Neonatal Seizures Induced Persistent Changes in Intrinsic Properties of CA1 Rat Hippocampal Cells

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We investigated the effects of repeated early-life seizures induced by flurothyl inhalation on intrinsic membrane properties of hippocampal pyramidal neurons from young rats (postnatal day 15–20). Intracellular recordings of CA1 and CA3 pyramidal neurons from flurothyl-treated and control rats revealed no significant differences in resting membrane potential, input resistance, membrane time constant, and action potential characteristics. In CA1 pyramidal cells from flurothyl-treated rats, the spike frequency adaptation and afterhyperpolarizing potential following a spike train were markedly reduced when compared with controls. In contrast, no significant alterations in the firing properties of CA3 pyramidal neurons were found. It is concluded that neonatal seizures lead to persistent changes in intrinsic membrane properties of CA1 pyramidal neurons. These alterations are consistent with an increase in neuronal excitability and may contribute to the behavioral deficit and epileptogenic predisposition observed in rats that experienced repeated neonatal seizures.

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Both clinical and experimental studies have emphasized the age dependency of epilepsy with regard to the seizure susceptibility. Thus, the incidence of seizures is highest during the first years of life in humans,[1–3] and animal studies have demonstrated periods of increase or decrease in seizure susceptibility during development.[4–6] However, there are controversies regarding the consequences of seizures in the developing brain.[7–9] From clinical studies, there are suggestions that seizures occurring during childhood are detrimental in terms of cognitive performances[10,11] and lead to morphological alterations in the brain.[12,13] However, animal studies show that the brain sensitivity to the deleterious effects of seizures may change with age, with fewer behavioral alterations and less brain damage in immature brains than in adults.[14–17]

The clinical situation is complicated by the difficulties distinguishing the deleterious effects caused by the seizures[18] themselves from the developmental disturbances that caused the seizures and from the potential direct effects of the antiepileptic medications.[19] Such variables do not occur in animal studies, suggesting that seizures in the developing brain are not associated with significant pathological changes. A further explanation for the discrepancy between clinical and experimental issues may arise from the type of seizures induced in animal models when compared with the clinical situation. Thus, most animal models are characterized by a single and prolonged status epilepticus, whereas children often experience briefer but recurrent seizures. In addition to the type of seizures, the relation of seizure activity to postnatal development must also be considered. Correlative ontogenic studies have proposed that the cerebral cortex of 7- to 10-postnatal-day-old rats is functionally comparable to that of full-term newborn human.[20–22]

In the present study, we used flurothyl (2,2,2-trifluoroethyl ether), a volatile inhalant that leads to brief and stereotyped seizures in neonatal rats.[23,24] To mimic the clinical situation, seizures were repetitively induced during the first or second week of postnatal life, corresponding to the late fetal and immediate postnatal stages of development in human. With this model, we have previously shown that repeated early-life seizures lead to several behavioral and morphological alterations.[25] These alterations included a decreased performance in learning test, a greater number of dentate granule cells, a sprouting of hippocampal mossy fibers, and a lowered seizure threshold. This last in vivo observation suggests that repeated seizures early

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in life cause a long-lasting increase in excitability. In the present study, individual neurons were intracellularly recorded in slices to investigate the consequences of recurrent early-life seizures on the intrinsic membrane properties of hippocampal pyramidal neurons. We report that repeated seizures early in life lead to a consistent reduction in spike frequency adaptation (SFA) and afterhyperpolarizing potential (AHP) following a train of action potentials in CA1 pyramidal neurons. In contrast, neonatal seizures have no effect on the intrinsic properties of CA3 pyramidal neurons. The functional and pathological consequences of such alterations in the firing properties of CA1 pyramidal neurons are discussed.

Methods

Experiments were performed on a total of 20 male Wistar rats treated in accordance with the guidelines set by the National Institutes of Health and Institut National de la Santé et de la Recherche Médicale for the human treatment of animals.

Flurothyl Treatment

Each rat was placed into a plastic box (380 ml). Liquid flurothyl (2,2,2-trifluoroethyl ether) was delivered through a syringe onto a filter pad in the center of the chamber, where it evaporated. The rats remained in the plastic box until they developed tonic extension of forelimbs and hindlimbs. They were then removed from the chamber and recovered before being returned to their dam. Animals were housed with their litter until postnatal day (P) 15 to 20. At that stage they were killed for electrophysiological recordings. Two groups of experimental rats were prepared. The two groups received five seizures per day for 5 days with a minimum of 2 hours between seizures. The treatment started at P0 (the day of birth) for the first group and at P6 for the second group. Control rats were placed in the chamber for the same time period but were not exposed to flurothyl. No evidence of hypoxia was observed with control rats, demonstrating that the oxygen content in the chamber was sufficient.

The behavioral manifestations of flurothyl-induced seizures have been described previously.[24–26] Briefly, the seizures were quite stereotyped and consisted of head bobbing that started 10 to 15 seconds after the beginning of the flurothyl exposure followed by swimming movements and loss of posture. This phase was followed by a severe tonic posturing with both forelimbs and hindlimbs extended. Urinary and fecal incontinence were usually observed. The rats completely recovered to baseline behavior within 10 to 15 minutes. The behavioral features of the seizures were age dependent. From P0 to P3, the onset of the tonic phase was faster and a mild cyanosis was noted. After P6, the rats experienced a short tonic phase without significant cyanosis.

