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·Original Article·

Uric formaldehyde levels are negatively correlated with cognitive abilities in healthy older adults

Jing Yu[1, 3,*], Tao Su[2,4,*], Ting Zhou[1], Yingge He[2], Jing Lu[2], Juan Li[1], Rongqiao He[2]

[1]*Center on Ageing Psychology, Key Laboratory of Mental Health, Institute of Psychology, Chinese Academy of Sciences, Beijing 100101, China*

[2]*State Key Laboratory of Brain and Cognitive Science, Institute of Biophysics, Chinese Academy of Sciences, Beijing100101, China*

[3]*Faculty of Psychology, Southwest University, Chongqing 400715, China*

[4]*The University of Chinese Academy of Sciences, Beijing 100049, China*

[*]These authors contributed equally to this work.

Corresponding authors: Juan Li and Rongqiao He. E-mail: lijuan@psych.ac.cn, herq@sun5.ibp.ac.cn

ABSTRACT

Recent studies have shown that the abnormal accumulation of endogenous formaldehyde could be a critical factor in age-related cognitive decline. The aim of this study was to estimate the correlation between uric formaldehyde and general cognitive abilities in a community-based elderly population, and to measure the extent and direction in which the correlation varied with demographic characteristics. Using a double-blind design, formaldehyde in human urine was analyzed by high-performance liquid chromatography (n = 604), and general cognitive abilities were measured using the Montreal Cognitive Assessment (MoCA). Demographic characteristics, in terms of age, gender, residential region, and education were taken into consideration. We found that uric formaldehyde levels were inversely correlated with the MoCA score, and the concentration varied with demographic features: higher odds of a high formaldehyde level occurred among the less educated and those living in old urban or rural areas. In cytological experiments, the level of cellular formaldehyde released into the medium increased as SH-SY5Y and BV2 cells were incubated for three days. Formaldehyde in excess impaired the processes of N2a cells and neurites of primary cultured rat hippocampal cells. However, removal of formaldehyde markedly rescued and regenerated the processes of N2a cells. These results demonstrated a negative correlation between the endogenous formaldehyde and general cognitive abilities. High formaldehyde levels could be a risk factor for cognitive impairment in older adults, and could be developed as a non-invasive marker for detection and monitoring of age-related cognitive impairment.

Keywords: uric formaldehyde; the Montreal Cognitive Assessment; education; learning; living region

INTRODUCTION

It is widely known that exogenous formaldehyde, as used in manufacturing/building materials and numerous household products, can cause many health-related problems[1] and cerebral dysfunction[2]. Both epidemiological and animal studies have shown that overload of exogenous formaldehyde is associated with impaired performance in learning, memory, and equilibrium, and even results in damage to the central nervous system[3, 4]. In addition, intentional ingestion of significant doses of formaldehyde

revealed a decline in the neuropsychological integrity of participants[5].

In recent years, a considerable literature has shown that the human body generates and degrades endogenous formaldehyde[6], and excessive endogenous formaldehyde leads to neurodegenerative diseases[7-12]. In 2002, Hua and colleagues showed that neuronal tau is vulnerable to formaldehyde and forms stable aggregates in SDS-PAGE[13]. Zhang and coworkers further showed that tau aggregation results from changes in the protein conformation induced by formaldehyde[14]. In humans, endogenous formaldehyde is typically present in the brain, blood, urine, and other tissues[15, 16]. According to the "formaldehyde stress" hypothesis[17], the abnormal accumulation of endogenous formaldehyde can cause abnormal changes in proteins, resulting in neuronal responses such as tau hyperphosphorylation[18], DNA damage[19], reduced long-term potentiation[20] and even cell death[21], followed by associated dysfunctions. The concentration of endogenous formaldehyde tends toward equilibrium under normal physiological conditions. However, under certain stresses like aging, the metabolism of endogenous formaldehyde becomes unbalanced, resulting in chronic cognitive impairment[22, 23].

Moreover, clinical trials have shown that uric formaldehyde concentrations are significantly higher in patients with Alzheimer's disease (AD) than in unaffected older adults[8, 24, 25]. In an autopsy study, researchers found that the hippocampal formaldehyde in AD patients is significantly higher than that in age-matched controls or young people[26]. Therefore, there may be a negative association between endogenous formaldehyde and cognitive abilities in age-related dementia[27]. One question that needs to be answered, however, is whether these findings based upon data from clinical trials and animal models apply to community-based populations, that is, whether the inverse correlation between endogenous formaldehyde and cognitive abilities persists in relatively healthy older adult populations. In all the studies reviewed so far, the role of the demographic characteristics of a population (such as age, gender, education, and residential region) has not been considered.

Other than sampling serum or the brain, uric formaldehyde measurement has several advantages[28], including the non-invasive nature of sample collection and the presence of fewer interfering proteins. Since proteins interfere with formaldehyde detection, urine was analyzed instead of serum which contains high protein levels. The key objectives of this study were to (1) estimate the correlation between uric formaldehyde and general cognitive abilities in a community-based elderly population; (2) measure the extent and direction in which the correlation varies with demographic characteristics (age, gender, education, and residential region); and (3) assess the impairment of processes and connections between neurons in the presence of formaldehyde.

