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Effect of folic acid diet on the behavior of female mice and ultrasonic vocalization of their F1 offspring

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ABSTRACT

Folic acid (FA) is an essential B vitamin that plays a pivotal in various physiological processes, including neural tube defects, brain functions, neurotransmitter synthesis, and cognition. Earlier studies have suggested FA's role during pregnancy and the development of newborns; however, the broader impact of FA diet on maternal and offspring health remains unclear. Herein, the effects of FA on the behavior of female mice and ultrasonic vocalizations (USVs) of their F1 offspring were evaluated. Briefly, mice were placed into control, 2.3 mg FA, and 8 mg FA. Folic acid was supplemented in female mice at concentrations of 2.3 mg/kg and 8 mg/kg for 6 weeks. Afterward, female mice behavior was assessed *via* open field test, novel object recognition test, and gait analysis as well as acetylcholinesterase activity were performed. Further, USVs of their F1 offspring on postnatal days (PND) 7, 9, 11, and 13 were measured. Results showed that the FA supplementation in female mice reduced locomotor activity, impaired memory, increased anxiety-like behavior, and altered gait (walking pattern). Meanwhile, alterations were also observed in the level of acetylcholinesterase activity, while the change did not attain statistical significance. On the other hand, F1 offspring born from FA 8 mg supplemented mice showed substantial changes in USVs like extended call durations, increased frequencies, and higher amplitudes compared to FA 2.3 mg supplemented female mice offspring. Also, F1 offspring of FA 2.3 mg supplemented mice showed higher vocalizations pattern compared to control F1 offspring. Such study is useful to understand the impact of FA during pregnancy and its potential transgenerational effects, and helpful to understand maternal and offspring health.

Introduction

Folic acid, a synthetic form of folate (also known as vitamin B9), is essential for physiological functions like nucleic acid synthesis, methylation reactions, neurodevelopment, fetal development, cognitive functions and epigenetic mechanisms (Bailey and Ayling, 2009; Greenberg et al., 2011; Caffrey et al., 2023). Studies have examined the transgenerational effects of maternal FA supplementation on offspring development and cognitive functions (Gao et al., 2016; Wang et al., 2018; Virdi and Jadavji, 2022). The potential effects of maternal FA supplementation extend beyond the gestational period and may have implications for subsequent generations (Barua et al., 2014). Earlier studies reported that the FA influences maternal behavior and offspring outcomes across generations and extensively investigated as a potential target for neuronal development and plasticity (Iskandar et al., 2010; Roza et al., 2010; Skórka et al., 2012).

FA deficiency during pregnancy has been associated with cognitive impairments, disruptions in neurotransmitter synthesis, and neurotoxicity, which can impair brain function and behavior in rodents (Smith et al., 2010). Moreover, maternal folate intake has long been recognized for its critical role in preventing neural tube defects (NTDs) and supporting neurodevelopment, especially during the early stages of pregnancy (Czeizel et al., 1992; Greenberg et al., 2011). Vocalization, a fundamental mode of communication in many species has been studied as an indicator of neurodevelopmental alterations in offspring (Branchi et al., 1998; Hofer et al., 2002; Yao et al., 2023). Recent evidence suggests the role of FA in epigenetic modifications could serve as a link between maternal nutritional status and offspring phenotypes (Ly et al., 2016; Joubert et al., 2016).

Despite a growing body of research (Gao et al., 2016; Wang et al.,

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2018; Caffrey et al., 2023), the impact of maternal folic acid supplementation on offspring communication remains underexplored. Adequate maternal folic acid intake during pregnancy positively influences offspring's cognitive and communication abilities, including better language development, and reduces the risk of speech delays (Roth et al., 2011). Insufficient folate levels can negatively impact mood and lower cerebrospinal fluid concentration of serotonin (5-hydroxytryptamine; 5-HT), a neurotransmitter essential for attention, memory, and language acquisition. Additionally, maternal FA supplementation has been shown to influence stress-related vocalizations in rodents. Moreover, evidence suggests that excessive supplementation during pregnancy may alter animal models' ultrasonic vocalizations (USVs) pattern. Mice use USVs to convey information about their emotional states and mediate social interactions. Further, various types of USVs were identified in laboratory mice, including isolation-induced, interaction-induced, and maternal-pup USVs (Zippelius and Schleidt, 1956; Holy and Guo, 2005; Premoli et al., 2021). For example, Zippelius and Schleidt first time defined "whistles of loneliness," which means the USVs emitted by pups during separation from the mother and the littermates (Zippelius and Schleidt, 1956; Premoli et al., 2021).

The changes in USV patterns have been documented in mouse models of neurodevelopmental disorders (NDDs) and autism spectrum disorders (ASD), indicating variations in the number of calls, duration, and frequency (Servadio et al., 2015; Hodges et al., 2017). As vocalization is a crucial aspect of early communication and social behavior, alterations in USVs patterns may reflect underlying neurological or behavioral changes (Portfors, 2007). Given the importance of vocalization in early development, study the impact of maternal FA supplementation on USVs and communication remains critical (Barua et al., 2014).