Hippocampal Slice Preparation

Experiments were performed on hippocampal slices obtained from P15–P20 control and flurothyl-treated rats. A total of 7 P0–P5 flurothyl-treated rats and 7 P6–P10 flurothyl-treated rats were used. To keep the interval between flurothyl treatment and electrophysiological experiments the same (ie, 10 days), slices from 5 P0–P5 treated rats were prepared at P15–P16, and slices from 5 P6–P10 treated rats were prepared at P19–P20. The remaining treated rats were killed at P18. Brains were removed under ether anesthesia and submerged in cold (0°C) artificial cerebrospinal fluid (ACSF) of the following composition: NaCl 126 mM, KCl 3.5 mM, $CaCl_2$ 1.3 mM, $MgCl_2$ 1.3 mM, NaH_2PO_4 1.2 mM, $NaHCO_3$ 25 mM, and glucose 11 mM, with pH 7.4 when equilibrated with 95% O_2/5% CO_2. Hippocampal slices (600 μm thick) were cut with a McIlwain tissue chopper, incubated for at least 1 hour in ACSF at room temperature (20–23°C), and transferred individually in a submerged recording chamber superfused with ACSF (2.5–3.0 ml/min, 34°C).

Intracellular Recordings, Data Measurement, and Analysis

Intracellular recording of pyramidal neurons were obtained using a sharp pipette filled with KCl (3M, 50–60 MΩ). Current clamp recordings were performed using an Axoclamp-2A amplifier (Axon Instruments). Intrinsic membrane properties were determined at the resting membrane potential of the cells in the presence of ionotropic glutamate and $GABA_A$ receptor antagonists (6-cyano-7-nitro-quinoxaline-2,3-dione [CNQX], D(−)2-amino-5-phosphonovaleric acid [D-AP5], bicuculline; Tocris). Data were stored on a personal computer and analyzed off-line with Acquis software (G. Sadoc, Paris, France).

To measure passive membrane properties, square negative current pulses (800 msec duration) of increasing intensity (0.1–0.5 nA) were applied to the recorded cells. The input resistance (R_{IN}) was provided by the slope of the linear regression line of the steady-state voltage versus current amplitude plot. The membrane time constant (τ_M) was determined by the exponential function fitting of the charging curve produced by application of square negative current pulses. SFA was determined by injecting cells with square positive current pulses (800 msec duration). SFA time constant (τ_{SFA}) was taken as the slope of the linear regression line fitted from the plot of the natural logarithm of the instantaneous firing frequency versus action potential latency (time from the onset of current injection) plot.[27] The amplitude of burst AHP evoked by the application of square depolarizing current pulses of 200 msec duration was measured at the peak.

Data are expressed as means ± SEM. Statistical analysis of the mean data was performed using Student's unpaired two-tailed t test. The χ^2 test was used to compare the percentage of CA1 pyramidal neurons that exhibit an active afterdepolarizing potential (ADP). The level of significance was arbitrarily chosen to be $p < 0.05$.

Results

Seven rats treated between P0 and P5, 7 rats were treated between P6 and P10, and 6 control rats were used in this study. A maximum of two slices per rat was used, and only one CA1 and one CA3 pyramidal neurons were recorded per slice. During the recording sessions, no spontaneous epileptiform discharges were observed in control ACSF. Intracellular recordings

from 37 CA1 and 28 CA3 pyramidal cells from control and flurothyl-treated rats, in the presence of ionotropic glutamate and $GABA_A$ receptor antagonists, revealed no statistical differences in resting membrane potential, input resistance, and membrane time constant (Tables 1 and 2).

Neonatal Flurothyl-Induced Seizures Have No Effect on Action Potential Characteristics of CA1 Pyramidal Cells

A single action potential was evoked by injection of a short positive current pulse of increasing intensity (0.1–0.5 nA). No significant differences in action potential amplitude, duration, or threshold were found between control and flurothyl-treated cells (see Table 1). Two types of ADPs were observed following a single spike[28]: a passive ADP defined as a monotonic repolarization from the peak of the fast AHP to baseline (Fig 1A), and an active ADP defined as a redepolarization from the fast AHP followed by a slow repolarization to baseline (see Fig 1B). In control tissue, a passive ADP that decline with an average time constant of 15.8 ± 2.3 msec was observed in 9 of 12 CA1 pyramidal cells (see Fig 1A). In the 3 remaining cells, an active ADP of 9 ± 2 mV from resting potential lasting 60 ± 4.5 msec was observed (see Fig 1E and F). Following neonatal treatment with flurothyl, the proportion of cells that exhibited an active ADP did not differ significantly from controls. An active ADP was observed in 5 of 12 and 6 of 13 CA1 pyramidal cells from, respectively, P0–P5 and P6–P10 ($p = 0.66$ and $p = 0.49$, χ^2 analysis) flurothyl-treated rats (see Fig 1B and D). The mean values of amplitude and duration of the active ADP were not affected either by neonatal seizures (see Table 1 and Fig 1E and F). On average the amplitude and duration of the active ADP was 10 ± 2 mV ($p = 0.8$) and 45 ± 5 msec ($p = 0.11$) in P0–P5 flurothyl-treated cells and 12 ± 3 mV ($p = 0.45$) and 52 ± 7 msec ($p = 0.44$) in P6–P10 flurothyl-treated cells. In the remaining cells, individual action potentials were followed by a passive ADP with an average time constant of 15 ± 1.7 msec (n = 7; $p = 0.9$) and 17.8 ± 3.2 msec (n = 7; $p = 0.8$) in P0–P5 and P6–P10 flurothyl-treated cells, respectively. In 1 P0–P5 and 3 P6–P10 flurothyl-treated cells in which an active ADP was observed, the amplitude reached the threshold to trigger a second action potential (see Fig 1C). In control cells a single action potential was always observed.

