J. Afr. Ass. Physiol. Sci. 4 (1): 41-47, July 2016

Journal of African Association of Physiological Sciences

Official Publication of the African Association of Physiological Sciences

http://www.jaaps.aapsnet.org

Research Article

Outcome of sub-acute insulin administration on long-term visuo-spatial and short-term working memory in mice

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Keywords:
insulin, long-term visuo-spatial memory, short-term spatial memory, working memory, sub-acute treatment

ABSTRACT

Background: In the past, insulin was considered a peripheral hormone, unable to affect the central nervous system. Now, it is well established that insulin occurs in the brain where it exerts regulatory and trophic effects. This study was undertaken to determine the effect of sub-acute insulin administration on long-term visuo-spatial and short-term working memory. **Methods:** Twenty four mice, weighing between 18 – 22 g, were used. Two groups of six mice each were used during elevated plus maze and Y-maze, to determine long-term visuo-spatial and short-term spatial working memory, respectively. Control group received deionized water, while insulin group received insulin at 10 I.U./kg/day, subcutaneously. **Results:** In the elevated plus maze, acquisition and retention latencies were the same ($P > 0.05$) when compared between the groups. In the Y-maze test, number of entries into arms was similar ($P > 0.05$) within and between groups. Time spent in the novel arm by mice in the insulin (103.83 ±7.4 seconds) and control (108.00 ± 13.6 seconds) groups was higher ($P < 0.05$) when compared to time spent in arm A (68.33 ± 10.0 and 74.50 ± 5.6 seconds, respectively) and B (59.17 ± 9.5 and 69.67 ± 10.7, respectively). Number of triads and percent alternations were also the same ($P > 0.05$) when compared between the groups. **Conclusion:** It was concluded, that sub-acute insulin administration did not affect long-term visuo-spatial memory and short-term working memory in mice.

INTRODUCTION

In the past, insulin was considered only as a peripheral hormone, unable to cross the blood-brain barrier (Laron, 2009). Now, it is well established that insulin occurs in the brain where it exerts regulatory and trophic effects (Blázquez *et al*., 2014). Specifically, insulin has been reported to affect cognition (Ghasemi *et al*., 2013). Although researches have been undertaking to investigate the effect of insulin on learning and memory, the reported findings are often conflicting (Moosavi *et al*., 2007a; McNay *et al*., 2010). In addition, there is dearth of information on the effect of sub-acute insulin administration on the various forms of learning and memory. Thus, this study was undertaken to determine the effect of sub-acute insulin administration on long-term visuo-spatial learning and memory using elevated plus maze (EPM); and short-term working memory using Y-maze (YM).

METHODS

Animal Conditions, Grouping and Treatment

Young, 34 – 42 days old mice of both sexes, weighing between 18 – 22 g, were used for the study. They were kept in large cages in the Animal House and allowed free access to feed and drinking water. They were maintained under the prevailing natural light-dark cycle (photophase: 6:22 – 18:11). Experimental protocols

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were approved by local Institutional Research Committee and were in accordance with the guidelines for animal research, as stated in the NIH Guidelines for the Care and Use of Laboratory Animals (National Academy of Sciences and National Institutes of Health Publications, 2011).

Insulin (Actrapid®, Solution for injection in vial, human, ATC code: A10AB01, Novo Nordisk A/S, Denmark) was reconstituted 1:3 with deionized water for ease of dosing; and administered for seven days (Francis *et al*., 2008) using insulin syringe daily between the hours of 8:00 – 9:00 am. Actrapid® is fast acting insulin that is immediately and slowly absorbed upon subcutaneous injection, and maximum plasma concentration is reached within 1.5 hours (Product Information, 2011). This was considered in timing of the neurobehavioural tests to ensure adequate blood concentrations during the trials. Two groups of six mice each (n = 6) were used during each of the two neurobehavioural paradigms. The mice were treated as follows: mice in the control group received deionized water, while those in the insulin group received insulin at 10 I.U./kg/day, subcutaneously (Sharma *et al*., 2007). Behavioural tests were commenced 30 minutes and 1 hour 30 minutes after the last insulin injection for Y-maze and elevated plus maze tests, respectively.

