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Experience-Specific Plasticity at Excitatory and Inhibitory Synapses onto Granule Cells in the Dentate Gyrus

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Abstract The hippocampal dentate gyrus has been identified to play a critical role in maintaining contextual memory in many mammalian species. To evaluate learning-induced synaptic plasticity of granule cells, we subjected male rats to an inhibitory avoidance (IA) task and prepared acute hippocampal slices. In the presence of 0.5 μM tetrodotoxin, we recorded miniature excitatory post synaptic currents (mEPSCs) and inhibitory post synaptic currents (mIPSCs) in male rats experiencing four groups: untrained, IA-trained, unpaired, and walk-through. Compared with the untrained, IA-trained, unpaired, and walk-through groups, the unpaired group significantly enhanced mean mEPSC amplitudes, suggesting the experience-induced plasticity at AMPA receptor-mediated excitatory synapses. For inhibitory synapses, both unpaired and walk-through groups significantly decreased mean mIPSC amplitudes, showing the experience-induced reduction of postsynaptic $GABA_A$ receptor-mediated currents. Unlike the plasticity at CA1 synapses, it was difficult to explain the learning-specific plasticity at the synapses. However, overall multivariate analysis using four variables of mE(I)PSC responses revealed experience-specific changes in the diversity, suggesting that the diversity of excitatory/inhibitory synapses onto granule cells differs among the past experience of animals include the learning.

In comparison with CA1 pyramidal neurons, granule cells consistently showed greater amplitude and frequency of mE(I)PSCs. Fluctuation analysis further revealed that granule cells provide more postsynaptic AMPA receptor channels and greater single-channel current of $GABA_A$ receptors of than CA1 pyramidal neurons. These findings show functional differences between two types of principal cells in the hippocampus.

Key words: AMPA receptor, $GABA_A$ receptor, contextual learning, synaptic plasticity, granule cells

Introduction

The first evidence of a Hebb-like synaptic plasticity event was discovered in the dentate gyrus (DG) of a rabbit hippocampus.[1] Brief tetanic stimulation of the perforant path has persistently strengthened the stimulatory response in the DG, and the long-term potentiation (LTP) came to be viewed as a synaptic model of learning and memory.

Both AMPA- and NMDA-type glutamate receptors mediate perforant path stimulation at the synapses on granule cells.[2] The LTP not only enhances the presynaptic release of

glutamate[3] but also increases tritium-labeled AMPA receptor binding in the DG and CA1.[4] NMDA receptors are known to play a significant role in LTP induction, as proved by results showing that a specific antagonist of this receptor can block LTP induction.[5] More importantly, bilateral blockade of LTP in the DG synapses also impairs hippocampal-dependent learning,[6] suggesting a role for LTP in learning. Although granule cells in the DG are considered essential to learning function by decorrelating overlapping input patterns *in vivo*,[7] conclusive evidence for learning and induced synaptic plasticity in these cells is still lacking.[8]

To evaluate the plasticity, we previously showed contextual learning-dependent plasticity in CA1 pyramidal neurons. Contextual learning strengthened excitatory synapses through the synaptic incorporation of AMPA-type glutamate receptors. It also strengthened $GABA_A$ receptor-mediated inhibitory synapses on hippocampal pyramidal CA1 neurons, thus generating a broad diversity of excitatory/inhibitory synaptic inputs in individual CA1 neurons.[9,10] Using the same experimental protocol, we evaluated the synaptic plasticity of granule cells in the untrained, IA-trained, unpaired, and walk-through animals to examine the learning-specific change.

Materials and methods

Animals

Male Sprague Dawley rats (postnatal 4 weeks of age) were obtained from Chiyoda Kaihatsu Co., Tokyo, Japan. Prior to the experiment, the rats were individually housed in opaque plastic cages lined with wood chips for a couple of days (40 cm × 25 cm × 25 cm). We kept a constant temperature of 23 ℃ ± 1 ℃ under a constant cycle of light and dark (lights on: 8:00 AM to 8:00 PM) with ad libitum access to water and food (MF, Oriental Yeast Co. Ltd, Tokyo Japan). All animal housing and surgical procedures followed the guidelines of the Institutional Animal Care and Use Committee of Yamaguchi University. These guidelines comply with the Guide for the Care and Use of Laboratory Animals published by the National Institute of Health (NIH Publication No. 85-23, revised 1996).

