“The good physician treats the disease; the great physician treats the patient who has the disease.”

*- William Osler*

"So we need two things: first, we need ways of predicting and detecting disease well before it becomes life threatening; and second, we need medicines that work for you and your unique body."

*- Pieter Cullis*

# Foreword

Dear reader,

Before you lies the first issue of the 13th volume of the Journal of Neuroscience and Cognition.

I am once again amazed by what the students showed in terms of work ethic and independence in preparing this issue. A few months ago this new board started out with no experience in "being on a journal board" and within a few weeks they familiarized themselves with their specific roles within the board and became an efficient and also very "gezellig" team.

My compliments also go out to the choice of the theme for this issue, personalized medicine. To me this concept is like Jekyll and Hyde. On the one hand personalized medicine is a new and very exciting field of study. It offers fantastic opportunities and possibilities for developing new prevention approaches and treatment approaches for a range of serious illnesses for example. On the other hand there are also risks associated with the technological advances that underlie personalized medicine. Risks in terms of privacy and the right of "not knowing", for example. Important ethical questions should be answered, but a main question is whether the technological advances are not bypassing us before we find appropriate answers to these ethical issues? I hope that the current issue of the Journal of Neuroscience and Cognition will help the readers in shaping their opinion on personalized medicine.

Yours,

Anouk Keizer
*Senior supervisor Journal of Neuroscience & Cognition*

# Editorial

Dear reader,

We, as the board of the Journal of Neuroscience and Cognition, proudly present to you the first issue of 2019. It has been a challenging process, with intriguing conversations, and countless emails to our contributors and reviewers. But this only made us more determined to make it a success, and it made us incredibly proud of the end product.

It did not take long to unanimously decide on a theme for this first issue: Personalized Medicine. This is an increasingly important topic in healthcare, ranging from fundamental research to the clinic, moving away from a 'one size fits all' approach of treatment. We have incorporated this theme into the cover of the journal, by creating four different colours to distribute to you! Furthermore, we have interviewed Jurjen Luykx, a clinical psychiatrist, on his personal thoughts on the topic. Additionally, we carried out a questionnaire on the ethics of Personalized Medicine to students and society, in and outside of our master, and discovered some interesting results.

Equally important are our four research and review articles by Mauri, Joeri, Heike and Josine, who gave us more insight into some fascinating topics. This journal also includes a detailed methodology on high resolution 2D proton MRSI, a 'Master in the Spotlight' written by Geert Ramakers, a book review, experiences of your fellow students abroad and on conferences, a PhD report, and some perspectives on careers after the master.

We hope you enjoy reading this journal, and wish you all the best for the upcoming time!

Yours sincerely,

Ilse van Rijssen
*Editor in Chief*

# The role of dopamine in control over behaviour: The prelimbic cortex, but not striatal regions, mediates control over behaviour in rats

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*The role of ventral midbrain dopamine neurons in movement, memory, and motivation is well established, yet comparatively less is known about the role of these neurons in control over behaviour. This study aimed to investigate if and how the main output regions of ventral midbrain dopamine neurons mediate executive control over behaviour. Data was collected using behavioural-pharmacological and chemogenetic experiments. No evidence was found for the involvement of midbrain dopamine nuclei in control over behaviour; neither chemogenetic activation of dopamine neurons in the ventral tegmental area nor pharmacological blockade of dopamine receptors in the nucleus accumbens altered executive control over behaviour. Following pharmacological inactivation of the prelimbic cortex, animals exhibited impaired executive control over their behaviour. Pharmacological inactivation of the nucleus accumbens, dorsomedial striatum, or dorsolateral striatum did not affect behavioural control, but decreases in motivation were observed. These results suggest that ventral midbrain dopamine neurons do not play a role in executive control over behaviour, but the prelimbic cortex might be involved in this process via projections to the nucleus accumbens.*

***Keywords**: behavioural control; dopamine; striatum; accumbens; prelimbic cortex; ventral tegmental area.*

Resource procurement from the environment is an essential aspect of survival and requires an individual to make complex decisions about how to maximize (potential) reward, whilst minimizing potential aversive consequences. Inability to use an optimal behavioural strategy whilst tip-toeing this balance can have serious consequences for one's well-being. For example, inability to maintain adequate food intake may lead to malnutrition, whereas unregulated intake may lead to obesity; both of these behaviors are leading causes of mortality in humans (Martins et al., 2011; Lee, Carter, Owen, & Hall, 2012). This example serves to show that in many cases, optimal use of environmental resources requires a certain degree of control over behaviour. Loss of control (LoC) refers to the inability to limit rewarding actions despite their negative consequences. As such, LoC is a feature of psychiatric conditions such as addiction (Thomas, Kalivas, & Shaham, 2009), eating disorders (Berridge, Ho, Richard, & Difeliceantonio, 2010), and obsessive-compulsive disorder (Volkow, Wang, & Baler, 2011), all of which have been associated with alterations in the mesolimbic dopamine system (Figee et al., 2011).

When control over behaviour is compromised, it is often that an immediate reward is pursued, not taking into account the future punishment following the reward. For example, a rat given access to palatable, highly rewarding food may consume a significant amount, not taking into account the health consequences of binge-eating. Such behaviour does not occur randomly, but is largely guided by the outcomes of previous behaviour, as first noted by Thorndike (1898). Pavlov (1927) further expanded upon this notion with his observations that dogs became conditioned to salivate at the sound of a bell because this stimulus predicted food. This association between an unconditioned response and a conditioned stimulus is known as operant conditioning and may occur via positive reinforcement by providing reward or via negative reinforcement, which achieves behavioural change through the absence or removal of punishment. As such, operant conditioning allows animals to adapt their behaviour to a wide range of unfamiliar environments. Effective adaptation requires not only knowledge of behavioural outcomes, but also the ability to suppress or inhibit inappropriate behaviour.

The ability to exert control over behaviour might be augmented by increased activity of the neurotransmitter dopamine, as is suggested by research on the behavioural effects of attention deficit hyperactivity disorder (ADHD) medication (Prasad et al., 2013). Methylphenidate, an indirect dopamine agonist often used for ADHD treatment, is associated with better academic achievement and task focus (Evans et al., 2001; Prasad et al., 2013). In addition, polymorphism in the dopamine transporter gene DAT1 has been associated with increased impulsivity in humans (Mata, Hau, Papassotiropoulos, & Hertwig, 2012).

Dopamine is produced by conversion of L-tyrosine to L-DOPA by the enzyme tyrosine hydroxylase (TH), L-DOPA is then converted to dopamine by the enzyme aromatic-L-amino-acid decarboxylase (Morales & Margolis, 2017). The production and storage of dopamine occurs in dopamine neurons, which can be identified by antibody-mediated detection of TH. Dopamine is a neurotransmitter involved in a wide range of functions, including: movement initiation (Barter et al., 2015; Panigrahi et al., 2015) and execution (Jin & Costa, 2015), working memory (D'Esposito & Postle, 2015; Vijayraghavan, Wang, Birnbaum, Williams, & Angsten, 2007), mental flexibility (Arnsten, Wang, & Paspalas, 2012; Costa et al., 2014), and motivation (Boekhoudt et al., 2018; Bromberg-Martin, Matstumoto, & Hikosaka, 2010; Lammel, Lim, & Malenka, 2014). In addition to these functions, dopamine is also crucial to reward and aversion learning. Schultz and colleagues were the first to demonstrate how dopamine neuron activity in the ventral midbrain responds to the discrepancy between expected and actual outcome (Schultz, Apicella, & Ljungberg, 1993; Schultz, Dayan, & Montague, 1997; Schultz, 1998), a finding corroborated by pharmacological and optogenetic recordings (Bayer & Glimcher, 2005; Chang et al., 2016; Fiorillo, Tobler, & Schultz, 2003; Stauffer et al., 2016). The dopamine neurons in the ventral midbrain are subdivided into two regions: the ventral tegmental area (VTA) consisting of the lateral A9 group, and the substantia nigra pars compacta (SNc) which is comprised of the medial A8 and A10 groups (Van den Heuvel & Pasterkamp, 2008).

The VTA is a heterogeneous structure with major dopaminergic projections to the nucleus accumbens (NAc) and the medial prefrontal cortex (mPFC) (Holly & Miczek, 2016; Morales & Margolis, 2017). The NAc is part of the ventral striatum and comprises of mostly (95%) gamma-aminobutyric acid (GABA) medium spiny neurons (MSNs) that act as the primary output neurons. The post-receptor effects of dopamine are contingent on the type of dopamine receptor located on these MSNs (Gerfen & Surmeier, 2011). Specifically, MSNs containing dopamine Type 1 receptors (D1R) are thought to be involved in mediating rewarding behaviours, whilst dopamine Type 2 receptors (D2R) are implicated in avoidance behaviours (Kravitz, Tye, & Kreitzer, 2012; Steinberg et al., 2014). The VTA also projects to the mPFC, an area receiving inputs from the amygdala and hippocampus which are thought to enable to mPFC to assess the motivational relevance of stimuli (Hoover & Vertes, 2007; Morales & Margolis, 2017).
The mPFC is thought to be important for control over behaviour as this area can initiate motor sequences via projections to the NAc (Moorman, James, McGlinchey, & Aston-Jones, 2015). Recent research has shown that pharmacological and optogenetic inactivation of a specific sub-region of the mPFC, the prelimbic (PL) cortex, increases premature responding in rodents on a response-preparation task (Hardung et al., 2017). The PL cortex receives projections from the hippocampus, prefrontal cortex, VTA, and sub-cortical areas and is thought to integrate this information to form comprehensive stimuli representations that facilitate the selection of goal-directed behaviour (Mannella, Gurney, & Baldassarre, 2013).

The SNc has dopaminergic projections to the dorsal striatum (Lerner et al., 2015), which can be divided along a ventromedial-dorsolateral continuum based on connectivity and functionality (Voorn, Vanderschuren, Groenewegen, Robbins & Pennartz, 2004). The dorsolateral striatum (DLS) has been implicated in inflexible habitual behaviour (Gremel & Costa, 2013; Ito & Doya, 2015; Thorn, Atallah, Howe, & Graybiel, 2010). Activity in the dorsomedial striatum (DMS) is thought to be related to goal-directed behaviour (Gremel & Costa, 2013; Hart, Leung, & Balleine, 2014). In addition, the dopaminergic neurotransmission in the DMS seems to be involved in motoric responses, as dopaminergic activation of D1R or D2R in this area improves or worsens response inhibition in a stop-signal task, respectively (Eagle et al., 2011).

In order to clarify the role of the main output regions of ventral midbrain dopamine neurons (the striatum and PL cortex) in control over behaviour in rats, we employed a newly developed 'loss of control' (LoC) task by combining it with behavioural pharmacology and chemogenetics. We show that pharmacological inactivation of different regions of the striatum impairs motivation, but not control over behaviour. Furthermore, we show that control over behaviour is not compromised after pharmacological blockade of mesolimbic dopamine receptors or chemogenetic activation of VTA dopamine neurons. Lastly, we find that inactivation of the PL cortex results in impairments of the animals to exert control over behaviour.

## METHODS

### Animals

In total, 73 male rats (Long Evans background) were used for the experiments which were either TH:Cre (bred in-house) or Rj:Orl animals (Janvier, France), weighing at least 250g at the start of the experiments. Animals were individually housed in a humidity- and temperature-controlled environment under a reversed 12-hour light-dark schedule (lights off at 07:00 A.M.). Rats were food-restricted with 15 g of regular chow (Special Diet Service, UK) per day during training phases.

During the behavioural training phase animals were food restricted to 15g of regular chow (Special Diet Service, UK). Food restriction during experiments

consisted of food removal from the cage 4-6 hours prior to testing. Animals always had ad libitum access to water and were provided a wood block as cage enrichment. All experiments were approved by the Animals Ethics Committee of Utrecht University and were carried out in accordance with Dutch laws (Wet op Dierproeven Revised, 2014) and European regulations (Guideline 86/609/EEC; Directive 2010/63/EU).

## Surgical procedures

Animals were given anesthesia via an intramuscular injection of a mixture of 0.315 mg/kg fentanyl and 10 mg/kg fluanisone (Hypnorm, Janssen Pharmaceutica, Beerse, Belgium). Next, animals were placed in a stereotactic apparatus (David Kopf Instruments, Tujunga, United States). A small incision was made along the midline of the skull and lidocaine spray was administered as a local anesthetic. Two small craniotomies were made above the brain region of interest. For striatal regions, two 23G single guide cannulas (Plastics One, United States) were bilaterally implanted. For the prelimbic cortex, 23G bilateral guide cannulas (Plastics One, United States), spaced 1.2 mm apart, were placed. Secure attachment of guide cannula to skull was ensured through screws, dental glue (C&B Metabond, Parkell Prod Inc., United States) and dental cement. Next, dummy injectors were put inside the guide cannulas to prevent contamination or obstruction of the cannula. Guide cannulas were placed based on the following coordinates:

| | | | |
|---|---|---|---|
| Dorsolateral striatum | AP +1.20mm | ML ±3.40mm | DV -4.10mm from skull |
| Dorsomedial striatum | AP +1.20mm | ML ±1.90mm | DV -4.10mm from skull |
| Nucleus accumbens | AP +1.20mm | ML ±2.80mm | DV -7.50mm from skull |
| Prelimbic cortex | AP +3.20mm | ML ±0.60mm | DV -2.60mm from skull |

In addition to guide cannulas, male TH:Cre+ (n=10) and TH:Cre- (n=9) also received a bilateral injection of 1 µl of AAV5-hSyn-DIO-hM3Dq-mCherry (2 * 1012 particles/ml) into the VTA (AP -5.40 mm, ML ± 2.20 mm, DV -8.90 mm from skull under a 10° angle). The virus was infused over a period of 5 minutes at a rate of 0.2 µl/min using an infusion pump, after which the needle remained at the injection position for 10 minutes, allowing the virus to diffuse into the tissue.

Post-surgery treatment consisted of suturing of the wound, subcutaneous injection of 5 mg/kg carprofen (once per day for 3 days) for pain relief, and a single subcutaneous injection of 10 ml saline for rehydration purposes. Animals were allowed a 7-day recovery period before continuation of behavioural training.

## Behavioural procedures

Animals were trained in operant conditioning chambers (30.5 x 24.2 x 21.0 cm; Med Associates Inc., United States) containing a house light, tone generator, sucrose receptacle dispensing 45 mg sucrose pellets (TestDiet, United States), food port with infrared movement detector, shock grid, and two cue lights. All behavioural training took place between 8 am and 8 pm, for 5-7 days per week.

## Loss of Control task

Each session of the LoC task consisted of 60 trials of 40 seconds each. Every trial started with delivery of one sucrose pellet into the food port. Trial type was pseudorandomly distributed so that 30 were 'no-stimulus trials' during which the animals were free to retrieve the pellet without negative consequences, and the remaining 30 trials were 'stimulus trials' during which pellet delivery was accompanied by a 12-second audiovisual stimulus signaling that the animal had to inhibit the urge to consume the pellet. The trial order was the same for all animals, as to allow for simultaneous training in the same room without sound interference between conditioning chambers.

Initial training sessions consisted of exclusively no-stimulus trials until animals consistently made 55+ trials. Next, stimulus trials were introduced where the stimulus consisted of two cue lights and a generated tone. Entry of the food port, and likely consumption of the pellet, was detected via an infrared movement detector. If animals entered the food port during the 12-second stimulus in the 'stimulus trials', the stimulus was terminated and a 0.3 ms foot shock was delivered via a shock box (Med Associates Inc., United States) connected to a grid rod floor (Med Associates Inc., United States). The intensity of the electric shock was set at 0.40 mA in the first sessions of stimulus training. If animals received many shocks during training, it was assumed that the current shock intensity failed to induce effective punishment and intensity was incremented by 0.05 mA or 0.10 mA. If an animal made many omissions foot shock intensity was decreased by 0.05 mA or 0.10 mA as the foot shock was assumed to be too intense to allow for effective training. Foot shock intensity was maintained once animals consistently succeeded in 20 out of 30 stimulus trials.

## Locomotor test

Behavioural activity levels of TH:Cre+ (n=10) and TH:Cre- (n=9) rats were assessed via a locomotor test. Animals were placed in 80 x 40 cm plastic boxes illuminated with white light. Locomotor activity was measured using video tracking software (Ethovision XT, Noldus, Wageningen, The Netherlands). Animals were given an intra-peritoneal injection of 0.5mg/kg CNO dissolved in saline after 10 minutes after the experiment commenced.

## Drugs

The DREADD ligand CNO was dissolved in sterile saline by placing this solution in an ultrasonic bath for 30-60 minutes. 20 minutes prior to testing on the LoC task,

and 10 minutes into the locomotor test, Cre+ and Cre- animals received an intra-peritoneal injection of 0.5 mg/kg CNO.

The GABAA receptor agonist baclofen (Sigma-Aldrich, Zwijndrecht, The Netherlands) and the GABAB receptor agonist muscimol (Sigma-Aldrich, Zwijndrecht, The Netherlands) were dissolved in sterile saline to create a baclofen muscimol cocktail. Infusions were performed in the same manner as described above. Animals were infused with the baclofen muscimol cocktail at a rate of 0.5 µl / min for 1 minute by gently inserting an injector into the guide cannula. The injector was connected via polyethylene tubing to an automatic micro-infusion pump (Harvard Apparatus, United States). After completion of the infusion, the injector remained in position for an additional 30 seconds to allow for the drug to diffuse into the tissue. Infusions occurred ±20 minutes prior to experimental sessions. A repeated measures, counterbalanced designed was used with 24 hours between experimental sessions as to allow for comparison of baclofen muscimol infusion to saline infusion in all animals.

### Immunohistochemistry

Animals received a lethal injection of sodium pentobarbital and were perfused with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde (PFA) in PBS. After dissection, brains were post-fixed in 4% PFA in PBS for 24 hours and stored in a 30% sucrose in PBS solution. The brain slices (40 µm) were stained for TH and hM3Dq-mCherry using Mouse anti-TH (EMD Millipore, 1:500) and Rabbit anti-dsRed (Clontech, 1:500), respectively. Brain slices were incubated overnight in a PBS solution containing these primary antibodies. Next followed a 2 hour incubation in a PBS solution with secondary antibodies (Goat anti-Rabbit 568 (1:500) and Goat anti-Mouse 488 (Molecular Probes, 1:1000) and 0.05% Tween. Placement of all guide cannulas has been histologically verified by an experimenter unaware of how animals performed on the LoC task (Fig. 2b).

### Data analysis

Data from the LoC task was analyzed using MATLAB R2017B (The MathWorks Inc.) and statistical analysis was performed using Graphpad Prism version 6.0 (GraphPad Software Inc.). To compare treatment (CNO or B/M) to control (saline) condition on the LoC task, statistical comparisons consisted of paired t-tests for each of the task parameters. Locomotor data was analyzed using a 2-way repeated measures ANOVA (with time bin as a within-subjects repeated measures factor and genotype as a between-subjects factor) and an unpaired t-test (for the cumulative distance moved; comparing Cre- to Cre+). To analyze the cFos data, multiple unpaired t-test were used, and were corrected for multiple comparisons using a false discovery rate set at 5%. In addition, an unpaired t-test was used to compare cFos between the no-stimulus and stimulus condition. Three animals were excluded from experiment 1; two animals showed unilateral DREADD expression and one animal had no expression. One animal was excluded from experiments 2 and 3 due to hydrocephalus.

## RESULTS

### The novel Loss of Control task

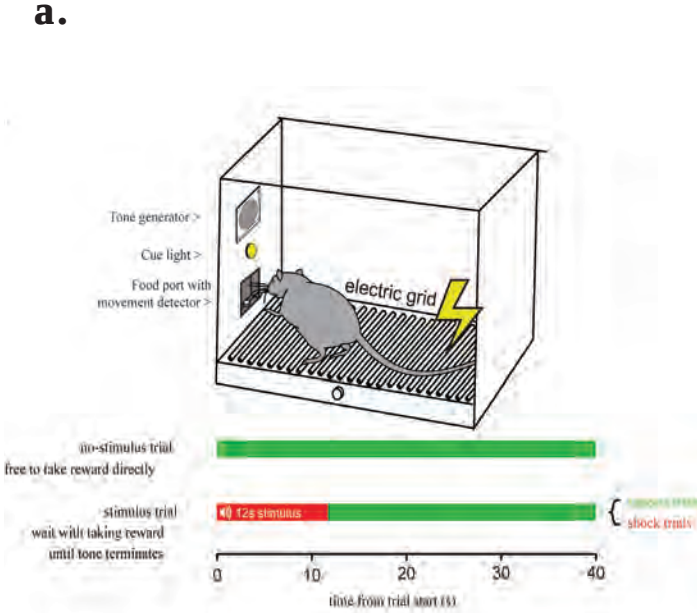
To study loss over behavioural control we developed a novel task for rats (Fig. 1a). This task comprises of sixty 40-second trials, a sucrose pellet being delivered into the food port of an operant chamber at the start of each trial. Half of all trials were so-called 'no-stimulus trials', during which animals were free to retrieve the pellet directly without negative consequences. The remaining 30 trials consisted of so-called 'stimulus trials', in which pellet delivery was accompanied by a 12-second audio-visual stimulus signaling that the animal had to inhibit the urge to consume the pellet until stimulus offset, i.e. a signal threatening punishment should behavioural control be compromised. An animal that entered the food port during the stimulus immediately terminated the stimulus and received a mild foot shock, these trials were regarded as 'shock trials'. If animal successfully waited for stimulus offset they were free to retrieve the pellet without negative consequences afterwards, these trials were regarded as 'success trials'. If an animal did not enter the food port during the 40-second trial, this was regarded as an 'omission' and this halted pellet delivery during future trials until the animal entered the food port. An indication of the amount of LoC was established via a so-called 'shock-index' (LoC = 100% * shock trials / (shock + success trials), which is a measure for the relative amount of shock trials within stimulus trials, after correcting for the number of omissions.

