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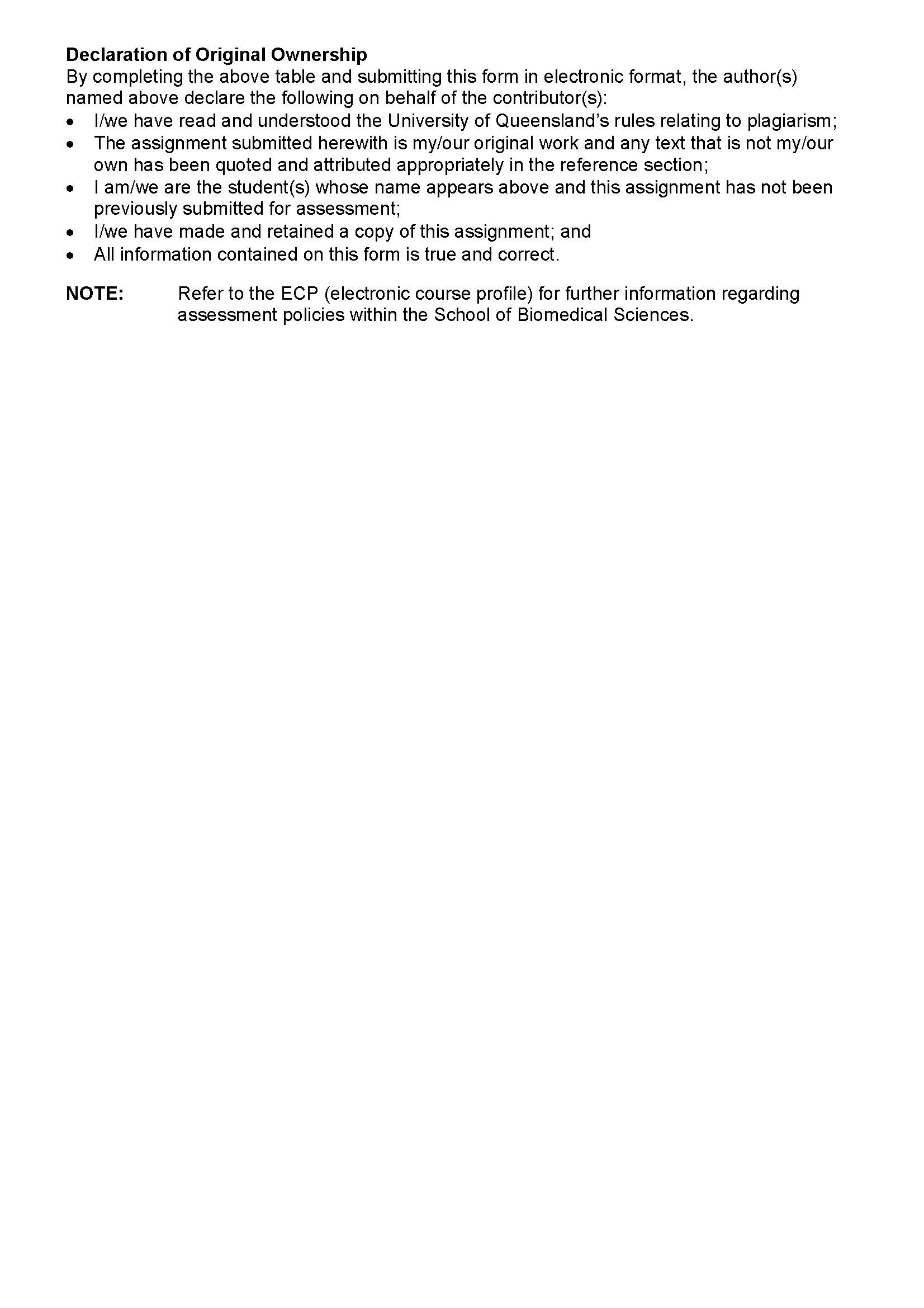
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Introduction

Diabetes is one of the most popular diseases worldwide, two types of diabetes are type 1 diabetes (T1D) and type 2 diabetes (T2D). Diabetes is characterized by a deficiency in insulin secretion and sensitivity. T1D is a chronic autoimmune disease that is genetically susceptible present in human. Human pancreatic beta cells are attacked by own immune system, and destroyed or damaging them leading to reductively production of insulin and followed by hyperglycemia (Tom et al., 2011) (Atkinson, 2012). T2D is distinguished with T1D, which the important features are the defective of receptor response in insulin and progressive pancreatic beta cell failure (Schock-Kusch, 2009). T1D is the major types of diabetes going to investigate in this study. T1D Patients are often diagnosed with the clinical signs, for instance, excessive thirst, frequently urination, and hunger. These often sign to blood hyperglycemia. (Tom et al., 2011). There are several complications that may result by hyperglycemia or glucotoxicity, which including microangiopathy (microvescular)(e.g. Neuropathy, nephropathy and retinopathy) and macroangiopathy (macrovescular)(e.g. cardiovascular disease and cerebral disease) (Chao et al., 2010).