Inhibitory avoidance (IA) task

Hippocampus-dependent IA training procedures were described previously.[9,11] The IA training apparatus (length: 33 cm; width: 58 cm; height: 33 cm) was a two-chambered box consisting of a lit safe side and a dark shock side separated by a trap door (Fig. 1A). For training, rats were placed in the lit side of the box, facing the corner opposite the door. After the trap door was opened, the rats could enter the dark box at will. The latency before entering the novel dark box was measured as a behavioral parameter (latency before IA learning, Fig. 1B). Soon after the animals entered the dark side, we closed the door and applied a scrambled electrical foot shock (1.6 mA, 2 s) via electrified steel rods placed in the floor of the box. The rats were kept in the dark compartment for 10 s before being returned to their home cage. Untrained control rats were not moved from their home cages, and injected with the same dose of anesthesia. The unpaired control rats (foot shock only) were housed in the shock cage and subjected to the same scrambled electrical foot shock without any contextual experience. The walk-through control rats were placed in the IA training apparatus and allowed to explore for 1 min, without shock (IA trained group: n = 10; walk-through group: n = 8; unpaired group: n = 7; untrained group: n = 10).

Thirty minutes after the procedure described above, the rats were placed in the lit side of the box. The latency before entering the dark box was measured as an indicator of learning performance (latency after IA learning).

Electrophysiological recordings

One hour after the paired foot-shock, rats were anesthetized with pentobarbital and acute brain slices prepared.[9,11] Preparation of brain slices using the mixture of ketamine (75-100 mg/kg sc) and xylazine (10 mg/kg sc) anesthesia did not affect the mE(I)PSC amplitude and frequency.

For the whole-cell recordings,[12,13] the brains were quickly perfused with ice-cold dissection buffer (25.0 mM $NaHCO_3$, 1.25 mM NaH_2PO_4, 2.5 mM KCl, 0.5 mM $CaCl_2$, 7.0 mM $MgCl_2$,

25.0 mM glucose, 90 mM choline chloride, 11.6 mM ascorbic acid, 3.1 mM pyruvic acid) and gassed with 5% CO_2/95% O_2. Coronal brain slices (target CA1 area: AP -3.8 mm, DV 2.5 mm, LM ± 2.0 mm) were cut (350 μm, Leica vibratome, VT-1200) in dissection buffer and transferred to physiological solution (22-25 ℃, 114.6 mM NaCl, 2.5 mM KCl, 26 mM $NaHCO_3$, 1 mM NaH_2PO_4, 10 mM glucose, 4 mM $MgCl_2$, 4 mM $CaCl_2$, pH 7.4) gassed with 5% CO_2/95% O_2). The recording chamber was perfused with physiological solution at 22-25 ℃. For the miniature excitatory post synaptic current (mEPSC) and inhibitory post synaptic current (mIPSC) recordings, we used the physiological solution containing 0.5 μM TTX to block Na^+ channels.

Patch recording pipettes (4-7 MΩ) were filled with intracellular solution (127.5 mM cesium methanesulfonate, 7.5 mM CsCl, 10 mM Hepes, 2.5 mM $MgCl_2$, 4 mM Na_2ATP, 0.4 mM Na_3GTP, 10 mM sodium phosphocreatine, 0.6 mM EGTA at pH 7.25). Under the IR-DIC microscope (BX51, Olympus, Tokyo, Japan), recording cells were randomly selected in the upper blade of the DG (Fig. 1C). Whole-cell recordings were obtained from the cells using an Axopatch-1D amplifier (Axon Instruments Inc., Union City, CA, USA). The whole-cell patch-clamp data were collected using a Clampex 10.4 instrument, and the data were analyzed using Clampfit 10.4 software (Axon Instruments).

