Preserved LTP and water maze learning in hyperglycaemic–hyperinsulinemic ZDF rats

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Abstract

Previous investigations have demonstrated that cognitive deficits as well as hippocampal dysfunctions are generated in animals presenting manifestations of Type 1 diabetes (T1D) mellitus. The present study examined whether such deficits can also be reproduced in the Zucker Diabetic Fatty (ZDF) rats after they developed symptoms of Type 2 diabetes (T2D). Learning and memory assessments were performed using the Morris water maze 5 weeks after the animals presented symptoms of Type 1 diabetes for Experiment 1 (Exp 1) and after 8 weeks for Experiment 2 (Exp 2). Testing in the water maze revealed that ZDF rats learned the task normally, although control rats were found to swim significantly faster after 5 or 8 weeks of untreated diabetes. From an electrophysiological perspective, we observed that the integrity of synaptic function was also preserved in ZDF rats as no alterations in long-term potentiation (LTP) were observed in the area CA1 of hippocampal slices. It is concluded that hyperglycaemia is not the only factor influencing water maze learning and LTP in this animal model of Type 2 diabetes (T2D). The experiments suggest that the resistance of ZDF rats to cognitive and electrophysiological dysfunctions might be related to the protective action of hyperinsulinemia. Indeed, measurements of the plasma insulin level at the end of testing were significantly superior in ZDF rats in comparison to control rats.

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1. Introduction

Numerous clinical studies have demonstrated that subtle but significant alterations in central nervous system (CNS) functions are often observed in diabetic patients [1–5]. Neurobehavioral studies have reported cognitive decline in subjects with diabetes [4,6], and neurophysiological investigations have revealed conduction abnormalities in the central auditory, somatosensory, and visual pathways in diabetic individuals [7,8]. On the other hand, people with diabetes, especially older adults, apparently face a greater risk of vascular dementia, with large population studies detecting an association between diabetes mellitus, dementia, and Alzheimer’s disease [9–12]. Following a population of over 6000 for up to 6 years, Ott et al. found that the risk of dementia is nearly doubled in diabetic subjects, an effect that cannot be accounted only through vascular factors [11].

Studies have revealed an association between Type 1 diabetes (T1D) and cognitive difficulties, particularly in diabetic individuals who expressed early onset of the disorder [13–16]. For instance, small deficits in attention, executive skills, and processing speed have been reported in studies of children with T1D. Six years after the onset of disease, children with T1D performed more poorly than
control subjects on measures of attention, processing speed, long-term memory, and executive skills. These neuropsychological defects in children with this form of diabetes were consistent with dysfunction of anterior and medial temporal brain regions and correlated with the history of hypoglycemic episodes [13]. Large-scale epidemiological investigations have also found poorer learning and memory performances to be also associated with Type 2 diabetes (T2D), impaired glucose tolerance, and/or hyperinsulinemia [2,16]. One of the cognitive functions that seems the most reliably altered in T2D is memory for verbal information [3]. As long-term memory for verbal information relies on a brain network that includes the hippocampus, this finding could suggest that T2D also has detrimental effects on this structure. However, the brain alterations could also be more diffuse as the effect on verbal memory was not only limited to long-term retention but also to short-term verbal memory, a form of memory with which the hippocampal formation has little to do. According to recent reports, cognitive alterations observed during T2D appear to require the interaction of ageing factors with diabetes [17–19]. Based on calculations made on a group of elderly women with T2D, Grodstein et al. of Harvard Medical School estimated that having diabetes is equivalent to ageing 4 years in terms of cognitive performance [20]. Recent investigations have indicated that diabetes-induced changes of brain properties might in fact share many properties with brain ageing [2,21,22]. Specific brain changes associated with T1D are corroborated by behavioral and cognitive changes observed in diabetic animals [16,23]. Most studies performed in animal models of T1D have shown that alterations of long-term potentiation (LTP) in the hippocampus, an electrophysiological model of learning and memory, might be an important factor contributing to diabetes-induced cognitive impairment ([16,24,25], but see Ref. [26] for discrepant findings). However, it is not clear yet whether cognitive and LTP defects are also associated with the development of T2D in animal models of this disease. To investigate this possibility, we studied hippocampal-dependent learning task and LTP formation in Zucker Diabetic Fatty (ZDF) rats, an animal model presenting the two main characteristics of T2D, i.e., development of hyperglycemia and hyperinsulinemia.

