

## BRIEF COMMUNICATION OPEN

## The antiepileptic drug levetiracetam improves auditory gating in DBA/2 mice

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Schizophrenia is associated with deficits in P50 gating. This deficit is preclinically modeled in the DBA/2 mouse by depth recordings in the hippocampus. Neurobiologically, the deficit may be due to dysfunction in inhibitory circuitry. It follows that anti-epileptic drugs which impact this circuitry, such as levetiracetam (LEV), may improve gating. To that end, the goal of this study was to evaluate the ability of LEV to normalize sensory gating in the DBA/2 mouse. Gating of the murine analog of the P50, the P20-N40, was evaluated from *in vivo* hippocampal recordings in 39 male DBA/2 mice. Gating effects were evaluated using four doses of LEV (3, 10, 30, and 100 mg/kg). The 10 mg/kg dose improved P20-N40 gating ( $P=0.016$ ). No other doses significantly affected gating. Low-dose LEV may improve P20-N40 gating in the DBA/2 mouse model of schizophrenia. Low-doses of LEV may improve P20-N40 gating in the DBA/2 mouse model of schizophrenia and warrant further investigation in the illness.

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## INTRODUCTION

The development of novel approaches for the treatment of schizophrenia, particularly for its cognitive symptoms, is among the greatest unmet needs in psychiatry. One efficient, cost-effective method of identifying new treatments is to examine the effects of potential therapeutic compounds on animal models that mirror human phenotypes.

A prominent feature of schizophrenia is patients' inability to filter, or 'gate,' brain responses to repetitive stimuli, as evidenced by poor inhibition of early (50 ms post-stimulus) evoked brain responses to the second of two closely paired, identical auditory click stimuli.<sup>1</sup> This 'P50 gating' deficit may predict cognitive dysfunction, including attention deficits, in the illness.<sup>1,2</sup>

Preclinically, auditory gating abnormalities are modeled using *in vivo* recordings of auditory-evoked potentials from the mouse hippocampus. The hippocampal model originates from work by Kornetsky and Mirsky as well as Venables who proposed that damage to the region would result in a failure to filter irrelevant stimuli as observed in schizophrenia.<sup>3,4</sup> Hippocampal filtering of auditory information is made possible by direct input from the auditory cortex to the parahippocampal cortex, which then projects to the hippocampus.<sup>5</sup> Hippocampal source localization of the deficit has since been verified in both schizophrenia patients and animal models of the disorder.<sup>6,7</sup> Several strains of mice, including the DBA/2 mouse, demonstrate poor gating of the rodent hippocampal analog of the P50 (the P20-N40).<sup>7</sup> Interestingly, drugs that improve P20-N40 gating in these mice (e.g., nicotinic agonists) have demonstrated similar effects in human patients,<sup>7</sup> supporting the utility of P20-N40 gating as a translational tool.

A prevalent model of gating suggests that deficits arise in part due to dysfunction in inhibitory neuronal circuitry.<sup>7</sup> It follows that drugs that enhance this circuitry, such as antiepileptics, may improve gating. Surprisingly, however, to our knowledge no study

has examined gating effects of these drugs in either schizophrenia patients or animal models of the disease. One of these drugs, levetiracetam (LEV), may improve cognitive dysfunction in neurological diseases, particularly at doses lower than prescribed for epilepsy.<sup>8,9</sup> Furthermore, in patients with epilepsy, relative to other antiepileptics, LEV has a favorable side-effect profile,<sup>10</sup> lower risk of pharmacokinetic interactions with antipsychotics,<sup>10</sup> and lower risk of cognitive side effects. Most pertinent to schizophrenia, hippocampal hyperactivity is associated with cognitive and positive symptoms in the illness,<sup>11–13</sup> and LEV has demonstrated the ability to reduce hippocampal hyperactivity in mild cognitive impairment during a pattern separation (a type of recognition memory) task.<sup>8</sup> Performance deficits during pattern separation have also been observed in schizophrenia, possibly indicative of hippocampal dysfunction common to both illnesses.<sup>14</sup> Therefore, further investigation into its effects in preclinical models is warranted. To that end, this study evaluated the effect of LEV in DBA/2 mice, hypothesizing that the agent would improve impaired gating in this mouse model of impaired sensory gating in schizophrenia.

## MATERIALS AND METHODS

Male DBA/2 mice (7–10 weeks old, 20–25 g) were purchased from Harlan and group housed until recording. The UCAMC IACUC approved the experimental protocols.

