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Seizure Susceptibility, Phenotype, and Resultant Growth Delay in the *nclf* and *mnd* Mouse Models of Neuronal Ceroid Lipofuscinoses

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Abstract

We examined flurothyl gas-induced seizure latencies and phenotype in 2 mouse models of neuronal ceroid lipofuscinoses: the *nclf* (*Cln6* mutant) variant late-infantile model and the *mnd* (*Cln8* mutant) Northern epilepsy model. *Mnd* mice on postnatal days 35 to 42 had increased latency to loss of posture compared with wild-type controls. *Nclf*, *mnd*, and wild-type mice on postnatal days 21 days to 25 displayed similar latency profiles during repeated seizure induction (kindling) and retesting; seizure phenotypes were different, however. Kindled wild-type mice re-exposed to flurothyl after a 28-day recovery displayed brainstem generalized seizures exclusively. Neuronal ceroid lipofuscinoses mutants demonstrated a lack of brainstem seizures at retesting after 28 days. Repeated induction of generalized seizures delayed weight gain in both *nclf* and *mnd* mice compared with wild-type mice. These and our previous results suggest abnormal seizure-related neuronal connectivity and/or plasticity are shared characteristics of the neuronal ceroid lipofuscinoses.

Keywords

Batten disease; epilepsy; flurothyl; neuronal ceroid lipofuscinoses; seizure induction latencies

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Author Contributions
David A. Pearce and Elizabeth Kriscenski-Perry designed the studies. Elizabeth Kriscenski-Perry, David A. Pearce, Elizabeth Kriscenski-Perry, and Attila D. Kovács analyzed the data and wrote the manuscript.

Declaration of Conflicting Interests
None of the authors of this paper has any conflict of interest relating to the publication.

Ethical Approval
All animal procedures were carried out according to the guidelines of the Animal Welfare Act, National Institutes of Health policies, and the University of Rochester Animal Care and Use Committee.

Introduction

Neuronal ceroid lipofuscinoses, also known as Batten disease, are the most prevalent pediatric neurodegenerative diseases, with an estimated incidence of 2 to 4 in 100 000 live births in the US (which translates to 80 to 170 new patients every year).[1] The occurrence of neuronal ceroid lipofuscinoses is the highest in the Scandinavian countries, Finland being the most affected, with an estimated incidence of 1 in 12 500 live births.[2] Neuronal ceroid lipofuscinoses are characterized histologically by abnormal lysosomal accumulation of autofluorescent storage material and loss of select neuronal populations.[3] This family of disorders comprises 8 distinct variants identified by unique patterns of onset and progression.[2] The different forms are caused by recessive mutations in at least 10 genes (*CLN1-10*).[2] Along with vision loss, cognitive decline, psychiatric complications, neurodegeneration, and premature death, seizures are a shared symptom of the neuronal ceroid lipofuscinoses.[3]

Seizure activity may be a critical driving force behind the progressive neurodegeneration seen with the pediatric-onset neuronal ceroid lipofuscinoses. This hypothesis is supported by studies that indicate that late onset of seizures, or their absence, correlates with milder progression and a better overall prognosis.[4] In addition, anecdotal information from parents of children with these disorders describes dramatic worsening of condition with repeated seizure activity.

We have previously demonstrated age-dependent alteration of flurothyl-induced seizure latencies and recovery in the *Cln3*$^{-/-}$ mouse model of juvenile neuronal ceroid lipofuscinosis,[5] and wished to determine whether similar seizure-related anomalies existed in 2 other mouse models, the *nclf* (*Cln6* mutant) model of variant late-infantile neuronal ceroid lipofuscinosis[6] and the *mnd* (*Cln8* mutant) model of Northern epilepsy.[7]

The *nclf* mouse has progressive retinal degeneration and motor paralysis.[8] The naturally occurring *mnd* mutant was originally a potential model of amyotrophic lateral sclerosis[9] but was later shown to have the mutation in the *Cln8* gene, providing a good model for Northern epilepsy/progressive epilepsy with mental retardation.[7] *Mnd* mice also display adult-onset progressive motor neuron degeneration, and both the *nclf* and *mnd* mice are characterized by accumulation of autofluorescent storage material.[8–9] Studies with the *mnd* mice have also demonstrated age-dependent changes in behavior that may be highly relevant to the disturbing psychiatric and behavioral symptoms that occur with progression of disease in the pediatric-onset human neuronal ceroid lipofuscinoses.[10]

Methods

Animals

Male C57BL/6J wild-type and male *nclf* and *mnd* mice on the C57BL/6J background were used for all experiments. Mice were group housed in the University of Rochester vivarium in a temperature-controlled room on a 12-h light/dark cycle. Food and water were provided ad libitum.

