参赛队员姓名: 周逸菲 YiFei Zhou.

中学: 上海包玉刚实验学校 Shanghai YK Pao School

省份: 上海

国家/地区: 中国

指导教师姓名: Mr. Steven Varty

指导教师单位: 上海包玉刚实验学校

论文题目: Uncovering Mirror Neuron's molecular and functional identity by single cell transcriptomic analysis

Uncovering MN's molecular and functional identity by single cell transcriptomic analysis

YiFei Zhou (Sophia)

Abstract:

Mirror Neurons (MNs) fire both when performing and seeing particular actions [1]. Those actions range from a hand grasping objects to a simple smile [2]. So, it's often been hypothesized that MNs play a vital role in social cognition namely empathy [3]. The active cortical regions in both execution and observation under neuroimaging are referred to as the Mirror Neuron System (MNS). It's composed of the Superior Temporal [4], Posterior Parietal Cortex, and Inferior Frontal Gyrus [5-8]. I aim to find MN neuronal identity. Such identification would lay the groundwork for future MN analysis and understanding of the mechanisms behind action understanding and empathy [9,10]. High-expression genes throughout the MNS became candidate genes.10 cell clusters fit MN's characteristic as a layer three pyramidal excitatory neuron in human cortical single cell sequencing data base. The cluster that has the highest sum of relative expression of candidate genes is cluster 85. With MN's molecular identity hypothesized, I wished to further explore the functional aspect. I employed single-cell sequencing and String to uncover connections between typical neural degenerative diseases, psychiatric disease and MN impairment. The ones related are Moebius Facial Syndrome, Autism Spectrum Disorder, and Amyotrophic Lateral Sclerosis.

摘要：

镜像神经元（MN）在执行和看到特定动作时都会被激发。那个动作可以简至抓东西，也可以是一个不起眼的笑容。因此许多人曾推断 MN 为公情感能力的基本机制。运用脑成像可见，在执行和观察中都活跃的脑区被称为镜像神经元系统。它由颞上沟、后顶叶皮质和额下回组成。我的目的是通过运用在 Allen Brian Atlas 中的 microarray 和 single cell sequencing 与 strings 来发现 MN 的特性包括在基因层面其所属的细胞族和神级通路层面相关的具体功能。找到 MN 的特性会为后期 MN 的研究奠定夯实的基础，帮助我们理解动作理解，共情和语言背后的机制。我使用 microarray，将所有镜像神经元系统的三个脑区都高表达的基因选出成为候选基因。MN 是一个第三层兴奋性神经元，而在 single cell 中有 10 个细胞簇符合此标准。在每个细胞族中，候选基因总相对表达量最高的是细胞族 85。为了进一步探索镜镜像神经元的功能方面的特性，我搜集了一些代表性神经退行性疾病与精神病的决定性基因，并运用 microarray 收集这些基因在细胞族 85 中的具体表达。其中，6 个被发现在细胞族 85 中具有似正态分布表达模式。这六个基因在 string 数据库中，三个有联系。分析整理后发现牟比士症候群，自闭症与渐冻症不仅与 MN 有关，互相之间也存在机理联系。

Key Word:

Mirror Neuron, single cell transcriptomics, Autism Spectrum Disorders, Moebius Facial Syndrome

Table of Contents

Background & Introduction ***4***

Method ***5***

1. Allen Human Brain Atlas *5*

2. String *5*

3. Heatmapper *6*

4. Pubmed -National Institute of Health (NIH)- *6*

Results ***6***

1. Brain region identification *6*

2. Differential gene analysis performed to uncover MN's molecular identity *7*

3. Uncovering connection between MN function and diseases *9*

Discussion ***13***

Works Cited ***15***

Acknowledgements ***20***

Background & Introduction

Imitation and empathy are common features of human beings. These higher cognitive neuro-activities are unique and intriguing but hard to actualize until the discovery of a type of neurons termed mirror neurons. Mirror neurons located in the parietal cortex and fire action potentials when subject observes a motion instead of performing it. They are first found in the area F5 of the ventral premotor cortex [11-13] and later also in the inferior parietal lobule [14-18] in the masque's brain.

Interestingly, the localization of human MNs slightly differs from that of *Rhesus Macaque*, potentially implicating the fact that humans are more sensitive to others' emotions. They were found in the Superior Temporal Sulcus (STS) for observation [19], Posterior Parietal Cortex (PPC) for motor processing and Inferior Frontal Gyrus (IFG) for goal and intention of imitation [20-23] using Functional Magnetic Resonance Imaging (fMRI). It detects blood oxygenation level dependent (BOLD) signals which share the same pattern with neural signals only delayed. These brain cortical regions are active in both observation and execution of a class of actions also referred to as the Mirror Neuron System as shown in Fig. 1 below. Another fMRI study shows that the amygdala and insula are both activated when exposed to disgusting smells or tastes and observing other faces that show disgust [24]. Additionally, the human anterior cingulate cortex (ACC) area activates when experiencing pain in the self and witnessing pain in the other [25]. Both studies reinforce MN's connection with emotion and empathy.

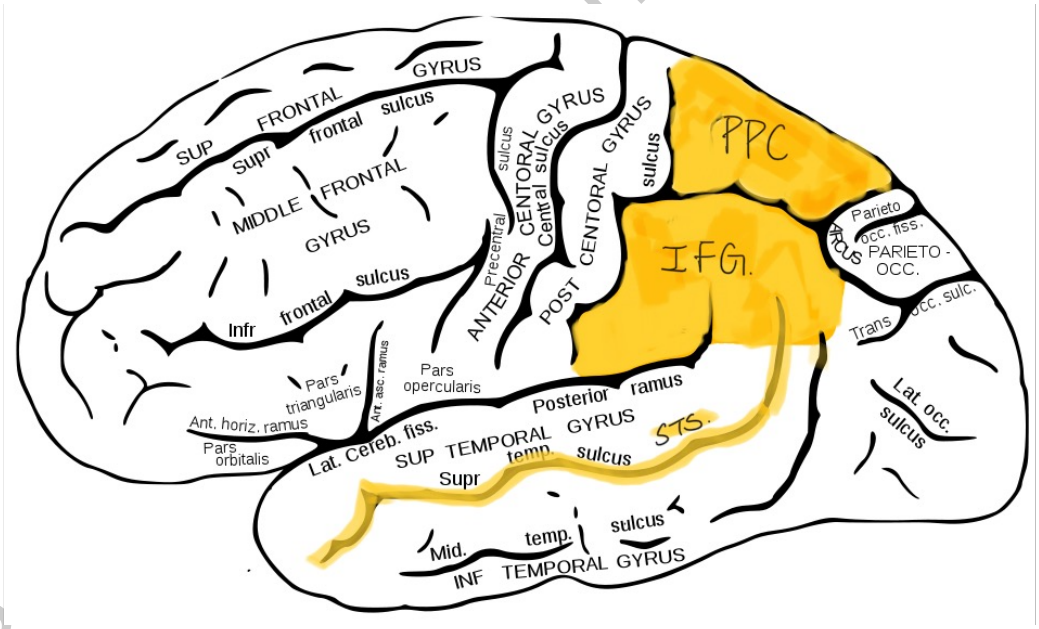

Fig. 1 The components of the Mirror Neuron System or the three cortical brain regions where collection of mirror neurons are found

There are two major hypotheses, congenital and acquired, regarding the controversial question of the origin of MNs [26,27]. The congenital hypothesis states MN originated from a gene mutation. It's kept and spread since it's more beneficial to survival and reproduction of human. In other words, this hypothesis view MN as an adapted gene. On the other hand, the acquired hypothesis proposes the property of MN to be majorly gained by associative learning

later on. The genetic evolution only provides significant non-specific background information. Studies lead by Rayson etl. [28] utilizing various tools like electroencephalography (EEG) has provided convincing backings. However, discussion backings lacked the inclusion of molecular or genetic basis of MN in both hypotheses. This report wants to mend this caveat.