For the miniature recordings, the mEPSCs (-60 mV holding potential) and mIPSCs (0 mV holding potential) were recorded sequentially for 5 min in the same neuron. The miniature events were detected using the Clampfit 10.4 software (Axon Instruments), and the events above 10 pA were used in the analysis. We recorded for at least 5 min, to determine the event frequency of mEPSCs or mIPSCs. The amplitudes of the events were averaged to obtain the mean amplitude. Bath application of an AMPA receptor blocker (CNQX, 10 μM) or $GABA_A$ receptor blocker (bicuculline methiodide, 10 μM) consistently blocked the mEPSC or mIPSC events, respectively. Although CNQX is a competitive antagonist of AMPA receptor and glycine site of NMDA receptors, extracellular Mg^{2+} is known to block NMDA receptors when the resting potential is less than -40 mV in CA1 pyramidal neurons.[14]

Nonstationary fluctuation analysis

AMPA receptor-mediated mEPSCs and $GABA_A$ receptor-mediated mIPSCs were analyzed by nonstationary fluctuation analysis.[10,15] To isolate fluctuations in current decay due to stochastic channel gating, the mean waveform was scaled to the peak of individual E(I)PSCs. The requirements for such analysis include a stable current decay time course throughout the recording and an absence of any correlation between the decay time course and peak amplitude. The relationship between the peak-scaled variance and the mean current is given by the following equation:

$$\sigma^2 = iI - I^2/N + b_l$$

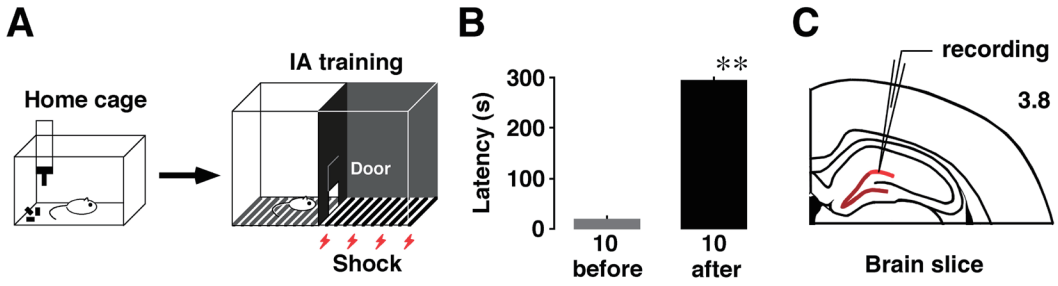

Fig. 1 (A) Schema of the light-dark box used for the inhibitory avoidance (IA) task. (B) Thirty minutes after the brief foot-shock, the rats consistently showed a longer latency before entering the dark side of the box. (C) We then prepared brain slices after the training and analyzed synaptic currents from granule cells in the suprapyramidal area of DG. The number indicates posterior coordinate from bregma. $^{**}P < 0.01$ vs. before. Error bars indicate ± SEM. The number of rats is indicated at the bottom of each bar.

where σ^2 is the variance, I is the mean current, N is the number of channels activated at the peak of the mean current, i is the unitary conductance, and b_I is the background variance. In our experiments, 31-69 EPSCs and 14-133 IPSCs were analyzed from selected epochs in each of the cells in which there was no correlation between current decay (63% decay time) and peak amplitude ($P > 0.05$, Spearman's rank-order correlation test). The weighted mean channel current can be estimated by fitting the full parabola or initial slope of the relationship. All the analysis was done using MATLAB software (MathWorks, MA, USA).

Statistical analysis

We used the paired t-test to analyze IA latency, and unpaired t-test to compare the miniature data between granule cells and CA1 pyramidal neurons. One of the four miniature parameters (i.e. mEPSC amplitudes) was analyzed by one-way factorial analysis of variance (ANOVA) where the variable was the experience. The ANOVA was followed by *post hoc* analysis with the Fisher's protected least significant difference test. Overall differences in four miniature parameters (mEPSC/mIPSC amplitudes and frequency) were analyzed using MANOVA with Wilks' Lambda distribution. Specific differences between two experiences were further analyzed using *pos-hoc* MANOVAs. $P < 0.05$ was considered statistically significant.

Results

IA task

To investigate learning-induced plasticity at DG synapses in granule cells, we subjected rats to an IA task (Fig. 1A).[12,16] In this learning paradigm, rats could cross from a light box to a dark box, where an electric foot-shock (1.6 mA, 2 s) was delivered. Half an hour after the IA task, we measured the latency in the illuminated box as representing contextual learning performance. The latency was much longer after training than before training (Fig. 1B; $t_9 = 27.607$; $P < 0.0001$) with paired foot-shock.