Assessment of long-term visuo-spatial memory using elevated plus maze.

An elevated plus-maze test was conducted as described by Komada *et al*., (2008). Briefly, the elevated plus-maze consisted of two open arms (25 × 5 cm) and two enclosed arms of the same size with 15-cm high wooden walls. The arms and central square were made of wood, elevated 55 cm above the floor. Each mouse was placed at the distal part of an open arm maze (5 × 5cm), facing away from the closed arms. On the training day (first day), each animal was placed at the end of one open arm, facing away from the central platform. The latency of the mouse to move from the open to the enclosed arms was recorded within 90 seconds. Following entry into the arm, the animals were allowed to explore the apparatus for 20 seconds. Twenty-four hours later, the second trial (retention test) was performed and the animals were observed for 90 seconds. Reduction in latencies between day one (acquisition) and day two (retention) indicates memory of the learned task. After each trial, the maze was wiped with a cloth dipped in 70% ethanol, and allowed to dry to remove any olfactory cue. An overhead video camera recorded movement of the mice for later quantification.

Assessment of Short-term Spatial Working Memory using Y-Maze

The test was conducted as described by Wright *et al.* (2006), and slightly modified. Briefly, the symmetrical Y-maze, as developed by Dellu *et al.* (1992), was used to assess hippocampal-dependent spatial recognition memory. The Y-maze consisted of three identical wooden arms (50 cm L, 16 cm W, 32 cm H) with multiple extra-maze cues (Conrad *et al*., 1996), located around the perimeter of the maze. The maze was rotated between training and testing. Hence, the arms, termed Novel, Start and Other, referred to the location of the arm in the room and not the actual arm. An overhead video camera recorded movement of the mice for later quantification, and the investigator stood in white coat in the same position during training and testing. Mice were tested on the Y-maze with a 1 hour delay between training and testing. Y-maze navigation relies upon a mouse's innate tendency to explore novel environments (Ennaceur and Delacour, 1988). In the present experiments mice that recognized and choose the Novel arm more than the other arms were defined as having intact spatial working memory, whereas those that entered the Novel and Other arms similarly were considered to have impaired spatial working memory.

During training, one arm (Novel) of the Y-maze was blocked with a shutter, allowing the mice to explore the Start and Other arms for 15 min. The shutter that blocked the novel arm was the height of the arms (32 cm), preventing mice from rearing and seeing into the novel arm or viewing the spatial cues, visible only from the novel arm. Following training, mice were returned to their home chambers during the 1 hour delay. After the delay, the shutter was removed, and mice were placed in the same Start arm, and allowed to explore the Y-maze for 5 min. Each mouse was given one trial during training and testing. A blind investigator unaware of the treatment groups determined the number of entries made into, and time spent (dwell) by each mouse in the Novel (arm C), Start (arm A), and Other (arm B) arm across all five minutes. An entry was counted, when the forearms of the mouse entered the arm. The number and the sequence of arms entered were also recorded. The parameters were activity, defined as the number of arms entered, and percent alternation, calculated as the number of alternations or triads (entries into three different arms consecutively) divided by the total possible alternations (i.e., the total number of arms entered minus 2) and multiplied by 100 (Sarnyai *et al*., 2000).

Statistical Analyses

All data were collated and analyzed using Statistical Package for the Social Sciences (SPSS) version 20.0. General Linear Model-repeated measures ANOVA was used to compare means of day 1 and day 2 latencies (EPM), time spent in and number of entries into arms (YM). Independent samples t-test was used to compare mean values of number of triads and percent alternations (YM). Bonferroni test was employed for

post-hoc multiple comparisons. Values of P < 0.05 were considered significant.

