Dnmt3a2/Dnmt3L

This study investigates how DNA methylation regulates motor behavior in single neuron type resolution. This topic is important for understanding that the behaviors like hyperactivity in relevant diseases are also regulated by epigenetic factors. Although accumulative researches have demonstrated that epigenetic factor is a potential regulator for brain function, the specific role of these factors in certain type of neurons is still unclear, especially in motor neurons, has not been studied. We aim to examine if the DNA methylation level of neurons are regulated by DNA methyltransferase and how is the consequence in locomotion.

The study generated a transgenic mouse model with overexpression of Dnmt3a2 and Dnmt3L, the DNA methyltransferase and its partner, in dopaminergic neurons which controls locomotor function. We found that the DNA methylation level was up-regulated in neurons with overexpression, and the spontaneous activity and exercise performance of the mice were increased significantly. Furthermore, the higher fire frequency and excitability of dopaminergic neuron were detected without dopaminergic biosynthesis change.

Dnmt3a2, a de novo DNA methyltransferase, is induced by neuronal activity and participates in long-term memory formation with the increased expression of synaptic plasticity genes.

1. Introduction

Accumulative studies have demonstrated epigenetic factors as a potent regulator for brain function in the nervous system. Epigenetics encompasses modifying changes in DNA and/or chromatin without altering the basic genetic code, involving DNA methylation, histone post-translational modifications, and post-transcriptional regulation by non-coding RNAs (ncRNAs)[1][2][3]. DNA methylation as one of the major epigenetic processes is mediated by DNA methyltransferases. These enzymes regulate many important cellular processes involved in neuronal activity and brain functions[4][5][6], such as neuron survival, differentiation, memory formation, and several neuropsychological impairments. De novo DNA methyltransferase Dnmt3a2, the shorter isoform of Dnmt3a[7], functions by adding a methyl group onto the C5 position of cytosine to form 5-methylcytosine, the dominant form of DNA methylation in mammals[8]. Dnmt3L, the non-enzymatic cofactor, directly enhances Dnmt enzyme activity[9] and specifically interacts with Dnmt3a2 in the nucleus to stimulate regional DNA methylation in mouse embryonic stem cells[10]. Moreover, Dnmt3L is specifically required by Dnmt3a2 upon functioning in mouse gonocytes[9], indicating the pivotal role of Dnmt3L for the function of Dnmt3a2. Recently, the role of Dnmt3a2 in the brain was revealed. It was reported that Dnmt3a2 expression can be transiently induced by neuronal activity, which in turn induces the expression of synaptic plasticity genes and memory formation[11][12][13]. Enhanced fear-conditioning in old mice was observed during transient over-expression of Dnmt3a2 in the hippocampus. In contrast, transient knock-down of Dnmt3a2 resulted in cognitive deficits[11], indicating that Dnmt3a2 could be a key modulator in regulating neuronal functions regarding learning and cognition. In addition, Dnmt1 and Dnmt3a1 double knock-out mice, as well as single Dnm3a knock-out mice have learning deficits[14][15]. A key regulator in specific synaptic changes is dopamine (DA), particularly in the hippocampal-prefrontal working memory network[16]. Authors therefore wanted to determine whether Dnmt3a2 with its cofactor Dnmt3L (Dnmt3a2/3L) influences DA activity. To this end, a dopaminergic neuron-specific Dnmt3a2/3L overexpression transgenic mouse line (Dnmt3a2/3LDat/wt) was generated. The dopaminergic neurons of the substantia nigra pars compacta (SNc) have been intensively studied and their electrophysiological properties are well known[17]. Their firing characteristics are homogeneous, therefore even subtle deviations from the common pattern can be detected.

2. Effect of Dnmt3a2/Dnmt3L Overexpression

The role of Dnmts were so far mostly investigated in mammalian development[18]. Previous studies demonstrated that Dnmts including Dnmt1 and Dnmt3a are required for plasticity in adult forebrain neurons[14], and that hippocampal Dnmt3a2 is essential for cognitive functions in adult mice[11][12][13]. In our study, we uncovered a novel role of Dnmt3a2/3L for regulation of locomotor function and spontaneous activity specifically in the population of dopaminergic neurons.

