Original Article

Chemogenetic activation of midline thalamic nuclei fails to ameliorate memory deficits in two mouse models of Alzheimer's disease

Short Running Title: Thalamic activation in dementia mouse models

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Abstract

One of the main features of Alzheimer's disease is the progressive loss of memory, likely due to pathological changes within brain regions such as the hippocampus and entorhinal cortex. These structures are embedded within the Papez circuit, an interconnected set of brain regions that are essential for episodic memory. The anterior thalamic nuclei (ATN) and thalamic nucleus reuniens (NRe) are both extensively and reciprocally connected with these important memory regions, so we sought to test the hypothesis that chemogenetically-enhancing neurotransmission through NRe and ATN would ameliorate memory deficits in two mechanistically-distinct mouse models of Alzheimer's disease. Using the hAPP-J20 mouse model of amyloidopathy and the Tg4510 mouse model of tauopathy, we carried out stereotaxic injections of viral vectors to transduce hM3Dq (Gq)_mCherry into NRe or the anterio-dorsal/anterio-ventral nuclei of ATN, using mCherry as a control. At nine months (hAPP-J20) or six months (Tg4510) of age, mice underwent a behaviour battery of open field (OF), novel object recognition (NOR) and radial arm maze (RAM), with DREADD agonist 21 administered 30min prior to each behaviour test. Tissue was collected post-behaviour to confirm injection site and virus expression. Both Tg4510 and hAPP-J20 mice show marked hyperactivity in the OF, significant deficits in recognition memory, and a significant impairment in spatial reference and spatial working memory. Unexpectedly, chemogenetic activation of ATN or NRe did not significantly improve these memory impairments or reduce the observed hyperactivity. This may be due to compensation elsewhere within the memory circuit, or that the pathological changes are too far advanced for behaviour reversal.

Keywords: Chemogenetics, thalamus, dementia

Abbreviations: AD/AV (Anterior-dorsal/Anterior-ventral); DREADDs (Designer Receptors Exclusively Activated by Designer Drugs); NRe (Nucleus Reuniens); WT (Wildtype)

Introduction

Alzheimer's disease is the most prevalent form of dementia, currently affecting an estimated 50 million people worldwide, with this number projected to triple by 2050.[1] The main features of Alzheimer's disease include the progressive loss of memory and general cognitive decline, accompanied by extensive neurodegeneration in multiple brain regions, but particularly the hippocampus and entorhinal cortex.[2] Given their well-established roles in memory and spatial navigation, these two regions have received considerable focus in preclinical dementia research. However, these regions sit within an extended hippocampal memory system that comprises regions of the Papez circuit, including prefrontal and retrosplenial cortices (RSC), and midline thalamic nuclei including nucleus reuniens (NRe) and the anterior thalamic nuclei (ATN).[3] NRe forms reciprocal connections with medial prefrontal cortex (mPFC) and subiculum, and projects to hippocampal region CA1 and entorhinal cortex.[4] Similarly, the ATN are extensively interconnected with prefrontal, hippocampal and parahippocampal regions as well as the RSC and mammillary bodies.[5] Thus, the thalamus is well-placed to act as a communication hub between key areas of the brain's extended memory circuit. Rodent studies show that NRe is required for goal-directed spatial navigation[6] and that altering its activity is sufficient to impair working memory.[7] Similarly, lesions of ATN have long been known to disrupt hippocampal-dependent memory tasks,[8] with the ATN having roles in contextual fear memory[9] and goal-directed behaviours.[10] Furthermore, recent advances suggest that both ATN and NRe could play a more general role in high-level information processing.[11] A recent review comparing function of the ATN with NRe concluded that the ATN support many aspects of hippocampal spatial encoding and performance while NRe aids a prefrontal, top-down control of cognition.[12] Altogether, the involvement of ATN and NRe in spatial memory is well-established, and a strong argument can be made for targeting these thalamic nuclei to treat memory disorders.[3]

The main pathological features for Alzheimer's disease, beyond synaptic and neuronal loss, are the accumulation of amyloid-β plaques (Aβ) and neurofibrillary tangles of hyperphosphorylated microtubule-associated protein tau (referred to as 'tau' hereafter). Accumulations of neurofibrillary tangles have been observed in both the anterior-dorsal sub-region of the ATN and NRe in *post-mortem* human tissue,[13] and such pathological changes within the thalamus are associated with the memory loss seen in the early stages of Alzheimer's disease progression in humans. This has not been widely studied in the myriad of animal models of Alzheimer's disease available, although there are some reports of accumulations of both Aβ plaques and hyperphosphorylated tau in the thalamus of some animal models, reviewed by Aggleton.[14] Nonetheless, irrespective of whether ATN and NRe show Alzheimer's disease-related pathology in mouse models, they present a compelling target for therapeutic interventions in this disorder, given their role as a hub for communication throughout the Papez circuit. Consequently, we sought to test this hypothesis using chemogenetic manipulation of ATN and NRe in preclinical models of dementia. We used two mechanistically-

distinct mouse models: the hAPP-J20 amyloidopathy model, which over-expresses a form of hAPP that incorporates the Swedish and Indiana familial Alzheimer's disease mutations,[15] and the Tg4510 tauopathy model that expresses the MAPT P301L mutation under the CamKIIa promoter, limiting the expression of mutant tau to forebrain glutamatergic neurons.[16,17] Although degeneration in the Tg4510 mouse model is unlikely to be wholly attributed to neurofibrillary pathology[18] it should not impede upon the ability to assess whether chemogenetic activation can improve spatial memory deficits in neurodegenerative disorders. In both mice, we expressed the hM3Dq excitatory Designer Receptor Exclusively Activated by Designer Drugs (DREADD)[19] into ATN and / or NRe and assessed changes in learning and memory performance. Remarkably, increasing the 'gain' of information flow through these nuclei did not lead to improvements in spatial memory in either mouse model.

Materials and Methods

Animals

One-hundred-and-eighteen male and female hAPP-J20 mice (internally bred and maintained on a C57BL/6J genetic background) and seventy-eight male Tg4510 mice (kindly provided by Eli Lilly, UK) were housed in groups of two or three in individually ventilated cages with food and water available *ad libitum* until food restriction, where they were individually housed in the same cage type. Mice were maintained in a temperature (21 ± 2 °C) and humidity (45 ± 15%) controlled environment on a 12 h light-dark cycle (lights on at 06:20h), with all experiments performed in the light phase. All procedures were conducted in accordance with the Animals (Scientific Procedures) Act 1986 and received approval from the University of Exeter Local Ethical Committee Review Board.

Drugs

DREADD agonist 21 (Compound 21) dihydrochloride and clozapine-n-oxide (CNO) was purchased from Hello Bio (Avonmouth, UK), dissolved in 0.154 M saline, and subcutaneously administered at a volume of 1 mg/kg. The appropriate DREADD agonist and dose was selected based on current literature showing agonist 21 to have good bioavailability, pharmacokinetic properties, and brain penetrability[20] without back metabolism to clozapine, a common problem with other DREADD agonist, CNO.[21]

To establish that compound 21 at this dose shows no compounding effects on behaviour, six-month old Tg4510 mice (n=20) were tested on three occasions at weekly intervals in a novel object recognition (NOR) test following a subcutaneous (s.c.) injection of saline vehicle, compound 21 or CNO in a pseudo-random order

to serve as their own control. All drugs were administered 30 min prior to each behaviour test; Supplementary Fig. 1.