Whether MN's genetic identity is a deterministic or is a non-specific background, finding it is a good starting point since the development environment is hard to be controlled. In order to uncover MN's genetic identity, I aim to find the most likely cell cluster(s) which MN belongs to via differential single cell gene expression analysis and MN correlated neurodegenerative disease(s) to gain further understanding of its function.

Method

1. Allen Human Brain Atlas

I employed Allen Brian Atlas for genetic analysis of the mirror neuron system. It's a comprehensive and easily accessed multi-modal mapping of gene expressions in the human brain [29]. It's a fundamental tool for neuroscientists worldwide. Specifically, I used two parts of this huge database.

One is the human single cell RNA sequencing. It is a scalable approach to provide gene expression of thousands of single-nucleus cells. This is achieved by micro-dissecting tissue from brain cortical layers or cell groups [30]. The data are clearly displayed via interactive heatmaps showing the general overview and specific gene expression at the same time.

The second part is the microarray database. The differential search enables me to find overlapping genes across various brain structures. Indeed, it contains more than 62000 gene probes per profile and around 500 samples per hemisphere across cerebrum, cerebellum and brainstem. The data are mapped with histology into unified 3-Dimensional anatomic framework based on neuroimaging.

http://human.brain-map.org/

2. String

I used String to find connections between likely candidate genes. It is an open database that finds and analyzes interactions between proteins. The interactions include both direct(physical) and indirect (functional). They are predicted based on computational modeling, knowledge transfer between organisms, and interactions collected from other databases [31]. More specifically, functional links are inferred from genomic association supported by a unique scoring framework based on benchmarks of the different types of associations against a common reference set. String is updated continuously and highly accurate. It currently covers 24,584,628 proteins from 5090 organisms [32].

As I input my list of candidate genes and choose the organism as Homo Sapiens, connection results are established. Since mirror neurons likely communicated with one another across layers and brain regions, String is a useful tool.

https://string-db.org/

3. Heatmapper

Heatmapper enables visualization of my collected data into six main forms of heatmap via graphic interface [33]. I attach the raw data set and add personal clustering and coloring to increase visual clarity.

It's used to create a simplified and zoom-in version of Allen Brain Atlas single-cell RNA sequencing data that consists of the likely candidate genes' name and relative expression.

http://www.heatmapper.ca/expression/

4. Pubmed -National Institute of Health (NIH)-National Clinical Assessment Tool (NCAT)

Pubmed is one of the most credible, global data-base available to bio-medical studies [34]. I employed this tool to gain a more in-depth understanding of prior knowledge of mirror neurons which aided me to decide the specific focus of this report and understand the concept of Mirror Neuron more fully.

PubMed is also used to form parts of the candidate pool, specifically the known related genes of neurodegenerative and psychiatric diseases. Indeed, one of NIH's primary purposes is to advance research of rare diseases [28].

https://www.ncbi.nlm.nih.gov/pmc/

Results

1. Brain region identification

The three known components of MNS as previously established are the Superior Temporal Sulcus (STS), Posterior Parietal Cortex (PPC) and Inferior Frontal Gyrus (IFG) [16-18]. STS, the observational component of the MNS is a sulcus instead of a particular brain region. This requires me to choose between the two neighboring areas: the Superior Temporal Gyrus and the Middle Temporal Gyrus (Fig. 2). The work of Molenberghs and his colleagues [35] proved a strong connection between the MTG and the MNS hence I chose MTG.

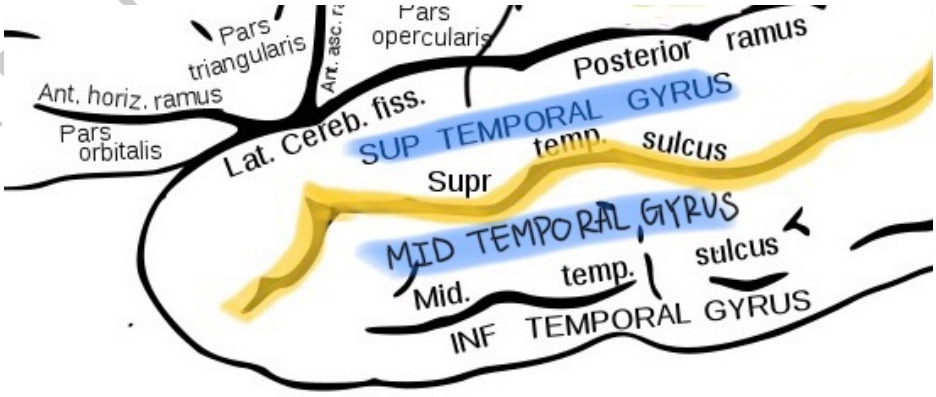

Fig. 2 Specific labeled cortical areas around the Superior Temporal Sulcus

The Allen Brain Atlas database doesn't define the region of PPC because it employs dissection or staining for specific cortical regions whereas the components of MNS uses electrophysiology specifically fMRI. So, after brief searches of cortical area diagrams, I found the PPC and paracentral lobule posterior part (PCLP) has the highest area of overlap (Fig. 3 And Fig. 4) [8].

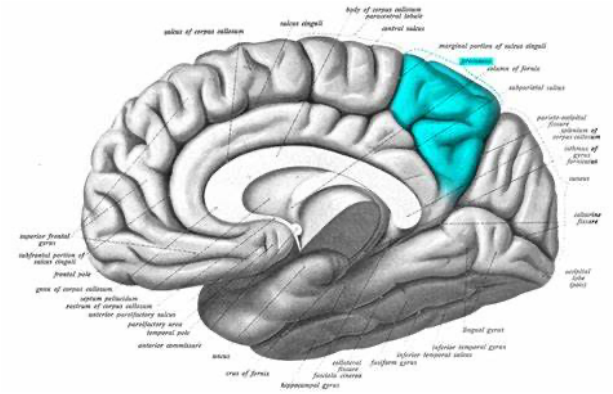

Fig. 3 Cortical diagram with the paracentral lobule posterior part (PCLP) labeled in blue [36]

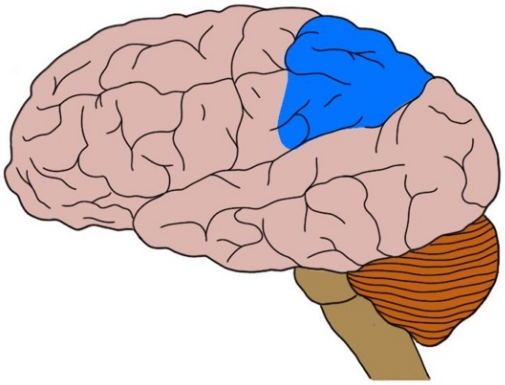

Fig. 4 Cortical diagrams with the Posterior Parietal Cortex (PPC) in blue [37]

With these mirror neuron-related brain regions identified as MTG, PCLP and IFG. I sought to further explore the neuronal identity of mirror neurons using microarray and single-cell sequencing data. The identity of MN contains two aspects (separated into two sections below): its belonging molecular cell cluster and its specific function.