It has been found in prior study that T1D patients with microangiopathy had diminished brain neural networks connection that worked in people’ s attention, memory, auditory and language processing, motor and visual process (Eelco et al., 2012). Additionally Epidemiology of Diabetes Interventions and Complications (EDIC) found cognitive performance could be affected by diabetic microangiopathy, as is known to be mediated by multiple interacting brain circuits and their connections (Eelco et al., 2012). Neurotransmitter and its receptor are the connection of each brain neural networks.

Glutamate is the predominant excitatory neurotransmitter in the brain, retina etc. α-amino-3-hydroxy-5-methyl-4-isoxazole propio-nate (AMPA) is one of the subtype ionotropic glutamate receptors that mediate the fast excitatory stimulation and ubiquitously disseminated throughout the central nervous system. The activation of AMPA receptors accelerated to the induction of long-term potentiation that is the major neural substrate in brain, which for the formation and development of learning and memory. The deficits in AMPA often result in cognitive impairment (Fowler et al., 2004). Glutamate receptor 3 (GluR3) is a protein that is encoded by the Gria3 gene that is on the long arm of the X chromosome. It has been showed in prior study that the partial tandem duplication of Gria3 in X chromosome on one allele in mother, would inherit to male offspring, and lead to the reductive expression of GluR3. It shows apparently that the mutations in Gria3 gene lead to reductive expression of GluR3 in male remarkably relate to mental retardation (cognitive impairment) (Lau et al., 2013) (Motyl & McCabe, 2009). Furthermore, without gene mutation, there are the other factor would cause the abnormal of GluR3 expression. The diabetes may be cause the loss of neuron cell by glutamate excitotoxicity and then allow large influx of Ca+ to damage the cell, which would cause the reduction of glutamate receptor as well. (Chiyonobu et al., 2007).

In the prior study, T1D may remarkably reduce the expression of ionotropic glutamate receptor in rat retina after 12 weeks STZ treatment, which would interesting that whether T1D with or without microangiopahty cause the effects on ionotropic glutamate receptor expression in brain, especially in Gria3 gene that relate cognitive progression. (Chiyonobu et al., 2007).

In this study, propose is going to investigate the effect of T1D on Gria3 gene expression in rat brain. Neuron dorsal root ganglia from streptozotocin (STZ) induced T1D rats had been used. STZ is a nitrosurea compound that enters pancreatic β cells and causes cellular toxicity and local immune responses that lead to hypoinsulinemia and hyperglycemia in animals. In addition, real-time polymerase chain reaction (qPCR) had been used as to quantify gene expression in the samples (Wu et al., 2007). The exceptive result is that the reductive expression of Gria3 in neuron dorsal root ganglia from STZ-rats.

Method

6-week old male Wistar rats were injected intraperitoneally with a single dosage of 65mg/kg STZ for 6 weeks treatmet and euthanized after T1D induced, tissues (kidney, brain, heart ventricles) had been collected, and RNA extracted, cDNA synthesized to perform qPCR to analysis the expression of gene. Gene of interesting (GOI) had been chose from ‘Top 100 genes’ database. Furthermore, database for annotation, visualization & integrated discovery (DAVID) was used to help find GOI from 100 genes database (Nature Protocols, 2009 & Nucleic Acids Res, 2009). The methods were to choose Gria3 as GOI in this study by using DAVID in ‘GSE34000’ neuron dataset and then found that Gria3 was downregulated in neuroactive pathway in brain, which relatively linked to type 1 diabetes model, and chose Gria3 gene in brain that had not been investigated in prior study. And then primer had been selected, which was R\_Gria3\_1.

Moreover, qPCR method had been performed to analysis the protein expression in diabetic brain samples (n=3). There were 6 samples were collected from three healthy rat and three STZ-diabetic mice. And cDNAs had been provided from tutors. In order to run PCR, forward and reverse primers were reconstitute with cDNAs, working stocks had been set up, at same time housekeeper gene (Beta-actin) as a reference gene to standardize across different cDNA samples, which was not affected by diabetic and expressed at a similar level in most cells. Master mixes had been made to prepare for running PCR and triplicated the mixes to 96-well plate, and transferred into PCR machine to read fluorescent signal.

After PCR running, the amount of protein had been calculated. It was by measuring how many cycles had been amplified to read the cycle threshold (CT), if more cDNA in sample which had fewer cycles to reach threshold. Normalisation of all samples was done and calculated delta-delta CT (ddCT). Two-tailed T-test and then had been used for statistic analysis of the result.

