Effects of developmental fluoride exposure on rat ultrasonic vocalization, acoustic startle reflex and pre-pulse inhibition


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Abstract. – Rats receiving fluoride during the whole pregnancy up to the 9th day of lactation showed, when isolated at 10th day of life, a reduced rate of ultrasonic vocalizations (UV) in male pups (NaF 5.0 mg) and, in 90th days male rats, an increase of the Pre-Pulse Inhibition (PPI) with a reduction of the Peak response to the Startle stimulus given alone. Newborn rat reactivity could represent a useful and validated model in anxiety studies which could be moored with the Acoustic Startle Reflex (ASR) and PPI, appropriate models to study, in adulthood, particular neurological and psychiatric disorders showing deficits in attention and sensory-motor gating (Tourette’s syndrome, obsessive compulsive disorders, Huntington’s disease and schizophrenia).

Key Words: Rat, Natrium fluoride, Development, Ultrasonic vocalization, Pre-pulse inhibition, Startle reflex, Sensorimotor gating, Anxiety, Neurologic, Psychiatric disorders.

Introduction

Fluoride, commonly used for dental caries prevention, could be recommended with a daily dose between 0.7-1.2 mg/litre in drinkable water as necessary substance to prevent caries1. Further fluoride sources, other than drinkable water, could be dietary supplements, foods in general, drinks, tooth paste, mouth rinse. It has been shown that prolonged daily ingestion of drinking water containing high levels of fluoride (>4 ppm) produces toxic effects in different tissues and organs of human and other mammals2-5 and in behavioural parameters6.

Data on the toxic effects of fluoride on the central nervous system (CNS) are controversial and not fully investigated. In fact, exposure to various sources of fluoride, not leading to evident CNS alterations or sensory deficits, will obviously leave unexplored eventual subtle brain disfunctions. Fluoride, as normal component of the cerebrospinal fluid7, is relatively able to cross the blood brain barrier. In fact, while a morphological study indicates that the nervous tissue remained totally unaffected by its exposure to fluoride8, in vitro studies on hippocampal neurons show that intracellular fluoride is able to modify the kinetic properties of calcium channels9 and that the chronic exposure to fluoride decreases the number of nicotinic acetylcholine receptors in rat brain10. Moreover, toxic effects were also evidenced in behavioural studies of rats perinatally exposed to fluoride where resulted behavioural deficits sex and dose related11. Recently, we also reported, in rats perinatally exposed to natrium fluoride (NaF), at levels resembling hu-
man daily exposure (2.5 and 5.0 mg/kg), behavioural alterations sex and dose specific which affected males more than females. In particular, significant impairments were observed in learning, memory, motor coordination, blood pressure and sexual behaviour. In the present investigation we analyze, in rat perinatally exposed to the same doses used in our above-mentioned study, the NaF effects on emotionality and sensory-motor gating throughout ultrasonic vocalization (UV), acoustic startle reflex (ASR) and pre-pulse inhibition (PPI).

Materials and Methods

Animals and Exposure Conditions

Animal experimentation was performed in accordance with the EU directive 86/609 EEC, with the guidelines released by the Italian Ministry of Health (D.L. 116/92 and D.L. 111/94-B), with the UK Animals Act 1986 and associated guidelines and with the "Guide for the Care and Use of Laboratory Animals", as adopted and promulgated by the National Institutes of Health (USA). According to the above guidelines, all efforts were made to minimize the number of animals and their suffering. Primiparous Wistar female rats (Harlan SRC, Milan, Italy), weighing 200-260 g were used. Animals were allowed free access to food and water, housed at constant room temperature (20-22°C) and exposed to a light cycle of 12 h day (08:00-20:00 h) for 2 weeks before the experiment. Pairs of females were placed with a single male rat in the late afternoon. Each female rat was inspected for vaginal smears on the following morning at 09:00 h. The day on which sperm were present was designated as day 0 of gestation. The procedure for sodium fluoride (NaF, SIGMA Aldrich, Italy) administration was fully described in the paper from Bera et al.

On the day following parturition (PND 1), 10 litters for each experimental group were reduced to a standard size of four male and four female pups per litter, when possible. All pups were tested on PND10 for UV and on PND 90 for ASR and PPI evaluations.