2. Differential gene analysis performed to uncover MN's molecular identity

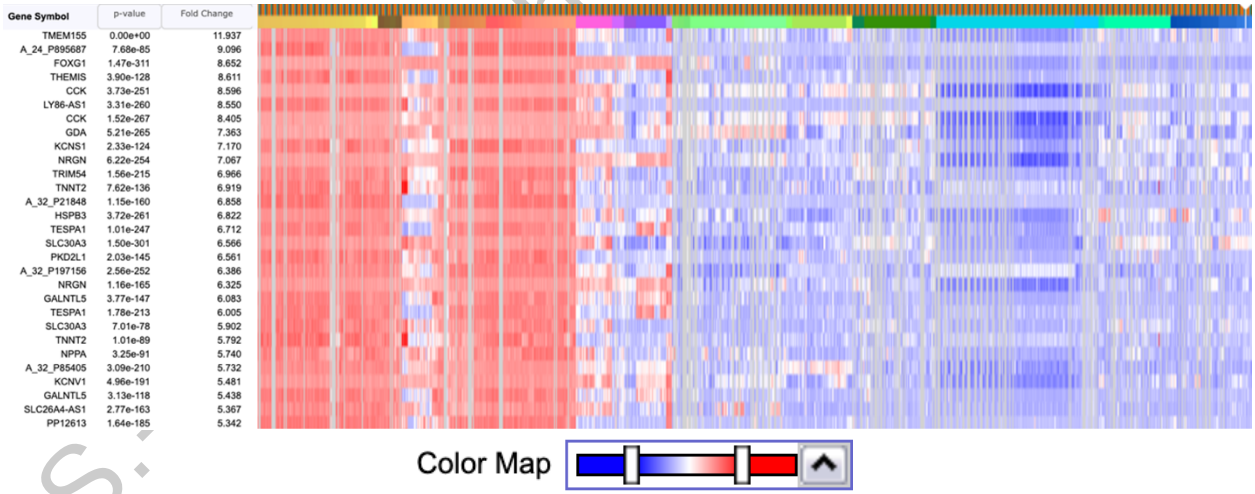

Fig. 5 differential research using microarray data in Allen Brain Atlas

The next question I want to explore is which cell identity at the molecular level presents MNs. To address this question, I first performed differential gene expression analysis of MNS with gray matter as control to find high expression genes uniquely expressed in the MNS comparative to the whole cortical area [19-23]. Using microarray data of the human brain, I found the relative expression levels of various genes in regions MTG, PPC, and IFG forming the result above (Fig. 5).

All the genes that have a fold of change higher than five are gathered for further analysis. This indicates these genes are highly expressed in all component of the MNS and resulted in a pool of 26 gene candidates. The genes that are repeated or have no or close to none (0.01-0.03) expression throughout the brain, namely NPAA, PP12613, PKD2L1, and GALNTL5, are ruled out. The relative expressions of candidate genes throughout the brain are found using the Allen brain atlas's human cortical single-cell database. Fig. 6 shows the results visualized. All candidate genes have a higher expression in the neuronal excitatory section in comparison to the inhibitory section. This fits MN's identity as an excitatory neuron reinforcing the rationale of my method. Except for CCK which is understandable because it's a gut hormone that improves digestion and acts as a satiety signal [38]. Hormones are known for their lack of specificity, so it's highly expressed in various clusters.

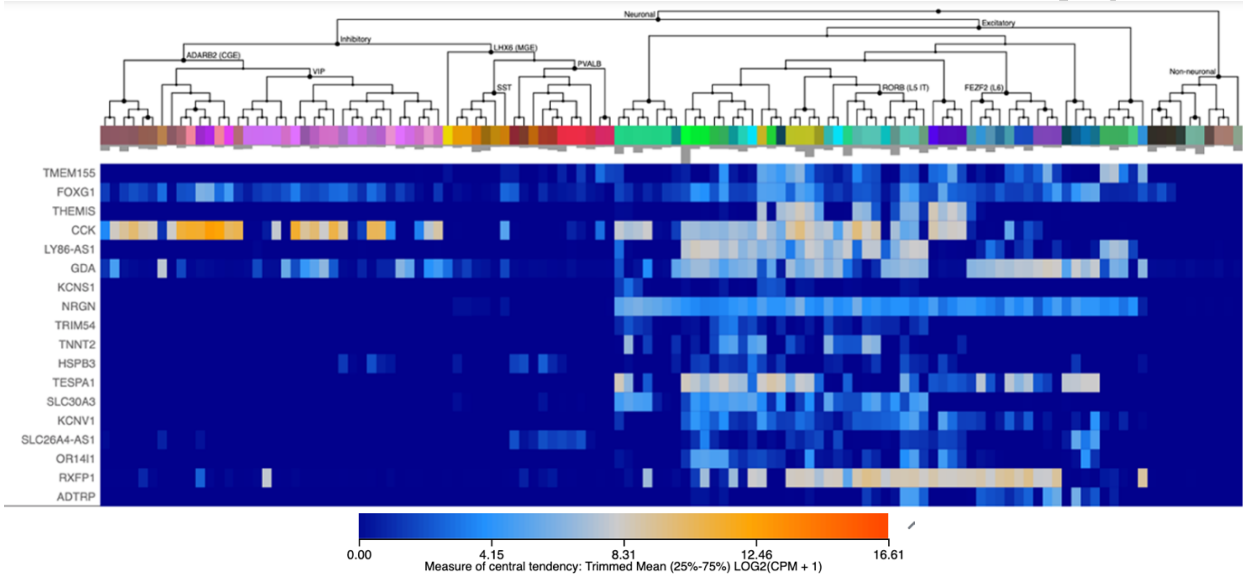

Fig. 6 micro-array identified genes in single cell data base

Then, I want to uncover MN belonging neuronal subtype (as defined by clusters in the single cell data). MN is a cortical excitatory neuron that falls within the realm of layer three pyramidal excitatory neurons [39]. So, the target cell groups for my search needs to contain layer 3. There is a total of 10 cell clusters fitting the characteristics of MNs within the Allen Brain Atlas human cortical single cell data base. I was able to separate them into four types according to their marker genes which are LINC00907, RORB, THEMIS and Fezf2. LINC00507 appear to be age-dependent, suggesting it may be involved in brain development of higher primates [40]. RORB is genetically associated with bipolar disorders [30], and THEMIS with mal-functioning T-cells [41]. Fezf2, on the other hand, is closely linked with motor functions [42]. In each of these areas the corresponding relative concentration or central tendency of each candidate genes is found and ranked in descending order, forming an excel table which is then transformed into a heatmap for direct visualization (Fig. 7).

