**Original Article**

# A pilot study on the changes of event-related potentials in school-aged children with iron deficiency anaemia

TH Shi MD, LF Yu MSc, LZ Huang MD, XH Ma MSc and QH Zhu MD

*Department of Neurology, Tongji Hospital of Tongji Medical University, Wuhan 430030, China*

Event-related potentials (ERP) were assessed in 70 school-aged children with a diagnosis of asymptomatic iron deficiency anaemia (IDA), based on low haemoglobin and either low serum ferritin or high free erythrocyte protoporphyrin levels. The IDA subjects were randomized into treatment and placebo groups of 35 cases each, and compared with a normal control group of 30 age- and gender-matched healthy subjects without iron deficiency. Further haematological and ERP assessment was carried out after 3 months, during which time the active group received iron supplementation with 10 mg ferrous sulphate, together with vitamin C, malic acid and folic acid. Pre-treatment, both IDA groups had prolonged P300 latencies in comparison with the non-IDA controls ($P < 0.01$). The proportion of cases with distorted wave appearance was more than twice as high in the IDA groups as in the non-IDA controls, although intergroup differences did not reach statistical significance. After treatment, the active treatment IDA group showed a significant increase in haemoglobin levels and shortening in P300 latencies. After treatment, neither value was statistically different from non-IDA controls. There was a decrease in the number of cases with abnormal waveforms in the active treatment group, compared with an increase in the number within the placebo group ($P = 0.002$). Testing of ERP shows promise as a non-invasive, sensitive and objective marker for assessing cognitive impairment in children with IDA.

**Key words: iron deficiency anaemia (IDA), school-aged children, event-related potentials (P300), cognitive function.**

## Introduction

Iron deficiency anaemia (IDA) is a widespread nutritional disorder and an enormous public health problem throughout the world.[1] It is estimated that the disorder affects 500–700 million people worldwide, while it is the most common disease reported among children, with prevalence being reported as being as high as 22% among East Asian children of school age.[2–5]

Early stage iron deficiency affects brain iron content distribution, resulting in neurotransmitter and brain metabolic alterations.[6,7] The most important manifestations of tissue iron deficiency in childhood are the impairment of psychomotor development and of cognitive function.[3] Although there are many ways to assess psychomotor development, such as intelligence quotient (IQ) testing, most tests are influenced by the child's level of cooperation.[8]

During the last few decades event-related potentials (ERP) have been extensively investigated in studies of neuroelectrophysiological correlates of human cognitive processes.[9] Long-latency evoked potentials (EP) related to aspects of cognitive processing are referred to as ERP or cognitive EP. Their components are labelled in numerical order, based on the polarity and/or average peak latency. The component commonly designated as P3 or P300 normally has a latency of about 300 ms and is maximal in amplitude in all the waves. It has been commonly used in research studies of psychomotor development, as it has been found to most closely represent high-ranking cognitive function in the cerebral cortex.[10–13] This includes simple recognition, short-term memory, simple measures of judgement, and attention.[10,12] We are not aware of any studies which utilize ERP testing as a measure of intellectual ability in IDA subjects. The present study was designed to assess the usefulness of this test in this context.

## Materials and methods

Seventy subjects with IDA were recruited from children aged 7–12 years attending a normal primary school in Huangpi County, a suburb of Wuhan City in China. All subjects met the diagnostic criteria for IDA set by the 1988 National Scientific Conference of Children's Blood Diseases.[14] These were low haemoglobin (Hb < 120 g/L) with either low serum ferritin (SF < 16 μg/L) or high free erythrocyte protoporphyrin levels (FEP > 500 μg/L). Apart from this diagnosis, the children were apparently well and showed no positive signs on physical examination. There was neither medical history nor family history of tuberculosis, hepatitis, ancylostomiasis, schistosomiasis, inherited and metabolic disorders, or anaemias other than IDA. There was no recent history of fever, diarrhoea or diseases of the central nervous system.

