“The new science of epigenetics rewrites the rules of disease, heredity and identity.”

- Ethan Watters

"Genes are equivalent to blueprints; epigenetics is the contractor. They change the assembly, the structure."

- Bruce Lipton

Content

Content

Foreword

Dear reader,

This is the 25th issue of the Journal of Neuroscience and Cognition. As you might have guessed after seeing the cover of this issue, the theme is epigenetics.

Key to epigenetics is that, through gene expression, changes in an organism occur, while the basis - the genetic code - remains intact. What I find fascinating about epigenetics is that research in this area focuses on both the past as well as the present.

For example, retrospective studies have been conducted in which the Dutch famine ("honger winter") during World War II is linked to certain health risks by comparing individuals who were born during the famine to their siblings who were born before or after the famine. In our current day and age obtaining food is no longer a challenge for the vast majority of the Dutch. The widespread availability of food in itself is even deemed problematic by some, as the Dutch are now facing the challenge of making healthy food choices in an environment in which nudges towards unhealthy food choices are strong. The Dutch are not all very successful in making healthy food and lifestyle choices, as is evident from the numbers on obesity. Epigenetics are currently playing an important role in research on obesity and the results of these studies play an important role in governmental policy making.

This is only one example of the possibilities that research on epigenetics offers to the field of neuroscience. More is highlighted in this issue of the Journal of Neuroscience and Cognition and I wish you a great time reading up on it all.

Yours,

Anouk Keizer
Senior supervisor Journal of Neuroscience & Cognition

Editorial

Dear reader,

This journal is the second and last product of this year's editorial board of the Journal of Neuroscience and Cognition. In the previous issue we provided you with information on huge datasets. In this issue, we wanted to go in the opposite direction by zooming into the small details of epigenetics. It is one of the things that connect our Master's ECN and CN tracks and we want to highlight its application in both disciplines. We did this by interviewing Peter Bos, from the department of experimental psychology and Marco Boks, from the department of psychiatry and epidemiology. Both researchers took the time to tell us about their respective fields and the importance of epigenetics within those fields. Furthermore, they were so kind to give you some tips for your future as researchers.

The rest of the journal is packed with informative, innovative and interesting news from your fellow students. The four articles from Coco, Docky, Sanne and Wouter will inform you on oral contraceptives, working memory, philosophy of neuroscience and actin cytoskeletons. We also feature concise descriptions of new methodologies, plus some reports on research in companies, like Nutricia. Meanwhile, many of you have been to various inspiring conferences, on which you kindly reported for us. Not in the least of course, our own Mind the Brain Symposium!

All of this is put together in the second issue of the Journal of Neuroscience and Cognition. That leaves me with thanking all our contributors, reviewers and you of course, for reading and supporting our final product.

I wish you a great read and the best of luck for the upcoming academic year!

Yours,

Maaike Dubbeldam
Editor in Chief

What was I doing here? Working memory in changing visual environments

Duncan, D. H.[1], van der Stigchel, S.[1]

[1]AttentionLab, Experimental Psychology, Utrecht University, Utrecht, the Netherlands

Congruent learning and test environmental contexts are known to have a positive effect on recall in long-term memory tasks. The effect of context on working memory is less understood; though being able to retain items in working memory while changing environments would seem to be a critical function of working memory. Several theories have recently proposed that context changes automatically draw attentional resources. These theories, in conjunction with new models that posit strong connections between working memory and attention, beg the question of whether stable contexts have a similarly positive effect on working memory performance as on long term memory. In a series of experiments, we first show that salient visual environmental changes at stimuli presentation boost change detection accuracy in a common working memory task, but these same shifts have no effect when they are not simultaneous with stimuli presentation. Secondly, we show that as the complexity of the environment increases, the effect of backgrounds changes to a congruency bias where participants were biased to perceiving stimuli presented on the same background as the same and stimuli presented on different backgrounds as different. Thirdly, we found that verbal memory was very important for keeping track of memory items in changing visual environments as when verbal memory was blocked by a verbal load, participants struggled to perform above chance levels on several of the experimental conditions. We conclude that incidental environmental changes do have an effect on visual working memory, which changes based on environmental complexity and can effectively be suppressed by utilising verbal working memory over visual working memory.

Keywords: working memory, attention, vision, context, change detection

INTRODUCTION

For many the following anecdote might be a familiar experience: you realise you need something from the store, so you leave your home and head to the supermarket. However, once you arrive you are struck by a total amnesia as to what it was that you came to buy. Or another common circumstance: you are in a conversation when something happens which draws your attention: maybe an external event or perhaps a new member joins the conversation briefly breaking the flow of talk. As you turn back to continue what you were previously saying, you are again struck by a curious inability to recall what you were just a moment ago deep in conversation about. Both these cases are examples of a failure of working memory, a system which is important for keeping track of temporarily important information, such as your daily tasks or conversation topics (Cowan, 2010). In both these cases you may find the most reliable way to recover your lost memory is to retrace the context you were in when you lost your memory, either by imagining the events leading up to you heading to the supermarket or the previous topics you had discussed, in hopes that some clues from your past environment will jog your memory into recollection. These examples illustrate a curious and familiar connection between memory and context, a relationship that, though recognisable from everyday life, is not well understood.

The effect of environment on memory is an old topic; perhaps many of us have heard the common academic advice that you should study for exams in a similar environment to that in which they will be tested, such as a quiet library. Unlike much of the life advice offered from pop-psychology, this one happens to have its footing in real experimental results. The positive effects of congruent learning and test environments have been familiar in the study of memory at least since the striking experiments of Godden and Baddeley in 1975 made a strong impression in the memory of many psychologists. In their experiment they used students at a diving school and demonstrated that if lists of random words were memorised and tested either on land or underwater then recall ws significantly aided when learning and test conditions were congruent. While learning lists of random words underwater is not a situation many of us have experienced, the results of this experiment have been widely replicated in all manners of environments. Perhaps the most relevant example for students is from of Dark et al. (1998) where students

studied for a test in either loud or quiet environments and were subsequently tested in either loud or silent environments. It was found that those who studied and were tested in congruent environments consistently scored better. These represent just two examples from a long line of inquiry into the effects of learning environment and recall (for a review see Smith & Vela, 2001) which collectively build a strong consensus that even seemingly irrelevant environmental conditions can influence how we remember things.

While the cause of these effects is unknown, it can reasonably be argued that these effects should not carry over to short-term working memory tasks for the simple reason that working memory would not be very useful if it abandoned its memory contents any time we changed our current environment. Despite this postulate, the research group of Radvansky and Zacks (2017) recently proposed their Event Horizon Model which included a demonstration that working memory can in fact be impaired following salient environmental changes. In a series of striking experiments, Radvansky and colleagues showed that if participants were tasked with remembering random shapes and colours for a short interval, they were more prone to forget these memory targets if they were made to walk through a doorway between learning and testing (Radvansky et al., 2011), a finding which seems to match anecdotal evidence as presented in the first paragraph of this paper (and I for one know the feeling of walking into a room and immediately wondering, "what was I going to do here?").

These findings usefully inform a recent line of research, which has proposed a close relationship between attention and working memory (Cowan, 2001; Theeuwes et al., 2009; de Fockert et al., 2001; Olivers et al., 2006). These works contribute to a new model which challenges our ideas that attention and working memory are two different cognitive systems, where instead attention is the direction of some cognitive mechanism outward to specific objects in the environment and working memory is the direction of that same cognitive mechanism inwards towards objects in our mind. It is possible then that salient environmental changes, like walking into a new room, automatically recruit attentional resources, and if working memory and attention do draw from the same discrete cognitive resource then it is possible that incidental environmental changes can degrade working memory retention?

While such a theory of attention and working memory seems reasonable, it faces a challenge from various lines of research which have found that attentional activity can in fact boost working memory accuracy. Baker and Levin (2015a/b) found that continuity errors in films (caused when shots from different takes of the same scene are edited together, sometimes leading to subtle abnormalities such as drinks becoming fuller or cigarettes inexplicably growing longer as a scene goes on) are better detected after salient environmental changes, such as when new characters enter a scene or the actors move into a new location. This boost of attentional sensitivity following environmental changes causes problems for a model of working memory and attention which asserts that attentional recruitment should always have negative consequences on working memory retention. This concern is further illuminated by research such as Konstantinou et al. (2014) who demonstrated that certain types of working memory loads can actually improve performance on attentional tasks, in their case visual memory loads helped participants ignoring irrelevant visual distractors.

On the surface, Radvansky et al.'s research should have solved such questions by demonstrating the negative effect of environment changes on working memory, but in actuality their task selection and test conditions were not based on any of the standard memory tasks familiar in the field of working memory research. To this end our line of research attempted to apply salient visual environment changes to a visuospatial working memory task based on the much-utilised paradigm first published by Luck and Vogel (1997). In our experiments our environment changes were changing background colours. We began with single colour background changes (Experiments 1 & 2) and scaled up to multi-colour background changes (Experiments 3 & 4) to investigate whether complexity of the environment influenced how environmental changes affected working memory.

EXPERIMENT 1:

Simple Environmental Changes Boost Change Detection

Simplicity was the guiding principle when designing our first experiment: we wanted to design the simplest possible working memory task which was both comparable to other memory tasks in the field of working memory research and incorporated changing environments. The paradigm we designed was a near copy of that used in Experiment 4 from Wheeler and Treisman's (2002) paper, which was in turn an adaptation of Luck and Vogel's (1997) widely used experimental design. Our task design required participants to remember arrays of four shapes presented in various random arrangements on a computer screen, where they needed to keep track of both visual feature information (the shapes) as well as spatial information (their exact locations). We chose this task because it used shape stimuli instead of colour stimuli, and by using shapes as our memory targets then colour features remained available for us to use as our background manipulation. To this end, environmental

changes in our experiments were operationalised as changing background colours. Backgrounds were always of one uniform colour which could either stay the same or change between learning and test screens. While the use of single colour backgrounds does not reflect the visual environments encounter in real life, we reasoned that we should first see if any effects relating to environment changes could be observed at such a low level of environmental complexity, before scaling up to more complex visual environments to see if any effects modulate in regard to this manipulation. Using simple single colour backgrounds, we did observe that background changes affected participant change detection accuracy in that trials which featured a background change had consistently higher accuracy than those that featured a constant background.

METHODS

Participants

13 participants were recruited (7 female). 6 participants were graduate students from the Neuroscience and Cognition master's program at Utrecht University and volunteered to participate for free. The remaining 7 participants were recruited through advertisements around the Uithof campus of Utrecht University as well as online through various paid participant recruitment websites. These participants were compensated 10 Euros for an hour and a half of participation.

Materials & Stimuli

Visual stimuli were presented on a 54.61 centimetre, 1920x1080 resolution LG Flatron W2261 monitor connected to a Macintosh MacPro version 10.10.5 running the Python library program Psychopy (v1.85.6). Participants were provided with a chin rest situated 60 cm from the test monitor. All experiments were conducted in a room with black walls with the lights turned off.

A set of nine possible shape stimuli where used as the memory items. The shapes were chosen due to their familiarity and were a: circle, triangle, diamond, trapezoid, pentagon, arrow, hexagon, star and cross. All shape stimuli were approximately 2 visual degrees in size and dark grey in colour. The locations that the shapes could appear were pseudo random in that the test screen was divided into an invisible 3x4 grid of evenly spaced locations approximately five centimetres away from each other and the screen edge. To make the shapes seem like they were not being placed on a grid, a random (x, y) value was added to each position when it was selected to hold a shape, making it seem that shapes appeared at random locations in space. Using a grid meant that spatial shifts were usually large enough that they should have been easy to detect, and minute spatial shifts rarely occurred (average shifts were 5°).

The background colours were all light in shade and highly luminous to ensure that the dark shapes were suitably visible regardless of what colour was used. Colours were selected for their discriminability and familiarity. The colours used were red (10 candelas), blue (11.4 cd), green (16.1 cd), yellow (18.5 cd), pink (10.8 cd), purple (10.1 cd), orange (12.4 cd) and grey (16 cd). The effect of different background colours is discussed in the results section of Experiment 2.

Procedure

The experiment lasted approximately one and a half hour including five breaks with a break occurring approximately every ten minutes. The length of the break was left up to the participant, though they were made aware that longer breaks would lead to later finish times and as a result many participants took very short breaks or even chose to skip breaks so as to end on time or early.

The experiment began with both a verbal description of the experiment as well as a comprehensive written description on the computer that included visual demonstrations of the types of stimuli and changes to expect. The experiment consisted of 576 trials broken up into six blocks of 96 trials each, with a break between blocks. Each trial began with a learning screen in which a four-shape array of memory targets were presented on a coloured background. The learning screen was presented for 2 seconds after which the memory items would disappear and a retention interval of 2-sec would follow. We determined the length of our retention interval by piloting for the shortest amount of time for which iconic memory was no longer useful in our task. During the retention interval the background would remain the same colour as during the learning screen and a black fixation cross would be centrally presented. Participants were never explicitly told to fixate on the cross. Following the delay, either a new shape array or a single shape probe was presented, as was the case in Wheeler and Treisman's (2002) experiments, with a 50% probability for each. The test screen would be presented for 2 -sec, then the stimuli would disappear and the subsequent answer screen would prompt participants to indicate whether they believed it was a change or no-change trial. Participants indicated their answers using the 'a' and 'l' keys corresponding to 'no change' and 'change' respectively. Additionally, for half the trials the background colour remained constant throughout the entire trial; for the other half background colour changed on the test screen. In all conditions a salient background flash was included between the retention screen and the test screen. The flash was very fast (~50ms) and was included so that in both same and different background conditions there would be some environmental activity,

ensuring that any effect observed was not caused simply by the fact that more activity was present in different background trials. The experimental paradigm is illustrated in Figure A.

The types of change possible were also taken from Experiment 4 from Wheeler and Treisman's (2002) paper and were position, shape or binding changes. In position change conditions one of the shapes would move to a new coordinate on the screen. In shape change conditions one of the shapes would change to a new shape not previously included in the test set. In binding change conditions then two of the shapes would swap positions, thus the shape and position sets were the same, but participants had to identify that the binding of these characteristics had changed. All change trials were evenly divided between the three change types (differences in accuracies between change types were similar through all experiments and are discussed only in the results and discussion sections of Experiment 4).

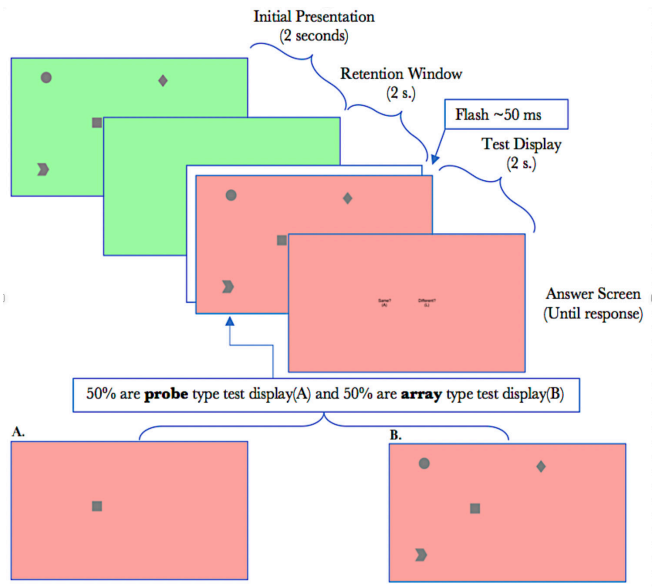


Figure A | The experimental flow of Experiment 1.
Experiment 2 was exactly the same as Experiment 1 except that the salient flash and background change occurred between the initial presentation and retention window instead of between the retention window and test display, effectively de-coupling background activity and stimuli presentation.

RESULTS EXPERIMENT 1

Of the 13 participants ran, one participant was excluded when it was found that he rated 97.92% of probe trials that featured a positional change as 'no change' trials as well as 93.75% of probe trials that featured a binding change. Given that guessing levels would have been 50% it is clear that the participant misunderstood the instructions that positional changes were being tested in probe trials and instead rated all probe trials that did not feature a new shape as 'no-change.'

We conducted a 2x2x2 repeated measures analysis of variance (ANOVA) using array/probe, background same/different conditions and trials in which a stimuli change had or had not occurred (change/no-change trials) as factors and overall accuracy as the dependent variable. First a main effect of background was found $F(1,11) = 11.510$, $p = 0.003$, $\eta^2 = 0.511$. We found that trials which featured a background change were reliably more accurate than background constant trials (82.49% and 79.11% respective accuracies). While the effect was only a 3% increase between conditions, it proved to be a very consistent effect with nine out of twelve participants showing the effect (average 4.63% difference between conditions), one participant scoring exactly the same between conditions and two participants seeing very slight improvement in the opposite direction (less than 1% difference in both cases). No other main effects were found ($F < 1.5$ for both).

Next, we found an interaction between background and array/probe conditions, $F(1,11) = 21.62$, $p < 0.001$, $\eta^2 = 0.663$. While probe trials were reliably more accurate when a change had actually occurred (90.51% accurate for change trials and 70.95% accurate for no-change trials, $F(1,11) = 26.07$, $p < 0.001$, $\eta^2 = 0.703$, array trials were in fact more accurate on average when no-change had occurred (84.55% accurate for no-change trials and 77.20% accurate for change trials), but unlike probe trials they were not reliably so ($F(1,11) = 3.64$, $p = 0.083$). These results indicate that participants were significantly more likely to report that probe trials were change trials and array trials were no-change trials, indicating a bias stemming from the type of test display used.

DISCUSSION

Background changes did affect participant accuracies but not in the same way changing environments affect long-term memory. If we were testing long-term memory, we would have expected participants to be more accurate when backgrounds stayed constant; instead, on our working memory task, they were more accurate when the background changed. These results first indicate that incidental environmental activity does affect working memory, a surprising finding if one assumed that the ability to retain information in working memory while changing environments should be a fundamental function of working memory. Furthermore, these findings do not seem to fit a unified attention and working memory model, which would suggest that if salient environmental changes are an attentional distractor, then environmental changes should only negatively affect working memory function as it would

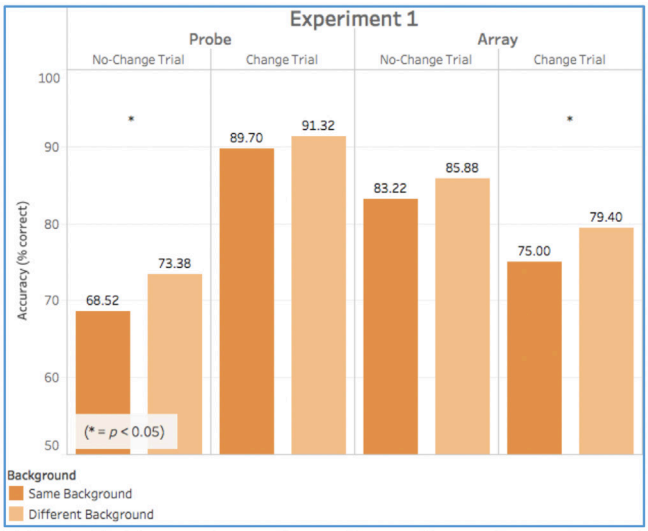


Figure B | Differences in Experiment 1 between average accuracies for probe and array trials, background same and different trials and change/no-change trials.

represent a redirection of cognitive resources away from working memory. These results do support a model of attentional arousal resulting immediately after salient environmental activity, as has been observed in studies on continuity errors in films (Baker & Levin, 2015a/b), suggesting that salient global changes sharpen change detection.