RESULTS

Assessment of Long-term Visio-spatial Memory using Elevated plus Maze for Memory

Acquisition and retention latencies

Acquisition latencies (seconds) were 39.13 ± 5.05 and 40.63 ± 5.79 for the control and insulin groups; while retention latencies (seconds) were 41.50 ± 9.77 and 56.00 ± 9.77 for the two groups, respectively. There was no significant difference between acquisition and retention latencies within each of the insulin-treated and control groups (Wilks' lambda = 0.928, $F_{(1, 15)}$ = 1.081, P = 0.316, multivariate partial Eta squared [Eta^2] = 0.072, n = 6) (Figure 1). The fact that the latencies did not increase or decrease between day 1 and day 2 suggests that there was no impairment or improvement of memory. There was also no significant difference in the latencies between the two groups ($F_{(1, 15)}$ = 1.069, P = 0.319, Eta^2 = 0.071, n = 6), indicating that insulin treatment had no significant effect on the treated animals.

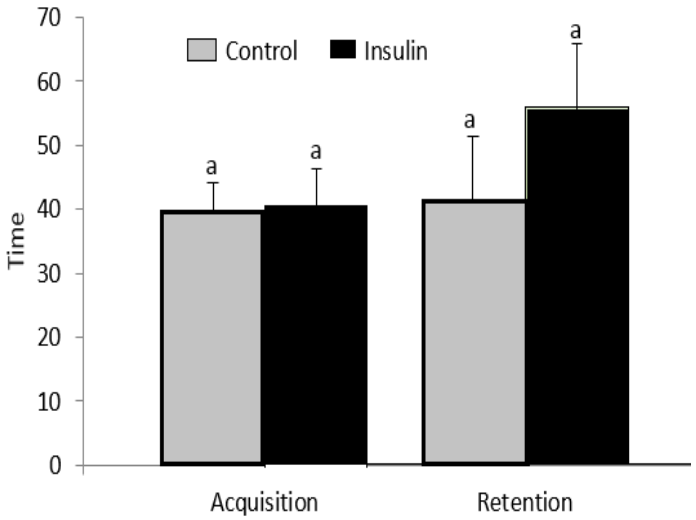

Figure 1: Acquisition and retention latencies (seconds) of control and insulin-treated mice during a 2-day elevated plus maze for memory task. [a] = Columns with the same superscript letters are not significantly different (P > 0.05). (Mean ± S.E.M, n = 6)

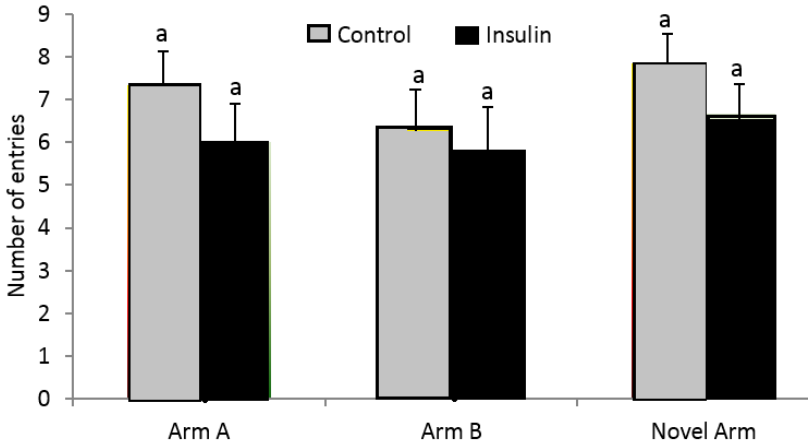

Fig. 2: Number of entries into each arm by control and insulin-treated mice during a Y maze task. [a] = Columns with the same superscript letters are not significantly different (P > 0.05). (Mean ± S.E.M, n = 6)

Assessment of Short-term Working Memory using Y Maze

Number of entries into each arm

Number of entries into arms A, B and C for the control and insulin groups were 7.33 ± 0.8 and 6.00 ± 0.9; 6.33 ± 0.9 and 5.83 ± 1.0; and 7.83 ± 0.7 and 6.67 ± 0.7; respectively. There was no significant difference in the number of entries into each arm within the insulin-treated and control groups (Wilks' lambda = 0.676, $F_{(2, 9)}$ = 2.159, P = 0.171, Eta^2 = 0.324, n = 6), as well as between the two groups ($F_{(2, 9)}$ = 0.915, P = 0.361, Eta^2 = 0.084, n = 6) (Figure 2). This indicates that both the insulin-treated and control mice did not show preference for the novel arm signifying inability to retain memory of exposure to the other two arms, and therefore not recognizing the novel arm.

