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The suprachiasmatic nucleus and Alzheimer's disease (AD): AD is the most frequently diagnosed form of dementia, with the total number of AD patients worldwide expected to triple by 2050 compared to 2015 (Prince et al., 2015). Despite years of research, much of the AD pathology remains unclear with no treatment or cure available. Besides its two hallmarks, amyloid-β (Aβ) plagues and hyperphosphorylated tau tangles, a distortion of circadian rhythms is commonly observed. Furthermore, poor sleep quality or trouble falling asleep are common AD symptoms, sometimes developing 10–15 years before cognitive symptoms associated with AD (Ju et al., 2014).

The suprachiasmatic nucleus (SCN), a small part of the brain located in the hypothalamus, has an important place in the biology of sleep (Foster, 2020). Individual SCN neurons possess the ability to generate circadian rhythms and synchronize the expression of several genes and proteins to the light-dark cycle. Consequently, the SCN is often quoted as the central or master circadian clock in mammals. Several studies toward AD patients found a disrupted circadian rhythm and neurodegeneration in the SCN (Wang et al., 2015). Therefore, it has been suggested that loss of integrity and neural dysfunction in the SCN could be the consequence or even the cause of AD development. In the SCN, γ-aminobutyric acid (GABA) is the principal neurotransmitter and is produced and received by almost all neurons in the SCN. It is known to play an important role in intercellular signaling and tissue-level rhythm synchronization (DeWoskin et al., 2015). Furthermore, the inflammatory inhibiting properties of GABA are generally accepted. Although its dense distribution in the SCN, the exact role GABA plays in the SCN is not fully understood. Among various possible scenarios for loss of integrity and neuronal dysfunction of SCN during AD progression, it is tempting to believe a dysregulation of GABA signaling in SCN during AD. The evidence for SCN abnormalities seen in AD is mainly derived from post-mortem studies. Sleep abnormalities and disturbed circadian rhythms have also been reported for AD animal models (Kress et al., 2018). Significant neurodegeneration and inflammatory responses such as an increase in reactive astrocytes have been reported in the SCN of AD mice, combined with circadian rhythmic disruption and sleep abnormalities (Roy et al., 2019).

It remains largely unknown how the SCN is affected by or prior to AD. Therefore, it is necessary to determine how and when integrity changes occur during AD. This requires non-invasive tools to (1) identify and follow subtle microstructural changes in SCN during AD development, and (2) dissect metabolic pathways in SCN for getting mechanistic connections between SCN dysfunction and AD.

In the first-ever studies of the SCN in AD mice using non-invasive *in vivo* magnetic resonance relaxation measurements (Roy et al., 2019) and *in vitro* metabolomics by ^{1}H HR-MAS NMR, supported by immunohistology (Eeza et al., 2021), our group found evidence of neuroinflammation in the SCN induced by dysregulated GABA metabolism (Roy et al., 2019). The following sections discuss some of the main findings of these studies.

***In vivo* MRI to probe microstructural changes in the SCN:** In MRI, the quantitative transverse relaxation time (T2) is an attribute of atomic spins, with its magnitude depending on the direct surrounding of the spin system. Consequently, T_2 is a powerful tool that can be used to examine microstructural changes in tissue such as, but not limited to, demyelination and astrogliosis. An MRI sequence applied for the determination of T_2 is the Multi-Slice Multi-Echo sequence, based on the Carr-Purcell-Meiboom-Gill sequence. In this sequence, the first 90° excitation pulse is followed by an echo train, consisting of equally distanced 180° pulses used to refocus transverse relaxation. For every voxel in the sample, the signal intensity is measured at every refocus, called the echo time. With every echo, the signal intensity is reduced and T_2 can be calculated from the decay curve created for every voxel. Multicomponent T_2 analysis can be applied to unmix and quantify multiple T_2 values contributing to a decay curve. Being sensitive to various water compartments, the value of multicomponent T_2 was proven by its ability to distinguish inflammatory processes from demyelination (Stanisz et al., 2004).