2. Methods

In Experiment 1 (Exp 1), nine ZDF rats and nine Zucker rats were tested in the Morris water maze. Testing began 5 weeks after ZDF rats presented clear signs of T2D symptomatology (blood glucose levels [BGLs]≥15.0 mmol/l, see Refs. [2,23,24]). In Experiment 2 (Exp 2), 27 ZDF rats and 27 Zucker rats were also evaluated in the Morris water maze. However, rats were tested after a longer exposure to diabetes as testing began after 8 weeks of untreated diabetes. In both experiments, diabetic rats and their controls were age-matched.

2.1. Subjects

All rats were acquired from Genetic Models International (Indianapolis, IN) [27]. For the entire duration of the experiment, the rats were housed on wood chips in group cages and were maintained under a 12:12-h-light–dark cycle. All rats were given continuous access to food and water. They were manipulated and tested during the light cycle of the day. Body weight and blood glucose concentrations were assessed weekly. Concentration of glucose was measured with a glucometer (Elite, Bayer), and blood was obtained by performing a small incision at the tail end. The first incision was made under light anaesthesia (Aeranne), whereas, in the following weeks, the experimenter gently scratched the scar to obtain the blood sample. At the end of both experiments, insulin level was evaluated by radioimmunoassay (Linco Research, St. Charles, MO). In Experiment 2, we notice that 13 of the 27 diabetic rats showed significant signs of cataract (a small to large white dot appearing in one or both of their eyes). Considering that navigation in the task is based on external visual cues, these rats, as well as their matched controls, were excluded from the analysis. The exclusion of these animals was also based on how they performed on the above water platform trials of the water maze (see below for a description). Indeed, all of them expressed impaired navigation even when salient visual cues were provided close to the platform itself.

2.2. Morris water maze

The water maze was similar to the one used in previous investigations on diabetic animals [16,23]. The maze was located in a room (292×359×252 cm) illuminated by white fluorescents situated right above the pool. The maze consisted of a white circular plastic pool (diameter=210 cm, height=50 cm), which was filled with opaque whitened water using nontoxic white gouache. The water temperature was maintained at 25 °C±1 °C, a condition sufficiently aversive to induce escape behavior from the water. The only alternative offered to the animal was a transparent platform on which the rat may stand. The platform was rectangular (15×10 cm) and was 29-cm high with respect to the bottom of the tank. A concrete block maintained the platform in place.

The maze was divided into quadrants of equal dimensions, in the center of which a mark was made to ensure proper placement of the platform. The marks were placed 55 cm from the edge of the pool. They were identified with a number from one to four: Position 1 was assigned to the northwest quadrant, 2 was the northeast quadrant, 3 was the southeast quadrant, and 4 was the southwest quadrant (see Fig. 1). The starting positions corresponded to the four
cardinal points (north, south, east, and west). A mark was placed on the edge of the pool to ensure that rats were dropped at the exact same position.

Experimental sessions were captured by a video camera (Computar FC-62C) placed above the maze. Trials were recorded (Panasonic AG-RT600A) and then fed to a detection system (HVS Image) which allowed to track the navigation path and quantify several parameters. The data were computed by the Water 2020 software configured on a PC-compatible computer (Seanix Pentium III, 600 MHz). All instruments were located in the same room as the maze.

2.3. Procedure

Learning and memory of the platform position were assessed through 15 consecutive sessions (1 session/day). In the first 10 sessions, the platform was submerged (1 cm below the surface of the opaque water), whereas, in the last five sessions, the tip of the platform was above the water line. When the platform was submerged, the rat had to position himself using external visual cues in order to reach it and to escape from the water (Fig. 2). The starting position varied from trial to trial. In the first five sessions, which we designate as Invisible 1 (I1), the platform was placed in Position 1. In the following five sessions, which was designated as Invisible 2 (I2), the platform was moved to Position 3. The location transfer of the platform between Sessions 5 and 6 was performed to assess whether rats learned to find the platform on the basis of external visual cues or on cues given by the submerged platform. Sessions with the Visible (V) platform allowed us to assess the presence of visual and/or motor deficits, which could explain impaired navigation behavior when the platform was submerged. In this condition, the platform emerged 5 mm above the water line and was surrounded by a 5-mm-wide black tape. During these visible sessions, the platform was returned to Position 1. In Exp 2, Positions 2 and 4 were used as platform locations in I and V trials.