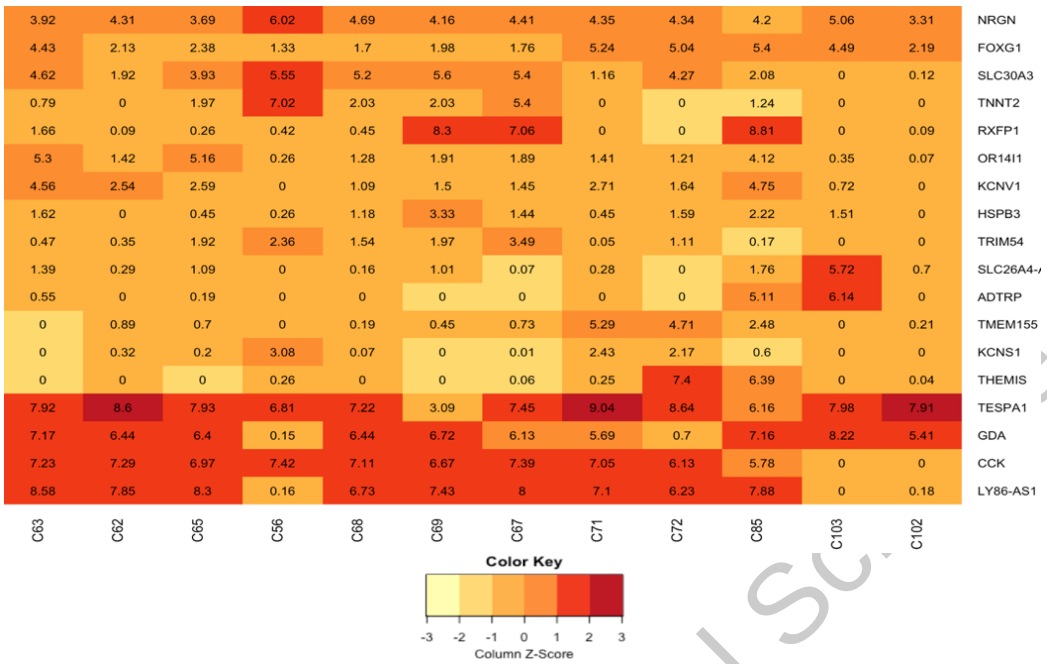

Fig. 7 Heatmap showing expression of mirror neuron candidate genes (x-axis) in specific clusters (y-axis) using single cell sequencing

In order to identify the most relevant cell types for mirror neuron from single cell sequencing clustering, I added up the relative expression of all the candidate genes within the same cell type. Cluster 85 is the significantly higher than all others having a sum of 76.31 whereas the second largest, cluster 67 is 62.14. The third in line being cluster 63 with 60.21. The difference between cluster 85 and 76 around 14 whereas the difference between cluster 67 and 63 is around 2. The high sum of candidate gene concentration in cluster 85 indicate it is the most likely region of MN presence.

3. Uncovering connection between MN function and diseases

MN's identity includes not only the molecular level of cell clusters but also the functional level. In order to uncover proof of MN related functions, I decide to find diseases related to MN dysfunction. First step is finding deterministic genes in typical neurodegenerative diseases and psychiatry disorders via literature search (Table 1). Note all of the illnesses' connection with MN remains unknown, they are selected due to their correlation with MN or representativeness in the disease field. Autism spectrum disorder (ASD) is positively correlated with MNs since its major symptom include lacking higher-order socio-cognitive abilities like imitation and empathy [43]. Moebius syndrome (MBS), dopa-responsive dystonia and ataxia are various types of facial control impairment caused by cranial nerve and muscle disfunctions [44]. They correlate with MN since mirroring of emotion require facial control. In the broader aspect of emotions, psychiatry disorders like depression are also included. Alzheimer's disease (AD), Parkinson's disease (PD) and Amyotrophic Lateral Sclerosis (ALS) are representative examples of neurodegenerative disease [45] Their correlation with MN hasn't yet been found. By selecting disorders with variable known correlation to MN function, I sought to decreases the likelihood of confirmation bias between MN function and these disorders.

Table 1. Genes related to neurodegenerative diseases and psychiatry disorders

Disease	Gene
Autism Spectrum Disorder (ASD) [46,34]	DDX3X, MECP2, AFF2, CDKL2, IQSEC2, NLGN4X, PCDH19, AGTR2, AP1S2, AR, ARHGEF9, ARX, ASMT, ATRX, CASK, *AVPR1a, DISC1, DYX1C1, ITGB3, SLC6A4, RELN, RPL10, SHANK3, PTEN*
Moebius syndrome (MBS) [47-50]	GATA2, EGR2, BASP1, TUBB3, PLXND1, REV3L, KLFL5, CCDC37, TMCC1, PODXL2, HOXB1, HOXA1
Dopa-responsive Dystonia [50-52]	*GCH1*
Ataxia [51]	APTX
Depression [55]	GNB3, MAOA, HTR2A, ACE, BDNF, DRD4, HTR1B, TPH1, SLC6A2, GABRA3
Alzheimer's Disease (AD) [56]	APOE, APP, MAPT, PSEN1, PSEN2
Parkinson's Disease (PD) [57]	SCN1A, LRRK1, LRRK2, PINK1, PARK7
Amyotrophic Lateral Sclerosis (ALS) [58]	SOD1, C9orf72, TDP43, FUS

Then I performed differential gene analysis on the 50 genes shown in Table 1 using human cortical areas single cell sequencing data base. Among them, HOXA1, HOXB1, GATA2, ERG2, PLXND1, KLF15, CCDC37, AGTR2, ASMT, AVPR1A, DISC1, DYX1C1, *ITGB3, SLC6A4, ARX,* MAOA, ACE, BDNF, DRD4, HTR1B, TPH1, SLC6A2, APOE, APP, PSEN2 have no expression in the neuronal excitatory section leading to a refined list of genes. The visualization of the relative expression of the 30 genes are shown in Fig. 8. The varying level of expression across cell clusters suggest neurodegenerative diseases and psychiatry diseases are caused by genetic operation across varying layers.

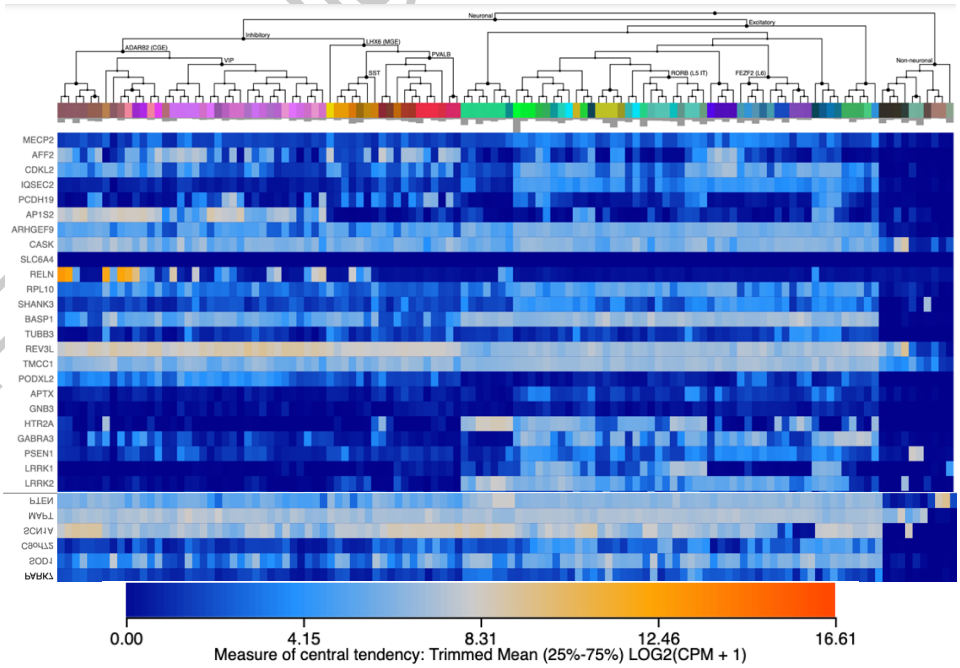

Fig. 8 Gene expression in neurodegenerative and psychiatry disease in single cell data base

