OTC, an active metabolite of oseltamivir, on LTD induction in the CA1 region. Oseltamivir is metabolized to OTC in the brain after passing the BBB,[20] and we previously found that OTC is more potent than oseltamivir in causing paired pulse facilitation of population spikes. This enhanced excitability results from altered dendritic propagation in hippocampal slices and does not require the presence of ethanol.[17] Therefore, in the following study, slices were pretreated with 3 μM OTC for 2 h prior to delivery of LFS consisting of 900 pulses at 1 Hz. In the pretreated slices, LTD was successfully induced without any significant difference from LTD in naïve slices (Figure 2A, EPSP slope 60 min after LFS: 71.0 ± 4.7% of control in pretreated slices, $N = 5$, and 67.1 ± 1.5% in naïve slice, $N = 5$). In pretreated slices, LTD was also induced when LFS was delivered in the presence of 100 μM caffeine (squares in Figure 2C, EPSP slope 60 min after LFS: 80.7 ± 3.9% in caffeine, $N = 5$) or 100 μM ephedrine (circles in Figure 2C, EPSPS slope: 60.4 ± 9.3% in ephedrine, $N = 5$). However, LTD was not induced in OTC-pretreated slices when LFS was delivered in the presence of both caffeine and ephedrine (Figure 2B, EPSP slope 60 min after LFS: 100.9 ± 3.6%, $N = 5$, $P < 0.01$ vs control LTD in both naïve and pretreated slices). In contrast, LTD was induced in the presence of both agents in naïve slices (Figure 2B, EPSP slope 60 min after LFS: 76.9 ± 5.4%, $N = 5$). Neither OTC alone nor the combination of OTC, caffeine, and ephedrine had an effect on induction of LTP induced by a single 100 Hz × 1 s HFS (EPSP slope 60 min after HFS: 140.1 ± 4.5% in slices treated with OTC alone, $N = 5$; 138.8 ± 1.0%, $N = 3$, in the presence of OTC, caffeine and ephedrine, data not shown).

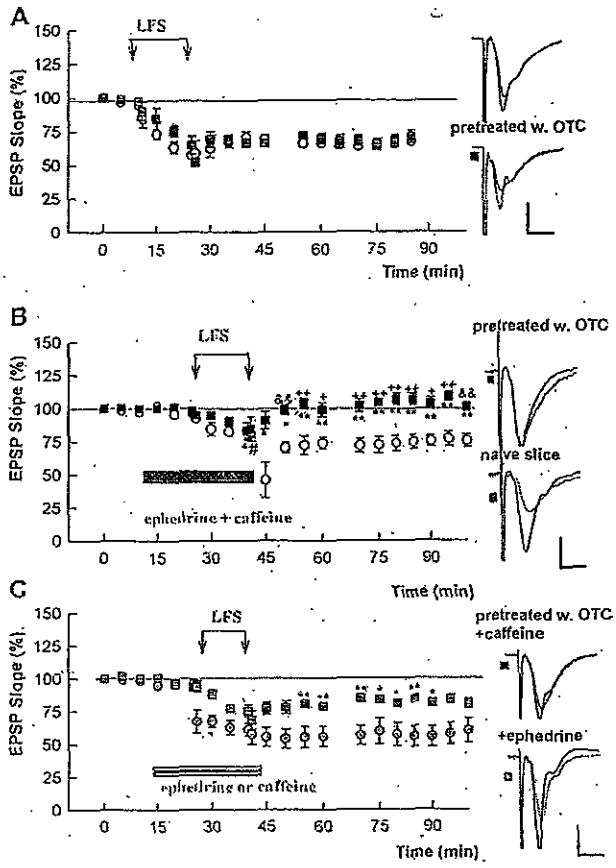

Figure 2 Impaired induction of hippocampal long-term depression (LTD) with ephedrine and caffeine in slices pretreated with 3 μM oseltamivir carboxylate (OTC). (A) Time courses of change in EPSPs in the CA1 region following LFS (arrows) in naïve slices (open circles) and slices treated with OTC (filled squares) ($N = 5$, each) are similar, suggesting that oseltamivir alone does not alter LTD. (B) Simultaneous administration of 100 μM ephedrine and 100 μM caffeine inhibits LTD in slices pretreated with OTC (filled squares) but not in naïve slices (open circles) ($N = 5$ in each). (C) In slices pretreated with OTC, caffeine alone (squares) or ephedrine alone (circles) failed to inhibit LTD induction. However, the inhibition was only partial with caffeine. Note that filled symbols in each graph denote pretreatment with OTC. $^{*}P < 0.05$, $^{**}P < 0.01$ by Student's *t*-test and $^{\#}P < 0.05$ by Mann–Whitney *U*-test against oseltamivir alone. In panel B, $^{+}P < 0.05$, $^{++}P < 0.01$ by Student *t*-test and $^{\&\&}P < 0.01$ by Mann–Whitney *U*-test against ephedrine plus caffeine without oseltamivir. Traces to the right depict EPSPs obtained before (solid line) and 60 min after (dotted line) LFS. Scales: 1 mV, 5 ms.