Result

The result showed in Figure 1 that no significant different of the expression of Gria3 gene between healthy rat brain and STZ-T1D rat brain (p>0.05).



Fig.1 Gria3 expression in rat brain (neuron dorsal root ganglia). N=3, ±mean=SED. No significant difference in Gria3 expression in brain between healthy rat and STZ-T1D rats (p>0.05).

Discussion

Based on the result that had been done in this study, which showed no significant different between healthy rat and STZ-T1D rat in the expression of Gria3 gene in brain. The expectant result is rejected.

According to previous study, long term-chronic hyperglycemia could cause the microangiopathy including neuropathy (Eelco et al., 2012), which means T1D has the possibility to damage the dorsal root ganglia in rat brain. Furthermore, some studies also mentioned that T1D without microangiopathy would also has some effects on neuron but not significant, which means 6 weeks treatment of STZ in rats may not sufficient to raise significant influence on neuron cell, as the high blood glucose in blood vessel affect the vessels first. It would be a plausible reason for result that did not give evidence about the effects on expression of Gria3 gene (Van et al., 2009).

Some research shown that GluR3 mRNA was expressed in the large neurons of the ganglion which innervate a sensory cell to use as an excitatory amino acid as a neurotransmitter (Safieddine et al., 1992). This might explain why the expression of GluR3 in STZ-T1D mice is a slightly higher than healthy mice in Figure 1, as its exits abundant in ganglion nerve. Nevertheless, in prior study, in rat retina, ganglion cells express Gria3, but after 2,4,6 weeks STZ exposure, no significant change in the expression of Gria3, even the strong evidence proved diabetes disrupts glutamate signalling in the retina and affects retinal neuron (Chiyonobu et al., 2007).

Further, the sample had been used in this study that was dorsal root ganglia in rat brain. The axon of dorsal root ganglion is located in the peripheral nervous system (PNS) that transfer sensory signal to central nervous system (CNS). Nonetheless, it was reported most of glutamatergic receptors are localized all through the CNS, and the distribution of subtype of the receptor still remains some unknown, which means GluR3 might not in the axons of doral root ganglion that in PNS, and also T1D might affect GluR3 in other part of brain but not only dorsal root ganglia. (Breese et al., 1996)

Therefore, there might have few possible reasons that lead to no significant different of the result in this study. It might was because 6 weeks were not sufficient to disturb the ganglia in rat brain. It might was considering as ample expression of Gria3 gene in ganglia cells, which may not generate the significant reduction. And it might was because dorsal root ganglia had been used in this study may not adequate for investigating Gria3 gene expression in this study. Besides, GluR3 used as probe for qPCR method in this study, and imprecise pipetting might bring the unreliable result.

Therefore, since T1D could accelerate the cognitive and mental impairment that is high relate to the expression of Gria3 gene, further investigation need to be considered. In the future direction, long-term chronic STZ-T1D rats need to be generated to examine the long-term effect on Gria3 gene expression in brain, which might enhance the possibility of microangiopathy complications risk in T1D rats, and might increase the risk of damage of brain. And also need to investigate the effect of the long-term hyperglycemia without microangiopathy on the expression of Gria3 gene in brain. Meanwhile, the brain sample would not only collect dorsal root ganglion, but also analysis the other part of brain such as neocortex, hippocampus (Breese et al., 1996) (Pellegrini-Giampietro et al., 1991). In addition, since only 3 healthy rat brain samples and 3 STZ-T1D rat brain samples had been used in this study, which may need more samples to improve the accurate, and decrease the variability.

In conclusion, GluR3 expression does not show significant different between healthy rat brain and STZ-T1D rat brain, but GluR3 is essential for the cognitive progression and neural network in brain, which mean it need to further investigate whether diabetes could affect the GluR3 expression or not, as diabetes can disrupt the glutamatergic signal transduction.

