Long-term consequences of ecstasy abuse
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GENDER DIFFERENCES IN ACUTE AND LONG-TERM HYPERTHERMIA AND SEROTONERGIC TURNOVER FOLLOWING MDMA IN RATS

Alinde E. Wallinga, Carolin Grahlmann, Ramon A. Granneman, Jaap M. Koolhaas, Bauke Buwalda
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ABSTRACT

Recreational use of ecstasy, or 3,4-methylenedioxymethamphetamine (MDMA), has become more widespread during the last decennia. There is much concern about the long-lasting consequences of MDMA use. Several studies in both animals and humans have indicated that MDMA selectively decreases 5-HT functioning in the brain. To investigate whether gender differences exist in the acute and long-term consequences of MDMA (ab)use, in the present research sexes are compared for their acute and long-term hyperthermic response and long-term serotonergic depletion after MDMA. Female and male rats were injected 3 times with 3 hours interval with saline, 0.3, 1, 3 or 9 mg/kg MDMA. MDMA was administered i.p. at an ambient temperature of 25 °C. Acute and long-term effects on body temperature were measured with telemetry. Four weeks after MDMA administration animals receiving 3 x 9 mg/kg were decapitated and brains processed for monoamine analysis. After the highest dose, MDMA elicited not only an acute but also a long-lasting hyperthermia, which was much higher in males than in females and persistent for males the whole 4-week period of sampling. Whereas no gender differences were found in the 5-HT depleting effect of MDMA, a striking difference was observed in 5-HIAA concentrations. In the majority of brain areas 5-HIAA levels decreased only in males, suggesting a lasting increase in 5-HT turnover in females. To exclude a role for the difference in hyperthermic response between both genders on the 5-HT depletion, in an additional experiment males and females were matched on the magnitude of the hyperthermic response. 5-HT depletion was not different between genders with similar hyperthermia. Also in this experiment 5-HIAA levels only decreased in males suggesting an increased 5-HT turnover in females 4 weeks after MDMA administration. In conclusion, males are more susceptible for the hyperthermic effects than females, but no long-term differences were found in the level of 5-HT depletion. Whether the lasting increase in 5-HT turnover in females is indicative of an increased vulnerability to the negative consequences of MDMA on 5-HT functionality has to be addressed in further studies.
INTRODUCTION

The popularity of the psychostimulant ecstasy (3,4-methylenedioxy-methamphetamine; MDMA) has increased during the last decades. One of the acute dangers of ecstasy (ab)use is the occurrence of a hyperthermia (for example see Schmidt et al., 1990; Nash, Jr. et al., 1988; Dafters, 1994; O’Shea et al., 1998; Malberg et al., 1996; Freedman et al., 2005), which may lead to lethality (Dowling et al., 1987; Chadwick et al., 1991). Maybe even more reason for concern is the long-lasting, possibly neurotoxic consequences of MDMA for the serotonergic system. Several preclinical studies have demonstrated that MDMA evokes a persistent decrease in serotonin and 5-hydroxyindoleacetic acid (5-HIAA), a reduction in tryptophan hydroxylase (TPH) activity and a reduction of serotonin transporter (SERT) activity and expression and long-term impairment of anterograde transport in serotonin axons (Battaglia et al., 1987; Hewitt & Green, 1994; Schmidt & Taylor, 1987; Stone et al., 1986; Xie et al., 2006; Sharkey et al., 1991; Stone et al., 1987b; Buchert et al., 2004; Ricaurte et al., 2000; Colado et al., 1993; Semple et al., 1999; Schmidt & Taylor, 1988; Callahan et al., 2001). Although clinical studies on this matter are less conclusive, there is increasing evidence that MDMA can be toxic for the human brain as well (Reneman et al., 2001; de Win et al., 2004; McCann et al., 2000; Reneman et al., 2006; Turner & Parrott, 2000; Grob, 2002).

In preclinical psychopharmacological research females are often not included because one has to control for the hormonal fluctuations due to the estrous cycle. However, preclinical and clinical studies emphasize the importance of sex differences in the pharmacokinetic and pharmacodynamic responses to many drugs of abuse, including alcohol, nicotine and psychostimulants like cocaine and amphetamine (Becker et al.,
Evidence suggests that females are more vulnerable than males to the reinforcing effects of psychostimulants (Liechti et al., 2001). In view of the evidence that the use of ecstasy increased significantly among females together with the general increasing popularity of ecstasy (Allott & Redman, 2007) it is important to consider gender as a modulatory factor in the long-lasting effects of MDMA on serotonergic functioning. Therefore, the present study aimed at the question of gender differences in the long-lasting serotonergic neurotoxic consequences.

