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Hippocampal Interictal Spikes Disrupt Cognition in Rats

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Abstract

Objective—Cognitive impairment is common in epilepsy, particularly in memory function. Interictal spikes are thought to disrupt cognition, but it is difficult to delineate their contribution from general impairments in memory produced by etiology and seizures. We investigated the transient impact of focal interictal spikes on the hippocampus, a structure crucial for learning and memory and yet highly prone to interictal spikes in temporal lobe epilepsy.

Methods—Bilateral hippocampal depth electrodes were implanted into fourteen Sprague-Dawley rats, followed by intrahippocampal pilocarpine or saline infusion unilaterally. Rats that developed chronic spikes were trained in a hippocampal-dependent operant behavior task, delayed-match-to-sample. Depth EEG was recorded during 5,562 trials among five rats, and within-subject analyses evaluated the impact of hippocampal spikes on short-term memory operations.

Results—Hippocampal spikes that occurred during memory retrieval strongly impaired performance ($p < 0.001$). However, spikes that occurred during memory encoding or memory maintenance did not affect performance in those trials. Hippocampal spikes also affected response latency, adding approximately 0.48 seconds to the time taken to respond ($p < 0.001$).

Interpretation—We found that focal interictal spike-related interference in cognition extends to structures in the limbic system, which required intrahippocampal recordings. Hippocampal spikes seem most harmful if they occur when hippocampal function is critical, extending human studies showing that cortical spikes are most disruptive during active cortical functioning. The cumulative effects of spikes could therefore impact general cognitive functioning. These results strengthen the argument that suppression of interictal spikes may improve memory and cognitive performance in patients with epilepsy.

Introduction

One of the most common yet troublesome co-morbidities in patients with temporal lobe epilepsy (TLE) is cognitive impairment¹⁻³. In particular, many patients have disturbances in memory, an essential higher cognitive function for continuity in time, personal history, and awareness. While both etiology⁴⁻⁵ and recurrent seizures⁶ have been implicated, an important controversy is whether interictal spikes (IIS) are a harmless biomarker of the disease or contribute to this dysfunction.

IIS are transient, abnormal focal neural discharges seen on electroencephalogram (EEG) recordings during periods between seizures. Lasting 50-200 milliseconds, they are a result of synchronous, paroxysmal depolarizations of neurons producing a rapid succession of action

potentials⁷⁻⁸. IIS usually occur close to the seizure focus⁹⁻¹⁰ and they are one of the most important factors in the diagnosis of epilepsy¹¹⁻¹². However, IIS may interfere with cognitive functions that are already jeopardized by disease, which would be particularly troublesome given the prominence of focal IIS in epileptic syndromes such as temporal lobe epilepsy and the epileptic encephalopathies¹³.

The effect of IIS on cognition is likely ephemeral, coinciding with timing of the event on the EEG. The clinical term transitory cognitive impairment has been used to describe brief disruptions in brain function concurrent with IIS¹⁴. This term encompasses impairments observed with both focal IIS and generalized spike-and-wave complexes. The latter have produced unquestionable demonstrations of transitory cognitive impairment, including impairments in reaction time¹⁵⁻¹⁷, perception¹⁶⁻¹⁹, verbal and spatial tasks²⁰⁻²², and even driving behavior²³. Furthermore, some studies suggest that the neuroanatomical site and timing of the EEG disturbance predicts the specific cognitive function affected. For example, disturbances localized to the occipital cortex briefly disrupt visual perception, particularly during a stimulus presentation^{17, 19}.

Focal IIS-induced cognitive disruption has been more difficult to identify than that associated with generalized spike-and-wave. This may be due to the use of scalp EEG in all studies of transient IIS effects thus far. Scalp EEG does not allow precise neuroanatomical localization of IIS, which weakens the determination of which cognitive function might be affected. Furthermore, non-cortical focal IIS, such as those in the hippocampus or other deep medial temporal lobe structures, are not effectively detected by scalp EEG²⁴. This presents problems in testing for effects of IIS on learning and memory, for which these structures are critical. Hence, mixed results in previous studies may be related to inconsistencies between IIS localization and the cognitive function tested.