Stereotaxic surgery

Mice were anaesthetised with isoflurane in O_2 and placed on a heat mat to maintain body temperature. Mice were placed in a stereotaxic frame and intracerebrally injected with AAV8-hSyn-hM3Dq(Gq)_mCherry or AAV8-hSyn-mCherry into the AD/AV thalamus (bilateral; AP -0.71, ML ± 0.8, DV 2.76 *from pia*) or NRe (unilateral; AP -0.8, ML 0.0, DV 3.8 *from pia*) respectively from Bregma (Paxinos and Watson). Representative images are shown in Supplementary Fig 2. Buprenorphine (0.03 mg/kg s.c.) was administered pre-surgery and Carprofen (1 mg/kg s.c.) was administered post-surgery. At the time of surgery hAPP-J20 mice were 7-7.5 months and Tg4510 mice were 4.5 months of age, respectively.

Behaviour

Following the initial study determining the effects of compound 21 on the NOR behaviour task, all mice underwent a behaviour battery of open field, NOR and RAM. All tests were performed by an experienced observer. Tg4510 mice were tested at six months and hAPP-J20 mice were tested at nine months of age, respectively. The nine month time point was chosen for hAPP-J20 mice, as they showed minimal behavioural deficits at six months (Supplementary Fig. 3). The experimenter was blind to mouse genotype and DREADD agonist administered in the pilot, and blind to mouse genotype, sex and viral vector group in subsequent studies.

Open Field (OF)

To assess novel arena induced ambulatory activity mice were placed into individual Perspex boxes (40 x 40 x 45 cm) for 1 h and recorded using Ethovision video-tracking software (Noldus).

Novel Object Recognition (NOR)

To examine recognition memory mice underwent a two-trial object discrimination task. On the test day, mice were re-acclimatized to the OF box for 10 min, returned briefly to their home cage while two identical objects (red Lego tower, glass bottles or grey canisters) were placed in opposite corners. The mouse explored both objects for 10 min during the first familiarisation trial and the time (s) spent exploring each object was recorded from Ethovision video-tracking software (Noldus). Animals were returned to their home cage for an inter-trial interval (ITI) of 24 h before reintroduction to the cage with one original (familiar) object and one novel object. During the second trial, exploration of each object was once again recorded separately. The location of the novel object was varied in a pseudorandom order within groups. Exploratory behaviour was

defined as sniffing, touching and direct attention to the object. Climbing on or chewing of the object was not counted. The Discrimination Index was calculated by the time spent exploring the novel object divided by the total time spent exploring both objects in the choice trial.

Radial arm maze (RAM)

To assess spatial working and reference memories, mice underwent the eight-arm RAM (Tracksys Ltd, UK). Each individual Perspex arm (38 x 7 x 15 cm, 45° from each other), originated from a central area used as the starting platform. 50% condensed milk was used as a food reward, and placed at the distal end of each arm. One-week prior mice were individually housed and food restricted to 90% bodyweight, which was maintained for the test duration. Two days before the test start, mice were habituated to the maze, allowed to explore freely, and consume the food reward. Successful habituation was deemed as consumption of all food rewards located in each arm, with the first reward consumed within 2 min of trial start. During the training sessions, four of the eight arms were baited for Tg4510 mice, and three of the eight baited for the hAPP-J20s. This was based on pilot data showing hAPP-J20 mice to have no significant impairment in the RAM using four-baited arms when tested at six months (Supplementary Fig. 3). The pattern of baited and un-baited arms remained unchanged throughout the entire procedure for each mouse. Each trial began by placing the mouse in the centre of the maze. Entry and re-entry into an un-baited arm were recorded as a reference memory (RM) error, and entry and re-entry into a previously visited arm was recorded as a working memory (WM) error. Trial ended when all food rewards had been consumed, to a maximum trial length of 5min, and was conducted over 14 days.

Tissue Collection

Post behaviour, mice were cervically dislocated with brains dissected and drop-fixed in 4% paraformaldehyde (Sigma-Aldrich, UK) to determine histological verification of correct stereotaxic injection placement and virus expression. One hAPP-J20 behaviour cohort however (n=25) received a s.c. injection with saline, agonist 21 or no injection, and underwent transcardial perfusion 90 min later (30 min following injection to allow brain penetrability and a subsequent 60min to allow for peak Fos production), to assess basal Fos activity and neuronal thalamic nuclei activity following DREADD agonist administration in mice stereotaxically injected with viral vectors in the ATN or NRe. For perfusions, mice were anesthetised using sodium pentobarbital (Pentoject XVD132; Covetrus) in saline (1:10; 200 mg/kg, i.p.) before transcardial perfusion with ice-cold PBS for 5min (5 ml/min), followed by ice-cold 4% paraformaldehyde for 10min (5 ml/min) using a peristaltic pump. All brains (drop-fixation and following perfusion) were dissected and stored overnight in 4% paraformaldehyde for 22 h, before cryopreservation in 30% sucrose in PBS for 3-4 days.

In total, fourteen hAPP-J20 mice and seven Tg4510 mice were excluded from the study due to injection misplacement, whilst two hAPP-J20 and four Tg4510s were excluded from the RAM due to lack of habituation.

Immunohistochemistry

Fos immunohistochemistry

Fos immunoreactivity was quantified as described elsewhere.[22] Briefly, coronal sections of 30 µm thickness were obtained using a microtome (SM2010R, Leica, Germany). Free-floating sections were rinsed with phosphate-buffered saline (PBS) and endogenous peroxidase activity was blocked by incubation in 0.3% v/v hydrogen peroxide (Sigma, UK) in PBS for 15 min. Sections were incubated in PBST (0.1M PBS, 0.2% Triton-X) and blocked in 0.03% v/v goat serum for 1 h before incubation in rabbit anti-c-fos monoclonal IgG primary antibody (1:500 dilution; #2250, Cell Signalling Technology) at 4 ^{o}C overnight for 20 h. Sections were then incubated with biotinylated goat anti-rabbit IgG secondary antibody (1:600 dilution, BA-1000, Vector Laboratories) for 2 h at room temperature. After reaction with avidin-biotin-horseradish peroxidase complex (Vectastain Elite ABC Standard Kit, Vector Laboratories) for 1 h, sections were developed using 0.04% 3, 3'-diaminobenzidine (DAB; HB0687, Hello-Bio Ltd, UK) and 0.05% nickel ammonium sulphate, in the presence of 0.04% v/v hydrogen peroxide in PBS. The reaction was stopped after sections were rinsed three times in PBS. Slices were mounted onto Superfrost Slides (#631-0108, VWR Int.) and dried overnight before dehydration using a graded series of alcohols and cleared using HistoClear (#Nat1334; Scientific Laboratory Supplies). Slides were cover-slipped using Histomount (#NAT1310; Scientific Laboratory Supplies) and left to dry for 2 days.

MC1 immunohistochemistry

The immunohistochemistry procedure was performed similar to Fos but the tissue sections were stained using the mouse-on-mouse (M.O.M.) immunodetection kit (VectorLabs; BMK-2202). Sections were blocked for 1 h in M.O.M.-blocking reagent followed by washing with PBS, and incubation with M.O.M.-diluent for 5 min, before incubation in mouse anti-MC1 (1:1000 dilution; a kind gift from Peter Davies) for 30 min at room temperature. Again, the sections were washed twice in PBS before incubation with M.O.M. Biotinylated IgG secondary antibody (1:250 dilution) for 10 min at room temperature. The reaction with ABC and DAB was performed as for Fos.