Discussion

This study shows that administration of a non-sedating dose of ethanol in combination with oseltamivir resulted in diminished behavioral activity and poor locomotion in rats. Evaluation of the changes in behaviors is difficult, but the pattern of response in the alternation task may represent a form of anxious or fearful behavior as manifest by altered exploration.[28] This behavioral restraint may result from risk assessment, the first line of defense against a threat, and appears as a decrease in environmental exploration and locomotion. Some rats were simply immobile without self-grooming, also suggesting an augmented risk-assessing behavior.[29] Immobility (freezing) is also believed to be a second level of defense and may involve assessment of a perceived threat from an undetermined source. If this is the case, random movement after injection of caffeine and ephedrine, which results in the impaired Y-maze performance, may represent a form of flight from an unknown threat as a third level of defense rather than environmental exploration. This may also reflect a form of a behavioral agitation.

It is also possible that oseltamivir alters cognitive processes maintained through synaptic plasticity. Of

importance is that oseltamivir, when combined with CNS stimulants, clearly impairs Y-maze performance. Thus, the random movements observed may have features similar to abnormal and agitated behaviors reported in humans following oseltamivir ingestion. Previous studies have indicated that Y-maze scores are correlated with induction of LTD, a form of hippocampal synaptic plasticity that may contribute to novelty-seeking behaviors.[27] We previously showed that administration of ethanol inhibits both LTD and Y-maze performance.[26] Impairment of LTD induction by the combination of OTC and CNS stimulants, which was observed in the present study, suggests that the combination of drugs affects the synaptic plasticity that may underlie this form of cognitive processes. It is also possible that the combination of drugs changes synaptic function and plasticity in other than regions of the CNS resulting in more profound changes in behavior.

Intentionally or unintentionally, patients with flu may consume CNS stimulants and other drugs. Alcohol, for example, is commonly used socially and may be ingested to relieve some flu-like symptoms by young teenagers.[23] Caffeine and related compounds are often included in soft drinks, nutritional supplements, and common cold regimens.[30] In Japan, ephedra is often taken by flu patients as part of a prescription of Chinese herbal medications that are believed to have antiviral effects.[31] Importantly, herbs such as ma huang (ephedra) are not free from adverse effects.[32] Just as with oseltamivir, suicides have been reported with abuse of ephedra.[33] Thus, it is possible that oseltamivir results in enhanced stimulant actions in the CNS and agitated behavior when combined with other stimulants. It is also possible that the interactions are more complicated during viral infections when there may be changes in the integrity of the BBB.[34,35] It has been reported that P-glycoprotein at the BBB plays an important role in accumulation of oseltamivir in the CNS.[20] In P-glycoprotein knock-out mice, oseltamivir concentration in the cerebrospinal fluid (CSF) is 5.5-fold higher.[36] Moreover, it was previously described that brain levels of oseltamivir were 1500 times those of adult animals exposed to the same dose.[19] The accumulated oseltamivir is likely converted to OTC in the CNS. Although it has been reported that OTC concentrations in the CSF in adult healthy volunteers administered 150 mg oseltamivir reach only approximately 0.1 μM,[37] much higher levels are expected if the BBB is immature or impaired.

The relation between use of oseltamivir and abnormal behaviors remain uncertain. It also remains unclear why these abnormal behaviors occurred primarily in Japan. This may simply reflect the frequency with which oseltamivir is used to treat influenza in Japan while oseltamivir use in the United States is relatively less common. We hypothesize that the combination of oseltamivir with CNS stimulants and/or alcohol could play a role in producing abnormal behaviors and accidental deaths. However, it is also possible that genetic variation resulting in reduced sialidase activities, which is detected in only some Asians, may account for the adverse effect of oseltamivir.[38] Taken together, these observations suggest that multiple factors are likely to contribute to the adverse effects of oseltamivir.[39] The present study suggests that oseltamivir, if combined with common neurostimulants, may alter a specific form of synaptic plasticity in the CNS; in turn, this could contribute to some of behaviors changes reported after use of oseltamivir. Further investigations, especially neurochemical analyses, will be required to elucidate the interactions of oseltamivir with other agents. This information will be important for determining the conditions under which antiviral agents can be used safely in humans given the potential need for widespread use of these drugs in event of an avian flu pandemic.

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