We showed that Dnmt3a2/3L overexpression in DA SNc neurons increases the firing frequency and excitability of SNc DA neurons from Dnmt3a2/3L$^{Dat/wt}$ mice. SNc neurons project to the striatum, forming the nigrostriatal pathway. A key feature of these neurons is their steady, autonomous pacemaking which maintains extracellular DA levels necessary for basal ganglia network operation[19]. We hypothesized that because of the higher SNc fire frequency, locomotor activity would be increased as well. Indeed, we found higher spontaneous motor activity of young adult Dnmt3a2/3L$^{Dat/wt}$ mice in cylinder and open field tests. This indicates that Dnmt3a2/3L overexpression regulates motor activity on the behavioral level through neuronal activity of nigrostriatal DA neurons.

We further expected that higher SNc fire frequency would increase tonic dopamine release and thus dopamine tissue concentration and/or metabolite concentration in Dnmt3a2/3L$^{Dat/wt}$ mice. Interestingly, ex vivo whole tissue analysis revealed that striatal DA concentration was the same in both groups. Furthermore, concentrations of the DA metabolites 3MT, DOPAC and HVA were significantly lower in Dnmt3a2/3L$^{Dat/wt}$ mice, indicating that long-term DA synthesis was not increased. Earlier studies, where a short-term increase of fire frequency was induced by electrical stimulation of the medial forebrain bundle, demonstrated a frequency-dependent release of dopamine [20], increased activity of tyrosine hydroxylase[19] and elevated tissue concentration of DOPAC[21]. However, DA tissue concentration remained constant[21]. A recent study employing deep brain stimulation of the medial forebrain bundle in rats confirmed that striatal DA release was elevated at onset of stimulation[22], but data on long-term effects are still lacking. DA release is strongly controlled by several mechanisms, including dopamine transporter activity (reuptake), D2 autoreceptor activity (inhibition of DA release), glutamatergic activity of neighboring synapses (H_2O_2 as transsynaptic messenger), and cholinergic activity (via muscarinergic receptors) [23]. Thus, DA fire frequency provides temporal information, while the quantity of DA release and therefore DA synthesis may be regulated independent from firing rate.

To examine DA synthesis capacity in vivo, we performed PET imaging with the tracer 6-[^{18}F]FMT. We have chosen this dopamine analog because it is not a substrate of the degrading enzyme catechol *O*-methyl transferase (COMT), and is only metabolized by amino acid decarboxylase (AADC; the enzyme that converts L-DOPA into DA) and subsequently monoamine oxidase (MAO)[24]. 30 min after injection, most of brain radioactivity is derived from the metabolite 6-[^{18}F]fluoro-3-hydroxyphenylacetic acid (6-[^{18}F]FPAC)[25]. Because 6-[^{18}F]FPAC does not cross the blood–brain barrier, it is trapped in the brain[25]. When peripheral AADC is blocked, e.g., by benserazide, it is assumed that 6-[^{18}F]FMT uptake mainly shows cerebral AADC activity with high image contrast[26]. Although AADC is not the rate-limiting step of DA synthesis, it has been shown that the enzyme is regulatable[27]. In the striatum, where dopaminergic input is dominating, AADC activity is thought to reflect presynaptic DA biosynthesis. In extrastriatal areas, such as the thalamus and midbrain, a considerable amount of 6-[^{18}F]FMT is taken up by other monoaminergic presynaptic terminals containing noradrenaline and serotonin as transmitters [28]. In addition, monoenzymatic AADC neurons, e.g., in the nucleus of the solitary tract[29], take up 6-[^{18}F]FMT as well. Although global 6-[^{18}F]FMT uptake is a mixture of DA and non-DA AADC activity, we found a tendency of increased global uptake in Dnmt3a2/3L$^{Dat/wt}$ mice. In the striatum, however, there was no significant difference between groups, indicating that DA biosynthesis was not increased in nigrostriatal presynaptic terminals of Dnmt3a2/3L$^{Dat/wt}$ mice. In contrast, we found a significantly elevated 6-[^{18}F]FMT uptake in the left ventral pallidum of Dnmt3a2/3L$^{Dat/wt}$ mice. The ventral pallidum receives DA afferents from both SNc and VTA and is involved in reward signaling[30]. Increased dopamine biosynthesis indicates stronger activation of reward pathways in Dnmt3a2/3L$^{Dat/wt}$ mice.