Behaviour on the LoC task can be divided into four phenotypes. First, if an animal loses control over behaviour this will be reflected by an increase in the number of shock trails and a simultaneous decrease in the number of success trials (Fig. 1b). Furthermore, latency to pellet retrieval might be decreased, as it could be that animals find it more difficult to maintain control and as such lose control earlier. The second phenotype pertains to the perception of the stimulus as a threat signal. If animals fail to comprehend the meaning of the stimulus, an increase in the number of shock trials and a decrease in the number of success trials are expected, as can be seen in Figure 1c. The difference with the LoC phenotype lies in the latency to pellet retrieval: during shock trials latency is shorter as animals are unable to perceive the difference between no-stimulus and stimulus trials whilst latency during success trials could be increased as animals fail to understand that stimulus

offset signals that the pellet can be safely retrieved. The third phenotype regards to a decreased motivation to obtain reward, which is characterized by an increase in the number of omissions (Fig, 1d) and increased latency to pellet retrieval during all trials. Lastly, a general disruption of behaviour is reflected by an increase in omissions during all trials and an increased number of shock trials (Fig. 1e). The latency to pellet retrieval could either increase or decrease during general disruption of behaviour.

## VTA dopamine neuron activation does not alter LoC

To study how stimulation of VTA dopamine neurons



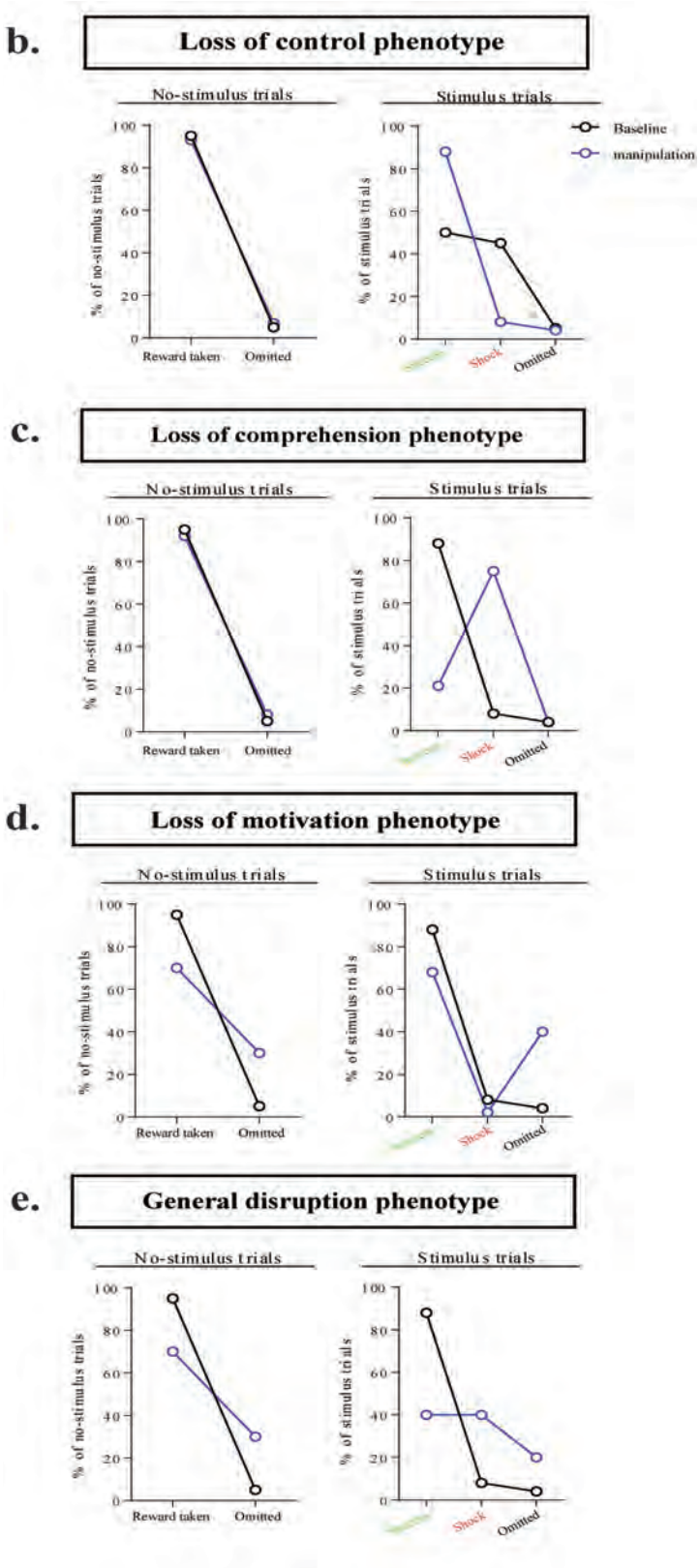




***Figure 1 | Task set-up in an operant chamber containing a tone generator, cue lights, food port with movement detector, and an electric grid.*** *a. Behavioural set-up used during the LoC task. b. The phenotype expected during loss of control consists increase in shock trials and a decrease in success trials during stimulus trials. Latency to pellet retrieval is expected to decreased, indicating earlier loss of control c. The expected phenotype for an animal failing to comprehend the stimulus is characterized by increased shock trials during stimulus trials. Latency during shock trials is decreased, whilst latency during success trial is expected to be increased. d. The phenotype expected if an animals loses motivation consists of increased omissions during both no-stimulus and stimulus trials. The latency is expected to increase. e. The phenotype expected if behaviour is disrupted in general is characterized by increased omissions during all trials and an increase in the amount of shock trials. The latency to pellet retrieval could both increase and decrease for this phenotype.*

influences behavior in the LoC task we injected a viral vector carrying a floxed Gq-DREADD in the VTA of TH:Cre rats. Prior to testing the animals on the task, we systemically injected the DREADD ligand Clozapine-N-Oxide (CNO; 0.5 mg/kg) to increase dopamine neuron activity in Cre+ rats. As shown in Figure 2c, CNO administration did not alter the number of omissions made during no-stimulus trials (paired t-test, t (5) = 1.151, p = .3019). The latency to pellet retrieval during no-stimulus trials was not affected by CNO injection, as shown in Figure 3a (paired t-test, t (5) = 1.387, p = .2240). CNO injection had no effect on the number of success trials (paired t-test, t (5) = 1.131, p = .3094), shock trials (paired t-test, t (5) = 1.168, p = .2956) or omissions made during stimulus trials (paired t-test, t (5) = 0.6532, p = .5425) (Fig. 2d). The latency to pellet collection during stimulus trials did not differ between saline and CNO conditions for both success trials (paired t-test, t (5) = 1.572, p = .1767) and shock trials (paired t-test, t (5) = 0.4143, p = .6959). No differences between CNO and saline injection were found on the shock index (paired t-test, t (5) = 1.241, p = .2698).

To ensure functionality of our DREADD, we injected the animals with CNO prior to testing them in a locomotor assay. A two-way repeated ANOVA comparing the genotype of the animals (Cre+ vs Cre-) showed a main effect of time bin on distance moved (two-way ANOVA, F (9, 117) = 4.538, p < .0001) and a main effect of genotype on distance moved (two-way ANOVA, F (1,13) = 47.26, p < .0001). Furthermore, the interaction effect between time bin and genotype was also significant (two-way ANOVA, F (9,117) = 9.802, p < .0001). Closer inspection of this interaction using a Sidak multiple comparison test revealed that the difference between Cre+ and Cre- rats on the locomotor test became apparent after CNO

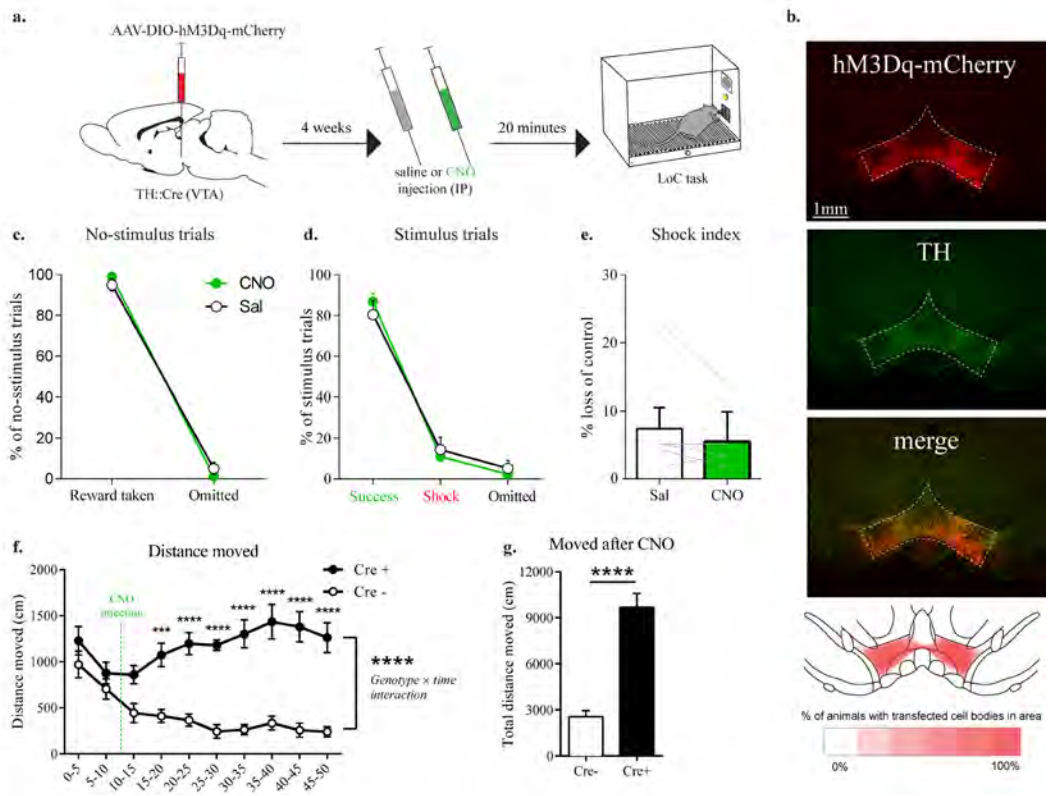



***Figure 2 | Behaviour on the LoC task after VTA dopamine neuron activation.*** *a. TH:Cre+ (N= x) and TH:Cre- (N= x) rats were injected with a floxed Gq-DREADD in the VTA, received saline or CNO injection and were tested on the LoC task. b. First, second, and third panels are a representative example of DREADD expression in dopamine neurons in the VTA of a TH:Cre+ animal, scale bar represents 1mm. The last panel is the percentage of DREADD expression in the VTA of all TH:Cre+ animals. c. CNO injection did not affect behaviour during no-stimulus trials. d. No differences between saline and CNO injection were observed for success trials, shock trials, and omissions. e. The shock index showed no effect of CNO on LoC. f. 10 minutes after CNO injection, Cre+ animals started moving a greater distance than Cre- animals. g. Cre+ animals move a greater total distance in comparison to Cre- animals.*
*#p < 0.1, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001*

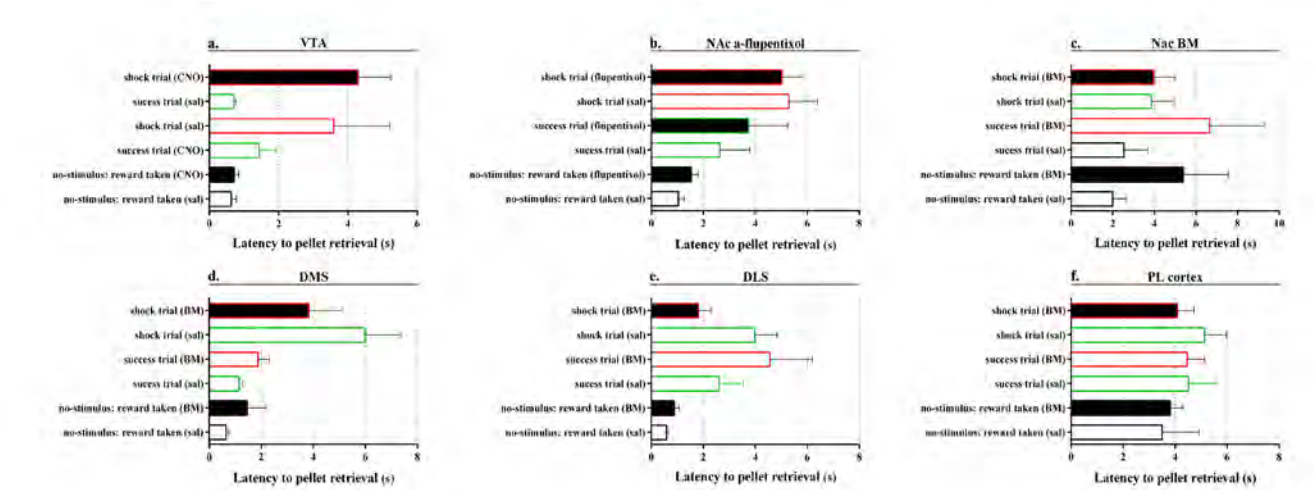



***Figure 3 | Latency to pellet retrieval for all experiments.*** *a. Latency following injection of CNO was not altered during no-stimulus and stimulus trials. b. Infusion of α-flupentixol in the NAc did not result in any changes of the latency to pellet retrieval during no-stimulus and stimulus trials. c. BM infusion in the NAc did not alter latency to pellet retrieval during no-stimulus and stimulus trials. d. Infusion of BM in the DMS did not result in any changes regarding the latency to pellet retrieval during both no-stimulus and stimulus trials. e. Following BM infusion in the DLS, latency to pellet retrieval remained unaffected during no-stimulus trials. During success trials latency was not affected by BM infusion, but during shock trials there is a trend towards increased latencies (paired t-test, t (10) = 1.991, p = .0745). f. BM infusion in the PL cortex did not affect latency to pellet retrieval during no-stimulus and stimulus trials.*

injection (Fig. 2f). These results indicate our DREADD to be functional at the time of testing. Therefore, these results show that increasing dopamine neuron activity in the VTA does not alter behaviour on the LoC task.

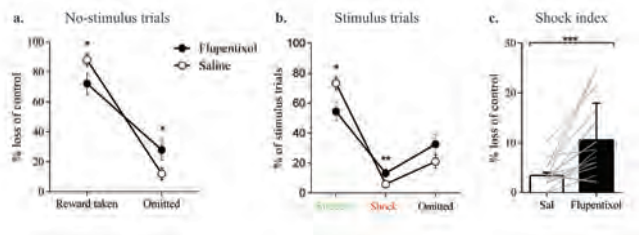



***Figure 4 | Dopamine receptor blockade in the NAc during the LoC task.*** *a. Infusion of α-flupentixol increased the number of omissions during no-stimulus trials; the behavioural phenotype is indicative of loss of motivation b. During stimulus trials α-flupentixol decreased the number of success trials, whilst there was increase in shock trials. Although an increase in shock trials can be observed, the phenotype shown during stimulus trials is suggestive of generally disrupted behaviour. c. The shock index shows increased LoC following α-flupentixol infusion.*
*#p < 0.1, *p < 0.05, **p < 0.01, ***p < 0.001.*

## Dopamine receptor blockade in the NAc does not alter LoC

To assess how dopaminergic neurotransmission in the NAc mediates behaviour in the LoC task, we infused the dopamine receptor antagonist α-flupentixol via guide cannulas placed above the NAc, after which animals were tested on the task. α-Flupentixol infusion increased the number of omissions during no-stimulus trials compared to saline-treated animals (paired t-test, t (16) = 2.584, p = .0200). Latency to reward retrieval during no-stimulus trials did not differ between α-flupentixol and saline treated animals (paired t-test, t (16) = 1.432, p = .1715). During stimulus trials, α-flupentixol decreased the number of success trials (paired t-test, t (16) = 2.651, p = .0174), whilst an increase in the number of shock trials was observed (paired t-test, t (16) = 3.911, p = .0012) (Fig. 4b). The number of omissions in stimulus trials was not affected by α-flupentixol infusion (paired t-test, t (16) = 1.611, p = .1267). The shock index increased following α-flupentixol infusion (paired t-test, t (16) = 4.363, p = .0005). No changes in the latencies of pellet retrieval were detected for success trial (paired t-test, t (16) = 0.6430, p = .5293) or shock trial latencies (paired t-test, t (14) = 0.1711, p = .8666). Although the shock index indicated increased LoC, the behavioural phenotype following α-flupentixol infusion was similar to the expected phenotype of motivation loss (Fig. 1c; Fig. 3a, b). Thus, these results suggest that dopaminergic neurotransmission in the NAc is not essential for control over behaviour.

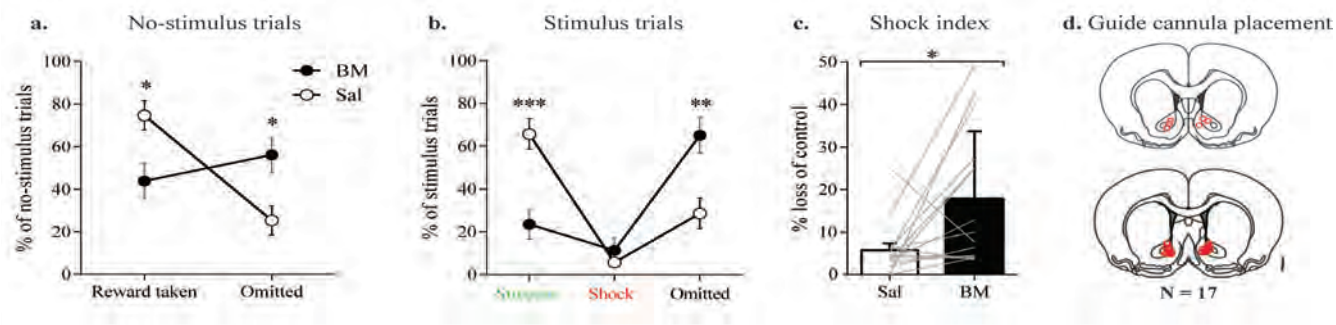



***Figure 5 | Pharmacological inactivation of the NAc during the LoC task.*** *a. Infusion of BM increased the amount of omissions during no-stimulus trials. b. BM infusion decreased the number of success trials, produced no differences on the amount of shock trials, and increased omissions during stimulus trials. c. The shock index showed an increase in LoC following BM infusion. Grey lines represent performance of individual animals. d. Red circles indicate histologically verified placements of guide cannulas in the NAc. #p < 0.1, *p < 0.05, **p < 0.01, ***p < 0.001.*

## Pharmacological inactivation of the NAc does not alter LoC

The purpose of this experiment was to examine how inactivation of the NAc affects behaviour in the LoC task. Prior to testing the animals on the task, we locally infused the GABA receptor agonists, baclofen and muscimol (BM), via guide cannulas placed above the NAc (Fig. 1d). Infusion of BM increased the number of omissions in no-stimulus trials (paired t-test, t (16) = 2.661, p = .0171), but no difference in latency to pellet retrieval was detected (paired t-test, t (14) = 1.284, p = .2201). During stimulus trials, BM decreased the number of successes (paired t-test, t (16) = 4.779, p = .0002) and increased the number of omissions (paired t-test, t (16) = 3.201, p = .0056). There was no difference in the number of shock trials following BM infusion compared to saline infusion (paired t-test, t (16) = 1.042, p = .3130). No significant differences were found on the latencies to pellet retrieval between saline and BM infusions for success trials (paired t-test, t (10) = 1.681, p = .1237) and shock trials (paired t-test, t (12) = 0.3866, p = .7058). Furthermore, infusion of BM increased the shock index (paired t-test, t (14) = 2.766, p = .0152) (Fig. 1c). However, the behavioural phenotype after BM infusion differs from the expected LoC phenotype (Fig. 1b; Fig. 2a, b). Hence, inactivation of the NAc did not evoke LoC.

## Pharmacological inactivation of the DMS does not alter LoC

To investigate how the DMS influences behaviour in the task, we pharmacologically inactivated this area through infusion of BM prior to testing. During no-stimulus trials, infusion with BM did not significantly affect the number of omissions (paired t-test, t (8) = 2.126, p =

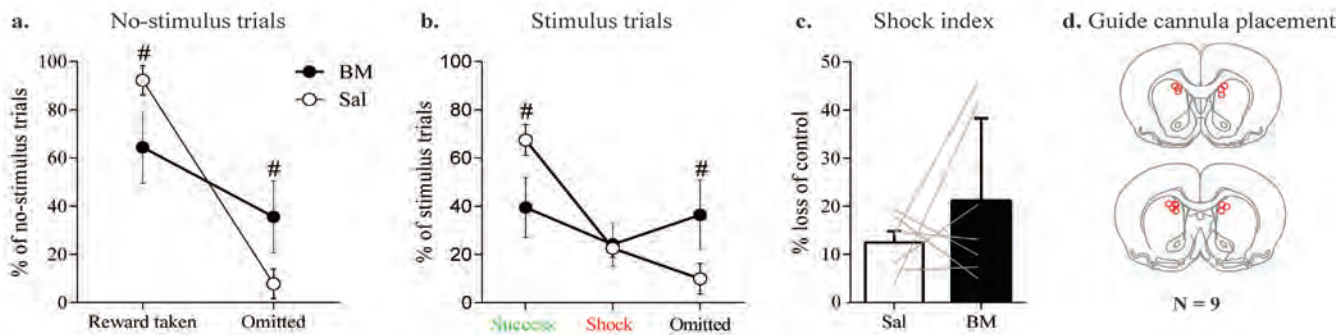



***Figure 6 | Pharmacological inactivation of the DMS in the LoC task****. a. Infusion of BM resulted in a trend towards increased omissions during no-stimulus trials. b. During stimulus trials, BM infusion produced no significant differences for success trials, shock trials, and omissions. However, there is a trend in the data of decreased success trials and increased omissions, producing a phenotype akin to that of loss of motivation. c. No differences between BM and saline infusions were observed on the shock index. d. Red circles indicate histologically verified placements of guide cannulas in the DMS.*
*#p < 0.1, *p < 0.05, **p < 0.01, ***p < 0.001.*

.0662), although a trend towards increased omissions is visible (Fig. 6a). Latency to pellet retrieval during no-stimulus trials did not differ after BM infusion (paired t-test, t (6) = 1.175, p = .2846). No differences were found during stimulus trials for the number of success trials (paired t-test, t (8) = 2.058, p = .0736), shock trials (paired t-test, t (8) = 0.1714, p = .8682), or omissions (paired t-test, t (8) = 2.266, p = .0532). In Figure 6b, is stands out that inactivation of the DMS seemingly results in an attenuation of the phenotype found following inactivation of the NAc (Fig. 5b). There were no significant increases in latency to reward collection similar to that expected for LoC (Fig. 1b). The latencies to reward collection during success trials (paired t-test, t (9) = 1.794, p = .1065) and shock trials (paired t-test, t (10) = 1.991, p = .0745) were not significantly affected by BM infusion, but shock trial latency reveals a trend towards earlier LoC. The shock index shows no significant difference between BM and saline infusion (paired t-test, t (10) = 2.090, p = .0631), although there is a visible trend of increased LoC following BM infusion (Fig. 7b). Overall, these results seem to suggest possible involvement of the DLS in control over behaviour.