Additionally, we observed a very strong bias effect depending on whether arrays or probes were tested. This finding surprised us, as to our knowledge such an effect has not been reported before in similarly designed working memory experiments. One possibility is that in array trials, three of the four shapes would always be the same as in the learning screen, meaning that if a participant only successfully remembered two or three of the memory items then there was a chance that all those items would also be present in the test screen. This could have caused a bias for participants to rate array trials as no-change trials as a result of the structure of the experimental design. This explanation additionally accounts for the bias to rate probe trials as change trials as if only a subset of memory items were remembered and only a single item was presented then there was a chance that the single item was not in the memorised set and thus appeared unfamiliar regardless of whether it was in the original test screen or not.

EXPERIMENT 2:

Onset Timing is Critical for Background Effects

The previous experiment motivated further investigation into the effect of context changes on working memory tasks by demonstrating a clear main effect of background changes on change detection accuracy. Our participants had improved memory accuracy when context did in fact change between learning and test screens, mirroring a line of results that has demonstrated heightened sensitivity to changes immediately following the onset of environmental changes in the case of scene changes in films (Baker & Lavin, 2015a/b). Under this model the co-pairing of environmental shifts with stimuli presentation is key to the observance of the effect as even a second after environmental changes then participants returned to regular levels of error detection. Thus, if the same effect was at play in Experiment 1, then by decoupling environmental changes with stimuli presentation, we would expect the positive effect to disappear and for the two conditions to be equivalent. We found that de-coupling stimuli presentation and background changes indeed the effect of background activity disappeared.

METHODS

11 participants were recruited for this experiment (3 female). Four of the participants were Neuroscience and Cognition graduate students who volunteered to participate for free. The remaining participants were recruited in the same way as the paid participants in Experiment 1.

The procedure for this and all subsequent Experiments was exactly the same as in Experiment 1, except for a change in the onset of environmental changes. In Experiment 1, when a new background was introduced, the onset of the background change would happen simultaneously with the presentation of the test stimuli. In this experiment the background changes happened earlier in the trial, thus decoupling background change and stimuli presentation. Background changes now would occur between the learning window and retention window, meaning that immediately after seeing the learning stimuli the participant would see a salient flash and the background would either be the same or a different colour during the retention window. This trial order will be referred to as early onset trials.

RESULTS EXPERIMENT 2

A 2x2x2 repeated measures ANOVA using background condition, array/probe conditions and stimuli change/no-change trial types as factors on participant accuracies found no significant main effects ($F < 1.5$ for all three). The average accuracy for same background and different background trials was 82.35% and 81.85% respectively. These results indicate that, onset of environmental changes indeed has a significant effect on change detection, in that when environment changes co-occur with stimuli presentation then participants have higher

change detection accuracy than when environment changes occur 2s earlier than stimuli presentation. The interaction between stimuli change/no-change and array/probe conditions remained significant in this experiment ($F(1,10) = 16.76$, $p = 0.002$, $\eta^2 = 0.626$), indicating that the bias effect of using array versus probe trials was independent of this onset effect.

Additionally, during this experiment we began tracking specific colour changes for each trial, in order to compare whether some colour changes were more difficult or easier than others. Using a Pearson's chi-squared test of independence we found no significant difference in accuracy for any one colour condition compared to the others (for colour same conditions, X^2 (8, n = 2.88) = 10.25, $p = 0.175$. And for each paired colour change, X2 (56, n = 2880) = 51.58, $p = 0.606$).

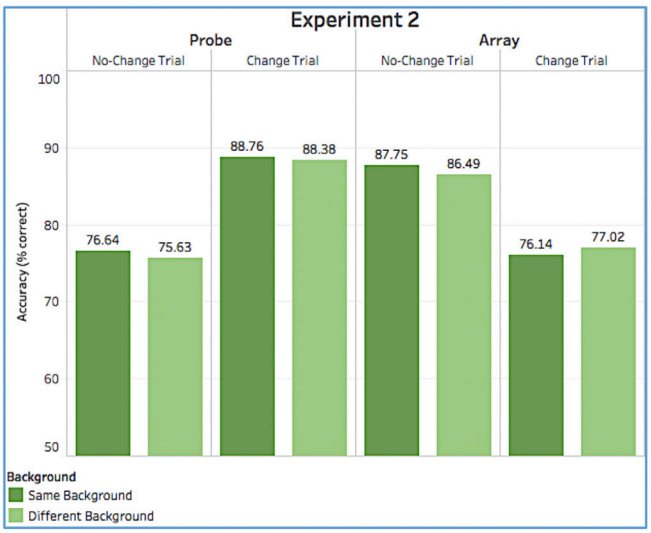


Figure C | Differences in Experiment 2 between average accuracies for probe and array trials, background same and different trials and change/no-change trials.

DISCUSSION

While Experiment 1 successfully showed a main effect of background changes on change detection accuracy, Experiment 2 showed that this effect is tied critically to the onset timing of the background change, where when salient background changes occurred 2s before stimuli presentation, no such difference in change detection accuracy was present any longer. These results further supported a model of attentional arousal immediately following salient environmental activity by demonstrating the critical temporal aspect of the background effect.

EXPERIMENT 3:

Complex Background Changes Cause Congruency Bias

Experiments 1 and 2 used simple single colour background designs to operationalise context changes for working memory. This design may have had low attentional salience as evinced by the many participants who, after completing the experiment, reported that they did not expect the background to have any effect as they were frequently able to ignore the incidental background activity. To this end, our next experiment introduced a new background design featuring two colours and a converging line vanishing point layout to see how performance was affected by more salient background conditions. For our new experiment we chose to include both early and late-onset change conditions, but also to exclude probe trials and use only array trials so as to incorporate the new within-subject condition, without needing to double the total number of trials to preserve statistical power. We chose to use array trials rather than probe trials as outside of Wheeler and Treisman (2002), most experiments using similar change detection paradigms use arrays exclusively as test stimuli. While the results we found were similar in that only late onset background changes had an effect, we found the effect changed from one of an attentional boost to that of a congruency bias.

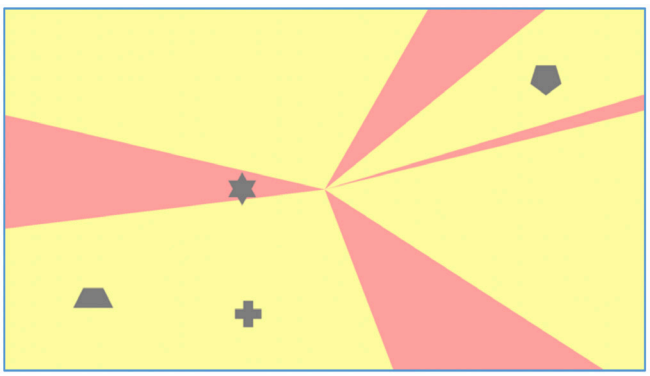


Figure D | An example of a background used for experiments 3 and 4.

METHODS

21 participants were recruited for this experiment (16 female). All participants were recruited in exactly the same way as previous experiments and compensated 10 Euro for an hour and a half of participation.

The background stimuli we used for this experiment differed from Experiments 1 and 2 in that now two colours were used and the screen was divided between

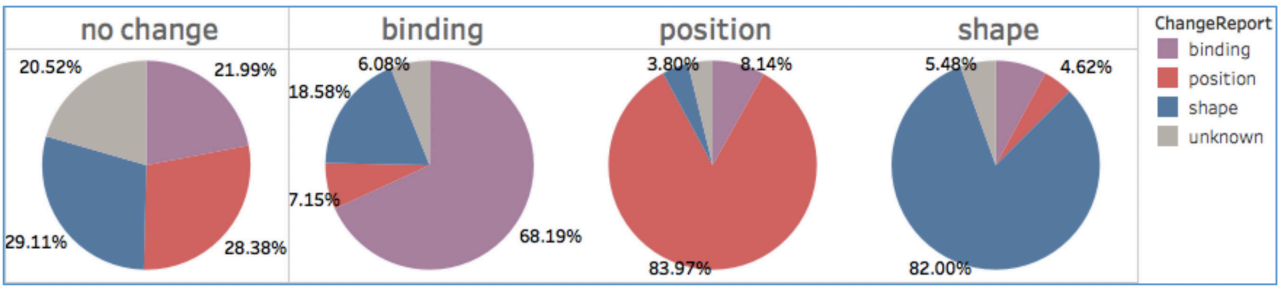


Figure E | A summary of the answers participants selected when asked what they think had changed following the marking of a trial as a change trial. *Trials in which participants indicated they did not believe the new shape array was different are not included in these visualisations.*

the colours in a vanishing point design (see Figure D).

Half of the trials in this experiment followed the exact same procedure as Experiment 1 (where the onset of background changes was late in the trial, immediately before the presentation of the test stimuli) and the other half copied the procedure of Experiment 2 (where the onset of background changes was early in the trial, immediately after the learning screen). These trial types were randomly mixed within blocks, so there was always a 50% chance that each trial could be early or late onset trials.

After running a subset of participants (not included in our analysis), we decided it was of interest to ask participants what they thought had changed in trials they had marked as different. We chose to do this because we observed a higher rate of false change detection than in our simple background conditions. One possible explanation for these false positives was, that our new background condition may have unintentionally caused spatial change illusions as it utilised a "vanishing point" design which featured moving lines. To test this, we included a new screen which only appeared on trials in which participants indicated that they believed a change had occurred and prompted them to indicate what type of change they believed had occurred. The options were 'don't know,' 'shape,' 'position,' or 'order' (which corresponded to binding changes but reflected the language used when participants learned the types of changes participants should expect). Participants chose from these four options using the 'down,' 'left,' 'right,' and 'up' arrow keys respectively. Because participants were now using the arrow keys to select what type of change they believed had occurred we chose also to use the 'left' and 'right' arrow keys to select whether trials were 'change' or 'no-change' trials on the test screen rather than the 'a' and 'l' keys as was used in previous experiments. This change was necessary as the experiment was conducted in a dark room using a chin rest and participants would have struggled to re-orient their hand mid trial.

RESULTS EXPERIMENT 3

The results of the change reports are shown in Figure E. This data shows that participants were generally able to correctly identify what type of change had occurred in true positive change detection trials, and in false positive trials participants did not significantly favour any one of the four options. If our background design had caused consistent unintentional spatial change illusions, then we would have expected 'position' reports to be overrepresented in the 'no-change' condition graph. Instead participants were just as likely to report that a spatial change had occurred as a shape change, indicating our background conditions did not engender spatial change illusions.

We conducted a 2x2x2 repeated measures ANOVA taking background same/different, early/late onset change and change/no-change trials as factors on participant accuracy. A main effect of change/no-change trials was found, $F(1,20) = 5.07$, $p = 0.036$, $\eta^2 = 0.202$, where participants were better at identifying no-change trials than change trials (83.26% versus 77.67% respectively). This tendency reflected our choice to use only array trials in this experimental design, which we saw in Experiments 1 and 2 to be inherently biased towards change rejection. Otherwise we found that in this new background condition there was no longer a main effect of background change or onset conditions ($F < 1.5$ for both).

Furthermore a three way interaction between onset timing, background condition and stimuli change conditions was found, indicating a complex system with multiple interacting factors was at play, $F(1,20) = 5.022$, $p = 0.037$, $\eta^2 = 0.201$. To simplify our illustration of this analysis we have broken down our 2x2x2 ANOVA into two separate 2x2 ANOVA's, by separating the onset timings conditions while keeping background and stimuli change conditions as factors. We chose to separate our

analysis based on onset timing both to better compare to Experiments 1 and 2 (which also were differentiated by onset timings) and also because this division usefully demonstrated two distinct task behaviours.

Early onset analysis

The results of the early onset background change trials very closely resembled the results found in the array trials of Experiment 2. The only main effect found was that between stimuli change/no-change conditions ($F(1,20) = 8.33$, $p = 0.009$, $\eta^2 = 0.294$), where no-change trials were easier to detect than change trials (84.12% and 76.12% accuracy respectively). This effect reflected the inherent bias for change rejection we identified for array trials in Experiments 1 and 2. No main effect of background was found ($F(1,20) = 2.73$, $p = 0.114$) nor an interaction between factors ($F < 1$). These results exactly mirrored those of Experiment 2 where we also found that background conditions had no effect on task behaviour.

Late onset analysis

While our early onset analysis mirrored that of Experiment 2, our late onset analysis differed greatly from that of Experiment 1. First, the main effect of stimuli change/no-change trial condition was found again (82.43% and 79.24% accuracy respectively, $F(1,20) = 4.88$, $p = 0.039$, $\eta^2 = 0.196$), but the main effect of background was no longer present ($F(1,20) = 1.478$, $p = 0.238$). However, a new interaction between the two had appeared ($F(1,20) = 9.34$, $p = 0.006$, $\eta^2 = 0.318$), indicating background conditions did continue to have an effect on task performance but in a different way than in the single colour background trials of Experiment 1. Rather than background change trials being consistently higher accurate than background same trials, we observed that when both the stimuli and background changed or when neither the background nor stimuli changed participants were more accurate. In other words, a congruency effect was now observed where stimuli looked different if presented on a different background than what it was learned on, and stimuli looked the same if presented on the same background used in the learning screen. For stimuli change trials the difference was 80.44% and 78.02% accuracy between background change vs. same respectively. For no-change trials the difference was 79.64% and 85.22% accuracy respectively (these results can be seen in Figure F, Experiment 3, Late Onset Background Change).

DISCUSSION

The preceding analysis illustrates a very complex interaction between three variables which requires some unpacking; of first note is that no-change trials were generally more accurate than change trials, reflecting the main effect of using array. Next, the behaviour of participants was different between early and late onset trials, where there was no effect of background activity in early onset trials but there was an effect in late onset trials. So far, these results sound very similar to those observed between Experiments 1 and 2, where participants were able to effectively suppress the background effect when background changes occurred early in the trials but were unable to do the same when the incidental activity occurred at the same moment as the stimuli were presented. However, in late onset trials, instead of background change trials being uniformly more accurate, a new congruency effect was observed where when both the background and the stimuli changed or when neither the background nor

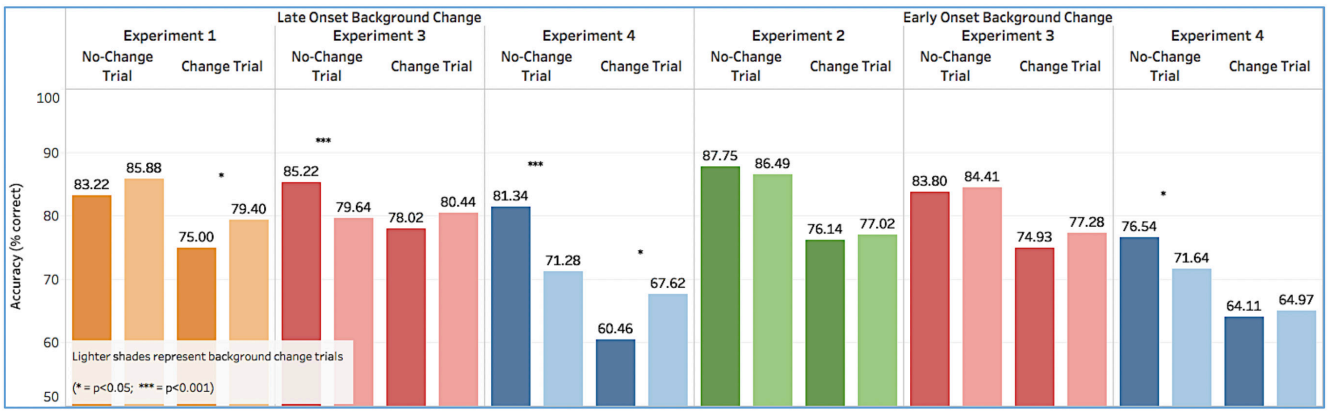


Figure F | Average condition accuracy in Experiments 1-4 seperated into the three relevant condition factors: background change onset timings, change/no-change trial type, and background conditions.
Probe trials in Experiment 1 and 2 have been excluded as Experiments 3 and 4 used only arrays. It is worth nothing that if position change trials are excluded from the analysis, then many of the change trial pairs become more significantly different (see discussion for Experiment 4 for further details).

the stimuli changed participants were more accurate. In trials where background and stimuli changes did not match (incongruent), participants struggled as they were biased to report that the stimuli behaved in the same way as the backgrounds. These results further support the attentional arousal effect immediately after salient incidental visual activity, but also indicates that participant's behaviour towards stimuli changes when faced by more complex backgrounds.

On the surface the congruency bias we observed makes sense as a method of reducing cognitive load: if we assume that global visual changes should be accompanied by changes to the minute details of our environment then we no longer need to devote limited attentional resources to track the specific changes of every item in a scene. This is especially true in the case of a non-changing environment, as if every time we looked down from our computer we automatically reappraised every item on our desk to ensure nothing has changed then certainly it would take much longer to finish anything. Furthermore, the bias to perceive the details of a scene as consistent is a reflection of the results familiar in change blindness research wherein unattended environmental features can often be changed without conscious awareness, revealing our tendency to trust in environmental consistency rather than exercising our cognitive resources to track every environmental factor (Rensink, 2002; Rensink et al., 1997). It is already well understood that less information enters conscious awareness than seems possible, given out subjective experience (O'Regan, 1992). These results speak to the extent that we depend on these processes of continuity to fill in the blanks and provide a stable perceptual experience of the visual world.

EXPERIMENT 4:

Verbal Memory Critical in Changing Complex Environments

While running Experiment 3, participants were asked during debriefing to describe the strategy they used to perform the task. It became clear that many participants depended on a nearly entirely verbal strategy to remember the shape items (a normal report would go something like: "I would say the names of the shapes as quickly as possible and then repeat them in my head and then check if the new shapes were the same"). This tendency led us to inquire what would happen if a verbal load was introduced to prevent participants from using a verbal rehearsal strategy to complete the change detection task. Verbal loads have been variously used in experiments using similar designs, including in Luck and Vogel's original 1997 paper, which tested the effect of verbal loads on the task and concluded that blocking verbal memory had only a marginal effect on change detection accuracy. Given our task was highly visual in nature, it was reasonable to expect that verbal loads shouldn't affect task performance beyond a slight decrease in global accuracy because performing dual tasks is more difficult than performing single tasks. We found that this was not the case as blocking verbal memory led to a significant decrease in change detection accuracy, with feature change detection specifically dropping to chance levels.

METHODS

17 participants (11 female) were recruited for this experiment in exactly the same way as in Experiment 3. The procedure used was exactly the same as that in Experiment 3 except that: 1) when participants identified a trial as a change trial, they were no longer asked to identify the type of change they believed had occurred, and 2) a new verbal load was introduced. The verbal load screen was presented at the beginning of every block and after every verbal load test. The screen was always a neutral grey colour with two large black numbers present. Participants were asked to repeat the numbers silently in their head. The numbers were presented until participants pressed the space bar key to indicate they had sufficient time to learn the numbers. Participants could randomly be tested on the numbers after one, two or three trials; this step was taken to shorten the experiment as it already moved much slower than Experiment 3, and we believed it was not necessary to test participants after every trial as verbal memory accuracy was not of actual interest to us. We expected participants to perform very well on the verbal tests, and indeed they were on average correct on 93.51% of verbal load tests.

Additionally, we eliminated a full block of trials in this experiment (total 480 trials rather than 576 as in Experiments 1-3) as the inclusion of a verbal load caused the experiment to move much slower than previous iterations and we felt that performance of this task for more than an hour and a half would lead to lower quality data due to fatigue.

