



## Short Communication

## Involvement of L-lactate in hippocampal dysfunction of type I diabetes

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## ABSTRACT

Hippocampal neurons play a crucial role in memory formation. Accumulating evidence raises the possibility that hippocampal sharp-wave ripples (SW-Rs) are involved in memory consolidation. Here, we examined in an animal model of diabetes and found the amplitude of SW-Rs in diabetic mice were smaller than control group and were rescued by acute application of L-lactate, a major neural energy source. The cognitive impairment in diabetic mice was alleviated by intracerebroventricular L-lactate treatment. Our results suggested that L-lactate is important for hippocampal dysfunction in diabetes.

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Patients with diabetes mellitus impair cognitive function compared to age-matched healthy subject.<sup>1</sup> Streptozotocin-treated animal, an animal model of type I diabetes, showed cognitive impairment.<sup>2</sup> Reduction of the synaptic strength and the number of synapse are suggested to be essential in learning and memory deficits in diabetic mice.<sup>3</sup>

Neurons obtain ATP from glucose and lactate, in order to maintain their synaptic activity.<sup>4</sup> Interruption of L-lactate provision impairs memory consolidation or storage.<sup>5</sup> Repetitive stimulation of primary cultures of mouse cortical neurons in the absence of any metabolic substrate decrease the number of active sites after the first cycle of vesicle loading/unloading.<sup>6</sup>

Memory consolidation is the post-training process that makes recently acquired information stable, and takes place mainly in the hippocampus.<sup>7</sup> Sharp wave ripple events (SW-Rs) generating in the hippocampus are implicated in memory consolidation.<sup>8</sup> Previous reports indicated that selective elimination of SW-Rs resulted in deterioration of memory consolidation in rats.<sup>9,10</sup>

In this study, we investigated the relationship between hippocampal functions and cognitive impairments in streptozotocin-induced diabetic mice. We established a role of the reduced L-

lactate in the aberrant hippocampal neural functions and impairment of memory consolidation in diabetic mice.

All of the experimental protocols used in the present study were approved by the Animal Care and Use Committee of Nagoya City University and University of Tokyo, and carried out in accordance with the guidelines of the National Institutes of Health and the Japanese Pharmacological Society.

Male ICR mice (4 weeks old; SLC, Shizuoka, Japan) were housed four to six per cage under controlled circumstance ( $23 \pm 2$  °C with a 12-h light dark cycle; lights on 07:00 to 19:00), and rendered diabetic by a single injection of streptozotocin (200 mg/kg, i.v.) prepared in 0.1 N citrate buffered saline at pH4.5. Age matched non-diabetic mice were injected with vehicle alone. Mice with serum glucose levels above 400 mg/dl were considered diabetic.

The novel-object recognition (NOR) task procedure consisted of 3 sessions: habituation, training, and retention. Mice were habituated to the box for 30 min in the absence of objects and returned to the home cage. Then, 2 objects (left and right) were placed into the open field, and each mouse was allowed to explore for 10 min (training session). The total time spent exploring each object was recorded. After either a short (8 min) or long (24 h) delay period, the animals were returned to the same box, in which one of the familiar objects used during training had been replaced by a novel object. The mice were then allowed to explore freely for 10 min, and the time spent exploring each object was recorded (test session). A preference index, a ratio of the amount of time spent exploring either one of the two objects (training session) or the novel object

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(test session) over the total time spent exploring both objects, was used to measure cognitive function.

Two weeks after the streptozotocin injection, mice were killed by cervical dislocation under anesthesia with isoflurane. A posterior brain block was cut into 400  $\mu\text{m}$  thick oblique slices at an angle of  $12.7^\circ$  in the front–occipital axis using a vibratome.<sup>11</sup> Local field potentials (LFPs) were recorded from CA3 stratum pyramidale using borosilicate glass pipettes (1–2 M $\Omega$ ) filled with aCSF. Signals were amplified by Multi Clamp 700B (Molecular Devices, Union City, CA, USA), digitized at 10,000 Hz and filtered with a band of 1–2000 Hz by pCLAMP 10 (Molecular Devices). Offline analysis was conducted using custom-made MATLAB routines (MathWorks, Natick, MA, USA). To detect SW-Rs, LFP traces were band-pass filtered at 2–30 Hz and thresholded at 4 times above the SD of the baseline noise.