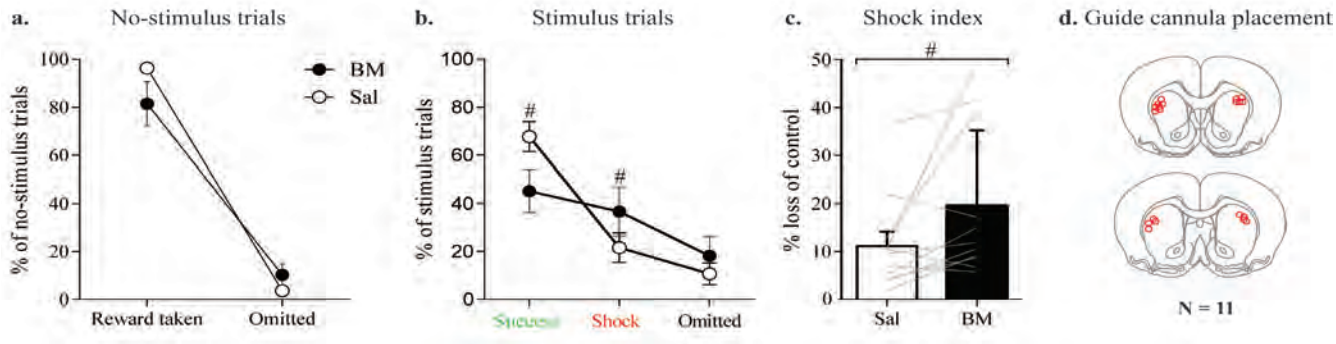



***Figure 7 | Pharmacological inactivation of the DLS in the LoC task.*** *a. Infusion of BM did not affect behaviour differently from saline during no-stimulus trials. b. Although not significantly different, a decrease in the number of success trials and an increase in the number of shock trials can be observed after infusion of BM. There is no difference in the amount of omissions. The phenotype following BM infusion appears similar to the expected LoC phenotype. c. There are no differences between saline and BM infusion on the shock index. d. Red circles indicate histologically verified placements of guide cannulas in the DLS.*
*#p < 0.1, *p < 0.05, **p < 0.01, ***p < 0.001.*

during success trials (paired t-test, t (6) = 2.029, p = .0888) or shock trials (paired t-test, t (6) = 1.103, p = .3125). We observed no increase in LoC on the shock index following BM infusion (paired t-test, t (6) = 1.109, p = .3099). Thus, these results suggest that the DMS does not directly mediate control over behaviour, but is possibly involved in motivation to obtain reward.

## Pharmacological inactivation of the DLS does not significantly increase LoC

With this experiment we sought to determine how the DLS is involved in task behaviour. To this end we inactivated the DLS via infusion of BM prior to the task. During no-stimulus trials, no differences were found in the number of omissions made by animals after treatment with BM (paired t-test, t (10) = .1424, p = .1424). Latency to pellet retrieval during no-stimulus trials was not affected by BM infusion (paired t-test, t (10) = 1.313, p = .2185). BM infusion did not change the number of success trials (paired t-test, t (10) = 2.106, p = .0615), shock trials (paired t-test, t (10) = 1.820, p = .0988) or omissions during stimulus trials (paired t-test, t (10) = 0.8804, p = .3993). As can be seen in Figure 7b, there is a trend towards a behavioural phenotype

## Pharmacological inactivation of the PL cortex increases LoC

To examine how the PL cortex influences behaviour on the LoC task, we pharmacologically inactivated this area prior to testing on the task. During no-stimulus trials, BM infusion did not affect the number of omissions (paired t-test, t (11) = 1.089, p = .2993) or the latency to pellet retrieval (paired t-test, t (11) = 0.1882, p = .8541). Animals treated with BM showed a decreased number of success trials (paired t-test, t (11) = 3.222, p = .0081), whilst there was an increase in the number of shock trials (paired t-test, t (11) = 4.270, p = .0013). The number of omissions during stimulus trials was not affected by drug infusion (paired t-test, t (11) = 1.009, p = .3346). Hence, BM infusion did increase the shock index (paired t-test, t (11) = 4.443, p = .0010). In Figure 7X, X, a phenotype similar to the phenotype expected during LoC can be discerned. During stimulus trials there were no differences between the latencies to reward collection between saline and BM treatment on success trials (paired t-test, t (11) = 0.0503, p = .9608) or shock trials (paired t-test, t (11) = 0.9173, p = .3787). Thus, these results suggest that the PL cortex directly mediates control over behaviour.

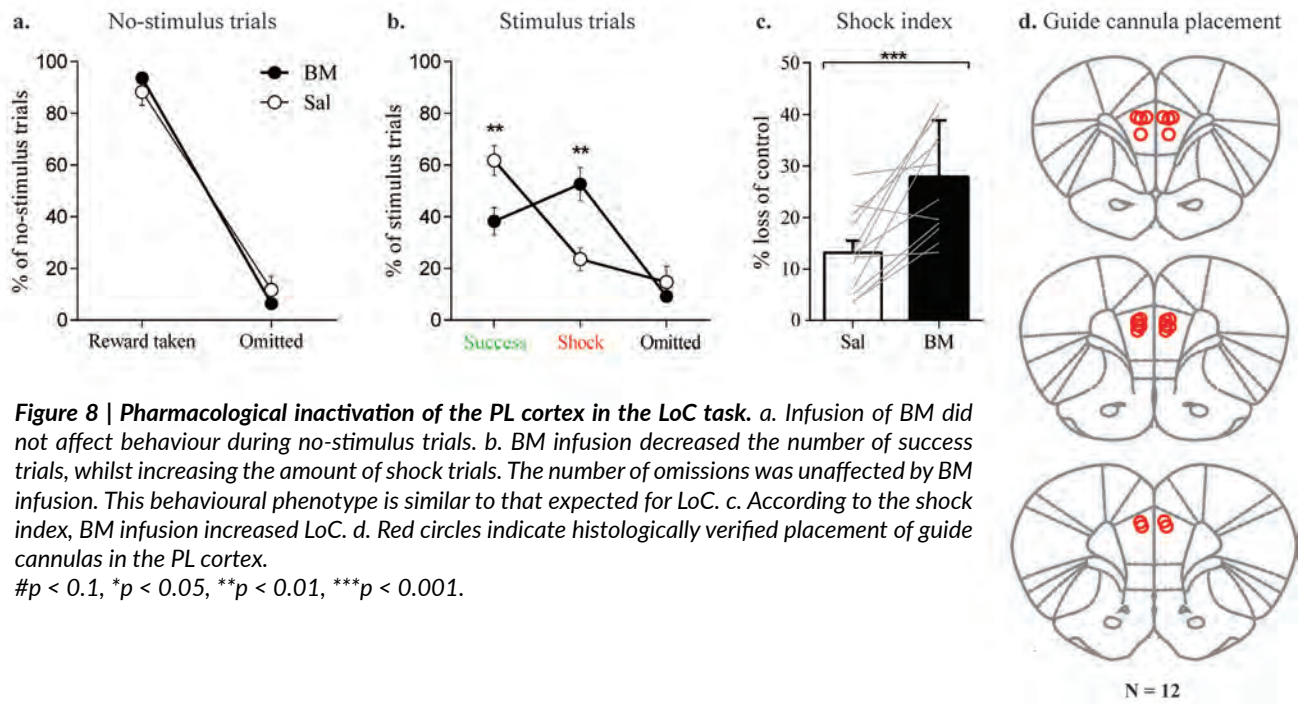



***Figure 8 | Pharmacological inactivation of the PL cortex in the LoC task.*** *a. Infusion of BM did not affect behaviour during no-stimulus trials. b. BM infusion decreased the number of success trials, whilst increasing the amount of shock trials. The number of omissions was unaffected by BM infusion. This behavioural phenotype is similar to that expected for LoC. c. According to the shock index, BM infusion increased LoC. d. Red circles indicate histologically verified placement of guide cannulas in the PL cortex.*
*#p < 0.1, *p < 0.05, **p < 0.01, ***p < 0.001.*

## DISCUSSION

The purpose of the current study was to determine if control over behaviour is mediated by activity in ventral midbrain dopamine nuclei and their target regions. We found no evidence for the involvement of midbrain dopamine nuclei in control over behaviour; neither chemogenetic activation of dopaminergic neurons in the VTA nor pharmacological blockade of NAc dopamine receptors impaired control over behaviour. Inactivation of striatal subregions, the NAc, DLS, and DMS, did not alter control over behaviour. Rather, inactivation of the NAc, and to a lesser extent, the DMS, resulted in decreased motivation. Lastly, inactivating the PL cortex resulted in impaired control over behaviour.

The diversity of excitatory inputs to the NAc, originating from the basolateral amygdala, the hippocampus, and the mPFC, suggest a role for the NAc in a wide variety of behaviours. It is well established that the NAc is an essential component of the neural circuitry associated with motivated behaviour. The NAc is a structure in the ventral striatum where a variety of behavioural drives converge (Floresco, 2015), along with motor control circuitry (Salgado & Kaplitt, 2015). Associative learning about reward- and aversion-related events is thought to be mediated by midbrain dopamine neurons (Floresco, 2015). Dopamine neurons increase or suppress their firing rates following a bigger or smaller reward than expected, respectively (Schultz et al., 1997; Schultz, 1998). These neurons do not respond to reward per se, if cue predicts the reward, then over time the changes in firing rate will shift over to the cue (Cohen, Haesler, Vong, Lowell, & Uchida, 2012). Given the dense VTA-NAc dopaminergic projections (Morales & Margolis, 2017) and the NAc being a structure where a variety of motivation-related inputs converge, the NAc has been proposed to act as a gating mechanism in which dopamine amplifies or attenuates the effects of excitatory inputs on the NAc (Floresco, 2015). It is likely that the NAc directs behaviour based on the motivational information it receives from the basolateral amygdala, hippocampus, and the mPFC. We found no evidence that a chemogenetic increase of VTA dopamine neuron activity increased control over behaviour. When we pharmacologically blocked dopamine receptors in the NAc, we did not observe impairments in control over behaviour. Rather, the behavioural phenotype following dopamine receptor blockade seems to indicate a loss motivation. These results suggest that ventral midbrain dopamine neuron activity does not mediate control over behaviour, but does have a role in goal-directed behaviour.

Following inactivation of the NAc, the shock index indicated an increase in Loc. However, this likely does not reflect impairment in control over behaviour as the equation was skewed by the few success trials during inactivation of the NAc. The observed behavioural phenotype (Fig. 3b) suggests a general disruption of behaviour, rather than increased LoC. Although these findings are in line with research showing the importance

of the NAc in motivated behaviours (Floresco, 2015), it appears that control over behaviour is not part of the behaviours mediated by activity in the NAc.

A different region of the striatum investigated in the current study pertains to the DLS. Existing literature relates activity in the DLS to inflexible habitual behaviour, as this behaviour is impaired following lesioning of the DLS (Gremel & Costa, 2013; Thorn et al., 2010). The DLS receives dopaminergic input from the SNc, which has been linked to impulsive choice behaviour (Tedford, Persons, & Napier, 2015). In our study we found no impairments of control over behaviour after inactivation of the DLS. However, the observed pattern of effects reflects an attenuated phenotype of generally disrupted behaviour, similar to the phenotype following NAc dopamine receptor blockade (Fig. 7b). We also examined the DMS, a region implicated in the acquisition of goal-directed behaviour (Gremel & Costa, 2013; Hart et al., 2014). After inactivation of the DMS, we observed an attenuated behavioural phenotype similar to that found following NAc inactivation, suggesting decreased motivation (Fig. 5b; Fig. 6b,). It is often assumed that the NAc, DLS, and DMS are functionally separated, however in our data similar effects can be observed after inactivation of these regions. We are not the first to notice a degree of shared functionality among striatal regions, Wendler et al. (2014) found, contrary to the literature, that DLS lesioning affected early phases of learning. In addition, Bergstrom et al. (2018) observed activity in the DLS from the outset of learning. It therefore seems that the functionality of the striatal regions might not be as straightforward as previously thought.

The inputs the PL cortex receives from the hippocampus, prefrontal cortex, VTA, and sub-cortical areas are thought to allow the PL cortex to form stimulus representations that facilitate the selection of goal-directed behaviour (Mannella et al., 2013). The PL cortex also has strong projections to the NAc (Stubbendorff, Molano-Mazon, Young, & Gerdjikov, 2018). In our study we found that inactivation of the PL cortex decreased control over behaviour. This could be explained by a disinhibition of inhibitory control from the PL cortex onto the NAc. An alternative explanation holds that inactivation of the PL cortex decreased control over behaviour via impairment in the processing of punishment-related cues (Orsini, Kim, Kanpska, & Maren, 2011; Sharpe & Killcross, 2015). Although this seems unlikely as the behavioural phenotype seen after inactivation of the PL cortex does not resemble the phenotype expected during comprehension loss (Fig. 8b).

Previous research has shown that post-receptor effects of dopamine in the NAc are contingent on D1Rs and D2Rs residing on NAc neurons (Kravitz et al., 2012; Steinberg et al., 2014). In our experiment we the aselective dopamine receptor antagonist α-flupentixol to block dopamine receptors in the NAc. Future research might examine how selective D1R and D2R agonist and antagonists mediate control over behaviour.

In summary, we found no involvement of ventral midbrain dopamine neurons in control over behaviour. Our results suggest that the striatal regions of the NAc, DLS, and DMS do not mediate control over behaviour. Important to note is that we found overlapping functionality of these striatal regions. Lastly, we demonstrate that the PL cortex is involved in control over behaviour.

## CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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# Topographically organized tuned responses to the amount of items encoded in visual short-term memory and relationships with numerosity in human association cortex

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*Neural tuning for numerosity, the number of objects in a visual group, has been demonstrated in several parts of human association cortex, with numerosity-tuned neural populations organized in topographic maps. Overlapping representations of numerosity and visual short-term memory (VSTM) load have been measured in several areas of the parietal cortex. However, the display numerosity changed in both tasks implying that task difficulty increased linearly with set size. Therefore, using functional magnetic resonance imaging and population receptive field modeling, previous methodological limitations are addressed by keeping numerosity of the visual display constant while measuring the brain's responses to the number of items held in VSTM. Then the cortical organization of these responses is determined and results are compared with the previously defined numerosity maps (Harvey & Dumoulin, 2017). Topographically organized tuned responses to the amount of items encoded in VSTM are demonstrated. Furthermore, these responses are organized in topographic maps throughout the human cortex. While representations of VSTM and numerosity show considerable spatial overlap, preferred VSTM set size and preferred numerosity within overlapping parts are not related. Therefore, it is speculated that enumerating sensory information and encoding these representations into VSTM are distinct cognitive processes that share common neural resources.*

***Keywords:*** *tuned neural responses, visual short-term memory (VSTM), functional magnetic resonance imaging (fMRI), population receptive field (pRF) modeling*

Perception of numerosity, the number of visual objects in a group, is implicated in many cognitive functions including decision making (Dehaene, 2003), dividing attention (Knops, Piazza, Sengupta, Eger, & Melcher, 2014) and multiple object tracking (Drew & Vogel, 2008).

Neurophysiological studies discovered neurons that selectively respond to certain numerosities in prefrontal and parietal cortex in both primates and humans (Nieder & Miller, 2004; Piazza, Izard, Pinel, Le Bihan, & Dehaene, 2004; Piazza, Pinel, Le Bihan, & Dehaene, 2007) and activity of numerosity-selective neurons seem to be closely related to behavioural performance (Nieder & Miller, 2003). Tuning curves of those neurons follow a logarithmic Gaussian distribution and show maximum activity in response to its preferred numerosity and decrease response amplitude with the logarithmic distance from their preferred numerosity (Nieder & Miller, 2004). The neuron's tuning width becomes progressively broader with preferred numerosity and therefore overlaps with the tuning curves of other neurons (Nieder & Miller, 2004). More recently, a network of numerosity-selective neural populations has been demonstrated in several parts of human association cortex, organized in topographic maps (Harvey & Dumoulin, 2017). Numerosity tuning widths increased with preferred numerosity in each map, with fewer preferred numerosities above five, suggesting more precise numerosity selectivity for fewer numerosities (Harvey & Dumoulin, 2017).

The rapid and accurate enumeration of groups of objects, referred to as subitizing, is accurate up to three or four items (Mandler & Shebo, 1982). Numbers higher than the subitizing range are estimated. The errors made while estimating increase linearly with numerosity, following the principle of Weber's law (Dehaene, 2003). Subitizing is a highly attention-demanding ability. Increasing attentional load during dual-tasks and attentional blink paradigms reduces behavioural performance precision within the subitizing range, but thresholds outside the subitizing range are unaffected by attentional manipulations (Burr, Turi & Anobile, 2010; Vetter, Butterworth, & Bahrami, 2008). In addition, subitizing is linked to visual short-term memory (VSTM), which is considered to be the ability to temporarily store a fraction of the visual scene that degrades over time (Cowan, 2008; Piazza, Fumarola, Chinello, & Melcher, 2011).

Like subitizing, VSTM shows similar capacity of three to four items, depending on the total number of objects and the amount of visual information (visual features or details) per object (Alvarez & Cavanagh, 2004). In addition, individual differences in working memory capacity are correlated with individual differences in subitizing capacity (Piazza et al., 2011). Performing both tasks simultaneously also reduces the capacity. Maintaining several items in visual working memory while enumerating visual objects reduces the behavioural subitizing range, and the enumeration task interferes with the visual working memory task in a similar manner (Piazza et al., 2011). Furthermore, salient items (manipulated by visual contrast and task relevance) are prioritized and recalled with enhanced precision, but reduces the subitizing range and VSTM resources for other, less salient items (Melcher & Piazza, 2011). The results of these experiments suggest that the accurate enumeration of small quantities and VSTM seem to share common resources that lead to a reduced capacity limit.

Multiple areas increase activity with the number of objects held in VSTM and asymptote after the capacity limit has been reached (Mitchell & Cusack, 2007; Todd & Marois, 2004). That is, bilateral areas in the posterior parietal cortex and superior occipital cortices increase activation until set size 3 or 4 and those activation patterns predict individual differences in VSTM capacity (Todd & Marois, 2004; Todd & Marois, 2005). A subsequent study found VSTM representations in inferior intraparietal sulcus (IPS) regardless of object complexity, and fewer representations in superior IPS and lateral occipital lobe as object complexity increased (Xu & Chun, 2006).

Several functional magnetic resonance imaging (fMRI) studies found overlapping representations while subjects performed simple perceptual tasks and a VSTM task (Mitchell & Cusack, 2007). The posterior IPS showed similar activity in response to these tasks, but only three set-sizes (1, 4, and 8) were used and do not cover the whole VSTM range. A recent fMRI study found differential activation patterns in posterior parietal cortex (PPC) during an enumeration task and a VSTM task (Knops et al., 2014). However, the numerosity of the visual display changed in both tasks, implying that task difficulty increased linearly with set size. Increasing task difficulty can lead to increased activation of areas that are initially involved in the task (Gould, Brown, Owen, & Howard, 2003), or the involvement of other cognitive processes (Barch, Braver, Nystrom, Forman, Noll, & Cohen, 1997; Tregellas, Davalos, & Rojas, 2006). Defining the brain substrates of a particular task could therefore be problematic if difficulty increases within the same task.

Having recently discovered a network of numerosity maps (Harvey & Dumoulin, 2017), the current study explored whether 1) tuned VSTM responses exist in the human brain, 2) whether these are topographically organized, 3) how tuning and organization of those responses relate to the numerosity maps, and 4) how task difficulty influences neural responses. Using ultra-high-field (7T) fMRI, previous methodological limitations (Knops et al., 2014; Mitchell & Cusack, 2007) and measure the brain's responses to the number of items encoded in VSTM while keeping the numerosity of the visual display constant. A population receptive field (pRF) modeling approach (Dumoulin & Wandell, 2008) is used to measure how well responses to VSTM load predict the observed fMRI responses and determine the cortical organization of these responses. Then the spatial overlap and organizational properties between VSTM tuned responses and representations of numerosity were compared (Harvey & Dumoulin, 2017).

## METHODS

Data and protocols from previous studies were used, since the fMRI numerosity data and pRF method allows to evaluate anatomical overlap and response preferences (Dumoulin & Wandell, 2008; Harvey & Dumoulin, 2017; Harvey, Fracasso, Petridou, & Dumoulin, 2015; Harvey, Klein, Petridou, & Dumoulin, 2013).

### Participants

Three male adults took part in the current study (28, 29 and 36 years, all right-handed). All subjects reported normal colour vision, visual acuity, and no history of neurological or psychiatric illnesses. All filled in the informed consent and experimental procedures were cleared by the ethics committee of University Medical Center Utrecht. All subjects were well trained in the task described below before scanning.

### Stimulus and task

Stimuli used for the VSTM task were generated in Matlab (Mathworks, Natic, MA) using the Psychophysics Toolbox (Brainard & Vision, 1997). VSTM capacity is determined both by a fixed number of objects and object complexity (Xu & Chun, 2006). Therefore, stimuli consisted of simple bars without surface textures, because storage capacity is higher for boundary features than for surface textures (Alvarez & Cavanagh, 2008).