In each of these 15 sessions, rats were given eight trials. At the beginning of a trial, the rat was randomly and gently dropped in the water at one of the four starting positions (north, south, east, and west). For each trial, the rat had 90 s to escape from the water by climbing on the platform. In case the rat did not find the platform within the allotted time, the experimenter placed it gently on the platform. Once out of the water, the rat remained on the platform for 15 s. Between trials, the rat was placed in a heated cage for another 15 s.

2.4. Hippocampal slice preparation and electrophysiology

The technique for preparing hippocampal slices was identical to the methods previously described by Kramar and Lynch [28]. Briefly, male control and ZDF rats from Exp 2 (5–6 months old) were anaesthetized with isoflurane and decapitated. The brain was quickly removed and placed in a cold oxygenated dissection medium containing 124 mM NaCl, 3 mM KCl, 1.25 mM KH2PO4, 5 mM MgSO4, 3.4 mM CaCl2, 26 mM NaHCO3, and 10 mM glucose. Transverse hippocampal slices (370-μm thick) from the middle third of the septo–temporal axis of the hippocampus were prepared using a McIlwain tissue chopper and placed on a nylon mesh at a liquid–gas interface in a recording chamber maintained at 34 °C; a humidified gas mixture (95% O2/5% CO2) was applied to the chamber at a constant flow rate of 165 ml/min. Slices were maintained in a preheated artificial cerebrospinal fluid (aCSF) consisting of 124 mM NaCl, 3 mM KCl, 1.25 mM KH2PO4, 2.5 mM MgSO4, 3.4 mM CaCl2, 26 mM NaHCO3, and 10 mM glucose. Recordings began following at least 1 h of incubation.

In all experiments, field excitatory postsynaptic potentials (fEPSPs) were recorded from stratum radiatum of CA1b using a single pipette filled with 2 M NaCl (with a resistance of 1–5 MU”) in response to orthodromic stimulation (twisted nichrome wires, 65 μm) of the Schaffer collateral–commissural projections in CA1 stratum radia-
tum. Pulses were delivered to the stimulation electrode at 0.033 Hz, with current test intensity adjusted to obtain 50–60% of the maximal fEPSP. LTP was generated by applying theta burst stimulation (TBS; 10 bursts of four stimulation pulses at 100 Hz), which were given at 200-ms intervals. fEPSP were digitized and analyzed using NAC 2.0 system (Theta Burst, Irvine, CA). In most cases, the slope of evoked fEPSPs was normalized to the mean fEPSP measured during the baseline recording period. These values are reported as percent changes from baseline and are given in the text as means ± S.E.

### 2.5. Data analysis

The navigation variables that were analyzed consisted of Swim Speed, Time Latency to reach the platform, and

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Age at the end (day)</th>
<th>Weight at the end (g)</th>
<th>Glycaemia (mmol/l)</th>
<th>Insulin at the end (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beginning</td>
<td>End</td>
<td>Beginning</td>
<td>End</td>
</tr>
<tr>
<td>Exp 1 ZDF (n=9)</td>
<td>184.02±9.44</td>
<td>420.11±48.18</td>
<td>8.11±2.39</td>
<td>24.51±8.08</td>
</tr>
<tr>
<td>Control (n=9)</td>
<td>184.02±9.44</td>
<td>397.45±33.91</td>
<td>5.67±0.32</td>
<td>6.0±0.42</td>
</tr>
<tr>
<td>Exp 2 ZDF (n=14)</td>
<td>198.54±11.67</td>
<td>443.12±26.89</td>
<td>7.87±1.35</td>
<td>25.00±3.80</td>
</tr>
<tr>
<td>Control (n=14)</td>
<td>198.54±11.67</td>
<td>401.54±41.83</td>
<td>5.64±0.32</td>
<td>5.39±0.45</td>
</tr>
</tbody>
</table>

* Difference between ZDF and control rats was found to be significant (t-test, p<0.01).
Navigation Distance. The Time Latency and Navigation Distance variables reflect the integrity of the hippocampus as memory for the location of the platform should translate in shorter latency and path distance. On the other hand, the Speed variable reflects the perceptive and motor capacities of the rats [29] and should not vary dramatically according to weight [30].