MATERIALS AND METHODS

Data Collection

One thousand and fifty-six participants were enrolled in the study. They were randomly selected from local residents aged 60 years and above in six communities in Beijing; among these, three communities were in Chao Yang District, one in Xi Cheng District, and two in villages in Chang Ping District. The Chao Yang, Xi Cheng, and Chang Ping Districts were selected to represent newly developed (new town), old downtown (old town), and rural areas (rural area) of Beijing, respectively. Among the 1056 participants, 256 did not agree to urinalysis, and another 85 agreed to bring a urine sample, but failed to do so for personal reasons. Eventually, 715 participants agreed and succeeded in providing suitable morning urine samples. To guarantee the reproducibility of analytical results, aliquots of the first urine in the morning were taken before participants had breakfast. Participants were asked to take a normal diet and avoid consumption of fatty and spicy foods for one week prior to sampling. The samples were stored in a sealed sterile container at −80°C until analysis. This study was approved by the Ethics Committee (H11036). Informed consent was given by each participant.

Participants

Experienced psychiatrists performed clinical diagnoses for all the participants based on history, physical exam, and the Neuropsychiatric Inventory (NPI)[29]. The Activities of Daily Life[30], Global Deterioration Scale[31], Clinical Dementia Rating[32], Hachinski Ischemic Score[33], and the Structured Clinical Interview for DSM Disorders (depression and anxiety parts only)[34] were used. Research assistants

with a background in psychology administered the neuropsychological battery, in which the Montreal Cognitive Assessment (MoCA)[35] score was included and used as the index for general cognitive abilities. The MoCA is more sensitive in detecting cognitive decline than the widely-used Mini-Mental State Examination[35-38]. All the research assistants and clinicians were intensively trained and high inter-rater reliability was obtained (>90%). The screening process was standardized with a comprehensive case report form recorded for each participant.

The morning urine samples were collected in the same week after the neuropsychological tests. Participants were excluded if they: (1) abused alcohol or other substances; (2) had central nervous system diseases or neurological disorders (n = 6); (3) were diagnosed with any form of emotional disorder (e.g., depression and anxiety) (n = 9); (4) were diagnosed with cognitive impairment (i.e., dementia and mild cognitive impairment) (n = 91); or (5) quitted the tests, thus did not have clinical diagnostic results (n = 5). The data from 604 older adults were included in the statistical analysis.

Analysis of Uric Formaldehyde by HPLC

Uric formaldehyde analysis and neuropsychological tests were carried out in a double-blinded manner. Uric formaldehyde was measured as previously described[39]. The urine sample (1 mL, thawed at 4°C) was pipetted into a 1.5-ml Eppendorf tube and centrifuged (12 000 rpm for 10 min at 4°C). A 0.4-mL aliquot of the supernatant was mixed with 2,4-dinitrophenylhydrazine (DNPH, final concentration 0.1 g/L in acetonitrile) and 0.1 mL trichloroacetic acid. Samples were vortexed vigorously for 30 s and then centrifuged (12 000 rpm for 10 min at 4°C). The supernatant was added to a 2-mL glass vial, heated in a 60°C water bath for 30 min, and then analyzed by high-performance liquid chromatography (HPLC). The HPLC system (LC-20A, Shimadzu, Japan) was equipped with an ultraviolet detector. The mobile phase was 65% acetonitrile-water solution. The flow-rate was 0.8 mL/min, and column temperature 35°C. The formaldehyde-DNPH derivative was eluted from the HPLC column at a retention time of 6–7 min at the maximum wavelength of 355 nm.

Cell Culture and Assay of Endogenous Formaldehyde

SH-SY5Y cells (a human neuroblastoma cell line)[40] and BV2 cells (a microglial cell line) were cultured in DMEM as described[41], and aliquots of their media were taken for measurement of formaldehyde at different times during culture. N2a cells (a Neuro-2a mouse brain neuroblastoma cell line) were cultured in the presence of 100 μmol/L formaldehyde for 4 h, then the medium was replaced with fresh DMEM without formaldehyde for another 4 h before observation under light field on an Olympus IX-71 inverted contrast microscope.

For holographic time-lapse microscopy, the HoloMonitor™ M4 digital holographic microscope captured 3D information from SH-SY5Y cells (inoculation, 5×10^5 per mL) treated with 0.5 mmol/L formaldehyde. Interfering wave-fronts were induced by a 0.8 mW HeNe laser (633 nm) and holograms digitally captured every minute as described by Molder *et al.*[42]. The holograms were analyzed and videos of dynamic cellular morphology were produced using HstudioM3™ software.

Cultures of hippocampal cells from 18-day embryos of Sprague-Dawley rats were established, as described previously[43]. To assess the impairment of neurites, a monoclonal antibody against MAP2 was used to stain neurites and Hoechst 33258 to stain nuclei. Images were captured using an Olympus IX-71 microscope as described previously[44, 45].

The formaldehyde concentration in pig brain was determined as follows. The fresh brains of adult domestic pigs (*Sus domesticus*) aged ~10 months were acquired from a slaughterhouse in Beijing. Samples were immediately taken from the parietal lobe, frontal lobe, temporal lobe, occipital lobe, hippocampus, cerebellum, and brainstem as described by Heffner and colleagues[46]. The concentrations of formaldehyde in these regions were then determined as described previously[39].