Time spent in each arm

Time spent (seconds) in arms A, B and C for the control and insulin groups were 68.33 ± 10.0 and 74.50 ± 5.6; 59.17 ± 9.5 and 69.67 ± 10.7; and 108.00 ± 13.6 and 103.83 ±7.4; respectively. The time spent in arms within the insulin-treated and control groups differed significantly (Wilks' lambda = 0.458, $F_{(2, 9)}$ = 5.316, p = 0.030, Eta^2 = 0.542, n = 6), but not between the two groups ($F_{(2, 9)}$ = 0.832, P = 0.383, Eta^2 = 0.077, n = 6) (Figure 3). The mice in both groups spent more time in the novel arm, indicating intact memory, but the

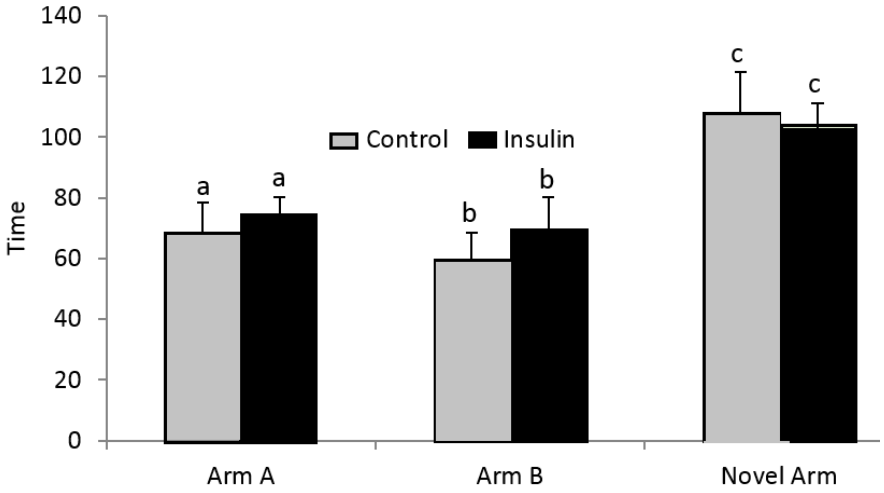

Fig. 3: Time (seconds) spent in each arm by control and insulin-treated mice during a Y maze task. [a,b,c] = $P < 0.05$. (Mean ± S.E.M, n= 6)

performance of the two groups were the same. This suggests that insulin administration had no effect on the treated mice.

Number of triads and percent alternations performed by the animals

The animals in the insulin and control groups performed 5.83 ± 0.9 and 4.50 ± 0.7 triads, respectively. Percent alternation was 29.51 ± 2.5 and 27.13 ± 1.5 for the mice in the control and insulin groups, respectively. There was no significant difference in the number of triads performed by the insulin-treated and control mice ($P = 0.435$) (Figure 4). There was also no significant difference in percent alternation performed by the animals between the groups ($P = 0.277$). The results of these parameters indicate that insulin treatment had no significant effect on the treated animals.

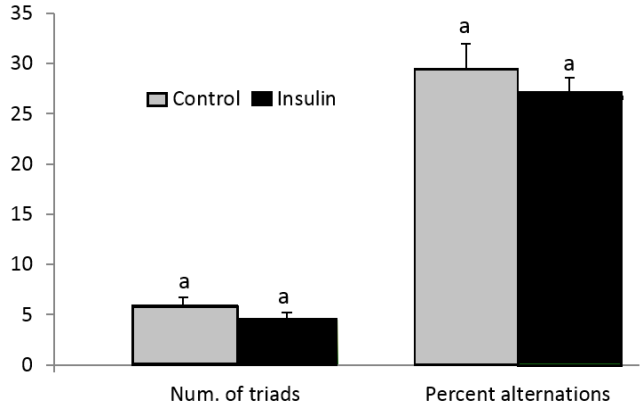

Fig. 4: Number of triads performed and percent alternations of control and insulin-treated mice during a Y maze task. [a] = Columns with the same superscript letters are not significantly different ($P > 0.05$). (Mean ± S.E.M, n = 6).

