Formaldehyde inhalation & open field behaviour in rats

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Background & objectives: It has been shown in animal studies that repeated exposure to formaldehyde vapour alters behaviour and memory. Since information is not available on the behavioural consequences of acute formaldehyde exposure, this study was conducted to investigate the influence of single inhalative exposure to formaldehyde on the explorative and locomotor behaviour of adult male and female rats.

Methods: Rats were exposed to different concentrations of formaldehyde vapour (0.5, 1.0, 2.5%, corresponding to inhalation chamber concentrations of 1.0, 2.5, and 5.0 ppm, respectively) for 2 h and an open field test was carried out 2 h after the end of exposure (first test) and repeated 24 h thereafter (second test). The parameters examined were crossing of floor squares, sniffing, grooming, rearing, climbing, and defaecation.

Results: In exposed male rats, significant reduction of crossed floor squares, grooming, and wall climbing, and increase in floor sniffing and rearing were observed in the first test. During the second test, males in the groups exposed to 2.5 ppm and 5 ppm crossed significantly higher numbers of squares when compared to controls. Air sniffing, wall climbing, and rearing were altered in all exposed males. Control males showed higher incidence of defaecation in comparison to the values of first test. The formaldehyde-exposed female rats crossed significantly decreased numbers of floor squares in the first test. In females in the 2.5 ppm and 5 ppm groups, decreased grooming and enhanced floor sniffing were observed. In the second test, all exposed females crossed higher numbers of floor squares than controls. Frequencies of air and floor sniffing were higher in females exposed to 2.5 ppm and lower in those exposed to 1 ppm. Defaecation was enhanced in females in the 2.5 ppm group in comparison to the first test.

Interpretation & conclusion: The results show that formaldehyde inhalation in the concentrations and duration of exposure used in the present experiments significantly influences the locomotor and explorative behaviour of rats after a single exposure in a gender-related manner and that various behavioural components in the exposed animals remains altered even after 24 h.

Key words Behaviour - exploration - locomotor activity - open field behaviour - rat

Several human studies demonstrate that chronic exposure to formaldehyde at the work place leads to neurovegetative complaints like headache, fatigue, indigestion, and nausea, and neurobehavioural symptoms such as sleep disturbances, somnolence, insomnia, loss of concentration, recent and remote memory deficits, instability of mood, and increased irritability. The effect of formaldehyde on memory has also been observed in experimental animals after repeated inhalative, intraperitoneal, or oral administration. Further, formaldehyde affects the open field behaviour in male and female rats after single intraperitoneal application or repeated inhalative exposure and influences the chimney test performance in mice. Since information is...
not available on the effects of an acute inhalative formaldehyde exposure on the behaviour of animals, the present study was undertaken to investigate the influence of formaldehyde on the locomotor and explorative open field behaviour of male and female rats after a single inhalative exposure. As many chemical agents have an immediate effect on central nervous system (CNS) function and the majority of the effects are reversible, we also studied the reversibility of the effects after withdrawal of the exposure agent.

**Material & Methods**

The animal experiments were conducted with the approval and permission of the Ministry of Nutrition, Agriculture, Forests, and Fisheries of Mecklenburg-West Pomerania, Germany. As per the guidelines of the US Environmental Protection Agency for neurobehavioural toxicity testing, LEW.1K rats of both sexes, aged 110-130 days and with a mean body weight of 300±12 g (males) and 220±7 g (females) were used in the experiments. The rats separated according to gender were kept in groups of 3-4 in transparent polycarbonate cages (52 x 32 x 20 cm) with metal grid tops in an animal holding cabinet (Life Island System, Type 110, France). The circadian rhythm (12h dark: 12h light) was regulated automatically. Room temperature was maintained at 21±2°C. The animals were provided with tap water and standard laboratory chow (ssniff R-Z extruded, ssniff® Spezialdiaeten GmbH, Soest, Germany) ad libitum, except during the inhalation and the open field test. Standard bedding (type lignocel 3-4 fibres, ssniff®, Soest, Germany) was used. Both the animal husbandry and the animal experiments were conducted at the Peter Holtz Institute for Pharmacology and Experimental Therapeutics, Ernst-Moritz-Arndt-University, Greifswald, Germany.