Image analysis

Bright-field images were taken as .TIFF files at a 10x magnification using a Nikon Eclipse 800 microscope using Qcapture (Qimaging) software. Fos-positive immunoreactivity was quantified in a blind manner using an automatic cell count, as described elsewhere,[22] during which images underwent a Fast Fourier transform bandpass filter, inverted, and run through the 3D object counter plugin using FIJI software.[23] Values were averaged between hemispheres, and three subsequent sections, which was divided by the area, to get one cell density value per animal.

Statistical Analysis

Data were checked for normality and homogeneity of variance using Shapiro-Wilk's and Levene's tests respectively, followed by a two-way or three-way Analysis of Variance (ANOVA) with viral vector, genotype, and sex as group factors, and where appropriate, time or test day as a repeated measure, using GraphPad Prism v9 (GraphPad Software Inc.) and IBM SPSS v25. Multiple comparison post-hoc tests were conducted where applicable. Data are presented as mean ± S.E.M. and $p < 0.05$ was considered significant.

Data Availability

All raw data that supports the findings herein are available from the corresponding author upon reasonable request.

Results

Following successful experiments to confirm compound 21 did not significantly alter baseline behaviour in the NOR paradigm in Tg4510 mice (Supplementary Fig. 1), mice underwent stereotaxic surgeries to inject viruses to deliver genes to express either hM3Dq_mCherry (with mCherry as a control), unilaterally or bilaterally, in the NRe or AD/AV thalamus, respectively. A representative image of excitatory DREADD expression, including confirmation of neuronal activation at the injection site can be seen in Fig. 1 (AD/AV thalamus) or supplementary Fig. 2 (NRe). hAPP-J20 mice injected in the AD/AV thalamus show a significant increase in Fos expression within this brain region following treatment with compound 21 (effect of drug treatment: $F_{2,8} = 24.49$, $p = 0.0004$), in mice expressing hM3Dq (effect of vector: $F_{2,8} = 19.44$, $p = 0.0023$; *two-way ANOVA, combining mice for genotype due to small population size*; Fig. 1). Similarly, hAPP-J20 mice expressing hM3Dq within the NRe also show increased Fos immunoreactivity within this brain area (effect of vector: $F_{1,6} = 11.58$, $p = 0.0145$), following treatment with compound 21 (effect of drug treatment: $F_{2,6} = 7.084$, $p = 0.0263$; *two-way ANOVA, combining mice for genotype due to small population size*); Supplementary Fig. 4.

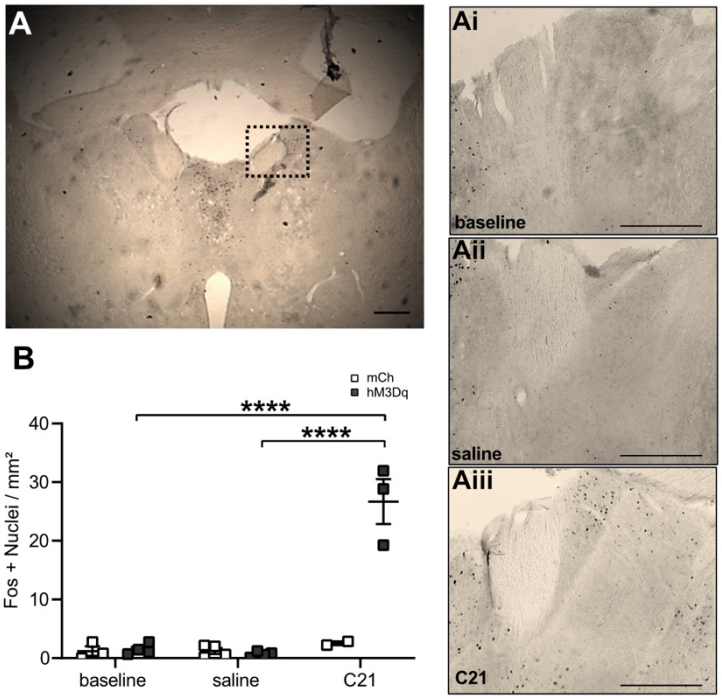

Figure 1: Fos immunoreactivity in mice intracerebrally injected with excitatory DREADDs in the AD/AV thalamus. A, Representative x10 magnification images of Fos counts across three treatment groups and **B,** Comparative differences (mean ± S.E.M.) in Fos density within the AD/AV thalamus, following injection with either saline vehicle or DREADD agonist compound 21 (C21; 1 mg/kg), in hAPP-J20 mice expressing excitatory DREADD (hM3Dq; AAV8-hSyn-hM3Dq(Gq)_-mCherry), or mCherry control (mCh; AAV8-hSyn-mCherry). Baseline animals left untouched in home cage. Scale bar = 100 µm. ****$p < 0.05$ Tukey post-hoc. n=9 mCh, n=10 hM3Dq.

Chemogenetic excitation of the AD/AV thalamus does not ameliorate cognitive behaviour deficits in Tg4510 mice.

During the OF, mice habituated to the arena with ambulatory counts progressively decreasing ($F_{1, 27} = 20.432$, $p = 0.0001$; Two-way repeated-measures ANOVA) over the 1 h time-course. There was a significant main effect of genotype in total distance travelled over the 1 h ($F_{1,30} = 19.470$, $p = 0.0001$), demonstrating that Tg4510 mice were significantly more active than WT controls, particularly at the 10 min ($p < 0.01$), 40 min ($p < 0.05$) and 50min ($p < 0.05$) time points following Tukey post-hoc analysis. Chemogenetic enhancement of AD/AV thalamic activity in Tg4510 mice failed to ameliorate this increased activity ($F_{1,30} = 2.579$, $p = 0.120$), with Tg4510 mice expressing hM3Dq also showing markedly increased activity compared to wildtype controls, that was significant at the 40 min and 60 min time points ($p < 0.05$; Tukey post-hoc); Fig. 2A and Fig. 2B. Indeed, chemogenetic activation of ATN in the Tg4510 may have exacerbated the hyperactive phenotype.