For each variable, we calculated the average of the eight trials in each of the 15 sessions. Then, we proceeded with a Group (ZDF or Zucker)×Session (1 to 5) analysis of variance (ANOVA), with repeated measures on the Session variable. When the interaction was deemed significant, we followed with an analysis of simple main effects as well as Tukey a posteriori mean comparisons tests when necessary (HSD, p<0.05). One set of analyses was run for each type of trial (I1, I2, and V).

In order to evaluate whether the relocation of the platform between Sessions 5 and 6 influenced the navigation behavior of the animals in Exp 1, we compared the Latency and Distance variables on the very last trial of I1 (Session 5, Trial 8) to what was observed on the first trial of I2 (Session 6, Trial 1). In Exp 2, we added a trial at the very end of the I1 sessions, in which the platform was withdrawn from the pool. The rats’ navigation paths were recorded during 90 s. This probe test is another means to evaluate whether learning and memory of the platform depended on allocentric processing. For this trial, we considered the proportion of time spent in Quadrant 2, which should, if appropriate learning took place, be superior to the time spent in the other three quadrants.

3. Results

3.1. Biochemical analyses

In Exp 1 and 2, upon their arrival at the laboratory, the average blood glucose of the ZDF rats was significantly higher than in control Zucker rats (Table 1). Blood glucose increased significantly during the experiment in ZDF rats only. ZDF rats were also significantly heavier than the controls. As stated earlier, we measured plasma insulin concentration in both groups of rats after sacrifice. We found that ZDF rats from both experiments showed a higher concentration of insulin plasma than the Zucker rats at sacrifice.

3.2. Analysis of the navigation behavior: Experiment 1

3.2.1. I1 sessions

The results of the Group (ZDF vs. Zucker)×Session (1 to 5) ANOVA applied on Speed yielded a significant Group effect, indicating that ZDF rats were significantly slower than the Zucker rats [F(1, 16)=5.95, p<0.05] (Fig. 3). The analysis also yielded a Session effect which indicated that speed increased over the course of the first five testing sessions [F(4, 16)=35.24, p<0.01]. The analysis revealed also a significant Group×Session interaction [F(4, 68)=7.97, p<0.01]. Analysis of simple main effects indicated that, on Sessions 3 to 5, ZDF rats were significantly slower than control rats [Session 3: F(1, 16)=8.49, p<0.05; Session 4: F(1, 16)=10.16, p<0.01; and Session 5: F(1, 16)=21.52, p<0.01].

The ANOVAs computed on Latency and Distance yielded a similar pattern of results. The two analyses indicated that there was no Group effect, but that the Session effect [Latency: F(1, 16)=22.00, p<0.01 and
Distance: $F(1, 16)=32.75, p<0.01$ and the Group×Session interaction reached significance [Latency: $F(4, 16)=2.54, p<0.05$ and Distance: $F(4, 16)=3.11, p<0.05$]. Surprisingly, ZDF rats had a shorter time latency and navigation path on the first test session, a difference that vanished on the other sessions [Time: $F(1, 16)=6.35, p<0.05$ and Distance: $F(1, 16)=10.87, p<0.05$].

### 3.2.2. I2 sessions

Again, the ANOVA applied on the Speed variable indicated that ZDF rats were significantly slower than the Zucker rats. The Session effect also reached significance [$F(4, 16)=50.12, p<0.01$] and so did the Group×Session interaction [$F(4, 68)=7.50, p<0.01$]. In fact, ZDF rats were significantly slower than the Zucker rats on Sessions 1 [$F(1, 16)=11.32, p<0.01$], 2 [$F(1, 16)=14.66, p<0.01$], and 5 [$F(1, 16)=2.56, p<0.05$].

The ANOVAs performed on the Latency and Distance variables both indicated that only the Session effect was statistically reliable [Latency: $F(4, 16)=3.15, p<0.05$ and Distance: $F(4, 16)=3.51, p<0.05$], and only the first session differed significantly from the others for both parameters, as demonstrated by Tukey post hoc comparisons. On the first session of testing, all rats covered more distance and needed more time to reach the platform.