The effects of maternal FA supplementation on offspring behavior and communication have gained attention from researchers (Roth et al., 2011; Bjørk et al., 2018; Sun et al., 2023). Folic acid is a crucial methyl donor in vivo and plays an important role in supporting brain development during early pregnancy, formation of the neural tube, DNA replication and methylation. Research has demonstrated that 5 mg/kg FA supplementation improves glucose metabolism in offspring exposed to prenatal stressors like lipopolysaccharide (LPS) (Sun et al., 2023). FA dose 2.3 mg/kg aligns with the rodent equivalent of the human recommended dietary allowance (RDA) (~400 μg/day), scaled for metabolic differences, and reflects levels used to model typical dietary supplementation (National Research Council US Subcommittee on Laboratory Animal Nutrition, 1995; Bailey and Gregory, 1999).

Adequate folic acid intake during preconception and early pregnancy has been linked to a reduced risk of severe language delays in children. However, further research is required to elucidate its direct impact on speech development. In a cohort of Norwegian mothers and children, maternal folic acid supplementation during early pregnancy was associated with a lower risk of severe language delay in children aged 3 years (Roth et al., 2011). These findings suggest the potential benefits of folic acid in supporting early speech development and warranting continued investigation in this area. Taken together, this study is helpful to understand the role of two different doses of 2.3 mg/kg and 8 mg/kg supplementation on maternal behavior and the USVs of their F1 offspring.

Material and methods

Animals, diet and groups

Swiss albino female mice 8 weeks old were purchased from ICMR-National Institute of Nutrition, Department of Health Research, Ministry of Health and Family Welfare, Hyderabad, India. All mice were housed under standard laboratory conditions at 25ºC± 1, 12 h light/dark cycle with *ad libitum* food and water. Based on the standard AIN-93G diet for rodents, female mice were randomly placed in three groups (n = 6): Control, FA 2.3 mg, and FA 8 mg. FA 2.3 mg mice were supplemented with AIN-93G food at a concentration of 2.3 mg of folic acid/kg, and FA 8 mg mice were provided with AIN-93G food with a modification of 8 mg of folic acid/kg for 6 weeks (Henzel et al., 2017; Lin et al., 2011). Further, after 6 weeks of FA supplementation, behavioral tests were performed in female mice, and thereafter, they were mated with male *Swiss albino* mice. The AIN-93G diet was procured from the ICMR-National Institute of Nutrition, Hyderabad. All experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC) of IGNTU, Amarkantak, Madhya Pradesh, India (Ref. No. IGNTU/IAEC/2021/13).

Behavior assessments of folic acid-supplemented female mice

Open field test (OFT)

OFT was performed to check anxiety and exploratory behavior of FA supplemented female mice. Briefly, mice were habituated in an open wooden box (47 cm × 47 cm × 40 cm) with base divided into sixteen equal quadrants for 2 consecutive days. On day 3 (test day), mice were placed in the center of the open box arena and allowed to explore for 10 min. Further, mice exploration and anxiety like behavior was measured as followed by total distance travelled, time spent in the peripheral zone and time spent in center zone using ANY-maze software (Version 7.4).

Gait analysis

Motor coordination and walking patterns of FA-supplemented mice were analyzed using the foot printing test (FPT). In FPT, mice were allowed to walk along a narrow, 60 cm-long white paper. After three training trials for 5 min each, the paws of the mice were painted using nontoxic watercolors: forepaws (orange) and hind paws (blue). Immediately after painting, mice were allowed to walk on the path covered with white paper. Three steps of each mouse were measured and analyzed for left front stride length, right hind and front stride length, fore base width (the width between the right and left forelimbs), and hind base width (the width between the right and left hind limbs).

Novel object recognition test (NORT)

A NOR test was performed as described previously to assess the recognition memory in folic acid-supplemented mothers (Moigneu et al., 2023). Initially, mice were habituated to an empty arena (47 cm × 47 cm × 40 cm). After that, each mouse was placed in an open arena with two familiar objects equidistant from the wall to explore the objects for 8 min. Then, all mice were placed back in their home cage. After 2 h, mice were placed back in the arena where one object was replaced with a novel object, and mice left for 8 min to explore. The time spent interacting with the novel object was recorded on camera and analyzed using Any-maze 7.4 software. The discrimination index (DI) was calculated as follows: DI= Exploration time of novel object - Exploration time of familiar object / Exploration time of novel object + Exploration time of familiar object.

Biochemical assay

Acetylcholinesterase (AChE) activity

FA-supplemented female mice were sacrificed by cervical dislocation for estimation of acetylcholinesterase activity. Brain homogenate was prepared using 0.1 M phosphate buffer (pH 8.0). The 0.1 M phosphate buffer was initially mixed with DTNB (5,5′-dithiobis-(2-nitrobenzoic acid) used as an oxidizing agent and brain tissue homogenate. Then after, Acetylcholine iodide (75 mM) was used as a substrate, and absorbance was measured in a spectrophotometer at 412 nm.