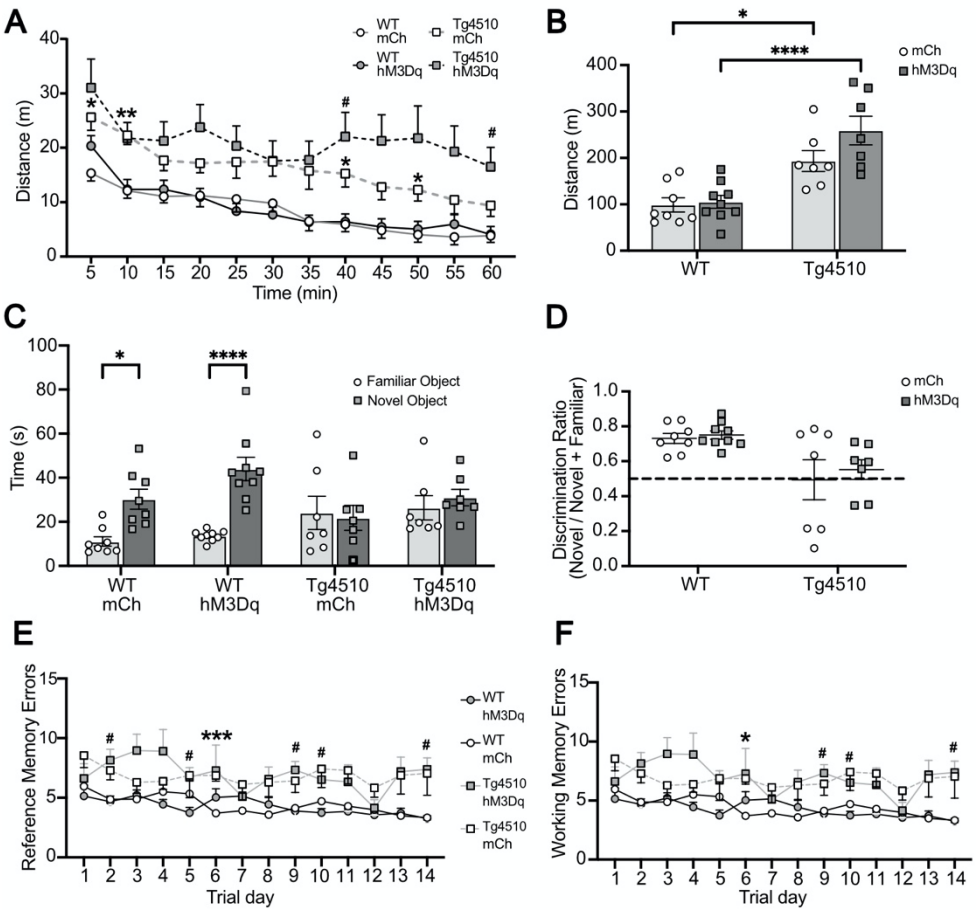

Figure 2: Effects of chemogenetic activation in the AD/AV thalamus in Tg4510 mice. Comparative differences (mean ± S.E.M.) in **A,** time-course of ambulatory activity (cumulative counts/5-min time-bins), *$p < 0.05$, **$p < 0.01$ WT mCh vs Tg4510 mCh, #Tg4510 hM3Dq vs WT hM3Dq; Tukey post-hoc. **B,** total ambulatory count, **C,** time spent exploring familiar vs. novel object, Sidak post-hoc **D,** discrimination ratio in NOR after a 24h ITI, **E,** spatial reference and **F,** working memory errors in the RAM over 14 days in six month male Tg4510 mice and WT controls expressing excitatory DREADDs (hM3Dq; AAV8-hSyn-hM3Dq(Gq)_mCherry) or mCherry control (mCh; AAV8-hSyn-mCherry) in the AD/AV thalamus following subcutaneous administration of compound agonist 21 (1mg/kg) 30 min prior to each test. *$p < 0.05$, ****$p < 0.0001$. AD/AV (anterio-dorsal/anterio-ventral thalamus), WT (wildtype), n=8 WT mCh, n=9 WT hM3Dq, n=7 Tg4510 mCh, n=7 Tg4510 hM3Dq.

In the NOR paradigm, a two-way ANOVA revealed that WT mice spent significantly more time exploring the novel, as opposed to familiar, object following a 24h ITI ($F_{1,27} = 13.106$, $p = 0.001$), which reached significance in both WT controls ($p < 0.05$) and WT expressing excitatory DREADDs ($p < 0.001$) following Sidak's post-hoc test; Fig. 2C. However, male Tg4510 mice show deficits in recognition memory as shown by the discrimination index ($F_{1, 27} = 10.860$, $p = 0.03$), which reached post-hoc significance ($p < 0.05$; Tukey post-hoc). This was not ameliorated by chemogenetic activation of the AD/AV thalamus via hM3Dq ($F_{1, 27} = 1.601$, $p = 0.217$; Fig. 2D). Similarly, Tg4510 mice show robust deficits in working and learning memory relative to WT controls; chemogenetic enhancement of AD/AV activity failed to ameliorate neither reference ($F_{1,28} = 65.247$, $p = 0.0001$) nor working ($F_{1,28} = 50.764$, $p = 0.0001$) memory deficits; two-way repeated-measures ANOVA; Fig. 2E and Fig. 2F. Due to limited availability of Tg4510 mice and the extensive neurodegeneration observed by the

end of our behavioural battery (Supplementary Fig. 5), plus additional caveats associated with this mouse that became apparent midway through our study,[18] we did not test chemogenetic activation of NRe in these animals.