Data from studies in humans suggest indeed a gender difference: females seem to be more susceptible than males to deficiencies in 5-HT functioning after ecstasy use (McCann et al., 1994; Buchert et al., 2004; Croft et al., 2001; Reneman et al., 2001). So far, animal studies have found no (consistent) difference between male and female rats in depletion of 5-HT and 5-HIAA levels after MDMA administration (Chu et al., 1996; Koenig et al., 2005; McNamara et al., 1995; Walker et al., 2007). Regarding gender differences in MDMA-induced changes in body temperature and behavioral locomotor response, conflicting results are reported (Walker et al., 2007; Palenicek et al., 2005; Koenig et al., 2005; Colado et al., 1995; McNamara et al., 1995; Fonsart et al., 2008).

The present study investigated whether male and female rats differed in the acute hyperthermic and long-term body temperature and 5-HT depleting effects of MDMA. The study was divided into two parts. In the first part of the experiment it was investigated whether there are differences in acute and long-term body temperature effects and in the long-term serotonergic depletion between male and female rats after MDMA administration. Different dosages of MDMA were administered and the MDMA-induced hyperthermic response was measured in both males and females. Body
temperature was measured using biotelemetry, to assure body temperature sampling in a stress-free manner. To control for interaction effects with body temperature, in a second experiment male and female rats were matched for their acute MDMA-induced hyperthermic response and it was investigated whether these male and female rats differed in the induced long-term serotonin depletion.

**MATERIALS AND METHODS**

**Subjects**
All experiments have been approved by the animal experiments committee of the University of Groningen. Wild-type Groningen rats were used. Their ancestors were originally wild-trapped animals, subsequently bred in our laboratory for more than 31 generations. Room temperature was 21 ºC, except during MDMA administration. Males and females were housed in separate rooms. All rats were individually housed in clear Perspex cages (31 x 15 x 14 cm) with sawdust bedding in a room with 12:12 light:dark cycle (lights on at 8:00h). Food (chow) and water was available *ad libitum* throughout the whole experiment.

**Experiment 1**
In the first experiment 45 female and 50 male Wild-type Groningen rats (*Rattus norvegicus*) of 10 weeks old (male 370 ± 3.8 g, females 210 ± 2.2 g at the moment of MDMA injection) were used.
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Telemetry

A radio-telemetry system (Data Sciences, St. Paul, MN) was used for stress-free monitoring of body temperature. At least 14 days before MDMA administration, in 28 males and 30 females transmitters (TA10TA-F40; sensitivity: 0.1 °C) were implanted intraperitoneally using O₂-isoflurane anaesthesia. Temperature was measured every ten minutes throughout the entire experiment.

The cages of the rats were placed on receiver plates and the signal was collected and analyzed using the DSI Dataquest® A.R.T. Acquisition System.

To determine the long-term effect of MDMA on body temperature, data averages of 12 hours (matching the light and the dark period) were calculated.

MDMA/saline injections

3,4-Methylenedioxymethamphetamine (± MDMA-HCl, 99.6% obtained from the Dutch Forensic Institute, The Netherlands) was injected intraperitoneally (i.p.) three times with intervals of three hours (“binge administration”) during the light phase. The first injection was given 1.5 hours after lights went on. MDMA was given to both females and males at concentrations of 0.3 (8 males, 9 females), 1 (9 males, 9 females), 3 (8 males, 9 females) and 9 (15 males, 9 females) mg/kg MDMA, solved in 1 ml ultra purified water. Control animals were saline injected (9 males, 9 females). Ambient temperature was 25 ± 0.5 °C), starting from 1.5 h before the first injection until 3 hours after the last injection.
Analysis of brain monoamine concentrations

It has been demonstrated that MDMA-induced 5-HT depletion only occurs when acutely a significant hyperthermic response could be observed (for review see (Green et al., 2003). Since the part of aim of the present study was to investigate whether there are gender differences in the serotonergic depletion after MDMA, only of male and female rats administered saline or 3 x 9 mg/kg MDMA (resulting in a clear hyperthermic response) monoamine concentrations were measured in the brain.