DISCUSSION

Diabetes-related cognitive impairment is one of the consequences of hyperglycaemia in type 2 diabetes (T2D) (Duarte *et al*., 2012). But hyperglycaemia in T2D and pre-diabetic states coexists concurrently with hyperinsulinaemia (Craft, 2005; WHO, 1999; Reaven, 2003). It is possible that some of the pathophysiological effects ascribed to hyperglycaemia could actually be due to hyperinsulinaemia. To resolve this, there is the need to study the effects of hyperinsulinaemia in non-diabetic normoglycaemic subjects without the interference of hyperglycaemia. Previously, hyperinsulinaemia was induced experimentally by administration of exogenous insulin (Somogyi, 1959; Patočková *et al*., 2003; Walrand *et al*., 2004; Rybicka *et al*., 2011). This experimental model was employed in the present study.

In short-term memory, a small amount of information is kept active for random and repeated access for some seconds up to a minute. It involves the ability to remember and process information at the same time (Davelaar *et al*., 2007). Working memory, assessed by Y-maze, is a form of short-term memory. It has been considered to be a core cognitive process that underpins a range of behaviors, from perception to problem solving and action control; and is closely related to measures of intelligence. It has recently been proposed that working memory might better be conceptualized as a limited resource that is distributed flexibly among all items to be maintained in memory (Wei *et al*., 2014). In the present study, 34 – 42 days old mice in the adolescent stage of life (Finlay and Darlington, 1995; Age Converter, 2016) were used for optimum learning and memory performance. The animals in insulin-

treated group showed no preference for the novel arm during the Y-maze task. This could indicate memory impairment (Ennaceur and Delacour, 1988). However, memory impairment here is unlikely because the animals in the control group also demonstrated lack of preference for the novel arm, suggesting that insulin treatment did not alter this behavior from the normal. In addition, both insulin-treated and control animals showed preference towards the novel arm by spending more time in it than in the other arms. This result indicates intact hippocampal-dependent memory (Sarnyai *et al*., 1997). The apparent discordance between these two parameters (number of entry and time spent in arms) may call for additional tests such as locomotor behaviour to compliment Y-maze test for spatial memory. Staying in and exploring the novel arm may arguably be a better index of showing preference than entering (and not staying in) it. This is because number of entry into arms is more likely to be affected by locomotor behavior which is influenced by other local events within the muscle itself. Spontaneous alternation behaviour is a typical manifestation of short-term spatial (working) memory measured in the Y-maze task (Kim *et al*., 2008; Mori *et al*., 2001; Sarnyai *et al*., 1997). Mice have natural tendency to alternate the arm they choose to enter in a Y-maze test (Dember and Richman, 1989). Alternation reflects the motivation of the animal to explore its environment and locate the presence of resources such as food, water, mates or shelter (Deacon and Rawlins, 2006). Animals do not need to be deprived of such resources to show alternation behavior; in this case it is called 'spontaneous alternation' (Dember and Richman, 1989). Alternation, whether rewarded or spontaneous, is superb at detecting hippocampal dysfunction, probably even better than the Morris water maze (Deacon and Rawlins, 2006). The time spent in the arms by the insulin-treated and control mice was similar. Number of triads (alternations) and percent alternation of the two groups was also similar (Faizi *et al*., 2012). Taken together, the results of Y-maze test indicate that insulin treatment did not affect short-term working memory. This is contrary to the findings of Kamal *et al*. (2013) who reported impairment of memory and defect in synaptic plasticity following hyperinsulinaemia.