Reference

1. Belle, T., Coppieters, K., & Herrath, M. (2011). Type 1 Diabetes: Etiology, Immunology, and Therapeutic Strategies. *Physiological Reviews,* 91, 79-118.
2. Atkinson, M. (2012). The Pathogenesis and Natural History of Type 1 Diabetes. *Cold Spring Harbor Perspectives in Medicine,* A007641-A007641.
3. Schock-Kusch, D., Sadick, M., Henninger, N., Kraenzlin, B., Claus, G., Kloetzer, H., ... Gretz, N. (2009). Transcutaneous measurement of glomerular filtration rate using FITC-sinistrin in rats. *Nephrology Dialysis Transplantation,* 24(10), 2997-3001.
4. Chao, E., & Henry, R. (2010). SGLT2 Inhibition — A Novel Strategy For Diabetes Treatment. *Nature Reviews Drug Discovery,* 551-559.
5. Eelco VD, Menno MS, Ernesto JSA, Richard GI, Annette CM, Frank JS, Christopher MR, Martin K, Michaela D, and Frederik B. (2012). Resting State Brain Networks in Type 1 Diabetic Patients With and Without Microangioathy and Their Relation to Cognitive Functions and Disease Variables. *Diabetes*, 61, 1814–1821.
6. Fowler IL, Whalley K, Murray T, O’Neill MJ, & McCulloch J. (2004). The AMPA receptor potentiator LY404187 increase cerebral glucose utilization and c-fos expression in the Rat*. Journal of Cerebral Blood Flow & Metabolism*, 24, 1098–1109.
7. Lau CMJ, Kroes RA, Moskal JR, Linsenmeier RA. (2013). Diabetes changes expression of genes related to glutamate neurotransmission and transport in the Long-Evans rat retina, *Molecular Vision*, 19, 1538-1553.
8. Motyl K and McCabe LR. (2009). STZ type 1 diabetes severity and bone, *Biological Procedures Online*, 11(1), 296-315.
9. Chiyonobu T, Hayashi S, Kobayashi K, Morimoto M, Miyanomae Y, Nishimura A, Nishimoto A, Ito C, Imoto I, Sugimoto T, Jia ZP, Inazawa J, and Toda T. (2007). Partial Tandem Duplication of GRIA3 in a Male With Mental Retardation, *American Journal of Medical Genetics Part A*, 143A, 1448–1455
10. Wu Y, Arai AC, Rumbaugh G, Srivastava AK, Turner G, Hayashi T, Suzuki E, Jiang Y, Zhang L, Rodriguez J, Boyle J, Tarpey P, Raymond FL, Nevelsteen J, Froyen G, Stratton M, Futreal A, Gecz J, Stevenson R, Schwartz CE, Valle D, Huganir RL, and Wang T. (2007). Mutations in ionotropic AMPA receptor 3 alter channel properties and are associated with moderate cognitive impairment in humans, *PNAS,* 104 (46), 18163–18168.
11. Nature Protocals (2009), 4(1), 44 & Nucleic Acids Res (2009), 37 (1),1
12. Van DE, [Klein M](http://www.ncbi.nlm.nih.gov/pubmed?term=Klein%20M%5BAuthor%5D&cauthor=true&cauthor_uid=19584309), [Schoonenboom NS](http://www.ncbi.nlm.nih.gov/pubmed?term=Schoonenboom%20NS%5BAuthor%5D&cauthor=true&cauthor_uid=19584309), [Hoogma RP](http://www.ncbi.nlm.nih.gov/pubmed?term=Hoogma%20RP%5BAuthor%5D&cauthor=true&cauthor_uid=19584309), [Moll AC](http://www.ncbi.nlm.nih.gov/pubmed?term=Moll%20AC%5BAuthor%5D&cauthor=true&cauthor_uid=19584309), [Snoek FJ](http://www.ncbi.nlm.nih.gov/pubmed?term=Snoek%20FJ%5BAuthor%5D&cauthor=true&cauthor_uid=19584309), [Stam CJ](http://www.ncbi.nlm.nih.gov/pubmed?term=Stam%20CJ%5BAuthor%5D&cauthor=true&cauthor_uid=19584309), [Diamant M](http://www.ncbi.nlm.nih.gov/pubmed?term=Diamant%20M%5BAuthor%5D&cauthor=true&cauthor_uid=19584309). (2009). Functional Brain Connectivity and Neurocognitive Functioning in Patients With Long-Standing Type 1 Diabetes With and Without Microvascular Complications, *Diabetes*, 58(10), 2335-43.
13. Safieddine S, Eybalin M. (1992). Co-expression of NMDA and AMPA/kainate receptor mRNAs in cochlear neurons, *Neuroreport*, 3 (12), 1145-8.
14. Breese CR, [Logel J](http://www.ncbi.nlm.nih.gov/pubmed?term=Logel%20J%5BAuthor%5D&cauthor=true&cauthor_uid=8968949), [Adams C](http://www.ncbi.nlm.nih.gov/pubmed?term=Adams%20C%5BAuthor%5D&cauthor=true&cauthor_uid=8968949), [Leonard SS](http://www.ncbi.nlm.nih.gov/pubmed?term=Leonard%20SS%5BAuthor%5D&cauthor=true&cauthor_uid=8968949). (1996). Regional gene expression of the glutamate receptor subtypes GluR1, GluR2, and GluR3 in human postmortem brain, *J Mol Neurosci,* 7(4), 277-89.
15. Pellegrini-Giampietro, D. (1991). Differential Expression of Three Glutamate Receptor Genes in Developing Rat Brain: An in situ Hybridization Study. *Proceedings of the National Academy of Sciences,* *88*(10), 4157-4161.