We also examined how the position transfer of the platform between Sessions 5 and 6 influenced navigation behavior. The analysis compared navigation parameters on...
two trials (before transfer and after transfer) and revealed that there was no Group effect but a significant difference between trials for Latency \[ F(1, 16)=5.64, p<0.05 \] and Distance \[ F(1, 16)=7.54, p<0.05 \] variables. When the platform was moved, rats took more time and navigated more to find it [last trial of I1 (Latency: \( M=3.92\pm 0.16 \) s and Distance: \( M=72.95\pm 70.94 \) cm); first trial of I2 (Latency: \( M=16.28\pm 19.42 \) s and Distance: \( M=342.56\pm 371.05 \) cm)].

3.2.3. V sessions

The ANOVAs indicated that there was a significant Session effect \[ F(4, 16)=19.32, p<0.01 \] and a Group effect \[ F(1, 16)=11.22, p<0.01 \] when speed was considered, meaning that both groups learned the task progressively and also that ZDF rats were slower overall than control Zucker rats (Fig. 4).

The analysis of Time Latency also yielded significant Session \[ F(4, 16)=30.32, p<0.01 \] and Group effects \[ F(1, 16)=10.00, p<0.01 \], indicating that ZDF rats took more time to reach the visible platform than the Zuckers. The ANOVA applied on the distance variable also yielded a significant Session effect \[ F(4, 16)=41.04, p<0.01 \], all rats covering less distance to reach the platform on Sessions 2 and 3, and a Group effect \[ F(1, 16)=6.32, p<0.05 \], ZDF rats covering more distance to reach the platform than the Zucker rats.

3.3. Analysis of the navigation behavior: Experiment 2

3.3.1. I1 sessions

The ANOVA computed on Speed indicates that the two groups differed significantly \[ F(1, 24)=49.87, p<0.01 \], diabetic rats being slower than controls (Fig. 5). The analysis also revealed a Session effect \[ F(4, 24)=27.20, p<0.01 \], which confirmed that rats were gradually faster to reach the platform as testing unfolded.

The ANOVA performed on Latency revealed that only the difference between sessions was reliable \[ F(4, 24)=57.36, p<0.01 \]. More precisely, there was a significant decrease in Sessions 2 and 3. Identical results were obtained for the Navigation Distance variable [Session: \( F(4, 24)=74.90, p<0.01 \)], indicating that both groups of rats learned to locate the platform.

3.3.2. I2 sessions

The set of analyses that we performed on these sessions yielded results that were identical to the first five sessions. Once again, a group difference was observed on the Speed variable \[ F(1, 24)=19.87, p<0.01 \], ZDF rats being slower than the control Zucker rats. There was improvement over the five testing sessions for all three dependent variables: Speed \[ F(4, 24)=33.87, p<0.01 \], Latency \[ F(4, 24)=22.43, p<0.01 \], and Distance \[ F(4, 24)=25.93, p<0.01 \]. No significant Group×Session interactions were observed.

As we expected, the analysis performed on the probe test, in which the platform was withdrawn from the water for one trial after completion of Session 5 of I1 trials, indicated that all rats searched for the platform in Quadrant 2 (\( M=43.87\pm 13.22 \% \)) more than they did in any of the other quadrant \[ M=15.41\pm 4.31 \%, F(1, 24)=96.04, p<0.01 \]. This result strongly suggests that the navigation towards the platform was based on allocentric cues. There was no difference between the two groups.
3.3.3. V sessions

The analysis applied on Speed revealed a Group effect \([F(1, 24)=49.96, p<0.01]\), once again, ZDF rats being slower than the Zucker rats (Fig. 6). The analysis also yielded a significant Session effect \([F(4, 24)=43.25, p<0.01]\), but the interaction was not deemed significant.

The ANOVA performed on Latency also indicated that ZDF rats took significantly more time to reach the platform than control animals \([F(1, 24)=10.54, p<0.01]\). There was also a significant difference between testing sessions as search time decreased significantly across sessions \([F(4, 24)=55.93, p<0.01]\). The ANOVA computed on the Navigation Distance variable to reach the platform indicated that the Session effect was the only one deemed significant \([F(4, 24)=56.27, p<0.01]\), meaning that all the rats performed better across testing sessions and that the Group effect observed on the Latency variable was caused by lower swimming speed in ZDF rats.