LEV (3, 10, 30, or 100 mg/kg, Sigma, St. Louis, MO, USA) was dissolved in saline (pH ~ 5.5, 80  $\mu$ l for every 20 g of body weight) and injected *i.p.*

Hippocampal recordings were conducted as described previously.<sup>15</sup> Briefly, mice were anesthetized, placed in a stereotaxic apparatus, and hippocampal evoked responses measured with a recording electrode. Electrode location was verified by the presence of complex action potentials typical of hippocampal pyramidal neurons.<sup>15</sup> Tones (3,000 Hz) were presented in pairs

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separated by 500 ms at 10-s intervals. Six sets of 16 pairs of recordings were taken before drug administration and 12 sets taken after administration.

The amplitudes of auditory-evoked P20-N40 responses (i.e., the first or 'conditioning' stimulus (S1), the second or 'test' stimulus (S2), and the S2/S1 ratio) were analyzed (Datawave Sciworks, Loveland, CO, USA) by repeated measures analysis of variance as described previously.<sup>15</sup> Significant main effects of time indicated drug effects in line with previous studies from our laboratory. In addition, Fisher's least significant difference *post hoc* tests were conducted for doses that showed significant main effects of time to compare baseline means to each post-drug time point.

## RESULTS

Consistent with previous studies, DBA/2 mice failed to suppress S2 amplitudes during baseline as evidenced by mean S2/S1 ratios of approximately 1 (Figure 1c,f).

We investigated the effects of LEV at doses of 3, 10, 30, and 100 mg/kg ( $n = 10, 10, 10,$  and  $9,$  respectively). Across all the doses, significant main effects of time were observed on S1 amplitude ( $F(17,595) = 10.3, P < 0.001$ ) and S2 amplitude ( $F(17,595) = 6.38,$

$P < 0.001$ ). A significant time  $\times$  dose interaction was observed for S2 amplitude ( $F(17,595) = 1.38, P = 0.04$ ).

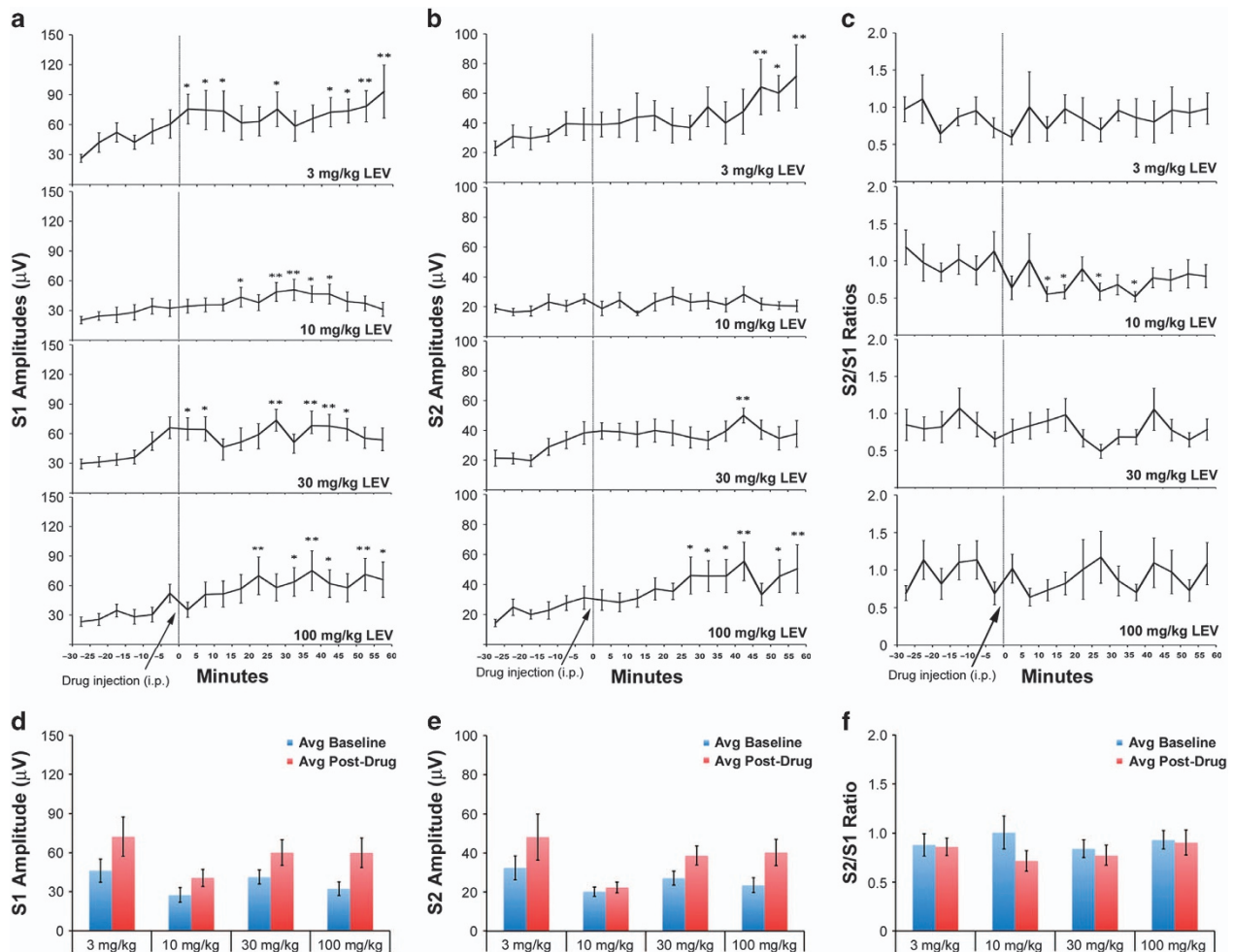
For S1 amplitude, significant main effects of time were observed for the 3 mg/kg ( $F(17,153) = 3.69, P < 0.001$ ), 10 mg/kg ( $F(17,153) = 2.48, P = 0.002$ ), 30 mg/kg ( $F(17,153) = 4.13, P < 0.001$ ), and 100 mg/kg ( $F(17,136) = 3.28, P < 0.001$ ) doses. All the doses increased S1 amplitude relative to baseline (Figure 1a,d).