The rats were randomly divided into four groups (groups A to D) of 15 males and 15 females each (calculated overall study power: 95%) and exposed to 1.0 (group B), 2.5 (group C), or 5.0 ppm (group D) formaldehyde vapour once for two hours. Controls (group A) inhaled distilled water vapour. The formaldehyde concentrations used were based on the fact that such concentrations could also occur in the human work place. The exposure took place in a transparent vitreous inhalation chamber (90 x 50 x 70 cm). The walls were provided with small holes to allow fresh air exchange. To generate and maintain the concentrations in the chamber air during the inhalation session, aqueous formaldehyde solutions of different concentrations (0.5% for group B, 1% for group C and 2.5% for group D) were prepared using a 37 per cent stock formaldehyde solution (Merck, Darmstadt, Germany) and added in volumes between 5-8 ml three to four times in an hour by means of a pipette (Transpipettor, Fa. Brand, Wertheim, Germany) into a flat dish which was located in the center of the bottom of the chamber. In order to avoid direct contact of the experimental animals with the formaldehyde solution, the whole bottom was covered with a 5 cm high perforated platform. To control the formaldehyde concentration in the chamber air, a Dräger tube combination was used in conjunction with Dräger activating tubes for formaldehyde (measurement range: down to 0.04 ppm) Drägerwerk AG, Lübeck, Germany). When air samples are sucked through the tube system, the indicating layer changes colour to pink in the presence of formaldehyde. The scale on the tube shows the formaldehyde concentration in ppm based on the height of pink coloured layer.

The measurements of formaldehyde levels were performed 16 times throughout the exposure session. At the end of exposure, the rats were returned to the cages and kept there for 2 h with free access to food and water before being exposed to the open field arena (first test). After conducting the test, the rats were placed back in the cages. The open field test was repeated after 24 h (second test). The single exposure regimen was carried out during the dark phase of the day on different days (group B on day 1, group D on day 3, group A on day 5, and group C on day 7) between 0800 and 1000 h (males) and 1100 and 1300 h (females).

**Test apparatus for open field test**: To perform the test, a wooden, rectangular, light brown coloured open field apparatus measuring 100 x 100 x 40 cm was used. The floor was divided into 25 rectangular squares by pencil lines. The experiment room was illuminated by a 40 Watt white light tube which was located 150 cm above the test apparatus. The following behavioural components were quantitatively examined in each animal for three minutes: ambulation (crossed squares): the number of floor squares entered by an animal; grooming: face cleaning, fur licking, and scratching; rearing: upright
standing with hind paws while forepaws are free; sniffing: the nose is held close to the floor (floor sniffing) or upwards in the air (air sniffing) while movements of the nasal skin take place; wall climbing: standing on hind paws with one or two forepaws placed against the surrounding wall; defecation: number of faecal boli dropped by an animal in the open field arena. Crossing of floor quadrants was registered automatically by means of infrared photocells which were built in the walls. Other behavioural parameters were registered manually by an independent observer, who was blind to the exposure regimen. All open field tests were performed during the dark phase of the day. The first open field test was carried out 2 h after the end of the single exposure and repeated 24 h thereafter (second test) (i.e., group B on days 1 and 2, group D on days 3 and 4, group A on days 5 and 6, and group C on days 7 and 8). Both tests were carried out during the day at the same time between 1200 and 1300 h (males) and 1500 and 1600 h (females). The floor of the test apparatus was thoroughly cleaned after each test.

Statistical analysis: The data were analyzed by means of the Kruskal-Wallis-test followed by the two-sided Mann-Whitney test. To find out the significant differences between the results of the first and the second trial in each animal group, the paired Wilcoxon signed rank test has been used. The dose-effect-relationship was assessed by the Jonckheere-Terpstra test (trends test). All statistical evaluations were carried out using the SPSS program for Windows 2000. A $P$ value of $<0.05$ was considered significant.

Results

The measurements of the formaldehyde concentrations in the inhalation chamber revealed the following mean values: group B: 1.01±0.29 ppm, group C:2.51±ppm, and group D: 5.04±0.27 ppm. During all exposure sessions, minor but not significant fluctuations in the formaldehyde vapour concentration were registered.

Immediately after the animals were placed in the inhalation chamber, they started to explore the new environment. An increase of the locomotor activity (walking, climbing) was evident. After the initial explorative phase which lasted for a few minutes (7-9 min), a decrease of the locomotor activity in both the control and the experimental groups was noticeable. No signs of irritation or intoxication, such as lacrimation, nasal secretion, or regurgitation could be seen either during the inhalation session or thereafter. Also no defensive or aggressive behavioural changes in the rats were evident. Food and water intake was unaffected by the inhalation.