Miniature of postsynaptic currents and frequencies events in granule cells

To analyze experience-induced synaptic plasticity, we recorded mEPSCs at −60 mV and mIPSCs at 0 mV sequentially in the same neuron in the presence of tetrodotoxin (0.5 μM) on both sides of the dorsal hippocampus (Fig. 2A). By changing the membrane potential, we sequentially recorded mEPSCs (at −60 mV) and mIPSCs (at 0 mV) from the same neuron, as has been reported previously.[9] The postsynaptic currents correspond to the response elicited by a single vesicle of glutamate or GABA. In contrast, the number of synapses affects event frequency.[17]

To evaluate the experience specificity, we plotted the four parameters in a four-dimensional virtual space in order to analyze both amplitude and frequency of mEPSC and mIPSC events in individual granule cells. We used one-way multivariate ANOVA (MANOVA) with experience as the between-group factor, which showed a significant main effect of experience (Figs. 2B and C; $F_{12,323} = 4.680$, $P < 0.0001$). *Post-hoc* MANOVA further showed differences between two specific experiences (Table 1), suggesting experience-specific synaptic plasticity of the DG granule cells.

As to the parameter specific change, we detected an overall difference in mEPSC amplitude among untrained, IA-trained, unpaired, and walk-through animals (Fig. 2D, $F_{3,125} = 4.431$, $P = 0.0054$). *Post-hoc* analysis showed that the experience of task significantly increased mEPSC amplitudes in the unpaired ($P = 0.00079$), but not in the trained or walk-through groups. Similarly, in mIPSC amplitude, we found an overall difference (Fig. 2D, $F_{3,125} = 6.516$, $P = 0.0004$). *Post-hoc* analysis showed that the experience significantly decreased mIPSC amplitudes in the unpaired ($P = 0.002$) or walk-through ($P = 0.001$) but not in the trained groups. Conversely, in mEPSC frequency, there is no overall significance among the four groups (Fig. 2E, $F_{3,125} = 0.168$, $P = 0.92$). In mIPSC frequency, we found an overall difference (Fig. 2E, $F_{3,125} = 6.521$, $P = 0.0004$), and *post-hoc* analysis showed that the experience significantly increased in the unpaired ($P = 0.0003$) or walk-through ($P = 0.0003$) groups.

