Auditory Startle Reaction is disinhibited in idiopathic Restless Legs Syndrome

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**Study objectives:** Because the auditory startle reaction is abnormal in disorders with substantia nigra pathology, we hypothesized that auditory startle responses (ASRs) might also be altered in restless legs syndrome (RLS).

**Design:** Neurophysiologic study of the auditory startle reaction.

**Setting:** Neurology departments of a university hospital and an affiliated local hospital.

**Patients and Participants:** Fifteen patients with idiopathic RLS (6 de novo, 9 untreated after a 7-day wash-out period of levodopa, mean duration of RLS symptoms 21.2 ± 17.9 years, mean IRLS severity score 23.5 ± 6.7) and 15 sex- and age-matched healthy controls were investigated.

**Interventions:** Not applicable.

**Measurements and Results:** ASRs were elicited by 8 high-intensity auditory stimuli differing randomly in tonal frequency and intensity. Reflex electromyographic activity was simultaneously recorded with surface electrodes from 8 facial, neck, arm, and leg muscles. In RLS patients, ASRs were significantly more frequent (541 of 960 possible responses; controls, 430 of 960), and ASR area under the curve was significantly larger (3812 ± 450 µVms; controls, 1756 ± 226 µVms). Analysis per body region revealed that ASRs were significantly more frequent in RLS patients than in controls in leg muscles (138/360 vs 55/360); ASR latencies to leg muscles were significantly shorter in RLS patients (129 ± 6 ms vs 160 ± 11 ms); ASR area under the curve was significantly larger in RLS patients in facial (7547 ± 1326 µVms vs 2982 ± 448 µVms) and leg muscles (1373 ± 308 µVms vs 541 ± 193 µVms).

**Conclusions:** Our data demonstrate disinhibition of reticulospinal pathways in RLS patients as compared to normal controls, likely originating from dysfunction rostral to the lower brainstem.

**Keywords:** auditory startle reaction, pathophysiology, restless legs syndrome

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**INTRODUCTION**

RESTLESS LEGS SYNDROME (RLS) IS A FREQUENT NEUROLOGIC DISORDER WITH A PREVALENCE OF APPROXIMATELY 10% IN THE GENERAL CAUCASIAN population. Essential features comprise the urge to move the legs, usually accompanied by unpleasant sensations; onset or worsening of symptoms during periods of rest or inactivity; partial or total relief by movement; and worsening of symptoms in the evening or at night. To date, few neurophysiologic studies have been published in patients with RLS. Bara-Jimenez et al reported increased spinal cord excitability based on disinhibited flexor reflex responses. Investigation of soleus H-reflex and H-reflex recovery curves yielded ambiguous results. Blink reflex and exteroceptive electromyographic suppression of temporalis and gastrocnemius muscles were found to be normal, as were somatosensory and auditory evoked potentials. Transcranial magnetic stimulation studies have shown normal motor excitability measures following single pulse stimulation but have revealed evidence of intracortical disinhibition in RLS.

The auditory startle reaction, a brainstem reflex that occurs in response to an unexpected loud stimulus, is generated in structures of the caudal brainstem, from where the reflex responses propagate up the brainstem and down the spinal cord along slowly conducting reticulobulbar and reticulospinal pathways. The nucleus reticularis pontis caudalis is the central structure that integrates the pattern of auditory startle responses (ASRs) in various muscles. This nucleus, along with other structures, is under basal ganglia control. ASRs have been examined in various disorders with basal ganglia involvement and have been reported to be absent or reduced in progressive supranuclear palsy, accelerated with a tendency toward higher probability in Parkinson disease, and exaggerated in multiple system atrophy. Because nigral neuronal loss is common to these different disorders and because recent neuropathologic and immunohistochemical studies in RLS have suggested impaired iron acquisition in neuromelanin cells of the substantia nigra, we hypothesized that ASRs might also be abnormal in idiopathic RLS.