Seizure Induction

Animals were transported from the vivarium to the laboratory a minimum of 45 minutes prior to experimental trials to allow for acclimatization. All trials were run between 10 am and 1 pm to minimize circadian influences. Seizures were induced by placing mice individually into a 2.4-liter closed Plexiglas chamber into which a 10% solution of flurothyl (2,2,2-trifluroethyl ether; Sigma-Aldrich, St. Louis, Missouri) was infused as previously described.[11] Animals were weighed immediately post-seizure. For kindling studies, generalized seizures were induced once a day for 8 consecutive days (Induction Phase) with subsequent retesting once a week for 4 weeks (Incubation Phase). Data shown are from the fourth retest, 28 days after the end of the kindling.

Generalized seizures were classified from grade 1 to 7 using a previously published scoring system.[12] Grades 1 through 2 represent forebrain seizures. Grade 3 through 7 seizures can be elicited in the absence of forebrain connections and are generally termed “brainstem seizures.” Generalized brainstem seizures are preceded by forebrain seizures and are designated as forebrain-to-brainstem seizures.[12]

Data Analysis

Means of latencies to loss of posture as well as means of body weight were compared using the Student unpaired *t* test.

Results

Age-Dependent Increase in the Latency to Flurothyl-induced Seizure in mnd Mice

Similarly to previous observations for the *Cln3*$^{-/-}$ mouse model of juvenile neuronal ceroid lipofuscinoses,[5] *mnd* male mice ages 35 days to 42 days demonstrated a statistically significant, 26% increase in latency to loss of posture in a single trial flurothyl exposure ($P < .05$). No latency differences were observed between *mnd* and wild-type mice ages 21days to 25 days or 6 months to 9 months (Figure 1). Seizure latencies for n*clf* mice were statistically identical to those of wild-type mice at all 3 ages (Figure 1).

Immature Wild-type, nclf and mnd Mice Show Similar Seizure Latencies but an Altered Seizure Phenotype During Kindling and Retesting

Wild-type, *nclf*, and *mnd* mice at 21 days to 25 days of age underwent kindling: seizure induction by flurothyl gas once a day for 8 consecutive days. Seizure induction latencies during the 8 days of kindling in wild-type, *nclf*, and *mnd* mice were very similar (Figure 2A). A trend toward increased latency in the *mnd* mice was observed but did not reach statistical significance on any but the eighth day ($P < .05$ by unpaired *t* test). No differences in seizure induction latencies were observed between groups during a 28-day post-kindling retest (once a week for 4 weeks; data for the 28th day retest are shown in Figure 2A). An expected shift from forebrain to forebrain-to-brainstem seizures was observed in the kindled wild-type mice upon retesting 28 days later (Figure 2B). Mutant strains, however, failed to show this phenotypic change (Figure 2B). None (0%) of the *nclf* or *mnd* mice, but all (100%) of wild-type mice, demonstrated a forebrain-to-brainstem seizure (Figure 2B). This phenotypic switch following flurothyl-kindling has been proposed to result from synaptic

reorganization.[11–12] An absence of this phenotypic switch in the *nclf* and *mnd* mice indicates there may be shared disruption and reorganization of seizure-related neuroanatomical pathways in the brainstem among the neuronal ceroid lipofuscinoses.

Evidence for Seizure-Related Growth Delay in nclf and mnd Mice

Daily seizure activity for 8 consecutive days (kindling), and subsequent, once-a-week seizure induction (retesting) for 4 weeks resulted in a statistically significant decrease in total body weight in *nclf* ($P < .01$) and *mnd* ($P < .001$) mice when compared with naïve same-strain controls (Figure 3). Repeated seizure activity (kindling and retesting for 4 weeks) did not change the weight of wild-type mice (Figure 3). Young adult *nclf* and *mnd* naïve mice were consistently bigger than wild-type naïve mice; therefore, we made weight comparisons to same strain naïve animals only.