Ultrasonic vocalizations (USVs) of offspring

To record ultrasonic vocalization, pups ($n = 4$) from distinct litters belonging to FA 2.3 mg, FA 8 mg, and control group were selected. To record isolation calls, pups from PND 7, 9, 11, and 13 were placed inside a soundproof Styrofoam box attached with an ultrasound microphone (Avisoft UltraSoundGate 116Hb condenser microphone capsule CM16, Avisoft bioacoustics, Germany) and ultrasonic frequency detector. The microphone was placed about 6 cm above the pups (sensitive to 20 Hz =135 kHz) and 6 min of vocalization patterns were recorded (Shu et al., 2005). Avisoft UltraSoundGate 116Hb condenser microphonecapsule CM16, Avisoft bioacoustics was used with settings samplingrate 250 kHz; format 16 bit.

USVs analysis

Recordings were transferred into Avisoft SASLab Pro (Version 5.3.2) and Fast Fourier Transformation (FFT) was conducted for acoustics analysis. Spectrograms were generated with an FFT length of 512 points and a time window overlap of 50 % (100 % frame size, Hamming window). The spectrogram was produced at a frequency resolution of 488 Hz and bandwidth of 635 Hz. Spectrograms of different types of USVs call patterns such as Chevron, Complex, Composite, Downward, Flat, Frequency steps, Harmonics, Shorts, Two-syllables, and Upwards produced by control F1 offspring have been shown in supplementary file (Fig S1). The number and duration of calls were analyzed for each test day. The cut-off frequency was set at 15 kHz to eliminate the external noise calls. Thereafter, an automatic threshold-based algorithm was used to detect desired calls with a hold time mechanism (hold time-10ms). After the detection by automated parameter measurements of software, calls were manually checked for the accuracy of call detection.

Statistical analysis

All the data are expressed as the mean ± SEM. Ordinary one-way ANOVA followed by Tukey's multiple comparisons were applied for all experiments. Further, Ordinary two-way ANOVA followed by Tukey's multiple comparisons were applied to analyze the gait test. Additionally, for USVs data, two-way ANOVA was performed with group (control, FA 2.3 mg/kg, FA 8 mg/kg) and time (postnatal days 7, 9, 11, 13) as independent variables. Interaction effects between group and time were explicitly tested. In all calculations, $p < 0.05$ was considered a significant difference between groups.

Results

Folic acid 2.3 mg/kg and 8 mg/kg diet alters the behavior of female mice

To assess the effects of FA on anxiety and exploratory behavior, cognitive functions, and walking patterns, different behavior tests were performed. In OFT, mice supplemented with 2.3 mg/kg and 8 mg/kg FA showed significantly reduced total distance traveled compared to controls (Fig. 1A: $p < 0.005$ and $p < 0.002$), indicating diminished exploratory activity and increased anxiety-like behavior. Time spent in the peripheral zone showed no significant differences across all the groups (Fig. 1B). Meanwhile, 8 mg/kg FA mice significantly spent less time in the center zone compared to control mice (Fig. 1C: $p < 0.008$). Additionally, ANY-maze track plots displayed activity patterns in groups (Fig. 1D-F).

Gait analysis was done to evaluate mice walking pattern behavior. Further, results revealed that 8 mg FA supplementation significantly reduced stride length compared to controls (Fig. 2A: $p < 0.04$), suggesting impaired motor coordination whereas, no significant differences were observed in FA 2.3 mg mice. Similarly, alterations were observed

