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Inhalative formaldehyde exposure enhances aggressive behavior and disturbs monoamines in frontal cortex synaptosome of male rats

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ABSTRACT

Formaldehyde (FA) exposure is known to be toxic to central nervous system of mammals. In this paper, we evaluated the aggressive behavior after repetitive inhalative FA exposure to male SD rats, and explored the potential mechanism. The rats, ranging from 160 to 180 g, were randomly designated into the orchiectomized (ORX) group, the control and the inhalative FA treatment groups. Eight rats underwent orchiectomy surgery. Three days after the orchiectomy surgery, the inhalative FA (monitored to be 13.5 ± 1.5 ppm) treatment began. We found that the male SD rats, those were exposed to FA showed more aggressive behavior compared to the control. And the ORX rats exhibited less aggressive behavior than the control. Furthermore, the dopamine increased and 5-HT decreased significantly in frontal cortex synaptosome after inhalative FA treatment. It is the first to evaluate aggressive behavior and identified monoamines disturbances in the frontal cortex synaptosome after the repetitive inhalative FA exposure to rodents.

Neurotransmitter release is considered to play decisive roles in multiple physiological and pathological events. As the most potent neurotransmitter storage pool, synaptosome attracts great concerns to explore the early and sensitive changes in it. Formaldehyde (FA), widely used in industry, is known to be toxic to mammals in mutagenicity, genotoxicity, sensory irritation or neurotoxicity, carcinogenicity, psychological and even memory impairment [2,13]. Since early, people have realized and made profound studies on FA and central nervous system (CNS) [18]. Epidemiological data and the following studies revealed memory and psychological impairments caused by FA exposure [5–7,16]. Furthermore, repetitive inhalative FA treatment could enhance anxiety-like behavior in rodents [10,11,20]. However, less study is concerned with aggressive behavior, which is so common and significant in the society, neither monoamines which mainly underlie aggression. Impairment of frontal cortex and the resultant loss of limitation to inhibit the subcortical emotional centers, amygdala and nucleus accumbens will lead to predisposition to aggression, which are mainly caused by disturbances of dopamine and serotonin (5-HT). This makes frontal cortex studies quite important in aggression.

In the current study, the repetitive inhalative FA treatment paradigm was conducted on the male Sprague–Dawley (SD) rats to evaluate the effects on aggressive behavior. The subsequent detection of monoamines in frontal cortex synaptosome explored the underlying mechanism. The intriguing results first indicated the aggressive behavior enhancement and increase of dopamine and decrease of 5-HT in frontal cortex synaptosome after repetitive inhalative FA treatment

Male SD rats (160–180 g of body weight), obtained from Institute of Animal care of Health Science Center of Peking University (Beijing, China), were group-housed in a standard animal care room (the temperature 22 ± 1 °C, the humidity 50 ± 5%, and 12-h light/dark cycle) with food and water ad libitum. The rats were randomly divided into the orchiectomy (ORX), the control and the inhalative FA treatment group with eight rats in each group for behavioral tests and another five rats in control and FA treatment group for monoamines detection. After habituation for 3 days, eight rats underwent bilateral ORX surgery with general anesthesia induced by intraperitoneal injection (i.p.) of chloral hydrate (350 mg/kg). Three days after surgery, the inhalative FA treatment began. FA gas was generated by evaporation of formalin solution (37%, Beijing Chemical Works) which was added onto the four lower insides of the static toxification chambers (54 cm × 31 cm × 34 cm) with three or four rats in a chamber during each inhalative FA treatment. The concentration of evaporated FA was monitored by Formaldehyde Detect Device (Interscan 4160-2) to be around 13.5 ± 1.5 ppm during the procedure. The treatment was conducted twice a day, 7:00–7:30 AM and 19:00–19:30 PM. After inhalative FA treatment for 14 days, the rats in the ORX, the control and inhalative FA treatment

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groups were tested in behavioral tests. And after further 14 days of inhalative FA treatment, the frontal cortex synaptosomes of the control and inhalative FA treatment group were separated to detect monoamines. When the treated group(s) were accepting inhalative FA treatment, all the other group(s) were also accepting the 30-min placebo treatment in which the formalin solution was absent.

The rats received territorial aggressive behavior test based on the resident–intruder paradigm 1 day after inhalative FA treatment. The smaller male SD rats (160–180 g of the body weight) served as intruders, whereas the treated rats (370–450 g of the body weight) served as residents. The resident rat was kept in a 460 mm × 300 mm × 160 mm clean rat cage individually for 30 min before the intruder was put into. We recorded the aggressive scores (contact time and frequency, as well as violent attack time and frequency) those the resident conducted to the intruder within the test of 3 min. The recordings of social contact and violent attack were based on the previous studies [9,12].