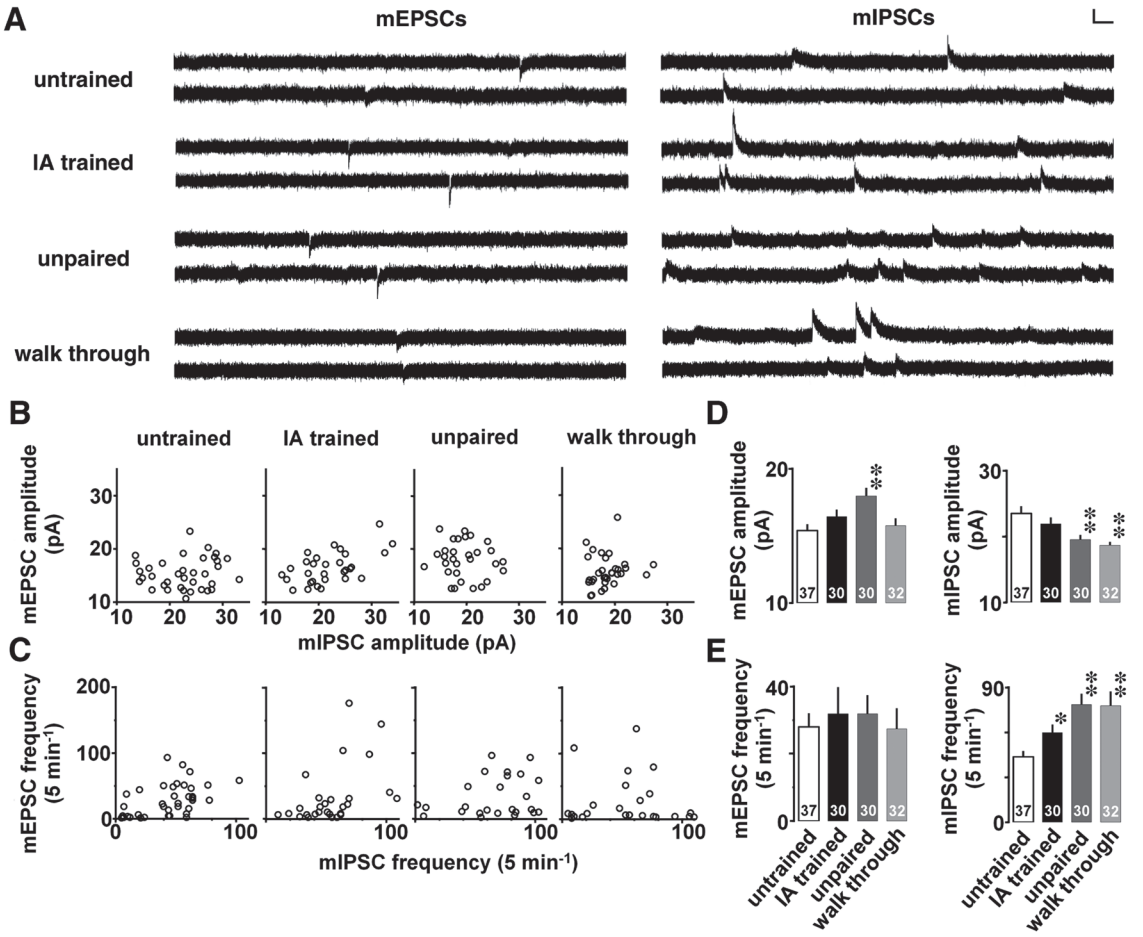

Fig. 2 (A) Representative traces of mEPSCs and mIPSCs. mEPSCs at −60 mV and mIPSCs at 0 mV were measured sequentially in the same neuron in the presence of tetrodotoxin (0.5 μM). Vertical scale bar, 10 pA; horizontal scale bar, 200 ms. (B and D) Plots of mean mEPSC/mIPSC amplitudes of individual granule cells in untrained, trained, unpaired, and walk-through rats. (C and E) Plots of mean mEPSC/mIPSC frequencies of individual granule cells in the four groups. Error bars indicate ± SEM. The number of cells is indicated at the bottom of each bar. $^{**}P < 0.01$ vs untrained.

Table 1 *Post-hoc* MANOVAs for Figure 2

Group	Untrained	IA-trained	Unpaired	Walk-through
Untrained	−	$F_{4,62} = 2.900$	$F_{4,62} = 9.566$	$F_{4,64} = 6.933$
IA-trained	$P = 0.0289$	−	$F_{4,55} = 4.166$	$F_{4,57} = 3.437$
Unpaired	$P < 0.0001$	$P = 0.0051$	−	$F_{4,57} = 2.007$
Walk-through	$P = 0.0001$	$P = 0.0138$	$P = 0.1057$	−

Comparison with CA1 pyramidal neurons

By comparing mE(I)PSC data in CA1 pyramidal neurons,[10] we found further cell-specific differences in untrained conditions (Table 2). At excitatory synapses, granule cells showed greater mEPSC amplitudes/frequency than CA1 pyramidal neurons. Further fluctuation analysis indicated greater number of postsynaptic open Na^+ channels without changing single channel currents of granule cells.

Also, at inhibitory synapses, granule cells were noted to exhibit greater mIPSC amplitudes/frequency than CA1 pyramidal neurons. Further fluctuation analysis indicated greater single Cl^- channel currents without changing the number of open channels of granule cells.

Discussion

A single granule cell is known to receives inputs of approximately 5600 excitatory[18,19] and 950 inhibitory synapses.[20] Although we detected variable experience-induced plasticity at excitatory or inhibitory synapses, it was difficult to clarify the learning-specific change at the synapses. If it is experience with IA apparatus, 3 experience groups (trained, unpaired, walk-through) should be different from the untrained group but be similar to each other. Alternatively, the experience could be "time spent in the dark box," where groups that spend more time on the dark box may promote plastic change at GABAergic inhibitory synapses. To extract context-specific or learning-dependent plasticity, input-specific and/or cell-specific analysis of the plasticity should be necessary in granule cells.