In our recent *in vivo* longitudinal T2 relaxation MRI study, we compared the SCN of AD mouse model Tg2576 with wild-type mice. In this study gender was also taken into consideration. We found a significant reduction of the T2 in AD mice occurring much earlier in female (after 12 months) than male mice (18 months) (Roy et al., 2019). Additionally, multicomponent T2 analysis was performed by the non-negative least square-based algorithm. Here, an additional slow relaxing component was found in the SCN of Tg2576 mice that was not present in wild-type mice. In general, the presence of this long T2 component suggests either axonal loss, inflammation, or demyelination. In the same study, reduced production of GABA was found in the SCN by quantifying glutamic acid decarboxylase, an enzyme involved in the production of GABA. The existence of an additional long T2 component, in combination with reduced production of GABA, suggested inflammatory responses contributing to circadian rhythmic changes and sleep disturbances. It has been further emphasized that reduced GABA and increased inflammatory responses could be at the root of marked sex disparities observed in AD subjects (Roy et al., 2019).

To identify metabolic pathways and reveal the underlying connection between GABA signaling, neuroinflammation, and clock dysfunction, a systematic metabolic study is indispensable. However, due to the small size of the SCN this is highly challenging, especially in AD animal models. In the past, mass spectrometry and reversed-phase ultra-high performance liquid chromatography-tandem mass spectrometry have been applied in metabolic studies of the SCN from control mice (Buijink et al., 2018). Although extremely sensitive, the sample preparation is rather time-consuming, labor-intensive, and more important, destructive by nature. Therefore, there is a great need for a non-destructive metabolic method to monitor metabolic changes in their native environment in the SCN of AD patients.

High-resolution magic angle spinning nuclear magnetic resonance (HR-MAS NMR)-based metabolomics of the SCN: HR-MAS NMR is a type of NMR spectroscopy that offers great potential to study metabolites in their native environment, impossible for many other metabolic profiling methods. It is based on the principle of spinning the sample by few thousands of Hertz at the magic angle (54.74°) to reduce the dipolar coupling and heterogeneous isotropic susceptibility of the sample (**Figure 1**). This allows the acquisition of high-resolution spectra of a very small volume (~50 μL) of heterogeneous samples such as intact cells, embryos, or tissues (Zuberi et al., 2019). A large amount of information on the whole metabolic profiles of a complex sample is obtained utilizing a single experiment with minimal or no sample preparation steps. This technique can be applied to monitor metabolic changes in the brain tissues of AD patients or AD animal models for identifying biomarkers associated with dysregulations of a wide range of biological processes such as disturbances in circadian rhythms or neurodegeneration. The direct measurement of certain metabolites such as GABA in intact tissue is very challenging due to its complex resonance pattern, showing extensive overlap with more intense resonance peaks. In an earlier study, our group performed the first successful attempt for direct measurement of GABA by HR-MAS NMR in intact brain tissue such as the hippocampus and cortex (Roy et al., 2018).

In our recent study, metabolomics by HR-MAS NMR was performed to identify metabolic pathways and revealing the underlying role of circadian clock disturbances occurring in the pathology of AD (Eeza et al., 2021). A broad view of metabolic changes in the SCN was obtained which helped to identify pathways connecting reduced GABA signaling, pro-inflammatory responses, and circadian rhythmic dysfunction. Quantitative analysis showed that several metabolites were significantly altered in the SCN of Tg2576 mice compared to wild-type mice (Eeza et al., 2021). First, GABA was significantly reduced, confirming reduced glutamic acid decarboxylase expression observed earlier (Roy et al., 2019). Besides a reduction of GABA, reduced levels of several metabolites involved in the syntheses of GABA, including glutamine and glutamate were observed. This, in combination with the observed reduction of several other TCA cycle metabolites including glucose, suggested an

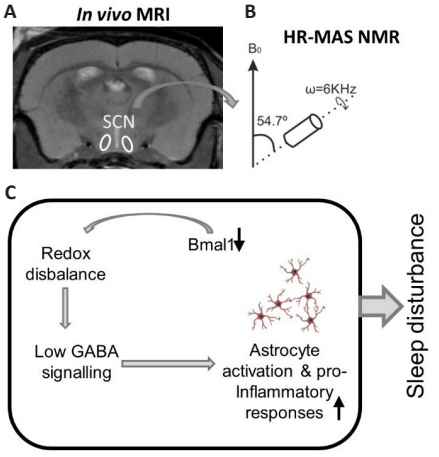

Figure 1 | **Magnetic resonance methods to probe neurode generation in the SCN of AD mouse model.**
(A) *In vivo* MRI relaxation measurements provide information of microstructural changes in SCN. (B) High-resolution magic angle spinning nuclear magnetic resonance (HR-MAS NMR) offers studying metabolomics in intact SCN by spinning sample at magic angle (54.74°) to reduce dipolar coupling and isotropic susceptibilities. (C) These magnetic resonance techniques provide the mechanistic understanding of interconnection between redox dysfunction, compromised GABA signaling, neuroinflammation and sleep disturbance in AD mice. AD: Alzheimer's disease; GABA: γ-aminobutyric acid; SCN: suprachiasmatic nucleus.