One day later, the rats were tested with spontaneous locomotive activity in an automated Tru Scan photo beam activity system (Coulbourn Instruments, Allentown, PA) [15].

All experiments were conducted following an approved protocol from animal care committee of the Peking University and performed in accordance with the animal care guidelines of the Chinese Council.

After further 14 days of inhalative FA treatment, the rats were decapitated after anesthesia by i.p. injection of chloral hydrate (350 mg/kg). Then the brain tissues, frontal cortex was dissected according to stereotaxin atlas of Paxinos and Watson [25]. Immediately afterwards, the frontal cortex were weighed and stored at −80 °C for further crude synaptosome (P2) preparation based on Dunkley et al. [4] with minor modifications as follows. The frontal cortex was centrifuged at 1000 × *g* at 4 °C for 10 min after isotonic homogenization. The supernatant was further centrifuged at 17,000 × *g* at 4 °C for 20 min to get the final P2 fraction. The P2 was protected with ice-cooled perchloric acid (250 μl/100 mg, 0.4 M) for further homogenization by using ultrasound (0.5 Hz) for 10-s and then centrifuged at 12,000 rpm at 4 °C for 20 min. The supernatant was passed through a 0.2 μm filter and kept at −80 °C until HPLC analysis. Dopamine and 5-HT were detected using reversed-phase ion-pair chromatography combined with electrochemical detection under isocratic conditions [22,23]. To be short, the six-channel detector potentials were set at + 50, 100, 200, 300, 400, and 500 mV using a glassy carbon electrode and an Ag/AgCl reference electrode. The mobile phase was delivered at a flow rate of 1 ml/min at 22 °C onto the reversed-phase column. Ten microliters of aliquots were injected by an autoinjector with cooling module set at 4 °C. Data were calculated by an external standard calibration.

Data were expressed as group means ± standard error of the mean (SEM). All the presented data in the paper which compared between the inhalative FA treatment group and the control, and between the ORX group and the control, were subjected to Student's *t*-test. Statistical difference was set at $P < 0.05$.

The findings from the studies were as follows. During the inhalative FA treatment, we observed that the FA treated rats exhibited more swearing, shaking, gathering and they produced more excretions (stool and urine) than control rats. In the aggressive behavior test, the FA treated rats earned more aggressive scores (contact frequency, violent attack frequency and violent attack time) with statistical significance of $P < 0.05$, $P < 0.001$ and $P < 0.01$ respectively (Fig. 1A, C and D). The contact time seemed comparable between the two groups (Fig. 1B). Meanwhile, the ORX treated rats earned less aggressive scores, violent attack frequency and violent attack time, than control rats with statistical significance of $P < 0.05$ (Fig. 1C and D). Subsequent locomotive activity demonstrated that spontaneous locomotive activities were similar in all groups (Fig. 2).

Then we further explored the dopamine and 5-HT in frontal cortex synaptosome by HPLC–ECD to the rats that received further 14 days of inhalative FA treatment. The results showed that dopamine in crude synaptosome of frontal cortex increased significantly with $P < 0.05$ (Fig. 3A), 0.1226 ± 0.0140 ng/mg (ng per mg frontal cortex weight) for control and 0.1718 ± 0.0147 ng/mg for inhalative FA treatment group. Meanwhile, the 5-HT decreased significantly with $P < 0.05$ (Fig. 3B) compared with the control, 0.0331 ± 0.0038 ng/mg