3.4. Hippocampal physiology in ZDF rats

It is well documented that Type 1 diabetes mellitus in rodents is associated with damages to synaptic transmission in the hippocampal formation. Hippocampal slices from control and diabetic rats were tested for changes in synaptic function at the end of Exp 2. We first conducted experiments in which we analyzed synaptic transmission evoked at different stimulus intensities in area CA1 of the hippocampus. As shown in Fig. 7a, the plots of fEPSP amplitude did not differ significantly between slices from control and ZDF rats \((n=7)\) when stimulus intensity was varied between 2 and 30 \(\mu\)A. To further evaluate the impact of Type 2 diabetes on brain function, hippocampal slices were also tested for LTP of synaptic transmission. Hippocampal slices prepared from control and diabetic animals were perfused for 1 h with aCSF before applying TBS to the Schaffer collateral–commissural projections in area CA1 of the hippocampus. Evoked responses were tested at 30-s intervals for at least 10 min prior to TBS and again for at least 70 min after TBS. Four evoked responses were averaged 1 and 60 min after TBS to determine possible effects on posttetanic potentiation (PTP) and LTP, respectively. As shown in Fig. 7b, ZDF rats exhibited almost the same increase in fEPSP slope at 1 min post-TBS as the controls (84 ± 9% for controls vs. 80 ± 7% for ZDF rats; \(n=7\)). Furthermore, there was no significant decrease in the magnitude of LTP in area CA1 of hippocampal slices prepared from the diabetes rats (54 ± 7%) when compared to controls (61 ± 5%; \(n=7\)). Paired-pulse facilitation, a phenomenon known to be sensitive to perturbations in neurotransmitter release probability, was not modified in ZDF rats at intervals of 20, 50, 75, 100, 150, 200, 300, and 400 ms (data not shown). From an electrophysiological perspective, these data suggest that synaptic transmission and plasticity are not substantially altered in ZDF rats.

Fig. 7. Electrophysiological properties of hippocampal slices in control and ZDF rats. (a) The graph shows the fEPSP amplitude evoked at different stimulus intensities. Note that synaptic transmission in area CA1 of hippocampal slices was not substantially altered in ZDF rats when compared to controls. Results are means±S.E. of seven separate experiments. (b) LTP responses of ZDF and control rats tested in Experiment 2. The degree of LTP in area CA1 of hippocampal slices (initial slope of response measure) was expressed as a function of time for responses recorded before and after theta burst stimulation (arrow). As shown, application of theta burst stimulation in control rats (●) produced an initial potentiation which after 10 min decayed to a long-lasting and stable 60% potentiation. The same stimulation resulted in an initial potentiation and stable LTP in ZDF rats (○). Results are means±S.E. of seven separate experiments. Representative field responses presented above the graph were taken 2 min before (1) and 60 min after (2) theta burst stimulation. The two responses were then superimposed. Calibration was as follows: 5 ms, 1 mV.
4. Discussion

The main goal of this study consisted in evaluating the effect of T2D on memory and learning processes relying on the integrity of the hippocampus through an allocentric-based navigation task. In two experiments, our results convincingly showed that the metabolic condition resulting from T2D, as expressed in ZDF rats, had no significant influence on the rats’ navigation behaviors in the Morris water maze. Diabetic animals and control rats presented similar learning curves in the two conditions when the platform was submerged, a condition that requested memory of the platform location based on allocentric processing of external visual cues. This finding was also confirmed by rats’ navigation paths when the platform was moved to a new location in Exp 1 and by the probe test in Exp 2. On the transfer trial, rats took more time to find the platform, whereas, in the probe test, rats concentrated their search in the quadrant where the platform was located in the previous sessions. The only difference that was observed recurrently between the ZDF and control rats concerns their swimming speed. Zucker rats swam faster than the ZDF rats. However, differences in swimming speed were not large enough to influence navigation behavior as no difference on search latency as well as on distance was observed when the platform was submerged.