Open field performance: The Kruskal-Wallis test revealed significant inter-group differences for the following parameters in males square crossing, air sniffing, floor sniffing and grooming ($P<0.001$), and rearing, and wall climbing ($P 0.04$). In females, the significant inter-group differences at the first test were square crossing, air sniffing, floor sniffing and grooming ($P<0.001$), and rearing ($P 0.002$). During the first test, the formaldehyde exposed males crossed significantly ($P<0.005$) decreased numbers of floor quadrants compared to the controls. Grooming and wall climbing were also depressed while air and floor sniffing and rearing were increased (Table I). Defaecation was not significantly affected, though a trend toward increased excretion was obvious. In the second test, males exposed to 2.5 ppm and 5 ppm formaldehyde crossed significantly higher numbers of quadrants than controls ($P<0.005$) while significant ($P<0.05$) lower frequencies of air sniffing and wall climbing as well as higher frequency of rearing ($P<0.005$) were seen in all exposed males (Table I).

In the first test with the female rats, formaldehyde inhalation caused a significant ($P<0.005$) decrease in the number of crossed floor squares (Table II). In the 2.5 ppm and 5 ppm groups, decreased grooming was observed while floor sniffing was significantly ($P< 0.005$) enhanced. Rearing was significantly ($P<0.05$) increased in the 1 ppm and 2.5 ppm female groups. Air sniffing was significantly depressed in the 2.5 ppm group and significantly enhanced in the 5 ppm group. In the second open field test, it was obvious that all females crossed significantly ($P<0.005$) higher numbers of floor quadrants than controls. Frequencies of air and floor sniffing were significantly higher in the 2.5 ppm and lower in the 1 ppm females. Defaecation was not affected in both tests when compared with controls.
Table I. Open field performance of male rats in first and second tests
(Data are mean ± SE)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>First open field test</th>
<th>Second open field test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>1 ppm</td>
</tr>
<tr>
<td>Crossed quadrants</td>
<td>44.27</td>
<td>16.60**</td>
</tr>
<tr>
<td></td>
<td>±6.45</td>
<td>±4.48</td>
</tr>
<tr>
<td>Air sniffing</td>
<td>9.20</td>
<td>18.67**</td>
</tr>
<tr>
<td></td>
<td>±1.21</td>
<td>±1.91</td>
</tr>
<tr>
<td>Floor sniffing</td>
<td>14.00</td>
<td>28.73**</td>
</tr>
<tr>
<td></td>
<td>±2.27</td>
<td>±2.05</td>
</tr>
<tr>
<td>Grooming</td>
<td>7.93</td>
<td>4.20</td>
</tr>
<tr>
<td></td>
<td>±1.75</td>
<td>±1.74</td>
</tr>
<tr>
<td>Rearing</td>
<td>3.33</td>
<td>4.27</td>
</tr>
<tr>
<td></td>
<td>±0.98</td>
<td>±1.33</td>
</tr>
<tr>
<td>Wall climbing</td>
<td>4.47</td>
<td>3.47*</td>
</tr>
<tr>
<td></td>
<td>±1.55</td>
<td>±0.92</td>
</tr>
<tr>
<td>Defaecation</td>
<td>0.73</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>±0.80</td>
<td>±0.85</td>
</tr>
</tbody>
</table>

$P**<0.005 *<0.05$ compared to the controls in the same trial
$P††<0.005 †<0.05$ compared to the results of the first trial within the same dose group

Males of all groups crossed significantly ($P<0.005$) less number of floor quadrants in the second test when compared to the first (Table I). Control males showed significantly ($P<0.005$) higher frequencies of air and floor sniffing, wall climbing and defaecation while grooming was significantly ($P<0.005$) depressed in the second tests when compared to the first. Males of the 1 ppm group showed in the second test lower frequencies of air and floor sniffing ($P<0.005$). However, floor sniffing was significantly ($P<0.005$) increased in the 2.5 ppm group.
Control females crossed significantly ($P<0.005$) decreased number of floor squares and showed enhanced frequency of air and floor sniffing and wall climbing ($P<0.005$), but decreased ($P<0.005$) grooming in the second test when compared to the first (Table II). In the formaldehyde exposed females, significant ($P<0.005$) enhancement in floor crossing, air sniffing, and wall climbing was seen. Floor sniffing was significantly ($P<0.05$) increased in the $1$ ppm and $2.5$ ppm groups. Incidence of faecal boli excretion was significantly ($P<0.005$) increased in the $2.5$ ppm female group in the second trial when compared to the first.