Fig. 9 Disease's gene expression in cell cluster 85 (8 likely co-expression genes and 2 controls)

The previous differential gene analysis of high expression genes in the MNS revealed cluster 85, Layer 3 to 5 THEMIS UBE2F to be the likely cell cluster of MN presence So, I extracted all the candidate gene's expression within cluster 85. Interestingly, the expression patterns of LRRK1, SOD1, CASK, MAPT, ATRX, TMCC1, REV3L, BASP1 are trending to normal distribution with relatively high average expression (>6.9) (Fig. 9). Their similar expression patterns indicate their potential co-expression within cluster 85. Their high expression specifically in MN-like cell cluster 85 implicates that their dysfunction may contribute to certain neurological and psychiatric disorders, including ALS, AD, MBS and ASD (Table 1). In comparative, PODXL2 and SHANKS

selected randomly served as controls. They have low average expressions and random distribution pattern. To check whether the eight identified disorder related genes are strongly associated, String analysis which shows the interactions between genes is performed on them (Fig 10).

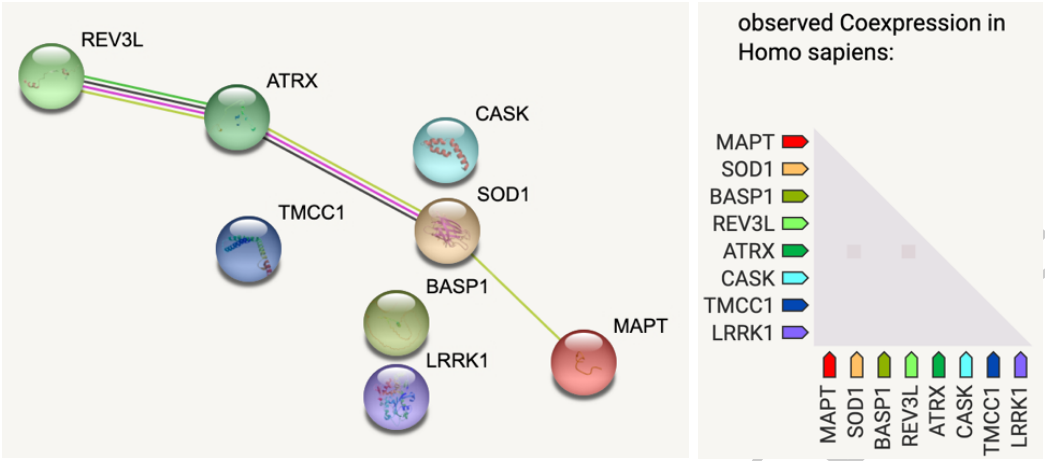

Fig. 10 interaction between similar-expression-pattern diseases' genes using the String network, overview (left) and co-expression (right)

The String analysis shows four genes are indeed interrelated. They are REV3L, ATRX, SOD1, and MAPT. REV3L is a catalytic subunit of the DNA polymerase zeta complex and is vital in MBS and MBS is a type of congenital facial palsy where the 6th and 7th cranial nerve disfunctions [59]. ATRX is a transcriptional regulator that facilitates DNA replication and is influential in ASD. From String analysis, REV3L and ATRX are co-expressed in human and various other organisms (Fig. 10 right). Based on RNA expression pattern and protein co-regulation, their co-expression score in humans is 0.083. In addition, SOD1 is well-known for ALS pathogenesis, which destroys toxic radicals produced in oxidative respiration. ALS is the progressive degeneration of motor neurons responsible for controlling voluntary muscle movements [60]. ATRX and SOD1 are also co-mentioned and co-expressed with a score of 0.046 (Fig. 10 right).

The result indicates MBS, ASD, and ALS are all connected with MN malfunction revealed by intrinsic characteristics of the genes and their interactions revealed by single cell sequencing and String. Such connection on a genetic level hasn't been found before. This discovered connection successfully fulfill the aim for this section: better understand the MN's function. MNs' connection with ALS indicates MN is linked to the voluntary motor system or the somatosensory pathway. MN's connection with ASD, on the other hand, shows connection to socio-cognitive ability or the limbic pathway. MN's connection with the MBS exhibits connection to a specified part of the limbic pathway, the facial emotion. This provides convincing backing to the two anatomical pathway Ferrari etc. state to be present in the mirror mechanism [61].

The diseases are not only all individually related to MN, but also connected to each other (Fig. 10 left). Co-expression of REV3L and ATRX indicates MBS is connected with ASD. This connection falls within rationality since facial palsy would hinder emotion delivery which is a vital part of social communication. ATRX and SOD1 are co-expressed which suggests ASD to be linked with ALS. A major symptom of ASD is repetitive and characteristic behavioral patterns [43]. Whereas

ALS patients show impairment in the voluntary motor system. ASD and ALS's shared deficit in the motor system provides justification for their connection.

Discussion

With multiple comprehensive analysis on the Allen Brain Atlas database, I fulfilled my aim and beyond. By employing of differential gene analysis within the three major components of the MNS and gray matter as control, I found cluster 85 (excitatory layer 3-5 THEMIS UBE2F) to be the most likely region MN is located in. I used human cortical single-cell sequencing to find expressions of deterministic genes in neurogenerative disease and psychiatry disorders within cluster 85. This caused me to discover MN related diseases: MBS, ASD, and ALS and gained some understanding of MN's functional identity. With the help of the STRING network, I also uncovered connections between these diseases.

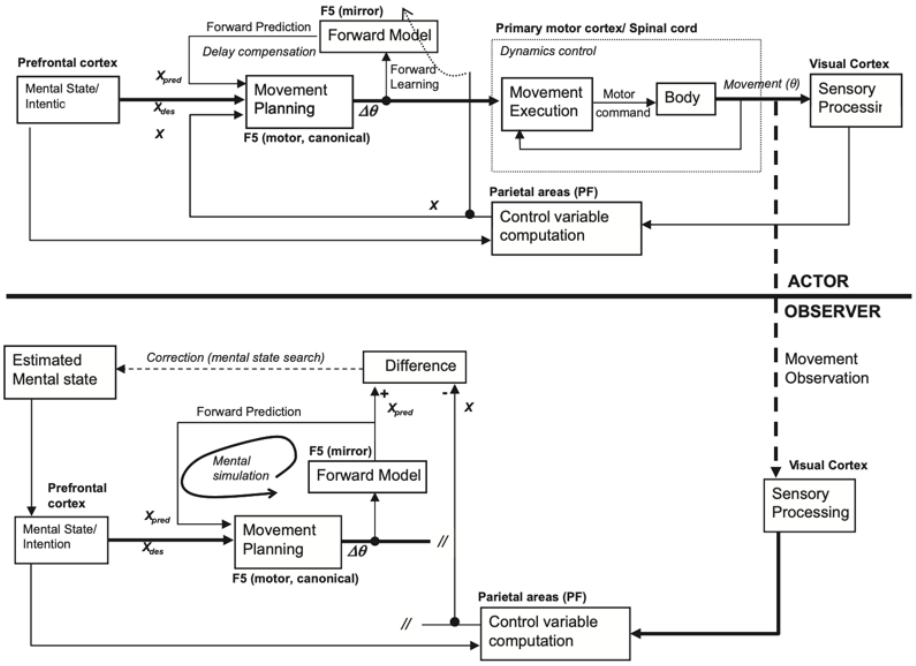

Fig. 11 Illustrated Mental State Inference (MSI) Model [62]