**METHODS**

**Patients and Controls**

Fifteen patients with the diagnosis of idiopathic RLS according to International RLS Study Group essential criteria were included. Secondary RLS was excluded by clinical examination; routine laboratory testing, including serum iron, ferritin, transferrin, transferrin saturation, thyroid function, vitamin B₁₂, and folate levels; and detailed electrophysiologic studies when necessary. Only pa-
patients with more than 15 points on the International RLS Scale were included. Nine patients had received previous treatment with levodopa, and experiments were performed after a 7-day wash-out period. The other 6 patients were naive to treatment. Fifteen sex- and age-matched healthy volunteers served as control subjects. All patients and controls granted written informed consent.

Auditory Startle Reaction

Patients and controls were studied in the supine position in a quiet semidarkened room between 8:00 and 10:00 am, when patients experienced no RLS symptoms. They were asked to remain awake and relaxed. Care was taken to avoid potential visual and auditory prepulse stimuli. After normal bilateral hearing thresholds were ascertained, ASRs were elicited by 8 binaurally presented tone bursts that differed randomly in tonal frequency and intensity (250 Hz, 90 dB; 500 Hz, 105 dB; 750 Hz, 105 dB; 1000 Hz, 110 dB nHL) in order to enhance the novelty of the stimulus. Consecutive stimuli were given at intervals of 2 to 3 minutes. Nonrectified surface electromyographic recordings were obtained simultaneously after each stimulus from right masseter, orbicularis oculi, sternocleidomastoid, biceps brachii, abductor pollicis brevis, rectus femoris, tibialis anterior, and soleus muscles. Silver-silver chloride cup electrodes were attached over the muscle belly and tendon except for orbicularis oculi muscle. Single sweeps of 1000 milliseconds, including 200-millisecond prestimulus delay, were recorded with filters set at 10 and 10.000 Hz.

Data Analysis

Traces with background activity of a mean amplitude exceeding 50 µV were rejected from further analysis. ASRs were accepted when electromyographic activity of at least 50 µV occurred in either of the 8 simultaneously recorded muscles at an appropriate latency. ASR latencies were measured manually from the time of stimulus delivery to ASR onset. The ASR area under the curve (AUC) of each response was calculated during the first 100 milliseconds following response-onset latency in order to avoid contamination with volitional movement.

Normal data distribution was ascertained with the Shapiro-Wilks-W test. The χ² test was used to compare the probability of responses between patients and controls. For each subject, mean ASR latency and mean ASR AUC following 8 stimuli were calculated for each muscle. These mean values were then compared between patients and controls for all muscles combined, for individual muscles, and for 3 separate body regions: face (masseter, orbicularis oculi, sternocleidomastoid), arm (biceps brachii, abductor pollicis brevis), and leg (rectus femoris, tibialis anterior, and soleus) using the Student t-test. Bonferroni corrections were applied for multiple comparisons. The Mann-Whitney U test was used to compare hearing thresholds between groups.

RESULTS

Patients and controls were of similar age and had similar hear-

| Table 1—Characteristics of Patients with Restless Legs Syndrome and Healthy Control Subjects |
|-----------------------------------------------|-----------------|-----------------|
| Patients | Controls | p Value |
| (n = 15) | (n = 15) | |
| Women/men, no. | 12 / 3 | 12 / 3 | NS |
| Age, y | 50.9 ± 12.2 | 51.1 ± 12.4 | NS |
| Weight, kg | 79.9 ± 17.4 | 64.7 ± 8.2 | p < .001 |
| Height, cm | 170.5 ± 9.3 | 167.9 ± 6.8 | p < .05 |
| Body mass index, kg/m² | 27.3 ± 3.8 | 22.8 ± 2.8 | p < .05 |
| Hearing threshold, dB | 12.8 ± 5.1 | 11.8 ± 6.1 | NS |
| Serum ferritin value, µg/L | 85.7 ± 83.4 | not available | |

Data are presented as mean ± SD unless otherwise indicated.