For S2 amplitude, significant main effects of time were observed for the 3 mg/kg ( $F(17,153) = 2.97, P < 0.001$ ), 30 mg/kg ( $F(17,153) = 2.30, P = 0.004$ ), and 100 mg/kg ( $F(17,136) = 2.88, P < 0.001$ ) doses. These doses increased S2 amplitude relative to baseline (Figure 1b,e).

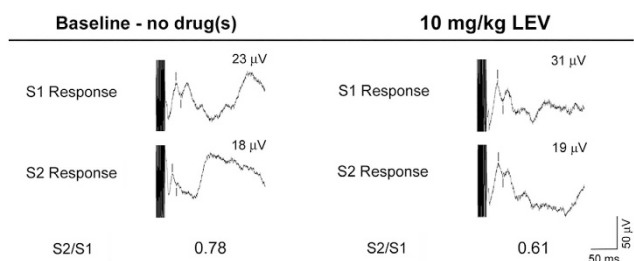
For S2/S1 ratio, a significant main effect of time was observed for the 10 mg/kg dose ( $F(17,153) = 1.98, P = 0.016$ ). This dose decreased S2/S1 ratio relative to baseline (Figures 1c,f and 2).

## DISCUSSION

The results of this preliminary investigation suggest that LEV may improve auditory gating. Gating enhancement was conveyed via (1) an increase in S1 amplitude and (2) no change in S2 amplitude at the 10 mg/kg dose.



**Figure 1.** (a–c) Effect of increasing doses of LEV (3, 10, 30, and 100 mg/kg, i.p.) on S1 response amplitudes (a), S2 response amplitudes (b), and S2/S1 ratios (c) as a function of time in DBA/2 mice. The first six points (–30, –25, etc.) refer to the baseline period of recording, before administration of drug(s). The last 12 points (0, 5, 10, etc.) refer to the post-drug administration period of recording. Asterisks mark those post-drug time points at which the S1 or S2 amplitude is significantly different from the average of the baseline S1 or S2 amplitudes, as determined using Fisher's LSD (\* $P < 0.05$ , \*\* $P < 0.01$ ). Data are mean  $\pm$  s.e.m. (d–e) Average baseline and post-drug S1 amplitude (d), S2 amplitude (e), and S2/S1 ratio (f) for each LEV dose. Data are mean  $\pm$  s.e.m. LEV, levetiracetam; LSD, least significant difference.



**Figure 2.** Representative waveforms demonstrating the effect of LEV (10 mg/kg) on P20-N40 gating in DBA/2 mice. Waveforms on the left side represent pre-drug (i.e., baseline) responses to the first (S1) and second (S2) stimuli. Waveforms on the right side represent post-drug responses to the first and second stimuli. Burst response artifacts represent the auditory stimuli, and tic marks denote the P20-N40 auditory-evoked potentials. In these representative examples, injection of LEV alone reduced S2/S1 ratio relative to baseline by increasing S1 amplitude while not affecting S2 amplitude. LEV, levetiracetam.