Depth electrode studies can help to resolve these issues, through visualization of focal IIS in subcortical structures that play a central role in memory. However, patients with intracranial electrodes present other difficulties for extended neuropsychological testing, mainly due to post-surgical discomfort and cognitive side effects of pain medication. We investigated the effects of IIS on operant behavior, a particularly useful tool to examine IIS in an animal model which enables rigorous assessment of a fleeting and potentially subtle disruption. Pilocarpine-treated rats with chronic IIS were trained to perform a delayed-match-to-sample task (DMTS), which relies largely on intact hippocampal function²⁵, and involves short-term memory encoding, maintenance, and retrieval. We used within-subject analysis to compare trials with IIS versus trials without IIS from the same animal, isolating the specific effect of IIS on cognition from differences that might otherwise vary between animals.

While cortical IIS have been shown to disrupt stimulus perception for later recall¹⁹, patients with TLE are not particularly prone to problems in perception²⁶, despite high incidence of IIS in temporal lobe structures including the hippocampus²⁷. However, neuronal firing and neurophysiological rhythms in the hippocampus are closely related to short-term memory, particularly the retrieval phase, and may be vulnerable to the electrophysiological impact of hippocampal IIS²⁸⁻³⁰. Therefore we hypothesized that focal IIS in the hippocampus momentarily disrupt short-term memory, specifically short-term memory retrieval.

We report that IIS in the hippocampus are associated with a transient disruption in function and an increase in response latency. This evidence illustrates that focal IIS in limbic structures, which are very common in epileptic conditions such as TLE, can provide an independent contribution to cognitive impairments.

Methods

Animals

All animal procedures were approved by the Dartmouth IACUC, under USDA and AAALAC-approved conditions, in accordance with National Institutes of Health guidelines. Fourteen Adult male Sprague-Dawley rats, approximately eight weeks old, were housed individually with a 12-hour light/dark cycle and *ad libitum* access to food and water. All rats underwent surgical implantations and infusion with either pilocarpine or saline, and were later recorded to screen for IIS. Specifically, nine rats were implanted and infused with pilocarpine at least two weeks before behavioral training began, while two rats were trained before surgery and pilocarpine infusion. Within-subject analyses accounted for any individual differences between rats such as the timing of training, therefore all behavioral data was pooled together in the analysis (see Data Analysis section).

Three control rats were implanted and infused with saline at least two weeks before training to provide a comparison to pathological EEG phenomena in pilocarpine-treated rats, and to verify that the surgical and infusion techniques alone did not produce IIS. Control rats were not involved in the behavioral analysis since this study aimed to use within-subject comparisons to assess the independent impact of IIS, which were not exhibited at any time following a saline infusion. Female rats were not utilized in this study to prevent the potential effects of fluctuating estrous cycle hormones on behavior and cognition³¹.

Surgery

Rats were anesthetized with isoflurane (2-3% in oxygen) and custom electrodes were implanted in three sites³² in each rat: bilateral ventral hippocampal CA1 (± 5.7 mm lateral, 5.4 mm posterior, and 6.0 mm ventral from the bregma skull fissure; Fig 1A), and prelimbic prefrontal cortex (0.6 mm lateral, 3.2 mm anterior, and 4.3 mm ventral). A 26-gauge guide cannula (Plastics One, Roanoke, VA) was bonded to the right ventral CA1 bipolar electrode to allow close proximity (<1 mm) of EEG recording to the infusion focus. A ground wire was soldered to a bone screw, and a reference wire was implanted in the cerebellum. All wires were plugged into an interconnect socket array (Mill-Max Mfg. Corp., Oyster Bay, NY), and encased in dental cement (Dentsply International Inc., Milford, DE), leaving only the gold pin sockets exposed to allow connection to the amplifiers (see Electrophysiology section).