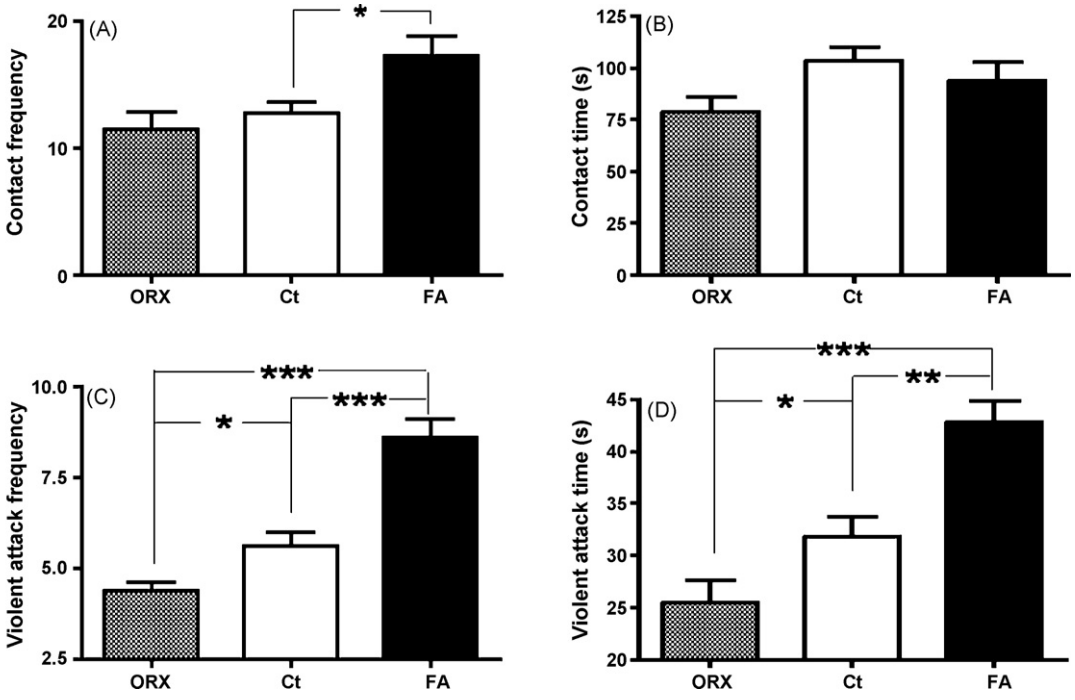

Fig. 1. Repetitive inhalative FA exposure enhanced aggressive behaviors in aggression test. (A) Contact frequency; (B) contact time (s); (C) violent attack frequency; (D) violent attack time (s). $N = 8$. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. Ct, control; FA, inhalative FA treatment; ORX, orchiectomy treatment.

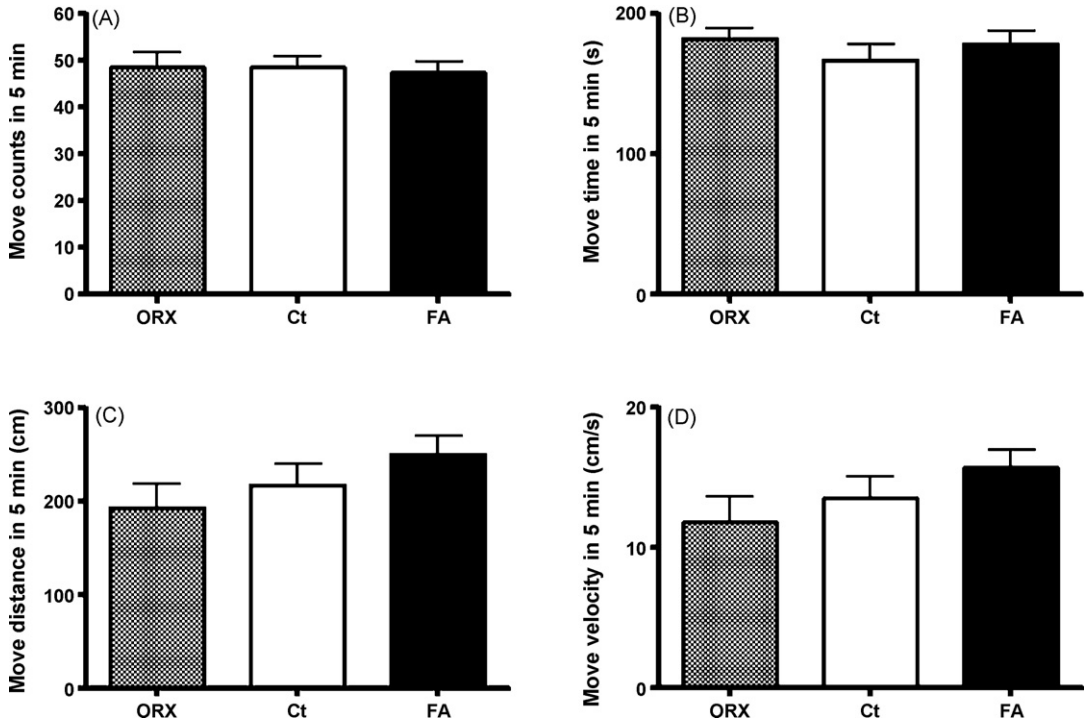

Fig. 2. Locomotive activity was similar in all groups. (A) Move counts; (B) rest time (s); (C) move distance; (D) move velocity (cm/s) in 5 min. $N = 8$. Ct, control; FA, inhalative FA treatment; ORX, orchiectomy treatment.

for control and 0.0192 ± 0.0031 ng/mg for inhalative FA treatment group.