Granule cells received three types of glutamatergic inputs. The layer II neurons of the entorhinal cortex projected to the cells *via* lateral and medial perforant paths,[21] and mossy cells in the *hilus* also projected to the cells.[22,23] Medial entorhinal area cells are known to contain several spatially modulated cell types, such as border cells, head cells, direction cells, victor cells, and grid cells. In contrast, lateral entorhinal area cells preferentially represent non-spatial information, such as objects and object-related spatial features, including egocentric bearing toward an object or previous object locations.[24] Optogenetic induction of LTP at perforant path synapses of engram cells in the DG restored spine density and fear-conditioned memory, suggesting a role for performant path synapses in memory.[25] Moreover, glutamatergic inputs from the mossy cells may be essential for learning because optogenetic inhibition of hilar mossy cell activity impedes the encoding of spatial context.[23]

Since the synaptic plasticity was learning-dependent in CA1 pyramidal neurons, we further compared the miniature currents between the two principal cell types (Table 2). Considering the results of fluctuation analysis, more postsynaptic AMPA receptor channels may contribute to form greater mEPSC amplitudes than CA1 pyramidal neurons. In

Table 2 A comparison of granule cells and CA1 pyramidal neurons

Parameters	Granule cells	CA1 neurons	*P*-value
mEPSC amplitudes pA	15.4 ± 0.5 (37)	12.8 ± 0.3 (56)	< 0.0001
mEPSC frequency per 5 min	28.0 ± 4.0 (37)	7.6 ± 0.9 (56)	< 0.0001
number of open Na^+ channels	32.6 ± 7.8 (16)	15.2 ± 2.1 (42)	= 0.046
single Na^+ channel currents pA	2.0 ± 0.4 (16)	2.9 ± 0.6 (42)	ns
mIPSC amplitudes pA	23.5 ± 1.1 (37)	16.3 ± 0.5 (56)	< 0.0001
mIPSC frequency per 5 min	43.7 ± 3.9 (37)	68.7 ± 5.8 (56)	< 0.0001
number of open Cl^- channels	28.0 ± 11.5 (23)	31.3 ± 3.6 (23)	ns
single Cl^- channel currents pA	6.2 ± 1.4 (23)	1.6 ± 0.5 (23)	= 0.004

Parentheses indicate the number of cells.

contrast, at inhibitory synapses, greater single-channel current of $GABA_A$ receptors may form greater mIPSC amplitudes than CA1 pyramidal neurons. Because the expression of individual $GABA_A$ receptor subunits in DG was different from that in CA1,[26] the composition of $GABA_A$ receptor subunits in DG may contribute to increase the single-channel current.

Regarding the number of synapses, the neuro-anatomical analysis estimated that single suprapyramidal granule cells (approximately 5600)[19] have smaller excitatory synapses than single CA1 pyramidal neuron (about 13,000 to 30,000).[27] In these present results, however, granule cells showed much higher mEPSC frequency than CA1 pyramidal neurons. Since the mE(I)PSC frequency seems to represent the number of functional synapses or the presynaptic release probability,[17] a great population of silent synapses in CA1 pyramidal neurons[28] may cause the low mEPSC frequency in CA1 neurons. Also, the high presynaptic glutamate release probability onto granule cells[3] may contribute to elevate mEPSC frequency in granule cells. Consequently, the difference may contribute to forming the typical firing pattern in DG granule cells. Spontaneous firing rates of granule cells were extremely low when rats were awaken,[7,29,30] which is quite different from the firings of CA1 pyramidal neurons.[31,32]

Conclusion

We sequentially recorded mEPSCs and mIPSCs from the same granule cells to obtain four variables (mEPSC/mIPSC amplitudes and frequency). Unlike the plasticity at CA1 synapses, it was difficult to identify learning-specific plasticity in granule cells. However, we detected variable experience-induced plasticity at excitatory or inhibitory synapses. Moreover, overall MANOVA with four variables revealed experience-specific plasticity at excitatory/inhibitory synapses, suggesting that the diversity of excitatory/inhibitory synapses differs among the past experience of animals.

In comparison with CA1 pyramidal neurons, granule cells exhibited greater mE(I)PSC responses, providing more postsynaptic AMPA receptor channels and greater single-channel current of $GABA_A$ receptors than CA1 pyramidal neurons. These findings show functional differences between the two principal cell types in the dorsal hippocampus.

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Conflict of Interest

The authors declare no conflict of interest.

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