The subjects were randomly divided into two equal-sized groups, one treatment and one placebo, and compared with 30 non-anaemic age- and sex-matched healthy children from the same school. The treatment group received a daily oral dose of a solidified beverage containing iron (10 mg in the form of ferrous sulphate), vitamin C (50 mg), malic acid (25 mg) and folic acid (250 μg), while the placebo group was

**Correspondence address:** Professor Shi Tinghui, Department of Neurology, Tongji Hospital of Tongji Medical University, Wuhan 430030, China.
Tel: 86 27 8361 4243; Fax: 86 27 8361 4243.
Email: thshi@tjh.tjmu.edu.cn
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given a beverage of similar appearance, odour and taste without any of these nutrients. Both active treatment and placebos were given for 3 months.

Event-related potentials testing was performed on all subjects on two occasions, before and after the treatment period. Event-related potentials testing was conducted according to International Federation of Clinical Neurophysiology standards using a binaural auditory tone stimulus.[13] Two easily discernible tones were presented in a random order using the so-called 'odd-ball' paradigm: that is, contrasting a 'frequent' tone of pitch, 1000 Hz, in 80% of the trail with a 'rare' tone of pitch, 2000 Hz, in 20% of the trail. Recordings were obtained from the vertex and referenced to linked-earlobe electrodes, using 0.5 Hz high pass and 30 Hz low pass filters with the sweep duration set at 750 ms. The subjects were required to count the number of rare tones mingled with a series of frequent stimuli. In normal subjects an N1-P2 complex should be identified in response to the frequent tone and an N1-P2-N2-P3 complex in response to the rare tone.[13]

At the same time as ERP testing was performed the subjects had an IQ test using the Chinese–Binet Intelligence scale as revised by Cunren Fan.[15] This revised version of the Binet test has been used extensively in China since it was published in 1992. The test provides an overall evaluation of development level along with a measurement of sub-areas of clinical interest, such as language, memory, concept, thinking, reasoning, visual perception and social skills. Average IQ was defined as being a score between 90 and 109.

The entire study was conducted in a double-blind fashion.

Averaged ERP for each subject were used for calculation. Three cases in the IDA group (two in the treatment group and one in the placebo group) were excluded from statistical analyses because of an absence of pretreatment P300 components.

## Results

The baseline data for the three sample groups are shown in Table 1.

### *Haematology*

Haemoglobin levels rose in the treatment IDA group such that they were no longer significantly different to those of the non-anaemic control subjects, with all treated subjects having haemoglobin levels greater than or equal to 120 g/L (meaning that all treated subjects had an increase in haemoglobin levels of more than 10 g/L over the original level). This was associated with corresponding improvements in other haematological parameters of anaemia. Hematological indices in the placebo group improved slightly, but remained statistically different to the control group ($P < 0.05$) (see Table 2).

### *Event-related potentials*

Prior to treatment, both IDA groups had prolonged P300 latencies in comparison with the control group ($P < 0.01$). Although there were differences in the proportion of cases with abnormal waveforms between each IDA group and non-anaemic controls (Table 3), this did not reach statistical significance (treatment group *vs* control group $\chi^2 = 3.09$, $P = 0.079$; placebo group *vs* control group $\chi^2 = 2.83$, $P = 0.092$). When the combined two IDA groups were compared with the control group the difference was of increased significance, although it was still not statistically significant ($\chi^2 = 3.63$, $P = 0.057$).

The differences in proportion of abnormal waveforms within each group comparing pre- with post-treatment testing did not reach statistical significance for any one group, although there was a trend to more abnormal waveforms in the placebo group ($P = 0.052$), and less abnormal waveforms in the treatment group ($P = 0.174$). The proportion of abnormal waveforms in the non-IDA control group did not change. However, the differences in proportion of abnormal waveforms between groups during post-treatment testing were of high statistical significance. There was a decrease in the number of cases with abnormal waveforms in the active treatment group, compared with an increase in the number within the placebo group ($P = 0.002$).