impairment of the glutamatergic and GABAeric glucose oxidation and neurotransmitter cycle in the SCN in AD mice. As said, a reduction in GABA levels could indicate inflammatory-induced neurodegeneration. Support of neuro-inflammation contributing to or causing neurodegeneration in the SCN was given by an observed increase of lactate, a well-recognized biomarker of inflammation in a wide range of conditions. Furthermore, a significant increase of *myo*-inositol (m-Ins) and a decrease of N-acetyl aspartate were observed. Decreased N-acetyl aspartate suggests a loss of neuronal integrity as it is found almost exclusively in neurites, axons, and the cell body of neurons (Moffett et al., 2007). Due to its occurrence in the cell body and branches of astroglia, an increase of m-Ins suggests an increase of reactive astrogliosis (Roy et al., 2019). This observation confirmed earlier observations suggesting neuroinflammation since astrogliosis is a known underlying event in neuroinflammation. Finally, a significant reduction of the redox co-factor nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate were observed (Eeza et al., 2021). As the pentose phosphate pathway is the foremost source of nicotinamide adenine dinucleotide phosphate, these results reflected a disturbance of the pentose phosphate pathway leading to redox disbalance in the SCN. A reduced level of glutathione, an antioxidant and redox regulator in cells, was also observed. It is known that reduction of glutathione can lead to increased levels of H_2O_2 in astrocytes and neurons, leading to the stimulation of inhibitory pathways and neuroinflammation responses as potential consequents. Immunohistochemical examinations confirmed several biological processes hypothesized by observed microstructural and metabolic changes. Immunohistology showed a significant decrease in the number of neurons and increased activation of astrocyte, confirming observed HR MAS NMR results of a decrease in N-acetyl aspartate and an increase in m-Ins. Co-localization of astrocyte and *Bmal1* staining showed reduced *Bmal1* expression in activated astrocyte in the SCN, indicating the development of neuroinflammation in association with low *Bmal1* expression. A recent study showed that loss of *Bmal1* in astrocytes causes astrogliosis (Lananna et al., 2018). Finally, through GABA transporter 1 and astrocytes immunostaining, it was concluded that GABA transporter 1 expression is significantly reduced in the SCN of Tg2576 mice. This suggests diminished GABA uptake and signaling in SCN. As GABA uptake by astrocytes is known to be involved in prohibiting inflammatory responses, a diminished GABA uptake by astrocytes may be at the root of inflammatory response seen in SCN during AD.

Summary and future perspective: In summary, the magnetic resonance techniques provide powerful non-destructive means to study the pathology of neurodegenerative diseases such as Alzheimer's disease. By applying T_2 and HR-MAS NMR analysis, one can monitor microstructural- and metabolic changes occurring in the native environment, impossible for many other methods. This, in combination with histochemical analysis, allows the identification of disrupted metabolic pathways and the underlying role of circadian rhythmic disturbances. A model has been proposed which advocate reduced *Bmal1* expression, redox dysregulation, compromised GABA signaling, pro-neuroinflammation responses, and neurodegeneration in the SCN of the AD mice leading to sleep disturbances (**Figure 1**). Dysregulation in GABA signaling, and uptake are considered the driving force of astrocyte activation and neuroinflammatory responses. Dysregulation of GABA is evidenced by 1) reduced total numbers of neurons, 2) reduced expression of glutamic acid decarboxylase and 3) reduced levels of several metabolites involved in the syntheses of GABA. A dysregulation in the redox balance could, according to this proposed model, function as a bridge between the observed reduction of core clock gene *Bmal1* and a dysregulation of the GABA cycle. In conclusion, the overall results from *in vivo* MRI and HR-MAS-based metabolomics in SCN significantly strengthens the hypothesis that neuroinflammation derived from GABA dysfunction plays a key role in sleep disturbance in AD.