Nonetheless, it's still unclear how MNs functionally interact with the other neurons in the MNS circuitry. Nishimoto etal. proposed the MSI model which is only one of the numerous hypothesized computational models to explain mechanism within the MNS [63]. Finding the data backing those models can be very challenging since MNS is a unique structure in humans within the higher cognitive domain. Even though *Rhesus macaque* shares some similarity with human in MN related functions, significant differences still exist. Various methods of brain imaging could compensate each other and form a partial picture for humans. Namely fMRI has high temporal resolution, low spatial resolution while EEG has the opposite. The two methods combined could be of great help in event-related potentiation (ERP) [64] . After more detail regarding MN identity is uncovered, fluorescent in situ sequencing (FISSEQ) could also be used to assess RNA transcriptomes, barcoded connectomes, and time series data [65]. Nonetheless all methods remain un-invasive, so they would lack spatial differentiation and precision. In comparative to invasive measures like ECoGs which detects electrical impulse received by the implanted electrodes on the surface of the

brain [66]. This would increase both resolutions drastically at the same time however would require open craniotomy which can't happen on live humans.

To investigate the mechanism even on the basis of the most precise data is hard since MN in the limbic pathway is a highly subjective experience affected by various confounding variables. This is why finding MN identity is of such high significance. It would enable investigation not only on the macro-aspect (interaction across cortical areas), but also the micro-aspect (specific role of the identified cell cluster or genomes. For example, using CRISP-CAS9 to knock out MN related genes within the identified cluster on mice and perform experiments to see if its imitation is still intact [67] . The knock-out mice could refine molecular identity and uncover connection between impaired MNs and tasks revealing MN's function and connection. For example, knock-out mice could witness its pair experiencing electrical shock and see if its ACC are still activated, as it did in normal MN-intact mice [28].

My study has limitations that qualify my conclusions and invite future investigation. Firstly, the identification of cluster 85 derived from the calculation of sum and needs to be reinforced. The likelihood of it being the location of MN could increase by applying the Wilcoxon signed rank test, Benjamin Hochberg correction for multiple testing, exact permutation testing and so on [68]. Secondly, building on top of the identified cluster, the strength of MN's connection with MBS, ASD and ALS calls for further investigation. For example, genetic test could be performed on individual with congenital genetic conditions leading to MBS, ASD or ALS and see whether the detected variation includes cluster 85. Additionally, finding connections between MBS, ASD and ALS. Performing large scale medical investigation to find the number of shared genetic associations and the extent to which their associated proteins interact would be exceedingly helpful. It provides better understanding of pathology of those diseases which in turn could improve prevention and treatment of the diseases.

Works Cited

1. Cook, R., et al., Mirror neurons: from origin to function. Behav Brain Sci, 2014. 37(2): p. 177-92.

2. R.C. Miall, Connecting mirror neurons and forward models, Neuroreport 14 (2003) 2135–2137.

3. L. Carr, M. Iacoboni, M.-C. Dubeau, J.C. Mazziotta, G.L. Lenzi, Neural mechanisms of empathy in humans: a relay from neural systems for imitation to limbic areas, PNAS 100 (2003) 5497–5502.

4. Molenberghs, Pascal, et al. "The Role of the Superior Temporal Sulcus and the Mirro NeuronSystem in Imitation." Human Brain Mapping, Wiley Subscription Services, Inc., A Wiley Company, 19 Sept. 2010, www.ncbi.nlm.nih.gov/pmc/articles/PMC6870593/.

5. Iacoboni M; Koski LM; Brass M;Bekkering H;Woods RP;Dubeau MC;Mazziotta JC;Rizzolatti G;, M. "Reafferent Copies of Imitated Actions in the Right Superior Temporal Cortex." Proceedings of the National Academy of Sciences of the United States of America, U.S. National Library of Medicine, 2001, pubmed.ncbi.nlm.nih.gov/11717457/.

6. PL; McGregor HR; Gribble. "Changes in Visual and Sensory-Motor Resting-State Functional Connectivity Support Motor Learning by Observing." Journal of Neurophysiology, U.S. National Library of Medicine, 1999, pubmed.ncbi.nlm.nih.gov/25995349/.

7. JC;, Molnar-Szakacs I;Iacoboni M;Koski L;Mazziotta. "Functional Segregation within Pars Opercularis of the Inferior Frontal Gyrus: Evidence from FMRI Studies of Imitation and Action Observation." Cerebral Cortex (New York, N.Y. : 1991), U.S. National Library of Medicine, 2005, pubmed.ncbi.nlm.nih.gov/15513929/.

8. Editors. "Paracentral Lobule." Paracentral Lobule - an Overview | ScienceDirect Topics, 2021, www.sciencedirect.com/topics/veterinary-science-and-veterinary-medicine/paracentral-lobule.

9. Rizzolatti G, Fogassi L, Gallese V (2001) Neurophysiological mechanisms underlying the understanding and imitation of action. Nat Rev Neurosci 2:661–670.

10. Rizzolatti G, Fogassi L, Gallese V (2001) Neurophysiological mechanisms underlying the understanding and imitation of action. Nat Rev Neurosci 2:661–670.

11. G. Di Pellegrino, L. Fadiga, L. Fogassi, V. Gallese, G. Rizzolatti, Understanding motor events—a neurophysiological study, Experimental Brain Research 91 (1992) 176–180.

12. V. Gallese, L. Fadiga, L. Fogassi, G. Rizzolatti, Action recognition in the premotor cortex, Brain 119 (1996) 593–609.

13. G. Rizzolatti, L. Fadiga, V. Gallese, L. Fogassi, Premotor cortex and the recognition of motor actions, Cognitive Brain Research 3 (1996) 131–141.

14. Rizzolatti G, Luppino G (2001) The cortical motor system. Neuron 31:889–901.

15. Rizzolatti G, Fogassi L, Gallese V (2001) Neurophysiological mechanisms underlying the understanding and imitation of action. Nat Rev Neurosci 2:661–670.

16. Borra E, Belmalih A, Gerbella M, Rozzi S, Luppino G (2010) Projections of the hand field of the macaque ventral premotor area F5 to the brainstem and spinal cord. J Comp Neurol 518:2570–2591.

17. Bonini L (2015) The extended mirror neuron network: Anatomy, origin, and functions. Neuroscientist 25:1–12.

18. Maier MA, Kirkwood PA, Brochier T, Lemon RN (2013) Responses of single corticospinal neurons to intracortical stimulation of primary motor and premotor cortex in the anesthetized macaque monkey. J Neurophysiol 109:2982–2998.