Long-term memory stores information over a long period of time. Short-term memories can become long-term memory through the process of consolidation, involving rehearsal and meaningful association. A group of studies suggests that erasing information from long-term memory might not typically occur (Storm *et al*., 2008). In EPM for memory task, which evaluated long-term memory, there was no significant change in transfer latencies for acquisition and retention phases within each group; and the performance of the insulin-treated group was similar to that of the control, indicating that insulin did not enhance or impair learning and memory in the treated mice. Because of anxiety for open space while performing on the EPM for memory, the mice in both control and insulin group tended to quickly escape from the open arm into the closed arm, as proposed by Blat and Takahashi (1998). The findings in this study agree with those of Moosavi *et al*. (2007a and 2007b), who found no significant effect of low-dose (0.5 and 6 MU), but not high-dose (12 MU) intrahippocampal insulin administration on memory deficit. The results also agree with others in human studies by Backeström *et al.* who showed that insulin is not associated with memory performance in middle aged women (Backeström *et al*., 2015).

From the literature, there is no debate on the fact, that insulin plays a definite role in cognitive function. There is evidence demonstrating the fact, that insulin signalling is required for normal memory process in *drosophila* (Chambers *et al*., 2015), *Xenopus* tadpols (Chiu *et al*., 2008), rats (Liu *et al*., 2013) and humans (Fernandez and Torres-Alemán , 2012). However, reports on the effect of insulin on long-term spatial learning and short-term working memory remain scanty and inconsistent, with some reporting improvement (Reger *et al*., 2006), others – impairment (Krikorian *et al*., 2013), and yet others – no effect (Backeström *et al*., 2015). As it is inherent in most biological processes, the effects vary based on many influencing circumstances.

Indeed, authors have continued to report incongruent findings on the effect of insulin on spatial learning and memory. For example, Adzovic *et al.* reported improved performance in the MWM task following intra-cerebro-ventricular insulin infusion into the brain of young rats, but no effect in aged ones (Adzovic *et al*., 2015). Earlier, Biessels *et al.* (1998) reported that insulin treatment may prevent, but not reverse deficits in water maze learning and LTP in streptozotocin-diabetic rats. The differences in the results may be due to dose, duration of exposure (acute, sub-acute or chronic) and route of insulin administration (including intraperitonial, intra-cerebro-ventricular, intrahippocampal), details of methodology (use of different paradigms, many variations and modifications of a single paradigm), or even subject used in the studies (age and specie variations).

CONCLUSION

The results suggest that sub-acute insulin administration did not affect long-term visuo-spatial memory and short-term working memory in mice. Time is required to accumulate sufficient data for meta-analysis on the effect of insulin on the different aspects of learning and memory in varied situations.

ACKNOWLEDGMENT

The authors wish to appreciate Tertiary Education Trust Fund (TETFund) and Bayero University Research Board for funding this work.

REFERENCES

Adzovic L, Lynn AE, D'Angelo HM, Crockett AM, Kaercher RM, Royer SE, Hopp SC, Wenk GL, (2015). Insulin improves memory and reduces chronic neuroinflammation in the hippocampus of young but not aged brains. *J. Neuroinflam*., 12: 1-10.

Age Converter, Mouse Age Calculator, available at file:///C:/Users/NCC/Desktop/Calculate%20mouse%20age%20in%20human%20years%20(equivalence).htm (accessed on 23/02/2016).

Backeström A, Eriksson S, Nilsson L, Olsson T, Rolandsson O, (2015). Glucose but not insulin or insulin resistance is associated with memory performance in middle-aged non-diabetic women: a cross sectional study. *Diabetol. Metab. Syndr*., 7: 20-28.

Biessels G, Kamal A, Urban IJA, Spruijt BM, Erkelens DW, Gispen WH, (1998). Water maze learning and hippocampal synaptic plasticity in streptozotocin-diabetic rats: effects of insulin treatment. *Brain Res*., 800: 125-135.

Blatt SL, Takahashi RN, (1998). Memory-impairing effects of local anaesthetics in an elevated plus-maze test in mice. *Braz. J. Med. Biol. Res*., 31: 555-559.

Blázquez E, Velázquez E, Hurtado-Carneiro V, Ruiz-Albusac JM (2014). Insulin in the brain: its pathophysiological implications for states related with central insulin resistance, type 2 diabetes and Alzheimer's disease. *Front. Endocrinol*., 5: 161-171.