| Table 2—Results of Auditory Startle Responses for Individual Muscles of RLS Patients and Healthy Controls |
|-----------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                | ASR frequency, ratio | ASR latency, ms | ASR area, µVms |
| RLS     | Controls | RLS     | Controls | RLS     | Controls | RLS     | Controls |
| Masseter | 91/120 (76) | 94/120 (78) | 72 (12) | 64 (3) | 3144 (634) | 1296 (249) |
| Orbicularis oculi | 118/120 (98) | 120/120 (100) | 34 (2) | 35 (2) | 10193 (1374) | 5078 (726) |
| Sternocleidomastoid | 90/120 (75) | 93/120 (78) | 70 (10) | 62 (4) | 7807 (2017) | 2682 (605) |
| Biceps brachii | 52/120 (43) | 33/120 (28) | 88 (5) | 118 (13) | 2811 (1105) | 1774 (954) |
| Abductor pollicis brevis | 44/120 (37) | 35/120 (29) | 96 (5) | 114 (11) | 2670 (791) | 1651 (504) |
| Rectus femoris | 59/120 (49) | 21/120 (18) | 118 (5) | 141 (15) | 1785 (461) | 389 (151) |
| Tibialis anterior | 39/120 (33) | 17/120 (14) | 131 (10) | 194 (14) | 1115 (239) | 659 (331) |
| Soleus | 40/120 (33) | 17/120 (14) | 127 (7) | 173 (12) | 1218 (425) | 516 (179) |

*Significant differences between restless legs syndrome (RLS) and controls after correction for multiple comparisons. Values for ASR probability are given as ratio (percentage), and for auditory startle response (ASR) latency and ASR area under the curve as mean (SEM).
ing thresholds, but body weight and height were significantly greater in the patient group (Table 1). Body weight, however, has no influence on ASRs in humans.29

ASRs in all muscles combined were significantly more frequent in patients (541 out of 960 possible responses) than in controls (430/960), \( \chi^2 = 25.7, p < .001 \). ASR probabilities in individual muscles are shown in Table 2. In patients, ASRs were significantly more frequent in rectus femoris, tibialis anterior, and soleus muscles. When analyzed per body region, ASR probability was significantly greater in leg muscles of patients than controls (138/360 vs 55/360, \( \chi^2 = 48.7, p < .001 \)). Figure 1 shows a representative example of ASRs in a patient with RLS and in a healthy control subject. Mean ASR latencies (± SEM) in individual extremity muscles were shorter in RLS patients, as compared with controls, but the differences did not reach statistical significance (Table 2). Comparison per body region, however, revealed significantly shorter latencies in patients’ leg muscles (129 ± 6 ms) than in controls (160 ± 11 ms), \( p < .05 \) (Figure 2A).

The mean ASR AUC (± SEM) of all muscles combined was significantly larger in patients (3812 ± 450 µVms) than in controls (1756 ± 226 µVms) (\( p < .05 \)). The mean ASR AUC tended to be larger in all individual muscles of RLS patients, as compared to controls, but did not reach statistical significance (Table 2). Analysis per body region revealed significantly larger areas in the face and leg in patients (7547 ± 1326 µVms and 1373 ± 308 µVms), as compared to controls (2982 ± 448 µVms, \( p < .01 \), and 541 ± 193 µVms, \( p < .05 \)) (Figure 2B). Disease severity, as measured by International RLS Scale and ferritin levels did not correlate with ASR probability, ASR latency, or ASR AUC. No ASR parameter differed significantly between pretreated (\( n = 9 \)) and untreated (\( n = 6 \)) patients.

**DISCUSSION**

The present study demonstrates disinhibition of the auditory startle reaction in patients with idiopathic RLS, as compared to sex- and age-matched healthy controls. Overall, the ASR probability was higher, ASR latencies were shorter, and ASR size in terms of AUC was larger in patients than in controls.

ASR latencies to arm and leg muscles were consistently shorter in the patient group, with statistically significant differences for all leg muscles combined. The lack of significant differences in cranial nerve-supplied and upper extremity muscles may be due to their shorter latencies in the presence of large intraindividual and interindividual variability; potential differences are more likely to be confounded by measurement noise, which would explain the substantial overlap, particularly in cranial nerve-supplied muscles between RLS patients and controls (Table 2). Similarly, only a trend for larger ASR responses was observed in individual muscles of RLS patients due to the inherent large variability of
ASRs. However, when analyzed per body region, RLS patients had significantly larger responses in the face and legs.