**Table 1.** Baseline data for the three sample groups (mean ± SD)

| Group | Age (month) | Weight (kg) | Height (cm) |
|---|---|---|---|
| Treatment ($n = 35$) | 111.10 ± 1.74 | 27.12 ± 5.68 | 130.40 ± 11.36 |
| Placebo ($n = 35$) | 111.90 ± 1.87 | 26.50 ± 5.48 | 130.80 ± 10.26 |
| Normal ($n = 30$) | 111.60 ± 1.68 | 27.30 ± 5.15 | 131.10 ± 9.84 |

Note: The distribution of boys and girls for each group was equal or within one case of each other.

**Table 3.** Cases with abnormal waveforms

| Group | Total *n* (%) | Pre-treatment No. cases with abnormal waves (%) | Post-treatment No. cases with abnormal waves (%) |
|---|---|---|---|
| Treatment (T) | 33 | 12 (36.4) | 7 (21.2)* |
| Placebo (P) | 34 | 12 (35.3) | 20 (58.8)† |
| Normal (N) | 30 | 5 (16.7) | 5 (16.7) |
| P-value | | Pre-treatment differences between groups | Post-treatment differences between groups |
| N-T | | > 0.05 | > 0.05 |
| N-P | | > 0.05 | = 0.001 |
| T-P | | > 0.05 | = 0.002 |

Pre- versus post-treatment in each group: *$P = 0.174$; †$P = 0.052$.

**Table 2.** Haematological analyses before and after iron supplementation

| Group | Hb (g/L) | | FEP (μg/L) | | SF (μg/L) | |
|---|---|---|---|---|---|---|
| | Before | After | Before | After | Before | After |
| Treatment | 105.80 ± 3.27 | 124.00 ± 3.71* | 771.40 ± 21.14 | 422.80 ± 11.20* | 13.32 ± 1.56 | 36.95 ± 1.76* |
| Placebo | 104.90 ± 1.87 | 112.20 ± 4.30† | 748.00 ± 24.56 | 533.00 ± 11.32*† | 11.70 ± 1.70 | 12.53 ± 1.92§ |
| Normal | 120.49 ± 4.29 | 123.20 ± 3.84 | 354.70 ± 11.46 | 345.80 ± 9.56 | 57.99 ± 1.46 | 37.59 ± 1.69 |

Pre- versus post-treatment within each group: *$P < 0.05$. Placebo versus normal groups post-treatment: †$P < 0.05$; §$P < 0.01$. Hb, haemoglobin; FEP, free erythrocyte protoporphyrin; SF, serum ferritin.

In relation to the severity of these abnormalities, the trend was towards increasing severity in the placebo IDA group compared with decreasing severity in the treatment IDA group (as illustrated in Fig. 1).

Table 4 shows the data on latencies and amplitudes for the three groups. Pre-treatment, the P3 absolute latencies of both IDA groups were significantly longer than those of non-anaemic controls ($P < 0.01$). Post-treatment, these differences were reduced but were still statistically significant ($P = 0.05$) in the case of the placebo IDA subjects compared with the non-IDA controls. However, the P3 absolute latencies were no longer significantly different between the treated IDA subjects and the non-IDA controls ($P > 0.1$).

There were clear reductions on N1 and P3 peak latencies in both treatment and placebo groups between the pre- and post-treatment testing, but this reduction was less in the placebo group than in the treatment group.

Table 5 shows the results for IQ testing. Despite a trend towards lower scores in both IDA groups compared with non-IDA controls pre-treatment, there were no statistically significant differences. However, the treatment group was significantly higher compared with the placebo IDA group after treatment ($P < 0.05$).