Four weeks after treatment these male and female rats were decapitated under brief CO₂ anesthesia in early light phase. From these animals, prefrontal cortex (PFC), striatum, hippocampus, cerebellum, brainstem (Bregma -8.50 mm to 16 mm), hypothalamus, parietal cortex and septum were dissected on a chilled plate, immediately snap frozen in Eppendorf vials in liquid nitrogen and stored at -80 ºC. For determination of 5-HT, 5-HIAA, dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and noradrenalin (NA) in these areas, High Performance Liquid Chromatography (HPLC) was used. For this, samples were homogenized in 0.5 ml 0.1 M perchloric acid and centrifuged at 14000 rpm for 10 min at 4 ºC. Supernatant was removed and analyzed for 5-HT, 5-HIAA, DA, DOPAC, and NA concentrations. For determination of the monoamines and their metabolites, 100 µl was injected onto a reversed phase Gemini C18 column (150 x 4.6 mm, 5µm particle size), connected to a detector (ESA coulochem model 5100A) with a 5011A detector cell. A difference in potential of 340 mV was set (the potential of one electrode was 0 mV and the other was 340 mV). The mobile phase consisted of 62.7 nM Na₂HPO₄, 40.0 nM citric acid, 0.27 mM EDTA, 4.94 mM HSA, 10% methanol at pH 4.1 with a flow of 0.5 ml/min. Known amounts of 5-HT, 5-HIAA, DA, DOPAC, HVA (Sigma Chemicals) and NA (Research
Biochemicals International) were run throughout the whole procedure for standardization. Monoamine levels were calculated as ng/g tissue.

**Experiment 2**

The second experiment was performed to control for possible difference on serotonergic depletion between genders due to a difference in their MDMA-induced hyperthermic response. Therefore body temperature of both sexes was matched on peak temperatures. We chose to match males to the hyperthermic response of females that received 3 x 9 mg/kg MDMA in the first experiment, instead of matching females to the hyperthermic response of males that receiving 3 x 9 mg/kg MDMA, since the hyperthermic response to the latter dose includes a high risk of lethality.

**Telemetry**

At least 14 days before MDMA administration, in 10 male rats transmitters (TA10TA-F40; sensitivity: 0.1 °C) were implanted intraperitoneally (i.p.) using O₂-isoflurane anaesthesia. Temperature was measured every ten minutes throughout the whole experiment. The cages of the rats were placed on receiver plates and the signal was collected and analyzed using the DSI Dataquest® A.R.T. Acquisition System.

**MDMA administration**

The 10 male rats (364 ± 8.7 g at the day of MDMA administration) were injected 6 mg/kg MDMA (i.p.) three times with intervals of three hours (“binge administration”) during the light phase. The first injection was given 1.5 hours after lights went on. Ambient temperature was 25 °C (± 0.5), starting from 1.5 h before the first injection until 3 hours after the last injection.
Analysis of brain monoamine concentrations

Four weeks after the injections animals were decapitated under brief CO₂ anesthesia in early light phase. From all animals, prefrontal cortex (PFC), striatum, hippocampus, cerebellum, brainstem, hypothalamus, parietal cortex and septum were dissected on a chilled plate. Brain areas were removed and snap frozen in liquid nitrogen and stored at 80 °C. 5-HT and 5-HIAA brain concentrations were measured as described previously.

Statistical analysis

SPSS 14.0 for Windows was employed to analyze the data statistically. Acute body temperature response (Area Under the Curve, AUC) was analyzed using a two-factor ANOVA with treatment (5 levels) and gender (2 levels) as between-subject factors. The long-term body temperature was analyzed using repeated-measures ANOVA with treatment (5 levels) and gender (2 levels) as between-subject factors. In case of significant interaction effects, post hoc analyses were performed using an independent t-test to reveal the differences between both genders for each dose. A one-way ANOVA was used to reveal for each gender separately the differences between the several doses.

Lethality rate of males and females for each dose was statistically analyzed using logistic regression. In experiment 1 and 2, monoamine concentrations were statistically analyzed using a two-factor ANOVA with treatment (2 levels) and gender (2 levels) as between-subject factors. In case of a significant interaction effect post hoc analysis was performed using an independent t-test to reveal the differences between both genders for each dose.