RESULTS EXPERIMENT 4

Two participants were excluded from our analysis; the first because they marked 89.06% of trials as change trials, a significant deviation from the average 44.55% for the rest of the participants. This participant likely either thought we were trying to deceive them by presenting only change trials or alternatively chose not to do the task. The other participant was excluded due to an accuracy of 57.30% on the verbal load test, a significant

deviation from the average accuracy of 93.51% for all other participants, indicating they were not performing the verbal load component of the task.

Again, a 2x2x2 repeated measure ANOVA was conducted using background, onset and change/no-change trial conditions as factors on overall accuracy and found no significant main effects of background ($F(1,14) = 2.91$, $p = 0.110$), onset ($F < 1$), or change/no-change trial types ($F(1,14) = 2.54$, $p = 0.133$). There was a significant interaction between the background and change/no-change conditions ($F(1,14) = 2.54$, $p = 0.133$). Again, participants were significantly better at congruent background and stimuli change/no-change trials than incongruent trials. There was also a very strong interaction effect between the three combined factors ($F(1,14) = 19.18$, $p < 0.001$, $\eta^2 = 0.578$), which was due to differences in the magnitude of the background effect, where background changes caused a larger difference in late onset background changes than in early onset background changes (the average difference between background trials was 2.89% in early onset trials and 8.38% in late onset trials). No other interactions between the factors was found ($F < 1$ for all remaining combinations).

Verbal memory revealed itself to be a critical mechanism in completing this memory task with complex changing backgrounds as overall accuracy in the task dropped from 80.00% to 69.75% between Experiment 3 and 4. The drop was especially pronounced when separating change trials between the types of changes possible: position, shape or binding. A 3x2 repeat measures ANOVA taking the three types of changes as factors, found a significant difference between the three change conditions, ($F(1,14) = 78.59$, $p < 0.001$, $\eta^2 = 0.849$). Position-change detection remained very high in this experiment (90.56%) just as it was in all previous experiments, however the other two types of changes featured a precipitous drop in accuracy (50.39% and 51.29% for shape change and binding change respectively). Comparing just shape and binding changes revealed that these two condition types were not significantly different ($t(14) = -0.496$, $p = 0.638$) meaning that the main effect of the previous ANOVA was due entirely to a difference of position-change detection. No-change trials remained relatively high in accuracy, perhaps reflecting the natural bias towards marking array trials as no-change trials, though an accuracy of 75.2% for no-change trials was still 10% lower than the average accuracy recorded from Experiments 1, 2 and 3.

DISCUSSION

Participants were much worse at our change detection task when they were unable to use verbal memory, a result which differs greatly from a long line of experiments incorporating verbal loads with similar tasks but without salient background changes. This result shows the critical role that verbal memory plays when faced with changing complex environments.

Participants continued to be better when background and stimuli change behaviour matched. However, unlike in Experiment 3, this background effect was now present in both early and late onset conditions, though the magnitude of the effect was stronger in late onset trials. A possible explanation is that participants were still able to ignore incidental visual activity using only visual memory, but they required significantly more time than when verbal memory was available, thus stretching the background effects temporal window to be longer. Under this explanation then the early onset conditions

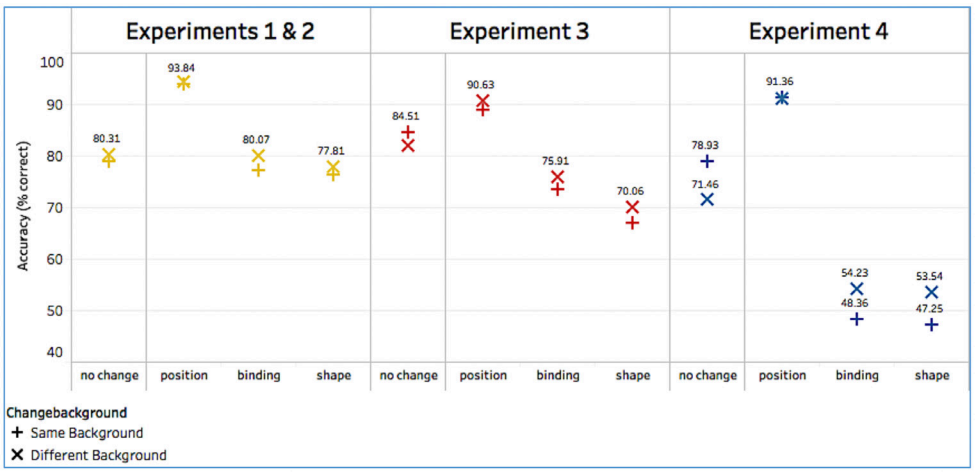


Figure G | A comparison of accuracies in Experiments 1 and 2 separated between no-change trials and the three types of changes that make up change trials. *40% accuracy is used as the floor on this graph as several conditions were slightly below 50% accurate.*

would have caught the tail end of the arousal effect, accounting for both its presence and lesser magnitude in these trials.

In previous experiments, position changes were consistently easier to detect than changes to visual features or binding changes, a result which was also observed in Wheeler and Treisman (2002). What is remarkable in this experiment is that, while shape and binding changes dropped to chance levels of accuracy, position changes remained highly detectable (see Figure G for a visual comparison of trial types), indicating that verbal memory was critical in visual feature memory but not spatial memory and further supporting a dissociation between spatial and visual memory already familiar in the field (Awh & Jonides, 2001). No-change trials remained well above change accuracy, though they were still much worse than no-change trials in Experiment 3 (75.2% and 83.44% respectively). It is possible that these trials remained relatively high in accuracy due to the inherent bias to perceive array trials as no-change trials as identified in Experiments 1 and 2, and that if we had used probe trials instead of arrays then the effects should have been opposite (where shape and binding accuracy may have risen to the 75% accuracy and no-change trials dropped to chance levels of accuracy).

One of the findings of Luck and Vogel's (1997) paper (Experiment 2) was that verbal loads should not significantly influence task performance on this experimental design. Another study investigating specifically the effects of verbal loads on performance on Luck and Vogel type visual memory tasks found that verbal load effects could be present but shouldn't exceed differences of about 2-10% accuracy depending on several features of the verbal load (Morey & Cowan, 2005). Our very different findings indicate that our use of changing backgrounds caused participants to depend on verbal memory to complete the task, leading to the significant decrease in accuracy when we subsequently blocked verbal working memory in Experiment 4.

GENERAL DISCUSSION

We began our line of inquiry by adopting a simple working memory task and adding changing background colours to see whether stable learning and testing background colours had an effect on working memory accuracy. In Experiment 1, we found that testing working memory targets on backgrounds that were of different colour than the backgrounds the stimuli were learned on, led to better working memory task performance, the opposite result from what is commonly observed in long term memory research using similar manipulations. We investigated this effect further in Experiment 2 where, we found that by moving the timing of background colour changes to earlier in the trial, the effect of backgrounds disappeared.

The relevant results of Experiments 1 and 2 are a demonstration that the positive effect of changing environmental factors, as observed in other experiments, is also present in simple change detection tasks (Baker & Levin, 2015a). While less ecologically valid than continuity error detection in movies, our simple paradigm identifies an easy methodology by which to investigate this positive change detection effect.

In Experiments 3 and 4 we changed our experimental paradigm to use more complex two-colour backgrounds to see how environmental complexity affected participant behaviour in our change detection paradigm and we found that participant behaviour changed dramatically. Trials which featured a background shift no longer scored consistently higher than those that featured constant backgrounds, but rather a congruency effect appeared: when background shifts occurred, participants were biased to perceiving that a change had also occurred with the stimuli and when backgrounds remained the same then participants were biased to report that the stimuli also remained the same. Experiment 4 revealed that this effect may have been due to a shift in strategy to one strongly favouring verbal working memory when faced with complex background activity. In Experiment 4 we changed our experiment to use a dual task design where, in addition to remembering the visual feature and spatial characteristics of a shape array, participants were also tasked with remembering a separate verbal load of two numbers which they had to repeat in their head while performing the visuospatial memory task. This design should have prevented participants from using verbal memory while leaving visuospatial working memory intact; a manipulation that has been shown in other experiments that featured no background activity to not interfere with their participants' abilities to perform the task. In our experiment we found this was not the case as when verbal memory was blocked, and visual and environments could change then participants were significantly impeded in their ability to perform the visual working memory task. Their ability to track changes to visual features specifically dropping to chance levels.

The relevant results of Experiments 3 and 4 are the illustration of a sensitive mechanism balancing attentional and memory tasks while trying to optimise operation of both. The first major observation from this series of experiments was the presence of a congruency bias, first identified in Experiment 3 and observed to even greater effect in the visual memory restricted Experiment 4. This bias may be a mirror to the mechanism underlying the inability to detect large changes familiar in the field of change blindness. Change blindness is an

inability to detect significant changes in complex scenes when the original and modified versions of the scene are presented sequentially with a salient flash in between presentations (Rensink, 2002). This effect is not unlike the same background trials in Experiments 3 and 4 that featured consistent complex backgrounds as well as a salient attentional flash similar to the flicker paradigm used in change blindness experiments. Although the standard change blindness paradigm does not have discrete memory items such as the four shapes present in our experiments, it is still reasonable to assume a person can only compare three to four features of one photo to the next at a time based on known capacity limits to working memory (Vogel, Woodman & Luck, 2001). Additionally, verbal memory would likely not be used in the complex scenes used in change blindness studies, as the points of comparison would be difficult to characterise verbally. We saw in Experiment 4 that participants were strongly biased to perceiving that no change had occurred when the incidental background information remained constant and verbal memory was not used. It is not too difficult to imagine how in change blindness trials, which are even more complex visual stimuli than what we used and did not limit attention to just four items, the bias effect could become even more powerful and eventually lead to strong perception of continuity in scenes which in fact feature drastic visual changes.

These findings also support an integrated model of visual working memory and visual attention, as context changes can be said to automatically engage some visual attentional resources that disrupt our maintenance of visual working memory items (Cowan, 2001, Theeuwes et al., 2009). The fact that this interference is only observable when verbal memory is blocked reflects a series of results in other working memory experiments which have found that the interaction of visual attention and visual working memory depends on the use of visual stimuli, and that allowing verbal working memory often leads to a disappearance of any effects (Olivers et al., 2006; Konstantinou et al., 2014).

Perhaps the most striking result we observed in Experiments 3 and 4 was the value of verbal working memory in dynamic visual environments, where participants were not able to track feature changes to sets of four simple visual objects when verbal memory was blocked (an average accuracy drop of 25% between Experiments 3 and 4). The dissociated functions of verbal and visual working memory are a central component of Baddeley's influential model of working memory (Baddeley, 1986), though the functional reason for this dissociation is not usually discussed, where most models seem to at least implicitly suggest that the appeal of having two memory systems is more total memory storage, not that one fails in certain conditions, necessitating the existence of the other. Our findings demonstrate exactly this; illustrating the role of verbal memory to preserve working memory items in the face of global visual environmental activity. It is important that we are able to preserve memory items in dynamic environments, but not at the expense of the ability to attention to potentially relevant activity in the environment. These results make clear that without verbal working memory, our lives would be very different when confronted with the complex and attentionally relevant environmental changes we encounter constantly in our daily lives. After all, if remembering a grocery list necessitated that we were no longer able to detect oncoming traffic then the structure of our daily lives would surely be very different.

CONFLICTS OF INTEREST

The authors declare no conflict of interest in the subject matter or materials discussed in this manuscript.

REFERENCES

Cowan, N. (2001). The magical number 4 in short-term memory: A reconsideration of mental storage capacity. Behavioural and Brain Sciences 24(1): 87-185

Godden, D. R., Baddeley, A. D. (1975) Context-Dependent Memory in Two Natural Environments: On Land and Underwater. British Journal of Psychol. 66(3): 325-331

Dark, V. J., Grant, H. M., Bredahl, L. C., Clay, J., Ferrie, J., Groves, J. E., McDormat T. A. (1998) Context-Dependent Memory for Meaningful Material: Information for Students. Applied Cognitive Psychology, 12: 617-623

Smith, S. M., Vela, E. (2001) Environmental context-dependent memory: A review and meta-analysis. Psychonomic Bulletin & Review, 8(2): 203-220

Radvansky, G.A., Zacks, J. M. (2017). Event boundaries in memory and cognition. Current Opinion in Behavioural Sciences, 17:133–140

Radvansky, G. A., Krawietz, S. A., Tamplin, A. K. (2011) Walking through doorways causes forgetting: Further explorations. The Quarterly Journal of Experimental Psychology, 64 (8), 1632–1645

Theeuwes, J., Belopolsky, A., Olivers, C. N. L. (2009). Interactions between working memory, attention and eye movements. Acta Psychologica 132: 106–114

de Fockert, J. W., Rees, G., Frith, C. D., Lavie, N. (2001). The Role of Working Memory in Visual Selective Attention. Science. 291(2): 1803-1806

Olivers, C. N. L., Meijer F., Theeuwes J. (2006). Feature-Based Memory-Driven Attentional Capture: Visual Working Memory Content Affects Visual Attention. Journal of Experimental Psychology: Human Perception and Performance. 32(5): 1243–1265

Baker, L. J., Levin, D. T. (2015). Event perception as a control process for visual awareness. Visual Cognition, 23:814-816

Baker, L. J., Levin, D. T. (2015). The role of relational triggers in event perception. Cognition 2015, 136:14-29

Konstantinou, N., Beal, E., King, J-R., Lavie, N. (2014). Working memory load and distraction: dissociable effects of visual maintenance and cognitive control. Attention, Perception and Psychophysics. 76:1985–1997

Luck, S. J., Vogel, E. K. (1997). The capacity of visual working memory for features and conjunctions. Nature, 390: 279–281

Wheeler, M. E., Treisman, A. M. (2002). Binding in short-term visual memory. Journal of Experimental Psychology: General. 131:48–64

Baddeley, A. D., Hitch, G. J. (1974) Working memory. In the Psychology of Learning and Motivation (Bower, G.A., ed.), pp. 47–89, Academic Press

Rensink, R. A., (2002). Change Detection. Annual Review of Psychology 53: 245–277

Rensink, R. A., O'Regan, J. K., Clark, J. J. (1997) To See or Not to See: The Need for Attention to Percieve Changes in a Scene. Psychological Science, 8(5): 368-373.

O'Regan, J. K., (1992). Solving the "Real" Mysteries of Visual Perception: The World as an Outside Memory. Canadian Journal of Psychology, 46(3): 461-488.

Morey, C. C., Cowan, N. (2005). When do Visual and Verbal Memories Conflict? The Importance of Working-Memory Load and Retrieval. Journal of Experimental Psychology: Learning, Memory and Cognition, 31(4): 703–713.

Vogel, E. K., Woodman, G. F., Luck, S. J. (2001). Storage of features, conjunctions, and objects in visual working memory. Journal of Experimental Psychology: Human Perception and Performance, 27: 92-114.

Awh, E., Jonides, J. (2001) Overlapping Mechanisms of Attention and Spatial Working Memory. Trends in Cognitive Sciences 5(3): 119-126.

A review of the relationship between oestrogen administration and depressive symptoms across the female life span

Börner, C.[1], Montoya, E.R.[1]

[1] Department of Experimental Psychology, Utrecht University, Utrecht, the Netherlands

Depressive symptoms are related to changes in oestrogen levels across a woman's menstrual cycle. Due to a high prevalence of disorders with depressive symptoms and a common use of hormonal contraceptives and oestrogen therapy in women, it is of clinical and societal relevance to investigate effects of oestrogen administration on depressive symptoms across the life span of women. This literature review indicates that the research to date is inconsistent with respect to effects of oestrogen administration on mental health ranging from being absent to beneficial or even detrimental. An explanation of the inconsistency of reported results in these analyses might be the ignorance of confounding factors like a woman's age (puberty, reproductive phase, menopausal transition, postmenopausal phase), individual differences amongst women of the same age (premenstrual symptoms, history of depression, prior negative experiences with oral contraceptive use), properties of oestrogen and its manner of administration as well as methodological factors. Most of these factors should be carefully considered when prescribing hormonal contraceptives or oestrogen therapy.

Keywords: oestrogen, depressive symptoms, menstrual cycle, hormonal contraceptives

Depressive symptoms are common in the general population: the life time prevalence of depression is approximately 17% (Böttcher, Radenbach, Wildt & Hinney, 2012); however, this prevalence is almost twice as high in women as in men (Kessler, McGonagle, Swartz, Blazer, & Nelson, 1993; Noble, 2005). These differences in prevalence might suggest an involvement of hormones in the vulnerability to, and development of depressive disorders. Research showed that prevalence differences of depressive symptoms correlate with the cyclicity of sex hormones, in particular oestrogen (Cheslack-Postava, Keyes, Lowe, & Koenen, 2015; Halbreich & Kahn 2001; Payne et al., 2007). More specifically, there are no gender differences in the prevalence of depressive symptoms before menarche (i.e., the first occurrence of the menstrual cycle). However, as hormonal fluctuations and instability start during puberty and continue throughout pre- and perimenopausal phases, differences in prevalence become obvious with women being at higher risk of developing depression (Halbreich & Kahn, 2001). As soon as hormonal levels stabilise at a low level after menopause (i.e., postmenopausal) this apparent gender difference in the prevalence of depressive symptoms disappears (Halbreich & Kahn, 2001; Payne et al., 2007). In addition, premenstrual symptoms are also relatively common within the general population: premenstrual dysphoric disorder (PMDD) includes physical and emotional symptoms (e.g., depression, anxiety, or irritability) in the luteal phase of the menstrual cycle, which improve with the start of menstruation, and its prevalence is approximately 3-9% in the population (Halbreich & Kahn, 2001; Payne et al., 2007). More strikingly, around 80% of women report experiencing mild to moderate premenstrual symptoms including depressed mood and mood swings, which constitute the premenstrual syndrome (PMS; Halbreich & Kahn, 2001; Payne et al., 2007). These findings show that depressive symptoms are prevalent in different phases of the menstrual cycle, as well as of the female life span.

Currently, approximately 80% of women in the US and over 100 million women worldwide are using hormonal contraceptives at some point during their reproductive period, especially in form of oral contraceptives (OCs; Keyes et al., 2013; Montoya & Bos, 2017). At a later stage in life, when approaching menopause and thereafter, many women participate in hormone replacement therapy (HRT) to treat menopausal symptoms like hot flashes. Both forms of external hormone administration are thought to suppress levels of sex hormones, especially oestrogen and progesterone levels, and their fluctuations across the menstrual cycle (Montoya & Bos, 2017). Since there is a high prevalence of depressive symptoms like PMS, PMDD and depressive disorders in women as well as a common use of hormonal contraceptives and HRT (Böttcher et al., 2012; Keyes et al., 2013; Montoya & Bos, 2017), it is clinically and societally relevant to investigate in which ways the use of hormonal contraceptives, in particular OCs, or HRT influence depressive symptoms throughout a woman's

life span. Research has increasingly tackled this problem during the last years; however, consistency of results is rare. Therefore, it is of importance to examine the effect of hormonal contraceptive use or HRT on depressive symptoms as well as when these effects occur during the life span to get a more complete picture of the current research progress. By reviewing available research, this paper explores the effects of external oestrogen administration (hormonal contraceptive use, HRT) on depressive symptoms across the life span of women (including puberty, premenopausal, perimenopausal and postmenopausal phases). Besides reviewing the directionality of effects, each section describes the hormonal levels throughout that life phase and the possible mechanisms underlying the effects of external oestrogen administration on depressive symptoms.

Puberty

During puberty, the menarche, which is the start of the first menstrual cycle, occurs on average at an age of 13 or 14 years. One menstrual cycle takes approximately 28 days and is divided into two stages. During the first stage - the follicular phase - hormonal levels including oestrogen are low, while they increase during the second stage - the luteal phase - creating cyclical fluctuations of hormone levels (Montoya & Bos, 2017).