Statistical Analysis

The uric formaldehyde level-related group differences in demographic variables and MoCA scores were assessed using one-way analysis of variance (ANOVA) or χ^2 analysis. Pairwise comparisons were further performed when necessary, with the significance levels adjusted by the Newman–Keuls method. Correlations between the uric formaldehyde concentrations, years of education, and general cognitive abilities measured by the MoCA were assessed using partial correlation methods. Linear

regression (stepwise method) was conducted to assess the influence of uric formaldehyde and demographic variables on the MoCA scores. Moreover, univariate analysis was used to assess the interaction between demographic variables and uric formaldehyde on the variance of general cognitive abilities. All statistical analyses were performed using SPSS 15.0 (IBM Corp., Somers, NY).

RESULTS

Demographic Characteristics and Formaldehyde Levels

Urine samples from participants were analyzed, and the absorbance peak area of the formaldehyde derivative of DNPH is shown in Figure S1. The samples were from 604 participants (55.8% women) ranging in age from 60 to 92 (70.16 ± 6.89 years; mean ± SD). Their education ranged from 0 to 24 (10.34 ± 5.31 years). Among them, 57% were from the new town, 25% the old town, and 18% the rural area. Their uric formaldehyde concentrations ranged from 1.22 to 36.30 μmol/L (11.16 ± 5.82). The participants were divided into three groups based on the uric formaldehyde concentrations: low (≤ 8.00 μmol/L, n = 192), medium (8.00–13.20 μmol/L, n = 200), and high (≥13.20 μmol/L, n = 212). Each group had similar numbers of participants to minimize statistical errors. Sample characteristics are summarized in Table 1. Age [F(2, 600) = 0.07, P = 0.935] and gender distribution (χ^2 = 0.72, df = 2, P = 0.698) were comparable across the three groups, while formaldehyde concentrations [F(2, 601) = 1134.27, P <0.001], education years [F (2, 597) = 27.05, P <0.001] and residential regions (χ^2 = 154.84, df = 4, P <0.001) were significantly different among the groups. *Post hoc* Newman–Keuls comparisons revealed that the low formaldehyde group had more education than the medium and high groups (P <0.050). Moreover, regarding the residential region, the low group had the highest proportion of participants living in the new town (91.67%).

Overall, those in the medium and high formaldehyde groups were less educated and more likely to live in the old town or rural area, but they had distributions of age and gender comparable with the low group.

Uric Formaldehyde Levels Correspond with General Cognitive Abilities

Cognitive abilities assessed by the MoCA test decreased considerably with increasing formaldehyde levels [F (2, 597) = 11.17, P <0.001] (Fig. 1A). Further *post hoc* Newman–Keuls comparisons revealed significantly higher MoCA scores in the low formaldehyde group than the medium and high groups (P <0.050).

Inter-correlations between Demographic Variables, MoCA Scores and Uric Formaldehyde Levels

Partial correlation analysis showed that the uric formaldehyde concentrations were inversely correlated with the MoCA scores (r = −0.191, P <0.001) and education

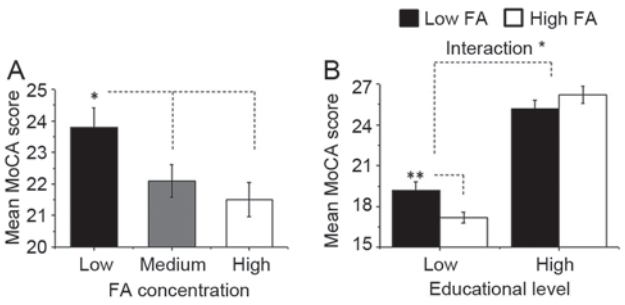

Fig. 1. MoCA scores in (A) groups with different uric formaldehyde (FA) levels and (B) FA groups with different educational levels. *P <0.050, **P <0.010.

Table 1. Demographic characteristics of healthy older adults in the uric formaldehyde (FA) groups

	Low FA	Medium FA	High FA	P-value
FA level (μmol/L)	4.67 ± 1.96	10.85 ± 1.47	17.33 ± 3.85	<0.001
Age (years)	70.10 ± 5.98	70.08 ± 7.29	70.30 ± 7.28	0.935
% Female	58.33	54.27	55.19	0.698
Education (years)	12.50 ± 5.06	9.77 ± 5.15	8.89 ± 5.06	<0.001
% New town [a]	91.67	48.50	32.55	<0.001

[a]Three regions were considered, new town, old town, and rural area.