Discussion

We have documented an age-dependent increased latency to flurothyl-induced generalized seizures in 2 out of the 3 neuronal ceroid lipofuscinosis mouse models we have examined (Figure 1 and [5]). In both *Cln3*$^{-/-}$ and *mnd* mice, this alteration of seizure induction threshold occurs only during a specific developmental window, at 35 days to 42 days postnatally, and is not present at earlier or later developmental time points. The findings suggests that abnormal synapse formation, pruning, and/or strengthening of synapses may occur. However, the evidence for this remains indirect and putative aberrant postnatal wiring might also result from earlier, even from embryologic, insults.

A high mortality rate in *Cln3*$^{-/-}$ mice following a forebrain-to-brainstem seizure[5] originally led us to hypothesize that absence of the neuronal ceroid lipofuscinosis-linked protein perturbs brainstem anatomy and/or postnatal nervous system maturation, and plasticity. This hypothesis is supported by our current findings that both *nclf* and *mnd* neuronal ceroid lipofuscinosis-mutant mice appear resistant to flurothyl kindling-induced remodeling (Figure 2B), which is a plasticity-dependent phenomenon. Behavioral studies in *mnd* mice have shown age-dependent changes in habituation, fear conditioning, and increases in both initiation and length of intruder-induced aggressive behavior.[10] These results are consistent with the idea that neuronal ceroid lipofuscinosis-related mutations may result in aberrant brainstem and limbic connectivity and/or altered plasticity. Glutamate receptors play an essential role in neuronal plasticity,[13–15] and therefore, abnormal glutamate receptor function can be an important contributor to disease development and progression. Aberrant glutamate receptor function have been demonstrated in the *Cln3*$^{-/-}$ mouse model of juvenile neuronal ceroid lipofuscinosis.[16–19] The mRNA expression of type 1 metabotropic glutamate receptor (mGluR1) was found to be downregulated in the brain of 10-week-old *Cln3*$^{-/-}$ mice.[16] Our previous studies also demonstrated that an abnormally increased AMPA-type glutamate receptor activity largely contributes to the motor coordination deficit in *Cln3*$^{-/-}$ mice as early as 1 month of age.[18–19]

Lastly, both *mnd* and *nclf* mice had highly significant decreases in total body weight after kindling compared with age-, sex-, and strain-matched mutants in which seizures had not been induced (Figure 3). It is not surprising that repetitive seizures in newly weaned (21- to

25-day-old) animals might delay or otherwise interfere with normal development. It is interesting, however, that this effect was limited to *nclf* and *mnd* mice; whereas wild-type kindled and naïve animals did not lose weight.

Taken together, our results support a working hypothesis that mutations in some (or all) of the neuronal ceroid lipofuscinosis-related proteins alter the biochemical and neuroanatomic pathways that mediate seizure initiation, propagation, and recovery.