Stimuli were displayed against a grey background. Two diagonal thin red lines crossed the entire display, which facilitates fixation accuracy (Schira, Tyler, Breakspear, & Spehar, 2009). Six bars were located at fixed positions and were placed uniformly on a non-visible circle with a radius of 1.2°. The center of the circle was placed on the intersection of the diagonal cross. The size of each individual bar was set to 0.8° x 0.2°. In some trials,

these were replaced by circles with the same surface area. The radius of these circles was set to 0.226°. A delayed match-to-sample task was used. Subjects had to remember a variable number of unique colour-orientation combinations (VSTM set size). The subject's task was then to decide whether a subsequently presented single bar of one of the unique sets was tilted in the same or a slightly different orientation.

Unique colour-orientation combinations were divided over the six bars (Fig. 1). The amount of unique combinations varied between one to five. To illustrate, a set size of three makes three unique colour-orientation combinations of each two bars (Fig. 1, set size three). A set size of five makes four unique sets of each one bar, and one unique set of two bars.

Each unique set and their corresponding bar(s) were tilted in a random angle between 0° and 180°. The orientation difference between each unique set was set to the maximum orientation difference possible. To illustrate, if three unique sets are presented, the maximum orientation difference between these sets is 60°.

Placing bars that belong to the same unique set next to each other might facilitate perceptual grouping. This refers to the process of determining which objects of the visual scene belong together (Treisman, 1982). Therefore, unique set alternated so bars of the same unique set were not placed next to each other. In other words, neighboring bars did not match in colour and orientation (Fig. 1).

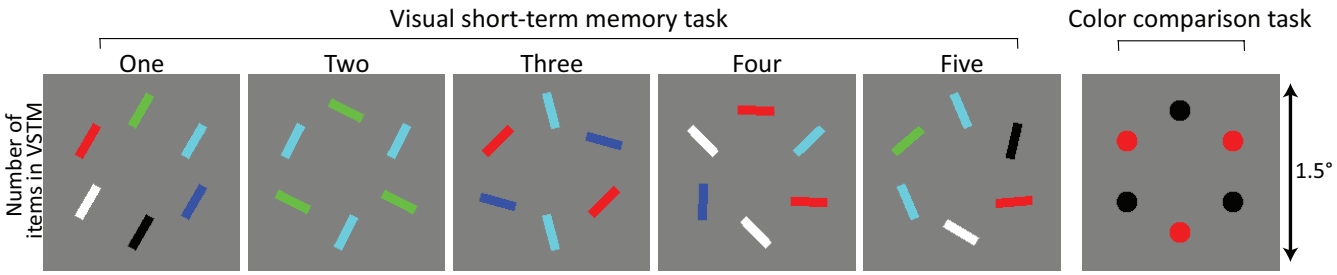


***Figure 1 | Example stimuli for the VSTM task and the colour comparison task.*** *Unique set of colour-orientation combinations (1-5) were divided over the six bars. Each unique colour-orientation combination was tilted in a random angle between 0° and 180°. Bars belonging to the same unique set were not placed next to each other and were separated by bars from another unique set. Set size one was presented in different colours to retain similar stimuli characteristics as set sizes two to five. Stimuli for the colour comparison task consisted of six circles, presented in two randomly selected colours which were each used for half of the circles. Colours alternated so neighboring circles did not match in colour.*

Colours for each unique set were randomly picked from a set of six discriminable colours (red, green, blue, cyan, black, and white). For set size one, and thus all bars in the same orientation, a different colour for every bar was used (Fig. 1, set size one). In this way, the same stimuli characteristics as higher set sizes were retained, since these were also presented in more than one colour.
Since resting is not an ideal condition for comparison to cognitive tasks, a baseline condition in the form of a simple colour comparison task was used, which was similar to the main VSTM task. Stimuli consisted of six circles at the same fixed positions. Two randomly selected colours from the six previously mentioned possible colours were each used for half of the circles, alternating so neighboring circles did not match in colour. Again, the subject had to remember the set and decide whether a subsequently presented circle was found in the set. However, the use of circles meant that subjects only had to remember object colours, and not orientations.

Visual stimuli were projected via a projector on a 15 x 9 cm magnetically shielded screen inside the MRI bore. Subjects viewed the display through prisms and mirrors attached on the head coil and foam padding was used to minimize head movement. Viewing distance was 41 cm, with a resolution of 1024x768.

## Procedure

A fixed number of tilted coloured bars were displayed simultaneously on a visual display. Throughout the scanning run the number of unique colour-orientation combinations (VSTM set size) were systematically varied, thereby changing VSTM load. Subjects were asked to fixate at the cross intersection of the display. Trials started with the presentation of unique sets of colour-orientation combinations divided over six bars and were displayed for 2300 ms. A mask appeared for 200 ms which consisted of a large circle with randomized black and white pixels. The mask then disappeared for 500 ms and a single test bar of one of the previously shown unique sets was presented at the cross intersection for

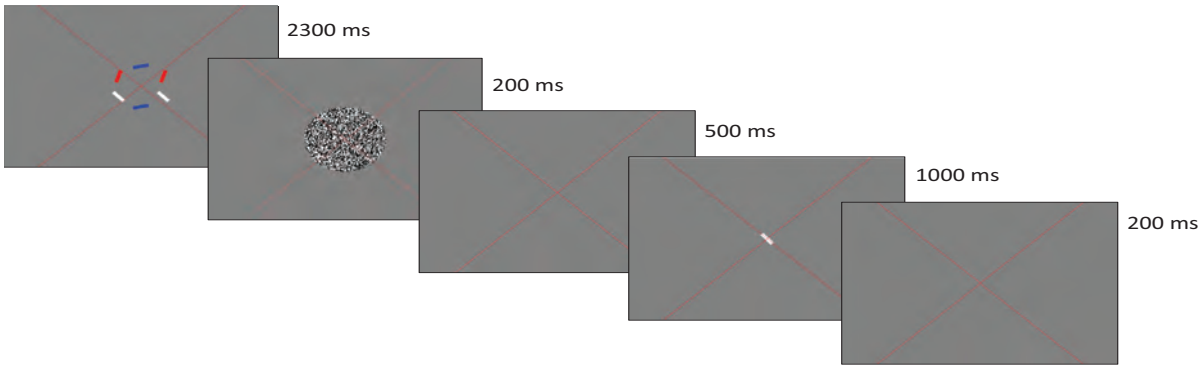


***Figure 2 | Schematic representation of a trial.*** *Sets of unique colour-orientation combinations divided over six bars were briefly presented (2300 ms). After the mask (200 ms) and delay (500 ms), a randomly selected single test bar from the previously shown sample bars was presented in either the same or a slightly different orientation (1000 ms). The next trial started after a delay (200 ms). The duration of a single trial was 4200 ms. In the trial depicted above, the sample bars display three unique sets of colour-orientation combinations. Subjects were instructed to remember the different orientations and corresponding coulors of the sample bars and to decide whether the same-coloured bar from the test display had changed orientation.*

1000 ms. The next trial started after a delay of 200 ms with a total duration of 4200 ms (Fig. 2). Intervals were based on the finding that VSTM is protected against masking and that effectiveness slowly decreases over the first few seconds, but not over the first 600 ms (Phillips, 1974). A 700 ms interval between the presentation of sample bars and the test bar would therefore minimize memory degradation.

Subjects were instructed to remember the unique colour-orientation sets of the sample bars and to decide whether the same-coloured bar from the test display had changed orientation. Orientations of the test bar could be identical, tilted clockwise or counterclockwise. Subjects responded with a button press for same (1) or different (2) and were required to give a response within 3500 ms of the test bar's onset.

## Training

Subjects had to judge whether the orientation of the test bar had changed with respect to the same-coloured bar of the previously presented sample bars. This was a demanding task, with a response required every 4.2 seconds, and during scanning subjects had to continue this at their 75% threshold for six runs of 8 minutes and 37 seconds, with only short breaks between runs. Therefore, subjects underwent extensive training before scanning, lasting around six hours until thresholds stabilized.

During training, a staircase procedure was used to ensure that the task was challenging enough for subjects by computing orientation difference thresholds. Those thresholds are defined as the minimum orientation difference between the sample bars and the test bar that yields a 75% correct performance on each VSTM set size. Auditory feedback was given immediately after button press, informing the subject if they responded correctly.

Once each subject's thresholds had stabilized (around 6 runs in the last hour of training), the 75% thresholds for each VSTM set size in each run were taken. For each VSTM set size, the median threshold across runs were selected and fit a linear function to these thresholds, which fit the thresholds closely. The resulting linear function at each VSTM set size were evaluated, and used this value as the orientation difference presented to each subject in the fMRI experiment. This allowed us to compensate for differences in orientation threshold between subjects and VSTM set sizes. For the baseline condition (where circles were presented and no orientation judgement was required), no orientation difference was applicable.

## fMRI task

Two task configurations were used to both control for and measure the influence of task difficulty on blood oxygen level-dependent (BOLD) responses (Barch et al., 1997; Gould et al., 2003; Tregellas et al., 2006). In the constant difficulty configuration, orientation differences were set to each subject's thresholds per set size obtained during training, such that performance was similar for all set sizes. In the constant orientation configuration, the orientation difference was the same for all set sizes, fixed to each subject's threshold at a set size of three, such that performance decreased as set size increased.

Coloured circles were used to contrast against measurements of VSTM. It was reasoned that during the presentation of circles, neurons that selectively respond

to a given VSTM set size would show minimal activation because such a simple colour judgement task with two easily discriminable colours would hardly load VSTM. Subjects were asked to judge whether the colour of the single test circle matched the colour from any of the previously presented sample circles. Performance of all subjects was 83% or higher in each scanning run.

Each VSTM set size (one to five) was presented over 4200 ms (2 repetition times, TR). In addition, each set size was presented twice before progressing in ascending order to the next VSTM set size. This was followed by four presentations of circles (16.8 seconds). Subsequently, each VSTM set size was presented in descending order (five to one), followed by another four presentations of circles (16.8 seconds). 56 fMRI volumes were obtained during this cycle sequence, with a duration of 117.6 seconds. This cycle sequence was repeated four times, so each functional scanning run consisted of 224 timeframes (470.4 seconds). The interval between runs was approximately one minute. Subjects participated in four to six functional runs within each session, in one of the two task configurations, with the other configuration tested on another day.

## MRI acquisition and preprocessing

Following the procedures of Harvey and Dumoulin (2017), functional data were acquired at University Medical Center Utrecht, the Netherlands on a 7T Philips Achieva scanner. T1-weighted anatomical MRI images were acquired with a voxel size of 0.75 x 0.75 x 0.8 mm (TR 10.029 ms, TE 2.84 ms, flip angle 8°), automatically segmented using Freesurfer (Dale, Fischl, & Sereno, 1999) and hand-edited afterwards using ITK-SNAP (Yushkevich, Piven, Hazlett, Smith, Ho, Gee, & Gerig, 2006) to reduce segmentation errors (Teo, Sapiro, & Wandell, 1997). This provided a very precise cortical surface model, used for further analysis.

Functional runs were each 230 time frames (483 seconds) in duration, of which the first six time frames (12.6 seconds) were discarded, allowing magnetization to reach a steady state. T1 images were obtained before each functional run for coregistration purposes. Blood oxygenation changes were monitored by using a T2*weighted 2D-EPI gradient echo sequence with a 32-channel head coil with a voxel size of 1.57 x 1.57 x 1.75 mm (TR 2.1 ms, TE 25 ms, flip angle 70°). Forty-one slices of 128 x 128 voxels were obtained, making the field of view 201 x 201 x 72 mm. The acquired volume covered occipital, parietal, posterior-superior frontal and temporal lobes. A single-shot gradient echo sequence with SENSE acceleration factor 3.0 and anterior-posterior encoding was used. Maximum gradient strength was 26mT $m^{-1}$, and maximum slew rate was 140 T $m^{-1}$ $s^{-1}$. A third-order image-based BO shim of the field of view of the functional scans was used (in-house IDL software, v6.3, RSI, Boulder, CO).

No spatial or temporal smoothing was applied, because spatial distortions would appear if functional activation patterns of different subjects would be mapped onto a single cortical surface. Head motion corrections were applied between and within functional scans. Then the subject's data was averaged across functional runs and aligned to the earlier obtained T1-weighted anatomical MRI image. Alignment was performed automatically using the algorithm of Nestares and Heeger (2000). Gray and white matter was labeled to accurately reconstruct the subject's cortical surface (Wandell, Chial, & Backus, 2000). Data from each task configuration was analyzed separately, as well as the average of both task configurations. Responses to numerosity and visual mapping stimuli followed the same scanning protocol and were acquired in separate sessions on different days (Harvey & Dumoulin, 2017).

## fMRI data analysis

Functional MRI data was analyzed in the mrVista software package for MatLab (http://white.stanford.edu/software/). As previously described for numerosity (Harvey & Dumoulin, 2017) and object size tuning (Harvey et al., 2015), a population receptive field (pRF) method was used to search for tuned neural responses to VSTM set sizes using. The pRF method is based on a forward model that estimates the amplitude and tuning width based on the stimulus time course and the measured BOLD response time series (Dumoulin & Wandell, 2008). The obtained models summarize VSTM set size tuning using both linear and logarithmic Gaussian functions with two parameters, namely preferred VSTM set size (mean of the Gaussian distribution) and tuning width (standard deviation of the Gaussian). Each VSTM model consists of 56 fMRI volumes.

The pRF modeling procedure starts with a forward model that predicts neural responses, based on a neural tuning model characterized by a candidate preferred VSTM set size (which gives the maximum response amplitude) and tuning width. For every stimulus time point, this function is evaluated at the displayed VSTM set size, resulting in a prediction of the neural response at each stimulus time point for this candidate neural tuning model. By convolving this with a hemodynamic response function (HRF), a predicted fMRI time course was generated. This process is repeated for a large set of candidate neural tuning models, all generating a prediction of the fMRI time course. For each recording site, the neural tuning model parameters are chosen that yield predictions which best fit the recorded data. Goodness of fit is quantified as the sum of squared differences ($R^2$, variance explained) between the predicted and observed fMRI time series.

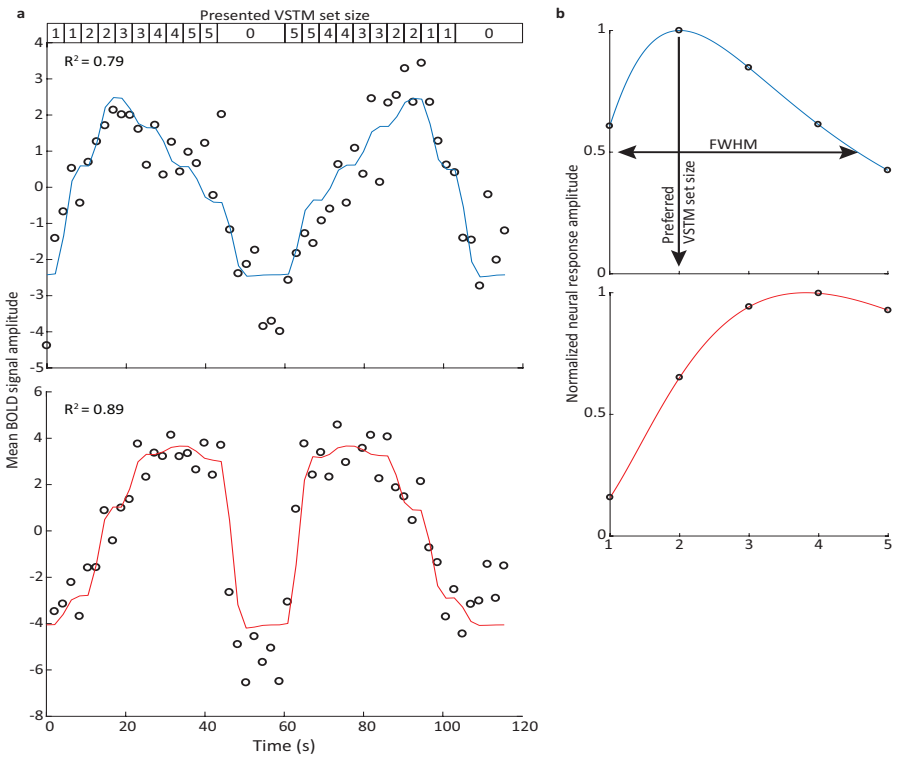


***Figure 3** | a) Two fMRI time courses of different recording sites located in the same VSTM map, with the hemodynamic response delay taken into account. Both recording sites changed their response amplitude, depending on its preferred VSTM set size. Black circles represent the mean BOLD signal amplitude. Blue and red lines show the model prediction, which captures more than 79% of the response variance across the time course. In the top panel, larger signal amplitudes were observed for lower set sizes. In the bottom panel, larger signal amplitudes were observed for higher set sizes. b) Neural model predictions of the recording sites' fMRI courses shown in A. The model describes VSTM set size tuning as a logarithmic Gaussian function with two parameters, namely preferred VSTM set size and tuning width. The tuning width is defined by the full-width at half-maximum (FWHM).*

## RESULTS

Data from both task configurations were initially analyzed separately. However, the two task configurations gave very similar responses. Therefore, analyses on both task configurations were performed separately, as well as on the average of both task configurations.

### Behavioural results

Subjects engaged in two task configurations to both control for and measure the influence of task difficulty. However, during some of the trials the subject did not respond within the given timeframe of 3500 ms. Because the answer of the subject is unknown, trials without a response are removed and not included in further behavioural analysis.

Behavioural performance decreased in the constant orientation task configuration and remained relatively stable in the constant difficulty task configuration. A MANCOVA was performed with the amount of correct answers as a dependent variable, VSTM set size as a covariate, and subject as a fixed factor. The MANCOVA explained a significant amount of the variance in the constant orientation configuration, implying an increase in task difficulty as set size increases, $F(1,11) = 37.081$, $p < 0.001$, $\eta p2 = 0.771$. The same MANCOVA did not reveal a significant amount of variance in the constant difficulty task configuration, implying a constant task difficulty across set sizes, $F(1,11) = 0.595$, $p = 0.457$, $\eta p2 = 0.51$.

Tuned responses to visual short-term memory set size fMRI responses to changing VSTM set sizes were measured during the experiment and summarized afterwards using VSTM set size population pRF models (Fig. 3). These models describe a Gaussian tuning function in both linear and logarithmic space with two parameters: preferred VSTM set size and tuning width. To measure whether a linear or logarithmic Gaussian

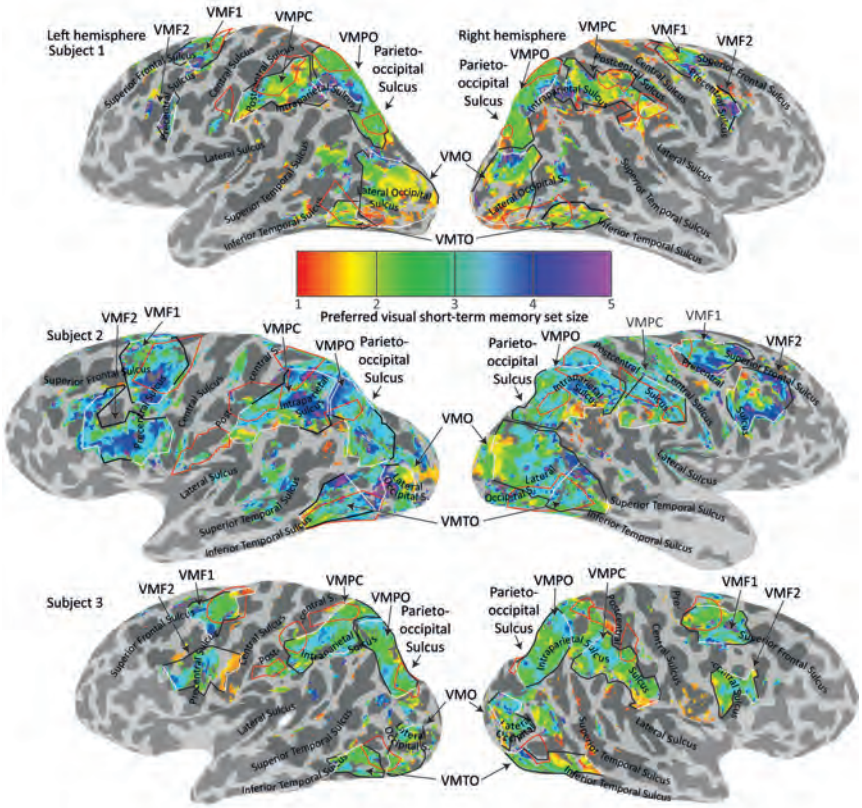


***Figure 4 |*** *Tuned responses to the amount of items encoded in VSTM and their organizational properties. Preferred VSTM set size from the VSTM tuning model (variance explained >30%) was projected on the subject's inflated cortical surface. This uncovered a topographic organization of the used VSTM set size range (1-5). Each hemisphere contained six consistent VSTM maps, covering occipital, parietal and frontal regions. VSTM maps are named according to their anatomical locations and preceded with 'VM' for visual short-term memory. The two white lines of each map represent borders with a constant minimum and maximum preferred set size of the range within that map. The two remaining black lines defined the edges of the map, where goodness of fit of the model decreased (variance explained <30%). Red lines represent borders of the previously described numerosity maps (Harvey & Dumoulin, 2017). Frontal and anterior temporal portions of each hemisphere fell outside the recorded fMRI volume (lighter gray-shaded region).*

function fitted the results better, the difference between the explained variance of both models was calculated. A paired-samples t-test was conducted and showed that a logarithmic Gaussian function explains slightly more response variance; M = 0.0076, SD = 0.0165, t(19945) = 65.0945, p < 0.001.