Data are expressed as the mean  $\pm$  S.E. Statistical significance was evaluated by two-tailed t test with Bonferroni correction after one-way analysis of variance for multiple comparisons, Student t test, or Kolmogorov–Smirnov (KS) test. Differences at  $P < 0.05$  were considered significant.

We examined cognitive performance in diabetic and non-diabetic mice using the NOR test. When memory retention was tested for 8 min after the training session, both non-diabetic and diabetic mice spent more time for exploring the novel object than they did exploring the familiar object (Fig. 1B and C). When memory retention was tested for 24 h after the training session, non-diabetic, but not diabetic mice spent more time for exploring the novel object ( $P < 0.01$ , Bonferroni test; Fig. 1B and C).

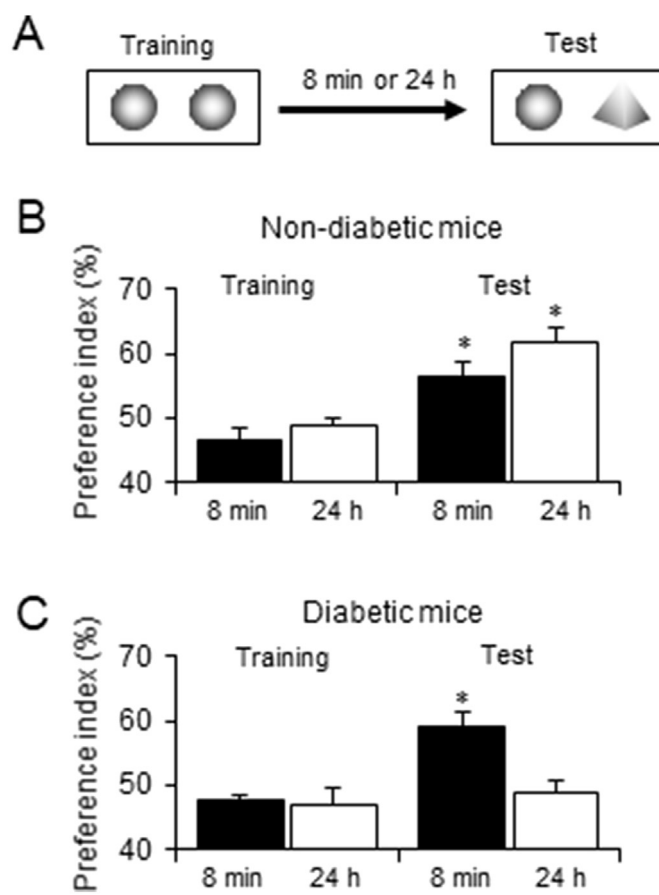
Although NOR test reportedly indicated the non-hippocampus dependent learning task, recent report indicated that hippocampus is crucial for consolidation of non-hippocampal learning during sleep.<sup>12</sup> Therefore, we recorded LFPs from hippocampal slice area, and investigated the SW-Rs in diabetic and non-diabetic mice (Fig. 2A). The frequency of SW-Rs was not changed in diabetic mice (Fig. 2B). On the other hand, the mean peak amplitude of the SW-R events was significantly smaller in diabetic than non-diabetic mice ( $P < 0.01$ , KS test; Fig. 2C). Since neurons require high energy to maintain their excitability, we examined the effect of  $\text{l-lactate}$  application on the aberrant SW-Rs in diabetic mice (Fig. 2D–F). Twenty minutes application of  $\text{l-lactate}$ <sup>4</sup> increased the amplitude of the SW-Rs in diabetic mice, but not in non-diabetic mice (Fig. 2F).

We examined whether  $\text{l-lactate}$  improves the cognitive impairment of diabetic mice. Single i.c.v. treatment with  $\text{l-lactate}$  (200 nmol) 3 h after training session increased the preference index in diabetic, but not non-diabetic mice ( $P < 0.05$ , Bonferroni test; Fig. 3B and C).