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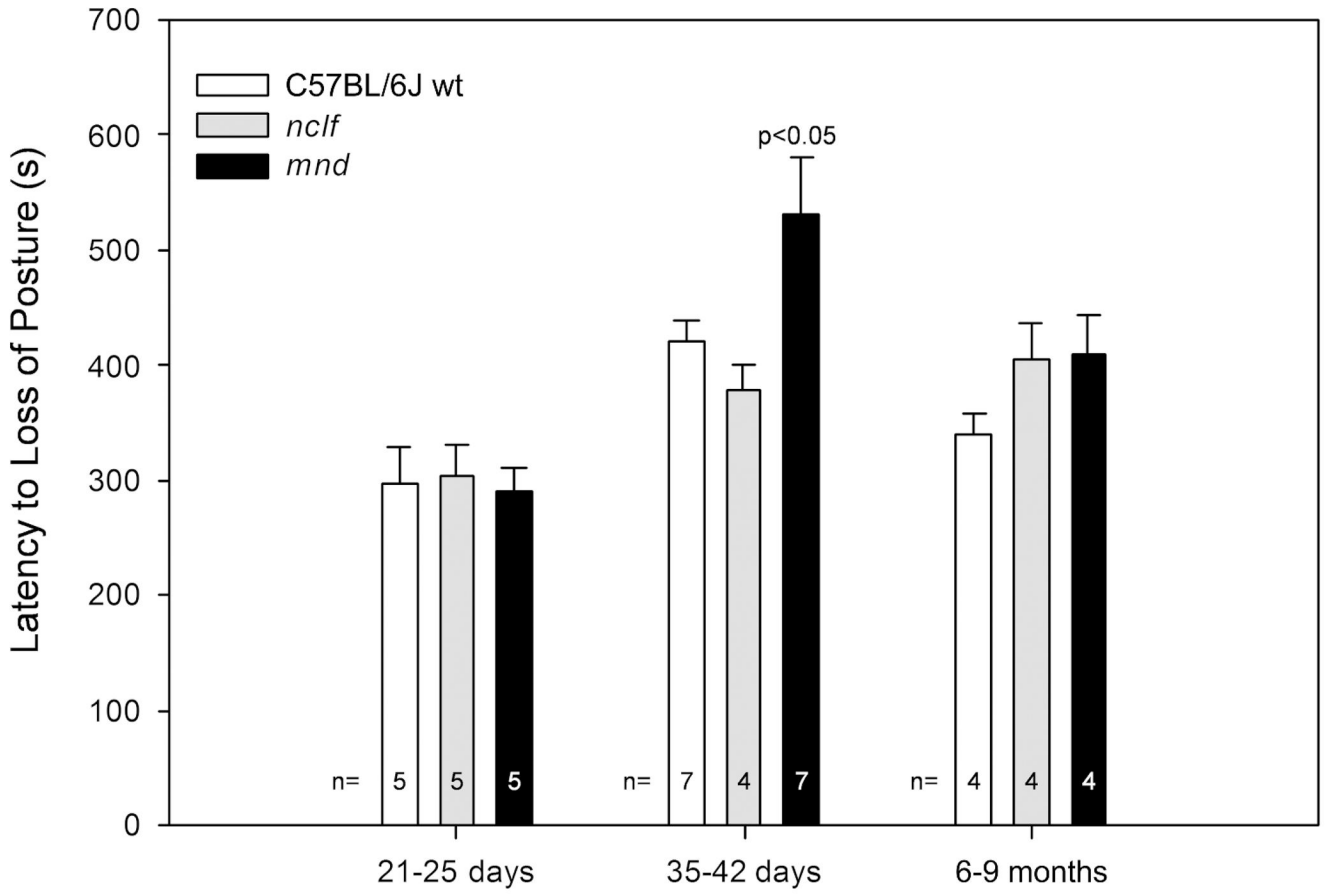

Figure 1.

Age-dependent increase in the latency to flurothyl-induced seizure in mnd mice. Male wild-type, *mnd*, and *nclf* mice at 3 different ages (21 days to 25 days, 35 days to 42 days, and 6 months to 9 months) were used in the experiments. Seizure was induced by inhalation of flurothyl gas, and the latency until loss of posture was measured. Columns and bars represent mean ± standard error of the mean (n = 4–7). At the age of 35 days to 42 days, the mean latency to flurothyl-induced loss of posture was 532.1 ± 47.8 s for *mnd* mice and 420.9 ± 17.6 s for 35-day to 42-day wild-type mice ($P < .05$ by unpaired *t* test). No significant differences were observed between *mnd* and wild-type mice at earlier (21 days to 25 days) or later (6 months to 9 months) developmental time points. No statistically significant differences existed between *nclf* and age-matched wild-type mice.

