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Original Article

Brainstem Evaluation in Children with Primary Nocturnal Enuresis

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We investigated the brainstem integrity in children with primary nocturnal enuresis (PNE) using auditory brainstem responses (ABR), blink reflex and exteroceptive suppression of the masseter muscle. We examined 23 children with PNE (16 male, 7 female; mean age: 10.4 years) and 19 control subjects (11 male, 8 female; mean age: 11.8 years). ABR parameters such as wave latencies, amplitudes and interpeak latencies and blink reflex parameters such as R1 and R2 amplitude and latencies were not significantly different between the 2 groups. Although S2 parameters of the exteroceptive suppression of the masseter muscle were easily and completely obtained from the control subjects, in the PNE group S2 onset latency and duration were not recorded in 26% of the study children (n = 6) ($P = 0.01$). S2 duration time was significantly lowered in the enuretic group (left side: $P = 0.001$ and right side: $P = 0.003$). S2 duration time changes in the enuretic group supports a possible brainstem dysfunction in children with PNE.

Key words: nocturnal enuresis, auditory brainstem response, blink reflex, exteroceptive suppression, brainstem neurophysiology

Primary nocturnal enuresis (PNE) is a genetically heterogeneous developmental disorder that is characterized by the persistence of involuntary bed-wetting beyond the age of 5 years [1]. Epidemiological studies suggest that approximately 5% of children aged 7 years old and 0.5% of the adult population wet the bed at least once a month [2]. Genetic and psychologic factors, sleep abnormalities, autonomic nervous system dysfunction and developmental delay are the main etiopathogenetic causes [3, 4]. PNE may be related to a functional immaturity of the central nervous system (CNS). Although micturition and bladder control centers are located in the pons, few studies have focused on the brainstem. In this study, we aimed to investigate the brainstem integrity in children with PNE using auditory brainstem responses (ABR), trigeminal blink reflex and exteroceptive suppression of the masseter muscle.

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Materials and Methods

Patients. We examined 23 children with PNE and 19 age- and sex-matched control subjects. All parents gave informed consent for their children to participate in the study, which was approved by the Mersin University ethics committee. The study group consisted of 16 males and 7 females (mean age, 10.4 years; range, 8-14 years), and the control group consisted of 11 males and 8 females (mean age, 11.8 years; range, 8-15 years). Routine

physical examination, pure tone audiometry and tympanometry were within normal limits. Although we did not investigate the quantitative electroencephalographic (EEG) changes, we found all of the routine EEG traces to be normal. Children and adolescents having PNE according to the criteria listed in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) were included in the study. Cases with mental retardation, neurologic disorder (*e.g.*, seizure disorder), any known metabolic disease, current urinary tract infections or psychiatric disorders (*e.g.*, pervasive developmental disorders, psychosis or attention deficit hyperactivity disorders) or those taking any medication within the prior 10 weeks were excluded from the study [5].

Electrophysiological Investigations. Auditory Brainstem Responses (ABR): ABRs were recorded in a quiet and dim room in our laboratory. All subjects were requested to sit on a comfortable armchair during the test. Also subjects were instructed to avoid eye and head movements during the test. Medelec Synergy EMG equipment was used for electrophysiological investigations. "Click" stimulations were used. First, a threshold level was determined for each side (each ear). The stimulus intensity was 90 dB for each ear. The stimulation frequency was 10 Hz. Recordings were performed using silver-plated surface electrodes. At least 1200 responses (between 1200–1500) were averaged for each analysis. In addition, 2 successively recorded ABRs were obtained and superimposed on each side. For recording, active electrodes were located on both mastoid bones (A1 and A2). These electrodes were referenced to a Cz electrode located according to the 10–20 system. The oscilloscope sweep time was 10 msec. The amplifier filter was set between 100 Hz and 2 kHz.

The latencies and amplitudes of the first (V1), third (V3) and fifth (V5) waves and the interpeak latencies of I-III, I-V and III-V were analyzed.