Table 2. Regression analysis of general cognitive abilities

Variables[a]	Model 1	Model 2	Model 3
Constant[b]	23.888***	50.441***	34.957***
FA[c]	-0.142**	-0.169***	-0.023
Gender[c]		-0.187***	-0.065*
Age[c]		-0.451***	-0.319***
Education[c]			0.533***
Adjusted R^2	0.018	0.244	0.480

[a]Stepwise linear regression to investigate the effects of different variables on MoCA scores. Model 1 only entered one variable as FA; model 2 entered FA, gender, and age; model 3 entered FA, gender, age, and education. [b]Unstandardized regression coefficients for models. [c]These numbers are standardized coefficients (Beta) for different variables. *P <0.050, **P <0.010, ***P <0.001, residuals of the model with *versus* without the variable.

years (r = −0.288, P <0.001), while the MoCA scores were positively correlated with education years (r = 0.581,

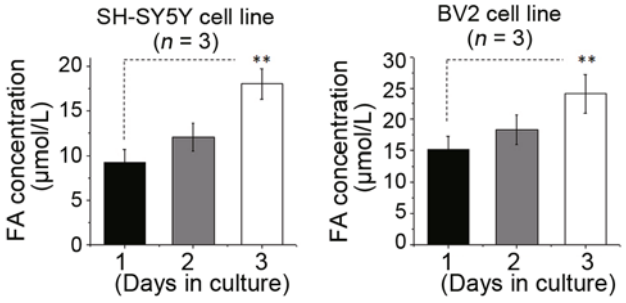

Fig. 2. Changes in the intrinsic concentrations of formaldehyde in cells. Determination of formaldehyde in the culture medium of SH-SY5Y cells (left) and BV2 cells (right) for 3 days using DNPH-HPLC[39]. Data are from three separate experiments. **P <0.010.

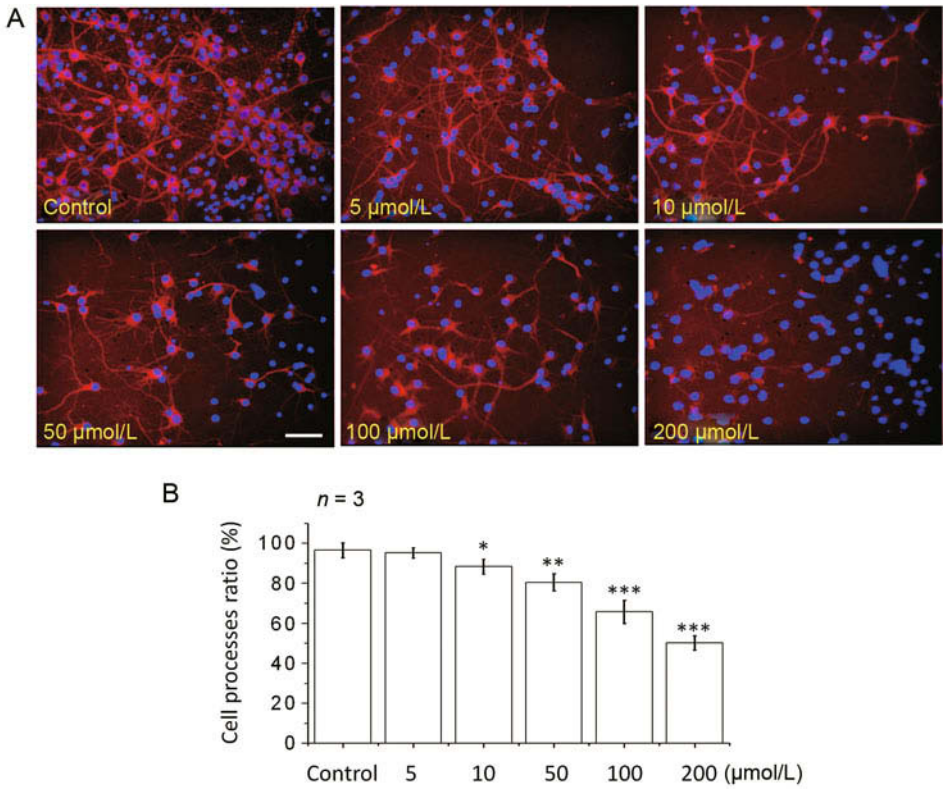

Fig. 3. Changes in neurites in the presence of different concentrations of formaldehyde. A: Immunocytochemical staining of primary cultured hippocampal neural cells with different concentrations of formaldehyde as indicated. Monoclonal anti-MAP2 antibody was used to stain for neurites and Hoechst 33258 for nuclei. B: The proportions of cells with axons among total cells. *P <0.050, **P <0.010, ***P <0.001. Scale bar, 40 μm.

P <0.001), controlling for age and gender.

In addition, linear regression analysis was used to assess the direction and strength of the association between these correlates and general cognitive abilities. The variable uric formaldehyde concentration was no longer significant after education was included in the models (Table 2), indicating a mediating effect of education on the association between uric formaldehyde concentration and cognitive ability.

Furthermore, to investigate how the demographic variables education and living region influence the correlation between uric formaldehyde and cognitive ability, we used univariate analysis, but did not find significant interactions between these variables in the whole sample. However, we found a significant education and uric formaldehyde interaction [$F(1, 168) = 4.30$, $P < 0.050$] in a sub-sample that had participants in the high and low formaldehyde level groups with either a very high education level (≥17 years, $n = 73$) or a very low education level (≤8 years, $n = 105$). Further analysis showed that participants in the low education sub-group had lower MoCA scores with high than low formaldehyde levels [$F(1, 174) = 15.22$, $P < 0.001$], whereas in the high education sub-group, participants with high formaldehyde levels had even better MoCA scores than those with low formaldehyde levels [$F(1, 174) = 7.71$, $P < 0.010$] (Fig. 1B).