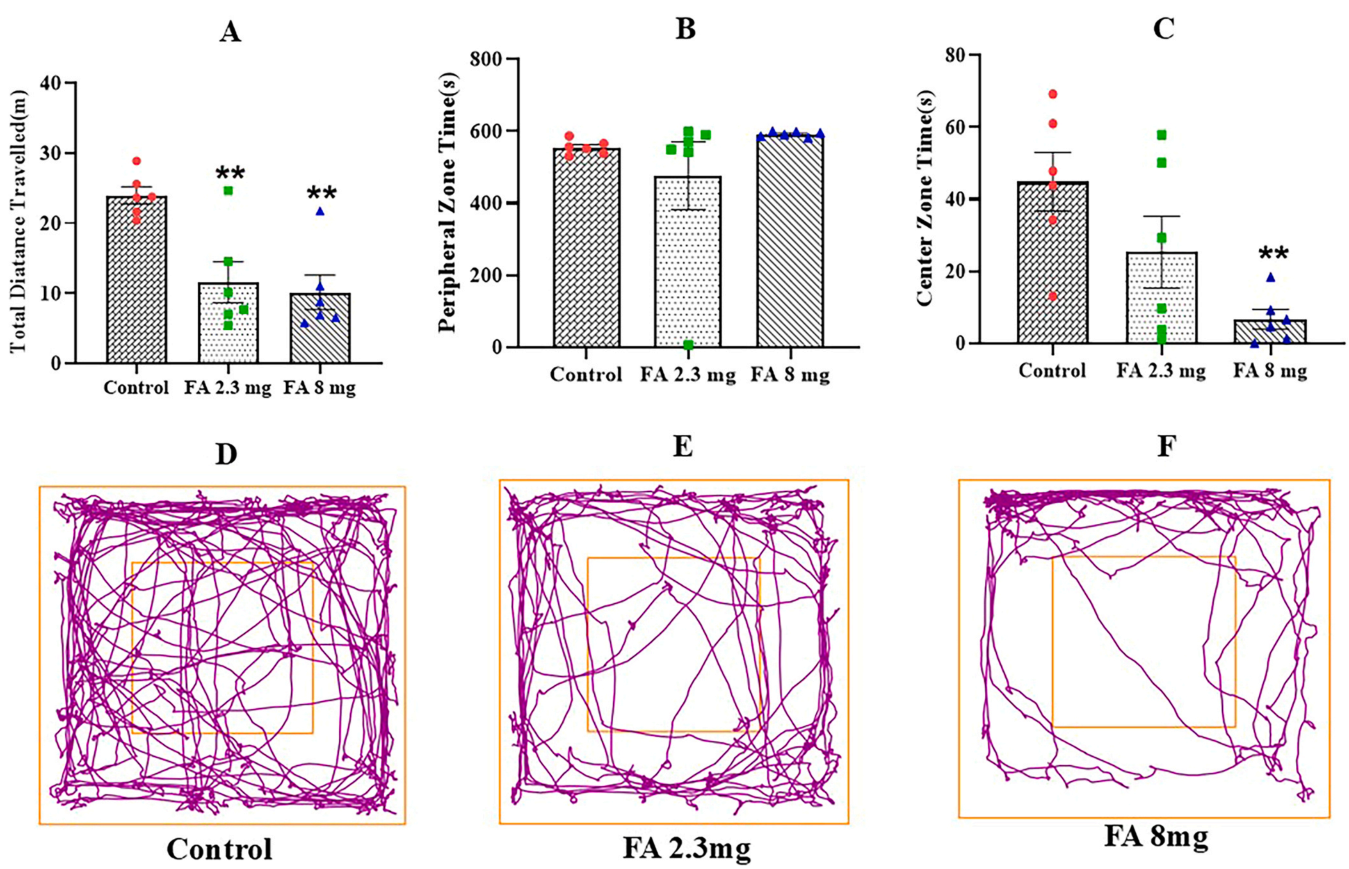

Fig. 1. Effects of folic acid in open field test (A) Total distance travelled in the open field arena. (B) Time spent in the peripheral zone. (C) Time spent in the center zone. (D-F) ANY-maze track plots displayed activity patterns. Data expressed as mean ± SEM ($n = 6$ mice/group). * *$p < 0.01$ vs. Control group.