During hippocampal SW-Rs, excitability in both pyramidal cells and interneurons reach highest synchrony,<sup>13</sup> presumably high energy provision is required. As we expected, the decrease in the amplitude of SW-Rs in diabetic mice was recovered by transient  $\text{l-lactate}$  application (Fig. 2F), suggesting that enough provision of  $\text{l-lactate}$  normalize the bursty firing in CA3 neurons of diabetic mice.

The SW-Rs events are involved in the consolidation of memory. Previous report indicated that selective elimination of SW-Rs resulted in deterioration of memory consolidation in rats.<sup>9</sup> Another study by Ego-Stengel and Wilson<sup>10</sup> indicated that disruption of SW-Rs during sleep after training resulted a slower leaning. In the present study, diabetic mice showed the impairment of only long-term memory (Fig. 1). Therefore, the aberrant SW-Rs events in diabetic mice are associated with the impairment of consolidating hippocampus-dependent memories.

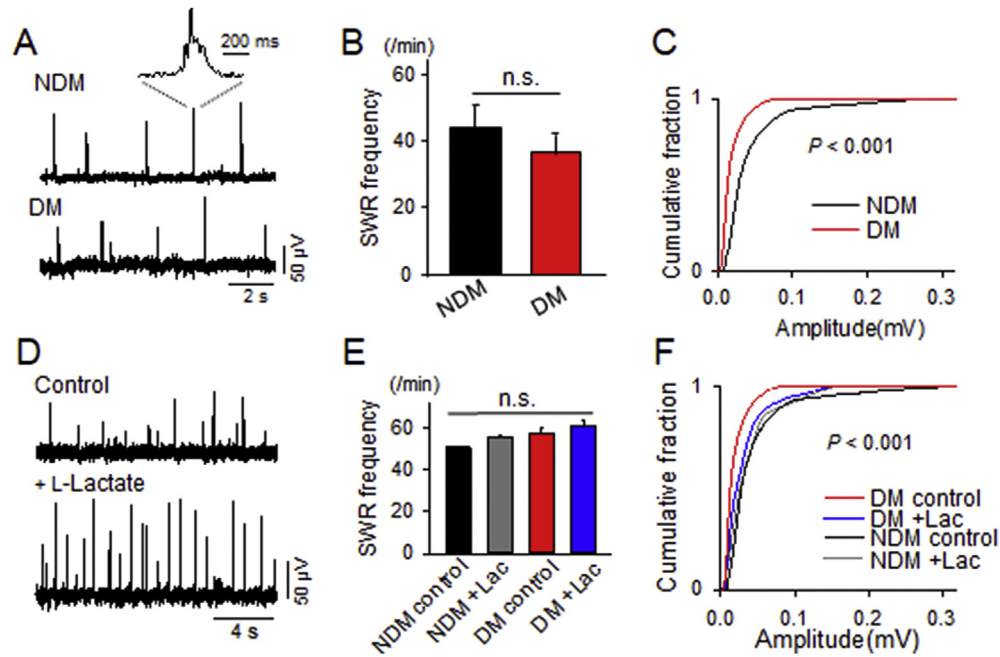
The  $\text{l-lactate}$ -induced improvement of diabetic cognitive impairment might be mediated by the recovery from structural