Blink reflexes: Subjects were requested to sit on a comfortable armchair for recording of blink reflexes. They were instructed to avoid any eyelid movement and to remain in a relaxed position during the test. The electrical stimulation of both supraorbital nerves was used to obtain reflex responses. The stimulation intensity was adjusted according to responses and fell between 40 and 90 mV. The same stimulus intensity was used for both sides. Recordings were performed using silver-plated surface electrodes from both orbicularis oculi muscles. The active electrode was placed over the belly of the muscle, and a reference electrode was placed on the arcus of the zygomatic bone. First, the right supraorbital nerve was stimulated, and thereafter the opposite side was stimulated. The reflex responses of both ipsilateral and contra lateral orbicularis oculi muscles were recorded simultaneously for each supraorbital nerve. The frequency filter of the amplifier was set between 10 Hz and 10 kHz. The oscilloscope sweep time was 100 msec; the gain was 0.1–0.5 mV/division.

At least 5 responses were recorded for each subject. We analyzed the latencies and amplitudes of R1 and R2 responses, and the signal that had the biggest reflex activity was used for analysis.

Exteroceptive Suppression of Masseter Muscle: Subjects were requested to remain lying down in a relaxed position. During the electrophysiological investigation, subjects were instructed to make a forceful tooth clenching, with stimuli applied during this contraction period. A stimulating electrode was placed over the midline of the jaw. Both of the mental nerves were stimulated simultaneously from the jaw with an electrical impulse. An active recording electrode was placed over both masseter muscles, and reference electrodes were placed on the arcus of the zygomatic bone. We used AgCl surface electrodes for recording. Initially the suppression patterns of both masseter muscles were recorded simultaneously. The oscilloscope sweep time was 200 msec. The amplifier filter was set between 10 Hz and 10 kHz; the gain was 0.2–0.5 mV/division. The stimulus duration was 0.2 msec. These stimulation parameters were relatively comfortable and not too painful for subjects. This method was described previously [6].

At least 10 successive responses were recorded and superimposed. When onset and end points of S2 suppression periods were equivocal, 20 stimuli were recorded and averaged. To avoid habituation, the minimal time interval between the 2 stimuli was 10 sec. The duration of S1 and S2 periods and onset latencies of the S1 and S2 periods were calculated. The determination of the onset and the end of the S2 period was accomplished by averaging 10 responses. Sometimes a few muscle activities were seen

Abbreviations

R1, first component of the trigeminal blink reflex; R2, second component of the trigeminal reflex; S1, first suppression period of exteroceptive suppression of the masseter muscle; S2, second suppression period of exteroceptive suppression of the masseter muscle.

during the S2 period. We measured the silent interval from the end of the intermediate EMG activity to the beginning of EMG activity as the S2 period. Any EMG activity exceeding 20% of maximal amplitude of the EMG signal was not considered to be a silent period. The determination of the S1 period was relatively easier than that of the S2 period because the S1 period was more stable than the S2 period and because the S1 period did not include any electromyographic activity. In addition, we calculated the interside differences in the duration and the onset latencies of the S2 period in healthy controls. Increased interside difference of the S2 period was considered to have ocurred when the difference of S2 duration between the right and left side exceeded + 2SD.

Statistics. Student's t test and Z approximation tests were used for statistical analysis. P value < 0.05 was accepted as significant.

Results

There was no statistically significant difference between the enuretic and control groups in ABR parameters such as latency, amplitude and interpeak latencies (IPL) or blink reflex amplitude and latencies (Table 1). Although S2 parameters of the exteroceptive suppression of the masseter muscle were easily and completely obtained from the control subjects, in the PNE group S2 onset latency and duration were not recorded in 26% (n = 6) of the children under optimum test conditions ($P = 0.01$). S2 duration time was significantly lowered in the enuretic group ($P = 0.001$ and $P = 0.003$) (Table 2 and Figs. 1, 2 and 3). We did not observe any significant difference between the patients with exteroceptive suppression abnormalities and children with no abnormalities with respect to ABR and blink reflex results, age or sex (Table 3).