Chambers DB, Androschuk A, Rosenfelt C, Langer S, Harding M, Bolduc FV, (2015). Insulin signaling is acutely required for long-term memory in Drosophila. *Front. Neur. Circ*., 9: 8-12.

Chiu SL, Chen CM, Cline HT, (2008). Insulin receptor signaling regulates synapse number, dendritic plasticity, and circuit function *in vivo*. *Neurone*, 58: 708-719.

Conrad CD, Galea LAM, Kuroda Y, McEwen BS, (1996). Chronic stress impairs rat spatial memory on the Y-Maze, and this effect is blocked by tianeptine pretreatment. *Behav. Neurosci*., 110: 1321-1334.

Craft S, (2005). Insulin resistance syndrome and Alzheimer's disease: age- and obesity-related effects on memory, amyloid, and inflammation. *Neurobiol. of Ageing*, 26: 65-69.

Davelaar EJ, Goshen-Gottstein Y, Haarmann HJ, Usher M, (2005). The demise of short-term memory revisited: empirical and computational investigation of recency effects. *Psych. Rev*., 112: 30–42.

Deacon RMJ, Rawlins JNP (2006). T-maze alternation in the rodent. Natu Protocols 1: 7-12. doi:10.1038/nprot.2006.2

Dellu F, Mayo W, Cherkaoui J, Le Moal M, Simon H, A (1992). Two-trial memory task with automated recording: Study in young and aged rats. *Brain Res*., 588: 132-139.

Dember WN, Richman CL. (1989). Spontaneous Alternation Behavior. Springer, New York, 36 pp.

Duarte AI, Moreira PI, Oliveira CR, (2012). Insulin in central nervous system: More than just a peripheral hormone, J. Ageing Res., 384017. doi: 10.1155/2012/384017.

Ennaceur A, Delacour J, (1988). A new one-trial test for neurobiological studies of memory in rats. 1: behavioral data. *Behav. Brain Res*., 31: 47-59.

Faizi M, Bader PL, Saw N, Nguyen TV, Beraki S, Wyss-Coray T, Longo FM, Shamloo M, (2012). Thy1-hAPPLond/Swe+ mouse model of Alzheimer's disease displays broad behavioral deficits in sensorimotor, cognitive and social function. *Brain Behav*., 2: 142-154.

Fernandez AA, I. Torres-Alemán I, (2012). The many faces of insulin-like peptide signalling in the brain. *Nat. Rev*., Neuroscie. 13: 225-239.

Finlay BL, Darlington RB, (1995). Linked regularities in the development and evolution of mammalian brains, Science, 268: 1578-1584.

Francis GJ, Martinez JA, Liu WQ, Xu K, Ayer A, Fine J, Tuor UI, Glazner G, Hanson LR, Frey II WH, Toth CC, (2008). Intranasal insulin prevents cognitive decline, cerebral atrophy and white matter changes in murine type I diabetic encephalopathy. *Brain*, 131: 3311-3334.

Ghasemi R, Haeri A, Dargahi L, Mohamed Z, Ahmadiani A, (2013). Insulin in the brain: sources, localization and functions. *Molec. Neurobiol*., 47: 145-71.

Kamal A, Ramakers GMJ, Gispen WH, Biessels GJ, Al-Ansari A, (2013). Hyperinsulinemia in rats causes impairment of spatial memory and learning with defects in hippocampal synaptic plasticity by involvement of postsynaptic mechanisms. *Exper. Brain Res*., 226: 45-51.

Kim YT, Yi YJ, Kim MY, Bu Y, Jin ZH, Choi H, Dore S, Kim H, (2008). Neuroprotection and enhancement of spatial memory by herbal mixture HT008-1 in rat global brain ischemia model. *Am. J. Clin. Med*., 36: 287-299.

Komada M, Takao K, Miyakawa T, (2008). Elevated plus maze for mice. *J. Visual Exper*., 22: 1088-1092.

Krikorian R, (2013). Metabolic disturbance and dementia: A modifiable link. *Curr. Psych*., 12: 17-23.