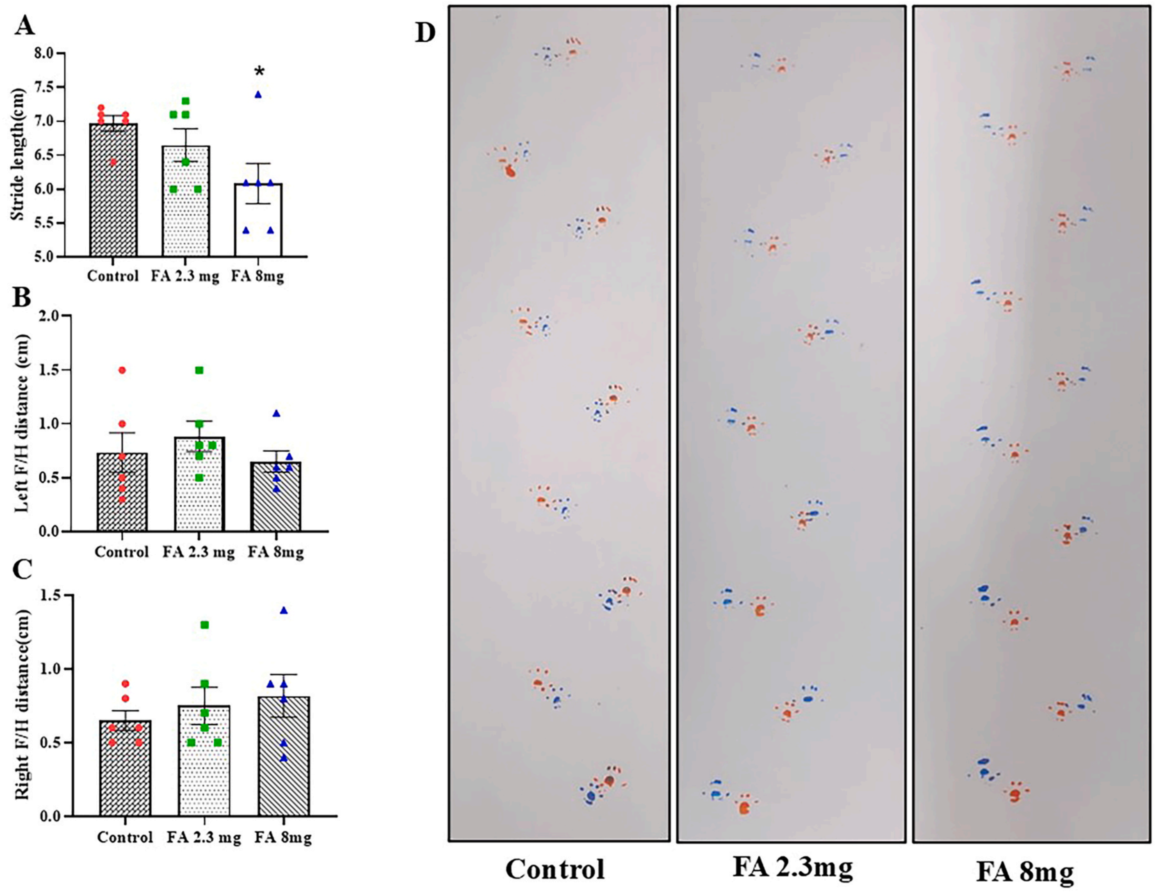

Fig. 2. Effects of folic acid in walking patterns. Gait analysis (foot printing test) (A) Stride length (B) Left forelimb/hindlimb distance (C) Right forelimb/hindlimb distance and (D) Representative footprints image from each group. Data expressed as mean ± SEM (n = 6 mice/group). *p < 0.05 vs. Control group.

in left front/ hind distance (Fig. 2B) and right front/ hind distance (Fig. 2C) while changes did not achieve statistical significance differences. Lastly, representative footprints image from each group were shown in (Fig. 2D).

To assess the recognition memory, a novel object recognition test was conducted. Results showed that FA 2.3 mg ($p < 0.001$) and FA 8 mg ($p < 0.001$) supplemented mice exhibited significantly low discrimination index compared to control mice (Fig. 3A). Additionally, to evaluate the effects of folic acid in acetylcholine, AChE activity was conducted and result showed no significance differences between the groups (Fig. 3B)

Effects of folic acid in offspring ultrasonic vocalizations (USVs)

The USVs of F1 offspring were assessed on PND 7, 9,11 and 13. The results revealed significant alterations in the total duration of calls

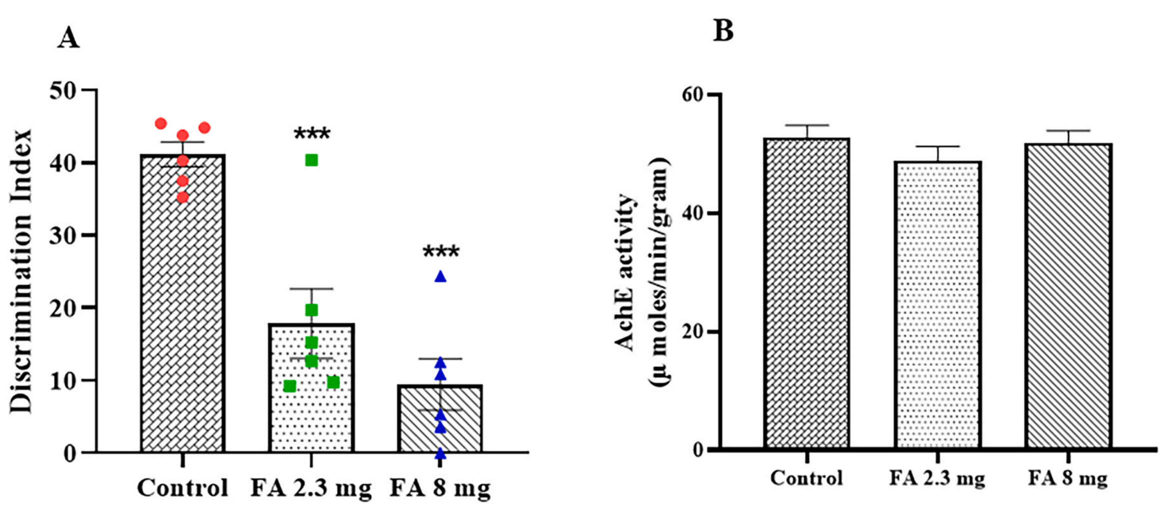

Fig. 3. Effects of folic acid on (A) Novel object recognition test and (B) Acetylcholinesterase activity. The data present the value of mean ± SEM from (6 mice/ group). * **p < 0.001 vs Control group.