Pilocarpine infusion

One week following surgery, pilocarpine dissolved in 0.9% saline (0.5 mg/ μ L; Sigma-Aldrich, St. Louis, MO) was infused into the hippocampus at a rate of 0.05 μ L/minute with concurrent depth-EEG recordings. To minimize mortality while ensuring IIS occurrence, pilocarpine infusions were terminated for a given animal when continuous EEG status epilepticus (SE) was observed in the hippocampus. The amount of solution infused varied from 0.9 μ L to 2.3 μ L (0.45-1.15 mg pilocarpine). Importantly, individual differences in infusion amount were accounted for by the within-subject design of the analysis (see Data Analysis section). Control rats received an infusion of 1-2 μ L of 0.9% saline, to provide later qualitative comparison of their long-term EEG activity to that of pilocarpine-treated rats. Rats were monitored on EEG for one half hour after SE began, and behavior was monitored for another 12 hours. To assess IIS development, 30-60 minute EEG recordings were made each day after the infusion for two weeks, and once per week for an additional 3 weeks.

Electrophysiology

Electrodes consisted of two insulated 25 μ m nichrome wires (California Fine Wire, Grover Beach, CA), twisted and inserted into a 25-gauge stainless steel guide tube (Small Parts Inc.,

Miami Lakes, FL). Operational amplifiers plugged directly to the rat connector to reduce mechanical artifacts. EEG signals, transmitted via a custom cable and a rotating commutator (Dragonfly Research & Development Inc., Ridgeley, WV), were amplified (10K) and acquired at 2.2kHz with an Axoscope analog/digital converter and software acquisition set-up (DigiData 1322, Axon Instruments, Foster City, CA).

Apparatus

Two operant conditioning chambers contained in sound-attenuating cubicles (Lafayette Instruments Inc., Lafayette, IN; Med Associates Inc., St. Albans, VT) were controlled with behavior software packages (ABET II; MED-PC IV). Two retractable levers were separated by a pellet dispenser on one of the walls. A stimulus light was located above each lever. An infrared photobeam nosepoke detector was placed on the opposite wall, with a stimulus light above it. A white incandescent house light bulb was illuminated during each trial and extinguished at its end. Three DC outputs from the behavioral software computer interface were sent directly to the EEG data acquisition system to additional input ports. Stimulus and response events were recorded as binary (on/off) combinations of these signals, enabling microsecond synchronization with EEG data.

Procedure

Rats were food-restricted and maintained at 85% of their *ad libitum* weight throughout operant behavior study. They were trained in operant chambers to lever-press and poke their nose into the infrared beam for food reward (45 mg Noyes food pellet; Research Diets, Incorporated, New Brunswick, NJ). Training sessions were run at approximately the same time each day for each rat. Once the rats reliably pressed levers and broke the infrared beam for food, the DMTS training paradigm commenced (Fig 1B). At the beginning of each trial, one lever (right or left) was randomly presented [Sample step]. Upon pressing the sample lever, rats were then required to poke their nose into an infrared beam [Delay step], which was positioned on the opposite wall of the chamber to prevent rats from relying on body positioning (non-hippocampal-dependent) strategies to gain reinforcers³³. Immediately after the beam was broken, both levers were presented [Match step]. A correct response was defined as pressing the same lever that was presented in the Sample Phase (i.e. match-to-sample), which produced a food reward. An incorrect response was pressing the lever that had not been presented, which produced no food reward. Daily sessions consisted of 60-100 trials, and when accuracy reached >85% for 3 continuous days, the delay period was increased. This was accomplished by designating nosepoke beam-breaks as unproductive during the delay step until a specified amount of time had elapsed. The delay length was increased for each rat until performance reached >80% accuracy on sessions with 6-15 second variable delays, and EEG data was recorded for all sessions thereafter. Rats with at least 10 IIS/hour were included in DMTS analysis. Five rats qualified under both performance and IIS prerequisites, generating a total of 5,562 trials for analysis.