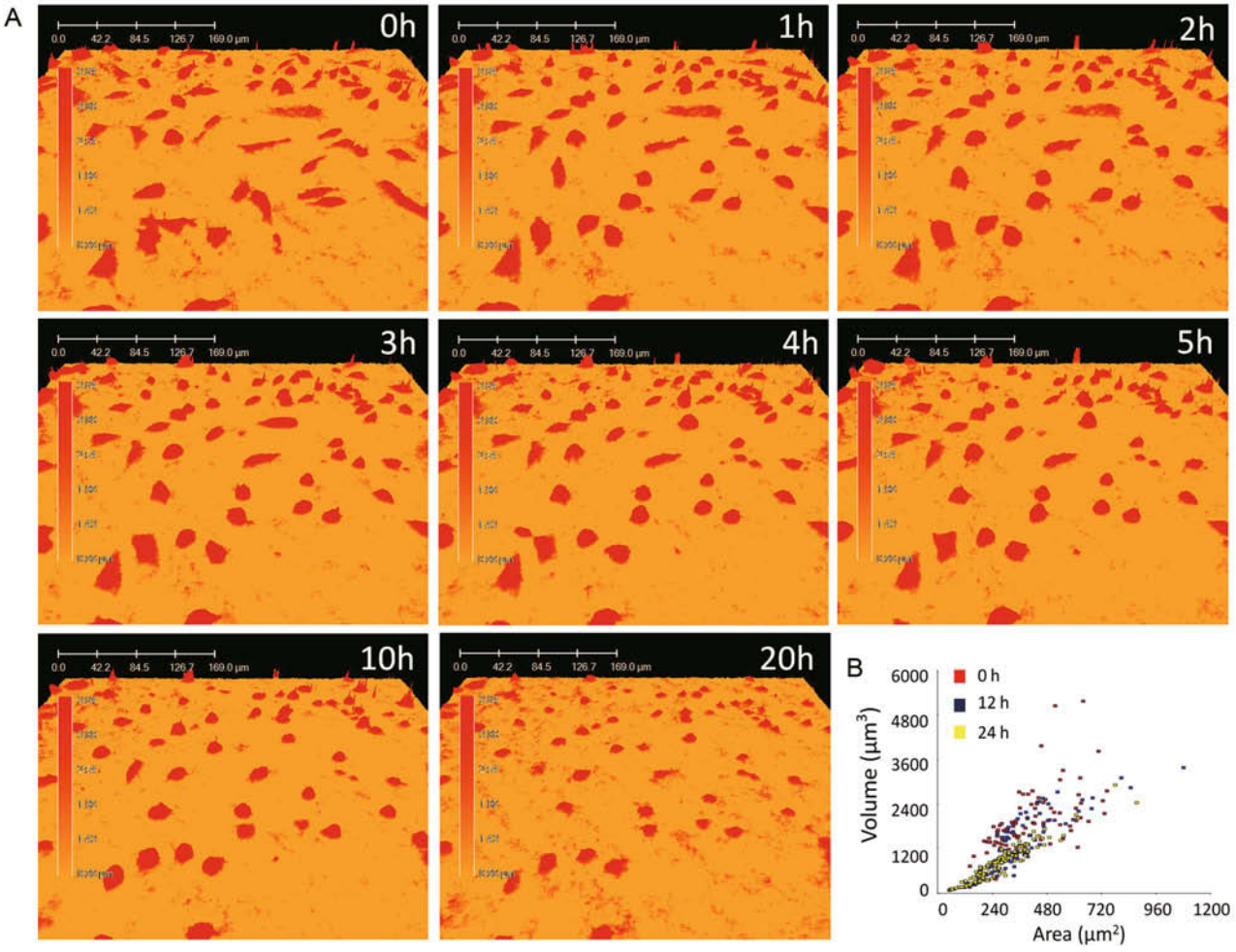

Fig. 4. Kinetic changes of cellular morphology in response to formaldehyde. A: Morphological changes in SH-SY5Y cells incubated with 0.5 mmol/L formaldehyde monitored and visualized with real-time holographic imaging. Cells began to shrink and the mean cell area decreased with time. B: Scatterplot showing decreases in cellular volume with cellular area at 0, 12 and 24 h. The slope represents cellular thickness.

Intrinsic Formaldehyde Increases with Aging

To demonstrate that increased formaldehyde results from intrinsic cellular metabolism, we evaluated the concentrations of formaldehyde in neuronal culture medium. The concentration increased in the medium of SH-SY5Y cells after 72 h in culture (Fig. 2, left). Note that the medium was not replaced with fresh medium during culture. Similar results were obtained for BV2 cells under the same conditions (Fig. 2, right). These findings indicated that intrinsic formaldehyde is produced and accumulates in the medium with time during culture.

Formaldehyde Impairs Processes and Neurites of Neurons

Connections between neurons are the basic structures for information transduction. Here, we stained primary-cultured hippocampal cells with MAP2 to observe changes in their neurites in the presence of different concentrations of formaldehyde and found that the neurites markedly decreased in 50 μmol/L formaldehyde for 24 h compared with those in the absence of formaldehyde (Fig. 3A). Neurites decreased in a formaldehyde concentration-dependent manner (Fig. 3B). Cell death occurred in the presence of 100 μmol/L formaldehyde or higher.