The present results indicate that, in spite of important metabolic and endocrine impacts caused by T2D [8] in ZDF rats, the learning and memory deficits, as well as decreased LTP associated with hippocampal impairment, were not observed after 5 weeks or even after 8 weeks of untreated T2D in ZDF rats. Considering that testing took 3 weeks to complete, meaning we studied the effect of T2D between Week 5 and Week 11 (if we combine the two experiments), our observations are quite different from what was observed in T1D animal models. In the later case, LTP alterations were observed after 6–8 weeks of untreated diabetes [24]. The present findings speak against our initial hypothesis and contrast with the well-known effects on the brain, learning, and memory caused by T1D in animal models [2,16,23,24]. Indeed, numerous studies have concluded that T1D significantly alters the central nervous system (CNS) both globally (cortical atrophy [31]) and specifically (alteration of LTP and AMPA glutamate receptors within the hippocampus [16,24]). The present observation that basal synaptic transmission and LTP are not impaired in ZDF hippocampal slices cannot yet totally exclude the possibility of functional alterations in this animal model of diabetes. Indeed, one could argue that longer exposure to standard aCSF made the ZDF slices more like the controls, which certainly warrants further studies to determine how in vivo LTP might be influenced in ZDF rats. On the other hand, the results do not rule out the possibility that other parameters of synaptic transmission, such as excitability of presynaptic axons, could be altered in the ZDF model. In future investigations, it will be interesting to examine such changes in axonal excitability by conducting experiments using CNQX to block postsynaptic AMPA receptor-mediated synaptic currents and picrotoxin to block feed-forward GABAergic responses. Responses recorded under these conditions have a very short latency and do not exhibit paired-pulse facilitation, characteristics expected of composite action potentials uncontaminated by synaptic currents.

Before reaching the conclusion that T2D, as expressed in ZDF rats, in itself does not induce notable cognitive changes, some alternative hypotheses need to be considered. The first and primary interpretation concerns the hyperinsulenic condition of ZDF rats. Interestingly, in both experiments, we noticed that ZDF rats manifested hyperinsulinemia, even after 11 weeks (which included 8 weeks of untreated diabetes before testing in addition to the 3 weeks necessary to perform testing) of elevated BGLs. Others observed hyperinsulinemia after 24 weeks of untreated diabetes in ZDF animals [32]. The initial and most prevalent phase of T2D is characterized by an important increase of insulin secretion in order to maintain homeostasis within the organism. The impact of hyperinsulinemia on the organism, and more specifically on the central nervous system, is still a matter of debate [16]. Insulin receptors were found in the brain, and it is also known that insulin can cross the blood brain barrier [33,34]. Moreover, when applied locally, insulin appears to have various effects on hippocampal pyramidal neurons [35]. From a behavioral perspective, Zhao et al. [36] noticed a significant concentration increase of insulin receptors after rats learned to find a platform in the Morris water maze. The hypothesis that hyperinsulinemia could play a protective role in ZDF rats by preventing neuronal degeneration receives indirect support from an examination of the autonomic nervous system in ZDF rats. Indeed, Schmidt et al. [32] observed that ZDF rats examined after 6 months of untreated diabetes did not show neuroaxonal dystrophy, a common nerve deterioration of the sympathetic autonomic system associated with this disease. In contrast, streptozocin (STZ)-induced diabetes in rats, which does not show the hyperinsulinemia condition, presented severe neuropathy. They proposed that insulin and/or insulin growth factor (IGF-I) could play a very important neurotrophic function and be responsible for the preservation of this system. More interestingly, they went on to suggest that ZDF rats could represent a valuable model of increased systemic insulin. Their striking finding fits quite nicely with recent observations regarding the involvement of IGF-I in the neurogenesis of hippocampal cells, which suggests that the presence of insulin, and for that matter IGF-I, could have a significant impact on the brain [37]. Additional support comes from findings indicating that reinstatement of insulin level partially compensates for the cognitive impairment caused by T1D [16,38–40]. Moreover, a study conducted by Biessels et al. [23] revealed that LTP in a T1D animal model was reestablished, at least partially, after an insulin treatment. However, this interpretation goes against findings in
the human literature suggesting that hyperinsulinemia could induce cognitive impairment [41]. Although this is all rather speculative, insulin (and/or IGF-I) appears to have a strong influence on the nervous system. For instance, it has been suggested that, in T1D, lower insulin secretion could have detrimental effects on the nervous system other than those resulting from hyperglycaemia [32]. At this stage, it remains to be determined how the central nervous system is altered by insulin, how and to what level it mediates cognitive functions such as memory, and specifically what metabolic context induces such conditions. To this regard, a thorough comparative study of various animal models of diabetes is requested.