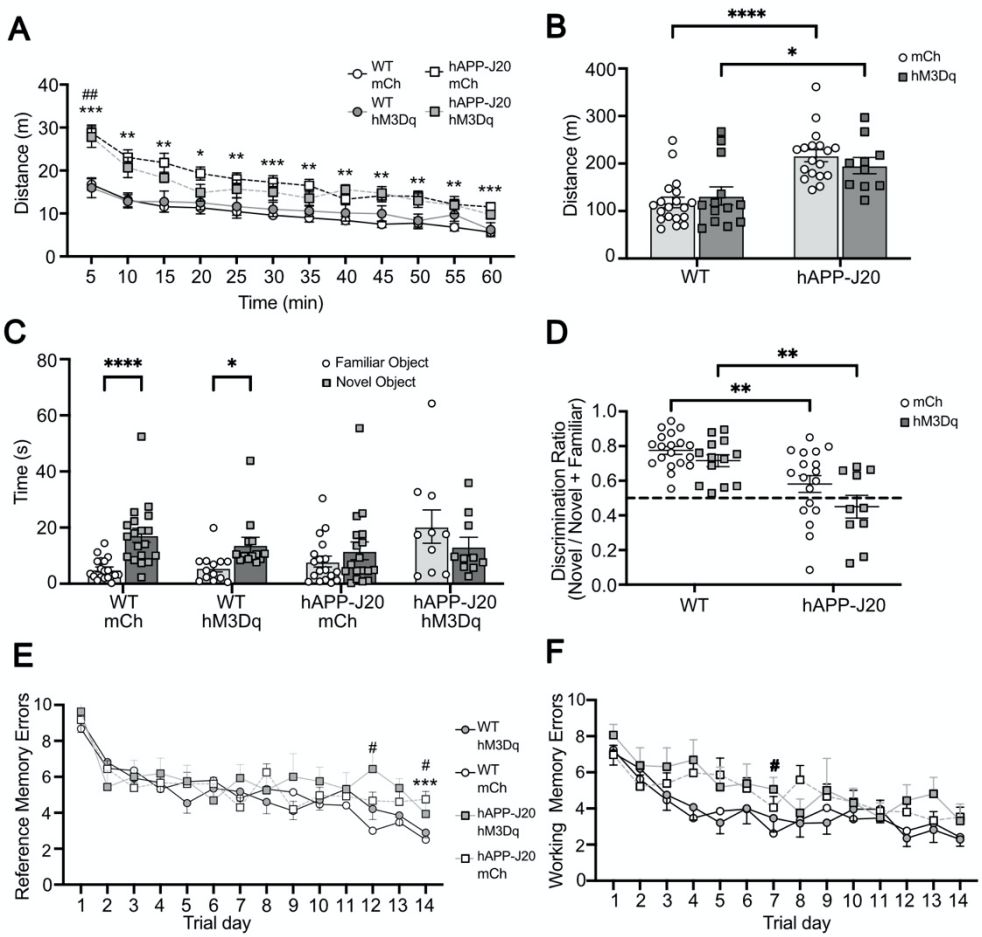

Figure 3: Effects of chemogenetic activation in the AD/AV thalamus in hAPP-J20 mice. Comparative differences (mean ± S.E.M.) in **A,** time-course of ambulatory activity (cumulative counts/5-min time-bins), *$*p < 0.05$, $**p < 0.01$, $***p < 0.001$* WT mCh vs hAPP-J20 mCh, $^{\#\#}p < 0.01$ WT mCh vs hAPP-J20 hM3Dq; Tukey post-hoc. **B,** total ambulatory count, Tukey post-hoc. **C,** time spent exploring familiar vs. novel object, Sidak post-hoc. **D,** discrimination ratio in NOR after a 24h ITI, Tukey post-hoc. **E,** spatial reference and **F,** working memory errors in the RAM over 14 days in nine month male and female hAPP-J20 mice and WT controls expressing excitatory DREADDs (hM3Dq; AAV8-hSyn-hM3Dq(Gq)_-mCherry) or mCherry control (mCh; AAV8-hSyn-mCherry) in the AD/AV thalamus following subcutaneous administration of compound agonist 21 (1mg/kg) 30min prior to each test. No sex differences were found and so data is combined for representation. *$*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$*. AD/AV (anterio-dorsal/anterio-ventral thalamus), WT (wildtype), n=19 WT mCh, n=16 WT hM3Dq, n=19 hAPP-J20 mCh, n=15 hAPP-J20 hM3Dq.

Chemogenetic excitation of the AD/AV thalamus does not ameliorate cognitive behaviour deficits in hAPP-J20 mice.

During the OF, mice habituate to the arena, with ambulatory counts progressively decreasing over time ($F_{1,52} = 25.753$, $p < 0.0001$; three-way repeated measure ANOVA) over the 1h time course. There was a significant main effect of genotype ($F_{1,59} = 25.823$, $p < 0.0001$) in the total distance travelled, with hAPP-J20 mice

exhibiting increased activity, with post-hoc significance for all time points; Tukey post-hoc. There were however no significant main effects in total distance travelled between male and female mice ($F_{1, 59} = 0.27$, $p = 0.869$) or following chemogenetic activation with excitatory DREADDs in the AD/AV thalamus ($F_{1, 59} = 0.0.457$, $p = 0.435$; Fig. 3A and Fig. 3B).

In the NOR paradigm, a three-way ANOVA revealed that WT mice spent significantly more time exploring the novel object in the choice trial ($F_{1,52} = 8.065$, $p < 0.05$) which also reached post-hoc significance for WT controls and those expressing hM3Dq in the AD/AV thalamus ($p < 0.0001$ and $p < 0.05$, *respectively*; Sidak post-hoc). No sex differences were observed in either WT or hAPP-J20 mice ($F_{1, 59} = 1.390$, $p = 0.244$), but hAPP-J20 mice show impaired recognition memory as shown by the discrimination index ($F_{1, 59} = 22.792$, $p < 0.0001$), which was not rescued by chemogenetic activation within the AD/AV thalamus ($F_{1, 59} = 2.862$, $p = 0.097$; Fig. 3C and 3D).

Within the RAM, hAPP-J20 mice also show impaired spatial reference ($F_{1, 49} = 4.242$, $p = 0.045$) and spatial working ($F_{1, 49} = 10.766$, $p = 0.002$) memory deficits compared to WT controls following a three-way repeated measures ANOVA. Similarly, no sex differences were seen in learning for either reference ($F_{1, 49} = 1.288$, $p = 0.262$) or working memory ($F_{1, 49} = 1.155$, $p = 0.288$) performance. Chemogenetic activation of the AD/AV thalamus did not influence learning in either WT mice, nor improve impaired learning deficits in hAPP-J20 mice for either reference ($F_{1,49} = 0.364$, $p = 0.549$), or spatial working ($F_{1,49} = 0.004$, $p = 0.953$) memory performance; Fig. 3E and 3F).

Chemogenetic excitation of NRe does not ameliorate cognitive behaviour deficits in hAPP-J20 mice.

During the OF, mice habituate to the arena, with distance travelled progressively decreasing over time ($F_{1,51} = 73.576$, $p < 0.0001$; three-way repeated measure ANOVA) over the 1h time course. There was a significant main effect of genotype ($F_{1,58} = 73.577$, $p < 0.0001$) in the total distance travelled, with hAPP-J20 mice exhibiting increased activity, with post-hoc significance reached for all time points; Tukey post-hoc; Fig. 4A). There were however no significant main effects in total distance travelled between male and female mice ($F_{1, 58} = 1.828$, $p = 0.182$) nor following chemogenetic activation with excitatory DREADDs in NRe ($F_{1, 58} = 0.619$, $p = 0.435$; Fig. 4B).