Spike Frequency Adaptation in CA1 Pyramidal Neurons Is Reduced following Neonatal Seizures

In control tissue, one-third of CA1 pyramidal cells (4/12) responded to positive current pulses (800 msec; 0.2–0.6 nA) with a rapid action potential discharge followed by a period of silence (not shown). In the remaining neurons (n = 8), spike firing with a marked adaptation was maintained for the duration of the current injection (Fig 2A). In flurothyl-treated rats, the relative proportion of neurons in which positive pulse led to rapid spike firing followed by a period of silence was similar to control tissue (4/12 neurons and 3/13 neurons from P0–P5 and P6–P10 flurothyl-treated rats, respectively). However, in contrast to controls, the remaining flurothyl-treated CA1 pyramidal cells exhibited a continuous discharge during current injection with no (see Fig 2B and D) or very little further (see Fig 2C and D) adaptation.

To quantify the SFA, τ_{SFA} was measured for each cells (see Methods) in response to a 0.5-nA positive current pulse. τ_{SFA} was consistently longer in flurothyl-treated cells than in control cells (see Fig 2E and Table 1). On average, the τ_{SFA} was 59 ± 9 msec in controls (n = 12), 118 ± 29 msec in P0–P5 flurothyl-treated cells (n = 12; $p = 0.037$ vs controls), and 157.7 ± 26 msec in P6–P10 flurothyl-treated rats (n = 13; $p =$

Table 1. Intrinsic Neuronal Properties of CA1 Pyramidal Cells in Control and Flurothyl-Treated Rats

	Control	P0–P5 Flurothyl-Treated	P6–P10 Flurothyl-Treated
No. of cells	12	12	13
RMP (mV)	−65.3 ± 1.7	−66.2 ± 1.9	−64.3 ± 1.6
R_{IN} (MΩ)	47.9 ± 3.9	42.4 ± 4.3	46 ± 2.3
τ_M (msec)	12 ± 0.88	13.8 ± 0.9	13 ± 1
AP amplitude (mV)	82.3 ± 3	87.1 ± 2.7	85.6 ± 2.5
AP half amplitude duration (ms)	1.13 ± 0.049	1.15 ± 0.057	1.01 ± 0.034
AP threshold (mV)	−58.1 ± 0.94	−59.2 ± 1	−58 ± 1.4
τ_{SFA} (msec)	59 ± 9	118 ± 29[a]	157.5 ± 26[a]
Active ADP amplitude (mV)	9 ± 2	10 ± 2	12 ± 3
Active ADP duration (msec)	60 ± 4.5	45 ± 5	52 ± 7

Values are means ± SEM.

[a] $p < 0.05$.

P = postnatal day; RMP = resting membrane potential; R_{IN} = neuronal input resistance; τ_M = membrane time constant; AP = action potential; τ_{SFA} = spike frequency adaptation time constant; ADP = afterdepolarizing potential.

Table 2. Intrinsic Neuronal Properties of CA3 Pyramidal Cells in Control and Flurothyl-Treated Rats

	Control	P0–P5 Flurothyl-Treated	P6–P10 Flurothyl-Treated
No. of cells	10	9	9
RMP (mV)	−67.5 ± 2	−66.1 ± 2.4	−65.6 ± 2.5
R_{IN} (MΩ)	62.1 ± 8	55 ± 9	59 ± 8
τ_M (msec)	22.6 ± 3.4	21 ± 5.4	18.7 ± 3.1
AP amplitude (mV)	78.5 ± 3.7	84.1 ± 3.3	82.5 ± 3
AP half amplitude duration (msec)	1.19 ± 0.16	0.98 ± 0.059	0.9 ± 0.035
AP threshold (mV)	−54.3 ± 2.1	−53.5 ± 6	−55.7 ± 2.7
τ_{SFA} (msec)	42.2 ± 7	30.5 ± 8	40 ± 7
Active ADP amplitude (mV)	11 ± 1	12 ± 5	9 ± 3
Active ADP duration (msec)	60 ± 6	70 ± 8	53 ± 10

Values are means ± SEM.

P = postnatal day; RMP = resting membrane potential; R_{IN} = neuronal input resistance; τ_M = membrane time constant; AP = action potential; τ_{SFA} = spike frequency adaptation time constant; ADP = afterdepolarizing potential.