Ultrasonic Vocalization

Recording sessions were conducted in an experimental room far from any source of noise. Each pup, removed from his nest, was placed in a deep plastic container filled with cotton wool and moved to the experimental site (Amplifon sound attenuating cabin: 1 m × 1 m × height 2 m) where was placed in a shallow glass dish (20 cm in diameter and 10 cm deep) 30 sec before the test. This limited the movements of the pup relative to the ultrasonic microphone (Condenser A-B CMPA/CM 16) which was supported vertically 15 cm above the dish and thus avoiding to handle them during each recording session lasting 30 sec. The ultrasonic microphone was connected to a Personal Computer (PC), running Windows XP, through an analogic-digital converter (A ultrasonic gate 116). Rat pup vocalizations were recorded with a PC based A Recorders software (multichannel triggering hard-disk recording system) and further analyzed with a SAS Lab Pro software (spectrograph, synthesizer and signal analyzer). The whole apparatus was provided by Eng. Raimund Specht, Avisoft Bioacoustics, Berlin, Germany.

All pups (4 males and 4 females) from each litter (10 litters/treatment group) were tested and then statistically analyzed with a one-way ANOVA. The rate of vocalization (number of calls/30 sec) was counted by the experimenter (with a tally counter) hearing, for each pup, its recorded ultrasonic by mean of a loudspeaker connected to the audible output of the PC during the replay. It was made at a lower scanning speed, in order to make them audible and, in the same time, monitoring them on the PC screen.

"Prepulse Inhibition" of the "Acoustic Startle Reflex"

ASR amplitude was measured and PPI calculated with the “Startle Reflex System ANL 925C” (Med Associates inc., St. Albans, VT, USA). The equipment included response platforms placed in a 0.64 × 0.40 × H 0.42 mt sound attenuating chamber. Each platform was calibrated with a spinner type calibrator (“Startle Calibrator”, Med Associates inc., St. Albans, VT, USA). A speaker was placed within the chamber midway on the long axis of the platform. The sound intensity of the speaker in each chamber was calibrated by the (“Digital Sound Level Meter”, Extech Instruments, Janesville, WI, USA). The diameter of the plexiglas cylinder, mounted on the platform, was calculated in order not to allow animals to turn around. The background white noise level was calibrated at 50 dB SPL. Each test session was conducted in 3 procedural blocks. Before the beginning of the test, each an-
imal was placed in the test chamber along a 5 min acclimation period.

Block 1 consisted of 10 trials white noise stimulus with 20 ms duration, 85 dB SPL intensity and 4 KHz Pure Tone frequency.

Block 2 consisted of 10 trials of 110 dB SPL startle stimulus with 100 ms duration and 5 KHz Pure Tone frequency.

Block 3 consisted of 10 trials each one formed with both block 1 and block 2 consequently supplied. Each acoustic stimulus had a 10 ms rise/fall time. The null period was 250 ms and the pre-pulse/startle delay was 50 ms (onset to onset). The whole test period lasted 30 min. Each experimental group consisted of 10 litters (4 males and 4 females/litter).

Statistical Analysis
The rate of ultrasonic calls emitted by 10 days rat pups and the values of ASR and PPI detected in 90 day old rats, were statistically analyzed by a two-way analysis of variance (ANOVA) and by Kruskal-Wallis ANOVA. Individual comparisons were performed by Tukey’s or Dunn’s Multiple Comparison tests, where appropriate.

Results
Results obtained from ultrasonic emissions at 10 days of age and those from PPI of the ASR at 90 days of age, show that perinatal exposure to NaF significantly affected only male rats and only at the higher dose level of NaF used in this study (5.0 mg/kg).

In fact, the number of ultrasonic calls emitted in 30 sec. by 10 day old male rats, perinatally exposed to NaF, resulted significantly lower with respect to the control rats only at the NaF dose of 5.0 mg/kg (one way ANOVA : F= 5.5682; df=1/96; p<0.01 (Figure 1).