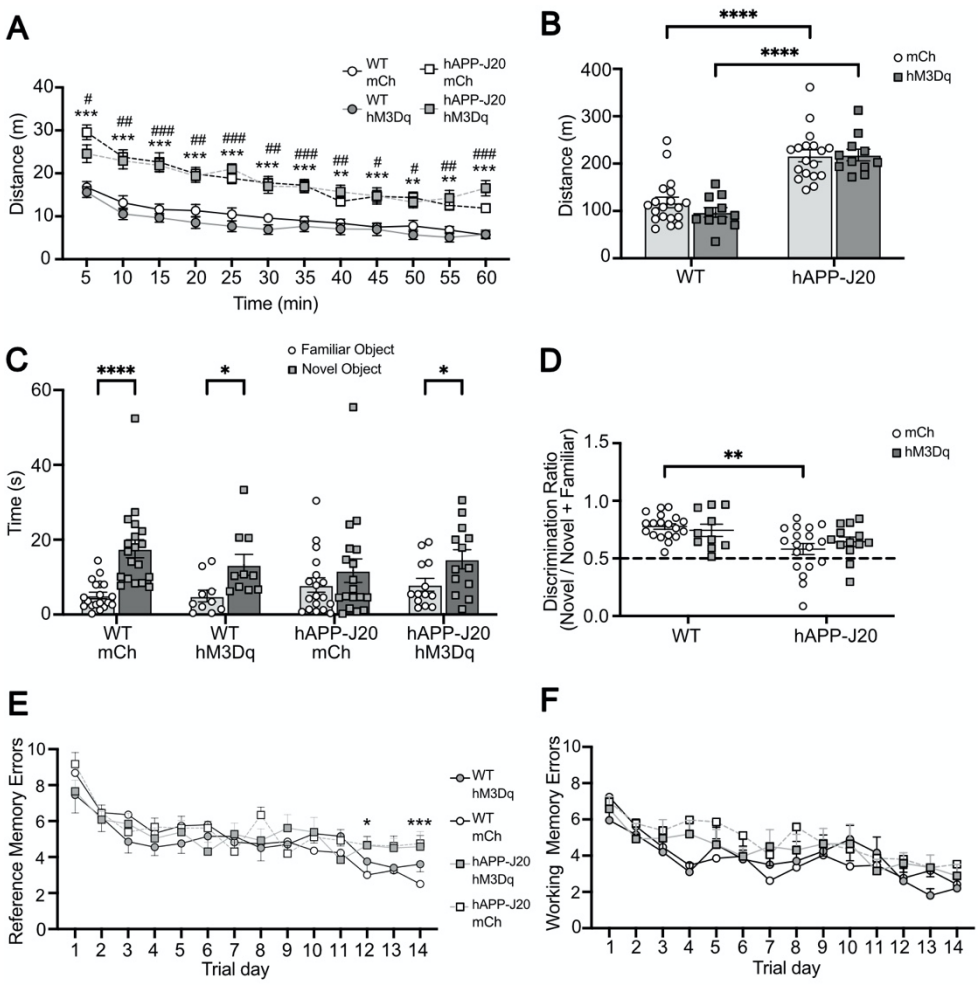

Figure 4: Effects of chemogenetic activation in the NRe thalamus in hAPP-J20 mice. Comparative differences (mean ± S.E.M.) in **A,** time-course of ambulatory activity (cumulative counts/5-min time-bins), ***p < 0.01, ***p < 0.001* WT mCh vs hAPP-J20 mCh, *#p < 0.05, ##p < 0.01, ###p < 0.001* WT mCh vs hAPP-J20 hM3Dq; Tukey post-hoc. **B,** total ambulatory count, Tukey post-hoc. **C,** time spent exploring familiar vs. novel object, Sidak post-hoc. **D,** discrimination ratio in NOR after a 24h ITI, Tukey post-hoc. **E,** spatial reference and **F,** working memory errors in the RAM over 14 days in nine month male and female hAPP-J20 mice and WT controls expressing excitatory DREADDs (hM3Dq; AAV8-hSyn-hM3Dq(Gq)_-mCherry) or mCherry control (mCh; AAV8-hSyn-mCherry) in the NRe following subcutaneous administration of compound agonist 21 (1mg/kg) 30min prior to each test. No sex differences were found and so data is combined for representation. **p < 0.05, **p < 0.01, ***p< 0.001, ****p < 0.0001*. NRe (Nucleus Reuniens), WT (Wildtype), n=19 WT mCh, n=15 WT hM3Dq, n=19 hAPP-J20 mCh, n=15 hAPP-J20 hM3Dq.

In the NOR paradigm, a three-way ANOVA revealed that WT mice spent significantly more time exploring the novel object in the choice trial ($F_{1,51} = 36.368$, $p = 0.001$) which also reached post-hoc significance ($p < 0.0001$ and $p < 0.05$; Sidak post-hoc) for WT controls and those expressing excitatory DREADDs in the AD/AV thalamus; Fig. 4C. No sex differences were observed in either WT or hAPP-J20 mice ($F_{1, 58} = 2.806$, $p = 0.100$), but hAPP-J20 mice show impaired recognition memory as shown by the discrimination index ($F_{1, 58} = 14.269$, $p < 0.0001$). In the choice trial, compound 21-treated hM3Dq-injected hAPP-J20 mice show increase exploration for the novel as opposed to familiar object, reaching post-hoc significance ($p < 0.05$; Sidak post-hoc) but this effect

was insufficiently large to see a significant main effect of chemogenetic activation in NRe ($F_{1,58} = 0.913$, $p = 0.344$; Fig. 4D).

Within the RAM, hAPP-J20 mice also show impaired spatial reference ($F_{1, 50} = 4.535$, $p = 0.038$) and spatial working ($F_{1, 50} = 8.002$, $p = 0.007$) memory deficits compared to WT controls following a three-way repeated measures ANOVA. Similarly, no sex differences were seen in learning for either reference ($F_{1, 50} = 1.615$, $p = 0.210$) or working memory ($F_{1, 50} = 2.854$, $p = 1.288$) performance. Chemogenetic activation of thalamic nucleus reuniens did not influence learning in either WT mice, nor improve memory deficits in hAPP-J20 mice for either reference ($F_{1,50} = 0.458$, $p = 0.502$), or spatial working ($F_{1,50} = 0.782$, $p = 0.381$) memory performance; Fig. 4E and 4F.

Discussion

This study set out to test the hypothesis that spatial memory impairments in mouse models of amyloidopathy and tauopathy could be improved by increasing the gain of information flow through the two midline thalamic nuclei known to act as a conduit for communication throughout the hippocampal-entorhinal-prefrontal network.[12] Although we found that chemogenetic activation of neurons in these nuclei gave robust Fos expression, indicating strong activation of hM3Dq, this neither ameliorated memory deficits in transgenic mice nor disrupted memory in WT animals. While this result was somewhat unexpected, it provides evidence that these thalamic systems are remarkably resistant to perturbation in both wildtype mice and those that model dementia.

The role of ATN in spatial memory

Much of our understanding of the role of ATN in spatial memory come from lesion studies, as has been reviewed by Aggleton and Nelson[8]; these rodent studies show that the ATN play an integral role in processing information from local and distant circuits during memory formation by coordinating and synchronising activity across hippocampal and cortical regions. This function has also recently been demonstrated in human epilepsy patients undergoing combined intrathalamic and scalp EEG recordings.[24] Lesions in the ATN have been shown to reduce activity within the RSC; an area also implicated in spatial and contextual memory formation.[25,26] In addition to lesion studies, it was recently reported that chemogenetic inhibition of ATN is sufficient to impair spatial memory in rats[27]; this study found that this effect could also be replicated by inhibiting the dorsal subiculum projections to ATN.

One possible explanation for the failure to find evidence of an effect in our experiment could be that pathology in hippocampal regions upstream of ATN could be too advanced for manipulating ATN to then have a positive

effect in rescuing spatial memory. This could certainly be true of the Tg4510 mouse line, which displays substantial neurodegeneration throughout the entire Papez circuit by six to seven months (Supplementary Fig. 5). While we are not aware of any published study that has used chemogenetic or optogenetic stimulation of ATN to rescue memory deficits, the connectivity of ATN and lesion studies suggest this should be a successful strategy, as also discussed by Barnett and colleagues.[28] Electrical stimulation of ATN in rats, when combined with corticosterone delivery, significantly improves performance in the delayed non-match to sample T maze task with a delay of 60 s, but significantly impairs performance at longer latencies.[29] In humans, long-term deep brain stimulation of ATN in epilepsy patients was found to improve performance on memory tasks.[30] We employed a chemogenetic strategy as we predicted that optogenetic stimulation in a massively interconnected network would disrupt memory, whereas chemogenetic stimulation would serve to amplify endogenous signalling. However, the success of electrical stimulation suggests that perhaps a stronger drive of ATN neurons is required to improve memory, so optogenetic stimulation may prove more fruitful at rescuing memory deficits.

The role of NRe in spatial and object memory

A similar argument may be made for NRe: acting as a hub for frontal and hippocampal interactions, NRe could also be an attractive target for treating memory impairments. Indeed, NRe contains head-direction cells[31] and lesions in this area also contribute to impaired spatial learning performance in the Morris Water Maze.[32,33] Transient inactivation of the NRe via $GABA_A$ agonist muscimol in rats has been shown to produce deficits in a T-maze task[34] via regulation of hippocampal-PFC synchrony,[35] and optogenetic inhibition of NRe afferents in CA1 is sufficient to impair goal-directed behaviour in mice.[6] In contrast to ATN, where inactivation disrupts spatial memory,[27] optogenetic stimulation in NRe has been reported to disrupt spatial memory.[7] Interestingly, while ATN tends to interact more with the dorsal hippocampus, NRe preferentially (but not exclusively) engages with ventral hippocampal regions, suggesting NRe may associated more with object-based, rather than spatial, information.[12] Our findings are consistent with this perspective: chemogenetic activation of NRe failed to alter performance in the eight-arm radial maze in either WT or hAPP-J20 mice in our study. The hAPP-J20 mice in our study also displayed impaired novel object recognition; while our overall analysis failed to find strong evidence of an effect of chemogenetic manipulation on novel object recognition, *post hoc* multiple comparisons (Fig. 4C) provided weak evidence of a modest improvement in novel object recognition in the compound 21-treated over vehicle-treated hAPP-J20 mice.