19. Rizzolatti G, Fogassi L, Gallese V (2001) Neurophysiological mechanisms underlying the understanding and imitation of action. Nat Rev Neurosci 2:661–670.

20. Iacoboni M; Koski LM; Brass M; Bekkering H; Woods RP; Dubeau MC; Mazziotta JC; Rizzolatti G;, M. "Reafferent Copies of Imitated Actions in the Right Superior Temporal Cortex." Proceedings of the National Academy of Sciences of the United States of America, U.S. National Library of Medicine, 2001, pubmed.ncbi.nlm.nih.gov/11717457/.

21. PL; McGregor HR; Gribble. "Changes in Visual and Sensory-Motor Resting-State Functional Connectivity Support Motor Learning by Observing." Journal of Neurophysiology, U.S. National Library of Medicine, 1999, pubmed.ncbi.nlm.nih.gov/25995349/.

22. JC;, Molnar-Szakacs I;Iacoboni M;Koski L;Mazziotta. "Functional Segregation within Pars Opercularis of the Inferior Frontal Gyrus: Evidence from FMRI Studies of Imitation and Action Observation." Cerebral Cortex (New York, N.Y. : 1991), U.S. National Library of Medicine, 2005, pubmed.ncbi.nlm.nih.gov/15513929/.

23. Editors. "Paracentral Lobule." Paracentral Lobule - an Overview | ScienceDirect Topics, 2021, www.sciencedirect.com/topics/veterinary-science-and-veterinary-medicine/paracentral-lobule

24. González-Burgos, Guillermo, et al. "Distinct Properties of Layer 3 Pyramidal Neurons from Prefrontal and Parietal Areas of the Monkey Neocortex." Journal of Neuroscience, Society for Neuroscience, 11 Sept. 2019, www.jneurosci.org/content/39/37/7277.

25. PL; McGregor HR; Gribble. "Changes in Visual and Sensory-Motor Resting-State Functional Connectivity Support Motor Learning by Observing." Journal of Neurophysiology, U.S. National Library of Medicine, 1999, pubmed.ncbi.nlm.nih.gov/25995349/.

26. Cook, R., et al., Mirror neurons: from origin to function. Behav Brain Sci, 2014. 37(2): p. 177-92.

27. Bonini L (2015) The extended mirror neuron network: Anatomy, origin, and functions. Neuroscientist 25:1–12.

28. Emotional Mirror Neurons in the Rat's Anterior Cingulate Cortex Carrillo et al., 2019, Current Biology 29, 1301–1312 April 22, 2019, Published by Elsevier Ltd. https://doi.org/10.1016/j.cub.2019.03.024

29.Rayson, H., Bonaiuto, J., Ferrari, P. et al. Early maternal mirroring predicts infant motor system activation during facial expression observation. Sci Rep 7, 11738 (2017). https://doi.org/10.1038/s41598-017-12097-w

30. Sacks D;Baxter B;Campbell BCV;Carpenter JS;Cognard C;Dippel D;Eesa M;Fischer U;Hausegger K;Hirsch JA;Shazam Hussain M;Jansen O;Jayaraman MV;Khalessi AA;Kluck BW;Lavine S;Meyers PM;Ramee S;Rüfenacht DA;Schirmer CM;Vorwerk D. "Multisociety Consensus Quality Improvement Revised Consensus Statement for Endovascular Therapy of Acute Ischemic Stroke." International Journal of Stroke : Official Journal of the International Stroke Society, U.S. National Library of Medicine, May 2018, pubmed.ncbi.nlm.nih.gov/29786478/.

31. Jones, A., Overly, C. & Sunkin, S. The Allen Brain Atlas: 5 years and beyond. Nat Rev Neurosci 10, 821–828 (2009). https://doi.org/10.1038/nrn2722

32. Christian von Mering, Martijn Huynen, Daniel Jaeggi, Steffen Schmidt, Peer Bork, Berend Snel, STRING: a database of predicted functional associations between proteins, Nucleic Acids Research, Volume 31, Issue 1, 1 January 2003, Pages 258–261, https://doi.org/10.1093/nar/gkg034

33. Kristoffer Forslund Peer Bork Michael Kuhn, Damian Szklarczyk Milan Simonovic Christian von Mering. STRING, Swiss Institute of Bioinformatics, European Molecular Biology Labitory, Novo Nordisk Foundation Center for Protein Research, 2021, string-db.org/cgi/about?footer_active_subpage=content.

34. Sasha Babicki, David Arndt, Ana Marcu, Yongjie Liang, Jason R. Grant, Adam Maciejewski, and David S. Wishart. Heatmapper: web-enabled heat mapping for all. Nucleic Acids Res. 2016 May 17 (epub ahead of print). doi:10.1093/nar/gkw419

35. Molenberghs, Pascal, et al. "The Role of the Superior Temporal Sulcus and the Mirror Neuron System in Imitation." Human Brain Mapping, Wiley Subscription Services, Inc., A Wiley Company, Sept. 2010, www.ncbi.nlm.nih.gov/pmc/articles/PMC6870593/

36. Neurosci. "Know Your Brain: Posterior Parietal Cortex." Neuroscientifically Challenged Neuroscientifically Challenged, 26 Jan. 2021, www.neuroscientificallychallenged.com/blog/know-your-brain-posterior-parietal-cortex.

37. Publishers. "Paracentral Lobule." Wikiwand, www.wikiwand.com/en/Paracentral_lobule.

38. publishers. "Cck Gene Cards ." GeneCards, 2021, www.genecards.org/cgi-bin/carddisp.pl?gene=CCK#:~:text=CCK%20(Cholecystokinin)%20is%20a%20Protein,receptors%20and%20Signaling%20by%20GPCR.

39. González-Burgos, Guillermo, et al. "Distinct Properties of Layer 3 Pyramidal Neurons from Prefrontal and Parietal Areas of the Monkey Neocortex." Journal of Neuroscience, Society for Neuroscience, 11 Sept. 2019, www.jneurosci.org/content/39/37/7277.

40. Rubinstein, Yaffa R, and Pamela McInnes. "NIH/NCATS/GRDR® Common Data Elements: A Leading Force for Standardized Data Collection." Contemporary Clinical Trials, Elsevier, 20 Mar. 2015, www.sciencedirect.com/science/article/pii/S1551714415000476.

41. Choi S; Cornall R; Lesourne R; Love. “THEMIS: Two Models, Different Thresholds.” Trends in Immunology, U.S. National Library of Medicine, 2017, pubmed.ncbi.nlm.nih.gov/28697966/.

42. Clare AJ; Wicky HE; Empson RM;Hughes. “RNA-Sequencing Analysis Reveals a Regulatory Role for Transcription Factor Fezf2 in the Mature Motor Cortex.” Frontiers in Molecular Neuroscience, U.S. National Library of Medicine, 2017, pubmed.ncbi.nlm.nih.gov/28936162/.

43. “Nimh " Autism Spectrum Disorder.” National Institute of Mental Health, U.S. Department of Health and Human Services, 2021, www.nimh.nih.gov/health/topics/autism-spectrum-disorders-asd.

44. publishers. “What Is Facial Palsy?” Facial Palsy UK, 2 June 2021, www.facialpalsy.org.uk/inform/what-is-facial palsy/#:~:text=The%20term%20facial%20palsy%20generallyin%20order%20to%20function%20properly

45. Editors. “Neurodegenerative Diseases.” National Institute of Environmental Health Sciences, U.S. Department of Health and Human Services, 2021, www.niehs.nih.gov/research/supported/health/neurodegenerative/index.cfm.