The observed findings indicate disinhibition of reticulospinal pathways, which may be due to alterations of neuronal function at various levels of the central nervous system. Bara-Jimenez et al studied flexor reflexes in patients with idiopathic RLS and periodic leg movements in symptom-free (during the day) as well as symptomatic (during sleep) conditions. They found less inhibition of flexor reflexes during symptomatic periods, greater spatial spread of responses, and occurrence of late and long-lasting electromyographic discharges with lower threshold, as compared to controls and to symptom-free conditions, indicating enhanced state-dependent spinal excitability. Because all of our examinations were performed in the morning during asymptomatic periods, ASR differences between patients and controls may actually have been underestimated.

Flexor reflexes clinically resemble periodic leg movements, and flexor reflexes and RLS share common dopaminergic mechanisms; thus, a common generator was hypothesized. However, because flexor reflexes also are under suprassegmental control via corticospinal, rubrospinal, and reticulospinal pathways, these findings may not be explained solely by alterations at the spinal level. Enhanced ASRs, which originate in the nucleus reticularis pontis caudalis and which are conveyed via reticulobulbar and reticulospinal pathways, are consistent with a supraspinal dysfunction in RLS.

Disinhibition of ASRs in RLS patients, as compared to healthy controls, may be caused by altered ASR integration or by loss of inhibitory control of higher hierarchical structures, eg, by reduced inhibitory influences of the basal ganglia on midbrain and brainstem nuclei engaged in the auditory startle reaction. The normal pattern of ASRs in RLS patients with more responses in face muscles and subsequently fewer responses in arm and leg muscles, as well as increasing latencies from face to leg muscles, suggests a normal function of the primary startle circuit. This finding is in accordance with the absence of macrostructural brainstem lesions in RLS patients. The nucleus reticularis pontis caudalis receives both excitatory and inhibitory cholinergic projections from the pedunculopontine tegmental nucleus, which regulates the excitability by predominantly inhibiting startle-related structures of the pontine reticular formation. The pedunculopontine tegmental nucleus in turn receives predominantly inhibitory basal ganglia input, mainly from globus pallidus and substantia nigra pars reticulata.

Studies in patients indicate basal ganglia involvement in the control of ASRs. Basal ganglia disease with substantia nigra pathology may result in disinhibition of the basal ganglia output, thus contributing to net facilitation of ASRs. Notably, this disinhibition is significantly more pronounced in the parkinsonian subtype of multiple system atrophy, as compared to the cerebellar subtype. A potential role of dopamine in ASR modification is supported by animal data; for example, in a rat model of Parkinson disease using 6-hydroxydopamine, neurons in the caudate nucleus and putamen become supersensitive to dopamine agonists after denervation of the nigrostriatal pathway, and supersensitive dopamine D receptors have been suggested to mediate enhanced ASRs. Recent neuropathologic studies in RLS have found impaired iron accumulation in neuromelanin cells of the substantia nigra, implying that this brain structure may contribute to the pathogenesis of RLS. These findings are also supported by midbrain sonography, which demonstrates hypoechogenicity in the substantia nigra, potentially indicating iron deficiency. Brain iron deficiency has previously been implicated in the modulation of ASRs in laboratory animals, in both directions and to various degrees. However, methodology of measuring ASRs differs greatly between rats and humans, which renders comparison difficult.

Exaggerated ASRs may also be encountered in diseases with cortical or subcortical alterations, e.g., stroke or hypoxia, causing loss of cortical control over the brainstem. Because transcranial magnetic stimulation studies have provided evidence for cortical disinhibition in RLS, cortical dysfunction may also contribute to enhanced ASRs in RLS. These results, together with our findings, are in accordance with a previously described general state of hypereexcitability in RLS.

In summary, this is the first study to demonstrate enhanced ASRs in patients with idiopathic RLS, as compared to healthy controls, suggesting disinhibition of reticulospinal pathways, likely originating rostral to the lower brainstem.

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