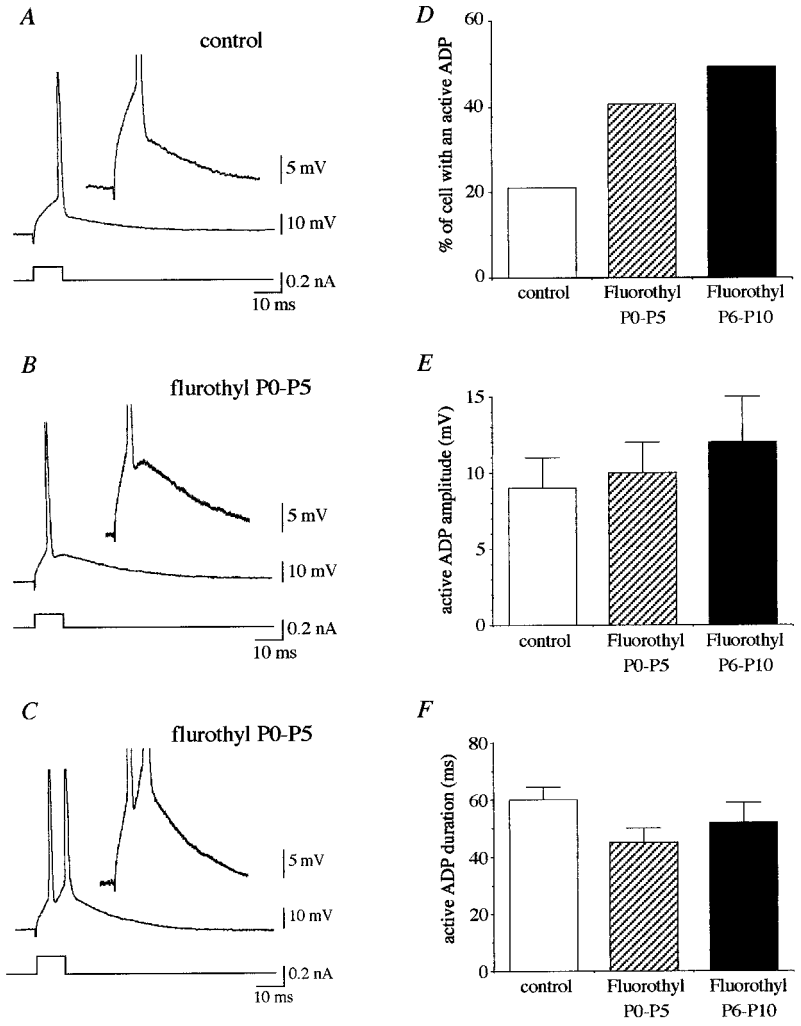

Fig 1. Spike afterdepolarization in CA1 pyramidal cells following neonatal seizures. (A–C) Records obtained from a control (A) and a postnatal day (P) 0 to 5 (P0–P5) flurothyl-treated (B and C) cell. In A and B, a single spike was evoked by injecting a brief positive current pulse. In C, the cell fired two spikes. Afterdepolarizing potentials (ADPs) are shown on a expanded voltage scale in the upper panels. (D–F) Pooled data from 12 control cells, 12 P0–P5 flurothyl treated cells, and 13 P6–P10 flurothyl-treated cells, depicting the percentage of cell with an active ADP (D), the mean amplitude (E), and duration (F) of the active ADP.

0.028 vs controls and $p = 0.36$ vs P0–P5 flurothyl-treated cells). As a result of decreased SFA, the number of action potentials generated by a positive current injection was significantly higher in flurothyl-treated cells (see Fig 2F). On average, a square positive current injection of 0.5 nA generates 10 ± 1 action potentials in control cells (n = 12), 17 ± 3 action potentials in P0–P5 flurothyl-treated cells (n = 12; $p = 0.008$), and 16 ± 1 action potentials in P6–P10 flurothyl-treated cells (n = 13; $p = 0.02$). To further quantify