Table 1 ABR and trigeminal blink reflex results

Parameter	Enuresis (n = 23)	Control (n = 19)	*P*
ABR			
I-III IPL			
Right	2.10 ± 0.15	2.13 ± 0.16	0.64
Left	2.09 ± 0.16	2.16 ± 0.11	0.11
III-V IPL			
Right	2.0 ± 0.18	1.9 ± 0.2	0.10
Left	2.03 ± 0.17	1.93 ± 0.21	0.11
I-V IPL			
Right	4.11 ± 0.17	4.05 ± 0.24	0.40
Left	4.12 ± 0.16	4.13 ± 0.23	0.87
Blink Reflex			
R1 latency			
Right	10.56 ± 0.94	10.09 ± 0.96	0.24
Left	10.35 ± 0.84	10.15 ± 0.66	0.40
R1 amplitude			
Right	0.30 ± 0.3	0.40 ± 0.2	0.24
Left	0.25 ± 0.15	0.31 ± 0.18	0.26
R2 latency			
Right	35.10 ± 6.21	34.37 ± 3.41	0.45
Left	36.70 ± 3.69	36.01 ± 3.52	0.51
R2 amplitude			
Right	0.27 ± 0.12	0.35 ± 0.14	0.07
Left	0.25 ± 0.13	0.31 ± 0.12	0.24

Discussion

Children with PNE have an increased incidence of various signs of developmental delay, including higher rates of mental retardation, physical handicap, reduced body length and soft signs of neurological delay [7, 8]. Electroencephalographic (EEG) studies have revealed a slightly higher rate of nonspecific changes in children with PNE, especially increased slow-wave activity in connection with delayed cerebral maturation [8, 9]. In this study, children with mental retardation or neurologic or psychiatric disorders were excluded. The relatively normal EEGs in our study may be related to superficial recording of the outer layers of the cerebral cortex. Although the micturition center is located in the pons, few studies have focused on the brainstem [8, 10]. The

Table 2 Exteroceptive suppression of the masseter muscle results

Parameter	Enuresis (n = 17)*	Control (n = 19)	*P*
S1 onset latency			
Right	12.18 ± 1.47	12.06 ± 0.79	0.75
Left	12.1 ± 1.57	11.84 ± 0.95	0.54
S1 duration			
Right	16.68 ± 4.49	16.91 ± 3.57	0.86
Left	16.49 ± 3.92	17.38 ± 3.08	0.43
S2 onset latency			
Right	49.48 ± 6.85	47.97 ± 3.38	0.41
Left	49.47 ± 7.54	47.8 ± 2.63	0.38
S2 duration*			
Right	33.35 ± 13.1	51.5 ± 19.43	0.003
Left	31.47 ± 14.72	53.29 ± 20.13	0.001

*This analysis was done after extracting the 6 cases whose responses could not be obtained (17 enuretic, 19 control children).

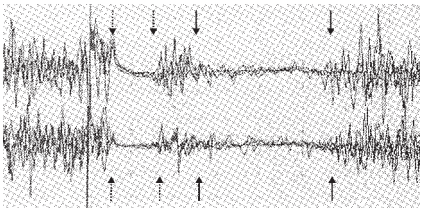


Fig. 1 Right (upper trace) and left masseter (bottom trace) exteroceptive suppression patterns obtained from a healthy control. Arrows indicate the onset and end points of the S2 period. Arrows with dotted lines show the S1 period.

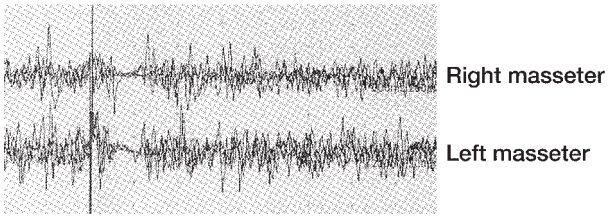
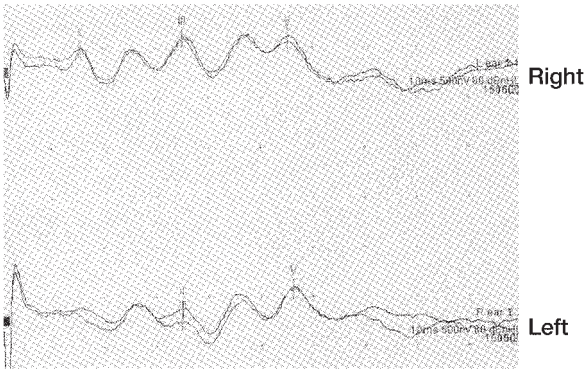
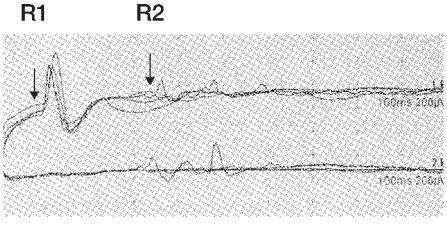


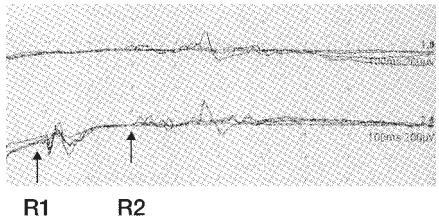
Fig. 3 Masseter exteroceptive suppression patterns obtained from a 10-year-old enuretic child. S2 suppression periods of both masseter muscles (right side, upper trace, left side, bottom trace) were absent.