A second interpretation associates the presence of cognitive deficits caused by T2D with old age [2]. This finding is supported by the negative influences of both old age and the metabolic changes on cognition [1–3]. Although rather mild up to 70 years of age, the cognitive deficits associated with T2D become more pronounced in the eighth and ninth decades [1]. In line with this interpretation, U’Ren et al. [42] have demonstrated that diabetes accelerates the appearance of cognitive deficits usually caused by normal ageing. They even observed a negative influence of T2D on short-term memory in an elderly population, a form of memory known to be relatively intact in older adults. These observations have been corroborated with a sample of older individuals expressing a weak tolerance to glucose without being diagnosed with diabetes [36]. Indeed, the authors noticed short-term memory deficit as well as atrophy of the hippocampus in their elderly sample. As mentioned previously, the array of medical conditions that are associated with T2D and old age could contribute to the alteration of cognitive functioning. This hypothesis also receives supports from the results of Gispen and Biessels [16] who found that aged rats suffering from T1D (STZ-induced diabetes) presented accentuated learning and memory deficits. Nevertheless, the age interaction interpretation, as an explanation of ZDF rats’ intact performance on the Morris water maze, does not appear as the primary interpretation because of the confounding effect of hyperinsulinemia.

One could also suggest that learning and memory impairments were not noticed in this experiment because ZDF rats were not exposed long enough to the negative influences of T2D. To our defence, it is important to highlight the fact that our ZDF rats presented a very high BGLs as well as some symptoms indicative of the harmfulness of untreated T2D. The first observation concerns the visual system of the ZDF rats after 8 weeks (Exp 2) of untreated diabetes. Almost half of the sample had to be excluded from the study because of the presence of cataract in one or both eyes. This condition prevented the animals from appropriately perceiving the surrounding external cues that are necessary to locate the submerged platform. When tested in the visible platform condition, their performance (time latency and distance traveled) was at least two-standard-deviations below the average. They searched rather randomly for the location of the platform, whereas the other rats followed a straight path to the platform. Their poor vision also explains why these rats could not learn the task when tested with the submerged platform. Because of their condition, it was impossible for us to tell whether this subgroup of rats expressed learning and memory deficits on top of their peripheral problems as navigation in the Morris water maze based on allocentric cues relies on intact vision. We believed that, if our ZDF rats sample would have been exposed to additional days of untreated diabetes, their condition would have deteriorated in such a way that it would have been impossible to test them using the Morris water maze. Based on an earlier report [32], ZDF rats show hyperinsulinemia even after 6 months of untreated diabetes. Thus, even if we had tested our animals after a longer exposure to diabetes, it would have been impossible to discard the hyperinsulinemia hypothesis.

As mentioned above, we observed that ZDF rats showed slower swimming speed. This finding clearly indicates that impaired motor functions did not influence learning on the Morris water maze task and needs to be dissociated from learning and memory mechanisms. Indeed, slower speed was not sufficient to influence the results on the other two variables. The slower speed of ZDF rats might be potentially explained by their overweight condition. However, this is unlikely inasmuch as others have observed that obese rats swim as fast as lean control rats [30]. Indeed, weight surplus is constituted of lipids in ZDF rats, which should facilitate floating. Their slower speed seems to depend more on the alteration of motor capacities. In fact, a study from Frisbee and Stepp [43] showed that the muscular tissues of ZDF rats deteriorate faster in comparison to control rats. Nevertheless, their altered muscular condition had little effect on their learning and memory capacities, as assessed with the water maze.

In conclusion, our results showed preservation of the processes underlying learning and memory in a spatial navigation task in ZDF rats exposed to 5 or 8 weeks of untreated T2D. Considering that other physiological symptoms of diabetes are typically observed in ZDF rats [44], we propose that the integrity of the CNS, and especially the hippocampal functions, may be maintained by their hyperinsulinemic condition. Increased levels of insulin and/or IGF-I may retard the apparition of CNS side effects caused by T2D in this animal model [16]. Yet we cannot rule out the possibility that the effect of T2D on CNS, learning, and memory might only appear in conjunction with the negative influences of old age.

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