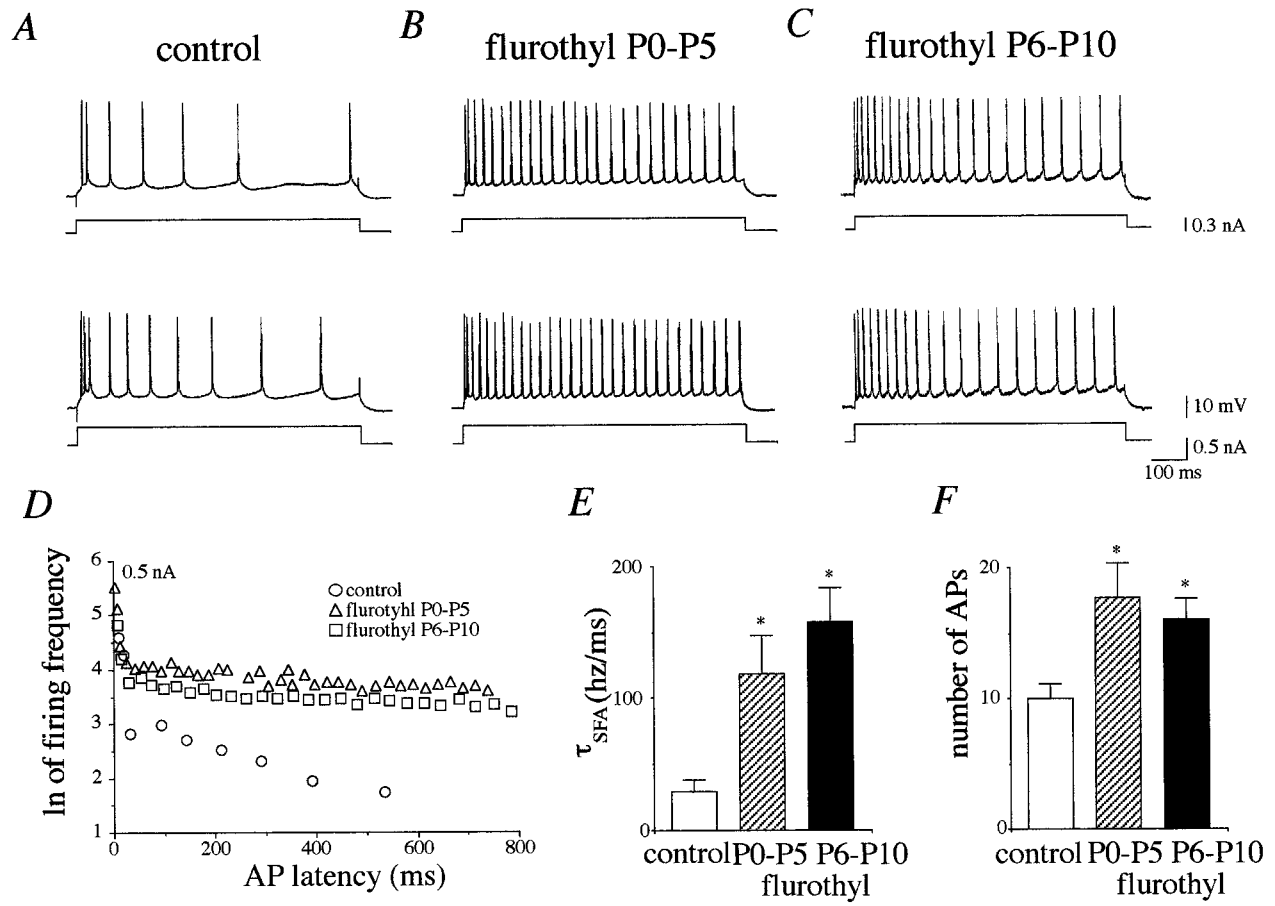

*Fig 2. Spike frequency adaptation (SFA) is reduced in CA1 pyramidal neurons following neonatal seizures. (A–C) Response of control (A), postnatal day (P) 0 to 5 (P0–P5) flurothyl-treated (B), and P6–P10 flurothyl-treated (C) CA1 pyramidal cells to positive current pulses of 0.3 nA (upper row) and 0.5 nA (lower row). (D) Plot of the natural logarithm (ln) of the firing frequency versus action potential (AP) latency obtained from the cells depicted in A, B, and C in response to a 0.5-nA depolarizing pulse. (E and F) Pooled data from 12 control cells, 12 P0–P5 flurothyl-treated cells, and 13 P6–P10 flurothyl-treated cells, depicting the mean value of the SFA time constant (τ_{SFA}; E) and the mean number of APs (F) in response to a 0.5-nA depolarizing pulse. *$p < 0.05$.*

SFA, we measured the mean firing frequency during a 100-msec time window at the onset of the depolarizing pulses (Fig 3B) and at 350 msec (see Fig 3C) and 700 msec (see Fig 3D) from the onset of the depolarizing pulses (see Fig 3A). As illustrated in Figure 3B and C, the early and medium mean firing frequency in flurothyl-treated cells did not differ significantly from controls except at some current intensity. A significant difference between flurothyl-treated and control cells appeared when comparing the late mean firing frequency (see Fig 3D).

Burst AHP in CA1 Pyramidal Neurons Is Reduced following Neonatal Seizures

AHP following a spike train was examined by injection with positive current pulses (200 msec) of increasing intensity (0.2–0.6 nA). AHP was reduced in flurothyl-treated CA1 pyramidal cells when compared with controls (Fig 4A and B). In control cells, the peak amplitude of the AHPs increases as a function of the number of action potentials, reaching a maximal value of 7.8 ± 1 mV (n = 12) in responses to about 10 or 11 action potentials (see Fig 4C). In CA1 pyramidal neurons obtained from flurothyl-treated rats, the amplitude of AHPs also increased as a function of the number of action potentials (see Fig 4C), but the maximal AHP amplitude was reduced to 63 and 61% of control values in P0–P5 and P6–P10 flurothyl-treated cells, respectively. On average, the AHP amplitude in responses to 10 or 11 action potentials was 4.9 ± 1 mV in P0–P5 flurothyl-treated cells (n = 12; p = 0.0017 vs controls) and 4.7 ± 1 mV in P6–P10 flurothyl-treated cells (n = 13; p = 0.001 vs controls and p = 0.4 vs P0–P5 flurothyl-treated cells).