In experiment 2 body temperature response was analyzed by repeated measurements ANOVA with gender (2 levels) as between-subject factor. In
case of a significant main and/or interaction effect, post hoc analysis was performed with an independent t-test to reveal the differences between both genders.

RESULTS

Experiment 1

Acute effect of MDMA on body temperature
MDMA induced a significant hyperthermic response in rats administered 3 x 9 mg/kg MDMA (main dose effect F(4,47)=30.954, p<0.0001). When taking also gender into account, the two factor ANOVA revealed a significant gender x dose interaction effect (F(4,47)=8.388, p<0.0001). As shown in figure 1a and 1b, the hyperthermic response was stronger in male than in female rats (t(9)=-4.990, p<0.001). Furthermore, 3 x 3 mg/kg MDMA only induced a significant hyperthermic response in males (F(4,23)=112.179, p<0.0001), which was lower than the hyperthermic response observed after 3 x 9 mg/kg MDMA. In addition, baseline body temperature was higher in males than in females (t(10)=-3.240, p<0.01).

Lethality
Employing logistic regression using the model \( Y = \log \left( \frac{p}{p-1} \right) = ax+b \), \( (p = \text{chance of a rat dying and a zero model was used for fit, with } y = \text{constant}) \) revealed that dosage \( (X^2 = 30.88, p <0.0001) \) of MDMA and gender \( (X^2 = 6.13, p <0.05) \) together determined survival rate, indicating that significantly more males than females died after receiving 3 x 9 mg/kg MDMA (see table 1).
Figure 1a) Average total body temperature response (+ s.e.m.) over the first nine hours after the first MDMA injection. Male and female rats were injected 3 x 0.3, 3 x 1, 3 x 3 or 3 x 9 mg/kg MDMA or saline. * p<0.05. b) Average body temperature (+ s.e.m.) of male and female rats over time. Rats were injected three times with 0 or 9 mg/kg MDMA. Injections were administered at time point 0, 3 and 6. Arrows indicated time of injection. * control male vs. 9 mg/kg male p<0.05; # control female vs. 9 mg/kg female p<0.05; $ 9 mg/kg male vs. 9 mg/kg female p<0.05; & control male vs. control female p<0.05.
**Table 1** Lethality of male and female rats after the highest dose (3 x 9 mg/kg) of MDMA.

<table>
<thead>
<tr>
<th>9 mg/kg</th>
<th>Alive</th>
<th>% survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>6 out of 15</td>
<td>40%</td>
</tr>
<tr>
<td>Females</td>
<td>8 out of 9</td>
<td>89%</td>
</tr>
</tbody>
</table>

**Long-term effect on body temperature**

MDMA treatment resulted in a differential body temperature response in male and female rats (treatment x sex interaction \((F(4,2544)=2.335, p<0.0001)\)). Post-hoc analysis revealed that male and female rats receiving 3 x 9 mg/kg MDMA were also hyperthermic the day after MDMA injections. Female rats receiving 3 x 9 mg/kg MDMA were hyperthermic in the light phase one day after repeated MDMA treatment, whereas male rats stayed hyperthermic until 2.5 days after MDMA administration (figure 2). Within these 2.5 days body temperature in the light phase was higher than in the dark phase in male rats. MDMA treatment of 3 x 9 mg/kg in male rats increased body temperature during the dark phase (active period of the day) for the whole registration period as compared to female rats.
Figure 2. Average body temperature (+ s.e.m.) of male and female rats per twelve hours during 28 days. At day zero repeated MDMA (3 × 9 mg/kg) or saline injections were given. Dark symbols represent the average body temperature during the dark phase. Light symbols represent the average body temperature during the light phase. *control male vs. 9 mg/kg male p < 0.05; #control female vs. 9 mg/kg female p < 0.05; $9 mg/kg male vs. 9 mg/kg female p < 0.05; &control male vs. control female p < 0.05.

Monoamine concentrations

3 × 9 mg/kg MDMA induced 5-HT in all brain areas (main treatment effect, F(1,28) > 9.018, p < 0.05). Male and female rats did not differ in the induced 5-HT depletion (no significant treatment x gender interaction, (F(1,28) < 2.808, p > 0.05) (figure 3a).