Our study evaluated the aggressive behavior of the rats after repetitive inhalative FA treatment and further explored the dopamine and serotonin in frontal cortex synaptosome. The results showed that the repetitive inhalative FA treatment enhanced aggressive behavior of rats. And further inhalative FA treatment increased dopamine and decreased 5-HT in frontal cortex synaptosome significantly, which might underlie the behavioral changes.

Frontal cortex exerts crucial role in aggressive behavior [14]. Impairment of frontal cortex will lead to loss of limitation to inhibit the subcortical emotional centers in cascade reactions. Aggression related dopamine and 5-HT, are stored and most potent in synaptosome. Only the neurotransmitters, those are stored in the synaptosome, will undergo the secretion or exocytosis to trigger the following events. In this paper, we found that the aggressive behavior increased after repetitive inhalative FA exposure to male SD rats. The HPLC assay revealed the increase of dopamine and decrease of 5-HT in frontal cortex synaptosome after further inhalative FA treatment. The results coincided with the previous study that the monoamines disturbances would lead to aggressive behavior changes. Study has identified dopamine increase and 5-HT decrease in prefrontal cortex 50 min before, during and 80 min after aggressive confrontation [24], even 1 day after the aggressive stimulation [3]. Meanwhile, the locomotive activity seemed unchanged in all groups. It was reminiscent of the synaptosome neurotransmitters which were more sensitive in behavioral changes. Studies have revealed that in most species, orchiectomy inhibited aggression [8]. And we identified aggressive behavior reduction after ORX treatment which further proved the fidelity of aggression paradigm in this paper.

Many studies have investigated mechanisms underlying the behavioral changes, such as neurotransmitter and neuroendocrine disturbances. Repeated formaldehyde exposure in rodents produced CNS plasticity manifest as greater sensitivity to dopaminergic drugs (cocaine) [19]. Inhalative FA exposure selectively regulated mRNA of N-methyl-D-aspartate (NMDA) receptor subunits

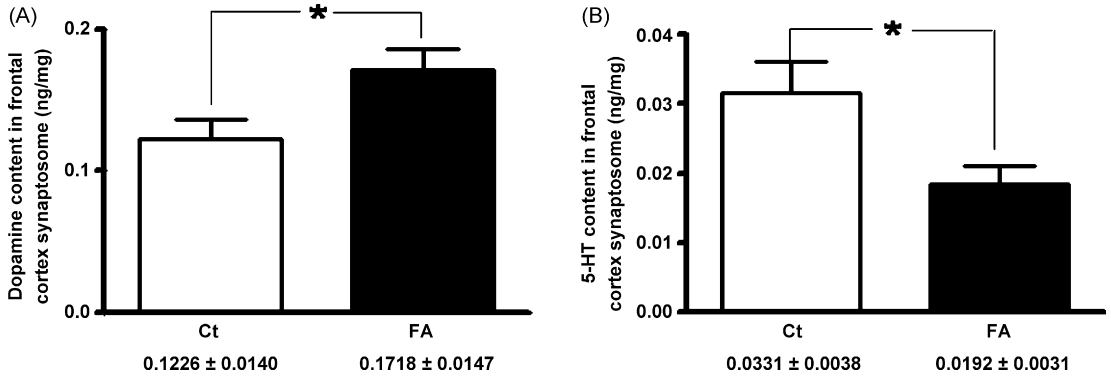

Fig. 3. Repetitive inhalative FA treatment disturbed dopamine and 5-HT in frontal cortex synaptosome. Dopamine increase (A) and 5-HT decrease (B) significantly in frontal cortex synaptosome after inhalative FA treatment. The underlying numbers indicate the weight ratio of monoamine and frontal cortex (ng per mg frontal cortex). $^*P < 0.05$. Control, $N = 5$; FA, $N = 5$. Ct, control; FA, inhalative FA treatment.

(NR2A), dopamine receptor subtypes D1 and D2 [1]. Furthermore, hypothalamus–pituitary axis, which was also impaired after FA exposure, played essential roles in psychology and psychiatry and even memory impairment [17,21].

In the current study, we investigated the aggressive behavior after repetitive inhalative FA exposure in male SD rats and explored the underlying mechanisms. We found that after repetitive inhalative FA exposure, the male rats showed cumulative enhancement of aggressive behavior. And further inhalative FA exposure increased dopamine and decreased 5-HT in frontal cortex synaptosome. As the usage of FA becomes more and more popular in people's life, it is important to clarify the side effects of FA on aggression which is so easily seen and crime related in the currency. Furthermore, our study provided an interesting study subject for people to make further explorations in the future.

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