A positive correlation between oestrogen levels and depression as well as increased mood variability was found for at least some stages of puberty (Balzer, Duke, Hawke, & Steinbeck, 2015). This association was most consistent during the phase of pubertal transition, in which oestrogen levels start to fluctuate with regard to the menstrual cycle, and it was suggested that the rapidity of hormonal changes may play a role in the development of mood symptoms (Balzer et al., 2015). However, it is apparent that there is a lack of longitudinal studies to support a causative effect of oestrogen on adolescent mood changes, since most studies are of correlational nature.

Given the possible influence of hormonal levels on mood variability and depressive symptoms in puberty, it is important to further consider how external oestrogen administration affects mood. With the start of the menstrual cycle, some female adolescents begin to take hormonal contraceptives, especially OCs. Thus, the effects of hormonal contraceptive use on mood should also be taken into account. One study found that females using hormonal contraceptives (including OCs) experience a higher risk for developing depression and this effect is more pronounced in adolescents than in women aged 20 to 34 years (Skovlund, Mørch, Kessing, & Lidegaard, 2016). In contrast, a three-month investigation of adolescents taking either an OC or a placebo found that both groups experienced similar types of OC side effects, including depression (O'Connell, Davis, and Kerns, 2007). The conclusion of the authors was that OC use does not cause depression or worsen depressive symptoms in adolescents.

In summary, at the time of puberty, gender differences in the prevalence of depressive symptoms become apparent, with females being at a higher risk (Halbreich & Kahn, 2001; Kessler et al., 1993; Noble, 2005), and research suggests that depressive symptoms are related to increases in oestrogen fluctuations. However, findings on the relationship between the use of hormonal contraceptives and depressive symptoms are conflicting. Thus, longitudinal studies are needed to determine a causative influence of hormones on the development of depressive symptoms. More importantly, future research should investigate whether OC use has detrimental effects on depressive symptoms in this age group (as suggested by Skovlund et al., 2016).

Premenopausal (reproductive) phase

The menstrual cycle continues throughout the reproductive years of a woman until the age of 45 or 50 years. As previously mentioned, oestrogen levels are highly fluctuating across the follicular and luteal phases of the cycle (Montoya & Bos, 2017). These oestrogen fluctuations are associated with an increased risk of depression in women (Halbreich & Kahn, 2001), leading to a twice as high prevalence as compared to men. Moreover, hormonal contraceptives are commonly used, since most women are sexually active during this life phase. Thus, it is of importance to study the possible effects of the use of hormonal contraceptives, including the effects on mood and depression.

Several studies suggest that there is no link between OC use and depression (Duke, Sibbritt, & Young, 2007; Joffe, Cohen, & Harlow, 2003). These findings are also consistent with a more recent study that did not find a significant relationship between OC use and the risk of a diagnosis for major depressive disorder (MDD; Cheslack-Postava et al., 2015). However, when looking at the difference between OC types with different dosage phases (i.e., mono- and multiphasic OCs), there is a trend, although insignificant, towards a reduced risk of MDD being associated with monophasic OC use and an increased risk of MDD being associated with multiphasic OCs. Thus, the type of OC might have an influence on the general relation between OC use and the risk for MDD development.

Other literature does suggest the presence of an association between the use of hormonal contraceptives and depression in the premenopausal phase. Older studies reported detrimental effects of OC use on mood including increased depressive symptoms (reviewed in Oinonen, & Mazmanian, 2002). However, reduced oestrogen dosages have been used in OCs since 1985 (Oinonen, & Mazmanian, 2002). Thus, findings from earlier studies might not be valid anymore for current OC formulations. More recent studies provide evidence that women using hormonal contraceptives experience a higher risk for developing depressive symptoms than non-users or placebo controls (Gingnell et al., 2013;

Skovlund et al., 2016). Moreover, Joffe et al. (2003) investigated women with a history of depression in order to further examine which factors may play a role in mood deterioration due to OC use. The results indicate that OC use is more likely to worsen premenstrual mood in women with a history of depression.

In contrast, other studies suggest a beneficial effect of OC use on mood, more specifically decreased variability in mood across the menstrual cycle (including less negative affect during menstruation; Oinonen, & Mazmanian, 2002) and reduced depressive symptoms (e.g., Bäckström, Hansson-Malmström, Lindhe, Cavalli-Björkman, & Nordenström, 1992; Young et al., 2007). A longitudinal study showed that the use of hormonal contraceptives is related to reduced depressive symptoms as well as fewer suicide attempts in young (25-34 years) and sexually active women (Keyes et al., 2013). This association was found for oestrogen/progesterone combinations and for progestin-only contraceptives and persisted after the authors also considered health and lifestyle factors in their analysis (e.g., prior hormonal contraceptive use and history of depression). In addition, Joffe et al. (2003) investigated women with early-onset premenstrual mood disturbance or dysmenorrhea to further examine which factors may be relevant for mood improvement due to OC use. The results indicate that OC use has beneficial effects on mood in women with early-onset premenstrual mood disturbance or dysmenorrhea. Thus, OC use is associated with an improvement in premenstrual mood in these women. This finding is consistent with studies reporting that OC use leads to mood improvements in women with PMS and PMDD (Freeman et al., 2001; Yonkers et al., 2005) and these effects were found for monophasic as well as for multi(tri)-phasic OC preparations (Bäckström et al., 1992). PMS and PMDD are thought to be caused by a drop in oestrogen levels during the luteal phase of the menstrual cycle. OC use suppresses these oestrogen fluctuations, which leads to a diminished decline in oestrogen levels in the luteal phase and consequently to reduced depressive symptoms (Payne et al., 2007). Thus, mood improvements, especially in women with PMS or PMDD, might be due to hormonal contraceptives stabilising mood across the cycle (Keyes et al., 2013).

In summary, oestrogen fluctuations across the menstrual cycle are thought to be related to an increased risk for the development of depressive symptoms in women during their reproductive years (Halbreich & Kahn, 2001). Findings from studies regarding the association between the use of hormonal contraceptives and depressive symptoms are contradictive; including no association, detrimental as well as beneficial effects of hormonal contraceptives on mood. Even though not entirely consistent, it seems that the use of OCs has little effects on mood in the majority of women, whereas it further deteriorates mood in women with a history of depression and improves mood in women with premenstrual mood symptoms like PMS or PMDD (e.g., Joffe et al., 2003). Depressive symptoms are thought to be (at least partly) caused by low oestrogen levels in the premenopausal phase (Young, Midgley, Carlson, & Brown, 2000) and suppressing these levels further by using OCs might induce more depressive symptoms in women with a history of depression. On the other hand, PMS and PMDD are suggested to be caused by a drop in oestrogen levels during the luteal phase of the menstrual cycle (Payne et al., 2007) and OC use suppresses these oestrogen fluctuations, which leads to a smaller decline of oestrogen levels in the luteal phase and consequently to reduced depressive symptoms.

Perimenopausal phase (menopausal transition)

Within the perimenopausal phase or menopausal transition (late 40s to early 50s), oestrogen levels highly fluctuate in an unpredictable manner, since the menstrual cycle starts to become more irregular (Halbreich & Kahn, 2001; Payne et al., 2007). These natural oestrogen fluctuations are thought to play a role in the increased development of depressive symptoms during this phase of life (Freeman, Sammel, Lin, & Nelson, 2006; Halbreich & Kahn, 2001; Morrison et al., 2004; Schmidt, Nieman, Danaceau, Tobin, Roca, Murphy, & Rubinow, 2000). The impact of this influence can be seen in a longitudinal study, which shows that development of depressive symptoms and diagnosed depressive disorders are significantly more likely during the menopausal transition than during the premenopausal phase (Freeman et al., 2006).

With regard to the effects of external oestrogen administration, studies show that oestrogen treatment has a beneficial effect on depression in perimenopausal women (Cohen et al., 2003; de Novaes Soares, Almeida, Joffe, & Cohen, 2001; Schmidt et al., 2000; Soares & Zitek, 2008). More specifically, it was suggested that transdermal oestrogen therapy has antidepressant effects in perimenopausal women with depression leading to remission in 68-80% of depressed women in the oestrogen treatment group compared to only 20-22% of the women in the placebo group (de Novaes Soares et al., 2001; Schmidt et al., 2000). Moreover, these beneficial effects were found in women with different severities of depression, including MDD, dysthymic disorder, and minor depressive disorder, which suggests a generalisable antidepressant effect of oestrogen treatment in perimenopausal women (de Novaes Soares et al., 2001). However, oestrogen treatment might have clinical risks and side effects in the long-term, like heart disease or breast cancer (Cohen et al., 2003). Thus, more data confirming the above-mentioned preliminary results are needed in order to examine whether the beneficial effects on depressive symptoms or the clinical side effects are more outstanding. Moreover, it is of

relevance to understand which mechanisms underlie the effects of oestrogen treatment on reducing depressive symptoms. One suggestion is that administering oestrogen might have a stabilising effect on oestrogen levels, which otherwise highly fluctuate during the menopausal transition (Halbreich & Kahn, 2001).
To summarise, the menstrual cycle gets irregular during the menopausal transition, which is associated with unpredictable oestrogen level fluctuations (Halbreich & Kahn, 2001; Payne et al., 2007). These fluctuations are thought to increase the risk for experiencing depressive symptoms in this age group (Freeman et al., 2006; Halbreich & Kahn, 2001; Morrison et al., 2004; Schmidt et al., 2000). Administering oestrogen therapy as a treatment is suggested to reduce depressive symptoms by stabilising oestrogen levels in perimenopausal women (e.g., Halbreich & Kahn, 2001; de Novaes Soares et al., 2001).

Postmenopausal phase

At times in women approaching menopause, hormonal levels, including oestrogen, decrease. This results in constant low levels of these hormones in the postmenopausal phase, which starts around the age of 50 to 55 years. These reductions in oestrogen levels are thought to be associated with an increase in depressive symptoms in postmenopausal women (Halbreich & Kahn 2001; Payne et al., 2007).

There is some evidence that external oestrogen administration leads to an improvement in mood, including a reduction in depressive symptoms (Scherwin, 1994). However, this evidence is contradicted by the finding that most postmenopausal women with depression did not respond to oestrogen therapy (Cohen et al., 2003; Morrison et al., 2004; Schleifer, Justice, & de Wit, 2002). In these studies, no clinically significant antidepressant effect was found: both placebo and oestrogen treatment groups showed improvements in depressive symptoms over time. As previously mentioned, oestrogen levels are constantly low in postmenopausal women, which might be the reason why oestrogen treatment cannot exert its stabilising effects and, thus, no antidepressant effect is observed (Soares & Zitek, 2008). However, the above-mentioned evidence needs further confirmation, since several factors could have influenced the results. First, these studies only included women with mild to moderate depression and it needs to be determined whether oestrogen treatment may have an antidepressant effect in postmenopausal women with more severe symptoms (i.e., major depressive disorder). Second, it was suggested that the antidepressant effect of oestrogen treatment in postmenopausal women might be depending on the dosage of oestrogen and the duration of exposure to oestrogen therapy (Schleifer et al., 2002). Hence, an ideal oestrogen dosage and duration of therapy may lead to antidepressant effects of oestrogen therapy.
In summary, oestrogen levels are stable at a low level during the postmenopausal phase, which is associated with an increased risk for depressive symptoms (Halbreich & Kahn 2001; Payne et al., 2007). While some studies suggest a beneficial effect of oestrogen treatment on depression (Scherwin, 1994), others show that most postmenopausal women with depressive symptoms do not respond to oestrogen therapy (Cohen et al., 2003; Morrison et al., 2004; Schleifer et al., 2002). This finding might be due to a failure of oestrogen therapy to stabilise oestrogen levels, since they may already be stable (Soares & Zitek, 2008).

DISCUSSION

This review summarised literature that suggests that the effects of the use of hormonal contraceptives, especially OCs, or oestrogen therapy on depressive symptoms vary throughout a woman's life span as well as within specific life phases. It appears that several factors influence the variability of responses to external oestrogen administrations and these factors will be discussed in the next sections.

Age

In women, age is one of the major impacting factors. At the time of puberty, oestrogen levels start to fluctuate across the menstrual cycle, which is related to an increased risk for experiencing depressive symptoms and causative for the apparent differences in the prevalence of depressive disorders between genders (Halbreich & Kahn, 2001; Kessler et al., 1993; Noble, 2005). However, the effects of the use of hormonal contraceptives during puberty are not clear: current evidence directs to either no (O'Connell et al., 2007) or detrimental (Skovlund et al., 2016) effects of OC use on depression, but more research is needed to define causality. During the premenopausal (reproductive) phase, oestrogen fluctuations continue across the menstrual cycle and thus, the increased risk for depression persists (Halbreich & Kahn, 2001). Even though findings are not entirely consistent, it seems that the use of hormonal contraceptives, especially of OCs, has little effect on mood in most women whereas it further deteriorates mood in women with a history of depression and improves mood in women with premenstrual mood symptoms like PMS or PMDD (e.g., Joffe et al., 2003). In the perimenopausal phase, however, the menstrual cycle becomes irregular, which is associated with unpredictable oestrogen level fluctuations and thus, an increase in experiencing depressive symptoms (Freeman et al., 2006; Halbreich & Kahn, 2001; Morrison et al., 2004; Schmidt et al., 2000). Treatment with oestrogen therapy has been found to effectively reduce depressive symptoms (e.g., Halbreich & Kahn, 2001; de Novaes Soares et al., 2001). After menopause, oestrogen levels are stable at a low level, which is related to an increased risk for depressive symptoms (Halbreich & Kahn 2001; Payne et al., 2007).

There is evidence that effects of oestrogen therapy on depressive symptoms might be beneficial (Scherwin, 1994), but most studies could not correlate positive effects of oestrogen therapy to depression (Cohen et al., 2003; Morrison et al., 2004; Schleifer et al., 2002).

The differential effects of external oestrogen administration on depressive symptoms across a woman's life span can be explained by age-related changes in hormonal levels, especially oestrogen levels. Effects in puberty have not been investigated thoroughly enough to draw causative conclusions. Thus, future research needs to investigate the relationship between depressive symptoms and hormonal levels, as well as the use of hormonal contraceptives in this age group. However, during the reproductive years depressive symptoms are thought to be (at least partly) caused by low oestrogen levels (Young et al., 2000). Therefore, especially women with a history of depression might have low oestrogen levels and suppressing these levels further by administering OCs might induce even more depressive symptoms (Joffe et al., 2003). On the other hand, PMS and PMDD are thought to be caused by a drop in oestrogen levels during the luteal phase of the menstrual cycle (Payne et al., 2007). OC use suppresses these oestrogen fluctuations leading to fewer declines of oestrogen levels in the luteal phase and consequently to reduced depressive symptoms. Hence, differences in oestrogen levels may explain why premenopausal women with a history of depression respond differently to hormonal contraceptives than women with PMS/PMDD.

Moreover, treating perimenopausal women with depression with oestrogen therapy effectively reduces depressive symptoms by stabilising oestrogen levels, since depression is thought to be related to fluctuations of oestrogen levels in this age group (e.g., Halbreich & Kahn, 2001; de Novaes Soares et al., 2001). In contrast, oestrogen therapy has no beneficial effect on depression in postmenopausal women, which might be due to the inability to stabilise oestrogen levels, as oestrogen levels are already stable and at a low level after the menopause (Cohen et al., 2003; Morrison et al., 2004; Schleifer et al., 2002; Soares & Zitek, 2008). Thus, oestrogen therapy may exert beneficial effects on depression due to stabilisation of oestrogen levels during menopause, but not afterwards. Nevertheless, it is striking that premenopausal women with depression are thought to have low oestrogen levels and OC use worsens depressive symptoms, while postmenopausal women also have low oestrogen levels but oestrogen treatment is found to have either a beneficial or no effect instead of detrimental effects. Therefore, future research is needed in order to examine why these divergent effects of external oestrogen administration emerge despite a suggested similar cause of the depressive symptoms (i.e., low oestrogen levels).

Individual differences amongst premenopausal women

In addition to differential effects of oestrogen administration across a woman's life span, women of similar age seem to respond differently to hormonal contraceptives or oestrogen therapy. For instance, OC use has no effect on mood, including depressive symptoms, in most women in their reproductive years (Joffe et al., 2003). However, a cohort of women in the same age range that have a history of depression report mood deterioration with OC use. In contrast, OC use seems to have a beneficial effect on mood in premenopausal women with PMS or PMDD. These women are more sensitive to hormonal changes since PMS and PMDD are thought to be a response to the drop in oestrogen levels during the luteal phase of the menstrual cycle. Use of OCs leads to a suppression of these oestrogen fluctuations and drops in the luteal phase and thereby reduces depressive symptoms (Keyes et al., 2013; Payne et al., 2007). These findings indicate that even within the same age cohort oestrogen administration can have divergent effects. Finally, some studies also found an increased risk for experiencing depressive symptoms with OC use (e.g., Gingnell et al., 2013). However, it should be considered that the study by Gingnell et al. (2013) included women with prior OC-induced negative effects on mood. They might have had negative expectations when re-exposed to OCs, which could have led to an over-reporting of depressive symptoms. Thus, these detrimental effects might be more specific to a subgroup of premenopausal women with prior problems with OC use rather than the general cohort. This is consistent with findings that OC use leads to reduced depressive symptoms in premenopausal women (Keyes et al., 2013) when only women without a history of depression or prior problems with OC use were included in the study. While women with depression are less likely to use hormonal contraceptives in first preference, women who experience mood side effects due to hormonal contraceptive use are more likely to discontinue the use (Gingnell et al., 2013; Keyes et al., 2013; Oinonen & Mazmanian, 2002). Thus, the population of women for whom hormonal contraceptives have a beneficial effect on mood might be restricted to women who do not experience major side effects of the contraceptives as well as to women without a history of, or current depression.

Properties of oestrogen and its manner of administration

Apart from the age of and individual differences amongst women, different properties of hormonal contraceptives or oestrogen therapies might also affect the different effects on depressive symptoms. Although only marginally significant, there is a trend to a reduced risk of MDD being associated with monophasic OC use and an increased risk of MDD being associated

with multiphasic OCs (Cheslack-Postava et al., 2015). Moreover, the use of both mono- and multi(tri)-phasic OC preparations was associated with mood improvements in women with PMS or PMDD (Bäckström et al., 1992; Freeman et al., 2001; Yonkers et al., 2005). In addition to phasic differences in OC preparations, the oestrogen and progestin combinations also vary across OCs. While the use of both oestrogen/progesterone combinations and progestin-only contraceptives was associated with reduced depressive symptoms in one study (Keyes et al., 2013), most studies did not particularly investigate the effect of different OC preparations and dosages on depressive symptoms (e.g., Joffe et al., 2003). These findings suggest that the type of OC preparation as well as oestrogen dosages are relevant factors to be considered when correlating OC use and depressive symptoms. Thus, future research should try to control for OC preparation types and oestrogen/progestin dosages to investigate their possible effects on depressive symptoms. Furthermore, different dosages of oestrogen are administered during oestrogen therapy; however, limited data is available comparing the effects of various dosages. Since it is apparent that dosage differences might have an influence on divergent effects on depressive symptoms with regard to OC use, future research should investigate this issue in various oestrogen dosages during oestrogen therapy. This is of importance, since variations in oestrogen administration might affect the hormone levels differently (i.e., suppression or stabilising of oestrogen), which consequently affects depressive symptoms.