*Correlation coefficient (Pearson's r) of set size preferences across the two task configurations*

| Hemisphere | Map | Subject 1 | Subject 2 | Subject 3 |
|---|---|---|---|---|
| Left | VO | .6** | .66** | .53** |
| | VTO | .18 | .62** | .26* |
| | VPO | .52** | .51** | .59** |
| | VPC | .42** | .59** | .66** |
| | VF1 | .63** | .29** | .8** |
| | VF2 | .15 | .36** | .31* |
| Right | VO | .52** | .56** | .64** |
| | VTO | .11 | .36** | .64** |
| | VPO | .42** | .40** | .67** |
| | VPC | .59** | .43** | .74** |
| | VF1 | .59** | .57** | .58** |
| | VF2 | .49* | .5** | .62** |

* $p < 0.05$. ** $p < 0.0001$. P-values are FDR-corrected.

The resulting minimum $R^2$ values where the goodness of fit of the model explained >30% of the response variance was projected onto the subject's inflated cortical surface. Following this, preferred VSTM set size from the VSTM tuning model was projected on the same inflated cortical surface. This uncovered a topographic organization of the used VSTM set size range (Fig. 4). For each VSTM map, lines were drawn across recording sites with a constant minimum and maximum preferred set size of the range within that map. The two remaining side borders defined the edges of the map, where goodness of fit of the models decreased (variance explained <30%). 36 maps in total were defined (6 maps x 2 hemispheres x 3 subjects). Maps varied in size and precise anatomical locations. However, locations relative to major gyri were found consistently across all subjects (Fig. 4).

As in visual field mapping- and numerosity mapping studies (Harvey & Dumoulin, 2017; Wandell, Brewer, & Dougherty, 2005), VSTM maps were named according to

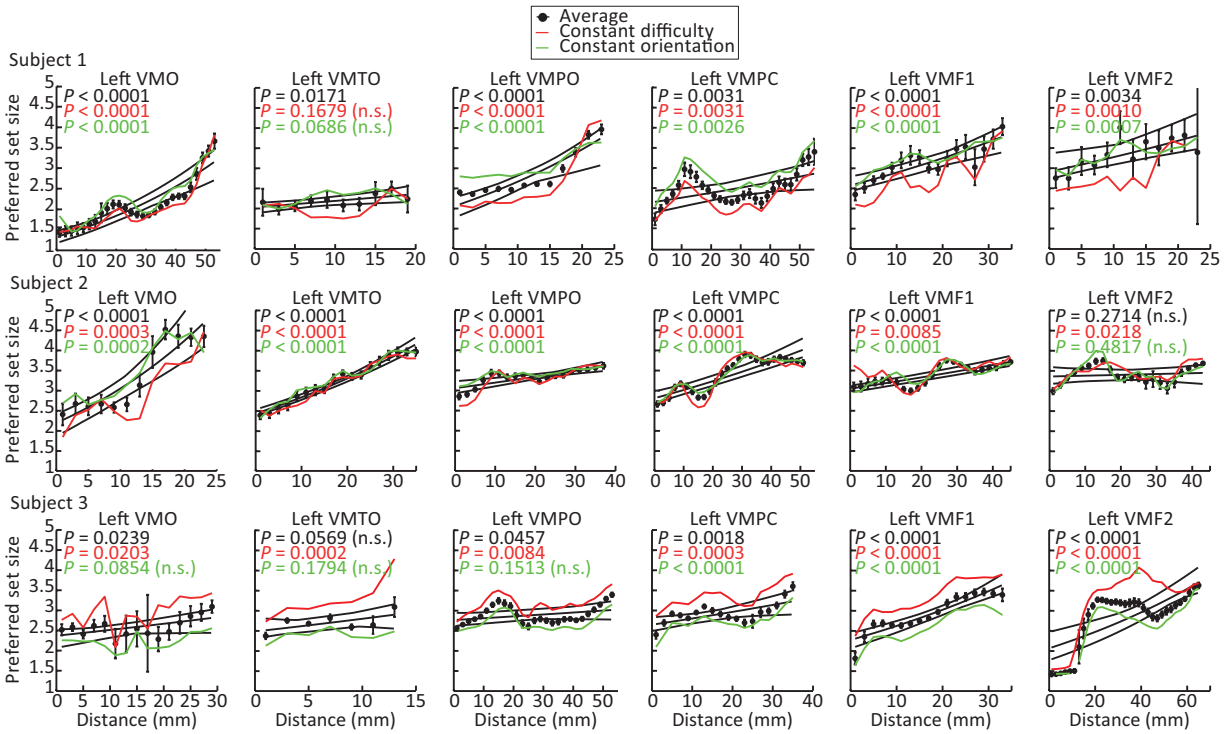


***Figure 5a*** *| Set size preferences changed in most the left hemisphere's VSTM maps. Bins were created by calculating the cortical distance of recording sites with respect to the white lines seen in Figure 4. Then preferred VSTM set size was plotted against this distance. Red and green lines represent the means of the task configuration's bins. Black dots represent the mean preferred VSTM set size for bins with a logarithmic function (black line). Error bars show the standard error of the mean for each bin. P-values are FDR-corrected.*

their anatomical locations. Each map's name is preceded with 'VM' for visual short-term memory (Fig. 4). The first VSTM map (VMO for 'VSTM occipital') is located in and around the lateral occipital sulcus. The second VSTM map (VMTO) covers the inferior lateral occipital lobe and a portion of the inferior posterior temporal lobe. The third VSTM map (VMPO) extends from the dorsal occipital lobe along the superior parietal lobule. The fourth VSTM map (VMPC) lies in and around the postcentral sulcus. The fifth and sixth map (VMF1 and VMF2) are located in and around the junction of the precentral sulcus, and superior and inferior frontal sulci respectively.

## Preferred VSTM set size progression within each VSTM map

To determine the organizational properties of each maps' preferred VSTM set size, bins were created by calculating the cortical surface distance of recording sites with respect to the white lines seen in figure 4. Then the preferred VSTM set size was plotted against this distance (Fig. 5a and Fig. 5b). Preferred VSTM set size changed significantly (2 task configurations x 6 maps x 3 subjects) in 34 of the 36 right-hemisphere calculations and in 30 of the 36 left-hemisphere calculations. P-values are corrected for the false discovery rate (FDR), which is a method to control for type I errors when conducting multiple comparisons (Benjamini & Hochberg, 1995).

## Relationship between set size preference and task configuration

Preferred set size changed consistently across the cortical surface in both task configurations (Fig. 5a and Fig. 5b). Following this, per map the recording sites' preferred VSTM set size of the constant orientation task configuration was correlated with the preferred VSTM set size of the constant difficulty task configuration. This revealed a significant positive correlation in 16/18 VSTM maps of the left hemisphere and in 17/18 VSTM maps of the right hemisphere (Table 1). P-values are FDR-corrected.

## Relationship with numerosity maps

To assess the relationship with the numerosity maps, both the VSTM maps and the numerosity maps were rendered onto the same cortical surface (Fig. 4). Previous fMRI data consisting of topographically organized numerosity maps from two of the subjects were used, and measured numerosity maps for the third subject using the experimental procedures of Harvey and

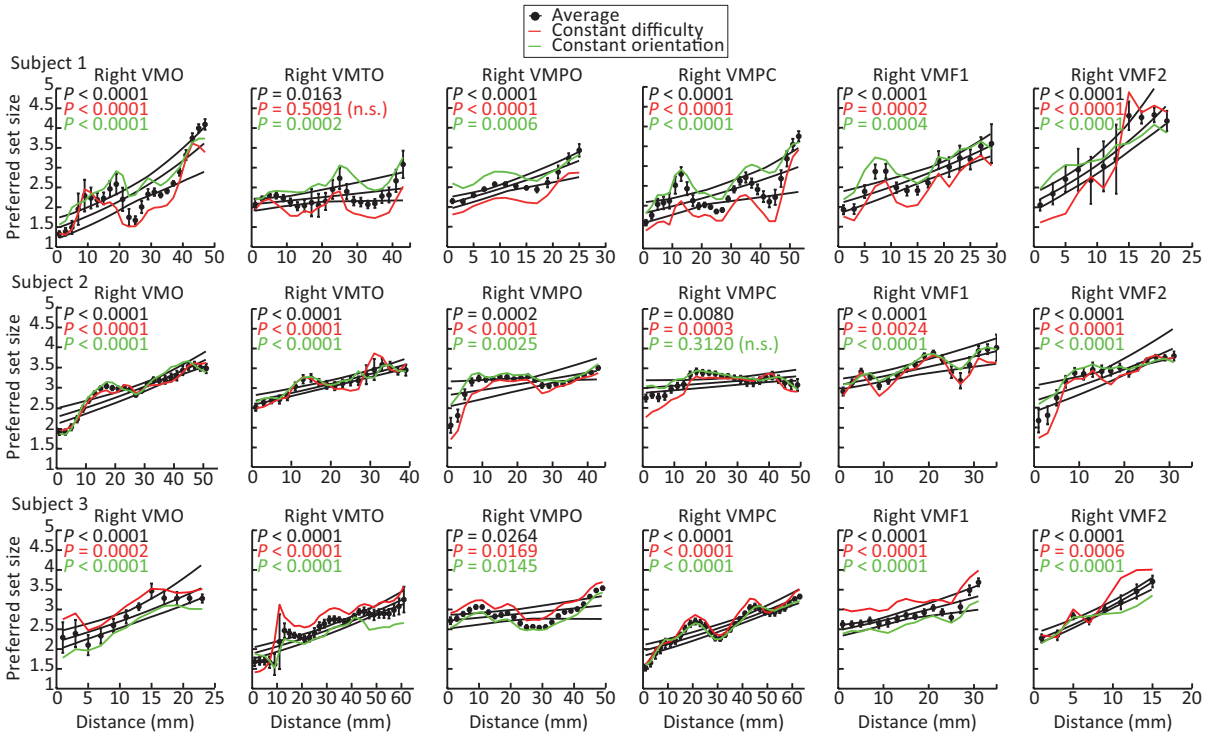


***Figure 5b*** *| Set size preferences changed in most the right hemisphere's VSTM maps. Bins were created by calculating the cortical distance of recording sites with respect to the white lines seen in Figure 4. Then preferred VSTM set size was plotted against this distance. Red and green lines represent the means of the task configuration's bins. Black dots represent the mean preferred VSTM set size for bins with a logarithmic function (black line). Error bars show the standard error of the mean for each bin. P-values are FDR-corrected.*

Dumoulin (2017). The VSTM maps largely include the numerosity maps. In addition, VSTM maps cover much larger areas of the frontal and parietal cortex (Fig. 4).

Lastly, areas were determined where the VSTM maps and the numerosity maps overlap and created combined maps. Both the logarithmic VSTM tuning model and the previously used logarithmic numerosity tuning model of Harvey and Dumoulin (2017) were used to calculate VSTM set size- and numerosity preferences within these combined maps. Following this, preferred VSTM set size was correlated with preferred numerosity. A few correlations were found, but not consistently for each map and across subjects. As such, VSTM and numerosity maps show anatomical overlap, but response preferences of areas where these maps overlap do not correlate.

## DISCUSSION

The present study examined tuned responses to the number of items encoded into visual short-term memory (VSTM). The current study explored whether 1) tuned VSTM responses exist in the human brain 2) whether these are topographically organized, 3) how tuning and organization of those responses relate to the numerosity maps, and 4) how task difficulty influences neural responses. Critically, this design kept the numerosity of the visual display constant across different set sizes, which allowed us to explore tuned VSTM responses to a particular set size in the absence of other confounding physical variables as a function of numerosity (Gebuis, Gevers, & Kadosh, 2014; Knops et al., 2014; Mitchell & Cusack, 2007). In addition, the design allowed to control for and measure the influence of task difficulty on VSTM sensitive blood oxygen level-dependent (BOLD) responses (Barch et al., 1997; Gould et al., 2003; Tregellas et al., 2006) using two task configurations. Tuned responses to the amount of items encoded into VSTM were found that are organized in topographic maps throughout the human cortex. VSTM set size preferences changed gradually in most of the VSTM maps.

To assess the relation between the tuning and organizational properties of VSTM and numerosity, previous functional magnetic resonance imaging (fMRI) data and experimental procedures were used from Harvey and Dumoulin (2017). The VSTM maps largely include the numerosity maps, but cover much larger

areas of the frontal- and parietal cortex. In addition, more VSTM maps might exist around the parieto-occipital- and postcentral sulci, as their preferred number of items progress without clear borders. While representations of VSTM and numerosity show considerable spatial overlap, organizational differences do exist. That is, preferred VSTM set size and preferred numerosity within overlapping areas are not related. It is known that the same neurons can change its functional properties according to the perceptual task that is being performed. For example, neurons can respond very differently to an identical visual stimulus under several visual discrimination tasks (Li, Piëch, & Gilbert, 2004). However, a single recording site contains hundreds of thousands of neurons and different neurons within the same recording site can respond, depending on the task at hand. Therefore, it is not clear whether the same neurons respond to both VSTM set size and numerosity, or whether different neurons within the same recording site respond to either VSTM set size or numerosity.

In contrast with the current study, subjects performed no task in the numerosity study (Harvey & Dumoulin, 2017). Yet, both VSTM and numerosity maps overlap. The observed anatomical overlap might implicate that the mere perception of numerosity and the further encoding of these items in VSTM are distinct processes that may share some neural resources. This proposition could also account for the similar behavioural capacity limits between numerosity and VSTM (Melcher & Piazza, 2011; Piazza et al., 2011). Additionally, there seem to be dissociable neural mechanisms for the mere selection of visual objects in the inferior intraparietal sulcus (IPS), and for the further processing of those objects in the superior IPS (Xu & Chun, 2009).

Behavioural- and brain imaging studies suggest that visual objects in the visual scene may be represented in anatomical maps, depending on the relative salience of each individual object (Fecteau & Munoz, 2006; Knops et al., 2014; Piazza et al., 2011). This is based on the concept of an attentional priority map that reflects the distribution of attention across the visual scene (Itti & Koch, 2001). However, attentional priority maps are generally considered with respect to the object's spatial position, and not for other features like VSTM set size.

Nonetheless, relationships between attention and VSTM do seem likely. Many cognitive models consider the mediating role of attention (Baddeley, 2000; Cowan, 1988) and others reviewed the extensive overlap between both abilities (Gazzaley & Nobre, 2012). Hence, both numerosity- and VSTM maps show considerable spatial overlap with the human dorsal frontoparietal attention network (Szczepanski, Pinsk, Douglas, Kastner, & Saalmann, 2013). Given the larger coverage of the VSTM maps in frontal and parietal areas, attentional resources might be differentially involved in the perception of numerosity and the encoding of items into VSTM. While the mere perception of numerosities might not stress the attention network as much, encoding these representations in VSTM might stress the dorsal frontoparietal attention network more. Hence, larger areas of the cortical surface might be devoted to encoding VSTM set sizes that could explain the observed size differences between numerosity- and VSTM maps.

The current findings are in accordance with previous fMRI studies that demonstrate numerosity- and VSTM related activation patterns in posterior parietal cortex (Knops et al., 2014; Mitchell & Cusack, 2007) and the occipital lobe (Todd & Marois, 2005), which are thought to be involved in VSTM storage and visuo-spatial attention (Marois & Ivanoff, 2005; Xu & Chun, 2006). The topographic VSTM maps found in the current study also show overlap with frontal areas that are implicated in decision making (Heekeren, Marrett, Bandettini, & Ungerleider, 2004), topographic maps of visuospatial attention (Silver, Ress, & Heeger, 2005), and visual responsive areas in the occipital cortices (Wandell, Brewer, & Dougherty, 2005). The involvement of the VSTM maps with these specialized areas further emphasizes the distributed role of VSTM, which is also argued for working memory (Christophel, Klink, Spitzer, Roelfsema, & Haynes, 2017).

The spatial resolution of the 7T fMRI scanner is superior to any other non-invasive imaging technique, but cerebral hemodynamic responses evoked by visual stimuli are delayed by one or two seconds and have a temporal width between four to six seconds (Menon & Kim, 1999). In the current experiment, subjects had to encode, maintain, and compare unique colour-orientation combinations within 4.2 seconds. The short timeframe makes it difficult to distinguish between the different measured VSTM components.

At the very least, however, evidence is provided for encoding a variable amount of items into VSTM, as the experiment did not require VSTM maintenance. A possible solution to isolate the maintenance component is to increase the retention interval. However, prolonging the retention interval could recruit the broader working memory (WM) system. It is generally assumed that WM is involved in the temporary maintenance and manipulation of information within a limited-capacity system using two storage systems (Baddeley, 1992). VSTM is used as a temporary buffer to briefly store visual information (Phillips, 1974). The second storage system is known as the phonological loop and is considered to be involved in the retention of information and rehearsal processes

(Baddeley, 1992). Increasing the retention interval, and thereby isolating the maintenance component, could evoke verbal rehearsal strategies to maintain encoded information. Indeed, other VSTM studies used auditory-presented digits that had to be remembered during the experimental task to minimize verbal rehearsal strategies (Todd & Marois, 2004; Xu & Chun, 2006). Another way to isolate the maintenance component of the task is to present unique items that all have to be encoded. Then a retro-cue is used to signal the subject which items they have to maintain for a longer period of time (delayed-response).

A final addition is the inclusion of an additional control experiment where a single sample bar has to be maintained in mind. Then six test bars appear and the subject has to decide whether the same-coloured bar from the test display had changed orientation. Fewer tuned responses are expected when a single item has to be kept in VSTM, as is the case in the current study.

The observed tuned responses to VSTM set size seem to agree with slot models of visual working memory. This proposition holds that items are each stored in three or four separate object slots (Luck & Vogel, 1997). However, this view has been challenged by studies examining the precision of recalled objects, rather than the amount of recalled objects (Bays, Catalao, & Husain, 2009). For example, Alvarez and Cavanagh (2004) showed that the behavioural VSTM capacity limit varies as a function of the number of objects, as well as increasing the amount of visual features or details of the objects. An fMRI study supports both views and found that representations in inferior IPS are fixed to about four objects, regardless of object complexity. In contrast, those in the superior IPS and the lateral occipital lobe varied, representing fewer than four objects as their complexity increased (Xu & Chun, 2006). Notably, the functional activation patterns were observed during both VSTM encoding and maintenance. In the current study relatively simple, two-featured visual objects were presented at fixed positions. Given the differential activation patterns found in the study of Xu and Chun (2006), it would be interesting to present objects with more than two features and measure whether preferred set size response functions vary as complexity increases.

To both control for and measure the influence of task difficulty on BOLD responses to each VSTM set size (Barch et al., 1997; Gould et al., 2003; Tregellas et al., 2006), two task configurations were used. As observed, the constant difficulty configuration led to a performance that was similar for all set sizes. In the constant orientation configuration performance decreased as set size increased. This is in line with other studies that report behavioural performance reductions while increasing attentional load (Burr et al., 2010; Vetter et al., 2008), object complexity (Alvarez & Cavanagh, 2004), numerosity (Knops et al., 2014), performing dual-tasks (Piazza et al., 2011), all of which have an inherent component of increasing difficulty. However, in the current study, no functional differences were observed between both task configurations.

Is performance a valid measure of task difficulty? A wide variety of processes that are not obviously related to the task could explain this invariability. For example, subjects could have encoded the display as well as possible, regardless of how difficult the task at hand is. Another possibility is that the observed responses could be responses to the number of groups of items, rather than the orientation differences. A related factor is the predictable cycle sequence, which allows subjects to anticipate the amount of upcoming set sizes. It has been shown that perceptual expectation can influence visuocortical areas (Gilbert & Li, 2013; Larsson & Smith, 2011). Presenting stimuli in a random order would resolve this issue and also ensures more robustness of the measured cognitive ability.

Taken together, the present study was designed to answer the question whether tuned responses to VSTM set size exist in the human brain and opens up exciting possibilities for further studies to elaborate on these questions.

## CONCLUSION

Tuned responses to the amount of items encoded into visual short-term memory (VSTM) were found that are organized in topographic maps throughout the human brain. Functional magnetic resonance imaging (fMRI) data and experimental procedures were used and adapted from Harvey and Dumoulin (2017). While no memory requirements were needed in the numerosity study, VSTM maps that largely include the numerosity maps were found, but cover much larger areas of the frontal and parietal cortex. While representations of VSTM and numerosity show considerable spatial overlap, preferred VSTM set size and preferred numerosity within overlapping parts are not related. In addition, no functional differences were observed between both task configurations, which brings into question whether performance is a good measure of task difficulty. Enumerating small quantities and encoding these representations into VSTM seem to be distinct cognitive processes that may share common neural resources. As suggested by others, the same brain areas might be involved in both the perception of objects and encoding these representations for a range of upcoming behaviours (Christophel et al., 2017; Gazzaley & Nobre, 2012; Mitchell & Cusack, 2007).

## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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# In-vivo imaging of the blood-brain barrier in Alzheimer's disease: A short review

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*The blood-brain barrier (BBB) has been the target of recent investigation of Alzheimer's disease (AD), due to its role in amyloid-ß removal. Animal studies have provided ample evidence of the involvement of BBB permeability and dysfunction in AD. This review presents support for the view that in-vivo imaging studies are in line with these findings, proving that subtle changes in the BBB of AD patients are seen, as compared to healthy controls. Moreover, the methodological advantages and disadvantages of the applied imaging techniques are discussed. Overall, in-vivo imaging has been able to support the neurovascular hypothesis of AD and implicate BBB impairments in AD pathology. However, the mechanisms of AD pathogenesis can only be further understood if in-vivo imaging is supplemented by the analysis of additional variables, such as vascular correlates or cerebrospinal fluid markers. Overall, the results of the discussed studies support the neurovascular hypothesis of AD, which suggests a causative role of multiple vascular factors in the pathogenesis and progression of AD.*

***Keywords:*** *Alzheimer's disease, blood-brain barrier, in-vivo imaging, magnetic resonance imaging, positron emission tomography*

***Abbreviations:*** *AD – Alzheimer's Disease; aß – amyloid-ß; ABC transporters – ATP-binding cassette transporters; BBB – Blood-Brain Barrier; CBF – cerebral blood flow; CSF – cerebrospinal fluid; DCE-MRI – dynamic contrast-enhanced MRI; MCI – mild cognitive impairment; MRI – magnetic resonance imaging; PET – positron emission tomography; Pgp – P-glycoprotein;* $PS_w$ *- trans-BBB permeability surface-area product to water;* $v_L$ *– leakage volume.*

Alzheimer's disease (AD) is a neurodegenerative disorder, primarily affecting elderly individuals and placing an increasing social and economic burden on society. Amyloid-ß (aß) plaques are one of the hallmarks of AD, and recent findings from animal studies indicate that the breakdown of the blood-brain barrier (BBB) is related to impaired aß clearance in AD (Zenaro, Piacentino, & Constantin, 2017). The BBB forms the border between the cerebrovascular system and the brain (Zhao, Nelson, Betsholtz, & Zlokovic, 2015). It is composed of endothelial cells, which are structurally supported by pericytes and astrocytic endfeet (Zhao et al., 2015). The tight junctions between the endothelial cells are highly selective, allowing only oxygen influx, carbon dioxide efflux, as well as passage for lipophilic molecules and drugs (Zhao et al., 2015). Due to this selective permeability at the tight junctions, the efflux and influx of other molecules is highly regulated by several transporter proteins (Obermeier, Danemann, & Ransohoff, 2013). These transport restrictions enable the BBB to sustain a homeostasis in the brain parenchyma, to prevent toxic substances or pathogens from entering the central nervous system (CNS), and to participate in active waste removal (Zhao et al., 2015).