Moreover, the peak intensity values of the ASR resulted significantly reduced with respect to the controls in 90 day old male rats, perinatally treated with NaF doses of 2.5 mg/kg (F= 2.083, df=218, p<0.01) and 5.0 mg/kg (F= 2.578; df=178; p<0.05) (Figure 2).

Furthermore, the latency to the peak intensity of the ASR, in 90 day old male rats perinatally treated with the higher dose of NaF (5.0 mg/kg), showed a significant reduction with respect to the controls (F=2.056; df=2/357; p<0.001) (Figure 3). Finally, the PPI peak values in 90 day old male rats, perinatally treated with the higher dose of NaF (5.0 mg/kg), resulted significantly increased with respect to the controls (F= 3.380; df= 2/357; p<0.001) (Figure 4) while the PPI latency values didn’t result statistically different.

In conclusion, perinatal exposure to natrium fluoride during the developmental period (from pregnancy day 1 (PD1) up to the postnatal day 9 (PND9), caused sex and dose specific behavioural alterations which affected males more than females, especially at the higher dose of NaF.

Discussion
In the present experiment, the most relevant results were observed at the higher dose of NaF.

Figure 1. UV rate in 10 days old rats.
(5.0 mg/kg) in which NaF induced significant sex related behavioural alterations affecting only male rats. These data are in agreement with a recent neurobehavioural study of our group\(^{12}\). In fact, in 10 day old male rats, perinatally exposed to NaF, we found a significant reduction in the number of ultrasonic emissions. This neurobehavioural approach represents now a validated feature in rat pups as a response to separation and/or cold stress, resulting in numerous experimental studies addressing this condition as an anxiety model to study both emotional and motivational aspects\(^{13,14}\). Moreover, in this study we also analyzed the ASR, which represents an useful model for the analysis of habituation, usually generalizing to other behaviours. The neuronal circuit of ASR of mammals is relatively simple and the neurons of the caudal pontine reticular nucleus are the principal key elements in this neuronal pathway\(^{15}\).

80-90 days old male rats, treated with the higher doses of NaF (5.0 mg/kg), showed a significant ASR reduction. Consequently, we could hypothesize a dysfunction of the ASR neuronal pathway, also
suggesting an ongoing neurologic and/or psychiatric pathologies. In fact, the ASR has been used as a behavioural tool to assess the neuronal basis of behavioural plasticity and to evaluate neuropathological deficits in attention and sensory gating related to human psychiatric (obsessive compulsive disorders, schizophrenia) and neurological (Huntington’s disease, Tourette’s syndrome) diseases.

Finally, in the present study we also found, in male rats treated with the higher dose of NaF, an increase of PPI, which represents a sensitive neurobehavioural model of sensory-motor adaptation in which the pre-pulse sensory stimulus reduces the reaction to the second quickly following stimulus.

The response to PPI of the ASR seems to be an excellent model for operational measure of brain mechanisms which prevent disruption of ongoing stimuli processing routines by other stimuli and which thereby avoid neurobehavioral interference. Therefore, much interest has been devoted to understand the PPI neural basis. Moreover, PPI indexes a basic neuronal adaptive response, which goes awry in a variety of neurological and psychiatric conditions including autism, Alzheimer disease, post-traumatic stress disorders and schizophrenia. In fact, several neurotransmitter systems, including dopamine, glutamate, serotonin and acetylcholine are known to be involved in PPI mechanisms. Our results suggest possible alterations in the brain regions (limbic cortex, striatum, pallidum, or pontine tegmentum) involved in the regulation of the PPI neuronal circuit not excluding a possible dysfunction in the neurotransmitter systems involved in the PPI.

Conclusion

Ultrasound vocalization represents a neurobehavioural validated feature to value the rat pup response to separation and/or cold stress as an anxiety model to study both emotional and motivational aspects. Special emphasis must also lay on the potentiality of ASR modulation in order to clarify human psychiatric and neurological diseases where the understanding of interactions among neurotransmitter systems underlying PPI, could not only help to explain the neural basis of this adaptive response but could also push forward in developing new therapeutic specific treatments.

In conclusion any result obtained by NaF treatment during pregnancy and/or lactation could simply suggest to always take in account a neurotoxic risk in the progeny.

References


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