46. Edited by Mindspec, Human Gene Search Results, 2017, autism.mindspec.org/autdb/submitsearch?selfld_0=GENE_CHR_NUM&selfldv_0=X&numOfFields=1&userAction=search&tableName=AUT_HG&submit=Submit%2BQueryy.

47. Patel RM, Liu D, Gonzaga-Jauregui C, et al. An exome sequencing study of Moebius syndrome including atypical cases reveals an indi- vidual with CFEOM3A and a TUBB3 mutation. Cold Spring Harb Mol Case Stud. 2017;3(2):a000984. https://doi.org/10.1101/mcs. a000984.

48. van der Zwaag B, Hellemons AJCGM, Leenders WPJ, et al. PLEXIN- D1, a novel plexin family member, is expressed in vascular endothelium and the central nervous system during mouse embryo-genesis. Dev Dyn. 2002;225(3):336-343. https://doi.org/10.1002/ dvdy.10159

49. Tomas-Roca L, Tsaalbi-Shtylik A, Jansen JG, et al. De novo mutations in PLXND1 and REV3L cause Möbius syndrome. Nat Commun. 2015; 6:7199. https://doi.org/10.1038/ncomms8199.

50. van der Zwaag, B., Burbach, J. P. H., Scharfe, C., Oefner, P. J., Brunner, H. G., Padberg, G. W., van Bokhoven, H. Identifying new candidate genes for hereditary facial paresis on chromosome 3q21-q22 by RNA in situ hybridization in mouse. Genomics 86: 55-67, 2005.

51. Hagenah J; Saunders-Pullman R; Hedrich K; Kabakci K; Habermann K;Wiegers K;Mohrmann K;Lohnau T;Raymond D;Vieregge P;Nygaard T;Ozelius LJ;Bressman SB;Klein C; “High Mutation Rate in Dopa-Responsive Dystonia: Detection with Comprehensive GCHI Screening.” Neurology, U.S. National Library of Medicine, 2005, pubmed.ncbi.nlm.nih.gov/15753436/.

52. Ichinose H; Suzuki T; Inagaki H; Ohye T; Nagatsu T; “Molecular Genetics of Dopa-Responsive Dystonia.” Biological Chemistry, U.S. National Library of Medicine, 1999, pubmed.ncbi.nlm.nih.gov/10661862/.

53. T Nagatsu. “[Molecular Mechanisms of Neurotransmission].” Rinsho Shinkeigaku = Clinical Neurology, U.S. National Library of Medicine, 2000, pubmed.ncbi.nlm.nih.gov/11464453/.

54. "Hypomimia." Medical Dictionary. 2009. Farlex and Partners 10 Aug. 2020 https://medical-dictionary.thefreedictionary.com/hypomimia

55. J.G. Goldman, in Encyclopedia of Movement Disorders, 2010

56. Publishers. “Alzheimer's Disease Genetics Fact Sheet.” National Institute on Aging, U.S. Department of Health and Human Services, 2021, www.nia.nih.gov/health/alzheimers-disease-genetics-fact-sheet.

57. “Parkinson Disease: MedlinePlus Genetics.” MedlinePlus, U.S. National Library of Medicine, 18 Aug. 2020,medlineplus.gov/genetics/condition/parkinson disease/:~:text=Familial%20cases%20of%20Parkinson%20disease,be%20sporadic%20(not%20inherited)

58. Ghasemi, Mehdi, and Robert H Brown. “Genetics of Amyotrophic Lateral Sclerosis.” Cold Spring Harbor Perspectives in Medicine, Cold Spring Harbor Laboratory Press, 1 May 2018, www.ncbi.nlm.nih.gov/pmc/articles/PMC5932579/.

59. “Moebius Syndrome.” Johns Hopkins Medicine, 2021, www.hopkinsmedicine.org/health/conditions-and-diseases/moebius-syndrome#:~:text=Moebius%20syndrome%20is%20a%20rare,for%20speech%2C%20chewing%20and%20swallowing.

60. “Amyotrophic Lateral Sclerosis (Als).” Mayo Clinic, Mayo Foundation for Medical Education and Research, 6 Aug. 2019, www.mayoclinic.org/diseases-conditions/amyotrophic-lateral-sclerosis/symptoms-causes/syc-20354022#:~:text=Amyotrophic%20lateral%20sclerosis%20(a%2Dmy,who%20was%20diagnosed%20with%20it.

61. S;, Ferrari PF;Gerbella M;Coudé G;Rozzi. “Two Different Mirror Neuron Networks: The Sensorimotor (HAND) and LIMBIC (FACE) PATHWAYS.” Neuroscience, U.S. National Library of Medicine, 2017, pubmed.ncbi.nlm.nih.gov/28687313/.

62. E. Oztop, D. Wolpert, M. Kawato, Mental state inference using visual control parameters, Brain Research. Cognitive Brain Research 22 (2005) 129–151.

63. MA;, Oztop E;Kawato M;Arbib. “Mirror Neurons: Functions, Mechanisms and Models.” Neuroscience Letters, U.S. National Library of Medicine, 2013, pubmed.ncbi.nlm.nih.gov/23063951/.

64. Eryilmaz HH;Duru AD;Parlak B;Ademoglu A;Demiralp T; “Neuroimaging of Event Related Brain Potentials (Erp) Using FMRI and DIPOLE Source Reconstruction.” Annual International Conference of the IEEE Engineering in Medicine and Biology Society. IEEE Engineering in Medicine and Biology Society. Annual International Conference, U.S. National Library of Medicine, 2007, pubmed.ncbi.nlm.nih.gov/18002723/.

65. Church, George M. “Brain: Innovative Neurotechnologies for Imaging and Therapeutics.” Dialogues in Clinical Neuroscience, Les Laboratoires Servier, Sept. 2013, www.ncbi.nlm.nih.gov/pmc/articles/PMC3811097/.

66. Hill NJ;Gupta D;Brunner P;Gunduz A;Adamo MA;Ritaccio A;Schalk G; “Recording Human Electrocorticographic (Ecog) Signals for Neuroscientific Research and Real-Time Functional Cortical

Mapping." Journal of Visualized Experiments : JoVE, U.S. National Library of Medicine, June 2012, pubmed.ncbi.nlm.nih.gov/22782131/.

67. Eryilmaz HH;Duru AD;Parlak B;Ademoglu A;Demiralp T; "Neuroimaging of Event Related Brain Potentials (Erp) Using FMRI and DIPOLE Source Reconstruction." Annual International Conference of the IEEE Engineering in Medicine and Biology Society. IEEE Engineering in Medicine and Biology Society. Annual International Conference, U.S. National Library of Medicine, Sept. 2020, pubmed.ncbi.nlm.nih.gov/18002723/.

68. Preisser JS, Sen PK, Offenbacher S. "Multiple Hypothesis Testing for Experimental Gingivitis Based on Wilcoxon Signed Rank Statistics." Europe Pmc, Europe PMC, 2011, europepmc.org/article/PMC/3186946.

Acknowledgements

Thank you, Mr. Varty for your useful suggestion regarding the language of this essay, particularly its grammar and clarity.