney, 1969; Olney *et al*., 1971) and neuronal retina degeneration causes blindness (Sand, 2005). MSG also induce ablation in hypothalamic arcuate nuclei that interfere with the function of hypothalamo-pituitary-target organ absis (Garattini, 2000) leading to disruption of endocrine function (endocrine disruption) including production and secretion of reproductive hormones (Camihort *et al*., 2005).

In relation to the effects of MSG on reproductive function, two days old male mice administered with 4 mg/g bw of MSG (equals to 30 - 240 mg/kg bw in human) results in the decrease in body weight, Sertoli and Leydig cells number per testis in puberty (Franca *et al*., 2005). The decrease in Leydig cell number per testis subsequently inhibits testosterone production. Hypogonadism that can be observed after administration of MSG is apparently due to the decrease of blood concentration of LH and FSH which are play a critical role in organ development and reproductive function (Franca *et al*., 2005). It was also reported that MSG causes decrease in seminal vesicle and epidydimis weight without significant changes in testicle histological structure (Giovambattista, 2003).

Both short- (15 days) and long-term (30 days) administration of MSG in rats resulted in decrease in testical weight (Vinodini *et al*., 2008; Ibrahim *et al*., 2012; Nossier *et al*., 2012) and seminiferous tubules diameter and the size of germinal cells (Nossier *et al*., 2012), and induce vacuolation of spermatogonia (Ibrahim *et al*., 2012) that implicated in the decrease in testicular concentration of sperm and the increase in the number of sperm with abnormal morphology (Nayanatara *et al*., 2008; Nossier *et al*., 2012). Because of the MSG administration is implicated in the increase of lipid peroxydation and the decrease of testicular concentartion of ascorbic acid (Vinodini *et al*., 2008), it could be concluded that MSG excert its effects by the mecanism of oxydative stress (Ahluwalia *et al*., 1996; Diniz *et al*., 2004; Diniz *et al*., 2005; Onyema *et al*., 2006; Pavlovic *et al*., 2007; Thomas *et al*., 2009; Okwidiri *et al*., 2012). Furthermore, it have been found that the short-term exposure of MSG induces a more extensive testicular oxydative detriments than the long-term one (Nayanatara *et al*., 2008; Vinodini *et al*., 2008). Young mature male mice administered with MSG for 90 consecutive days are experienced in a serious damage in seminiferous tubules histology and abnormality of germ cells in several steps of

spermatogenesis (Ismail, 2012). The administration of MSG during neonate period causes germ cells accumulation in primary spermatocyte pachytene step that subsequently decreases testicular spermatogenic cell number (Das and Ghosh, 2010). Even, in mature mice, daily dose of 4.5 mg MSG/g bw for 35 days could effectively induce a perturbation in spermatogenesis which is indicated by the decrease in the number of both pachytene spermatocytes and spermatids (Sukmaningsih *et al.*, 2011). These all indicate that both neonatal and young mature (or mature) periods are critical for disorder of germ cells structure and function or spermatogenesis due to the presence of MSG.

The presence of MSG induces not only disorders in germ cells structure and function but also in testicular somatic cells and tissues or in organs related to the reproductive system (Das and Ghosh, 2010; Fernandes *et al*., 2012; Sukhorum *et al.,* 2012). Long term administration of MSG to neonate or mature rats resulted in epididymides and seminalis vesicle hypertrophies (Fernandes *et al*., 2012; Sukhorum *et al.,* 2012), prostat gland (Fernandes *et al*., 2012) and vas deferens (Sukhorum *et al.,* 2012) that implicated in the decrease in sperm quality in epididymis and testis (Fernandes *et al*., 2012; Sukhorum *et al.,* 2012), and hypertrophy of interstitial Leydig cells in neonate mice (Das and Ghosh, 2010). Interstitial cell hypertrophy even is followed by the increase in testosterone due to the decrease of plasm level of FSH (Fernandes *et al*., 2012).