The cytotoxicity of formaldehyde depends on both its concentration[47] and the inoculation density of cells[18]. To clarify this, we performed experiments at different inoculation densities and with different concentrations of formaldehyde. Addition of 500 μmol/L formaldehyde to 5×10^5 cells, or 100 μmol/L formaldehyde to 1×10^5 cells, induced a distinct decrease in the viability of N2a cells (Fig. S2). Furthermore, pig brain contained similar concentrations of formaldehyde (~100 μmol/L) in different regions (Fig. S3). Though the concentration of hippocampal formaldehyde was higher than those in other regions, the difference was not significant ($n = 5$, $P > 0.050$).

To clarify how formaldehyde induces cell death, holographic time-lapse microscopy was used to monitor the kinetic changes in cellular morphology. Formaldehyde (0.5 mmol/L) was added to SH-SY5Y cells (5×10^5/mL) followed by recording of 3D information on cellular morphology with a HoloMonitor™ M4 digital holographic microscope as described by Molder and coworkers[42]. Decreases in cellular volume with cellular area were found (Fig. 4B,

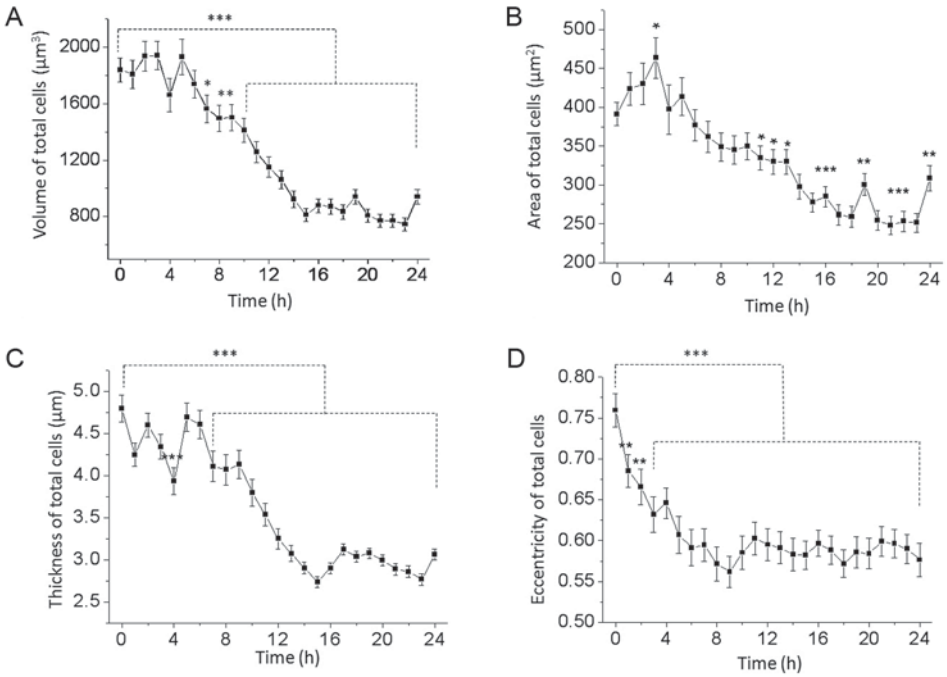

Fig. 5. Changes in morphology of N2a cells with time in the presence of formaldehyde. Conditions were as in Fig. 4. Changes in cellular volume (A), area (B), thickness (C), and eccentricity (D) at different times. $^{*}P < 0.050$, $^{**}P < 0.010$, $^{***}P < 0.001$.