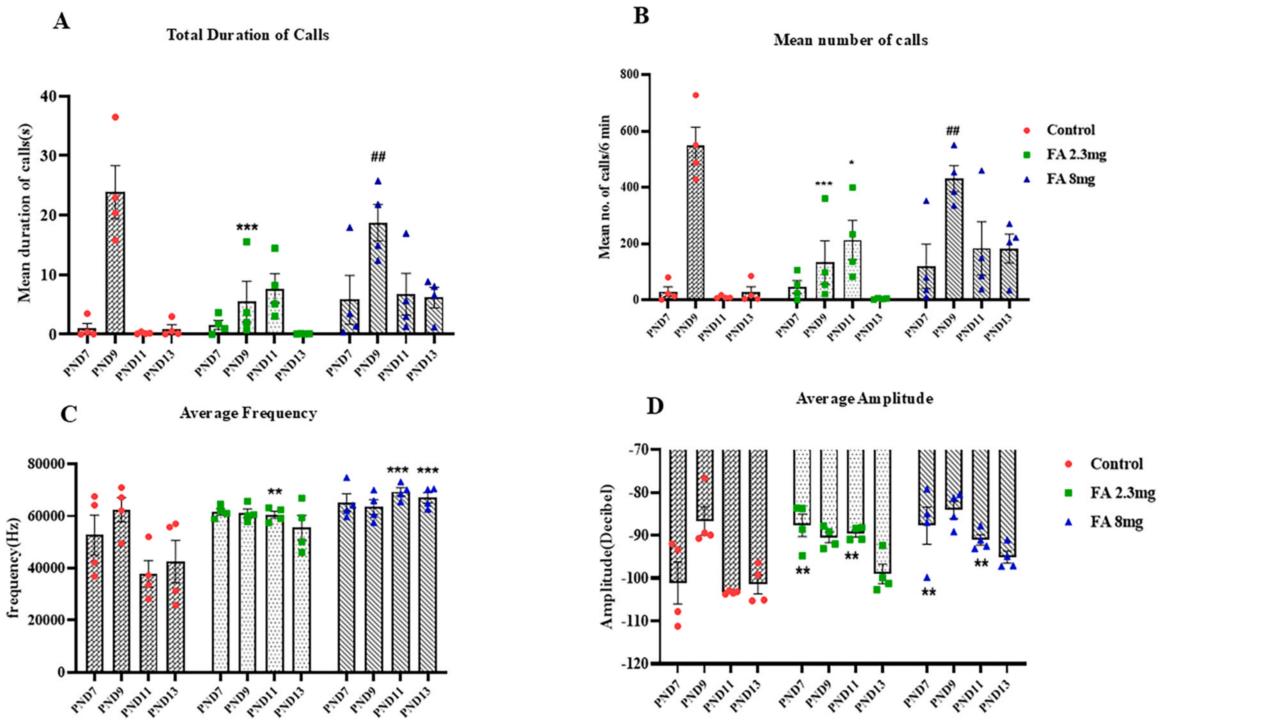

Fig. 4. Effects of FA in ultrasonic vocalizations of F1 offspring (A) Total call duration (B) Number of calls (C) Average call frequency (kHz) (D) Average call amplitude (dB). Data expressed as mean ± SEM (n = 4 pups/group). * **p < 0.001, * *p < 0.01, *p < 0.05 vs Control group. ##p < 0.01vs FA 2.3 mg group.

(Fig. 4A) and number of calls in FA 2.3 and FA 8 mg groups at PND 7, 9 and 13 compared to the control group. On PND 9, total duration of calls was significantly reduced in FA 2.3 mg group compared to the control group (P < 0.001). Additionally, FA 8 mg group showed significantly increased call duration relative to FA 2.3 mg group (P = 0.001). Regarding the number of calls (Fig. 4B), the FA 2.3 mg group exhibited a significant decrease in calls on PND 9 (P < 0.001), whereas a significant increase in calls was observed on PND 11 (P = 0.02) when compared to the control group. On PND 9, the number of calls was significantly higher in the FA 8 mg group compared to the FA 2.3 mg group (P = 0.001), indicating that different levels of FA supplementation affect the number of calls. No significant differences in call frequency and amplitude were observed on PND 7, 9, and 13. However, on PND 11, the call frequency was significantly increased in the FA 2.3 mg group compared to the control group (P = 0.03). On both PND 11 and PND 13, call frequencies were significantly higher in the FA 8 mg group compared to the FA 2.3 mg group (P < 0.01) (Fig. 4C). Furthermore, the average amplitude of calls was significantly reduced in both the FA 2.3 mg and FA 8 mg groups on PND 7 and PND 11 compared to the control group (P = 0.002) (Fig. 4D).