## Discussion

Iron is an essential trace element in the human body. It is well established that although mild IDA often has few obvious clinical symptoms it can adversely affect higher cerebral function, particularly in children during their vigorous and vulnerable stages of psychomotor and physical development.[16–22] The present study was undertaken to explore whether any evidence of cognitive disturbance could be found through neurophysiological assessment (such as ERP) or by psychological evaluation (such as IQ testing). Event-related potentials have already been used in the diagnostic assessment of various diseases other than IDA; for example, epilepsy, Down syndrome, schizophrenia, Parkinson's disease and diabetes mellitus.[12,23–26]

Our data show that IDA is associated with significant changes in latency prolongation of P300 and distortion of P300 waveforms, suggesting that IDA causes cognitive disturbances in children. It was particularly noteworthy that

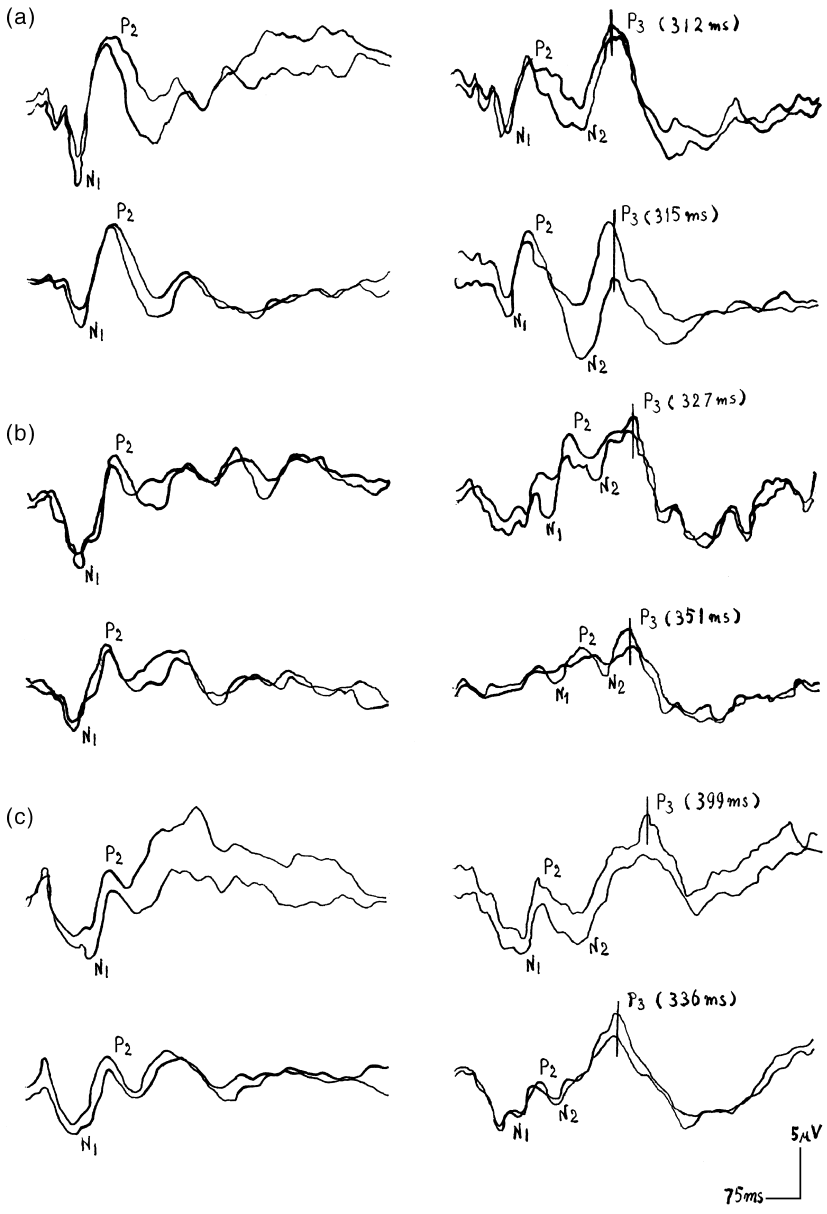


**Fig**. 1. Auditory event related potentials recorded in a normal subject (a), a subject with iron deficiency anaemia (IDA) in the placebo group (b), and a subject with IDA in the treatment group (c) to frequent 1000 Hz tones (left) and rare tones (right). Each trial consisted of approximately 20% rare and 80% frequent stimuli with two trials superimposed. Calibration marks are 75 ms and 5 μV. Upper and lower traces for each group show the first and second evaluations, respectively. From a, there are no distinct changes between two traces. From b, the lower trace shows prolonged P3 latency and more poorly defined P3 amplitude as compared with the upper trace. From c, the lower trace shows a more shortened P3 latency and a relatively better P3 component than does the upper trace.

**Table 4.** Latencies and amplitudes of event-related potentials (ERP) (rare stimuli)

| ERP | Treatment group | | Placebo group | | Normal group | |
|---|---|---|---|---|---|---|