Midline thalamus as a therapeutic target in dementia?

We used two mechanistically-distinct mouse models of Alzheimer's disease in an attempt to determine whether the midline thalamus could serve as a novel therapeutic target to treat memory impairment. Our results indicate that acute treatment with excitatory DREADD agonists was unable to attenuate behaviour deficits in two different models despite robustly activating thalamic neurons. However, this does not mean that future progress in this area is outwith the realm possibility. It may be that more specific targeting of individual projections through the thalamus is needed, as opposed to activating all outputs from the thalamic nuclei to their target regions (many of which are also interconnected through direct projections). Recent work by Nelson and colleagues[27] suggests that selectively activating the dorsal subiculum to ATN projection could yield positive results. This could be achieved using an intersectional strategy, by delivering Cre-recombinase into a retrograde virus into ATN and using a Cre-dependent effector in dorsal subiculum.

Although both mouse models used in our study have been well characterised, further work is required to understand which circuit mechanisms are impaired, and thus amenable to being targeted by chemogenetic tools. For example, extracellular field recordings in the CA1/subiculum of hAPP-J20 mice show reduced theta-gamma cross-frequency coupling, along with reduced intrinsic excitability of parvalbumin interneurons.[36] As inhibitory parvalbumin cells play an important role in coordinating hippocampal theta and gamma oscillations,[37] and both these oscillations play a pivotal role in spatial learning,[38] using chemogenetic approaches to instead recruit GABAergic populations within local hippocampal circuitry, could improve memory. Indeed, previously we found that perturbation of excitatory inputs onto parvalbumin interneurons exacerbated deficits in gamma oscillations in the APPswe/PS1DE mouse model.[39]

Another potential explanation for our failure to observe a positive result could be that any manipulation to thalamic excitability needs to occur before the onset of behavioural changes in the mouse models that we used. In particular, the Tg4510 mouse model displayed severe widespread neurodegeneration at the time point used (Supplementary Fig. 5). Long-term ATN stimulation improved memory in human epilepsy patients[30] so it may be that chronic activation of ATN, perhaps via inclusion of DREADD agonists in the rodent chow, is needed to reverse dementia-related dysfunction in the Papez circuit. The thalamus shows pathological changes within Alzheimer's disease, and our mouse models of dementia suggest similar pathological changes.[14] This may therefore be a limitation of currently available mouse models, and modelling robust thalamic pathologies may require combined interactions between amyloid and tau pathologies, and even glial interactions that are not currently captured. For example, post-mortem tissue from Down syndrome patients diagnosed with dementia, revealed fewer glial cells, in addition to neuronal loss and reduced volume in the ATN (incorporating the anterior-ventral and anterior-medial nuclei of the thalamus), compared to age-matched controls.[40] Glial loss in the ATN of hAPP-J20 mice has not been well characterised, however increased GFAP-positive astrocytes are observed in the hippocampus, alongside neuronal loss in 9 month mice.[41]

Furthermore, there is scarce literature investigating thalamic projections to and from key regions within the Papez circuit in hAPP-J20 mice, and so synaptic functions in the ATN and its connections may not be sufficiently disrupted before the appearance of cognitive impairment. If this is true, it may be of benefit to instead reduce thalamic excitability in the same brain regions by the use of inhibitory DREADDs, and determine whether this would influence cognitive behaviours in either WT or transgenic mice. It may also be of interest to use modern-approaches combining wireless *in vivo* recording technology alongside behaviour and decision-making tasks. This would provide more insight as to how circuit manipulation via specific sub-regions can influence behavioural outcomes and neuronal firing in real-time.

Conclusions and Future Directions

Surprisingly, we found that chemogenetic activation of ATN was unable to reverse spatial memory deficits in mouse models of dementia, nor was it sufficient to disrupt spatial memory in wildtype mice. The thalamus receives key inputs from the hippocampal formation and mammillary bodies, with cortical and hippocampal projections modulating neuronal activity in the anterior dorsal ATN region.[42] Excitatory DREADDs may be activating cells within the ATN and NRe, but this does not necessarily spread to wider cortical and subcortical circuits involved in spatial navigation and memory. It may also be that widespread activation of a strongly interconnected circuit was too broad to have the desired effect. Although projections to and from the ATN and NRe play a role in spatial working memory, improving neurotransmission through these central hubs was not sufficient in attenuating a strong behavioural impairment. Potentially, this style of stimulation or increased excitability needs to be introduced at a younger age in our mouse models, before strong pathological and behavioural changes are observed. Future studies will need to record neuronal network activity during such behaviour tasks, to provide more insight into how excitatory DREADDs may influence circuit-level neuronal oscillatory activity, and how these changes can influence behavioural outcomes in mice.

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MTC and SK conceived and designed the study, and drafted the manuscript. SK carried out behavioural and immunohistochemistry experiments. SK, LA, GMS, EB and MTC optimised and carried out stereotaxic surgeries and viral injections. SK, LA, GMS and EB maintained animal colonies. All authors discussed the results and critically revised the manuscript for intellectual content. We would also like to thank Dr Eusike Koya and Gabriella Margetts-Smith for use of their Macro programming code to quantify c-fos cell counts, developed at the University of Sussex (UK). Tg4510 mice were kindly provided by Eli Lilly (UK), and the MC1 antibody was kindly provided by Peter Davies, Albert Einstein College of Medicine (USA).

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Competing Interests

The authors declare no competing interests.

References

1. Collaborators GD. Global, regional, and national burden of Alzheimer's disease and other dementias, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *The Lancet Neurology.* 2019;18(1):88-106.
2. Braak H, Braak E. Evolution of the neuropathology of Alzheimer's disease. *Acta neurologica Scandinavica Supplementum.* 1996;165:3-12.
3. Aggleton JP. Looking beyond the hippocampus: old and new neurological targets for understanding memory disorders. *Proceedings Biological sciences.* 2014;281(1786).
4. Dolleman-van der Weel MJ, Griffin AL, Ito HT, et al. The nucleus reuniens of the thalamus sits at the nexus of a hippocampus and medial prefrontal cortex circuit enabling memory and behavior. *Learning & memory (Cold Spring Harbor, NY).* 2019;26(7):191-205.
5. Jankowski MM, Ronnqvist KC, Tsanov M, et al. The anterior thalamus provides a subcortical circuit supporting memory and spatial navigation. *Frontiers in systems neuroscience.* 2013;7:45.
6. Ito HT, Zhang SJ, Witter MP, Moser EI, Moser MB. A prefrontal-thalamo-hippocampal circuit for goal-directed spatial navigation. *Nature.* 2015;522(7554):50-55.
7. Duan AR, Varela C, Zhang Y, et al. Delta frequency optogenetic stimulation of the thalamic nucleus reuniens is sufficient to produce working memory deficits: relevance to schizophrenia. *Biological psychiatry.* 2015;77(12):1098-1107.
8. Aggleton JP, Nelson AJ. Why do lesions in the rodent anterior thalamic nuclei cause such severe spatial deficits? *Neuroscience and biobehavioral reviews.* 2015;54:131-144.
9. Marchand A, Faugère A, Coutureau E, Wolff M. A role for anterior thalamic nuclei in contextual fear memory. *Brain structure & function.* 2014;219(5):1575-1586.
10. Alcaraz F, Naneix F, Desfosses E, Marchand AR, Wolff M, Coutureau E. Dissociable effects of anterior and mediodorsal thalamic lesions on spatial goal-directed behavior. *Brain structure & function.* 2016;221(1):79-89.