In addition, 6-[^{18}F]FMT uptake was also increased in the hypothalamus and the pituitary gland indicating increased DA signaling in the tuberoinfundibular pathway of Dnmt3a2/3L$^{Dat/wt}$ mice. This pathway originates in the hypothalamic arcuate nucleus and projects to the pituitary gland, where DA inhibits prolactin release[31]. Prolactin is involved in the regulation of energy and metabolic homeostasis[32], and increases proportionally to exercise intensity[33].

The other areas where 6-[^{18}F]FMT uptake was significantly increased in Dnmt3a2/3L$^{Dat/wt}$ mice (piriform and sensory cortex) receive only weak DA inputs. We suspect that 6-[^{18}F]FMT uptake changes in these brain regions reached statistical significance more easily because of low baseline AADC activity, in contrast to regions where AADC activity is already very high in wildtype mice (e.g., striatum). Taken together, in vivo 6-[^{18}F]FMT PET results support our ex vivo tissue analysis finding that striatal DA biosynthesis was not increased in Dnmt3a2/3L$^{Dat/wt}$ mice. We can only speculate about how increased nigrostriatal firing may have led to higher locomotor activity without increasing DA synthesis. When

inter-spike intervals are shorter than 200 ms, DA cannot be completely cleared from the synaptic cleft before the the next release[34]. This may lead to an overstimulation of postsynaptic receptors, facilitating movement[35]. Further experiments are needed to investigate the relationship between SNc neuronal activity and locomotion.

Next, we wanted to investigate if neuronal activity in motor regions including the basal ganglia and/or the hypothalamus were altered in Dnmt3a2/3L$^{Dat/wt}$ mice. To this end we used metabolic PET imaging with the tracer [^{18}F]FDG, which reflects synaptic activity[36][37][38]. We compared [^{18}F]FDG uptake during two behavioral settings, continuous treadmill running and staying in the home cage. This was done with all animals pooled to describe the basic motor patterns. During running, we found increased activity in the medial part of the cerebellum in both groups. This region corresponds to the cerebellar motor region, which is involved in the control of rhythmic movements[39]. The dorsal hippocampus was also activated during running, probably caused by an increase of theta frequency during locomotor behavior[40]. Striatum and sensory cortices were more active during home cage activity, which may reflect random non-automatic motor behaviors such as sporadic walking, grooming, and gnawing. The motor cortex-basal ganglia loops are important for initiation of movement[41], but are no longer needed to maintain rhythmic automatic activity such as running, which leads to a lower activity in the treadmill condition.

For group comparison, we analyzed the difference images "treadmill minus homecage". They correspond to the pattern described above, i.e., they reflect the individual activity changes between the two behavioral conditions. No significant group differences were present in the areas described above. While this indicates that general motor patterns were the same in Dnmt3a2/3L$^{Dat/wt}$ mice and controls, we found significant differences between groups in other areas. A negative cluster emerged in the right deep cerebellar nuclei and the periaqueductal gray, indicating that "treadmill minus home cage" was greater in controls compared to Dat-Dnmt3a2/3L mice. This was driven by a group difference in the home cage condition, where the deep cerebellar nuclei and the periaqueductal gray were more active in Dnmt3a2/3L$^{Dat/wt}$ mice during their home cage stay compared to controls. However, no group difference was seen in these brain areas during running. This result indicates that brain activation patterns were different between groups when the animals engaged in random home cage activity. These differences disappeared when the mice were involved in treadmill running. The home cage differences may therefore merely reflect the individual behaviors of the mice during the 40 min uptake period, rather than inherent activity differences related to the transgene.