The Junckheere-Terpstra test (trends test) revealed significant exposure-related effects both in male and female rats. In the first test, the formaldehyde effects were dose-dependent in males for quadrants crossing ($P<0.03$), air sniffing ($P<0.005$), floor sniffing ($P<0.005$), grooming ($P<0.005$), and climbing ($P<0.005$), and in the females for floor sniffing ($P<0.005$) and rearing ($P<0.05$). During the second open field test, dose-effect-relationship has been assessed for following investigated parameters in males: squares crossing ($P<0.005$), grooming ($P<0.005$), climbing ($P<0.005$), and rearing ($P<0.05$), and in females: squares crossing ($P<0.005$), and floor sniffing ($P<0.005$). However, these effects did not exhibit linear trends with respect to the applied formaldehyde concentrations. Different sex-related behavioural response to formaldehyde exposure was evident. For instance, the exposure of male rats to $1$ ppm formaldehyde vapour caused in the first test significant ($P<0.05$) alterations in air and floor sniffing, grooming, and wall climbing when compared to controls while these parameters remained statistically unaffected in the females of the corresponding exposure group. Further, males of the $2.5$ ppm group exhibited significantly ($P<0.005$) higher air sniffing frequencies during the first test, where in the $2.5$ ppm females, this parameter was depressed.

Formaldehyde-exposed male rats crossed during the second open field test significantly ($P<0.005$) less floor squares in comparison to the first test while the formaldehyde-exposed females crossed significantly ($P<0.005$) higher numbers of floor quadrants. In the treated males, wall climbing frequencies in the first test were comparable to those of the second test while wall climbing frequencies in the second test were significantly ($P<0.005$) increased when compared to the results of the first test.

Discussion

The results of the present study show that single formaldehyde inhalation in the concentrations and exposure duration used affects the exploratory and locomotor activity of male and female rats in the open field. Our findings on decreased locomotor activity in the inhalation chamber are in agreement with the findings of other investigators$^{13}$. After inhalation, formaldehyde is primarily absorbed in the upper respiratory tract$^{14}$ and metabolized to formic acid. It has been suggested that formic acid is the metabolite responsible for the deleterious effects of formaldehyde$^{15}$.

Since the half-life for formaldehyde elimination from the blood is about $1$ min$^{15}$ and because of its ability to pass through the blood-brain-barrier$^{16}$, it is suggested that small amounts of free formaldehyde could reach the nervous system and directly interact with the cells$^{2}$. It has been shown that formaldehyde causes several pathological alterations in the structure of the nervous system like increase in neurofilaments$^{9}$, intraaxonal swelling, and mitochondrial disruption$^{17}$. Exposure to formic acid vapour has been shown to affect numerous neurochemical parameters and to induce cerebral hypoxia$^{18-19}$. Thus, the behavioural effects observed in the experimental animals in the present study cannot be attributed only to the effects of formaldehyde but presumably also of formic acid. Hypoxia induced inhibition of locomotor and exploratory activity in the rats open field behaviour has also been observed by other workers$^{20}$. The hazardous effects of formaldehyde on the trigeminal nasal sensory system$^{21}$ and on the respiratory epithelium$^{22}$ may also account for the effects seen in the experimental animals. However, since the trends test did not show linear correlation in the dose-effect-relationship, we conclude that the formaldehyde induced effects on locomotor and explorative behaviour observed in the experimental animals seem not to be dose-specific. These findings are in agreement with the results of other investigators$^{13}$, who compared the locomotor activity of rats after exposing them to $5$ ppm or $10$ ppm formaldehyde vapour. However, linear trends in dose-effect-relationship have been observed after repeated formaldehyde exposure$^{1,22}$. The results demonstrate clearly the impact of a single formaldehyde
inhalation on the behaviour of male and female rats in the open field situation and show that most of the behavioural parameters were still altered 24 h after exposure. The terminal half life for radioactive [14C] formaldehyde after inhalative exposure was estimated to be 55 h14 which may explain the behavioural changes observed in the animals 24 h later.

The significant differences in control values for various parameters in the second open field test in comparison to the first test have also been observed in other animal species and were attributed to the learning and memory effect23,24.

The gender specific differences in behavioural response to formaldehyde exposure observed in the present experiments may be attributed amongst other to the sex-specific metabolic capacities of the experimental animals25. Male and female sex hormones have also been implicated for such differences26. Gender-related differences in open field behaviour evidenced in the control rats in the present study were also reported by other investigators27, who considered the Lewis (LEW) rat strain as a useful genetic model for the study of behaviour and emotionality.

Though data from animal experiments cannot be directly extrapolated to human conditions, the results obtained in the present study demonstrate that formaldehyde inhalation causes significant alterations in locomotor and explorative activities in rats and emphasize that the recommended permissible formaldehyde levels for living and occupational sites have to be strongly adhered to.

References


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