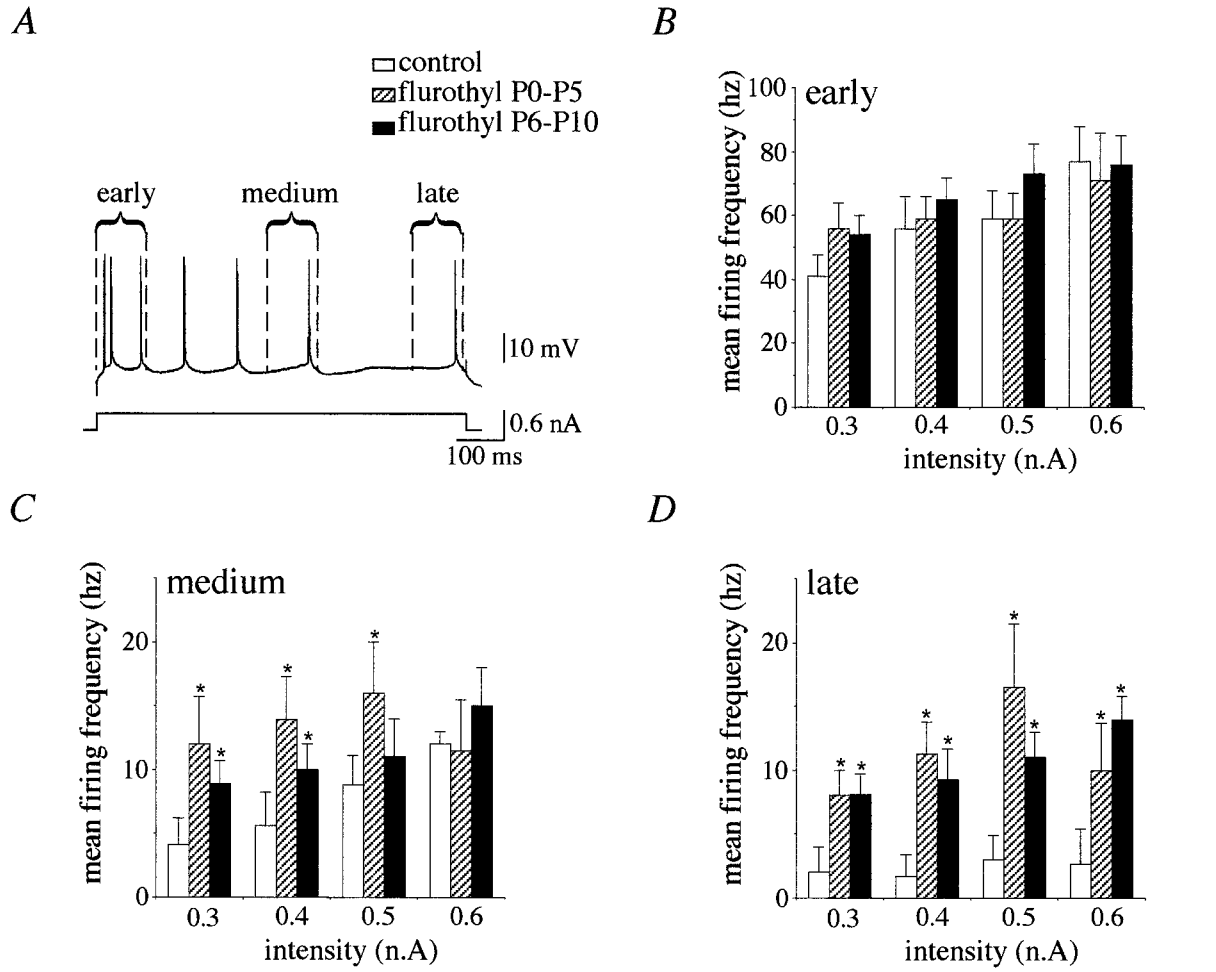

Fig 3. Spike frequency adaptation is reduced in CA1 pyramidal neurons following neonatal seizures. (A) Reference points used to measure the early, medium, and late mean firing frequency in response to the application of a positive current pulse. (B–D) Pooled data depicting the mean early (B), medium (C), and late (D) firing frequency obtained from 12 control cells, 12 postnatal day (P) 0 to 5 (P0–P5) flurothyl-treated cells, and 13 P6–P10 flurothyl-treated CA1 pyramidal cells in response to positive depolarizing pulses of increasing intensity. $^{*}p < 0.05$.

Neonatal Flurothyl-Induced Seizures Have No Effect on Electrophysiological Features of CA3 Pyramidal Cells

No significant differences in action potential amplitude, duration, or threshold were found between control and flurothyl-treated cells (see Table 2). The relative proportion of cells that exhibited an active ADP in flurothyl-treated rats was not different from that of controls. Thus, in 50% (5/10) of the recorded cells, a single action potential was followed by an active ADP of 11.5 ± 1 mV amplitude and 60 ± 6 msec duration (n = 5). In the 5 remaining cells, a single action potential was followed by a passive ADP. An active ADP was observed in 5 of 9 P0–P5 flurothyl-treated cells and in 4 of 9 P6–P10 flurothyl-treated cells. The mean amplitude and duration of the active ADP were, respectively, 12 ± 5 mV ($p = 0.9$) and 70 ± 8 msec ($p = 0.4$) in P0–P5 flurothyl-treated cells and 9 ± 3 mV ($p = 0.4$) and 53 ± 10 msec ($p = 0.2$) in P6–P10 flurothyl-treated cells.

We also investigated the effects of neonatal flurothyl treatment on SFA and AHP in CA3 pyramidal neurons. Both control and flurothyl-treated CA3 pyramidal cells responded to a positive current injection by a burst of action potentials followed by a period of silence (Fig 5A). No significant differences in τ_{SFA} and AHP were observed between the control and flurothyl-treated CA3 pyramidal cells (see Table 2). On average, the τ_{SFA} was 42.2 ± 7 msec in control cells (n = 12), 30.5 ± 8 msec in P0–P5 flurothyl-treated cells (n = 12; $p = 0.08$), and 40 ± 7 msec in P6–P10 flurothyl-treated rats (n = 13; $p = 0.25$). In both control and flurothyl-treated CA3 pyramidal cells, the AHP amplitude increased as a function of the number of action potentials, reaching a value of 14 ± 0.8 mV, 13 ± 1.7 mV, and 13 ± 0.8 mV for 10 or 11 action potentials