However, this explanation does not account for the hypothalamus. Dnmt3a2/3L$^{Dat/wt}$ mice showed a lower hypothalamic activity in the home cage condition, statistically significant in the medial preoptic nucleus and the ventromedial hypothalamus. During treadmill running, hypothalamic activity was higher compared to controls, significant in the lateral preoptic nucleus. These results suggest that hypothalamic activity was more tightly linked to motor behaviour in Dnmt3a2/3L$^{Dat/wt}$ mice compared to controls. With 6-[^{18}F]FMT we have found a higher dopaminergic activity in the hypothalamus, which was correlated to the metabolic activity changes observed with [^{18}F]FDG. Hypothalamic dopamine plays a crucial role in central fatigue mechanisms. The serotonin-to-dopamine ratio in the hypothalamus is positively correlated with the time to fatigue[42]. Higher dopamine concentrations, particularly in the preoptic area, block the signal for exercise cessation[43] and lead to higher motor performance [44]. The higher motor activity of Dat-Dnmt3a2/3L mice may therefore be caused by increased dopamine signaling in the hypothalamus rather than in the nigrostriatal system.

References

1. Jones, M.J.; Goodman, S.J.; Kobor, M.S. DNA methylation and healthy human aging. Aging Cell 2015, 14, 924–932.

2. Greer, E.L.; Blanco, M.A.; Gu, L.; Sendinc, E.; Liu, J.; Aristizábal-Corrales, D.; Chih-Hung, H.; Aravind, L.; Chuan, H.; Yang, S. DNA methylation on N 6-adenine in C. elegans. Cell 2015, 161, 868–878.

3. Weber, M.; Hellmann, I.; Stadler, B.M.; Ramos, L.; Svante, P.; Rebhan, M.; Schubeler, D. Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human genome. Nat. Genet. 2007, 39, 457.

4. Gibney, E.R.; Nolan, C.M. Epigenetics and gene expression. Heredity 2010, 105, 4–11.

5. Bayraktar, G.; Kreutz, M.R. Neuronal DNA Methyltransferases: Epigenetic Mediators between Synaptic Activity and Gene Expression? Neuroscience 2017, 24, 171–185.

6. Cui, D.; Xu, X. DNA Methyltransferases, DNA Methylation, and Age-Associated Cognitive Function. Int. J. Mol. Sci. 2018, 19, 1315.

7. Chen, T.; Ueda, Y.; Xie, S.; Li, E. A novel Dnmt3a isoform produced from an alternative promoter localizes to euchromatin and its expression correlates with active de novo methylation. J. Biol. Chem. 2002, 277, 38746–38754.

8. Moore, L.D.; Le, T.; Fan, G. DNA methylation and its basic function. Neuropsychopharmacology 2013, 38, 132–146.

9. Suetake, I.; Shinozaki, F.; Miyagawa, J.; Takeshima, H.; Tajima, S. DNMT3L stimulates the DNA methylation activity of Dnmt3a and Dnmt3b through a direct interaction. J. Biol. Chem. 2004, 279, 27816–27823.

10. Nimura, K.; Ishida, C.; Koriyama, H.; Hata, K.; Yamanaka, S.; Li, E.; Ura, K.; Kaneda, Y. Dnmt3a2 targets endogenous Dnmt3L to ES cell chromatin and induces regional DNA methylation. Genes Cells 2006, 11, 1225–1237.

11. Oliveira, A.M.M.; Hemstedt, T.J.; Bading, H. Rescue of aging-associated decline in Dnmt3a2 expression restores cognitive abilities. Nat. Neurosci. 2012, 15, 1111.

12. Oliveira, A.M.M.; Hemstedt, T.J.; Freitag, H.E.; Bading, H. Dnmt3a2: a hub for enhancing cognitive functions. Mol. Psychiatry 2016, 21, 1130–1136.