Following spontaneous seizures, rats tended to return to normal behavioral activity levels 5-15 minutes. Previous studies from our laboratory show that post-ictal effects are rather minimal after three hours, in terms of hippocampal function and related cell firing³⁴. Therefore, if an animal happened to have a spontaneous seizure before a DMTS session, the session was delayed at least 3 hours to help minimize influences of post-ictal impairment on general performance. In addition, the within subject design of our analysis, which compared trials with and without IIS in the same session, was designed to negate such influences.

Data Analysis

Custom IIS detection software, based on White *et al.*³⁵, was written using MATLAB (The Mathworks, Inc., Natick, MA). Briefly, potential IIS were detected each time the derivative of the EEG recording (re-sampled at 222 Hz) was greater than five times the standard deviation. Potential waveforms were then classified as IIS, artifacts, or false-detections of normal physiological waveforms³⁶ based on comparison EEG sessions from saline-treated rats (Fig 2C). IIS differentiation was based on morphology, by plotting the characteristic features of the waveforms against one another (e.g. amplitude). IIS included in the analysis had a peak amplitude of at least 1 mV, and lasted less than 50 ms (examples in Fig 2B), with the entire transient lasting less than 200 ms if including the subsequent slow wave component. We evaluated the effectiveness of our IIS detection programs by comparing their performance to the evaluation of an experienced epileptologist (GLH) in three randomly chosen sessions. Comparison of manually detected IIS to those detected by the program revealed a sensitivity of >95% and a specificity of >99%. Using the binary input signals from the behavioral software, the exact timings of IIS within trials were documented relative to behavioral events, to designate each trial as one with or without an IIS (Fig 1C and D).

DMTS performance as a function of delay length and timing of IIS occurrence was analyzed using logistic regression in Stata 10 (StataCorp LP, College Station, TX). Each DMTS trial was treated as an individual observation and standard errors were adjusted for within animal effects (N=5,562, clustered for five rats), to control for differences between animals such as pilocarpine dose, lesion extent, and general cognitive ability. Three stages of short-term memory processing were inferred in the DMTS task: encoding (4 seconds surrounding the sample press), maintenance (2 seconds after the sample press to 1 second before the match lever presentation), and retrieval (1 second before the match lever presentation to 1 second before it was pressed). The timing of these epochs was based on rat behavior in short-term memory tasks²⁹ and the pronounced effects in neuronal firing produced in the first second following a hippocampal IIS³⁰. Additionally, Pearson correlation coefficients were used to assess the timing of IIS within trials versus trial delay lengths, as well as test for any relationships between IIS frequency and general performance across sessions (defined as percent correct in a given session).

The effect of hippocampal IIS on response latency was assessed by quantifying the amount of time taken by the rat to press one of the match levers, following their extension and stimulus light illumination after the last nosepoke. This length of time was compared in trials in which no IIS occurred versus trials in which an IIS occurred during this period, using a Mann-Whitney U test.

Hippocampal theta oscillations have been related to greater vigilance during behavioral tasks, and would likely associate with improved performance²⁸. Likewise, IIS occur largely in the absence of hippocampal theta rhythm, i.e. during non-attentive states³⁷. To rule out the possibility that vigilance could account for effects of IIS on DMTS performance, a measure of vigilance ratio was calculated (sum of theta band (4-11Hz) power divided by the sum of delta band (0.5-3Hz) power)³⁸⁻⁴¹, and compared between trials with versus without IIS, using a Mann-Whitney U test.