Discussion

Folic acid plays an important role in various physiological processes, including epigenetic modifications, DNA synthesis, and neurodevelopment (Bailey and Ayling, 2009; Greenberg et al., 2011; Caffrey et al., 2023). Several studies have supported the transgenerational effects of folic acid supplementation during pregnancy on both offspring development and maternal cognitive functions (Greenberg et al., 2011; Gao et al., 2016; Wang et al., 2018; Virdi and Jadavji, 2022). Moreover, the study of USVs have been widely examined from an ethological perspective and gained significant attention in the field of neurological and psychiatric disorders, beginning with those characterized by social interaction and communication deficits, such as neurodevelopmental NDDs and ASD (Moy and Nadler, 2008; Premoli et al., 2021). Hence, the present study investigated the effects of FA-supplementation on female mice behavior and USVs of their F1 offspring.

Supplementation of 4 mg/kg diet of folic acid throughout pregnancy resulted in impaired memory and increased anxiety and depression-like behavior (Silveira et al., 2022). Further, to check the exploration and anxiety like behavior OFT was performed. OFT result indicated that both FA supplementation groups (2.3 mg/kg and 8 mg/kg) exhibited reduced exploration compared to the control group. This finding suggests that folic acid supplementation may influence exploratory behavior and general activity levels in female mice. On the other hand, the gait analysis revealed a significant decrease in the stride length of the left forelimb in the 8 mg/kg folic acid group compared to the control. This finding suggests that high-dose FA supplementation might affect motor function or coordination. Moreover, embodiment is a reciprocal interaction between the mind and body. Alteration of gait is clinically well-known as the motor phenomenon for neurological disorders. Embodiment has been highly relevant to comprehending cognitive and emotional processes in depression (Nisha and Paramanik, 2024; Tiwari and Paramanik, 2025). FA supplementation affects the motor function of rats in a dose-dependent manner (Shooshtari et al., 2012). However, the lack of significant differences in other gait parameters indicates that this effect may be subtle or limited to specific aspects of motor function.

Previous studies generally associated with folic acid improved cognitive functions (Roza et al.,2010; Iskandar et al., 2010). In this study, NOR results exhibited a significantly lower discrimination index in both FA-supplemented groups when assessing recognition memory compared to the control group. The discrepancy might be associated with differences in dosage, timing of supplementation, or specific cognitive domains assessed. Moreover, during pregnancy, adequate folic acid intake is crucial for preventing neural tube defects and other cognitive abnormalities in the developing fetus (Blom, 2009). Adequate folate levels are essential for normal neurodevelopment and cognitive function (Araújo et al., 2014). Some research indicates that excessive FA intake might unexpectedly affect behavior and neurological outcomes (Morris, 2003; Wiens, 2016).

Additionally, the cholinergic system, including acetylcholinesterase activity, plays a crucial role in cognitive functions like learning, memory, and attention (Hasselmo, 2006). FA plays an important role in one-carbon metabolism and the production of S-adenosylmethionine (SAM), a universal methyl donor (Miller, 2008). SAM is involved in various methylation reactions, including synthesizing phosphatidylcholine, a precursor for acetylcholine (Blusztajn et al., 1979). For instance, rats fed a folic acid-deficient diet showed decreased acetylcholine levels in the brain, suggesting that FA status may influence cholinergic neurotransmission (Crivello et al., 2010). However, in this study no changes were observed in the AChE activity. Interestingly, the study reported that FA deficiency in mice led to increased activity of acetylcholinesterase in certain brain regions, particularly in aged animals (Jadavji et al., 2015; Irwin et al., 2016; Virdi and Jadavji, 2022).

Meanwhile, communication is linked to social behavior, so the USVs study has become a valid assay in behavioral readout and monitoring (Premoli et al., 2021; Premoli et al., 2023; Yao et al., 2023). USVs of mice are an interesting theme in the communication research and social behavior that is still under an investigation. Branchi et al. (1998) preliminary characterized USVs in infant of laboratory mice under different conditions, establishing a foundation for understanding these early communicative behaviors. Wöhr and Schwarting (2013) emphasized the importance of USVs for emotions and motivation in rodents. In this study, maternal folic acid supplementation revealed significant changes in USVs of their F1 offspring. Pups from the 8 mg/kg group demonstrated longer call durations than the control and 2.3 mg/kg groups. Additionally, both FA-supplemented groups showed a significant increase in the number of calls. These findings suggest that maternal FA intake can influence early communicative behavior in offspring, potentially reflecting alterations in neurodevelopment or stress responsivity.

A total number of calls is a critical indicator of arousal, distress, and social motivation in neonatal mice. An increased number of calls is generally associated with heightened maternal attachment-seeking behavior, while a reduced number of calls may reflect impairments in neurodevelopment or anxiety-related responses. Call duration has been proposed as a marker of emotional and communicative intent (Mun et al., 2015). Longer call durations are often associated with more significant distress or a strong drive to elicit a maternal response. In contrast, shorter calls may indicate deficiencies in vocal motor control or neurodevelopmental delays (Mun et al., 2015). Additionally, postnatal day (PND) 7–13 corresponds to the early neonatal phase, during which isolation-induced USVs are robustly expressed as pups rely heavily on vocalizations to communicate distress and solicit maternal care. These vocalizations reflect sensory, motor, and emotional maturation (Hofer et al., 2002; Portfors, 2007). Not only that, this period spans the transition from reflexive vocalizations (PND 7) to more coordinated, context-dependent calls (PND 13), coinciding with the onset of auditory system maturation and social behavior precursors (Wöhr and Schwarting, 2013). Prior studies demonstrate that maternal diet and environmental factors during this period can significantly alter USV patterns, providing a window to assess the transgenerational effects of folic acid (Branchi et al., 1998; Virdi and Jadavji, 2022).