13. Karaca, K.G.; Kupke, J.; Brito, D.V.C.; Zeuch, B.; Thome, C.; Weichenhan, D.; Lutsik, P.; Plass, C.; Oliveira, A.M.M. Neuronal ensemble-specific DNA methylation strengthens engram stability. Nat. Commun. 2020, 11, 1–13.

14. Feng, J.; Zhou, Y.; Campbell, S.L.; Le, T.; Li, E.; Sweatt, J.D.; Silva, A.J.; Fan, G. Dnmt1 and Dnmt3a maintain DNA methylation and regulate synaptic function in adult forebrain neurons. Nat. Neurosci. 2010, 13, 423–430.

15. Morris, M.J.; Adachi, M.; Na, E.S.; Monteggia, L.M. Selective role for DNMT3a in learning and memory. Neurobiol. Learn. Mem. 2014, 115, 30–37.

16. Jay, T.M. Dopamine: A potential substrate for synaptic plasticity and memory mechanisms. Prog. Neurobiol. 2003, 69, 375–390.

17. Krashia, P.; Martini, A.; Nobili, A.; Aversa, D.; D'Amelio, M.; Berretta, N.; Guatteo, E.; Mercuri, N.B. On the properties of identified dopaminergic neurons in the mouse substantia nigra and ventral tegmental area. Eur. J. Neurosci. 2017, 45, 92–105.

18. Okano, M.; Bell, D.W.; Haber, D.A.; Li, E. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell 1999, 99, 247–257.

19. Guzman, J.N.; Sánchez-Padilla, J.; Chan, C.S.; Surmeier, D.J. Robust pacemaking in substantia nigra dopaminergic neurons. J. Neurosci. 2009, 29, 11011–11019.

20. Manley, L.D.; Kuczenski, R.; Segal, D.S.; Young, S.J.; Groves, P.M. Effects of frequency and pattern of medial forebrain bundle stimulation on caudate dialysate dopamine and serotonin. J. Neurochem. 1992, 58, 1491–1498.

21. Roth, R.H.; Murrin, L.C.; Walters, J.R. Central dopaminergic neurons: Effects of alterations in impulse flow on the accumulation of dihydroxyphenylacetic acid. Eur. J. Pharmacol. 1976, 36, 163–171.

22. Klanker, M.; Feenstra, M.; Willuhn, I.; Denys, D. Deep brain stimulation of the medial forebrain bundle elevates striatal dopamine concentration without affecting spontaneous or reward-induced phasic release. Neuroscience 2017, 364, 82–92.

23. Cachope, R.; Cheer, J.F. Local control of striatal dopamine release. Front. Behav. Neurosci. 2014, 8, 188.

24. DeJesus, O.T.; Sunderland, J.J.; Nickles, J.R.; Mukherjee, J.; Appelman, E.H. Synthesis of radiofluorinated analogs of m-tyrosine as potential L-dopa tracers via direct reaction with acetylhypofluorite. Int. J. Radiat. Appl. Instrument. Part A. Appl. Radiat. Isot. 1990, 41, 433–437.

25. Jordan, S.; Bankiewicz, K.S.; Eberling, J.L.; VanBrocklin, H.F.; O'neil, J.P.; Jagust, W.J. An in vivo microdialysis study of striatal 6-[18 F] fluoro-l-m-tyrosine metabolism. Neurochem. Res. 1998, 23, 513–517.

26. DeJesus, O.T.; Endres, C.J.; Shelton, S.E.; Nickles, R.J.; Holden, J.E. Evaluation of fluorinated m-tyrosine analogs as PET imaging agents of dopamine nerve terminals: comparison with 6-fluoroDOPA. J. Nucl. Med. 1997, 38, 630–636.

27. Hadjiconstantinou, M.; Neff, N.H. Enhancing aromatic L-amino acid decarboxylase activity: implications for L-DOPA treatment in Parkinson's disease. CNS Neurosci. Ther. 2008, 14, 340–351.