Furthermore, MDMA evoked a 5-HIAA depletion in all brain areas except septum, cerebellum and brain stem (main treatment effect, F(1,32) > 7.107, p < 0.05). When gender was also taken into account, significant larger 5-HIAA depletion was found in the hippocampus, septum, cerebellum, brainstem and hypothalamus of male rats (treatment x gender interaction
(F(1,28)>4.739, p<0.05) (figure 3b). Baseline concentrations of 5-HT did not differ between genders in all brain areas (t(16)<1.529, p>0.05) (table 2). Female rats had a significant higher baseline 5-HIAA concentration in hippocampus (t(16)=3.456, p<0.05, septum (t(16)=2.728, p<0.05, cerebellum (t(16)=2.2886, p<0.05 and brain stem (t(16)=2.290, p<0.05 (table 2). MDMA did not induce a depletion of DA, DOPAC, HVA and NA levels in any of the measured brain areas (data not shown).

**Table 2** Baseline 5-HT and 5-HIAA tissue concentrations (± s.e.m.) for male and female rats administrated three times saline are presented. * =p<0.05 males vs female.

<table>
<thead>
<tr>
<th></th>
<th>5-HT concentration (ng/g tissue)</th>
<th></th>
<th>5-HIAA concentration (ng/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PFC</td>
<td>Hippocampus</td>
<td>Parietal cortex</td>
</tr>
<tr>
<td>Male</td>
<td>553.1 ± 62.83</td>
<td>320.59 ± 18.33</td>
<td>63.97 ± 29.36</td>
</tr>
<tr>
<td>Female</td>
<td>522.0 ± 50.04</td>
<td>311.38 ± 17.76</td>
<td>84.57 ± 40.43</td>
</tr>
<tr>
<td></td>
<td>310.2 ± 32.06</td>
<td>296.81* ± 13.63</td>
<td>257.4 ± 15.37</td>
</tr>
<tr>
<td></td>
<td>347.6 ± 32.31</td>
<td>366.48 ± 14.85</td>
<td>244.1 ± 23.11</td>
</tr>
</tbody>
</table>
Figure 3 Average % of a) 5-HT and b) 5-HIAA tissue concentration (+ s.e.m.) relative to control were measured in eight different brain areas in male and female rats, four weeks after repeated saline or MDMA injections (3 × 9 mg/kg).
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Experiment 2
The results of experiment 1 showed that MDMA induced a higher acute hyperthermia in males than in females and that no sex differences were found in serotonin markers in the brain. As explained in the materials and methods, in this experiment we aimed to match males and female for their hyperthermic response to MDMA and investigated the long-term effect on the serotonergic system in the brain. Since in experiment 1 MDMA only resulted in a depletion of 5-HT and 5-HIAA concentrations, DA, DOPAC, HVA and NA concentrations were not analyzed.

Matching of the acute body temperature response of male and female rats
MDMA induced a hyperthermic response in male and female rats, with males having a higher body temperature than females (gender x dose interaction effect (F(1,1488)=2.214, p<0.0001)) at the time points indicated in figure 4. Importantly, no gender differences in peak temperature responses were observed.

5-HT and 5-HIAA analysis
Statistical analysis revealed that MDMA induced a significant depletion in 5-HT concentrations in all brain areas except in PFC, hypothalamus and brain stem (main treatment effect, F(1,32)>4.571, p<0.05). Similar to experiment 1, the magnitude of the 5-HT depletion did not differ between both genders except in the PFC and hypothalamus (gender x treatment interaction effect, F(1,32)=14.388, p<0.01; F(1,32)=7.455, p<0.01 respectively) (figure 5a). Post hoc analysis revealed that MDMA induced only a 5-HT depletion in females in the PFC (t(16)=3.841, p<0.01) and hypothalamus (t(16)=658, p<0.0001) (figure 5a). MDMA induced a 5-HIAA depletion in all brain
areas except PFC and brain stem (main treatment effect $F(1,32)>5.031$, $p<0.05$). The magnitude of the induced 5-HIAA depletion differed between both genders in the hippocampus, septum, cerebellum, brain stem and striatum (gender x treatment interaction $F(1,32)>4.697$, $p<0.05$) (figure 5b). Post hoc analysis revealed that in these brain areas MDMA only induced a depletion of 5-HIAA levels in males ($t(16)>2.585$, $p<0.05$) (figure 5b).