Results

Seizures and IIS

During intrahippocampal pilocarpine infusion, all rats exhibited electrographic seizures initiating in the ipsilateral hippocampus and eventually generalizing to both the contralateral hippocampus and the ipsilateral prefrontal cortex leads. The initial SE lasted from 3-7 hours

for all rats, corresponding to Stage III-V behavioral seizures⁴². In the subsequent weeks following infusion, seven rats had witnessed spontaneous seizures. Because long-term monitoring was not performed, we could not verify the presence of spontaneous seizures in the other animals with IIS. It should be noted that our use of the term “interictal” when referring to IIS is based on the pathological waveform characteristics (deCurtis & Avanzini, 2001), and not the apparition of previous and subsequent seizures. IIS developed within the first few days following infusion, subsequently increasing and reaching stable rates after approximately two weeks. IIS in the left hippocampus (contralateral to infusion site) tended to be more frequent than right hippocampal IIS in the first two weeks (Fig 2A), while prefrontal cortex IIS were seen in only one rat. Ten of eleven pilocarpine treated rats developed hippocampal IIS following pilocarpine infusion, ranging from 0.5 to 256.8 IIS per hour. IIS morphology and lateralization was variable between rats as illustrated in Figure 2B and C, although these features did not predict the degree of impairment induced. IIS included in the analysis were distinct from normal physiological waveforms seen in both pilocarpine- and saline-treated rats (Fig 2C). Electrode placements were verified in the majority of rats (N=9) to verify our surgical procedures, using thionin staining techniques of coronal sections⁴³. Electrodes were found to be in the hippocampus, in or very near the CA1 sub-region (Figure 1A). Although there was some variability in the amount of pilocarpine-induced cell loss, the hilus and CA1 appeared most affected by the pilocarpine. As shown in Figure 1A, cell loss tended to be greater ipsilateral to the injection site.

IIS-related impairment

Increasing delays produced overall decreases in DMTS accuracy (odds ratio (OR) – 1.08; 95% CI – 1.03 to 1.14, $p < 0.01$), confirming findings by Hampson and colleagues²⁵. Furthermore, IIS were associated with additional impairments in performance in a trial-specific or transient manner (Fig 3). IIS that occurred during the retrieval epoch of trials were related to markedly impaired performance, with rats more than 3 times as likely to make an error in those trials (OR – 3.19; 95% CI – 2.34 to 4.35, $p < 0.001$). However, IIS during the encoding epoch (OR – 0.97; 95% CI – 0.59 to 1.59, $p = 0.91$) or the maintenance epoch were not associated with impaired performance (OR – 1.06; 95% CI – 0.85 to 1.33, $p = 0.61$). The number of IIS in a trial was not a significant predictor of accuracy, although this was likely due to a statistical paucity of trials with multiple IIS. There was a greater likelihood of IIS occurring in longer delays rather than shorter delays ($p < 0.001$), but there was no relationship between the length of the delay and the epoch within which IIS happened ($r = 0.003$, $p = 0.92$). Vigilance ratio (see Methods) did not differ in trials with and without IIS ($p = 0.96$). Finally, we found no relationships between the frequency of IIS and overall accuracy across sessions ($r = 0.02$, $p = 0.78$), nor between the frequency of IIS and the average performances of individual rats ($r = 0.24$, $p = 0.69$).

Response latency

The median response latency to press the match lever after it was extended into the chamber was 2.28 seconds in trials without IIS, whereas if an IIS occurred anywhere during this period, the median increased to 2.76 seconds ($p < 0.001$; Fig 4). This addition in reaction time did not differ between correct or incorrect trials.

Discussion

We demonstrate that hippocampal IIS are associated with a dramatic alteration in on both accuracy and response latency in a rodent model. We were able to show this effect due to our use of depth electrodes, which have not previously been used to study the transient impact of IIS. Therefore, the transitory effects from cortical IIS described previously have a corollary in a deep brain structure. Furthermore, transient impairments in short-term

memory were related to focal IIS in the hippocampus, illustrating that a more generalized discharge is not necessary to disrupt this cognitive function.

The hippocampus is required for accurate performance of the DMTS task, particularly in trials with long delays²⁵. Using this cognitive paradigm, we found that the effect of depth-detected hippocampal IIS is contrary to the effect of scalp-detected IIS¹⁹. Specifically, hippocampal IIS had no effect on accuracy if they occurred during the encoding epoch (analogous to stimulus presentation in human neuropsychological studies), or during the maintenance epoch. However, hippocampal IIS during the memory retrieval epoch were associated with severe transient impairments, probably because of the intimate role of the hippocampus in this function²⁸⁻²⁹. These selective effects reveal that hippocampal operations may be devastated if hippocampal IIS occur during their neural processing windows.