Methodological factors

Besides factors concerning the female cohorts and external oestrogen administration, some methodological factors might also influence the variability in observed effects of hormonal contraceptives or oestrogen therapy on depressive symptoms. Many studies, for instance, did not include longitudinal data. Another drawback, especially in studies focusing on puberty and adolescence, is the focus on correlational studies rather than on placebo-controlled studies. Both factors introduce bias with respect to the causative effect of external oestrogen administration on depressive symptoms and may lead to misinterpretation of the results. Probably, this explains why some studies reported beneficial effects of oestrogen administration while other studies found no or even detrimental effects.

Of importance is, that the term 'depressive symptoms' was inconsistently used across studies: some studies used structured interviews and diagnostic criteria to judge the presence of depressive symptoms. For instance, Skovlund et al. (2016) categorised women as experiencing depressive symptoms if they were treated with antidepressants or were diagnosed at a psychiatric hospital. Thus, this study mainly included women with major depression, while mild or moderate depressive symptoms were not considered. Other studies relied on self-report measures of depressive symptoms (e.g., Schleifer et al., 2002). These studies examined women who could either have major depression or milder symptoms that do not necessarily require treatment. Although it is relevant to investigate effects of oestrogen administrations on depressive symptoms of different severities, findings from studies with different conceptualizations of depressive symptoms are difficult to compare. Thus, future research should indicate which severities of depressive symptoms are studied to enable conclusions on different outcomes across studies.

CONCLUSION

This review investigated literature on the effects of external oestrogen administration (hormonal contraceptive use, oestrogen therapy) on depressive symptoms across the life span of women. It shows that these effects can be either beneficial, detrimental, or absent. Since it is of clinical relevance to examine the cause of these divergent effects, this paper suggested some confounding factors, which should be considered when prescribing hormonal contraceptives (e.g., OCs) or oestrogen therapy. In this regard, age and individual differences amongst women are especially important. While the use of hormonal contraceptives does not affect most premenopausal women (i.e., no effect), women of the same age group with PMS/PMDD or a history of depression report mood improvement (i.e., beneficial effect) or mood deterioration (i.e., detrimental effects; Joffe et al, 2003). Moreover, an increase in depressive symptoms due to the use of hormonal contraceptives was observed in women with prior experience of OC-induced negative effects on mood (Gingnell et al., 2013). Therefore, clinicians should be aware of these different effects and check a woman's history of PMS/PMDD, prior OC-induced negative experiences, and depressive symptoms before prescribing hormonal contraceptives. In addition, oestrogen therapy was found to be effective in reducing depressive symptoms during the menopause (i.e., perimenopausal; Halbreich & Kahn, 2001; de Novaes Soares et al., 2001), but not afterwards (i.e., postmenopausal; Cohen et al., 2003; Morrison et al., 2004; Schleifer et al., 2002). This again suggests that age is a confounding factor and it should be taken into account when oestrogen therapy is considered a suitable treatment. Besides age and individual differences, the properties of the oestrogen administration, for instance OC preparations or oestrogen dosages in OCs and oestrogen therapy, might also induce different effects on depressive symptoms. Thus, it is important to further investigate which hormonal preparations and dosages are well tolerated (i.e., not inducing depressive symptoms) and can be prescribed as hormonal contraceptives or oestrogen therapy.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

REFERENCES

Bäckström, T., Hansson-Malmström, Y., Lindhe, B. Å., Cavalli-Björkman, B., & Nordenström, S. (1992). Oral contraceptives in premenstrual syndrome: a randomized comparison of triphasic and monophasic preparations. Contraception, 46(3), 253-268. doi:10.1016/0010-7824(92)90006-F

Balzer, B. W., Duke, S. A., Hawke, C. I., & Steinbeck, K. S. (2015). The effects of estradiol on mood and behavior in human female adolescents: a systematic review. European journal of pediatrics, 174(3), 289-298. doi:10.1007/s00431-014-2475-3

Böttcher, B., Radenbach, K., Wildt, L., & Hinney, B. (2012). Hormonal contraception and depression: a survey of the present state of knowledge. Archives of gynecology and obstetrics, 286(1), 231-236. doi:10.1007/s00404-012-2298-2

Cheslack-Postava, K., Keyes, K. M., Lowe, S. R., & Koenen, K. C. (2015). Oral contraceptive use and psychiatric disorders in a nationally representative sample of women. Archives of women's mental health, 18(1), 103-111. doi:10.1007/s00737-014-0453-4

Cohen, L. S., Soares, C. N., Poitras, J. R., Prouty, J., Alexander, A. B., & Shifren, J. L. (2003). Short-term use of estradiol for depression in perimenopausal and postmenopausal women: a preliminary report. American Journal of Psychiatry, 160(8), 1519-1522. doi:10.1176/appi.ajp.160.8.1519

Duke, J. M., Sibbritt, D. W., & Young, A. F. (2007). Is there an association between the use of oral contraception and depressive symptoms in young Australian women?. Contraception, 75(1), 27-31. doi:10.1016/j.contraception.2006.08.002

Freeman, E. W., Kroll, R., Rapkin, A., Pearlstein, T., Brown, C., Parsey, K., ... & Foegh, M. (2001). Evaluation of a unique oral contraceptive in the treatment of premenstrual dysphoric disorder. Journal of women's health & gender-based medicine, 10(6), 561-569. doi:10.1089/15246090152543148

Freeman, E. W., Sammel, M. D., Lin, H., & Nelson, D. B. (2006). Associations of hormones and menopausal status with depressed mood in women with no history of depression. Archives of general psychiatry, 63(4), 375-382. doi:10.1001/archpsyc.63.4.375

Gingnell, M., Engman, J., Frick, A., Moby, L., Wikström, J., Fredrikson, M., & Sundström Poromaa, I. (2013). Oral contraceptive use changes brain activity and mood in women with previous negative affect on the pill—a double-blinded, placebo-controlled randomized trial of a levonorgestrel-containing combined oral contraceptive. Psychoneuroendocrinology, 38(7), 1133-1144. doi:10.1016/j.psyneuen.2012.11.006

Halbreich, U., & Kahn, L. S. (2001). Role of oestrogen in the aetiology and treatment of mood disorders. CNS drugs, 15(10), 797-817. doi:10.2165/00023210-200115100-00005

Joffe, H., Cohen, L. S., & Harlow, B. L. (2003). Impact of oral contraceptive pill use on premenstrual mood: predictors of improvement and deterioration. American journal of obstetrics and gynecology, 189(6), 1523-1530. doi:10.1016/S0002-9378(03)00927-X

Kessler, R. C., McGonagle, K. A., Swartz, M., Blazer, D. G., & Nelson, C. B. (1993). Sex and depression in the National Comorbidity Survey I: Lifetime prevalence, chronicity and recurrence. Journal of affective disorders, 29(2), 85-96. doi:10.1016/0165-0327(93)90026-G

Keyes, K. M., Cheslack-Postava, K., Westhoff, C., Heim, C. M., Haloossim, M., Walsh, K., & Koenen, K. (2013). Association of hormonal contraceptive use with reduced levels of depressive symptoms: a national study of sexually active women in the United States. American journal of epidemiology, 178(9), 1378-1388. doi:10.1093/aje/kwt188

Montoya, E. R., & Bos, P. A. (2017). How oral contraceptives impact social-emotional behavior and brain function. Trends in cognitive sciences, 21(2), 125-136. doi:10.1016/j.tics.2016.11.005

Morrison, M. F., Kallan, M. J., Ten Have, T., Katz, I., Tweedy, K., & Battistini, M. (2004). Lack of efficacy of estradiol for depression in postmenopausal women: a randomized, controlled trial. Biological psychiatry, 55(4), 406-412. doi:10.1016/j.biopsych.2003.08.011

Noble, R. E. (2005). Depression in women. Metabolism, 54(5), 49-52. doi:10.1016/j.metabol.2005.01.014

de Novaes Soares, C., Almeida, O. P., Joffe, H., & Cohen, L. S. (2001). Efficacy of estradiol for the treatment of depressive disorders in perimenopausal women: a double-blind, randomized, placebo-controlled trial. Archives of general psychiatry, 58(6), 529-534. doi:10.1001/archpsyc.58.6.529

O'Connell, K., Davis, A. R., & Kerns, J. (2007). Oral contraceptives: side effects and depression in adolescent girls. Contraception, 75(4), 299-304. doi:10.1016/j.contraception.2006.09.008

Oinonen, K. A., & Mazmanian, D. (2002). To what extent do oral contraceptives influence mood and affect?. Journal of affective disorders, 70(3), 229-240. doi:10.1016/S0165-0327(01)00356-1

Payne, J. L., Roy, P. S., Murphy-Eberenz, K., Weismann, M. M., Swartz, K. L., McInnis, M. G., ... & Nurnberger, J. I. (2007). Reproductive cycle-associated mood symptoms in women with major depression and bipolar disorder. Journal of affective disorders, 99(1), 221-229. doi:10.1016/j.jad.2006.08.013

Schleifer, L. A., Justice, A. J., & de Wit, H. (2002). Lack of effects of acute estradiol on mood in postmenopausal women. Pharmacology Biochemistry and Behavior, 71(1), 71-77. doi:10.1016/S0091-3057(01)00638-4

Schmidt, P. J., Nieman, L., Danaceau, M. A., Tobin, M. B., Roca, C. A., Murphy, J. H., & Rubinow, D. R. (2000). Oestrogen replacement in perimenopause-related depression: a preliminary report. American journal of obstetrics and gynecology, 183(2), 414-420. doi:10.1067/mob.2000.106004

Sherwin, B. B. (1994). Sex hormones and psychological functioning in postmenopausal women. Experimental Gerontology, 29(3), 423-430. doi:10.1016/0531-5565(94)90021-3

Skovlund, C. W., Mørch, L. S., Kessing, L. V., & Lidegaard, Ø. (2016). Association of hormonal contraception with depression. JAMA psychiatry, 73(11), 1154-1162. doi:10.1001/jamapsychiatry.2016.2387

Soares, C. N., & Zitek, B. (2008). Reproductive hormone sensitivity and risk for depression across the female life cycle: a continuum of vulnerability?. Journal of psychiatry & neuroscience: JPN, 33(4), 331.

Yonkers, K. A., Brown, C., Pearlstein, T. B., Foegh, M., Sampson-Landers, C., & Rapkin, A. (2005). Efficacy of a new low-dose oral contraceptive with drospirenone in premenstrual dysphoric disorder. Obstetrics & Gynecology, 106(3), 492-501. doi:10.1097/01.AOG.0000175834.77215.2e

Young, E. A., Kornstein, S. G., Harvey, A. T., Wisniewski, S. R., Barkin, J., Fava, M., ... & Rush, A. J. (2007). Influences of hormone-based contraception on depressive symptoms in premenopausal women with major depression. Psychoneuroendocrinology, 32(7), 843-853. doi:10.1016/j.psyneuen.2007.05.013

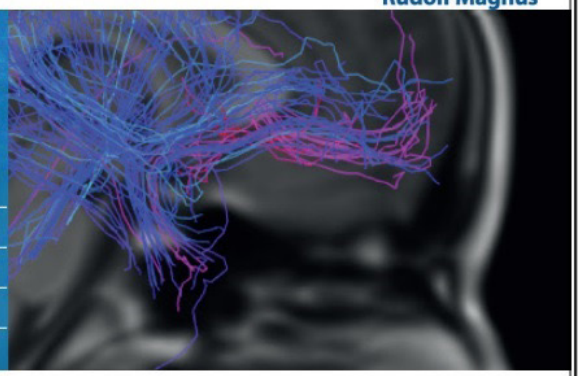
Young, E. A., Midgley, A. R., Carlson, N. E., & Brown, M. B. (2000). Alteration in the hypothalamic-pituitary-ovarian axis in depressed women. Archives of General Psychiatry, 57(12), 1157-1162. doi:10.1001/archpsyc.57.12.1157

Brain Center Rudolf Magnus

Brain Center
Rudolf Magnus

The approaches take place across all areas

	Stroke	Epilepsy	Neuromuscular disorders	Psychotic disorders	Developmental disorders
Genetic risks	✓	✓	✓	✓	✓
Environmental risks	✓	✓	✓	✓	✓
Structure and connections	✓	✓	✓	✓	✓
Translational research	✓	✓	✓	✓	✓
From research to care	✓	✓	✓	✓	✓



Research topics

- **Neurodevelopment & its disorders**
- **Psychosis**
- **Stroke**
- **Neuromuscular diseases**

Rudolf Magnus Young Talent Fellowship

- Each year 1-2 **competitive fellowships**
- For postdocs with (current) Veni or clinical fellow
- **2 years of salary and 100.000 Euro**

Utrecht, the Netherlands

- PhD program **Clinical & Experimental Neuroscience**
- 4 years of **PhD training**
- In English, no tuition fee
- PhD training can be combined with **clinical training**

Information http://www.braincenterrudolfmagnus.nl
Contact and information:
Dr. Mariken de Krom, m.dekrom-3@umcutrecht.nl

Brain Center
Rudolf Magnus

The actin cytoskeleton as a meshing maze: Exploring a novel function of actin in dendritic spines

Droogers, W.J.[1], MacGillavry, H.D.[2]

[1] Department of Translational Neuroscience, UMC Utrecht Brain Center Rudolf Magnus, the Netherlands
[1] Department of Biology, Cell Biology Faculty of Science, Utrecht University, Utrecht, the Netherlands

The actin cytoskeleton has multiple functions in dendritic spines that underlie synaptic plasticity and is therefore essential for learning and memory. These include morphological functions, barrier functions, transport, endo- and exocytosis and facilitation of functional interactions. Several studies show that the actin cytoskeleton can also influence localisation of proteins by affecting their diffusion. Actin was able to decrease the diffusion of Ca2+/calmodulin-dependent protein kinase II (CaMKII) and potassium chloride cotransporter 2 (KCC2) in dendritic spines. A hypothesis that can be proposed is that the actin cytoskeleton can localise proteins in specific areas in the spine by serving as a meshing maze. This hypothesis can be tested for actin-binding proteins using super-resolution microscopy. The influence of the actin cytoskeleton on the diffusion of CaMKII and its targets, needs to be investigated to test the meshing maze hypothesis. Further research should resolve whether this theory can be extrapolated to other proteins and other parts of the neuron, and whether a potential meshing maze effect is functionally relevant. In summary, the actin cytoskeleton as a meshing maze is a novel concept and needs more evidence for confirmation of the theory.

Keywords: spines, PALM, actin cytoskeleton, diffusion, meshing maze

Our brains adapt to our environment, which allows us to learn new things and remember these and other memories for a short or a long period of time. The changes in connectivity of neurons underlying learning and memory are the product of synaptic plasticity, in which synapses undergo alterations in an activity-dependent manner. Many processes are involved in these changes via both structural and molecular interactions. A protein that influences both types of mechanisms is filamentous actin (F-actin, further denominated as actin) (Kandel, Schwartz, Jessell, Siegelbaum, & Hudspeth, 2013). In dendritic spines, this can be present as the actin cytoskeleton and as free, unbound actin monomers. Actin can be nucleated, elongated, capped (to prevent elongation), crosslinked and bundled by several actin-binding proteins, indicating its dynamic properties and ability to form highly branched and dense networks at specific locations (Figure 1) (Kandel et al., 2013; Tatavarty, Kim, Rodionov, & Yu, 2009; Urban, Willig, Hell, & Nägerl, 2011). One of the major functions of the actin cytoskeleton in synaptic plasticity is its structural role in dendritic spines and its effect on their morphology (Cingolani & Goda, 2008). Actin can also act as a barrier in the spine neck which is necessary to prevent the exchange of certain proteins between the spine and the dendritic shaft (Wang, Dumoulin, Renner, Triller, & Specht, 2016). Furthermore, it has been shown that the actin cytoskeleton in the spine is required for the transport of mRNA and mitochondria to specific locations in the spine (Huang, Chotiner, & Steward, 2007; Ligon & Steward, 2000). Moreover, the actin cytoskeleton can create a cytoplasmatic flow to propel vesicles in a certain direction and it can transport vesicles via myosin motors (Hanley, 2014). In summary, these processes show that the actin cytoskeleton is involved in a wide variety of mechanisms that underlie and support synaptic plasticity.

The known functions of the actin cytoskeleton in spines have been unravelled relatively recently. Evidence from 2014 suggests a novel function of the actin cytoskeleton that is still highly hypothetical (Lu, MacGillavry, Frost, & Blanpied, 2014). Namely, the ability of the actin cytoskeleton to be dynamically heternogeneous and to be able to form a dense network at a specific location on a specific moment (Figure 1), could allow actin to localise a protein as if it is trapped in a meshing maze (Tatavarty et al., 2009; Urban et al., 2011). In this way, the actin cytoskeleton can regulate the location of a protein, which can affect the ability of the protein to execute its function(s). As can be seen in Figure 1, the actin cytoskeleton has certain hotspots in the spine, which could serve as meshing mazes. These hotspots are analogous to microdomains in spines and in many cases can overlap with these subcompartments (Colgan & Yasuda, 2014). This trapping mechanism is comparable to the effect the *postsynaptic density* (PSD) has on the localisation of *α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid* (AMPA) receptors. Li et al. (2016) showed that AMPA receptors diffuse more slowly inside the PSD due to the dense structure of proteins

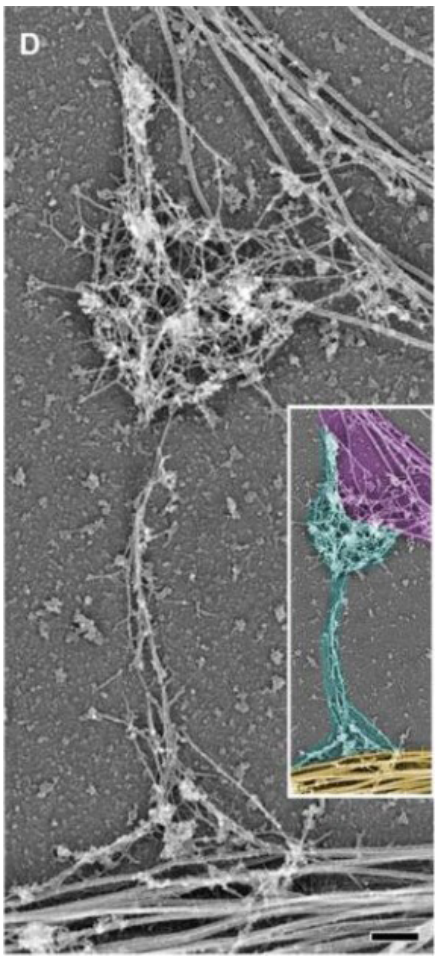


Figure 1 | The actin cytoskeleton imaged by high resolution electron microscopy (EM). *The interior of a spine visualised with EM after removal of the membrane. In the shrunken version of the picture, the actin filaments are indicated in blue, the microtubules in the dendritic shaft in orange and the position of an axon in purple. The thin structure of the spine neck and the heternogeneous organisation of the actin cytoskeleton in the spine head are illustrated. Scale bar is 200 nm. Modified from Korobova et al. (2010).*

in the PSD and the interactions between the AMPA receptors and the scaffold proteins of the PSD (T. P. Li, Song, MacGillavry, Blanpied, & Raghavachari, 2016). This results in a relatively high concentration of AMPA receptors at the PSD. This principle could also underlie the mesh-like function of the actin cytoskeleton in spines, in this case applied in a 3D environment. For *Ca2+/calmodulin-dependent protein kinase II* (CaMKII) and *potassium chloride cotransporter 2* (KCC2) it has already been shown that the actin cytoskeleton influences its diffusion rate (Chamma et al., 2013; Khan, Reese, Rajpoot, & Shabbir, 2012; Lu et al., 2014). It is therefore interesting to ascertain whether the functional localisation of CaMKII and KCC2 in the spine depends on the actin cytoskeleton.