| | Before | After | Before | After | Before | After |
| | | | Latencies (ms) | | | |
| N1 | 124.17 ± 24.12 | 109.17 ± 20.37† | 120.77 ± 27.08 | 108.70 ± 21.54* | 115.23 ± 33.59 | 103.37 ± 26.17 |
| P2 | 171.70 ± 23.25 | 169.87 ± 26.65 | 167.69 ± 31.23 | 161.30 ± 26.71 | 167.77 ± 36.83 | 161.69 ± 35.42 |
| N2 | 232.13 ± 32.05 | 224.60 ± 31.57 | 226.37 ± 36.02 | 221.17 ± 28.15 | 222.20 ± 35.60 | 217.47 ± 32.84 |
| P3 | 343.30 ± 31.48 | 325.13 ± 29.65‡ | 343.07 ± 41.46 | 329.87 ± 38.36* | 314.07 ± 37.89 | 311.87 ± 31.90 |
| | | | Amplitudes (μg) | | | |
| N2 | 7.74 ± 4.82 | 8.09 ± 2.97 | 8.77 ± 4.88 | 7.95 ± 4.91 | 7.88 ± 3.79 | 7.63 ± 4.51 |
| P3 | 13.14 ± 5.70 | 13.16 ± 6.19 | 13.63 ± 4.67 | 12.18 ± 5.73 | 14.62 ± 4.71 | 13.49 ± 5.84 |

Pre- versus post-treatment periods in each group: *$P < 0.05$; ‡$P < 0.002$; †$P = 0.001$.

**Table 5.** The changes in IQ at different periods

| Period | Treatment group | Placebo group | Normal group |
|---|---|---|---|
| Pre-treatment | 95.51 ± 8.47 | 97.12 ± 8.92 | 99.48 ± 8.39 |
| Post-treatment | 101.22 ± 8.86* | 97.12 ± 9.51 | 100.24 ± 8.54 |

Treatment versus placebo groups: *$P < 0.05$.

three IDA subjects had a complete absence of P300 waveforms despite their having been able to maintain an accurate count of the rare stimuli, something that was not seen in any of the non-anaemic controls. This phenomenon has been reported previously in the literature in patients with lesions of the auditory areas of the cerebrum and the neocortex,[27,28] where it has been suggested as a sign of compromised cognitive processing.[27]

The data also show some association between ERP results and IQ test scores. Previous studies have shown an inverse correlation between P300 latency and total IQ scores, such as in epileptic children with disrupted cognitive function where prolonged P300 latency is accompanied by low IQ scores,[12] and in gifted children where higher IQ scores are found together with short P300 latency.[29] Our findings are consistent with this association.