In our study, PND11 pups from both folic acid groups exhibited higher average call frequencies, with the 8 mg/kg group showing the most pronounced increase. The amplitude of calls was also significantly higher in both FA groups compared to the control. Alterations in call characteristics could indicate changes in the development of the pups' vocal production system or emotional/arousal states (Simola and Granon, 2019). These differences were most evident on PND 11, which might be related to age-dependent or specific developmental milestones. Amplitude variations can indicate differences in vocal strength and lung capacity, which may be affected by prenatal nutrient availability. FA supplementation has been implicated in neuroprotective effects, which could influence pups' motor coordination and vocalization control (Premoli et al., 2023).

Further, maternal FA supplementation is associated with increased call frequency and duration, suggesting a positive impact on early-life communication. Folate enhances neuroplasticity and potentially improves vocalization patterns (Wagner and MacDonald, 2021). Frequency modulation in USVs have been linked to sensorimotor integration and social communication (Brudzynski, 2013). Studies have suggested that the alterations in call frequency may be associated with changes in neural circuitry, particularly in neurotransmitter systems (e.g., glutamatergic and GABAergic pathways) which influenced by FA metabolism (Duman et al., 2019). Similarly, research on folate receptor alpha antibodies suggests that both deficiency and excess of FA can modulate USVs, influencing offspring's social and emotional behaviors (Boyles et al., 2011). This aligns with our findings that FA supplementation at 8 mg/kg resulted in distinct vocalization patterns compared to 2.3 mg/kg, likely due to differential effects on neurotransmitter systems. Our results contribute to growing evidence that prenatal nutrition significantly affects early neurodevelopment and FA might be associated with early vocalizations or vocal communication abilities in F1 offspring. This study suggests the potential transgenerational effects of folic acid supplementation on maternal behavior and their F1 offspring communication.

Implications and future directions

Our findings highlight the complex and multifaceted effects of FA supplementation on maternal behavior and offspring development vocalization. While, FA plays a crucial role in preventing neural tube defects and supporting fetal development (Czeizel and Dudás, 1992), our results suggest that its effects may extend beyond these known benefits. Specifically, observed changes in maternal behavior, particularly in learning and memory, raise questions about the optimal dosage of FA supplementation and potential between different health outcomes. The alterations in offspring USVs are fascinating, as they suggest that maternal FA intake can influence early communicative behavior in the F1 offspring of FA-fed female mice. This finding may have implications for understanding the role of maternal nutrition in offspring neurodevelopment and social behavior. Future research may investigate whether these early differences in USVs correlate with later social behavior or cognitive outcomes in the F1 offspring.

Studies have begun to elucidate the mechanisms by which FA influences neurodevelopment and cognitive function across the lifespan. McGarel et al. (2015) highlighted the emerging roles of folate and related B vitamins in brain health and emphasized their importance during early development and maintaining cognitive function throughout life. Additionally, maternal FA supplementation during pregnancy improved neurobehavioral development in rat offspring (Wang et al., 2018). Interestingly, in early life-stage FA deficiency delayed neurobehavioral development and cognitive function in rat offspring by hindering de novo telomere synthesis (Zhou et al., 2022). In conclusion, this study demonstrates that FA supplementation in female mice can impact their behavior and influence the vocalizations of their F1 offspring. Future research should aim to clarify the dose-dependent effects of FA with potential interactions of other nutrients, and investigate long-term outcomes in F1 offspring. Translational studies are needed to determine the evidence-based recommendations for FA supplementation before and during pregnancy.

CRediT authorship contribution statement

Paramanik Vijay: Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Mourya Vineet Kumar:** Writing – original draft, Methodology, Data curation. **Nisha:** Writing – review & editing, Visualization, Validation, Methodology, Formal analysis. **Tiwari Sneha:** Writing – review & editing, Visualization, Validation, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Declaration of Competing Interest

The author(s) declared no potential conflicts of interest. All the experiments were performed as per the guidelines of the Institutional Animal Ethics Committee (IAEC) of IGNTU, Amarkantak, Madhya Pradesh, India (Ref. No. IGNTU/IAEC/2021/13).

Author Contributions Statement

VP conceived the hypothesis and designed the study. VM performed the experiments prepared figures and tables and wrote the manuscript, whereas ST and NS checked and edited the manuscript. VP supervised the complete work, and all listed authors read and approved the manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ibneur.2025.03.007.

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