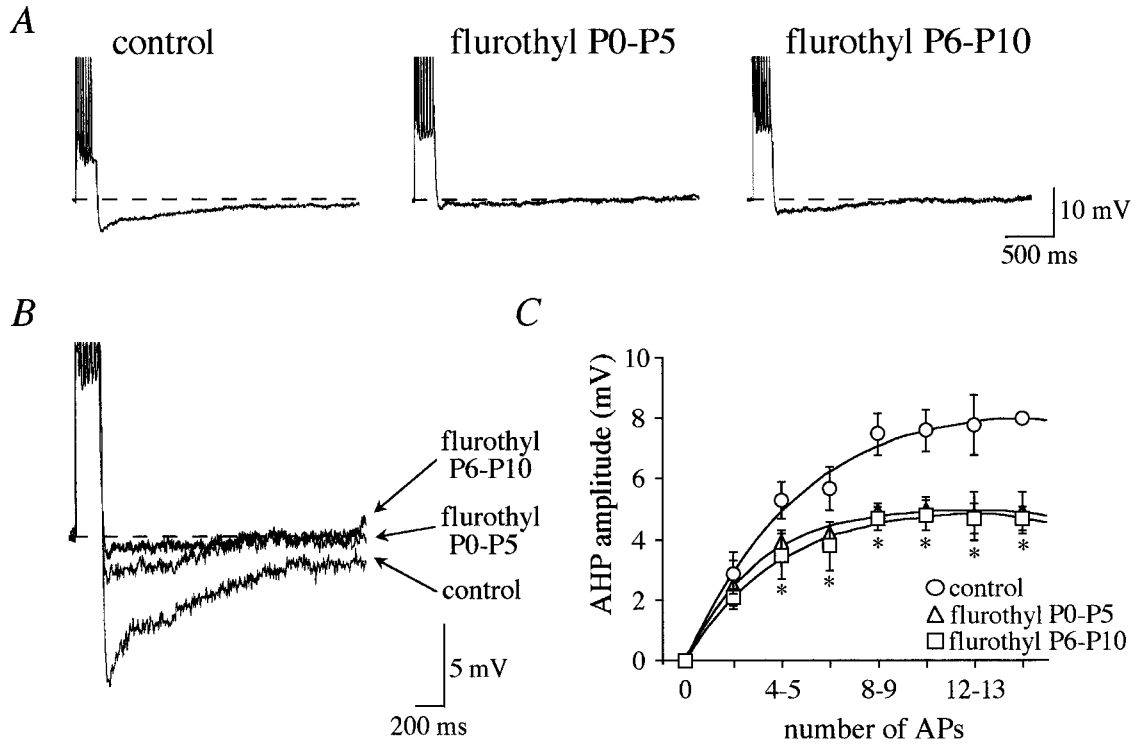

Fig 4. The afterhyperpolarizing potential (AHP) is reduced in CA1 pyramidal cells following neonatal seizures. (A) For each cell, a train of action potential was evoked by injecting a positive current pulse. (B) The AHP is depicted at an expanded voltage scale. (C) Pooled data obtained from 12 control cells, 12 postnatal day (P) 0 to 5 (P0–P5) flurothyl-treated cells, and 13 P6–P10 flurothyl-treated CA1 pyramidal cells depicting the mean AHP amplitude versus the number of action potentials triggered by the injection of positive current pulse. $^{*}p < 0.05$.

in control (n = 10), P0–P5 flurothyl-treated (n = 9; $p = 0.9$), and P6–P10 flurothyl-treated (n = 9; $p = 0.8$) CA3 pyramidal cells, respectively (see Fig 5B and C).

Discussion

The present data demonstrated significant alterations in the firing properties of hippocampal CA1 pyramidal neurons from rats that experienced repeated seizures early in life. Specifically, we have shown that the CA1 pyramidal neurons from flurothyl-treated rats show a marked reduction in SFA and amplitude of AHP following a spike train. In contrast, the intrinsic properties of CA3 pyramidal neurons were not affected by the repeated neonatal seizures.

Neonatal Seizures Lead to Persistent Changes in the Firing Properties of CA1 Pyramidal Neurons

The alterations in the firing properties of CA1 pyramidal neurons are likely due to the seizures induced early in postnatal life since (1) the standard passive membrane properties of CA1 pyramidal neurons did not differ from those of controls, indicating that cell injury did not contribute to the observed differences, and (2) the changes were observed 10 days after the end of the treatment, when flurothyl was no longer present. A hypothesis that cannot be completely excluded is that the alterations observed in P0–P5 flurothyl-treated cells may result from hypoxia/ischemia since mild cyanosis was observed from P0 to P3 during the seizures. This appears unlikely, however, because no signs of hypoxia were noted during seizures induced from P5 to P10, when alterations in neuronal firing properties were similar to those of P0–P5 treated cells.

The mechanisms underlying the persistent changes in the firing properties of CA1 pyramidal neurons require further study. However, the fact that the late phase of AHP and SFA were affected suggests common mechanisms. Thus, both are mediated by a voltage-sensitive M (muscarine-sensitive) potassium current and a calcium-activated potassium current,[29] and drugs that reduce these currents have been shown to decrease SFA and AHP.[29–32] Therefore, the reduction of AHP and SFA observed in flurothyl-treated CA1 pyramidal cells could be accounted for by a loss in the functionality of one or both of these potassium currents. Only these potassium currents are affected, however, as suggested by the fact that neither the resting membrane potential nor the action potential duration or early SFA was affected.