In-vivo imaging methods allow for the assessment of the BBB structure and function, however, only few studies have been conducted on the topic due to several methodological limitations, such as insensitivity of outcome measures to subtle permeability changes of the BBB (van de Haar et al., 2015). Nevertheless, clarifying the relevance of BBB damage in AD in human patients is important for two reasons. Firstly, should the BBB indeed be impaired in AD patients, early detection of changes in the BBB could lead to improved treatment for delaying disease onset. Secondly, any pharmacological treatment working within the brain parenchyma needs to pass the BBB to be effective. An altered BBB structure, thus, needs to be assessed to evaluate the efficacy of any possible pharmaceutical treatment in AD patients.

This review will present evidence provided by in-vivo imaging studies for the hypothesis that BBB damage is a feature of AD. Furthermore, it will discuss whether or not in-vivo imaging is a useful tool for understanding the mechanisms of AD. In general, there are two ways in which an impairment in the BBB may occur: either its permeability increases by damage to, or deterioration of, the tight junctions, or the active regulation of molecular transport is impaired. Van de Haar and colleagues (2015) defined the former as BBB leakage or permeability, and the latter as BBB dysfunction. In this review, the same

Table 1
*Overview of Studies Discussed in this Review.*

| | | n | | | | |
|---|---|---|---|---|---|---|
| Author (Year) | Subjects | Control | MCI | AD | Technique | Results |
| Freeze et al. (2017) | Human | 26 | 33 | 15 | MRI | No statistical group differences. |
| Van de Haar et al. (2017) | Human | 17 | - | 16 | MRI | Larger leakage volume in AD patients. |
| Van de Haar et al. (2016b) | Human | 17 | - | 16 | MRI | Larger leakage volume in AD patients. |
| Van de Haar et al. (2016a) | Human | 18 | - | 16 | MRI | Reduction in CBF correlates with increasing leakage rate. |
| Montagne et al. (2015) | Human | 42 | 21 | - | MRI | Accelerated, age-dependent BBB breakdown of the hippocampus in MCI. |
| Starr et al. (2009) | Human | 15 | - | 15 | MRI | BBB permeability in both, AD patients and healthy controls. |
| Deo et al. (2014) | Human | 9 | - | 9 | PET | Pgp activity significantly lower in some brain areas in AD patients. |
| Van Assema et al. (2012a) | Human | - | - | 18 | PET | No effect of microbleeds on Pgp in AD patients. |
| Van Assema et al. (2012b) | Human | 14 | - | 13 | PET | Pgp activity is compromised in AD. |
| Dickie et al. (2018) | Rat | 5 | - | 7 | MRI | Subtle BBB leakage in AD rats. |

Note: AD = Alzheimer's disease; BBB = blood-brain barrier; CBF = cerebral blood flow; MCI = mild cognitive impairment; MRI = magnetic resonance imaging; PET = positron emission tomography; Pgp = P-glycoprotein.

terminology will be employed. The literature search was conducted in PubMed using three-word search strings with the key words BBB, in-vivo imaging, MRI, PET, AD, and dementia. This review is not meant to be comprehensive, but rather to give an overview of the most recent advances in the field. Table 1 lists the authors, the methodological details and a short summary of the results of the studies included in this review.

## Imaging Passive Leakage with Dynamic Contrast-Enhanced Magnetic Resonance Imaging

Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) is the most widely used imaging method to assess passive BBB leakage (Heye et al., 2016). For this method, a paramagnetic contrast agent, typically gadolinium-based, is injected into the bloodstream of the participant. Gadolinium decreases the relaxation time of water protons, thus enhancing the signal wherever it is present (Heye et al., 2016). Should there be BBB leakage, signal enhancement by the contrast agent should not only be seen in the vascular system, but also in the brain parenchyma. Recording multiple $T_1$ images consecutively then allows for a temporal assessment of the distribution of the contrast agent. Commonly, the value $K^{Trans}$ is calculated to quantify BBB permeability. $K^{Trans}$ is a constant, which indicates the rate at which the contrast agent leaks into the extravascular space per voxel and overall plasma concentration (Heye et al., 2016). However, studies using leakage rate, as quantified by $K^{Trans}$, often failed to find a significant difference between healthy subjects and AD patients (Freeze et al., 2017; Starr, Farrall, Armitage, McGurn, & Wardlaw, 2009). The lack of significance could be ascribed to an insensitivity of the calculated outcome measure $K^{Trans}$.

### Alternative Outcome Measures of BBB leakage with DCE-MRI

Van de Haar and colleagues (2016b) used overall leakage volume ($v_L$) as a measure of BBB permeability. That is, instead of looking at the amount of tracer that leaks into the brain parenchyma, the overall number of voxels showing signal enhancement is determined. The authors report differences in $v_L$ between healthy participants and AD patients in grey matter, but also in white matter and deep grey matter regions (van de Haar et al., 2016b; van de Haar et al., 2017). By contrast, the conventional $K^{Trans}$ measure described above only showed a significant group difference within cortical grey matter regions. This indicates a

higher sensitivity of the new measure $v_L$ to subtle BBB changes. Importantly, $v_L$ does not require longer scanning times or additional injections, but can be extracted from the same images that are used to calculate KTrans (van de Haar et al., 2017).

Alternatively, Dickie and colleagues (2018) developed an MRI technique for measuring the transport of endogenous water molecules into the brain parenchyma. The signal of water exchange is enhanced by the injection of a contrast agent that shortens the relaxation time of blood, thus decreasing the blood signal and enhancing the water signal in MRI images. They term their measure trans-BBB permeability surface-area product to water ($PS_w$) (Dickie et al., 2018). Their study demonstrated a significant difference between healthy mice and transgenic AD mice, with a higher $PS_w$ in AD mice. Importantly, the group difference was not found in the same mice when looking at $K^{Trans}$ (Dickie et al., 2018). Thus, PSW also seems to be more sensitive to subtle BBB permeability changes than $K^{Trans}$. However, due to the long scanning time needed to calculate $PS_w$, it is questionable if it will be implemented into research or clinical practice in the near future (Dickie et al., 2018).

### Imaging BBB Dysfunction with Positron Emission Tomography

Whereas MRI offers the possibility to observe passive leakage at the BBB, positron emission tomography (PET) enables researchers to assess the active transport mechanisms at the BBB in-vivo. In AD, the ATP-binding cassette (ABC) transporters at the BBB have been investigated frequently (Deo et al., 2014; van Assema et al., 2012a). These transporters are important for the efflux of molecules, such as toxins, pathogens or waste products, including aß. Aß has been shown in animal studies to be inappropriately removed in AD (Zenaro, Piacentino & Constantin, 2017). P-glycoprotein (Pgp) is one ABC transporter previously shown to be responsible for aß removal (van Assema et al., 2012a). Pgp can be imaged in PET using the tracer [$^{11}$C]verapamil. [$^{11}$C] verapamil enters the brain passively, and is known to bind to Pgp from the inside. An increased amount of non-binding [$^{11}$C]verapamil in the brain parenchyma therefore indicates a lower amount of active Pgp. Indeed, a decrease in Pgp activity in AD patients compared to healthy controls has been found in two independent studies (Deo et al., 2014; van Assema, 2012a). Unfortunately, other efflux or influx transporter proteins at the BBB have not been studied with PET.

Vascular Correlates of BBB Damage

The BBB is part of a larger system, namely, the neurovascular unit (van de Haar et al., 2016a). Thus, it is interesting to see whether other vascular factors are related to BBB breakdown. More specifically, in-vivo imaging studies of the BBB have looked at measures of hypoperfusion (van de Haar et al., 2016a), microbleeds (van Assema at el., 2012b), and white matter hyperintensities (van de Haar et al., 2016b). Hypoperfusion is commonly found in patients with mild cognitive impairment (MCI) and AD patients (Zlokovic, 2005), and it has been shown that a decrease in overall CBF is related to an increase in BBB permeability (van de Haar et al., 2016a). In contrast, neither microbleeds (van Assema et al., 2012b) nor white matter hyperintensities (van de Haar et al., 2016b) could be related to BBB permeability or dysfunction. A consistent inclusion of more vascular markers in in-vivo imaging studies of the BBB has the potential to clarify the interactions within the neurovascular unit.

Only one of the previously discussed studies collected cerebrospinal fluid (CSF) samples from participants to assess levels of the soluble platelet-derived growth factor receptor ß (sPDGFRß). An increase in sPDGFRß in the CSF is a proxy for BBB pericyte death. The study showed sPDGFRB was heightened in MCI patients compared to controls (Montagne et al., 2015). Furthermore, the level of sPDGFRß correlated positively with BBB permeability (Montagne et al., 2015). As briefly mentioned in the introduction, pericytes provide stability to the BBB (Zhao et al., 2015). Therefore, decreased stability of the BBB could result from pericyte death, in turn causing increases in permeability. Due to the invasiveness of retrieving CSF from participants through a lumbar puncture, this procedure is not often performed for research purposes. However, the novel marker sPDGFRß identified by Montagne and colleagues (2015) might prove valuable for measuring BBB breakdown in the future.

## DISCUSSION

The results of the studies discussed in the present review suggest that there is subtle BBB leakage in AD patients compared to controls (e.g., van de Haar et al., 2016b). Furthermore, a change in active transport mechanisms has been identified, thus demonstrating BBB dysfunction (e.g., van Assema et al., 2012a). It has been shown that there are clear technical advances in in-vivo imaging of the BBB, such as increases in the sensitivity of measuring subtle changes in BBB permeability (Dickie et al., 2018; van de Haar et al., 2016b). Despite this progress in the field, in-vivo imaging studies are only rarely able to propose a mechanism by which BBB damage occurs. While they can provide descriptive evidence in favour or against the presence of BBB damage, the mechanisms by which these impairments occur can only be clarified if imaging data is combined with additional biomolecular or vascular measurements.

Most importantly, the findings from the presented studies provide support for the neurovascular hypothesis of AD (Zlokovic, 2005). More precisely, this

hypothesis proposes that one or more vascular events damage the BBB and cause hypoperfusion (Zlokovic, 2011). Hypoperfusion, in turn, leads to increased aß production, and BBB damage leads to decreased aß removal, causing formation of aß plaques within the brain parenchyma. Both aß accumulation and hypoperfusion may then influence the formation of tau-protein tangles. Aß plaques and tau-protein tangles are the pathological hallmark of AD, including neurodegeneration (Zlokovic, 2011).

Pericytes have been proposed as a common mechanism for both, changes in BBB damage and hypoperfusion. On the one hand, pericyte-deficient mice show increases in BBB permeability and changes in active transport proteins at the BBB (Armulik et al., 2010). On the other hand, pericytes are involved in the expansion and contraction of the blood vessels, and a reduction in pericytes results in hypoperfusion (Kisler, Nelson, Montagne, & Zlokovic, 2017). Moreover, in-vivo imaging research has shown that BBB breakdown in the hippocampus correlates with concentration of sPDGFRß, a marker of pericyte death (Montagne et al., 2015). Furthermore, van de Haar and colleagues (2016a) have correlated increased hypoperfusion with increased leakage rates. This emphasizes anew how correlating in-vivo imaging results with additional markers of AD could increase our understanding of the pathogenic mechanisms.

To conclude, in-vivo imaging studies of the BBB in AD have provided additional support for the neurovascular hypothesis. Overall, in-vivo imaging research presents an excellent addition to animal models, and to date it is the only way to examine the BBB in-vivo in humans. Future in-vivo imaging studies should try to go beyond assessing the presence of BBB impairments to understand the mechanisms of AD pathogenesis. This can be achieved by using more advanced imaging techniques and analysis methods, as well as combining imaging approaches with biomolecular, vascular, and cognitive measurements.

## CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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# High-resolution 2D proton magnetic resonance spectroscopic imaging

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Proton magnetic resonance spectroscopy ($^1$H-MRS) is a technique that enables in-vivo quantification of brain-metabolites, which is performed using an MRI-scanner. In conventional MRI-acquisitions, the signal originating from water molecules overwhelms signal from several brain-metabolites with a signal that is approximately 10.000 times as strong. By suppressing water signal during the acquisition, the brain-metabolites become detectable.

The acquired signal is conventionally represented as a spectrum, depicting the signal intensity as a function of the resonance frequency (Figure 1). The differences in frequencies that protons resonate on (i.e. chemical shift) are dictated by their chemical environment, meaning that protons from the same molecules generally resonate at the same frequencies. However, signals from different metabolites occasionally (partially) overlap as well. This can be disentangled by linearly combining predetermined basis-functions, resulting in a least-squares error approximation of the spectrum (Hofmann et al., 2002). Thereby making it possible to quantify the signal of individual metabolites (Bhogal et al., 2017), which is usually achieved by dividing the metabolite signal by a reference signal (e.g. total creatine) to amend acquisition-related variation (Buonocore & Maddock, 2015).

Spatial coverage using the conventionally used single-voxel (SV) $^1$H-MRS is narrow, and repeating the same acquisition for different locations in the brain is time demanding (Buonocore & Maddock, 2015). On the other hand, 2D $^1$H-MRSI produces a distribution of metabolite concentrations from a single slice across the brain in significantly less time (Nassirpour, Chang, & Henning, 2018).

Improvements of MR hardware have been made in recent years, including the use of ultra-high-field strength magnets (7$^+$ Tesla) (Henning, 2017), and novel lipid suppression techniques (Boer, Van De Lindt, Luijten, & Klomp, 2015). This enabled improvements in sensitivity and resolution within reduced scan time (Godlewska, Clare, Cowen, & Emir, 2017), facilitating the acquisition of high spatial-resolution $^1$H-MRSI (Figure 2).

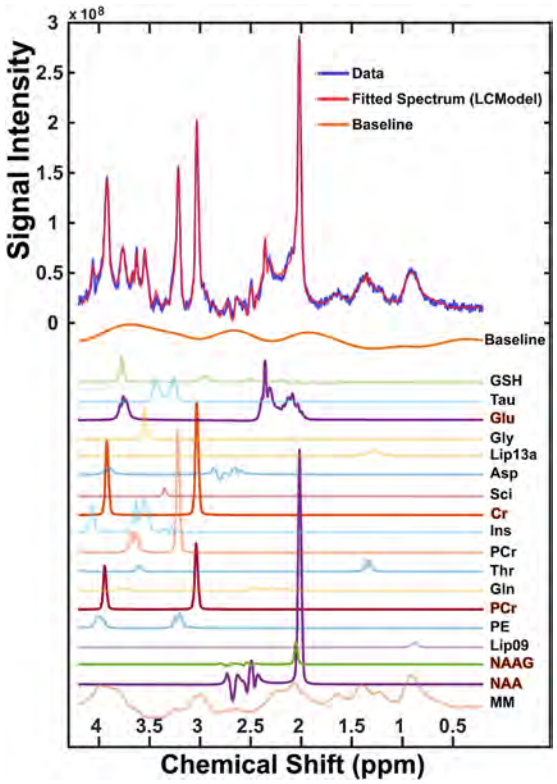


***Figure 1 |*** *In this figure, the raw signal spectrum that is attained using single-voxel proton magnetic resonance spectroscopy (SV 1H-MRS) is presented (top, blue). The y-axis depicts the signal intensity, whereas the x-axis illustrates differences in resonance frequency. The spectrum shows a water- and lipid-suppressed metabolite spectrum. Additionally, the fitted spectrum (top, red) is presented, and a baseline (top, orange). The fitted spectrum is composed of a combination of predetermined metabolite-specific basis-functions (bottom). The image is retrieved from (Bhogal et al., 2017).*

Despite the ability to detect subtle changes in metabolite concentration across entire slices of the brain (Bustillo, 2013), clinical usage of $^{1}$H-MRS has been limited. However, in light of recent developments, usage of 2D $^{1}$H-MRSI seems promising for analysing neurological and psychiatric disorders. The technique is perfectly suitable for studying metabolic biomarkers from entire slices across the brain, which in turn might act as predictor for at-risk individuals or as indicator for treatment response in diseased individuals.

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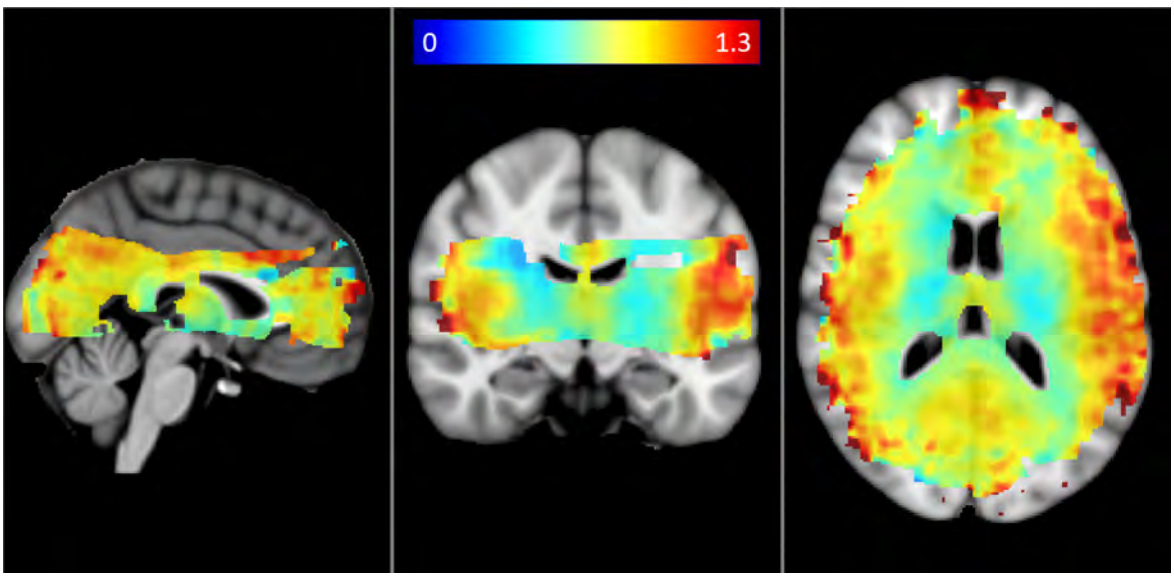



***Figure 2 |*** *In this figure, the raw signal spectrum that is attained using single-voxel proton magnetic resonance spectroscopy (SV 1H-MRS) is presented (top, blue). The y-axis depicts the signal intensity, whereas the x-axis illustrates differences in resonance frequency. The spectrum shows a water- and lipid-suppressed metabolite spectrum . Additionally, the fitted spectrum (top, red) is presented, and a baseline (top, orange). The fitted spectrum is composed of a combination of predetermined metabolite-specific basis-functions (bottom). The image is retrieved from (Bhogal et al., 2017).*

# Personalized medicine interview

# Jurjen Luykx

*Dr. Jurjen Luykx is a psychiatrist and a neuroscientific researcher. His scientific research focuses on the genetic foundations of psychiatric disorders, in particular the applicability of genetics to the diagnosis and treatment of patients with psychiatric disorders.*

***We know that you are a researcher and are also practising psychiatry. Can you tell us a bit about your background?***

I trained to become a psychiatrist at the department of psychiatry in Utrecht. Then I did a short training in neurology for a year, and then I combined my residency in psychiatry with a PhD program. The advantage of this combination is that you can collect samples as you move through your residency, so in the end you do statistical analysis and wrap up. The disadvantage though is that doing the combination of the PhD with the residency isn't very 'A to Z', so it doesn't always give you the complete picture.

My work week, nowadays, is mostly dedicated to education which is intertwined with a residents program that I direct in Apeldoorn. The residents are trainees for psychiatry: doctors training to become psychiatrists. I supervise and tutor about 20-25 residents, and make sure their educational and clinical program is all set up which takes up almost 30% of my work week. Other than that, I also have clinical activities which also take up about 20-30% of my week, and then there's research which makes up my remaining time in the week. They are almost one-third each, divided between Utrecht University and the hospital at Apeldoorn. My week illustrates how a doctor does research. Many doctors doing research try and balance all these educational, residency directing, clinical and research activities. That can be a challenge but is also a lot of fun and keeps you alert.

***How did you get into personalized medicine?***

My PhD was not about this. It was about genetic determinants of cerebrospinal fluid constituents. So it was a much more neuroscientific topic, with healthy patients and not with neurological or psychological

disorders. It was a very nice challenge and it was nice to set up such a project, but I really wanted to switch to patients thereafter.

Personalized medicine to me is really about the patient; it's about tailoring your treatment to the individual patient. I think there's a lot that we, as psychiatrists, already do really well. We generally tend to gauge what the patient and their family members would want and then tailor the treatment to that. Some of us may be more aware than other specialists that if you don't tailor the treatment to the patients well, the patient will not take your medication. I think 'shared decision making' is a very important part of this as well, which is involving the patient in the therapeutic decisions. However, there is a caveat here. If you share all the decisions, especially with a schizophrenia patient for example with cognitive impairment, sometimes it isn't practical. I would usually tell the patient where

we stand and what decisions lie before us, and I try to phrase that in very easy terms, which can be a challenge of course, and try and gauge whether the patient would be more confident or whether he or she is very opinionated about a certain treatment or not. It can also be along very simple lines: usually in psychiatry a very important topic is – do you want pills or do you not want pills? That's one of the first things I try to estimate. You can tell that from the sentences they are saying or the questions they are asking – that usually already illustrates whether the patient would want medication or not. So as a profession, I think we're already very used to personalized medicine, but it can still be increased.