The specific effect of IIS on retrieval but not encoding or maintenance may also relate to the transient nature of the hippocampal process involved in memory recall²⁹. Memory encoding and maintenance may involve a longer time-span of processing, and may be buffered by structures in addition to the hippocampus⁴⁴, and thus less vulnerable to a transient focal disruption. Future studies will be required to delineate the mechanism of the process-specific impact on hippocampal function, potentially utilizing multi-structure depth recordings.

Response latency was increased in trials with hippocampal IIS by approximately half a second. These additions are a fraction of the total latency to respond, and might be easily overlooked in a clinical context. However, they may be cumulatively detrimental to patients, particularly those with frequent IIS¹⁷. Similar increases in response latency were seen in both correct and incorrect trials, suggesting that this additional time does not reflect a hesitancy of the rat due to faulty memory recollection. Rather, in addition to their deleterious effect on informational integrity, hippocampal IIS may mark a temporary offline state of the brain, manifested by this delay in responding.

IIS-related memory impairments may be a consequence of the incorporation of local neurons into a synchronized burst⁴⁵. Drawing neurons away from their normal processing may disorder the representation of pertinent information within a hippocampal neuronal assembly⁴⁶, causing inaccurate recall of which lever was pressed just seconds ago. In addition, local changes in cellular environment immediately following an IIS⁷ may be sufficient to briefly depress neuron firing³⁰ and local oscillations⁴⁷, and this delay in brain processing could manifest as an increase in response latency.

IIS were observed throughout the first two weeks following pilocarpine infusion and reached relatively stable levels afterward, illustrating pathophysiological activity during the supposed “latent period” before the generation of spontaneous seizures⁴⁸⁻⁴⁹. The development of IIS contralateral to the infusion site was an unexpected finding, and may indicate a bilateral epileptogenic process following unilateral intrahippocampal pilocarpine infusion. The laterality of the IIS did not affect our results. Whether the spike occurred in either hippocampus, or propagated from one to the other, impairments were still observed.

Correlations between the frequency of IIS and cognitive impairment in patients have been controversial, and the clinical significance of such findings has been questioned¹³. For example, deficits in IQ and school performance among patients with Benign Epilepsy with Centro-Temporal Spikes have been correlated with the frequency of IIS but not seizure frequency in some but not all studies⁵⁰⁻⁵¹. Differentiating the transient effects of IIS from the underlying pathological factors is challenging in clinical settings since factors such as anti-epileptic drugs and seizures may contribute to cognitive deficits. IIS rate and general measures of performance were not related in the current study (see also Chauviere et al.,

2009⁵²). However, we show that the timing of IIS may be decisive in producing adverse cognitive effects, since complex functions are often supported by multiple brain structures with differential time windows of processing⁴⁴. Therefore, IIS in other brain structures may be equally disruptive and clinically evident if both the timing of the IIS, and the putative neurological substrate of the function tested, are taken into consideration.

While extrapolating results from rats to humans must be done cautiously, our study strongly suggests that hippocampal IIS produce intermittent hippocampal dysfunction. It is therefore not unreasonable to be concerned that cumulative influences of transient IIS-related disruptions may account for a portion of the cognitive impairment seen in epilepsy¹⁴. By extension, IIS-related disruptions could be devastating in children with epileptic encephalopathies, in whom high numbers of IIS may systematically obstruct learning functions in the brain, hence impeding normal cognitive development¹⁹.

Further studies are required using other tasks that also require an intact hippocampus, since this brain area contributes to a multitude of neural processing functions⁵³. Human studies of focal hippocampal IIS are also warranted, to elucidate the extent of their impact on more complex cognitive functions, and to determine whether therapy improves those cognitive functions if IIS can be effectively suppressed.

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FIGURE 1.