11. Huo Y, Chen H, Guo ZV. Mapping Functional Connectivity from the Dorsal Cortex to the Thalamus. *Neuron.* 2020;107(6):1080-1094.e1085.
12. Mathiasen ML, O'Mara SM, Aggleton JP. The anterior thalamic nuclei and nucleus reuniens: So similar but so different. *Neuroscience and biobehavioral reviews.* 2020;119:268-280.
13. Braak H, Braak E. Alzheimer's disease affects limbic nuclei of the thalamus. *Acta neuropathologica.* 1991;81(3):261-268.
14. Aggleton JP, Pralus A, Nelson AJ, Hornberger M. Thalamic pathology and memory loss in early Alzheimer's disease: moving the focus from the medial temporal lobe to Papez circuit. *Brain : a journal of neurology.* 2016;139(Pt 7):1877-1890.
15. Mucke L, Masliah E, Yu GQ, et al. High-level neuronal expression of abeta 1-42 in wild-type human amyloid protein precursor transgenic mice: synaptotoxicity without plaque formation. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* 2000;20(11):4050-4058.
16. Wes PD, Easton A, Corradi J, et al. Tau overexpression impacts a neuroinflammation gene expression network perturbed in Alzheimer's disease. *PloS one.* 2014;9(8):e106050.
17. Blackmore T, Meftah S, Murray TK, et al. Tracking progressive pathological and functional decline in the rTg4510 mouse model of tauopathy. *Alzheimer's research & therapy.* 2017;9(1):77.
18. Gamache J, Benzow K, Forster C, et al. Factors other than hTau overexpression that contribute to tauopathy-like phenotype in rTg4510 mice. *Nature communications.* 2019;10(1):2479.
19. Roth BL. DREADDs for Neuroscientists. *Neuron.* 2016;89(4):683-694.
20. Tran FH, Spears SL, Ahn KJ, Eisch AJ, Yun S. Does chronic systemic injection of the DREADD agonists clozapine-N-oxide or Compound 21 change behavior relevant to locomotion, exploration, anxiety, and depression in male non-DREADD-expressing mice? *Neuroscience letters.* 2020;739:135432.
21. Manvich DF, Webster KA, Foster SL, et al. The DREADD agonist clozapine N-oxide (CNO) is reverse-metabolized to clozapine and produces clozapine-like interoceptive stimulus effects in rats and mice. *Scientific Reports.* 2018;8(1):3840.
22. Margetts-Smith G, Macnaghten AI, Brebner LS, et al. Acute, but not longer-term, exposure to environmental enrichment attenuates Pavlovian cue-evoked conditioned approach and Fos expression in the prefrontal cortex in mice. *The European journal of neuroscience.* 2021.
23. Schindelin J, Arganda-Carreras I, Frise E, et al. Fiji: an open-source platform for biological-image analysis. *Nat Methods.* 2012;9(7):676-682.
24. Sweeney-Reed CM, Zaehle T, Voges J, et al. Corticothalamic phase synchrony and cross-frequency coupling predict human memory formation. *eLife.* 2014;3:e05352.
25. Todd TP, Bucci DJ. Retrosplenial Cortex and Long-Term Memory: Molecules to Behavior. *Neural plasticity.* 2015;2015:414173.
26. Powell AL, Vann SD, Olarte-Sánchez CM, et al. The retrosplenial cortex and object recency memory in the rat. *The European journal of neuroscience.* 2017;45(11):1451-1464.
27. Nelson AJD, Kinnavane L, Amin E, O'Mara SM, Aggleton JP. Deconstructing the Direct Reciprocal Hippocampal-Anterior Thalamic Pathways for Spatial Learning. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* 2020;40(36):6978-6990.
28. Barnett SC, Perry BAL, Dalrymple-Alford JC, Parr-Brownlie LC. Optogenetic stimulation: Understanding memory and treating deficits. *Hippocampus.* 2018;28(7):457-470.
29. Hamani C, Stone SS, Garten A, Lozano AM, Winocur G. Memory rescue and enhanced neurogenesis following electrical stimulation of the anterior thalamus in rats treated with corticosterone. *Exp Neurol.* 2011;232(1):100-104.
30. Oh YS, Kim HJ, Lee KJ, Kim YI, Lim SC, Shon YM. Cognitive improvement after long-term electrical stimulation of bilateral anterior thalamic nucleus in refractory epilepsy patients. *Seizure.* 2012;21(3):183-187.
31. Jankowski MM, Islam MN, Wright NF, et al. Nucleus reuniens of the thalamus contains head direction cells. *eLife.* 2014;3.

32. Hallock HL, Wang A, Shaw CL, Griffin AL. Transient inactivation of the thalamic nucleus reuniens and rhomboid nucleus produces deficits of a working-memory dependent tactile-visual conditional discrimination task. *Behavioral neuroscience.* 2013;127(6):860-866.
33. Loureiro M, Cholvin T, Lopez J, et al. The ventral midline thalamus (reuniens and rhomboid nuclei) contributes to the persistence of spatial memory in rats. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* 2012;32(29):9947-9959.
34. Viena TD, Linley SB, Vertes RP. Inactivation of nucleus reuniens impairs spatial working memory and behavioral flexibility in the rat. *Hippocampus.* 2018;28(4):297-311.
35. Hallock HL, Wang A, Griffin AL. Ventral Midline Thalamus Is Critical for Hippocampal-Prefrontal Synchrony and Spatial Working Memory. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* 2016;36(32):8372-8389.
36. Mondragón-Rodríguez S, Gu N, Manseau F, Williams S. Alzheimer's Transgenic Model Is Characterized by Very Early Brain Network Alterations and β-CTF Fragment Accumulation: Reversal by β-Secretase Inhibition. *Frontiers in cellular neuroscience.* 2018;12:121.
37. Pelkey KA, Chittajallu R, Craig MT, Tricoire L, Wester JC, McBain CJ. Hippocampal GABAergic Inhibitory Interneurons. *Physiol Rev.* 2017;97(4):1619-1747.
38. Bott JB, Muller MA, Jackson J, et al. Spatial Reference Memory is Associated with Modulation of Theta-Gamma Coupling in the Dentate Gyrus. *Cerebral cortex (New York, NY : 1991).* 2016;26(9):3744-3753.
39. Xiao MF, Xu D, Craig MT, et al. NPTX2 and cognitive dysfunction in Alzheimer's Disease. *eLife.* 2017;6.
40. Perry JC, Pakkenberg B, Vann SD. Striking reduction in neurons and glial cells in anterior thalamic nuclei of older patients with Down syndrome. *Neurobiology of aging.* 2019;75:54-61.
41. Wright AL, Zinn R, Hohensinn B, et al. Neuroinflammation and neuronal loss precede Abeta plaque deposition in the hAPP-J20 mouse model of Alzheimer's disease. *PloS one.* 2013;8(4):e59586.
42. Petrof I, Sherman SM. Synaptic properties of the mammillary and cortical afferents to the anterodorsal thalamic nucleus in the mouse. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* 2009;29(24):7815-7819.