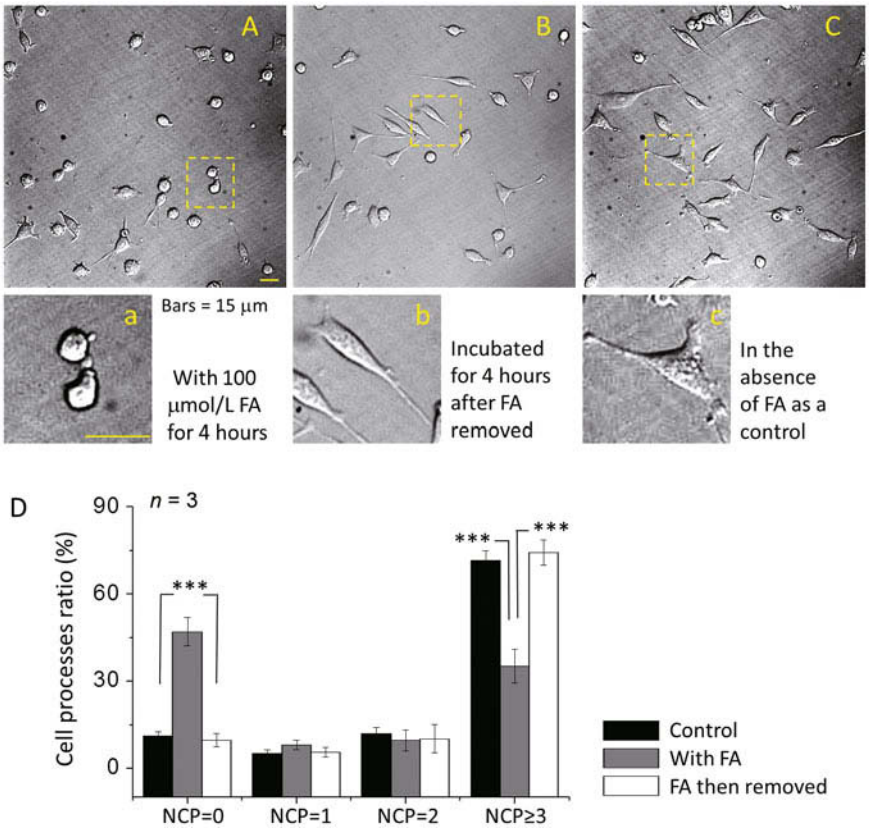

Fig. 6. Changes in processes of N2a cells in the presence and absence of formaldehyde. N2a cells (1 × 10^6/mL) were cultured for 12 h, and then exposed to 100 μmol/L formaldehyde. Aliquots were taken at 4 h of formaldehyde incubation (A, a). Then formaldehyde was replaced with fresh medium and cells were cultured for another 4 h (B, b). Cells in the absence of formaldehyde were used as controls (C, c). B: Cells were classified according to the number of cellular processes (NCP) in randomly-selected fields. The cell process ratio was the number of processes divided by the total cells in the field. ***P <0.001.

Fig. S4, and Supplementary Video1). Changes in cellular eccentricity (morphology of a neuron with its processes) were sensitive to the formaldehyde treatment and changes in volume, area, and thickness were distinctly visible (Fig. 5). These results indicate that formaldehyde at low concentrations impairs the connections between neurons.

To demonstrate that formaldehyde impairs the connections between neural cells, we added formaldehyde to the medium and recorded the changes in the processes of N2a cells. We found that the processes were shrunken and the cells rounded up after 4-h incubation with 100 μmol/L formaldehyde (Fig. 6A and a). However, the processes self-regenerated after replacement of the medium containing formaldehyde with that without formaldehyde (Fig. 6B and b). This suggests that scavenging the excess formaldehyde is beneficial to the regeneration of neural processes.

DISCUSSION

The results of the present study provide community-based data demonstrating a negative association between uric formaldehyde concentration and general cognitive abilities, which has been demonstrated in clinical trials[27] and animal experiments[48]. Concentrations of uric formaldehyde varied with demographic variables: high concentrations were more likely to be found in older adults with less education and living in the old town or rural area. Further analysis showed that the correlation between formaldehyde concentrations and cognitive abilities also varied with education, showing no cognitive decline in the highly-educated group even with high formaldehyde levels, indicating the strong protective

effect of learning (education) on cognitive abilities during normal aging.

Uric Formaldehyde Inversely Correlated with General Cognitive Abilities

Prior studies have reported that humans live in an environment containing both exogenous and intrinsic formaldehyde[49]. As reviewed earlier[27], a negative correlation between the endogenous formaldehyde concentrations and general cognitive abilities has been reported in clinical trials and animal experiments[50]. In this epidemiological study, both ANOVA and correlation analyses revealed that, even without a strict control for everyday diet and regular living habits of participants, the relationship still persisted, indicating that the correlation between these two variables is strong and stable.

Formaldehyde is produced constitutively in the human body and is in equilibrium under normal physiological conditions *via* synthesis and degradation[51], but increases with aging[17, 23]. Animal experiments have shown that the abnormal accumulation of formaldehyde promotes Aβ deposition, a main characteristic of AD[52-54] in addition to the neurofibrillary tangles[27, 55, 56], and induces memory decline and cognitive impairment[12, 26]. Therefore, elevation of endogenous formaldehyde may be one of the important risk factors for sporadic AD during aging[57]. Here, we found that the negative correlation between uric formaldehyde concentration and general cognitive abilities still existed in healthy older adults. For the purpose of dementia prevention, in the future, the uric formaldehyde concentration could be used as a biomarker to monitor the cognitive decline of older adults, and to warn of cognitive impairment with the risk of developing dementia. However, in this study we did not have follow-up data to test this prospective hypothesis. Along with this longitudinal project , we will collect the follow-up data from the same population to test whether the high formaldehyde group in this base-line investigation has higher probability for cognitive decline, and whether changing the formaldehyde concentrations negatively correlates with changes of cognitive scores between the base-line and follow-up investigations.

Risk and Protective Factors for the Uric Formaldehyde Effect

As described previously[23], several factors may lead to formaldehyde stress, inducing an imbalance between its production and degradation, such as aging[26], chronic dehydration[58], genetic factors[59], diet[51], tumors[60, 61], and environmental pollutants[23, 62].