The present study showed that iron supplementation was effective in treating anaemia, although the more contentious issue is whether iron supplementation is effective in reversing any cognitive disturbances which may be present. Walter reported that iron therapy was unable to reverse cognitive disadvantages, reflected in psychological tests, even after complete correction of haematological parameters in a majority of infants with IDA.[6] However, Vega-Franco *et al*., looking at older children aged 6 to 11 years, found that 12 weeks of iron therapy had a favourable effect on the attention spans of IDA subjects.[19] Further, Soewondo found that the alterations in cognitive processes in children aged 3 to 6 years with IDA could be reversed with iron treatment.[20]

Our data tend to support the more positive view. Treatment of IDA children in the present study was associated with a highly significant reduction in the latency of P300 ($P < 0.002$) such that the treated IDA group was no longer statistically significantly different to the non-IDA controls. This group also showed a significant enhancement of their IQ test scores ($P < 0.05$). There are a number of possible explanations for these positive outcomes. Firstly, the children in this study were school-aged rather than infants. Secondly, their iron deficiency and cognitive impairment were not severe.

These results also highlight the advantages of ERP testing as a test of cognitive function in children with IDA in comparison with IQ testing. The determination of IQ is influenced by a number of subjective elements, particularly in children, whereas auditory ERP are objective and require only minimal cooperation of the subject. They offer quantitative measurement of the extent and severity of cognitive dysfunction,[30,31] and the present results show that this is a useful measure in mild cases of IDA such as were seen here.

In summary, the present study suggests that ERP may be a very useful tool in diagnosis of minor cognitive disturbance in school-aged children with asymptomatic IDA. Event-related potentials were related to IQ scores, and these results support the conclusion of other authors that ERP may be the method of choice for longitudinal assessment of pathological effects on cognitive function.[32] Used early in the process of cognitive damage in children with IDA, ERP may help in the prevention of irreversible brain damage. It is also possible to administer this test to infants with IDA in order to confirm whether there are any cognitive disturbances, given that P300 can be elicited from infants with auditory stimuli through a passive tone sequence paradigm.[33]

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**References**

1. Cook JD. Iron deficiency anaemia. Ballieres Clin Haematol 1994; 7: 787–804.
2. DeMaeyer E, Adiels-Tegmen M. The prevalence of anaemia in the world. World Health Stat Sc Q 1985; 38: 302–316.
3. Cook JD, Skikne BS, Baynes RD. Iron deficiency: the global perspective. Adv Exp Med Bid 1994; 356: 219–228.
4. Nielsen VR, Valerius NH. Idiopathic pulmonary hemosiderosis. A cause of severe iron deficiency anaemia in childhood. Ugeskr-Laeger 1995; 157: 176–178.
5. Darnton-Hill I, Cavalli-Sforza LT, Volmanen PVE. Clinical nutrition in East Asia and the Pacific. Asia Pacific J Clin Nutr 1992; 1: 27–36.
6. Walter T. Effect of iron deficiency anaemia on cognitive skills in infancy and childhood. Baillieres Clin Haematol 1994; 7: 815–827.
7. Tanaka M, Kariya F, Kaihatsu K *et al*. Effects of chronic iron deficiency anaemia on brain metabolism. Jpn J Physiol 1995; 45: 257–263.
8. Rosenberg LA. Psychometrics. In: Oski FA, DeAngelis CD, Feigin RD *et al*. eds. Principles and practice of pediatrics, 2nd edn. Philadelphia: J.B. Lippincott Company, 1994: 753–760.