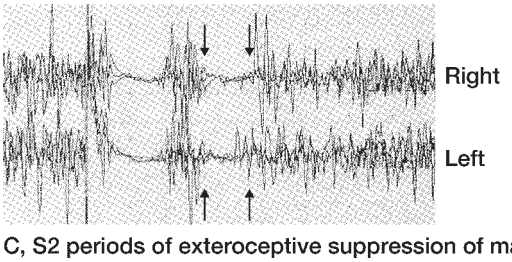
A, ABR trace of both ears.



B1, Right blink reflex.



B2, Left blink reflex.



C, S2 periods of exteroceptive suppression of masseter.

Fig. 2 Electrophysiological recordings obtained from an enuretic patient. ABR (**A**) and blink reflexes (**B1** and **B2**) accepted as normal. S2 periods of exteroceptive suppression obtained from both masseter muscles were shortened (**C**). Arrows indicate the onset and end points of S2 periods (**C**).

Table 3 ABR and blink reflex results in children with abnormal and normal exteroceptive suppression of the masseter muscle in the enuretic group

Parameter	Abnormal ESMM* (n = 16)	Normal ESMM (n = 7)	*P*
ABR			
I-III IPL			
Right	2.09 ± 0.14	2.13 ± 0.16	0.7
Left	2.011 ± 0.17	2.16 ± 0.11	0.2
III-V IPL			
Right	2.09 ± 0.11	1.9 ± 0.2	0.1
Left	2.07 ± 0.15	1.93 ± 0.21	0.1
I-V IPL			
Right	4.20 ± 0.15	4.05 ± 0.24	0.4
Left	4.18 ± 0.12	4.13 ± 0.23	0.7
Blink Reflex			
R1 latency			
Right	10.35 ± 0.27	10.09 ± 0.96	0.1
Left	10.45 ± 0.75	10.15 ± 0.66	0.5
R1 amplitude			
Right	0.27 ± 0.3	0.40 ± 0.2	0.15
Left	0.26 ± 0.2	0.31 ± 0.18	0.3
R2 latency			
Right	35.50 ± 5.48	34.37 ± 3.41	0.5
Left	36.30 ± 4.2	36.01 ± 3.52	0.5
R2 amplitude			
Right	0.26 ± 0.1	0.35 ± 0.14	0.09
Left	0.25 ± 0.15	0.31 ± 0.12	0.17

*ESMM, exteroceptive suppression of masseter muscle.

pontine micturition center includes medial (M region) and lateral (L region) components; the M region is part of the locus coeruleus (the initiator of micturition), and the L region is part of the locus subcoeruleus (the inhibitor of micturition) [8]. This center is also inhibited by the bladder over the periaquaductal gray, pedunculopontine tegmental nucleus and nucleus reticularis pontis oralis [1]. The noradrenaline projection network originates from the locus coeruleus; this system is responsible for arousal [11].

These neuroanatomical relationships also support the possible abnormalities involving sleep pattern and arousal mechanisms. Several studies have demonstrated a significant association between PNE and obstructive sleep apnea syndrome, especially due to adeno-tonsillar hypertrophy [4]. Ornitz *et al.* described the inhibitory effect of a weak peripheral input (auditory, visual or tactile) before a strong startling stimulus and demonstrated that prestimulation 120 milliseconds before the main stimulus results in significantly less inhibition of the blink reflex in patients with PNE than in children with attention deficit hyperactivity disorder and healthy controls [1, 11]. According to Ornitz *et al*, the sensory inhibition of the startle reflex is mediated by the pedunculopontine tegmental nucleus [1]. Also Von Gontard *et al.* evaluated 22 children with PNE using the Zurich Neuromotor Test Battery and pre-pulse inhibition of the startle blink and found similar results to those of Ornitz *et al.* [8]. They concluded that the pre-pulse inhibition, as well as inhibition of bladder emptying during sleep, are regulated by brainstem centers in close proximity to the pontine micturition center and that PNE represents a general neuromotor delay and specific brainstem dysfunction [8]. Our aim was to investigate the brainstem integrity, especially the blink reflex pathways, using the blink reflex and exteroceptive suppression of the masseter muscle in children with PNE. We measured ABR waveforms, which include a series of fluctuant far-field potentials appearing within the first 10 milliseconds following an acoustical stimulation and are used to evaluate the normality of the lower auditory system [12]. Auditory nerve and brainstem centers (the cochlear nucleus and superior olivary complex) are the main sources of the waveforms [12]. Our results showed that auditory brainstem pathways were not involved in PNE.