Laron Z, (2009). Insulin and the brain. *Arc. Physiol. Bioch.*, 115: 112-116.

Liu P, Zou L, Jiao Q, Chi T, Ji X, Qi Y, Xu Q, Wang L, (2013). Xanthoceraside attenuates learning and memory deficits via improving insulin signaling in STZ-induced AD rats. *Neurosci. Lett*., 543: 115-120.

McNay EC, Ong CT, McCrimmon RJ, Cresswell J, Jonathan S, Bogan JS, Sherwin RS, (2010). Hippocampal memory processes are modulated by insulin and high-fat-induced insulin resistance. *Neurobiol. Learn. Mem.*, 93: 546-553.

Mori K, Togashi H, Ueno KI, Matsumoto M, Yoshioka M, (2001). Aminoguanidine prevented the impairment of learning behaviour and hippocampal long-term potentiation following transient cerebralischemia. *Behav. Brain Res*., 120: 159-168.

Moosavi M, Naghdi N, Choopani M, (2007a). Intra CA1 insulin microinjection improves memory consolidation and retrieval. *P E P T I D E S*, 28: 1029-1034.

Moosavi M, Naghdi N, Maghsoudi N, Zahedi AS, (2007b). Insulin protects against stress-induced impairments in water maze performance. *Behav. Brain Res*., 176: 230-236.

Patočková J, Marhol P, Tůmová E, Kriak M, Rokyta R, Típek S, Crkovská J, Anděl M, (2003). Oxidative stress in the brain tissue of laboratory mice with acute post insulin hypoglycemia. *Physiol. Res*., 52: 131-135.

Sarnyai Z, Sibille EL, Pavlides C, Fenster RJ, McEwen BS, Tóth M, (1997). Impaired hippocampal-dependent learning and functional abnormalities in the hippocampus in mice lacking serotonin1A receptors. *Proc. Nat. Acad. Sci. USA*, 97: 14731-14736.

Sharma S, Chopra K, Kulkarni SK, (2007). Effect of insulin and its combination with resveratrol or curcumin in attenuation of diabetic neuropathic pain: participation of nitric oxide and TNF-alpha. *Phytother. Res*., 21: 278-283.

Reaven GM, (2003). Insulin resistance/compensatory hyperinsulinemia, essential hypertension, and cardiovascular disease. *J. Clin. Endocrinol. Metab*., 88: 2399-2403.

Reger MA, Watson GS, Frey II WH, Baker LD, Cholerton B, Keeling ML, Belongia DA, Fishel MA, Plymate SR, Schellenberg GD, Cherrier MM, Craft S, (2006). Effects of intranasal insulin on cognition in memory-impaired older adults: Modulation by APOE genotype, Neurobiol. *Ageing*, 27: 451-458.

Rybicka M, Krysiak R, Okopień B, (2011). The dawn phenomenon and the Somogyi effect - two phenomena of morning hyperglycaemia. *Polish J. Endocrinol*., 62: 276–283.

Somogyi M, (1959). Exacerbation of diabetes by excess insulin action. *Am. J. Med*., 26: 169-191.

Storm BC, Bjork EL, Bjork RA, (2008). Accelerated relearning after retrieval-induced forgetting: the benefit of being forgotten. *J. Exp. Psych*., 34: 230-236.

Walrand S, Guillet C, Boirie Y, Vasson M-P, (2004). In vivo evidences that insulin regulates human polymorphonuclear neutrophil functions. *J. Leuk. Biol*., 76: 1104-1110.

Wei JM, Masud H, Paul MB, (2014). Changing concepts of working memory. *Nat. Neurosci*., 17: 347–356.

World Health Organization (1999). Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications. Part 1: Diagnosis and Classification of Diabetes Mellitus. Department of Non Communicable Disease Surveillance, Geneva.

Wright RL, Lightner EN, Harman JS, Meijer OC, Conrad CD, (2006). Attenuating corticosterone levels on the day of memory assessment prevents chronic stress-induced impairments in spatial memory. *Eur. J. Neurosci*., 24: 595-605.

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