Although the negative impacts of MSG are reversible or can be minimized by administering antioxydants (Amir and Davis, 1982; MacDonald and Wilkinson, 1990; Onyema *et al*., 2006; Nossier *et al*., 2012), but considering the daily consumption of MSG by human being is high enough, varied between 0.55 - 3 g (NAS, 1979; Rhodes *et al*., 1991; Prawirohardjono *et al*., 2000), then the possibility emerge of its impacts on reproductive functions is constantly high. Multigeneration study that enables treated animals continually exposed to MSG from gestation to mature periods of life is very limited. Infact, human being is continually exposed to MSG during its life span, from intra uterine period, i.e. by means of placenta, neonatal period, i.e. by means of lactation, to mature and senescence periods of life by direct consumption. It is widely known that MSG molecules can easily penetrate the placental barrier (Gao *et al*., 1994; Yu *et al*., 1997; Hermanussen and Tresguerres, 2003). For that reason, the study on the impact of MSG exposure during life span, from intra uterine, lactation to post natal periods, on reproductive function would be very important to carried out in order to understand the real figure of its impacts, especially on mating behavior. The present study was designed to investigate the effects of long term exposure of MSG, from intra uterine to mature periods of life, on mating behavior of male mice.

METHODOLOGY

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Experimental animals. Male (10) and oestrous female (30) Swiss Webster mice (*Mus musculus*), aged two months, were used in this study. All the animals were housed in metal lid covered-plastic cages and fed commercially prepared feed ad libitum. Drinking water was provided. The animals were kept for one week acclimatization period before the commencement of the experiments.

Dose of MSG. A single dose of monosodium L-glutamate monohydrate (MSG) (Roth Company, Germany), 4 g/kg body weight, was used in this experiment. The dose was in the range of previews doses of 2 – 8 g/kg bw applied in mice and rats as reported by studeis (Nayanatara *et al*., 2008; Bojavic *et al*., 2009; Das and Ghosh, 2010; Sukmaningsih *et al*. 2011; Ibrahim *et al*., 2012; Nossier *et al*., 2012). MSG was dissolved in distilled water and administered daily with the maximum volume of 0.5 ml per animal. The same volume of distilled water was gavaged to the control animals.

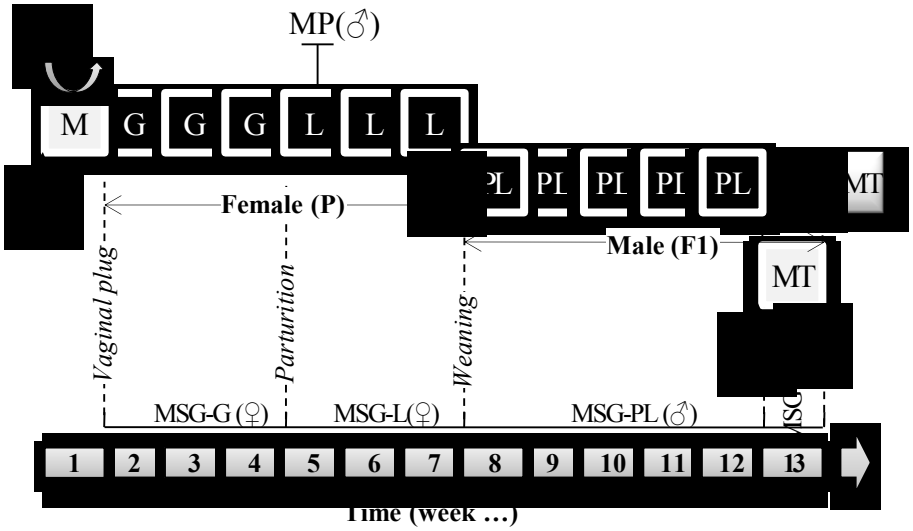

Figure 1. Research procedure. After one week of mating period (M), the vaginal plug positive P female mice were gavaged daily with MSG (4 g/kg bw, in 0.5 ml aquadest) during gestation (MSG-G) and lactation periods (MSG-L). At the third day after parturition all female litters were removed and the number of males were uniformed into 3 - 4 per litter (MP♂). After weaning, all F1 males were daily gavaged, for 5 consecutive weeks, with the same dose and volume of MSG solution (MSG-PL). All MSG-treated F1 males were then housed with oestrus mature female (MT), for one week, during which all males were continually treated with MSG (MSG-MT). During mating period (MT) all males were observed for mating behavior (AMT) as described in Materials and Methods. Control group was treated with the same procedure, except for the MSG that was changed with aquadest. G, L, and PL respectively indicate gestation, lactation, and post-lactation periods.