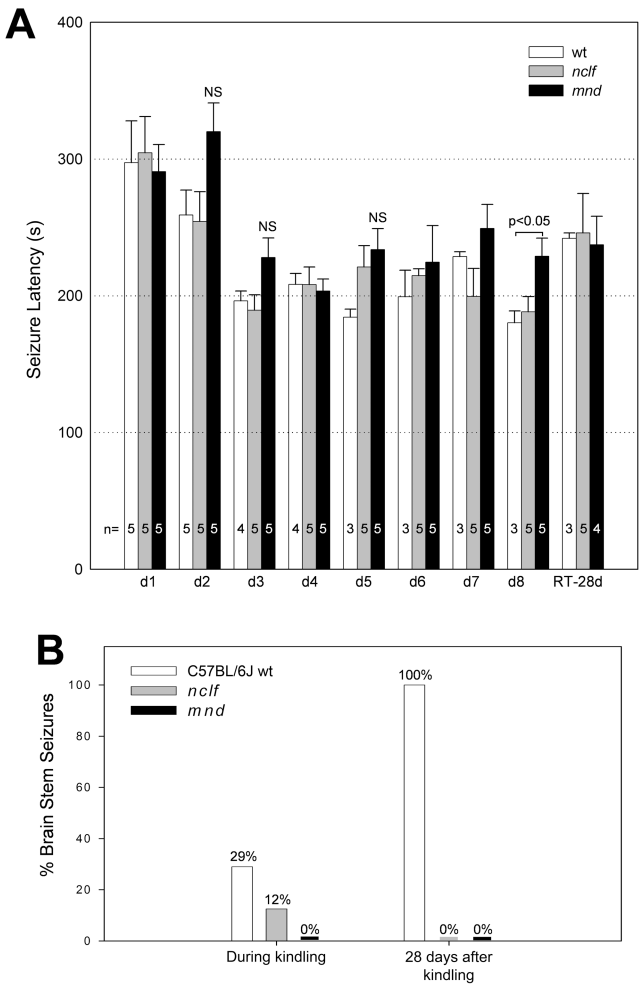

Figure 2.

Immature wild-type, *nclf*, and *mnd* mice show similar seizure latencies but an altered seizure phenotype during kindling and retesting. Wild-type, *nclf*, and *mnd* mice at 21 days to 25 days of age underwent kindling (seizure induction by flurothyl once a day for 8 consecutive days) and subsequent retesting (seizure induction by flurothyl once a week for 4 weeks). Columns and bars represent mean ± standard error of the mean (n = 3–5). (A) Seizure induction latencies during the 8 days of kindling (day 1 through day 8) and in the 4th (28th day) post kindling retest (RT-28 days). Statistical analysis was performed using unpaired *t* test: $P < .05$ as compared with wild-type; NS: not significant. (B) Seizure phenotypes during the 8 days of kindling and in the 4th (28th day) post kindling retest. Eight-day kindling in wild-type mice produced the expected shift from cortical to brainstem seizures when the animals were retested 28 days later. All (100%) wild-type mice demonstrated a brainstem seizure, whereas none (0%) of the *nclf* or *mnd* mice converted to the expected phenotype.

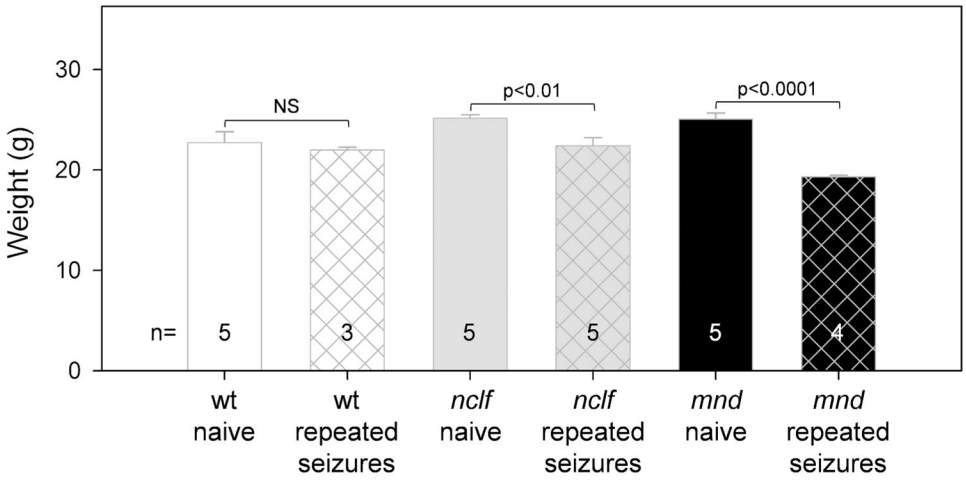

Figure 3.

Repeated flurothyl-induced seizure activity inhibits the growth of *nclf* and *mnd* mice. Wild-type, *nclf*, and *mnd* mice at 21 days to 24 days of age underwent kindling (seizure induction by flurothyl once a day for 8 consecutive days) and subsequent retesting (seizure induction by flurothyl once a week for 4 weeks). After the last retesting (at 57 days to 60 days of age), the mice were weighed. Age-matched naïve (no seizure induction), same-strain control mice also were weighed. Columns and bars represent mean ± standard error of the mean (n = 3–5). Repeated seizure activity significantly decreased the total body weight of *nclf* and *mnd* mice when compared with naïve same-strain controls ($P < .01$ for *nclf* and $P < .001$ for *mnd* by unpaired *t* test). Wild-type animals showed normal growth despite repeated seizure activity (NS: not significant by unpaired *t* test).