To give you an example, in depression, there are a lot of anti-depressants that you can prescribe and patients are very able to say what side-effects they fear most and those which are really important to them. Some patients may say they are skinny enough and don't worry about gaining weight, or others may say they want a certain pill to make them a little drowsy because they have sleeping difficulties. However, another might not want that because they want to go to work in the morning. Thus, the side-effects are a very important factor in this debate, because many pills have the same efficacy profiles so the side-effects can be used to tailor the treatment. These are examples of personalized medicine within a clinical setting – with your out-patient in front of you, which is a very important topic. In research, it's a completely different discussion. But that should enable physicians within their clinical settings to see what is most personal and adaptive to the patient.

As I just mentioned, in research, personalized medicine is a completely different discussion. My research, personally, is about genetics in psychiatry. For example, the study we did was on quality of life. Polygenic risk scores can be computed for any type of trait or disorder for which we have summary statistics available, and with that we are able to take a scan of the genome. This data can give us information about thousands of traits. This is not only for the genetically significant ones, but also the low-threshold ones which contribute to a certain risk. So everyone has a personalized risk profile for different things such as eye colour, height, even schizophrenia.

They can't predict a disease a 100%, but as the data becomes better, the prediction also becomes more accurate. In the clinic, for example, take bipolar disorder, a patient comes in with a manic episode.

***"Personalized medicine to me is really about the patient; it's about tailoring your treatment to the individual patient. I think there's a lot that we, as psychiatrists, already do really well."***

The guidelines say, in some cases you put the patient on lithium for a few years, but sometimes you just treat the single episode, and then follow up without medication. So there's a big difference here. We can use these polygenic risk scores to check the severity of the course of illness and see if the patients need to be more aggressively treated or given medication. Therefore, if you're able to determine polygenic risk scores, which give you a better understanding of the patients who have a poor quality of life, in the future you may be able to take a drop of blood, do a genome scan and get an output a profile in a couple of minutes. Then that profile can actually help you personalize medicine related choices. If a patient is, for example, genetically predisposed to low quality of life, then maybe you can optimize psychosocial therapies for that patient. You can take that into account in management. I think there are two important factors in research here: there's the genetics which is very important, as DNA is stable, and thus very powerful and not confounded by all these environmental circumstances. I think in psychiatry these distinctions are more important than in other fields. For instance, about 70-90% schizophrenia patients smoke nicotine, many of them smoke pot, many have had life stress. Those are all factors which may impact a range of measures, like neuroimaging; these may be influenced by these state-dependent events. But DNA is very stable and gives you a lot of information which is generalizable to a lot of patients, but can hopefully be used for the individual patient in the near future, with the previously mentioned drop of blood and genome scan.

That's not to say that the phenotypic information, which is the second part, is not important. Research should focus on the genetics which is stable, but also on the phenotypic part where we have a lot of

variables which we can assess in different cohorts. This allows you to gauge which variables are associated with which illness. Looking at the phenotype is more pragmatic, because you usually have this information already, but for a fuller understanding of an illness, we need both.

Precision medicine is a similar concept to personalized medicine, which is also about using data of a specific patient to optimize treatment and prognosis in that individual patient. It's also a term encompassing tailoring of medication and treatment management. An example of this is an outpatient clinic that we're setting up here currently at the department of psychiatry. It's about Psychiatric Genetic Counselling. We've adopted this concept from Vancouver, Canada and their model, which is to do psychiatric genetic counselling in patients who have questions ranging from their genetic risks and genetic mechanisms for them or their kids for a specific disorder, for example, but also what environmental factors and what experiences increase risk and how we can go about them. Are there also protective experiences that lower personal risk? I think this is a very good example of how we can become very personal with the patient, because you can assess the aspects which are very important for the patient, given his or her situation. We hope to set up this clinic this year and see patients. It would be a new concept in the Netherlands.

***You have experience both as a clinician and as a researcher. Do you think there's enough communication between the two worlds?***

The residents whom I teach in Apeldoorn are from a non-research setting and the whole idea of research is very 'far-away' for some of them. There are educational sessions for methodologies of course, but they are focused towards clinical trials so that's slightly different. They are usually very interested in research, but there isn't enough exposure within this clinical setting and some doctors may get their PhD degree primarily because that will allow them to get into their specialty. There really should be more 'cross-pollination' between the two, definitely, and the question is: how can we achieve this? What I'm trying to do more in the coming years is applied psychiatric genetics and counselling, and we're currently running some pharmacogenomic studies where we're trying to predict anti-psychotic induced weight-gain, or clozapine induced neutropenia, as a side-effect. These techniques allow us to adopt a more personalized approach to each patient, and will hopefully also make the research more accessible to residents and physicians who might not grasp the more complex genetic data.

So, there should definitely be more cross-talk between the two. There are many good initiatives world-wide, like patient associations for example, so I think we're heading into the right direction. But it would be really nice for people in the clinics to have a better understanding of the research, so they can inspire us with forthcoming research questions. And on the other hand, people in research settings should show a little more interest to actually see the patient and listen to their personal stories, for perspective.

***Would you like to say something about ethics in regards to personalized medicine? Many people are still not comfortable with sharing their genetic data.***

We do genetics-related work in clinical trials in various places, including Israel, for example. We find that consent for genetic studies really differs between countries since the percentage of people consenting to participate highly varies. In Belgium, for example, almost everybody participates. It's almost the same in the Netherlands, only slightly less. But in Israel, much fewer people consent and there are more strict ethical issues. The new data law "Algemene verordening gegevensbescherming" protects people's privacy really well. I think we should stick to a certain standard,

which is not that difficult to conceive in genetics. You can do more and more with summary statistics and these cannot be traced back to a specific person. But if you do a GWAS, and you publish the results, then possibly that individual level GWAS can actually predict what kind of eye-colour, hair-colour or ethnicity a person has, and that becomes tricky because you can use bioinformatics to say that that data pertains to that person, and that should not be the case. I think in genetics, it's basically all about storing individual level data really well and trying to almost always share summary data.

***There are also websites which offer to scan your genome using only a sample of your saliva. What do you think about those tests?***

There are actually some companies that don't do a lousy job at that. That is also why I'm currently setting up the aforementioned clinic, because patients sometimes get their results after giving their saliva and ask you what that means. The way these companies were set up a couple of years ago was really bad. They would just genotype one or two variants and tell you your risk for arthritis based on just that information, and that is not right. Nowadays, they use polygenic risk scores and those can be really helpful.

But there's definitely a demand for this right now, and people want to know. You can't stop it, so you learn from it. You take it to your advantage, like we set up the clinic. As people in Netherlands are doing it more and more, and then psychiatric patients can come to us and we can try and explain. In that way, we can start a conversation about the etiology of the disorder and empower the patient; there's actually research on how genetic counselling can empower patients and improve quality of life and autonomy.

***You already talked about the clinic you're setting up, but otherwise do you think a lot is happening here at UU in terms of genetics based personalized medicine, like pharmacogenomics?***

I know for epilepsy, for example, researchers are trying to use genetics to make new compounds. It's maybe not that personalized because it is kind of general for that group of patients, in a translational approach. Many of them focus on large numbers and disease etiology, but the interesting thing is there's a very close relationship between that and personalized medicine. For example, Jan Veldink does a lot of great ALS research, and he finds certain variants associated with ALS. But that, for example is only found in a certain number of patients. However, you can use that data to zoom in to specific genetic variations. So it's not very black and white, but it's a scale where you go from large data and you move on to individual patients. The larger the data, the more you can say about the individual.

Longitudinal studies are required for this kind of research, which sometimes go on for a decade. One of our projects this year is going to be to discern whether polygenic risk scores can help to predict clozapine response.

> ***"In research, personalized medicine is a completely different discussion."***

***What do you think is the next big step that needs to be taken to improve personalized medicine?***

In psychiatry, there's the aspect of the abundant usage of medication. The trials are generally small, because it usually takes years and about 50 countries at least to include 400 patients, but that number is still often not sufficient for genetic analyses, so there is a tension there. I think what could be very helpful, and is one of my goals for the future, is to be able to accrue people from all these different randomized-control trials, pile their genetic data, and see if we can predict efficacy and response, and see which genetic variants are associated with response to one agent or the other. That's a challenge.

Also, a worry that I personally have is that the medication that has come in the market in psychiatry for the last couple of years is the result of very scant research. There are very few compounds coming out. That's rather worrying in this research. In psychotherapy, there's more development; ramifications of certain therapies go on to prove their efficacies for different individuals. Whereas, in psychopharmacology, there is not a lot of new stuff. Thus, one of my concerns is that we can't proceed as much as we would like to, because we need more medication to more precisely tailor the medications to the individuals. I really hope new medications with less side-effects will be developed, to be able to make more of these decisions based on the specific phenotypic profiles of the patients.

# Outbound

## *"Being in Hong Kong was a mind-blowing experience."*

**NAME**
*Rachida Ganga*

**HOST INSTITUTION**
*Chinese University of Hong Kong*

**TOPIC**
*Differences in cortical processing of melodic patterns*

After running a Neurolinguistics experiment at the UU's Utrecht Institute of Linguistics OTS, I decided to deepen my knowledge on EEG methodology at the Chinese University of Hong Kong. Even as a teen, I wanted to go to South-East Asia to learn more about the different cultures that are so rich and colourful. When my first internship supervisor mentioned her connections with good researchers in Hong Kong, I took my chance. There, I researched the differences in cortical processing of melodic patterns that are shared in Dutch and Mandarin and the developmental change in the representation of Cantonese tones in the brainstem.

Being in Hong Kong was a mind-blowing experience. It is a highly developed, crowded city that is always moving and changing. Constructions are started and finished within weeks. Walking through a crowd requires you to dodge and weave to get to your destination. Anytime you look up, you either see huge concrete skyscrapers or huge mountains completely covered in greenery. Even though Hong Kong had been a British colony for a long time, I was often unable to speak with the locals as they could not or were too afraid to speak English. This was surprising and forced me to learn to communicate in other ways than spoken words!

One important difference between Hong Kong and the Netherlands is that the workload in Hong Kong is much higher. People often come in at 8-9 a.m. and stay until 8 or even 10 p.m. They cannot refuse this and are not paid more for working after office hours. Fortunately, as a foreign student, my lab was flexible and they did not ask the same from me. Thus, my working hours were similar to the UU's and I had plenty of time to explore Hong Kong, Macau, Taiwan, etc. However, I did feel that I had to work much harder to receive interesting work and appropriate training as their focus was put more on the newly hired research assistants. I felt like I was "just a visiting student", which was very different from what I was used to at the UU where I felt like I was part of the team and was even allowed to run my own experiment.

Overall, my internship was a blast. I visited old friends, made many new friends, experienced the Lunar New Year celebrations, had amazing food and so on. However, as a foreign student you are required to protect yourself. If your lab wants you to work at night and during weekends, be proactive and refuse if you do not want to. Also, asking never hurts. Hong Kong is known for its immensely high rental prices. I could not find housing on my own, so I asked my lab for help. They organized very affordable, comfortable housing on campus. I have learned that asking is a privilege that you should definitely make use of. Finally, an important question for anyone considering doing an internship abroad: Why do you want to go abroad? Is it because you want to or because others are considering it? My time in Hong Kong was also difficult without my loved ones. I believe that without the intrinsic motivation I would not have managed to stay as long as I did.

If you are considering an internship abroad and you do not shy away from a challenge, I highly recommend going to Hong Kong!

# Outbound

## *"Living abroad has made me more independent, resilient and open-minded."*

**NAME**
*Minke Nota*

**HOST INSTITUTION**
*APC Microbiome Ireland, Cork, Ireland*

**TOPIC**
*Understanding the gut-brain axis – alterations of the brainstem due to changes in gut microbiota*

I never had any question in my mind about whether I wanted to do my minor research project abroad. If there ever was an opportunity to experience living in a different country, this was the perfect time for it! Besides, I thought an internship abroad would be the best way to see the differences in research between countries. I was very interested in effects of diet on brain function and heard through a PhD student at the lab of my major internship about the Cryan lab in Cork, Ireland. This lab focusses on the gut-brain axis, a topic that was new to me but matched my interest really well. Apparently, Professor Cryan had visited Utrecht before and was acquainted with the lab of my major research project, which made it easy for me to establish a first connection.

I was put in touch with a postdoc in the Cryan lab, with whom I skyped to discuss what kind of project I could work on, and when I would be able to start. We decided that my project would be kept broad, so that I could let anything I liked fall under it. I preferred this because I was more interested in learning as many different techniques as possible, after having done a behaviour-based major research project. This way, I felt like I could get the most broad experience out of my master's and decide better what type of research suits me. The Cryan lab especially was a good place for a varied internship: there are a lot of people, with many projects and collaborations, weekly lab meetings to share work and ideas, and regular seminars on different topics.

One of my favourite things about both the lab and Ireland in general was how open and friendly everyone was. I was happy to arrive in a big lab full of internationals, and with almost everyone being away from home, it was always easy to find someone to do things with outside the lab. We would regularly go for drinks or dinner after work, organise a movie night or arrange a weekend trip somewhere. I loved to explore Ireland with others who were new to the lab, and I formed many close friendships during my time in Cork. I hardly ever felt homesick!

The only major downside about living in Cork is trying to find a good place to live. There is a shortage of affordable housing in the city, and many places are quite cold, damp and mouldy. I joined every Facebook group and housing site that I could find, and eventually found a room just before I left. Unfortunately, once I got there, the house turned out to be awful so I still looked for another place after arrival. If you're going to Cork, don't expect to find accommodation well in advance (and be wary of scams)!

Looking back, of course I gained a lot of research-related knowledge during the internship, but I think all the other experiences I had have been much more valuable. Living abroad has made me more independent, resilient and open-minded, and I have learned some of the best life lessons chatting over a cup of coffee with people from all sorts of different backgrounds. I wouldn't trade it for the world!

# The past and future of the Neuroscience and Cognition master's programme

## according to Dr. Geert Ramakers

*Since this journal is managed by the students of the master Neuroscience and Cognition, we thought it would be nice to ask Geert Ramakers, the coordinator of this master's programme, to tell a bit more about the history, the future plans and the uniqueness of this programme.*

The master Neuroscience and Cognition started when the Bachelor-Master structure was introduced at Utrecht University in 2002. The first directors of the programme were Gerda Croiset (Medicine) and Edward de Haan (Social Sciences) and the first coordinators were Pierre de Graan and Albert Postma. In the first year, the programme had two starting moments (in April and September), and the first cohort consisted of only 20 students. However, after the first try-out year it was decided that the programme continued with only one starting moment (in September) and the master programme was labelled 'prestigious master'. This label allowed strict selection and the opportunity to decide about scholarships. The label 'prestigious master' was ended after a few years, but the master programme remained highly selective.

Marian Joels became director of the programme and Mariken de Krom assisted her. Pierre and Albert remained coordinators. In 2010 I joined them in becoming an overall coordinator. Before this, I was coordinator of the Molecular and Cellular Neuroscience part of the Fundamentals of Neuroscience and Cognition course. In 2012, we completely revised this course, resulting in the programme as it is now. The positions of Albert and Pierre were taken over by Elly Hol and Stefan van der Stigchel, and Peter Burbach became director of the master when Marian left for Groningen. Since 2014, this team is in charge of the master programme Neuroscience and Cognition and Elly became director of the programme in 2018.

The initial concept developed by Gerda, Edward, Pierre and Albert is still intact: the master Neuroscience and Cognition studies the brain in all its aspects. The strength of the programme is using the strong aspects of neuroscience and cognition in Utrecht, and lies also in the quality of the selected students, the excellent research groups involved and the joined interests of students and scientist in trying to understand the functioning of the brain. The total number of students that have graduated in the 16 years of this master programme is about 800 and graduates have spread out all over the world.

The master's programme gets improved every year with the help of the evaluation forms that students fill in after completing the course. Also, these evaluation forms enable me to make future plans for the master's programme and they also measure the student satisfaction about this programme. The evaluation of this year's Fundamentals of Neuroscience and Cognition course was on average very positive! The new format we developed in 2012 enables us to make changes in specific weeks without having to change the complete concept of the course. So, for next year, a few changes in the content of three weeks will take place. Student satisfaction is also monitored in exit evaluations and these are very positive (> 7.5).

For the master programme in total we have developed new elective courses in the last years and the intention is to develop three more courses in the next two years. I would like to use this opportunity to ask you (students) which courses you would like to have as elective courses.

There are a lot of Neuroscience master programmes in the Netherlands and all over the world, but what makes the master's programme in Utrecht unique compared to other master's programmes? The special aspect of the master Neuroscience and Cognition programme lies in the 'and': in Utrecht we try to enable students to acquire knowledge about neuroscience and cognition. This is a benchmark for Utrecht, other programmes focus on specific aspects of the brain, training students to become specialists in one area.

For the future, I aim to keep up the high quality of all aspects of the programme (selected students, participating research groups and courses). Finally, we want to increase the awareness amongst our N&C master students about their future prospects and career opportunities.

# PhD Experience

*Femke Lammertink*

How often do you feel fortunate? Fortunate because of the impact your work might have on others, but also the work of others impacting you. Fortunate for meeting inspiring scientists who share their visions and values that drive young researchers. But also fortunate for doing something that feels meaningful and what (hopefully) makes a difference to the lives of others. When choosing the path of studying for a doctorate, we should all walk it with determination and satisfaction. But in reality, a doctorate is also characterized by challenges, setbacks, and a high dose of competition.

During my first research internship at the Behavioral Science Institute (Radboud University), I took part in a study on stress and inhibitory control. From this very first moment, I was intrigued by the dynamicity with which stress influences human behavior. We exposed participants to an acute stress-task, after which they had to complete a stop-signal task. This internship turned out to be a great experience, as I got to know a lot of researchers in the field of experimental psychopathology, but also affective neuroscience. One wonderful scholar for whom I have great professional and personal respect, and who really stood out to me, was Karin Roelofs.

I further explored the field of stress and automatic behaviors during my second internship, at the Experimental Psychopathology and Affective Neuroscience group led by Karin Roelofs. During this internship I studied the genetic underpinnings of human freezing behavior, which is a defensive response to threatening and stressful stimuli. I greatly enjoyed the multidisciplinary as well as international aspects of this project. These experiences made me realize that I enjoy collaborating with different research fields, to "dive" into the unknown, and to quickly adapt. I then applied for a Travel grant from the Honours Academy in order to meet researchers in a completely different academic culture, namely England. I visited Queen Mary University (London) and conducted another research project at the department of Biological and Experimental Psychology.

A couple months after graduating, I started with my PhD at the department of Neonatology, Wilhelmina Children's Hospital. I feel extremely fortunate to continue my stress research in a clinical population, namely extremely preterm born children. I chose this path, and certainly walk it with determination and satisfaction. I specifically enjoy the days where I can dive into the literature, keeping up-to-date with state-of-the-art developments in stress research. I also value the more applied part of doing a doctorate, such as data-collection. Being able to design your own procedures, and making sure that every detail is acted out as planned, can be very satisfying (but also frustrating). Seeing a direct pay-off of your hard work, in the extra megabits or even gigabytes of data. Maybe most importantly, and at the heart of academia, is to share your academic findings, to discuss your implications or limitations, but also to make mistakes and to grow from these experiences.

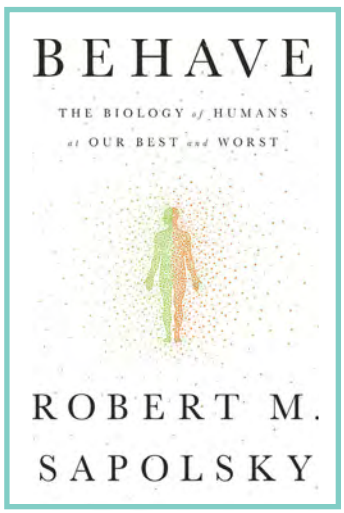
To be completely honest, taking an academic path is not necessarily a logical choice. The short-term contracts, the financial and geographical instabilities, but also the high degree of competition and mental health problems experienced in trainee academics, makes one wonder whether it is all worth it. I am not trying to make it appear as a tremendous sacrifice, because for me, that isn't the case. But for some it is. I often like to think that playing the, so-called, long game, and making some sacrifices now and then, will pay-off further down the line. Just as long as you do it with determination and satisfaction. If not, then it might not be the right path for you. And that is ok. Not pursuing a career in academia, after completing your Master's, is not a failure. A failure is actually doing something that makes you feel miserable. Be happy.