28. Brown, W.D.; DeJesus, O.T.; Pyzalski, R.W.; Malischke, L.; Roberts, A.D.; Shelton, S.E.; Uno, H.; Houser, W.D.; Nickles, R.J.; Holden, J.E. Localization of trapping of 6-[18F] fluoro-L-m-tyrosine, an aromatic L-amino acid decarboxylase tracer for PET. Synapse 1999, 34, 111–123.

29. Ugrumov, M. V Non-dopaminergic neurons partly expressing dopaminergic phenotype: distribution in the brain, development and functional significance. J. Chem. Neuroanat. 2009, 38, 241–256.

30. Stout, K.A.; Dunn, A.R.; Lohr, K.M.; Alter, S.P.; Cliburn, R.A.; Guillot, T.S.; Miller, G.W. Selective enhancement of dopamine release in the ventral pallidum of methamphetamine-sensitized mice. ACS Chem. Neurosci. 2016, 7, 1364–1373.

31. Moore, K.E. Interactions between prolactin and dopaminergic neurons. Biol. Reprod. 1987, 36, 47–58.

32. Tovar, S.; Diéguez, C. Prolactin and energy homeostasis: pathophysiological mechanisms and therapeutic considerations. Endocrinology 2014, 155, 659–662.

33. Hackney, A.C.; Davis, H.C.; Lane, A.R. Growth hormone-insulin-like growth factor axis, thyroid axis, prolactin, and exercise. Sports Endocrinol. 2016, 47, 1–11.

34. Sulzer, D.; Cragg, S.J.; Rice, M.E. Striatal dopamine neurotransmission: regulation of release and uptake. Basal Ganglia 2016, 6, 123–148.

35. Korchounov, A.; Meyer, M.F.; Krasnianski, M. Postsynaptic nigrostriatal dopamine receptors and their role in movement regulation. J. Neural Transm. 2010, 117, 1359–1369.

36. Schwartz, W.J.; Smith, C.B.; Davidsen, L.; Savaki, H.; Sokoloff, L.; Mata, M.; Fink, D.J.; Gainer, H. Metabolic mapping of functional activity in the hypothalamo-neurohypophysial system of the rat. Science 1979, 205, 723–725.

37. Raichle, M.E.; Mintun, M.A. Brain work and brain imaging. Annu. Rev. Neurosci. 2006, 29, 449–476.

38. Dienel, G.A.; Behar, K.L.; Rothman, D.L. Cellular origin of [18F] FDG-PET imaging signals during Ceftriaxone-stimulated glutamate uptake: astrocytes and neurons. Neuroscience 2018, 24, 316–328.

39. Manto, M.; Bower, J.M.; Conforto, A.B.; Delgado-García, J.M.; Da Guarda, S.N.F.; Gerwig, M.; Habas, C.; Hagura, N.; Ivry, R.B.; Mariën, P. Consensus paper: roles of the cerebellum in motor control—The diversity of ideas on cerebellar involvement in movement. Cerebellum 2012, 11, 457–487.

40. Li, J.-Y.; Kuo, T.B.J.; Hsieh, I.-T.; Yang, C.C.H. Changes in hippocampal theta rhythm and their correlations with speed during different phases of voluntary wheel running in rats. Neuroscience 2012, 213, 54–61.

41. Friend, D.M.; Kravitz, A. V Working together: Basal ganglia pathways in action selection. Trends Neurosci. 2014, 37, 301–303.

42. Meeusen, R.; Watson, P.; Hasegawa, H.; Roelands, B.; Piacentini, M.F. Central fatigue. Sport. Med. 2006, 36, 881–909.

43. Cordeiro, L.M.S.; Rabelo, P.C.R.; Moraes, M.M.; Teixeira-Coelho, F.; Coimbra, C.C.; Wanner, S.P.; Soares, D.D. Physical exercise-induced fatigue: the role of serotonergic and dopaminergic systems. Braz. J. Med. Biol. Res. 2017, 50.

44. Martyn, C.N.; Osmond, C.; Edwardson, J.A.; Barker, D.J.P.; Harris, E.C.; Lacey, R.F. Geographical relation between Alzheimer's disease and aluminium in drinking water. Lancet 1989, 333, 59–62.