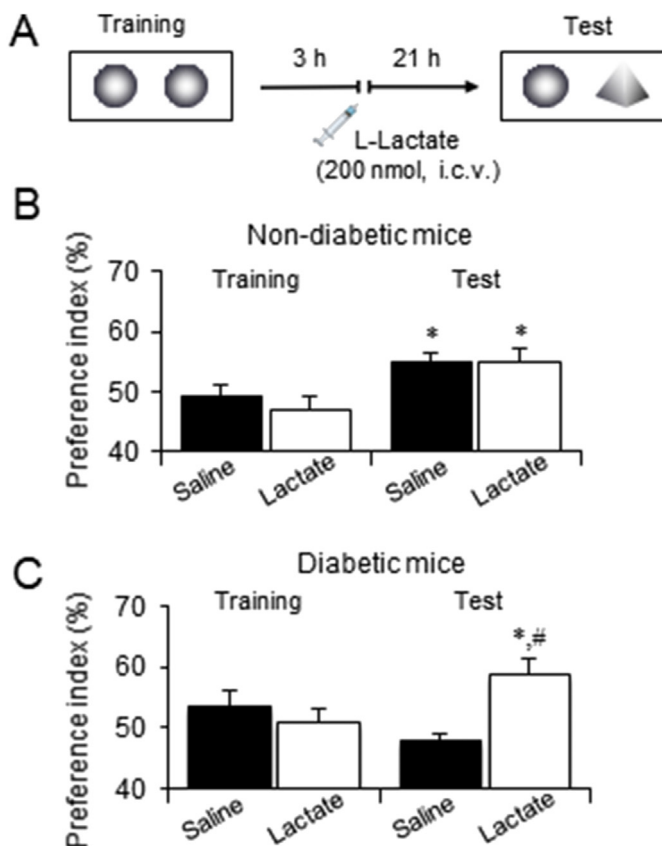
**Fig. 1.** Diabetes impairs long-term, but not short-term cognitive function. (A) A schematic representation of the novel-object recognition test. In the training session two identical objects shown as sphere were presented to a mouse. In the retention test (8 min or 24 h after the training session), one of the familiar objects used during the training sessions was replaced by novel object shown as triangular pyramid. Short-term (left panel) and long-term (right panel) memory in non-diabetic and diabetic mice (left panel). Short- and long-term memory was assessed 8 min and 24 h after the training session, respectively. (B and C) Preference index in non-diabetic (B) and diabetic (C) mice tested at 8 min or 24 h after the training session. Each column represents the mean with S.E.M. of 10 mice. \* $P < 0.05$  vs Training.

synaptic alteration, such as synaptic abnormalities.<sup>3</sup> The mechanisms of this  $\text{l-lactate}$  effect could be resulted from the improvement of energy shortage. In addition, it has been reported that  $\text{l-lactate}$  protects the glutamate-induced neurotoxicity.<sup>14</sup> Since STZ-induced diabetes has reportedly showed the glutamate toxicity in hippocampus,<sup>15</sup> it is plausible that the recovery of cognitive impairment in diabetic mice after  $\text{l-lactate}$  treatment might be mediated by not only mere improvement of energy shortage, but also the neuroprotective effect. However, since  $\text{l-lactate}$  induces multiple reactions including intracellular signaling activation, the detailed mechanisms how  $\text{l-lactate}$  affects diabetic cognitive impairment and hippocampal SW-R abnormality remain unclear. Further studies are still required for greater certainly.

In conclusion, the cognitive impairment in diabetic mice may be due to deficits in memory consolidation process during SW-R events, which is recovered by  $\text{l-lactate}$  treatment. The fundamental mechanism may be applied to the other cognitive deficits, including aging, dementia, and Alzheimer's disease.



**Fig. 2.** Diabetic mice showed reduction of SW-Rs. (A) Representative LFPs recorded from CA3 stratum pyramidale in a hippocampus slice prepared from non-diabetic (upper; NDM) and diabetic (lower; DM) mice (B) SW-Rs frequency in the hippocampal slices from non-diabetic (Left) and diabetic (Right) mice. Error bars indicate S.E.M. of 10 slices. (C) Cumulative distribution of the amplitude of SW-Rs in non-diabetic (black) and diabetic (red) mice. (D) Representative LFPs recorded from CA3 stratum pyramidale in a hippocampus slice prepared from diabetic (lower) mice before (Top) and after (Bottom) transient bath application of 50  $\mu$ M L-lactate. (E) SW-Rs frequency in slices perfused with L-lactate in non-diabetic and diabetic mice. Error bars indicate S.E.M. of 10 slices. (F) Cumulative distribution of the amplitude of SW-Rs in diabetic mice before and after L-lactate application.



**Fig. 3.** L-Lactate improves cognitive functions in diabetic mice. (A) A schematic representation of the novel-object recognition test in L-lactate treatment experiment. (B and C) Effects of i.c.v. treatment with L-lactate 3 h after the training session on preference index of non-diabetic (B) and diabetic (C) mice. Each column represents the means with S.E.M. of 10 mice. \* $P < 0.05$  vs. respective training session. # $P < 0.05$  vs. respective saline-treated group.

#### Conflict of interest

The authors declare no financial or other conflicts of interest in this study.

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