The blink reflex has 2 components, R1 and R2. The R1 component is a more stable reflex response and is evoked on the stimulation side; it is a pontine reflex [13]. R2 is recorded bilaterally with unilateral stimulation. This component has a complex route including the pons and lateral medulla [13]. We did not find any significant changes in R1 or R2 components, possibly because of the high number of synapses in the reflex circuit, especially R2 responses, which are relatively unstable and strongly modulated by suprasegmental influences, cortical and basal ganglia dysfunction, disorders of consciousness and cognition [14]. Exteroceptive suppression of the masseter muscle by electrical stimulation of the trigeminal nerve (the jaw opening reflex) has been especially studied in patients with headache [6, 15]. There are 2 different suppression periods after electrical stimulation of the trigeminal nerve; the first period (S1) starts at about 10–12 milliseconds, and the second period (S2) begins at about 45–55 milliseconds [6, 15]. Previous studies concluded that the S1 and S2 periods are generated by the activation of inhibitory neurons located in the brainstem [15, 16]. These neurons are probably located at the bulbar reticular formation [17]. These interneuronal

relationships are shown in Fig. 4. Our results clearly demonstrated meaningful changes affecting primarily S2 duration time. In 6 cases with PNE, S2 duration time was not obtained under optimum test conditions, but this parameter was recorded in all control subjects. We retrospectively reevaluated the patient recordings whose S2 durations could not be obtained but did not find any significant difference between their data and that of the patients with S2 duration time regarding age, sex, co-morbidity, severity of disease or response to treatment. We believe that this point should be studied further in a large group of patients. We also noted that S2 duration time was significantly lowered in children in the enuretic group.

Our data supports a possible brainstem dysfunction in children with PNE. It seems that altered activity of bulbar inhibitory neurons plays some role in PNE pathogenesis.

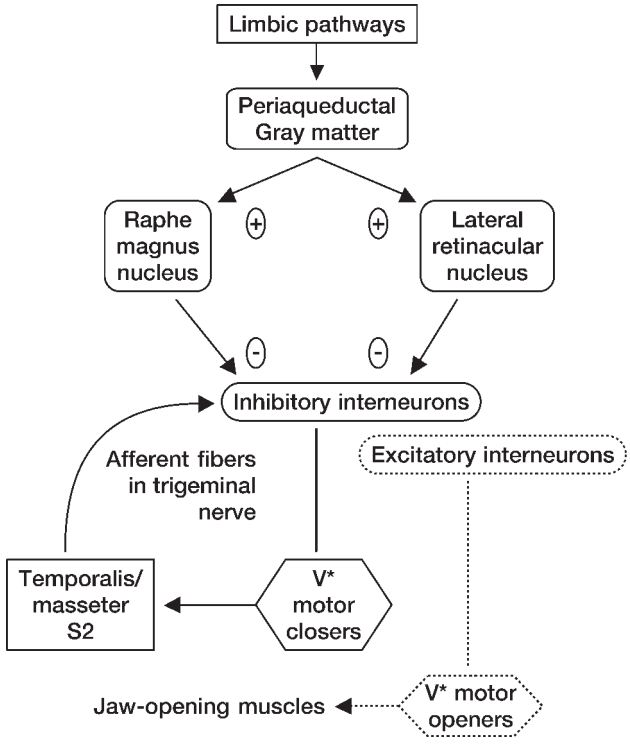


Fig. 4 Schematic diagram showing the brainstem interneuronal relations known to mediate the masticatory reflexes (adapted from Schoenen J, Headache (1993) 33:3–17). *V, trigeminal nevre.

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