Procedures. Experimental procedures is summarized in Figure 1. During one week mating period (M), female mice were checked daily (in the morning, at 08.00) for vaginal plug (day of plug was assigned as day 0 of pregnancy). The vaginal plug-positive P female mice were then gavaged daily with MSG (4 g/kg bw, volume 0.5 ml). The administration of MSG to pregnant P mice was continued during gestation period (G). At the parturition day, all F1 female

litter was retrieved from the mother and the remained male litter was uniformed to be 4 – 5 offspring/mother. During lactation period (L), approximately 3 consecutive weeks, the MSG was continually gavaged to the mother mice. After weaning (or during post lactational period, PL) all male F1 mice received directly the same dose and volume of MSG, with the same way of administration for 5 consecutive weeks. All male F1 mice were then caged with the same age and estrous females for four days mating test period (one male for each female). The adminstration of MSG to the male was ceased at the day of vaginal plug of its female counterpart. During mating period, male mice were observed for mating behavior. The control group were treated with the same treatment but received no MSG.

Mating behavior. Mating behavior of F1 male mice observed including mounting frequency, length of time needed by the male to copulate its female counterpart, and mating success. Mounting frequency is the number of times of F1 male attempts to mount its female mating pair in 30 minutes time periods. Mounting frequency was observed manually during dark period of the first day of mating test, from 18.00 h to 20.00 h. The length of time (in hours) needed by the male to copulate its mating pair was counted from the commencement time of male and female joined until the appearance of vaginal plug in female. Visual indicator for capulation is the appearance of vaginal plug or sperm-positive vaginal smear. After the joining of F1 male and its pairing mate, females were checked for vaginal plug or sperm-positive smear every day, in the morning (08.00-10.00), for four consecutive days. Mating succes (in %) was calculated as the ratio of number of female with vaginal plug and the total number of female mated.

Statistical analyses. Means and standard errors were calculated for each group of observations. The data obtained were statistically analyzed using t-test (SPSS 11.0 Statistical Package of SPSS Inc. USA).

RESULTS AND DISCUSSION

Results. Mating behatior of F1 male mice treated chronically with MSG from intrauterine development, lactation, post lactation (weaning) and adult periods of life is presented in Table 1. Table 1 clearly shown that chronic and two generations exposure to MSG induce the decrease in mating behavior quality in F1 males as indicated by the decrease in mounting frequency and the lengthening of the copulation time. As the consequence, the copulation rate or mating success of males is also significantly decreased.

Tabel 1. Mating behavior of F1 male mice after chronic administration of MSG during intrauterine development, lactation, post lactation (weaning) and adult periods of life.

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No.	Mating behaviors	Control group	Treatment group
1.	Mounting (*frequency/30 minutes*)	12.7 ± 3.2	9.5 ± 6.7*
2.	Length of time for vaginal plug (*hours*)	32.8 ± 10.5	48.6 ± 15.4*
3.	Mating success or copulation rate (%)	90	70*

*) indicates significantly different from control group (t-test, $p < 0.05$)

Discussion

In this study, the F1 male mice is exposed to the MSG by two different ways, i.e. indirectly by means of placenta (for 3 weeks of intrauterine development) and of milk secreted by the mother to nourish the litter (for 3 weeks of lactation period) and directly by oral gavage (during post lactation to adult period of life, approximately 5 weeks) (Figure 1). Therefore, the total length of F1 males exposed to MSG before tested for mating behavior was approximately 11 weeks.