The meshing maze hypothesis

Only a small number of studies have thus far been performed on the possible meshing maze function of the actin cytoskeleton. A recent study on CaMKII can be used as an example to determine the feasibility of the proposed function of the actin cytoskeleton. CaMKII is involved in multiple processes that underlie learning and memory, such as synaptic plasticity and *long term potentiation* (LTP) (Coultrap & Bayer, 2012; Giese, Fedorov, Filipkowski, & Silva, 1998; Lisman, Yasuda, & Raghavachari, 2012). Lu et al. (2014) investigated the localisation of CaMKII in spines and dendrites of hippocampal neurons during normal conditions and after *N-methyl-D-aspartate* (NMDA) receptor stimulation, which can induce LTP (Lu et al., 2014). The influence of the actin cytoskeleton on the localisation and diffusivity of CaMKII was also determined, which is relevant regarding the meshing maze theory. Single-molecule tracking *photoactivated localisation microscopy* (PALM) was used to visualise the localisation and movement of the CaMKII proteins (Lu et al., 2014; Manley, Gillette, & Lippincott-Schwartz, 2010). The diffusion rate of every protein was calculated afterwards. CaMKII was visualised in dissociated hippocampal neuron cultures using the CaMKIIα subunit tagged with mEos2, which is a photoactivatable fluorescent protein that can be green or red fluorescent (McKinney, Murphy, Hazelwood, Davidson, & Looger, 2009). The luminescent phase of mEos2 varies temporally, which makes it possible to distinguish between two proteins in small proximity of each other after reconstruction of the image (Figure 2). This core principle of PALM was used by Lu et al. (2014) to visualise CaMKII and track its diffusional path. The results of these experiments can shed light on the way actin can localise proteins in spines.

The functional differences between the CaMKII subunits can be used to investigate the influence of the actin cytoskeleton on the diffusion of the protein as a whole. The CaMKIIα subunit of CaMKII binds weakly to F-actin, which makes it insufficient to study the interaction between CaMKII and the actin cytoskeleton (Khan, Conte, Carter, Bayer, & Molloy, 2016). However, another subunit of CaMKII, CaMKIIβ, has a higher affinity for F-actin and can therefore mediate the interaction between CaMKII and the actin cytoskeleton (Khan et al., 2016; Lisman et al., 2012). To study the effect of the actin cytoskeleton on CaMKII mobility and localisation, Lu et al. (2014) transfected dissociated hippocampal neuron cultures with either CaMKIIα or both CaMKIIα and CaMKIIβ. The researchers compared the diffusion of CaMKIIα between the two groups of transfected neurons, hypothesizing that the CaMKIIβ would reduce the diffusion of the CaMKIIα by binding to the actin cytoskeleton. To analyse these results, the CaMKII proteins were divided in three groups depending on their diffusion rates. The first group showed very low diffusion and were likely immobile. The second group also showed relatively low diffusion rates but were

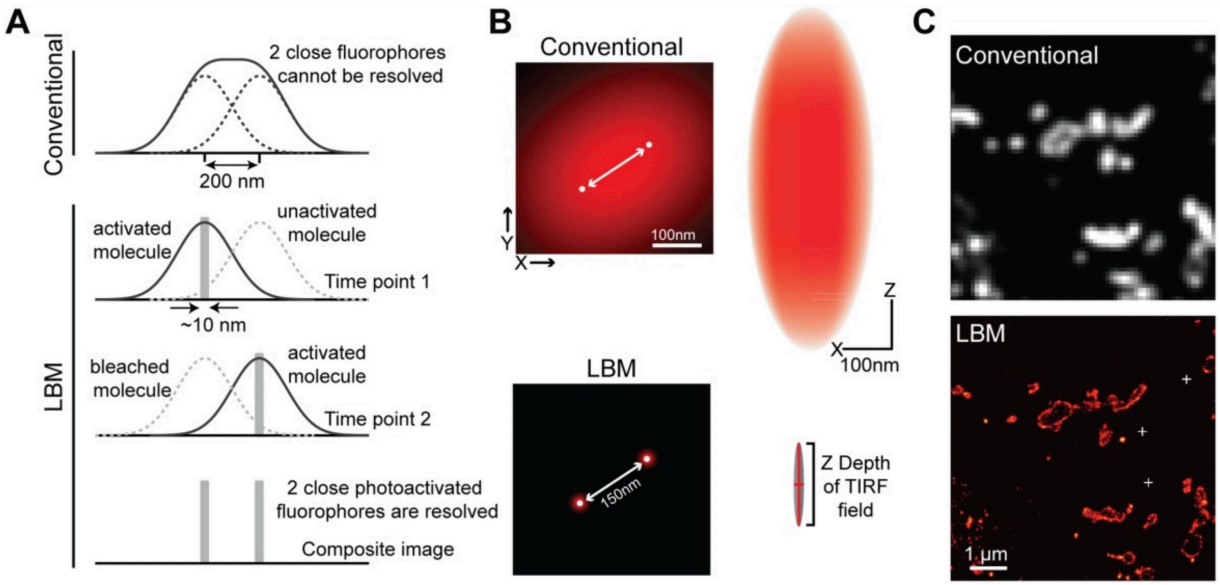


Figure 2 | The main difference between conventional and localisation-based microscopy (LBM). *(A) The place and intensity of the illumination of two fluorophores is presented for conventional microscopy and LBM, a form of super resolution microscopy. In conventional microscopy, the observed illumination of the fluorophores overlaps (upper part). If the illumination of the fluorophores is temporally separated as in LBM (lower part), the location of the fluorophores can be more accurately perceived in the composite image. (B) The optical differences in the observed image of conventional microscopy and the composite image of LBM are illustrated. The Z depth of TIRF field indicates how deep the light is scattered. (C) Two images using the different methods are displayed, clearly indicating the difference in resolution. From Zhong (2015).*

not immobile and were hence called the intermediate population. The other proteins had high diffusion rates and were part of the fast population. Lu et al. (2014) showed that in the double-transfected neurons, the fast population of CaMKIIα was significantly reduced, while the intermediate population was significantly increased. This observation indicates that the ability of the CaMKII enzyme to bind to actin makes it more inclined to diffuse at intermediate speeds. The decrease in the diffusion of CaMKII via binding to the actin cytoskeleton implies that actin could potentially influence the localisation of CaMKII inside spines.

Lu et al. (2014) suggest that the actin cytoskeleton, which is highly branched and very dense in spines, can serve as a sieve for CaMKII (Korobova & Svitkina, 2010; Lu et al., 2014). Interactions with other proteins that bind to the actin cytoskeleton could add to this function of actin. These interactions of CaMKII with actin makes the enzyme immobile for a only short period of time, because the binding between CaMKII and actin is relatively weak (Khan et al., 2016). CAMKII can therefore move freely between interactions with actin filaments. The diffusion of CaMKII is the average of the (actin-)bound and free state, which means that the enzyme is neither immobile nor fast diffusing when it is in close proximity to a dense actin network (Figure 3). This is observed as the intermediate population and can also be described as the confined population (Chazeau et al., 2014). The immobile proteins, however, are held at one spot via strong interactions with rigid structures. The most important difference between the groups is that the immobile proteins are localised in a very small confinement zone compared to the intermediate proteins. In another study it was investigated whether the lateral diffusion of the membrane protein *potassium chloride cotransporter 2* (KCC2) was influenced by actin (Chamma et al., 2013). KCC2 is involved in chloride homeostasis in neurons and binds to the actin cytoskeleton via its C-terminal domain and an actin-linker protein (H. Li et al., 2007; Watanabe & Fukuda, 2015). Chamma et al. (2013) showed that the lateral diffusion of KCC2 was comparable to a confined population in normal conditions, but shifted to a more free-diffusing population after actin depolymerisation (Chamma et al., 2013). In the article it is speculated that the actin cytoskeleton could support the clustering of KCC2 by confining these proteins in the same zone (Chamma et al., 2013). The last experiment supporting the meshing maze hypothesis was published in a recent article by Yamazaki et al. (2018). The results of this experiment indicate that drebrin, which binds actin, localises CaMKIIβ in the spine without making the protein immobile (Shirao, 1995; Yamazaki, Sasagawa, Yamamoto, Bito, & Shirao, 2018). Moreover, depolarising

the actin cytoskeleton and removing drebrin both increase the immobile fraction of CaMKIIβ (Yamazaki et al., 2018). These conclusions suggest that CaMKIIβ is confined but not immobilised in actin hotspots via interactions with drebrin. Whether the trapping of proteins by the actin cytoskeleton is functionally relevant has not been investigated conclusively, although some of the experiments imply a function. It is therefore only possible to speculate on the meshing maze theory.

The meshing maze theory can be substantiated by evidence of the functions of CaMKII. For example, it is known that CaMKII is important in the execution of activity-induced LTP (Coultrap & Bayer, 2012; Kandel et al., 2013; Lisman et al., 2012). In this process, CaMKII is activated as a result of excessive calcium influx through NMDA receptors after multiple synaptic transmissions in a short period of time (Coultrap & Bayer, 2012; Kandel et al., 2013). Calcium binds to calmodulin in the spine, after which this complex can bind to and activate CaMKII (Coultrap & Bayer, 2012; Giese et al., 1998; Kandel et al., 2013). This complex binds only briefly, but longer activation of CaMKII is possible by reaching a certain calcium concentration (Kandel et al., 2013). This process facilitates autophosphorylation of CaMKII (Giese et al., 1998; Kandel et al., 2013). Actin trapping CaMKII increases the chance of an interaction between multiple CaMKII proteins, which may facilitate autophosphorylation. However, in case CaMKII is non-phosphorylated, its binding to actin will result in less autophosphorylation (Khan et al., 2011). Furthermore, the actin cytoskeleton could aid CaMKII in the execution of its functions in several ways. For instance, CaMKII could be trapped just under the PSD, where the actin cytoskeleton is relatively extensive, in order to execute its role in AMPA receptor trafficking more efficiently (Chazeau et al., 2014; MacGillavry & Hoogenraad, 2015; Poncer, Esteban, & Malinow, 2002). Moreover, some substrates of CaMKII are further away from the PSD, which could indicate that CaMKII needs to be specifically localised near these substrates (Colbran, 2004). An interaction between CaMKII and its substrate is more likely if the enzyme is in close proximity to its substrate for a relatively long period of time. The actin cytoskeleton could therefore, via its potential function as a meshing maze, slow down and localise CaMKII near its substrate (Lu et al., 2014). These examples illustrate the usefulness of actin's ability to serve as a meshing maze in dendritic spines.

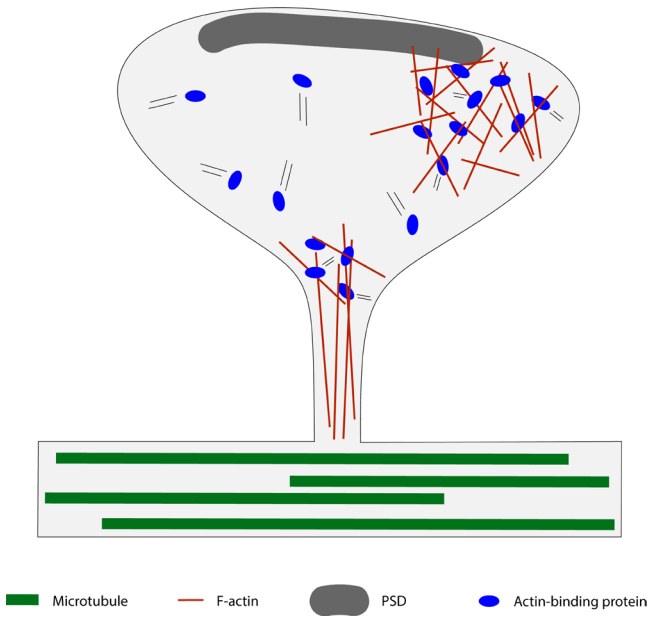


Figure 3 | Schematic illustration of the meshing maze hypothesis. *The diffusion of an actin-binding protein inside a dendritic spine is schematically displayed. Microtubules in the dendritic shaft, actin in the neck of the spine and the spine head, the post-synaptic density (PSD) and an actin-binding protein are portrayed in a hypothetical situation. The spine has in this case a mushroom shape, indicating that it is mature. Diffusion rates of the proteins are illustrated by relative differences in the size of their trails. Differences in the diffusion rates of the proteins that are either distant (fast diffusing population) or close (intermediate diffusing population) to the actin cytoskeleton, result in a situation where a bigger proportion of the proteins are inside the dense network of actin. Microtubules: green, actin: red, PSD: grey, the actin-binding protein: blue.*

DISCUSSION

This review summarised literature that suggests that the effects of the use of hormonal contraceptives, especially OCs, or oestrogen therapy on depressive symptoms vary throughout a woman's life span as well as within specific life phases. It appears that several factors influence the variability of responses to external oestrogen administrations and these factors will be discussed in the next sections.

The actin cytoskeleton has many functions in dendritic spines. A novel function of actin was suggested by experiments done by Lu et al. (2014), which indicated a role of the actin cytoskeleton in influencing the diffusion rates of CaMKII. By decreasing the diffusion of CaMKII, the actin cytoskeleton could hold the protein in a confinement zone as if trapped in a maze. Such localisation could ultimately influence the performance of CaMKII in executing its functions. Other studies imply a similar role of actin for KCC2 and drebrin. These experiments serve as a basis for the meshing maze theory and might be applicable for other (synaptic) proteins as well.

There is some evidence of the novel function of the actin cytoskeleton in dendritic spines as a meshing maze. Future research should focus on testing the hypothesis and to further uncover the functionality of the actin cytoskeleton in synaptic plasticity. An obvious aim of further research is to assess whether the performance of synaptic proteins such as CaMKII can be influenced through localisation by actin. Moreover, the potential meshing maze effect of the actin cytoskeleton should be confirmed by investigating endogenous CaMKII as well as other endogenous synaptic proteins. Tracking endogenous proteins is now possible using the recently developed *homology-independent targeted insertion* (HITI) approach, which can in principle be used for synaptic proteins (Suzuki et al., 2016). This method uses the CRISPR-Cas9 system to tag endogenous proteins with fluorescent markers (Suzuki et al., 2016). Multiple endogenous synaptic proteins should be investigated with experiments similar to experiments done by Lu et al. (2014), in order to confirm their confinement by actin. Moreover, it should be tested whether the functional output of these proteins is influenced by actin depolarisation. Furthermore, a meshing effect of actin could also be confirmed in a mimicking experiment, in which a non actin-binding synaptic protein should be able to bind to actin artificially resulting in intermediate diffusion rates. This would show if and how both localisation and function of that protein could be altered through transient binding with actin. After confirmation of the theory, it should be considered whether this function of actin plays a role in the pathology of mental disorders. A subtle change in the actin cytoskeleton hotspots could have drastic effects on the functional localisation of proteins that bind actin transiently or stably, ultimately leading to mental disorders (Yan, Kim, Datta, Lewis, & Soderling, 2016).

On the other hand, possible objections to the meshing maze hypothesis need to be considered. For example, it could be that the meshing maze effect is a by-product of the structure the actin cytoskeleton has in spines (Figure 1). This would mean that the results show localisation of a protein as a consequence of the dense actin network in that area, but it does not have any effect on the functions of that protein. In other words, it would be a coincidence that the protein colocalises with actin hotspots. Furthermore, critics could generalise the ability of the actin cytoskeleton to functionally localise proteins, combining the immobilisation and meshing functions. However, confining a protein in a rigid structure will influence the function of that protein differently than when it is still able to diffuse. Refuting these objections and executing the previously described experiments for multiple proteins, can lead to substantial evidence for the meshing maze hypothesis.

Lastly, the actin cytoskeleton does not only have functions in dendritic spines. It is therefore interesting to investigate whether actin might serve as a meshing maze in other parts of the neuron, such as the pre-synapse. The meshing maze hypothesis would be strongly favoured if similar experiments on boutons of axons would have corresponding results to those performed in spines. Moreover, the regulatory mechanisms behind the heternogeneous and dynamic structure of the actin cytoskeleton still needs to be further unravelled. To create such a network of the actin cytoskeleton the nucleation, elongation, capping, branching and bundling of actin need to be applied in different combinations, both spatially and temporally (Chazeau et al., 2014; MacGillavry & Hoogenraad, 2015; Spence & Soderling, 2015). The execution and regulation of these processes can orchestrate the actin network, which affects the location of potential meshing mazes. These regulatory processes should be targeted with therapies for mental disorders in which actin is implicated. In summary, the factors that govern the timing and location of the processes that regulate actin can ultimately determine the functional localisation of proteins by the actin cytoskeleton, according to the theory as if they are meshed in the actin maze.

CONFLICTS OF INTEREST

No potential conflict of interest was reported by the authors.