In this study, we included some of these factors. (1) Aging. The metabolic efficiency slows during aging[63], which affects the degradation of formaldehyde, and the formaldehyde levels are markedly higher in aged animals and humans than in the young[20]. However, in our study, we did not find age differences among the three groups of formaldehyde levels in this elderly population. First, this may be due to the relatively narrow age-range of our participants, as they were all 60 years old or above. It is also possible that there is a ceiling effect at some point in aging, so further aging no longer significantly correlates with formaldehyde concentration. Second, it may be due to the sampling bias, for in our study there was a disproportionately larger highly-educated population in the old-old age group (≥ 75 years old) compared with the young-old age group (≥ 60 and <75 years old). Education was negatively associated with the concentration of uric formaldehyde, which could neutralize the age effect. (2) Residential region. From the results of ANOVA, correlation, and regression analyses, we found that the residential region is significantly correlated with the level of uric formaldehyde, shown as older adults living in the old town and rural area having higher uric formaldehyde levels than those living in the new town. The old town was a heavily-industrialized area and most of the participants were workers, whereas most of the participants living in the new town were intellectuals or government employees. So the participants living in the new town had less chance of exposure to environmental pollutants than the participants working in factories or outdoors on farmland, due to China's rural modernization[64]. Previous research has shown that environmental pollutants such as mercury induce formaldehyde accumulation[65], and are believed to be pathogenic factors for AD[1, 66]. (3) Education. The uric formaldehyde of participants negatively correlated with their education, as participants with high education had low formaldehyde concentrations. Education is one of the most powerful demographic variables and has great influence on many aspects of life, such as interests, diet, living habits, and socio-economic status. The "brain battering" hypothesis proposed by Hachinski and colleagues[67] states that

individuals with higher previous education and associated higher socio-economic status are exposed to fewer toxins, have a healthier lifestyle, and have greater access to quality health care, all of which protect against brain lesions[68]. Older adults with higher education could, subjectively, pay more attention to and be more knowledgeable about a healthy diet, and objectively, be more capable of obtaining a healthier diet, which could protect them from pathological accumulation of formaldehyde compared with less educated older adults. Diet is one of the influential factors for the physiological and/or pathological accumulation of formaldehyde, as deficiencies of vitamin B12 or folate in the diet lead to dysfunction of one-carbon metabolism in AD patients[69], while formaldehyde participates in the "one-carbon cycle"[51].

Another interesting finding is that education was even able to counteract the negative effects of endogenous formaldehyde on neural connections or plasticity in sub-sample analysis, as in participants with higher education, though they had elevated formaldehyde concentrations, this elevation had no apparent negative effect on their cognitive abilities. These results are consistent with the "scaffolding theory of aging and cognition" which states that scaffolding is a normal process involving the use and development of complementary, alternative neural circuits to achieve a particular cognitive goal, and extra learning, cognitive training, and exercise promote or repair the scaffolding during the aging process[70]. In the present study, we did find that higher education, which is most probably related to more learning, more engagement in intellectual activities, and more participation in cognitive training, counteracted the impairment of cognitive abilities caused by elevated endogenous formaldehyde and rescued the scaffolding noted here as neuroplasticity. Combining these results, we believe that learning or education is one of the protective factors against the abnormal accumulation of formaldehyde and the corresponding cognitive decline (Fig. 7). However, it should be noted that given the broad spectrum of age-related cognitive decline, it is unlikely that any single biochemical mechanism can fully explain the effect across all individuals[71]. Therefore, the endogenous formaldehyde hypothesis is not an exclusive path, but rather a putative biochemical mechanism explaining the cognitive decline. On the other hand, well-controlled cellular experiments provide much clearer results, suggesting that uric

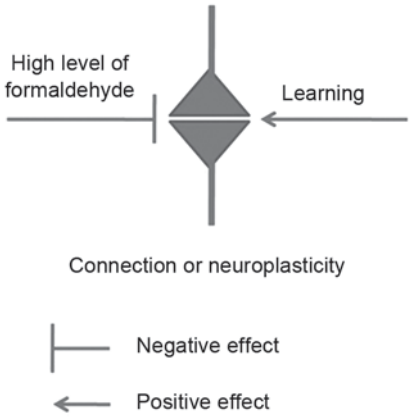

Fig. 7. Schematic of the putative mechanism for the protective effect of learning on cognitive function against high levels of formaldehyde. Learning enhances the connections or neuroplasticity to protect against a high level of endogenous formaldehyde[69].

formaldehyde could function as a non-invasive marker for AD.

We attempted to determine the plasma concentrations of formaldehyde; however, it is difficult to precisely determine the concentration because plasma contains many proteins that interfere with formaldehyde reactions. On the other hand, analysis of uric formaldehyde concentration is repeatable and reliable because urine contains little protein to disturb the analysis of formaldehyde[73]. In fact, uric formaldehyde comes from blood, and formaldehyde easily passes through the blood-brain-barrier[74]. Probably, determination of the level of uric formaldehyde reflects the metabolism of formaldehyde *in vivo*.

In conclusion, to date, this is the first community-based study to investigate the negative correlation between endogenous formaldehyde and general cognitive abilities. A high endogenous formaldehyde level is a risk factor for older adults' cognitive decline, and its evaluation in older adults may be used as a non-invasive approach to monitoring age-related cognitive decline in future. Another important finding to emerge from this study is that demographic variables, in terms of residential regions and education, are critical correlates of the formaldehyde concentrations, shown as high levels in participants with less education living in the old town and rural area. Moreover, the evidence from this study also suggests

that education could be the protective factor against the formaldehyde effect on cognitive abilities.

SUPPLEMENTAL DATA

Supplemental Data include four figures and one video, and can be found online at http://www.neurosci.cn/epData.asp?id=169.

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