The most effective dose in the present study, 10 mg/kg, is equivalent to human doses lower than typically prescribed for epilepsy (assuming 90 kg body weight, the dose is 900 mg without correcting for surface area, and 73 mg after a 1/12 correction for surface area<sup>16</sup>). When prescribed for epilepsy, LEV is administered in doses of 1,000–3,000 mg/kg depending on patient response.<sup>10</sup> Along with a previous study that showed clinical efficacy using a 125 mg/kg b.i.d. dose in patients with mild cognitive impairment during a pattern separation task,<sup>8</sup> these results suggest that LEV may have clinical benefit in other disease populations at doses much lower than typically given for epilepsy. The findings that schizophrenia patients also show performance deficits during pattern separation,<sup>14</sup> as well as generalized hippocampal hyperactivity,<sup>12</sup> further suggests that LEV is worthy of additional investigation in schizophrenia and animal models of the disease.

The mechanism(s) by which LEV decreases neuronal hyperexcitability are complex and not fully understood. Possibilities include (1) inhibition of neurotransmitter release, (2) inhibition of ryanodine and IP<sub>3</sub>-receptor mediated calcium release from intracellular stores, (3) blockade of Zn<sup>2+</sup>-mediated inhibition of GABAergic signaling.<sup>17</sup> On the basis of these mechanisms, it may be surprising that LEV consistently increased S1 amplitude in the present study. It is possible that the inhibitory effects of LEV increase neuronal synchrony, enhancing response to the first stimulus. Indeed, schizophrenia is associated with deficits in stimulus-evoked synchrony, in part, due to loss of inhibitory signaling.<sup>18</sup> Furthermore, the finding that LEV increased S2 amplitude in a similar manner at the 3, 30, and 100 mg/kg doses, but not the 10 mg/kg dose, suggests that it may have competing mechanisms of action that converge at a maximally efficacious point (10 mg/kg).

The present results must be considered in the context of study limitations. Due to the relatively high S1 and S2 variability at baseline, we caution against overinterpretation of the respective contributions of S1 and S2 to the observed effects. S2/S1 ratios were, however, stable at baseline, suggesting that the gating improvement observed at the 10 mg/kg is not due to drift over time. Furthermore, a previous study from our laboratory found no effect of saline on S2/S1 ratio across an equivalent period of time (90 m),<sup>19</sup> suggesting that changes in ratio are specific to drug effects and not time effects.

Another study limitation is that we cannot rule out the possibility that the anesthetic used (chloral hydrate) may influence the observed results. Anesthesia also reduces the contribution of environmental factors, potentially limiting the generalizability of the findings. Several factors may, however, mitigate these

concerns. First, drugs that affect P20-N40 gating in anesthetized DBA2 mice (such as nicotinic agonists) often have similar effects in human schizophrenia patients who are not under anesthesia.<sup>7,20,21</sup> In addition, the  $\alpha 7$  receptor partial agonist 3–2,4-dimethoxybenzylidene anabaseine (DMXB-A) improves P20-N40 gating in both anesthetized mice and freely moving rats under similar dose ranges.<sup>22,23</sup> Finally, LEV has minimal (< 10%) protein binding,<sup>10</sup> suggesting that it is unlikely to directly interact with chloral hydrate or pyrazole. Nonetheless, to expand upon the preliminary findings presented here, future studies should examine the effects of LEV on freely moving animals to control for anesthetic effects, in both gating paradigms and other behavioral/cognitive assays.

Due to the favorable side effect profile of LEV, as well as its potential as a cognitive enhancer, we chose to focus on the effects of LEV as opposed to other anti-epileptic drugs in the present study. Given the theoretical GABAergic-basis of sensory gating deficits, however, future investigation of pro-gating effects using other antiepileptics with favorable side effect profiles (e.g., the GABA Transporter blocker tiagabine<sup>24</sup>) is warranted.

In conclusion, in this study we demonstrate that a low dose of LEV may improve auditory gating in the DBA/2 mouse model of gating deficits in schizophrenia. Although a limited number of studies have shown promising effects (e.g., LEV for mild cognitive impairment), research into the effects of low-dose antiepileptic drugs for diseases outside of their main indication (epilepsy) is currently in its infancy, and hypotheses into their effects by necessity must be drawn by inference from common biological markers across diseases. Hippocampal hyperactivity is a promising candidate biomarker in schizophrenia that we hypothesize may be targeted by LEV without severe side effects. We must caution, however, that the mechanisms that underlie hyperactivity in schizophrenia may be different from mild cognitive impairment, and that drug effects, therefore, are not necessarily generalizable. Nonetheless, we hope that this study will help foster more research into the effects of LEV in schizophrenia.

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## COMPETING INTERESTS

The authors declare no conflict of interest.

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