REFERENCES

Chamma, I., Heubl, M., Chevy, Q., Renner, M., Moutkine, I., Eugene, E., ... Levi, S. (2013). Activity-Dependent Regulation of the K/Cl Transporter KCC2 Membrane Diffusion, Clustering, and Function in Hippocampal Neurons. Journal of Neuroscience, 33(39), 15488–15503. http://doi.org/10.1523/JNEUROSCI.5889-12.2013

Chazeau, A., Mehidi, A., Nair, D., Gautier, J. J., Leduc, C., Chamma, I., ... Giannone, G. (2014). Nanoscale segregation of actin nucleation and elongation factors determines dendritic spine protrusion. The EMBO Journal, 33(23), 2745–64. http://doi.org/10.15252/embj.201488837

Cingolani, L. A., & Goda, Y. (2008). Actin in action: the interplay between the actin cytoskeleton and synaptic efficacy. Nature Reviews Neuroscience, 9(5), 344–356. http://doi.org/10.1038/nrn2373

Colbran, R. J. (2004). Targeting of calcium/calmodulin-dependent protein kinase II. The Biochemical Journal, 378(Pt 1), 1–16. http://doi.org/10.1042/BJ20031547

Colgan, L. A., & Yasuda, R. (2014). Plasticity of Dendritic Spines: Subcompartmentalization of Signaling. Annual Review of Physiology, 76(1), 365–385. http://doi.org/10.1146/annurev-physiol-021113-170400

Coultrap, S. J., & Bayer, K. U. (2012). CaMKII regulation in information processing and storage. Trends in Neurosciences, 35(10), 607–618. http://doi.org/10.1016/j.tins.2012.05.003

Giese, K. P., Fedorov, N. B., Filipkowski, R. K., & Silva, A. J. (1998). Autophosphorylation at Thr286 of the alpha calcium-calmodulin kinase II in LTP and learning. Science (New York, N.Y.), 279(5352), 870–3. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9452388

Hanley, J. G. (2014). Actin-dependent mechanisms in AMPA receptor trafficking. Frontiers in Cellular Neuroscience, 8, 381. http://doi.org/10.3389/fncel.2014.00381

Huang, F., Chotiner, J. K., & Steward, O. (2007). Actin Polymerization and ERK Phosphorylation Are Required for Arc/Arg3.1 mRNA Targeting to Activated Synaptic Sites on Dendrites. Journal of Neuroscience, 27(34), 9054–9067. http://doi.org/10.1523/JNEUROSCI.2410-07.2007

Kandel, E. R., Schwartz, J. H., Jessell, T. M., Siegelbaum, S. A., & Hudspeth, A. J. (2013). Principles of neural science. Harvard University Press. Retrieved from https://books.google.nl/books/about/Principles_of_Neural_Science_Fifth_Editi.html?id=s64z-LdAIsEC&redir_esc=y

Khan, S., Conte, I., Carter, T., Bayer, K. U., & Molloy, J. E. (2016). Multiple CaMKII Binding Modes to the Actin Cytoskeleton Revealed by Single-Molecule Imaging. Biophysical Journal, 111(2), 395–408. http://doi.org/10.1016/j.bpj.2016.06.007

Khan, S., Reese, T. S., Rajpoot, N., & Shabbir, A. (2012). Spatiotemporal maps of CaMKII in dendritic spines. Journal of Computational Neuroscience, 33(1), 123–139. http://doi.org/10.1007/s10827-011-0377-1

Khan, S., Zou, Y., Amjad, A., Gardezi, A., Smith, C. L., Winters, C., & Reese, T. S. (2011). Seques-

tration of CaMKII in dendritic spines in silico. Journal of Computational Neuroscience, 31(3), 581–594. http://doi.org/10.1007/s10827-011-0323-2
Korobova, F., & Svitkina, T. (2010). Molecular Architecture of Synaptic Actin Cytoskeleton in Hippocampal Neurons Reveals a Mechanism of Dendritic Spine Morphogenesis. Molecular Biology of the Cell, 21(1), 165–176. http://doi.org/10.1091/mbc.E09-07-0596
Li, H., Khirug, S., Cai, C., Ludwig, A., Blaesse, P., Kolikova, J., ... Rivera, C. (2007). KCC2 interacts with the dendritic cytoskeleton to promote spine development. Neuron, 56(6), 1019–33. http://doi.org/10.1016/j.neuron.2007.10.039
Li, T. P., Song, Y., MacGillavry, H. D., Blanpied, T. A., & Raghavachari, S. (2016). Protein Crowding within the Postsynaptic Density Can Impede the Escape of Membrane Proteins. Journal of Neuroscience, 36(15), 4276–4295. http://doi.org/10.1523/JNEUROSCI.3154-15.2016
Ligon, L. A., & Steward, O. (2000). Role of microtubules and actin filaments in the movement of mitochondria in the axons and dendrites of cultured hippocampal neurons. The Journal of Comparative Neurology, 427(3), 351–61. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11054698
Lisman, J., Yasuda, R., & Raghavachari, S. (2012). Mechanisms of CaMKII action in long-term potentiation. Nature Reviews. Neuroscience, 13(3), 169–82. http://doi.org/10.1038/nrn3192
Lu, H. E., MacGillavry, H. D., Frost, N. A., & Blanpied, T. A. (2014). Multiple Spatial and Kinetic Subpopulations of CaMKII in Spines and Dendrites as Resolved by Single-Molecule Tracking PALM. Journal of Neuroscience, 34(22), 7600–7610. http://doi.org/10.1523/JNEUROSCI.4364-13.2014
MacGillavry, H. D., & Hoogenraad, C. C. (2015). The internal architecture of dendritic spines revealed by super-resolution imaging: What did we learn so far? Experimental Cell Research, 335(2), 180–186. http://doi.org/10.1016/j.yexcr.2015.02.024
Manley, S., Gillette, J. M., & Lippincott-Schwartz, J. (2010). Single-particle tracking photoactivated localization microscopy for mapping single-molecule dynamics. Methods in Enzymology, 475, 109–20. http://doi.org/10.1016/S0076-6879(10)75005-9
McKinney, S. A., Murphy, C. S., Hazelwood, K. L., Davidson, M. W., & Looger, L. L. (2009). A bright and photostable photoconvertible fluorescent protein. Nature Methods, 6(2), 131–133. http://doi.org/10.1038/nmeth.1296
Poncer, J. C., Esteban, J. A., & Malinow, R. (2002). Multiple mechanisms for the potentiation of AMPA receptor-mediated transmission by alpha-Ca2+/calmodulin-dependent protein kinase II. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 22(11), 4406–11. http://doi.org/20026449
Shirao, T. (1995). The roles of microfilament-associated proteins, drebrins, in brain morphogenesis: a review. Journal of Biochemistry, 117(2), 231–6. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7608104
Spence, E. F., & Soderling, S. H. (2015). Actin Out: Regulation of the Synaptic Cytoskeleton. Journal of Biological Chemistry, 290(48), 28613–28622. http://doi.org/10.1074/jbc.R115.655118
Suzuki, K., Tsunekawa, Y., Hernandez-Benitez, R., Wu, J., Zhu, J., Kim, E. J., ... Belmonte, J. C. I. (2016). In vivo genome editing via CRISPR/Cas9 mediated homology-independent targeted integration. Nature, 540(7631), 144–149. http://doi.org/10.1038/nature20565
Tatavarty, V., Kim, E.-J., Rodionov, V., & Yu, J. (2009). Investigating Sub-Spine Actin Dynamics in Rat Hippocampal Neurons with Super-Resolution Optical Imaging. PLoS ONE, 4(11), e7724. http://doi.org/10.1371/journal.pone.0007724
Urban, N. T., Willig, K. I., Hell, S. W., & Nägerl, U. V. (2011). STED nanoscopy of actin dynamics in synapses deep inside living brain slices. Biophysical Journal, 101(5), 1277–84. http://doi.org/10.1016/j.bpj.2011.07.027
Wang, L., Dumoulin, A., Renner, M., Triller, A., & Specht, C. G. (2016). The Role of Synaptopodin in Membrane Protein Diffusion in the Dendritic Spine Neck. PLOS ONE, 11(2), e0148310. http://doi.org/10.1371/journal.pone.0148310
Watanabe, M., & Fukuda, A. (2015). Development and regulation of chloride homeostasis in the central nervous system. Frontiers in Cellular Neuroscience, 9, 371. http://doi.org/10.3389/fncel.2015.00371
Yamazaki, H., Sasagawa, Y., Yamamoto, H., Bito, H., & Shirao, T. (2018). CaMKIIβ is localized in dendritic spines as both drebrin-dependent and drebrin-independent pools. Journal of Neurochemistry, 146(2), 145–159. http://doi.org/10.1111/jnc.14449
Yan, Z., Kim, E., Datta, D., Lewis, D. A., & Soderling, S. H. (2016). Synaptic Actin Dysregulation, a Convergent Mechanism of Mental Disorders? The Journal of Neuroscience, 36(45), 11411–11417. http://doi.org/10.1523/JNEUROSCI.2360-16.2016

Scientific finetuning of the truth by Bayesian integration of theoretical models

Böing, S.[1]

[1] Multisensory Space Lab, Dept. of Experimental Psychology, Helmholtz Institute, Utrecht University, Utrecht, the Netherlands

Acquiring knowledge is the aim of science, and is highly esteemed in our society. Philosophers of science introduced a variety of perspectives to look at the way in which science should be carried out and defined 'scientific growth' accordingly. This thesis describes and compares the most influential philosophies of science. It further proposes a statistical inference framework that serves as a foundation for decision making, the Bayesian framework, to optimally integrate existing scientific theories to finetune our knowledge. This Bayesian framework is increasingly applied to multisensory spatial research. The main point of the Bayesian framework is that it takes the reliability of unisensory estimates into account to arrive at optimal multisensory localisation. The more reliable the sense, the more weight it is attributed in the final estimate of the multisensory percept. The same principles can be applied to the philosophy of science as well: different theories can produce outcomes that are more or less probable, rendering the theory more or less reliable. Just as with the Bayesian optimal integration in localisation, the weighted average 'belief' in a specific theory will be shifted towards the more reliable one. Eventually, either one of both theories can become reliable enough to dominate the other theory, or an integrated model would develop around the weighted average.

PHILOSOPHY OF SCIENCE: AN INTRODUCTION

Philosophy, derived from the Greek word φιλοσοφία meaning 'love of wisdom', studies a wide variety of topics ranging from existence to beauty, mind and knowledge. Acquiring knowledge is the aim of science, and is highly esteemed in our society (CBS, 2018; Chalmers, 1999). It is not always clear what exactly we consider good science and how science should be performed. Philosophers of science introduced a variety of perspectives to look at the way in which science should be carried out. Methodology and reasoning approaches were topic of discussion and accordingly, various definitions of 'growth of science' were formulated.

To perform science one should be aware of the different interpretations of 'good practice'. To ensure that good practice is performed in line with a standard interpretation, universities in the Netherlands comply with a Code of Conduct (VSNU, 2012). Historically speaking, the definition of 'good practice' differs across the school of thoughts introduced by various philosophers of science. For example, as described in later paragraphs, Thomas Kuhn favoured a standard research paradigm, whereas Paul Feyerabend argued that science is better performed when there is no golden method for conducting research. Unfortunately, nowadays both students and education programs do not seem to appreciate the value of history of science until the Master's degree or even later. As Christoph Lüthy (2007), dean and professor of the Faculty of Philosophy, Theology and Religious Studies at Radboud University Nijmegen, stated in his inaugural lecture, practitioners of the history of science have become an endangered species in the Netherlands.

Nevertheless, history of science is still regarded as a field of great importance. Contemporary practice of models, theories and paradigms are directly and indirectly derived from models, theories and paradigms from the past (Chalmers, 1999; Lüthy, 2007). To understand the nature of equations, models and theories, one must dive into history and link contemporary practice to earlier findings. This is the way to gain insight into the development of (neuro)science and to understand the principles of significance and strengths and weaknesses, resulting in critical thinking and methodological precision, which are, at least in my opinion, definitely part of 'good practice'.

There are several key points that contribute to practicing good science. To start off, it is important to understand the principles of induction and deduction and their strengths and weaknesses. In induction, one makes claims about general laws by the observation of a sequence of single facts that comply with one another, whereas in deduction the formation of general laws and principles lead to conclusions and predictions about individual events (Chalmers, 1999; Frank, 1957). According to physicist, mathematician and philosopher Philipp Frank (1957, p. 301) "the task of science has been to infer from the observational material the general

principles that are made of symbols and connected by logical operations". Both induction and deduction are involved in scientific reasoning. If laws and theories are established by observation and induction, the statements and predictions about single events can be made using deductive arguments (Chalmers, 1999). Theories established by induction are, however, subject to vagueness and inadequacy (Chalmers, 1999). Karl Popper, seen as one of the most influential philosophers of the twentieth century, discarded inductive theories as being too flexible as they could account for any instance that was observed. Furthermore, the theories could not rule out anything, and so, were not informative (as cited in Popper, 2005; Chalmers, 1999).

From Popper's point of view, hypotheses needed to be falsifiable, that is, they should be specific enough to be eliminated when a non-compatible observation is done in experimental tests. A new speculative conjecture then follows. The main point of Popper's view is that only the theories that are tested and can withstand falsification survive. It does not mean, however, that the surviving theories are to be seen as the truth or facts. They are simply the best theories currently available. For theories to be falsifiable there is need for existence of an observable event and an accompanying falsifying statement. If this contradicting event is observed, the initial theory is falsified by the falsifying hypothesis. A famous example is the initial generalisation "All swans are white". This theory is falsified by falsifying statement "Not all swans are white" and the observation "At place x at time t, two black swans swam in the river" (See Figure 1 for schematic view). An example of a non-falsifiable theory would be the existence of gnomes, since they are only visible when you do not look at them. To arrive at the point of falsification, a theory needs to make definite falsifiable claims. This means that the more specific statements a theory includes, the more it is falsifiable, and the more it is informative. Thus, according to Popper, scientific growth is achieved by trial and error, because eliminating falsified hypotheses can bring us closer to the truth. This "asymptotic approach to the truth" is the essence of science ("The logic of", 2012). The modified hypothesis needs to be more falsifiable than the one before. If the modified hypothesis resists falsification it contributes to knowledge. Especially when novel predictions derived from wild guesses are confirmed, there is progression in the eyes of the falsificationists. After all, wild guesses that are confirmed, lead to establishment of a completely new theory. This is seen as a big leap forward towards the currently best available theory.

Thomas Kuhn challenged the falsificationist approach by emphasising the revolutionary character of scientific progress (Chalmers, 1999; Kuhn, 1970). This perspective approaches science by looking at the theoretical framework in which research is conducted. Kuhn believed that growth of science is established by replacing existing theoretical structures by other incompatible ones, a process that is captured by the Kuhnian cycle (Figure 2): Initially an immature science exists before becoming structured and stable. Normal science is established once a standard paradigm is used in which research in that field is performed. This paradigm consists of a general agreement on laws, principles and methods used in the field. However, inevitably, researchers will encounter observations that do not fit in the ruling paradigm: an anomaly (or falsification) is detected which, if evidence cumulates, can lead to a crisis. Researchers will react to this crisis by replacing the old paradigm with a new one, thereby causing a so-called scientific revolution, since paradigms determine how scientists explain the world. The new paradigm will be adopted by the scientific community if it appears to be successful. Former fundamental beliefs are replaced (Chalmers, 1999; Gholson & Barker, 1985; Kuhn, 1970). Kuhn argued that science progresses in a step change manner, and not gradually. In Kuhn's view, this revolution is essential for the growth of science.

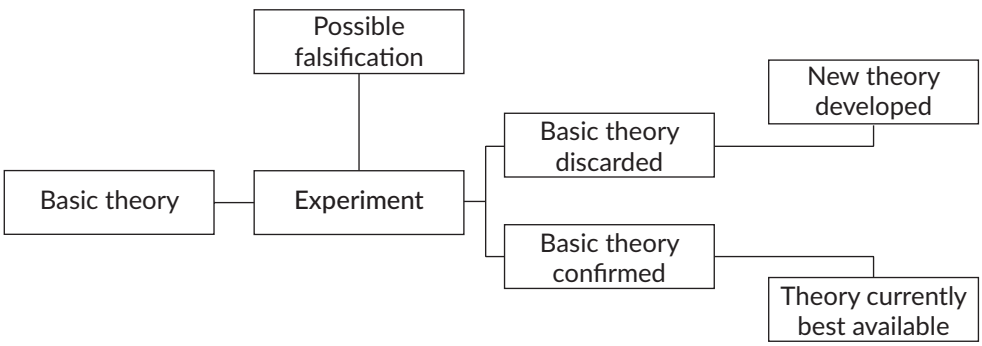


Figure 1 | Popper's model of falsificationism. *A basic, falsifiable theory is stated. This theory should be falsifiable by the observation of a falsifying claim. An experiment will reveal which theory is best in predicting the outcome. Once a falsifying observation is made, the initial theory is falsified. The initial theory is discarded and replaced by a new, more informative theory. If the falsifying hypothesis is not confirmed, the initial theory withstood falsification and is regarded as currently the best available.*

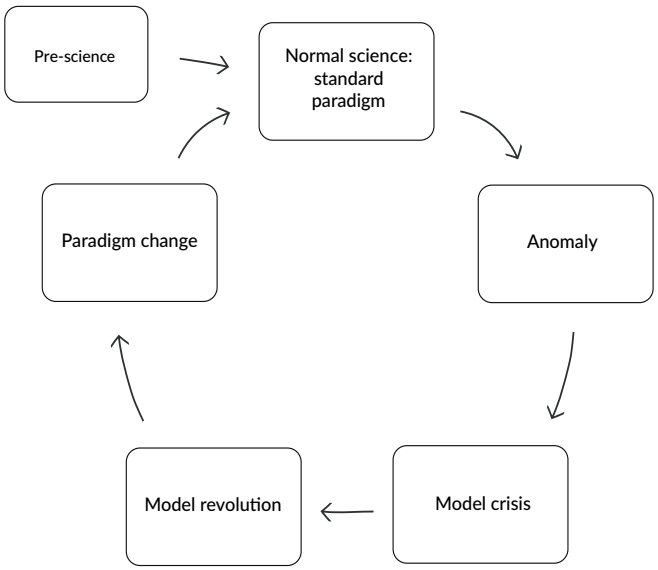


Figure 2 | The Kuhnian cycle. *Before becoming structured and stable, an immature pre-science exists. Once a standard paradigm is used to perform research, normal science is established. Even within this standard paradigm, anomalies can be encountered that are not compatible with normal science beliefs. If the observation of anomalies is increasingly encountered, this can lead to a crisis in the research field. In the model revolution, the old paradigm will be replaced by another paradigm that can account for the observed anomalies. The new paradigm becomes the new standard paradigm if it appears to be successful.*

Imre Lakatos, philosopher of mathematics and science, pilloried Kuhn's theory as being historic-sociological. Furthermore, he discarded Popper's falsificationism. He claimed that falsificationism could not explain exactly what factors contributed to the falsification of a theory and that falsificationism denies that certain empirical observations can be transmitted to theories by induction (Lakatos, 1970). Lakatos suggested that at least some part of the theory stayed quite continuous along the way and was not to be blamed for the falsification. A series of core assumptions remained intact (Gholson & Barker, 1985). Lakatos refers to this part as the hard core, or the fundamental principles, of a research program. The protective belt surrounding the hard core consists of supplementary laws and principles and protects the hard core from being falsified (see Figure 3). To guide research towards discovery, Lakatos proposed positive and negative heuristics, implying what scientists should or should not do within a research program. With this, Lakatos directly differs from falsificationism in that he regards verifications as keeping the program going (Lakatos, 1970, in Walker, 2018, p.437). With regards to the definition of 'growth of science', in Lakatos' view science advances when a research program becomes more productive and useful than another, leading to a 'progressive problem shift' (Chalmers, 1999; Lakatos, 1970). As such, Lakatos presumed that multiple research programs could exist simultaneously, whereas the Kuhnian idea was that a discipline in science could only contain one paradigm at a specific moment. Their view on 'growth of science' differed in the sense that Lakatos considered newly developing theories that were relatively progressive as growth, whereas Kuhn pleaded an 'all-or-nothing' approach (Gholson & Barker, 1985).

Paul Feyerabend presented an anarchistic account of science in which creativity thrives (Feyerabend, 1970; Feyerabend, 1993). He stated that science is not superior to other forms of knowledge, since there is no golden method for conducting scientific research. He noted that the formerly described movements (as introduced by Popper, Kuhn and Lakatos) built on the development of science over history. Feyerabend challenged the movements exactly on the ground of history: the schools of thought could not explain the course of events in Galileo's contribution to science. Galileo's claim ('the earth is not stationary) was, after all, not compatible with observations ('a stone falls down at the base of the tower and not some distance away') and were built on a methodology (the telescope) that was not straightforward (Chalmers, 1999). Nevertheless, his theory was a huge contribution to scientific progress. Feyerabend, therefore, claims that a liberal practice in which the scientist is not bound by specific rules or even breaks them is 'absolutely necessary for the growth of science' (Feyerabend, 1993). Feyerabend, thus, advocates scientific freedom with no scientific rules. As Feyerabend (1993, p. 39) states himself: "It will become clear that there is only one principle that can be defended under all circumstances and in all stages of human development. It is the principle: anything goes".

To sum up, Popper, Kuhn, Lakatos and Feyerabend all introduced their own philosophy of science. Whilst there may be some general agreement on how 'good practice' can be achieved (e.g. in a code of conduct), there is still no general agreement on the perspective one should adopt to arrive at 'growth of science'. In the next section, another perspective will be proposed. This perspective advocates the optimal integration of existing scientific theories to finetune our knowledge. Still, regardless of which perspective will appeal most to you, let it be clear that science is not self-evident, as evidence and the gathering of evidence are already a matter of debate.