9. Kropotov JD, Ponomarev VA. Subcortical neuronal correlates of component P300 in man. Electroencephalogr Clin Neurophysiol 1991; 78: 40–49.
10. Oken BS. Endogenous event-related potentials. In: Chiappa KH, ed. Evoked potentials in clinical medicine, 2nd Edn. New York: Raven Press, 1990: 563–584.
11. Courchesne E, Elsmasian R, Yeung-Courchesne R. Electrophysiological correlates of cognitive processing: P3b and NC, basic, clinical, and development research. In: Halliday AM, Butler SR, Paul R, eds. A textbook of clinical neurophysiology. Chichester: John Wiley & Sons, 1987: 645–676.
12. Naganuma Y, Konishi T, Matsui M *et al*. The relationship between P300 latencies, and WISC-R and Wechsler memory scale results in epileptic children. No To Hattatsu [Brain and Development] 1993; 25: 515–520.
13. Goodin D, Desmedt J, Maurer K *et al*. IFCN recommended standards for long-latency auditory event-related potentials. Report of an IFCN Committee. Electroencephalogr Clin Neurophysiol 1994; 91: 18–20.
14. Qingkui Liao. The diagnostic criteria and suggestions on the prevention and treatment of children with IDA (Chinese National Scientific Conference of Children's Blood Diseases) (In Chinese). Chinese J Pediatrics 1989; 27: 159–160.
15. Cunren Fan. The manual of Chinese–Binet intelligence scale. Psychological Research Institute of Chinese Academy of Sciences, Beijing, 1992.
16. Martin PL, Pearson HA. Iron deficiency anaemia. In: Oski FA, DeAngelis CD, Feigin RD *et al*. eds Principles and practice of pediatrics, 2nd edn. Philadelphia: J.B. Lippincott Company, 1994: 1657–1658.
17. Howell D. Significance of iron deficiencies: Consequences of mild deficiency in children. Extent and meaning of iron deficiency. In: USA proceedings of workship of food and nutrition board. Washington DC: National Academy of Sciences, 1971.
18. Evans DIK. Cerebral function in iron deficiency: a review. Child Care Health Dev 1985; 11: 105–112.
19. Vega-Franco L, Robles-Martinez B, Mejia AM. Effect of iron deficiency on attention capacity among school children. Gac Med Mex 1994; 130: 67–71.
20. Soewondo S. The effect of iron deficiency and mental stimulation on Indonesian children's cognitive performance and development. Kobe J Med Sci 1995; 41: 1–17.
21. Hong Q, Yao KN, Liu L *et al.* Case control study of intellectual status in school children with iron deficiency. Child Health Care China 1994; 2: 183–185.
22. Shen XM, Yan CH, Xu JD *et al*. The effect of iron deficiency anaemia on learning, memory and behavior in rats. Chinese J Paediatrics 1994; 32: 267–268.
23. Kaneko WM. Auditory event-related potentials in fetal alcohol syndrome and Down's syndrome children. Alcohol Clin Exp Res 1996; 20: 35–42.
24. Bougerol T. Interest of recording event related potentials (ERP) in the knowledge of schizophrenic disorders. Ann Med Psychol (Paris) 1995; 153: 19–32.
25. Tachibana H, Aragane K, Kawabata K *et al*. P3 latency change in aging and Parkinson disease. Arch Neurol 1997; 54: 296–302.
26. Kurita A, Mochio S, Isogal Y. Changes in auditory P300 event-related potentials and brainstem evoked potentials in diabetes mellitus. Acta Neurol Scand 1995; 92: 319–323.
27. Obert Ad Cranford JL. Effects of neocortical lesions on the P300 component of the evoked response. Am J Otol 1990; 11: 447–453.
28. Musiek FE, Baran JA, Pinheiro Ml. P300 results in patients with lesions of the auditory areas of the cerebrum. J Am Acad Audiol 1992; 3: 5–15.
29. Martin F, Delpont E, Suisse G *et al*. Long latency event-related potentials (P300) in gifted children. Brain Dev 1993; 15: 173–177.
30. Rappaport M, Clifford Jo Jr, Winterfield KM *et al*. P300 response under active and passive attentional states and uni- and bi-modality stimulus presentation conditions. J Neuropsychiatry Clin Neurosci 1990; 2: 399–407.
31. Naganuma Y, Konishi T, Murakami M *et al*. Age-related changes of auditory event-related potential (P300) in children. No To Hattatsu [Brain and Development] 1991; 23: 94–99.
32. Kugler CF, Taghavy A, Flatt D. The event-related P300 potential analysis of cognitive human brain aging: a review. Gerontology 1993; 39: 280–303.
33. McIsaac H, Polich J. Comparison of infant and adult P300 from auditory stimuli. J Exp Child Psychol 1992; 53: 115–128.