The facts that MSG can cross the placental barrier in rats and mice have been well documented (Toth *et al*., 1987; Gao *et al*., 1994; Yu *et al*., 1997). Yu *et al*. (1997) reprorted that maternal oral administration of MSG during late pregnancy (i.e. 19 to 21 days of pregnancy) in mice has been shown to accumulate at twice the maternal level in the brains of fetuses. The other researchers documented that MSG given subcutaneously to pregnant rats caused acute necrosis of the acetylcholinesterase-positive neurons in the area postrema of both mother and fetuses (Toth *et al*., 1987). This observation raises the possibility of transplacental toxicity, especially neuronal toxicity, in human fetuses after the consumption of glutamate-rich food by the mother. In mice, transplacental neuronal toxicity mainly characterized by swollen cytoplasm, dark pyknotic nuclei and loss of neurons both in arcuate nucleus and ventromedial nucleus of hypothalamus (Gao *et al*., 1994). All these results clearly indicates that MSG ingested by mother during pregnancy are effectively penetrated the placental barrier, reached the fetuses, toxified the neuronal cells in hypothalamus, and finally may be adversely affect the brain function of the fetuses.

In relation to the effect of MSG ingestion on lactation and breast-fed infants, the lactating women who consumed MSG at 100 mg/kg bw has no effect on the level of glutamate in her milk, and therefore, there is no effect on the infant's intake of glutamate (Filer and Stegink, 1994). However, a higher susceptibility of neonatal male rats to glutamate-induced dysfunction of sexual and ingestive behaviors than females has been reported by (Sun *et al*., 1991).

The decrease of mating behavior quality observed in this study maybe resulted from hormonal disturbance due to the chronical intake of MSG as reported by Haffner *et al*. (1993). Sun *et al*. (1991) reported the presence of sex difference in the mechanism of dysfunction of sexual behavior, i.e., decreased copulatory activity in male rats is mainly due to CNS damage, in contrast to decreased blood progesterone level in female rats.

Hormonal disturbance could be originated from the disorders in structure and function of hypothalamo-hypophyseal-testes axis. MSG is an amino acid readily utilized by glutamate receptors throughout the mammalian body. These glutamate receptors are present in the central nervous system as the major mediators of excitatory neurotransmission and excitotoxicity. One of the most susceptible target of the MSG in this axis is nuclei arcuate of the hypothalamus. Subcutaneous injections of MSG in four day old rat pups caused a high degree of cell necrosis in the arcuate nuclei of the hypothalamus (Rascher and Mestres, 1980). Lesion in arcuate nuclei of hypothalamus after MSG treatment have also been widely reported in mice (Onley, 1969; Onley *et al*., 1971; Holzwarth-McBride *et al*., 1976a; 1976b), rat (Rodriguez-Sierra *et al*., 1980), hamster (Tafelski and Lamperti, 1977), and in any other mammals (see Garattini, 2000). According to Tafelski and Lamperti (1977), a single subcutaneous injection of 8 mg MSG/g bw on day 8 of the neonatal period induces alterations in the ovarian histology, hypothalamic neurohistochemistry and the levels of gonadotropins in the plasma and pituitary glands of hamster, following the lesion of the arcuate nucleus of the hypothalamus.

Cellular defect in hypothalamus could be implicated in the disorders of function of hypothalamo-hypophyseal-testis axis as a whole system in synthesis and/or secretion of hormones that regulate body functions, including reproductive functions. In male, for example, hypothalamic damage could be interfered with testicular function in testosteron synthesis and/or secretion. One of the consequence for this testosteron synthesis and/or secretion is the disruption of reproductive behavior and capacity, for example mounting, copulation capacity and mating success as have been observed in this study.

In conclusion, chronic administration of MSG during intrauterine development, lactation, post lactation (weaning) and adult periods of life can interfere with the male mice mating behavior. The interferences can be implicated in lower mounting frequency, longer time to copulate female counterparts and decreases mating succes of the males.

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