# Behave: The biology of humans at our best and worst

## Robert M. Sapolsky

*Roël Vrooman*

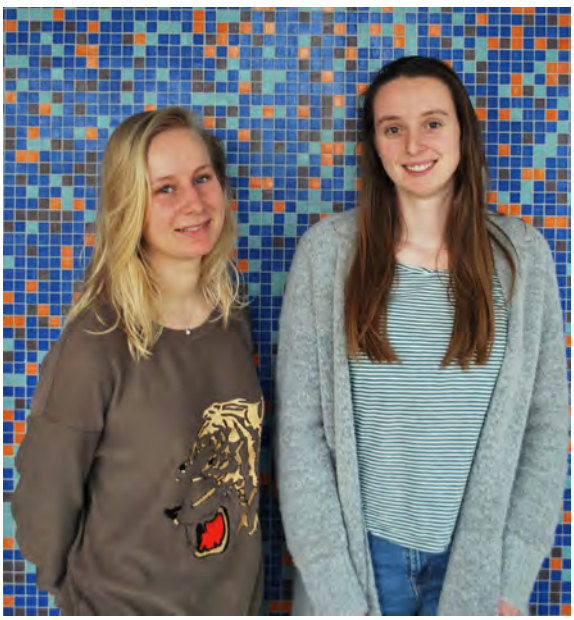
In Behave, Robert Sapolsky lays down the current knowledge on human behaviour, focussed on our best, such as affection and cooperation, and our worst, such as aggression and violence. Robert is currently a Professor at Stanford University and has a background in primatology and neurology, with a focus on neuroendocrinology. This is readily noticed as he tries to cover the subjects in the book from different angles. He recognizes that the age old discussion of nature vs. nurture is not one to have in this book. Instead he looks at all possible explanations for human behaviour, whether they are cultural or biological, if this distinction can even be made, since both influence each other. For this reason, the first half of the book concentrates on showing all these different effects on behaviour. From the firing pattern of neurons, to the evolution of behaviour, all perspectives are taken. The second half of the book talks about how all these effects on behaviour are translated into our worst and best. How they can form our view of Us vs. Them, our view of hierarchy and politics, our view of morals and view of war and peace to name a few of the topics touched upon in this book. Although Roberts is not shy to give his own hypothesis on different subjects in the book, he is also very careful in showing different arguments and research results of everything he talks about. For anybody scared of being a lay-man or just feeling like that genetics course was a while ago, Robert has been very helpful to add three appendages to the book. These appendages give a good basis for Neuroscience, Endocrinology and the Central Dogma of Biology. The only weakness of this book I can think of would be that it is pretty hefty, being 700 pages long, including the appendages. But his style of writing, which is light, insightful and funny at the right moments, though serious at others, definitely makes up for this. Anyone interested in gaining a basis in the understanding of human behaviour should absolutely consider this book. In any case it is a perfect addition to every neuroscientist's bookshelf.



# The future of developmental neuroscience

*Manon Veerkamp, Ida van de Water*

On December 12, a few of the interns from the Neonatology department at the Wilhelmina Children's Hospital went to a congress in Nijmegen on 'The Future of Developmental Neuroscience'. The symposium was free, and the program was very interesting as it covered multiple aspects. The basic point of the congress was to consider the future of Developmental Neuroscience, and what should be our focus of research in this field. A main topic that was discussed was the consequence of stress: both parental stress and early life stress. Related to this, there was a talk that debated the issues caused by severe deprivation in the life of a child. How does this influence the development of the brain and the functioning of a child later in life? The lectures were roughly divided into psychological and biological sides, which made it interesting for all of us. For instance, there was a presentation about research that focuses on the biological brain organization, and another one concerning statistical models behind cognitive processes that relate to one another (such as mutualism). There also was a very interesting talk about brain connectivity and the use of imaging (DTI) in this research.



**WHERE** Nijmegen, Netherlands
**WHEN** December 12th, 2018

After the morning program we had a very nice luch. This gave us the opportunity to talk things through with all the speakers in an informal setting. The lunch break was followed by another fascinating talk about how to translate all the knowledge that we have to clinical practice. It is nice to have all these data and conclusions, but how are we going to put them to use? This made us think about the value of the research that is going on in the field. There were panel discussions in which it was discussed how to connect these fields of research. Those were challenging and interesting, because sometimes it seems almost impossible to understand how one research outcome is related to the other on a totally different level. For instance, it was questioned if the models of neurocognitive development could match the idea of gradients in the brain instead of complete parcellation in the brain. These panel discussions made us think about the challenges in neuroscience and how to deal with them in the future.

Overall, the symposium gave a good overview of different research topics and where everyone is currently standing in their fields. Since we were with so many interns from the same department, it was nice that we could discuss our points of view and interests together. The combination of multiple fields at one symposium leads to the benefit of getting a broad view and new insights, which made it all very interesting!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# What do YOU think about personalized medicine?

For this issue of the Journal of Neuroscience and Cognition, we were looking for a way to include a bit of everyone's view and unique take on the topic. Therefore, we chose not to keep this issue's topic a secret, but on the contrary, let people think about it before reading the journal. What is your opinion on personalized medicine and how do you feel about genetic testing? We were really happy with the massive response to the survey, as almost a hundred people responded! Personalized medicine is a broad concept. As you have read in the interview with Jurjen Luykx, it aims to align the treatment with the patient. What does the patient want and what does he or she need? It endeavors to base the decision for treatment or medication on multiple aspects, such as DNA, the type of side effects, specific phenotypic profile of patients and their background. Patients are then often separated into different groups based on their genomic profiles. This way, medical decisions and interventions are tailored to the patients based on the genetic cause of disease or their predicted risk for diseases. The concept of personalized medicine comes with a lot of ethical questions. All these questions were included in the small survey we launched at the end of January. For example, to be able to choose which medicine suits a patient best, it might be necessary to do a genetic test. It is a great advantage that we are able to do that nowadays, but what about privacy?

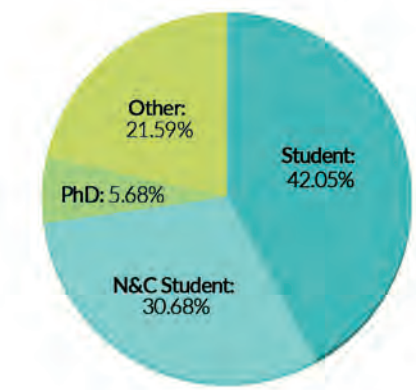


***Figure 1 | What is your current position?***

Respondents to the survey were mostly students (almost 80%!) and about 40% of them are currently following our master Neuroscience and Cognition (Fig.1). The other participants were mostly acquaintances of our current journal board. 70% of the participants of this survey were female and 30% male. From the non-student participants about 73% had never heard of personalized medicine, whereas only 1 student of the N&C program was not familiar with this concept (Fig.2). This is an interesting pattern to see. Such a big development in the medical field does not seem to have reached the general public yet. However, although many are unfamiliar with personalized medicine, only 2% of the participants have negative feelings after reading what personalized medicine is (Fig.3).

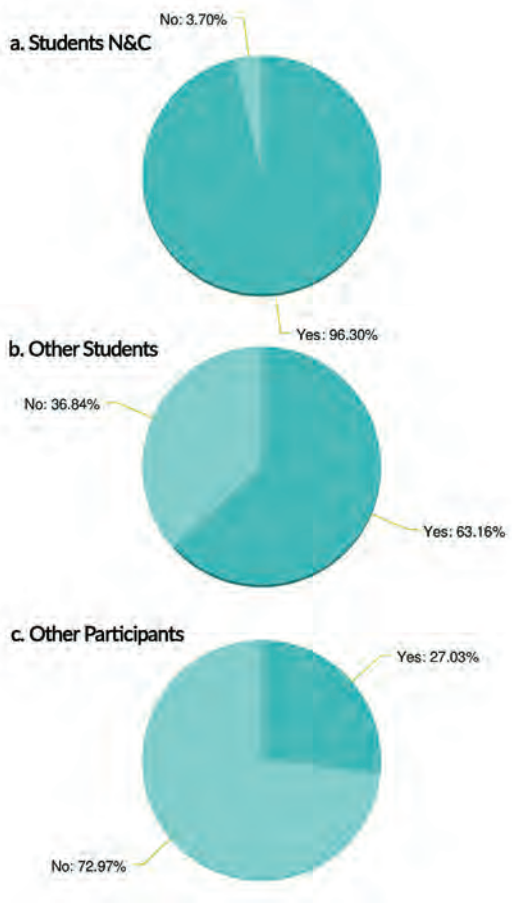


***Figure 2 | Have you ever heard about personalized medicine?*** *Answers for a) N&C Students, b) Other Students, and c) Other participants.*

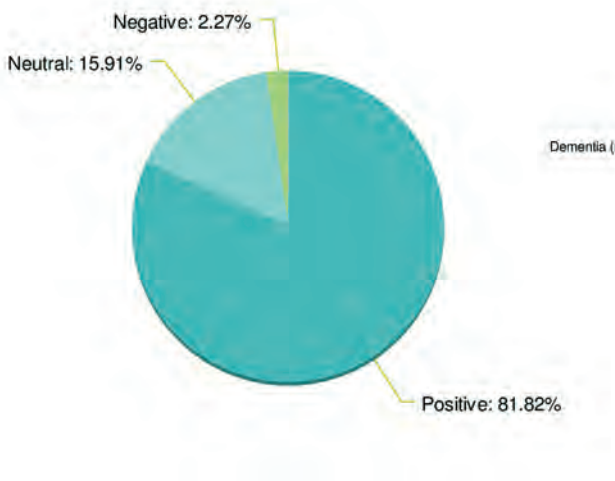


*Figure 3 | How do you feel about personalized medicine?*

## Genetic testing

When we asked our participants if they only wanted to be genetically tested for curable or preventable diseases, the opinions were really diverse (Fig.4). About one third of the respondents disagreed. This means they either do not want to be tested at all or that they also want to be tested for non-curable and non-preventable diseases. More than one third of the participants agreed on both questionsand only wants to be genetically tested for curable and preventable diseases. When we looked at the N&C students, there is a similar pattern between the 'curable' and 'preventable' diseases. About half of the N&C students agreed that they only want to be tested if this promises that they can be cured or that the disease can be prevented.

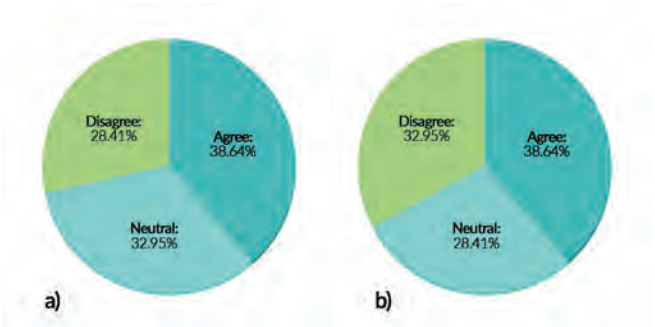


***Figure 4 | I only want to be genetically tested for predispositon if I can prevent or cure the disease.*** *a) Answers for curable diseases. b) Answers for incurable diseases.*

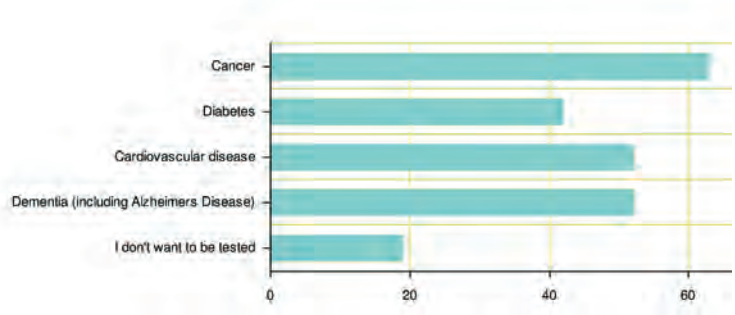


***Figure 5 | Concerning genetic tests, I would want to be tested for my risk of developing the following diseases.***

**PARTICIPANT QUOTE**

***"Genetic testing should become a mandatory screening, for instance like the one that is done for screening breast cancer at a certain age"***

Further on, we gave a list of diseases and asked what diseases you would want to be tested on, regardless of the possibility for treatment (Fig.5). Most of the respondents wanted to participate in genetic testing if this could predict the risk for developing cancer. About 20% of the participants did not want to be genetically tested for any of the diseases. Here an ethical question arises; what happens if you know the risk for a certain disease? After all, it is only a risk indication and does not give any certainty about actually getting the illness. That is why, in a later question, we asked if one still wanted to be genetically tested, realizing that this can actually predict the chances of certain non-curable diseases (Fig.6). Looking at the answers to this question it is not surprising to see that instead of 20% (Fig.5), now 30% do not want to be tested when they realize this might mean going through life knowing the risk for a disease (Fig.6). Another concern when it comes to genetic tests is privacy. In the comments at the end of the survey multiple respondents mentioned the risk of healthcare insurances not covering their medical bill if the risk for certain diseases is high based on genetic tests. Despite this, 60% of the participants do not see privacy as a reason for not participating in genetic tests.

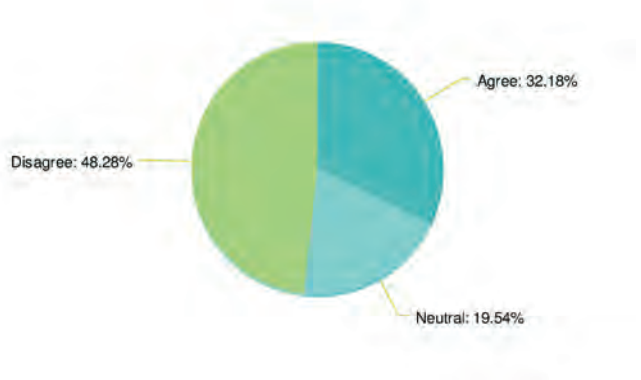


*Figure 6 | I do not want to participate in genetic testing, because I do not want to know results that might predict diseases I get in the future.*

**PARTICIPANT QUOTE**

***"A lot of people might think it's scary to know the outcomes of genetic testing and would rather take the risk of not knowing"***

## What do we expect from information of genetic tests?

Personalized medicine definitely has certain promising prospects. We asked what people expected from information gained by genetic tests. A vast majority (about 75%) agreed that genetic testing can help prevent a disease or helps in choosing the best available medicine. Besides, the respondents believe it helps reduce trial and error medicine and can decrease side effects. About half of the participants think that the results of genetic testing can lead to less invasive procedures. However, when we asked our respondents what they would pay to get their genome sequenced, less than 5% were willing to pay the actual amount of money that sequencing a whole genome costs nowadays (around 1000 Euros)(Fig.7.). More than a third reported they would not pay at all. Furthermore, two third of our participants thought that health insurances should cover all the costs of personalized medicine in the future. In short, even though our respondents think that personalized medicine and genetic testing have a lot of benefits, they are not willing to pay a large amount of money out of their own pockets.


*Figure 7 | How much, if anything, would you be willing to pay out of your own pocket to have your whole genome sequenced today?*

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**PARTICIPANT QUOTE**

***"Genetic testing might be seen as a scary thing whenever people mistake it for 'certain measurements' instead of 'possibility measurements'. Nonetheless, I feel like privacy concerning this kind of information should never be underestimated and be taken very seriously as misuse of this kind of information is a high risk."***

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# From academia to industry

## Careffect

*Elisa Timmer-Voets*

### *"Creating your own career consists of constantly making choices."*

My name is Elisa Timmer-Voets, I am 25 years old and currently a consultant for the company Careffect. After completing my bachelor Biomedical Sciences in 2014 I got selected for the Master program Neuroscience and Cognition at the University of Utrecht. At the start of the master I was convinced that one day I was going to be that scientist who would contribute to the creations of new drugs for schizophrenic patients. Only 5 years later I am working as a consultant at the Psychiatry department of Zaans Medisch Centrum. What has happened in those years?

In 2014, dopamine neurons, receptors and pathways were all I could think about. The research of Linde Boekhoudt, a PhD student at the lab of Prof. Dr. Roger Adan, already got my interest during my bachelor. Therefore, I made sure we could work together for twelve months during my first internship where we chemogenetically activated dopamine neurons in the ventral tegmental area or substantia nigra in rats using DREADD. For my second internship, I decided to continue doing behavioral research at the lab of Associate Prof. Garret Stuber in North Carolina, America. This is where everything changed. Science started to give me less and less satisfaction. Behavioral research often consists of repetitive tasks that are sometimes carried out for a longer period of time. At the same time, the article from my first internship was published. This made the doubts even worse. While everyone told me it was a big deal to publish and congratulated me, I had hoped to make an impact on society! However, everyday life stayed the same and I did not help any patients.

A secret interest in business and consultancy has always been there, only for me the order was always first PhD and then consultancy. What would happen if I would just skip my PhD? In which field could I become a consultant? Before I left America, I signed up for the course Organizational Dynamics in Life Sciences and became the chairman of the Education Evaluation Report (OER) to get more clarity on what I wanted to do next. Is conducting research and advising the university in the field of education, which OER does, something I am looking for? Once in the Netherlands, I decided to take part in the Utrecht University Business Course, an extracurricular program focused on entrepreneurship and consultancy. Combined, these experiences have ensured me that giving advice is what suits me the most.

After graduation I started as a market researcher at Rijnstate hospital, a job where I analyzed market (demographics, prevalence, trends), customer and competitor data, wrote reports and gave advice to management. Yet, the consultancy world continued to attract me: different clients, a lot of challenges and deadlines. That is why I took everything I had learned in the eight months at Rijnstate to my next job, a consultant at Careffect, where I started in March 2018.

Careffect strives to make a maximum contribution to a qualitatively affordable health care system. All our activities are focused on ensuring that the operations of organizations in the healthcare sector function effectively, so that the healthcare professionals can focus on their primary processes. As market researcher

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at Rijnstate and as consultant at Careffect I got the chance to speak with, and advice, many doctors. Since I started as a consultant at Careffect I got the possibility to see five hospitals, give training to new doctors at the UMC Utrecht and help with the HiX implementation at the Noordwest Ziekenhuisgroep Alkmaar. When there was a possibility to work at the Psychiatry department of Zaans Medisch Centrum via Careffect I was determined to get the job. Currently I work there for four days a week and the remaining day I work on internal projects. One project I am working on at the moment is organizing "Het Zorgtalent 2019", an in-house day especially for master students with an interest in healthcare and consultancy. This year we are looking to collaborate with Rijnstate hospital in Arnhem, a leading hospital in the field of marketing and communication. Moreover, it is possible to create your own product at Careffect. The product which I am working on helps me to combine all my current knowledge and the knowledge I will receive from my bachelor Business Marketing which I started in February this year. Especially nowadays, it is important for hospitals to refocus on the future and determine how target groups develop, which geographic area the hospital will focus on and what products they will offer. Therefore, in the future Careffect will offer portfolio- and market analyses, which will help healthcare organizations to determine their organizational strategy.

Creating your own career consists of constantly making choices. Although I have doubted about almost every choice in my career, I have not regretted any decision so far! That is something that I would have liked to know years ago, so that I had not always imposed myself with so much pressure. You probably would think that me focusing on marketing in hospitals does not have anything to do with Neuroscience and Cognition anymore, but actually, you could not be more wrong. I am combining the things I loved most about the master: doing research, predicting behavior and writing reports. Though, the reports I am writing now have an immediate impact and will help make strategic choices for big hospitals. My journey shows that you do not necessarily need to know now what you want to become. I strongly believe that everyone will end up wherever they belong eventually.

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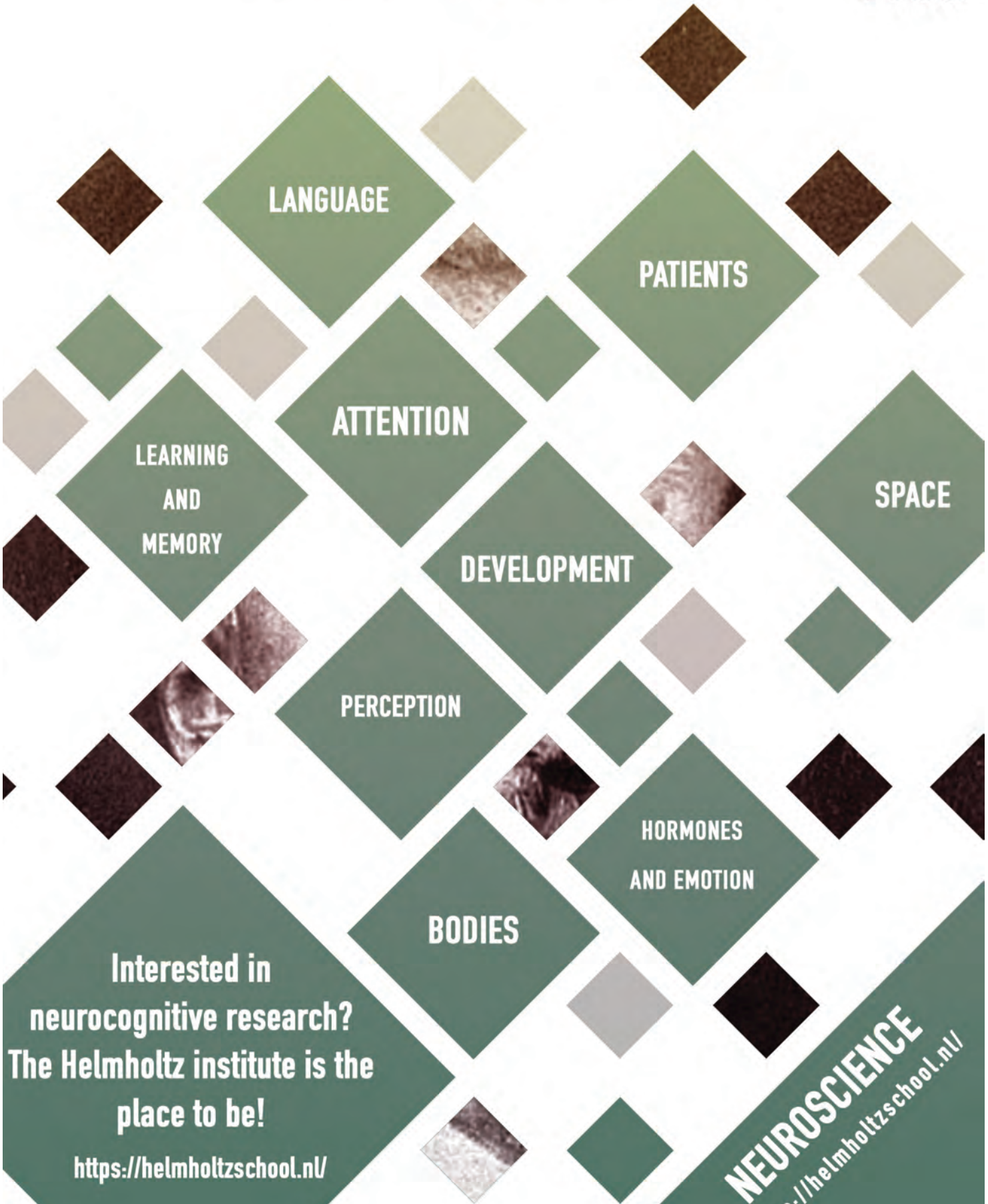

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