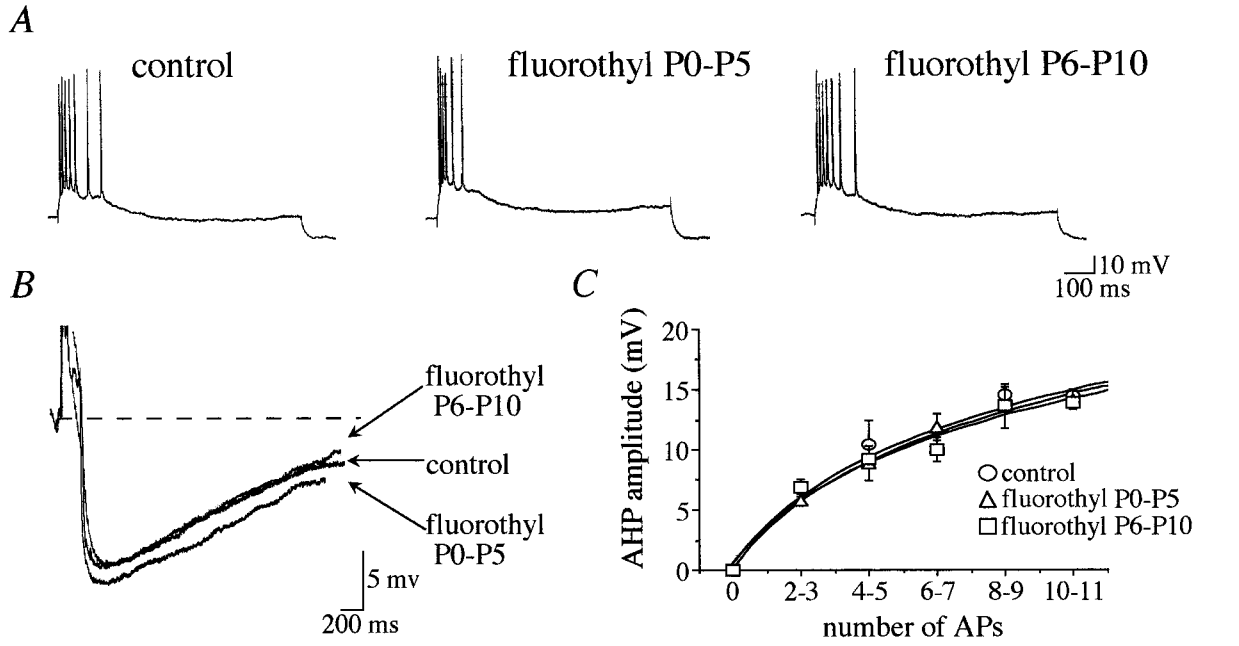

Fig 5. Lack of effect of neonatal seizures on the intrinsic properties of CA3 pyramidal cells. (A) Each cell fired a burst of action potentials in response to a positive current injection (0.5 nA). (B) Afterhyperpolarizing potentials (AHPs) evoked by a burst of four action potentials in response of a positive current injection. (C) Pooled data depicting the mean AHP amplitude versus the number of action potentials evoked by the application of positive current pulses obtained from 10 control cells, 9 postnatal day (P) 0 to 5 (P0–P5) flurothyl-treated cells, and 9 P6–P10 flurothyl-treated CA3 pyramidal cells.

Whatever the exact mechanisms leading to persistent changes in the firing properties of CA1 pyramidal neurons, they do not depend on the developmental stages at which the seizures were induced. Thus, we did not find significant differences whether the rats were treated during the first or the second postnatal week. In addition, similar alterations have been reported in CA1 pyramidal cells following kainate-treatment in adult rats,[33] an experimental model of human temporal lobe epilepsy. Our results have also shown that only CA1 pyramidal neurons were affected by neonatal seizures. Similar to our results, no significant alterations in the firing properties of CA3 pyramidal neurons have been observed in hippocampal slices from adult rats that experienced tetanus toxin–induced seizures as pups.[34] The reason for the difference between CA1 and CA3 pyramidal neurons is not known, but it may be related to differences in the type or density of potassium channels, as suggested by the different patterns of action potentials discharge generated by these two types of cells.

Single versus Prolonged Seizures: Effect on the Developing Brain

In an attempt to determine the effects of early-life seizures on developing brain, earlier studies have used pilocarpine or kainate as a model of status epilepticus. It appears that, even though the electrophysiological and behavioral manifestations of seizures are more severe in pups than in adult rats,[14] a single and prolonged seizure fails to produce any detectable histological changes[35–37] or even to alter seizure susceptibility in adulthood.[36,38] In contrast, results obtained from kindling model have shown that young animals subjected to recurrent seizures have a lower seizure threshold in adulthood.[39,40] Similarly, several other models of early-onset epilepsy have clearly demonstrated that recurrent seizures early in life can lead to transient histological alteration[41] as well as persistent abnormalities in hippocampal neuronal circuit. Thus, neonatal hyperthermia-induced seizures have been reported to cause a selective increase in inhibitory synaptic transmission in adulthood,[42] and rats pups that experienced hypoxia-induced seizures have a lowered seizure threshold.[43,44] Taken together with our findings, it is becoming clear that brief recurrent seizures during early development can alter the neurophysiology of hippocampal neurons.

Physiological and Pathological Consequences of Early-Life Seizures

Our study provides evidence that despite the lack of cell loss,[25] repeated early-life seizures result in physiological changes that in all cases promote hyperexcitability. These changes may, at least in part, underlie seizure predisposition observed in vivo.[25] Thus, while epilepsy is a network phenomenon, the firing properties of the neuronal constituents can affect the overall behavior of the network. For instance, intrinsically

burst-firing pyramidal cells located in the neocortical layer 5 play a key role in the generation of spontaneous cortical network–driven oscillations observed under free-magnesium conditions.[45] The modifications in the firing properties of CA1 pyramidal neurons may also alter the neuronal outputs from the CA1 hippocampal area and as a result may affect the behavior related to hippocampal function. The finding that flurothyl-treated rats demonstrated impairment in the water maze test is in agreement with this hypothesis.

An important issue concerns the relevance of the present observations to the human situation. The extrapolation and comparison of the results from rodents to children is complex at least in part because the correlation of developmental stages is approximate. In addition, the etiology, the type and number of seizures, and the antiepileptic medications have to be taken into account in humans. However, there are indications that recurrent seizures early in life can have detrimental effects on subsequent learning and behavior.[10,11] While it is speculative to suggest that changes in SFA and AHP are responsible for seizure-induced changes in cognition and seizure susceptibility in humans, our study does demonstrate that the hippocampus network can be significantly altered by seizures that fail to result in any discernible cell loss.

Conclusion

In summary, we have found that recurrent, brief, early-life seizures result in significant changes in membrane properties of CA1 pyramidal cells. Our findings demonstrate that while neonatal seizures may not result in any significant cell loss, lasting changes in firing patterns of hippocampal neurons can be altered for weeks after the last seizure. These alterations in firing patterns may play a role in the altered seizure susceptibility and cognitive impairment that occurs following a series of seizures during early development.

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