**Figure 4** Average body temperature (+ s.e.m.) of male and female rats over time. Rats were injected three times with 0 or 9 mg/kg MDMA. Injections were administered at time point 0, 3 and 6 hours. Arrows indicated time of injection. *control male vs. 6 mg/kg male $p<0.05$; #control female vs. 9 mg/kg female $p<0.05$; $\ddag$6 mg/kg male vs. 9 mg/kg female $p<0.05$; &control male vs. control female $p<0.05$. Control male, control female and female 9 mg/kg were already presented in figure 1b.
Figure 5 Average % of **a)** 5-HT and **b)** 5-HIAA tissue concentration (+ s.e.m.) relative to control were measured in eight different brain areas, four weeks after repeated saline or MDMA injections (3 × 9 mg/kg in female and 3 × 6 mg/kg in male rats).
DISCUSSION

The present study aimed to reveal gender differences in acute hyperthermia, lasting temperature regulation and monoamine depletion after MDMA treatment, using several dosages of MDMA. MDMA induced a much stronger acute hyperthermic response in male rats compared to female rats following treatment with $3 \times 9 \text{ mg/kg}$ MDMA and a higher lethality rate. This indicates that male rats are more vulnerable for the acute hyperthermic and lethal effects of MDMA than female rats. Our finding is in agreement with previous studies (Koenig et al., 2005; Fonsart et al., 2008; Wyeth et al., 2009). Furthermore, the long-lasting increase in body temperature observed in only in male rats receiving the highest dose of MDMA indicates that male rats are also more vulnerable for the long-term hyperthermic effects than female rats.

Furthermore, male and female rat do not differ in the magnitude of the MDMA-induced 5-HT depletion, which suggests there is no gender difference in the neurotoxic effects. A striking observation, however, was that 5-HIAA depletion was only observed in males. This result suggests that MDMA induced a long-lasting increase in 5-HT turnover in females. The implication of this increased turnover in females with regard to gender specific vulnerability to the possible neurotoxic effects of MDMA remains to be investigated.

Body temperature is known to fluctuate during the day. Normally, body temperature in rats is higher during the active period (dark phase) of the day and lowers during the inactive period (light phase) of the day. Strikingly, up to 3 days after MDMA treatment in both genders the elevated body temperature is higher in the light-phase than in the dark phase. This might indicate that MDMA induces a temporal shift in circadian rhythmicity.
Indeed, there is evidence indicating that MDMA can induce abnormalities in sleep and circadian patterns (Balogh et al., 2004; McCann & Ricaurte, 2007) in humans and rodents.

Two points for consideration come up with regard to the performed experiment. Firstly, mortality rates show that 2/3 of the males died after receiving 3 x 9 mg/kg MDMA. This might have caused a bias in the monoamine analysis. It is possible that the male rats that died after the MDMA injections are the rats that would also have the largest 5-HT depletion. The surviving rats might be relatively resistant to the MDMA-induced 5-HT depletion. If this would be true, it would result in an underestimation of the serotonergic depletion in males that received 3 x 9 mg/kg MDMA and a biased conclusion regarding the possible gender differences in vulnerability for MDMA induced neurotoxicity. Importantly, after administration of 3 x 6 mg/kg MDMA to male rats all rats survived, resulting in an unbiased gender comparison.

Another point may be the difference in body weight between males and females. In the present study male rats are heavier than females (about 160 gram difference). This might have resulted in higher peripheral and brain concentrations of MDMA in males. Higher circulating concentrations of MDMA might account for the observed difference in the acute hyperthermic response. However, recent evidence indicates difference in the thermogenic response. Females seem to be less vulnerable for the induction of a hyperthermic response by MDMA due to differences in their thermoregulatory abilities (Wyeth et al., 2009).

In conclusion, males seem to be more at risk of developing an acute, potential lethal, MDMA-induced hyperthermic response than females. With and without matching of the hyperthermic response genders seem to be equally vulnerable for the evoked 5-HT depletion. However, it seems that
females compensate for the initial loss of 5-HT by increasing the 5-HT turnover. It remains to be addressed in further studies what the functional consequences are of a lasting increase in serotonin turnover in an MDMA affected 5-HT system.

ACKNOWLEDGEMENTS

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