Electrophysiology and behavior setup. (A) Upper figures show an example of pilocarpine-induced cell loss in the hilus (*arrows*) and CA1 (*arrowheads*). Cell loss tended to be greater on the side ipsilateral to the pilocarpine infusion (*right-hand frame*) than the contralateral side. The lower figure shows an example of bilateral electrode placement in the hippocampus (*arrows*). Infusion area is seen adjacent to electrode track on right side (*larger arrow*). (B) Delayed-Match-To-Sample paradigm. In the Sample step, one of two levers was randomly presented (right or left) and was pressed by the rat. Then, in the Delay step, the rat had to poke its nose into a hole in the opposite wall for a random length of time (6–30 seconds). After this time period had elapsed, the first nosepoke into the hole turned off the stimulus light above and extended both levers. Then, in the Match step, the rat had to remember which lever he pressed during the sample phase, and press that same lever again to procure a food reward. Hippocampal interictal spikes (IISs) are hypothesized to disrupt short-term memory in this task, manifested by an increased likelihood of pressing the wrong lever if an IIS occurred during a trial. (C) Example of depth-electroencephalogram (EEG) traces recorded during one of the trials in which no IISs occurred (*top trace*: right hippocampus; *bottom trace*: left hippocampus). Trial events are marked with black vertical lines, denoted as “S” for the moment the rat pressed the sample lever, “D” for the first nosepoke following the delay simultaneously extending the match levers), and “M” for the moment of the match lever press. (D) Example of a trial in which an IIS occurred (*dotted box*).

FIGURE 2.

Interictal spikes (IISs). (A) Daily development of IIS in rats treated with pilocarpine ($n = 11$) over the first 14 days and 3 subsequent weeks post-status epilepticus (SE). IIS rate $\pm$ standard error of the mean (SEM) is shown for the right (solid line) and left (dashed line) hippocampus. IIS rates stabilized before behavioral training, which began at least 2 weeks following pilocarpine infusion. The majority of IISs developed in the left hippocampus, contralateral to the original pilocarpine infusion, although there was marked variability between the rats. IISs were apparent during most of the “latent period” before rats developed spontaneous seizures. A timeline is seen above the graph, indicating the periods of daily EEG recordings for 14 days, followed by food restriction and behavioral training/testing in the subsequent weeks. (B) Three examples of IIS in pilocarpine-treated rats, illustrated by concurrent EEG recordings from the right (top) and left (bottom) hippocampi. The first example corresponds to the IIS outlined in Fig 1D. (C) Example of an IIS and a subsequent normal physiological sharp wave, commonly seen in recordings from both pilocarpine- and saline-treated animals.

FIGURE 3.

Interictal spike (IIS) impact on accuracy in delayed match-to-sample (DMTS) trials of varying delays. Means $\pm$ standard error of the mean (SEM) are shown, which are based on predicted values using logistic regression analysis ($n = 5,562$ trials adjusted for five individual rats). Error bars are corresponding SEM estimates for the representative points shown. An epoch-specific breakdown of trials with IIS is shown. Among trials in which an IIS occurred during the encoding or maintenance epoch of short-term memory, accuracy did not differ from trials without IISs anywhere. However, IISs during the retrieval phase produced a marked decrease in accuracy. ($p < 0.001$). Increasing delays produced decreases in accuracy, regardless of IIS epoch timing ($p < 0.01$). No IIS (solid line), IIS during encoding (dotted line), IIS during maintenance (dash-dot-dash line), and IIS during retrieval (dashed line).

FIGURE 4.

Increase in response latency due to interictal spike (IIS). The length of time between the presentation of the match lever and pressing of that lever by the rat was quantified as response latency. The figure shows histograms of the relative frequencies of response latency in trials without IIS (thick solid line) and trials with IIS (thick dashed line). The median response latency to press the match lever was 2.28 seconds in trials without IIS (thin solid line), whereas if an IIS occurred the median increased to 2.76 seconds (thin dashed line; $p < 0.001$; $n = 4,609$ trials adjusted for five individual rats).