Future studies monitoring the rhythmic pattern of key metabolites throughout the circadian cycle in the early and late stages of AD will be necessary to get a full understanding of functional aberration in the SCN metabolome and its association with sleep disturbance. Further comparison of existing single cell sequencing of SCN and SCN metabolome will be very informative. The mechanisms linking neuroinflammation to circadian rhythm disturbance will stimulate interest in targeting inflammatory pathways as part of the strategy to prevent AD-related sleep disturbance. In addition, monitoring sex-specific differences in circadian rhythmicity during AD progression will help to understand the root of marked sex disparities in sleep disturbance observed in AD subjects.

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References

Buijink MR, van Weeghel M, Gulersonmez MC, Harms AC, Rohling JHT, Meijer JH, Hankemeier T, Michel S (2018) The influence of neuronal electrical activity on the mammalian central clock metabolome. Metabolomics 14:1-11.
DeWoskin D, Myung J, Belle MD, Piggins HD, Takumi T, Forger DB (2015) Distinct roles for GABA across multiple timescales in mammalian circadian timekeeping. Proc Natl Acad Sci U S A 112:3911-3919.
Eeza MNH, Singer R, Hofling C, Matysik J, de Groot HJM, Robetaner S, Alia A (2021) Metabolic profiling of suprachiasmatic nucleus reveals multifaceted effects in an Alzheimer's disease mouse model. J Alzheimers Dis 81:797-808.
Foster RG (2020) Sleep, circadian rhythms and health. Interface Focus 10:1-18.
Ju YE, Lucey BP, Holtzman DM (2014) Sleep and Alzheimer disease pathology--a bidirectional relationship. Nat Rev Neurol 10:115-119.
Kress GJ, Liao F, Dimitry J, Cedeno MR, FitzGerald GA, Holtzman DM, Musiek ES (2018) Regulation of amyloidbeta dynamics and pathology by the circadian clock. J Exp Med 215:1059-1068.
Lananna BV, Nadarajah CJ, Izumo M, Cedeño MR, Xiong DD, Dimitry J, Tso CF, McKee CA, Griffin P, Sheehan PW, Haspel JA, Barres BA, Liddelow SA, Takahashi JS, Karatsoreos IN, Musiek ES (2018) Cell-autonomous regulation of astrocyte activation by the circadian clock protein BMAL1. Cell Rep 25:1-9.
Moffett JR, Ross B, Arun P, Madhavarao CN, Namboodiri AM (2007) N-Acetylaspartate in the CNS: from neurodiagnostics to neurobiology. Prog Neurobiol 81:89-131.
Prince M, Wimo A, Guerchet M, Ali GC, Wu YT, Prina M (2015) World Alzheimer report 2015- The global impact of dementia. Alzheimers Dis Int l:1-87.
Roy U, Stute L, Hofling C, Hartlage-Rubsamen M, Matysik J, Robetaner S, Alia A (2018) Sex- and age-specific modulation of brain GABA levels in a mouse model of Alzheimer's disease. Neurobiol Aging 62:168-179.
Roy U, Heredia-Munoz MT, Stute L, Hofling C, Matysik J, Meijer JH, Rossner S, Alia A (2019) Degeneration of the suprachiasmatic nucleus in an Alzheimer's disease mouse model monitored by in vivo magnetic resonance relaxation measurements and immunohistochemistry. J Alzheimers Dis 69:363-375.
Stanisz GJ, Webb S, Munro CA, Pun T, Midha R (2004) MR properties of excised neural tissue following experimentally induced inflammation. Magn Reson Med 51:473-479.
Wang JL, Lim AS, Chiang WY, Hsieh WH, Lo MT, Schneider JA, Buchman AS, Bennett DA, Hu K, Saper CB (2015) Suprachiasmatic neuron numbers and rest-activity circadian rhythms in older humans. Ann Neurol 78:317-322.
Zuberi Z, Eeza MNH, Matysik J, Berry JP, Alia A (2019) NMR-based metabolic profiles of intact zebrafish embryos exposed to aflatoxin B1 recapitulates hepatotoxicity and supports possible neurotoxicity. Toxins (Basel) 11:258-275.

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