MY PHILOSOPHY OF SCIENCE

Interacting research fields

Even though the brain is regarded as the seat of cognitive functions for a long time already, both neuroscience and experimental psychology are relatively young fields of research (Finger, 1994; Mandler, 2007). Neuroscience

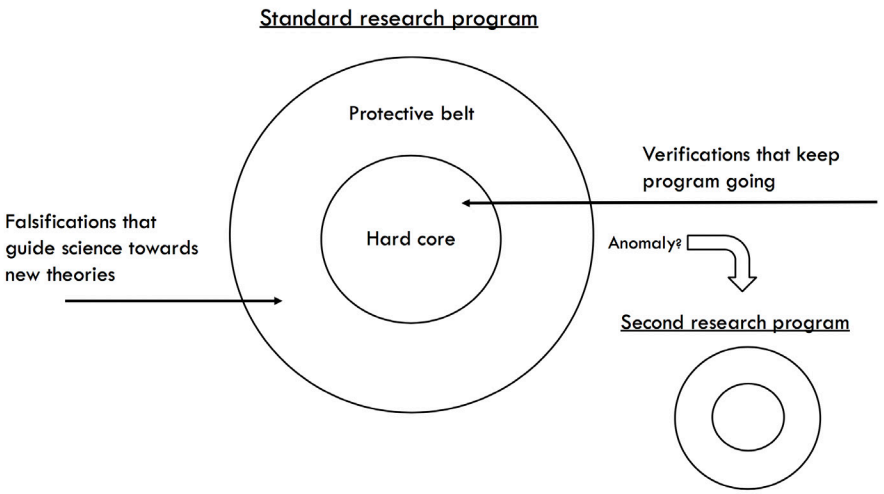


Figure 3 | Lakatos' research programs.
Fundamental principles in the hard core are protected by assumptions in the protective belt. Verifications of the hard core theories keep the program going and falsifications of protective belt assumptions facilitate development of new, more finetuned assumptions. An encounter with an anomaly that is not compatible with the hard core can pave the way for a second research program. Science progresses as one program becomes more productive than another.

and experimental psychology interact continuously, and the principles found in one can be linked to principles in the other (Bickle, 2003; Kalat, 1980). From this statement, I derived my philosophy of science.

In my philosophy of science, two or more distinct research fields can run in harmony. As in neuroscience and experimental psychology, the research fields produce scientific results that are compatible with one another. In my philosophy of science, the research fields investigate the same hard core assumptions using their respective paradigms, thereby opposing Kuhn's view on the existence of a single ruling paradigm.

In neuroscience and cognition this harmony is found in the study of multisensory integration. On a neuronal level, the principle of multisensory integration is reflected by 'a statistically significant difference between the number of impulses evoked by a cross-modal combination of stimuli and the number evoked by the most effective of these stimuli individually' (Stein & Meredith, 1993; Stein & Stanford, 2008). On a behavioural level, multisensory integration is reflected by, e.g., shorter saccadic reaction times to multisensory stimuli (sound and light) as compared to reaction times in unisensory conditions (sound or light only; Hughes, Reuter-Lorenz, Nozawa & Fendrich, 1994; Stein & Meredith, 1993). Both fields work on the same hard core assumption, but use another perspective, and so another methodology. The take-home message here is that it is possible that two research fields, and thus paradigms, contribute to knowledge about the same hard core assumptions and protective belt assumptions. In fact, when neuronal observations are compatible with behavioural results and vice versa, we can speak of a growth spurt: hard core assumptions are verified in not one, but two research fields, and protective belt statements can be specified even further until they become hard core assumptions. The interdisciplinary interplay between verifications and falsifications of those assumptions directs us to a more finetuned truth.

Development of hard cores

A research program evolves based on experience, outcomes and paradigm changes. Current programs are shaped by earlier falsifications, historical context and paradigm revolutions (Figure 4, 1). In this way, hard core 1 (HC1) and protective belt 1 (PB1) are established (2). This HC1 yields a corresponding methodology for experiments. Therefore, HC1 has a direct effect on (interdisciplinary) data collection (3). The collected data might falsify or verify PB assumptions (4). Through experimentation and data collection, research program HC1 is finetuned over time.

However, data collection can yield results that are not directly compatible with assumptions in HC1 (5). As a result, data collection does not feed to HC1, but instead redirects to a funnel of falsifications, historical context and paradigm revolutions (1). A new, slightly adapted research program with hard core 2 (HC2) develops (6). The same cycle will start for research program HC2. Note that HC1 is not fully discarded, since earlier verifications of HC1 assumptions and falsifications of PB1 assumptions are still informative. At this point, multiple hard cores are present, but they can contain slightly incompatible series of theories. The question is which research program is more reliable.

Bayes and optimal integration

According to Lakatos, science progresses as one program gets more productive and becomes a better predictor of novel phenomena (Chalmers, 1999). To judge which program is most successful, the predictive value of the

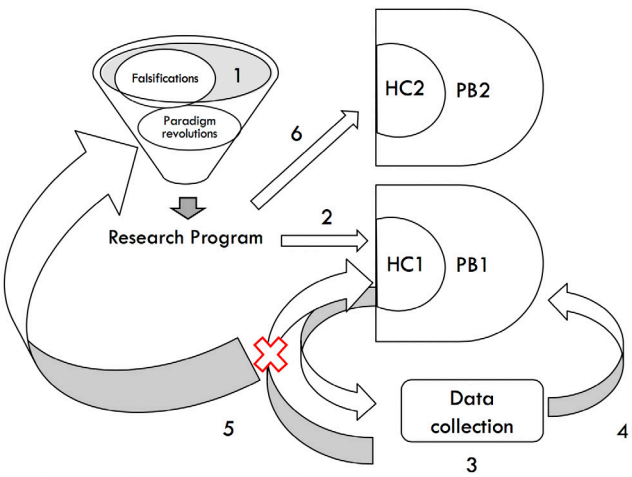


Figure 4 | My philosophy of science: Research program development. *(1) A research program is established by earlier findings and paradigm revolutions. (2) Hard core 1 and protective belt are established. (3) Data collection occurs according to a corresponding methodology of doing research. (4) Observations are made that verify hard core assumptions or lead to finetuning of protective belt assumptions. (5) Data collection can also yield results that are not compatible with hard core 1 assumptions. (1 + 6) A new, slightly adapted research program develops. The same cycle will start over.*

program and the reliability of such a prediction should be taken into account. At this point, I would like to introduce the Bayesian framework. This is a statistical inference framework that serves as a foundation for decision making (Bernardo & Smith, 2009; Howson & Urbach, 2006; Oaksford & Chater, 2007). In statistics, measurement uncertainty (error) receives special attention. By doing observations, a vast amount of values is obtained. From those values a distribution with a mean, median and variance can be derived. A small distribution width means that observed values are close to one another, while a broader distribution displays larger variability. This variability in observations reflects uncertainty about the estimated underlying distribution. To generate 'rational' choices, we must take into account this uncertainty. This uncertainty can vary across situations and decisions (Bernardo & Smith, 2009; Lee, 2012).

The application of Bayesian models of integration is increasing in multisensory research (Battaglia, Jacobs & Aslin, 2003; Ernst, 2006). To illustrate the function of the Bayesian theorem, I will introduce the topic of my major research project: multisensory spatial perception. In spatial perception, the visual system processes input directly in a high-resolution retinotopic representation (at least around the fovea; Grill-Spector & Malach, 2004; Purves, Cabeza, Huettel, LaBar, Platt & Woldorf, 2013; Wolfe et al., 2015). The auditory system more indirectly determines the location of a sound from interaural differences in a head-centred reference frame (Blauert, 1997; Frens, Van Opstal & Van der Willigen, 1995; Mills, 1958). The visual system is thought to be the most accurate and precise sense in terms of localisation, whereas the auditory system is regarded as less accurate and less precise. Therefore, the visual system is seen as the most reliable sense in localisation. Still, both senses contribute to the multisensory percept. As the unisensory estimate of a spatial location gets more reliable, however, it would be optimal to have a preference for this sense and attribute a higher weight to it when combining input from different senses. The weighted average (corresponding to the reliability of the sensory estimates) gives rise to an average perceived position of the multisensory stimulus. This is called Bayesian optimal integration. Now let's assume that someone perceives a light somewhere at a given time, and at the same moment that person perceives a sound somewhere nearby, but not at the exact same location. According to the Bayesian framework, the most optimal estimate of the spatial location of the audiovisual event would be to attribute more weight to the visual input because of the reliability of the visual system. The perceived spatial location would then be shifted more towards the seen rather than towards the heard location (Alais & Burr, 2004; Battaglia et al., 2003). When you are watching a TV-show, for example, you will perceive the sound of the voices more to come from the moving lips you see, instead of coming from your speaker.

The principles described above can be applied to the philosophy of science. Let us consider the research programs as the senses: HC1 will represent the auditory system and HC2 will represent the visual system. Both research programs will yield more and more observations leading to two separate distributions indicating the evidence for HC1 or HC2 (Figure 5a). At a certain moment, outcomes generated by research program 1 will be more variable than those generated by research program 2. This difference arises when one theory is not able to predict behaviour (or outcomes in general) as consistently as the other due to different assumptions. The program with less variability will generate more probable and frequent observations. This way, research program 2 becomes more reliable as a research program. Just as with Bayesian optimal integration, the weighted average 'belief' in a specific research program will be shifted towards the more reliable program (Figure 5b). As testing continues, it is possible that this program will yield such reliable outcomes, that it dominates the old one: all the weight is attributed to this program. Of course, within this dominating research program, finetuning continues as well by testing assumptions in both the protective belt and the hard core. If falsifications of the hard core assumptions are found again, the same process starts over. The current program is not able to

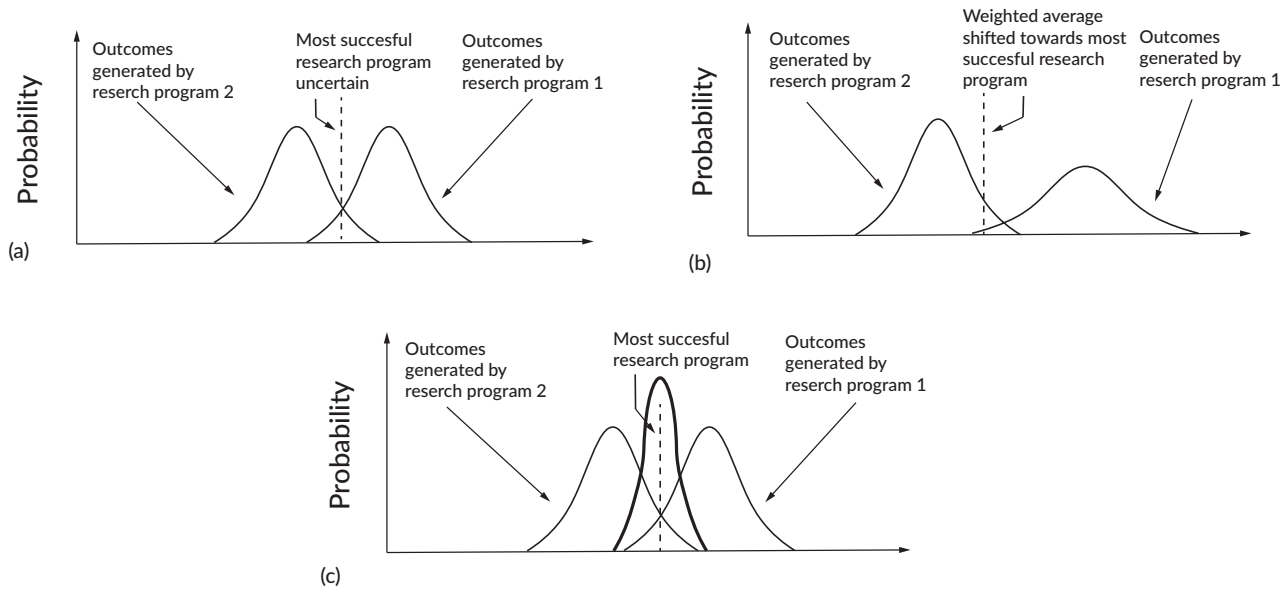


Figure 5 | Bayesian integration of research programs. *(a) Both research programs yield comparably probable results. The most successful research program is uncertain. (b) Outcomes generated by program 2 are more probable and frequently made. Program 2 is thus more reliable and successful. Therefore, the 'belief' in a program will be shifted towards this program (dotted line). (c) When two programs yield comparable results, a hybrid model would optimise results. A new model would develop around the weighted average. Adapted from "Bayesian integration of visual and auditory signals for spatial localization," by Battaglia, Jacobs & Aslin, 2003, Journal of the Optical Society of America A (JOSA A), 20(7), 1392. Copyright [2003] by Optical Society of America.*

predict outcomes consistently any more, so the need for a new research program will arise (as visualised in Figure 4).

Another possibility is that both research programs yield comparably probable results. To achieve highest reliability, a 'hybrid' theoretical model would optimise results, since it takes the best of both programs. Ideally, an integrated model would develop around the weighted average (Figure 5c).

MY PHILOSOPHY OF SCIENCE: APPLIED TO MY FIELD OF RESEARCH

Bayesian integration of theoretical models in multisensory spatial perception

In my current field of research, multisensory spatial perception, my philosophy of science shows as follows: as explained earlier, when a sound and a light do not come from the same position in space, the auditory and visual signal are in a so called conflict, just as research programs can be. Various models have been suggested to explain how the localisation judgement of an observer is established in case a conflict is present.

The first model goes by what is described as 'visual capture'. It proposes that the signal that is most reliable – in localisation: vision – dominates the observer's judgement in a winner-takes-it-all fashion. For example, a ventriloquist effect can occur when someone perceives the voice of the puppet player to come from the puppet itself (Bertelson & De Gelder, 2004; Pick, Warren & Hay, 1969; Welch & Warren, 1980). Based on the visual capture theory (HC1), researchers performed experiments that could either verify or falsify the hard core assumption that the visual system operated in a winner-takes-it-all manner. However, when collecting data, researchers stumbled upon observations that were not directly compatible with the hard core assumption of the visual capture model. Along the way, a new research program (HC2) would develop (as visualised in Figure 4).

This second research model is the maximum-likelihood estimation (MLE) model. This theory states that perceptual judgements are based on a combination of sensory information coming from multiple modalities. As mentioned before, a sense is regarded as more reliable when the variance of a sensory estimate is small, and less reliable when the observed variance of a sensory estimate is large. The MLE model proposes that, for example, spatial information of the conflicting modalities is averaged based on the independent reliabilities of the senses involved. Proportional to the reliabilities of each sensory estimate a weight is attributed to the available information (Ghahramani, 1995; Battaglia,

Jacobs & Aslin, 2003). Visual capture would then be a case wherein the visual system is assigned a value of 1 and the auditory system a value of 0.

Now, two models co-exist (Figure 4; Figure 6a). To evaluate which model could best predict observer's localisation judgements, Battaglia el al. (2003) investigated the extent to which observers use visual and auditory information when visual information is made less reliable by adding noise. If the principle of visual capture would best predict the outcomes, localisation would be solely based on visual information. If the MLE model would best predict outcomes, more weight would be attributed to the auditory system with increasing visual noise levels. Experiments were performed to test which assumption would hold. As it appeared, observations generated by model 2 (MLE; the auditory system has an influence as well) became more probable and frequent than those generated by model 1 (visual capture; the visual system dominates). Research program 2, therefore, became more reliable. The weighted average of 'belief' in one of the two models shifted towards research program 2 (Figure 6b). Nevertheless, research program 2 could not fully explain the outcomes. Indeed, in line with the MLE model, auditory input was used more when visual noise was added. However, according to Battaglia et al. (2003) the MLE model structurally underestimated the extent to which visual information was used, indicating that the data partly fit the visual capture model predictions as well. In sum, the researchers found that both models were partially correct.

Battaglia et al. (2003) resolved these results by applying a model that combined the two existing models. This new hybrid model would develop around the weighted average of program 1 and program 2, and would, thus, 'integrate' the two models in a Bayesian fashion. The optimal integration (Figure 6c, average, dotted line) of the two programs would yield the most reliable results. In this new model, the MLE model would be modified slightly by adding a visual bias. The older models would extinguish eventually when scientists adopt the new one: the new hybrid model would become so reliable that it would beat the visual capture and MLE model.

Zoom in: my research program

To zoom in a bit, I would like to focus on the research program I am involved in. In the field of multisensory perception, there is a general hard core assumption:

'Multisensory stimulation can enhance perception'

This assumption requires clarification. First, a definition of 'enhancement' is needed. Second, one needs to be cautious about the fact that perception entails a lot of aspects. Third, how do we measure perception? Do we use accuracy in signal detection, decreased neuronal spike thresholds or shorter reaction times as a reflection

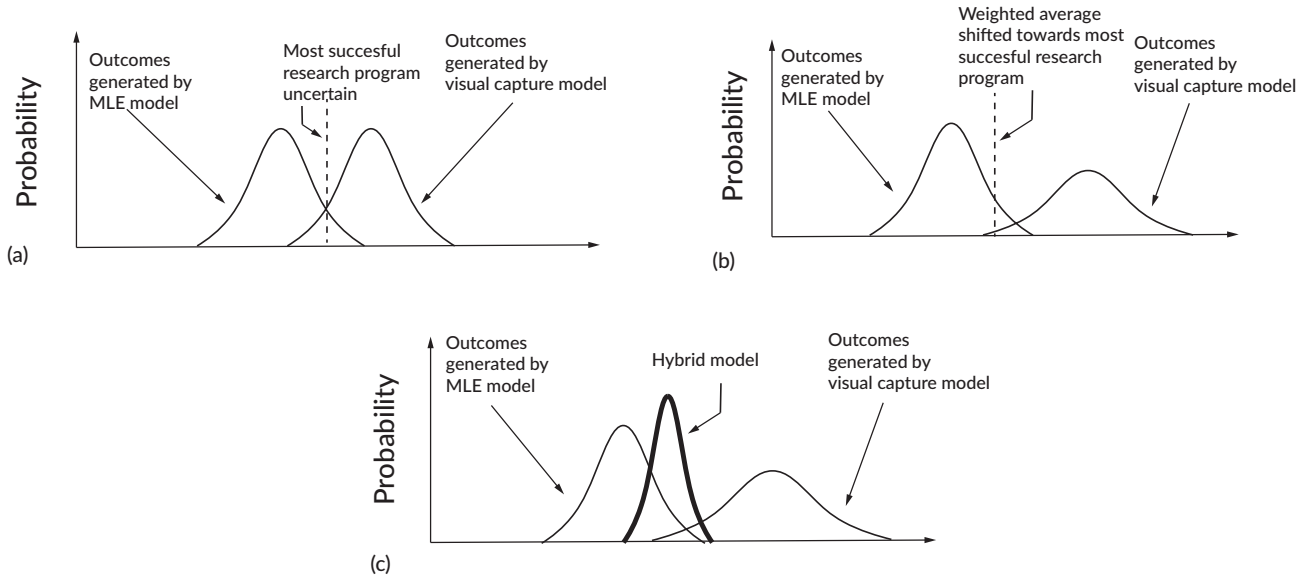


Figure 6 | Bayesian integration of localisation models. *(a) Two theories were proposed: the visual capture model and the maximum likelihood estimation (MLE) model. The most successful theory is unknown. (b) Observations were relatively more compatible with the MLE model. The MLE model was regarded as more reliable. The weighted average of 'belief' shifts towards the MLE model. (c) A hybrid model could account best for observations made. The reliability of both theories was taken into account and a new model was created around the weighted average. Adapted from "Bayesian integration of visual and auditory signals for spatial localization," by Battaglia et al., 2003, Journal of the Optical Society of America A (JOSA A), 20(7), 1392. Copyright [2003] by Optical Society of America.*

of enhanced perception? Last, under what circumstances can multisensory stimulation enhance perception? In this example, I will focus on those issues in multisensory spatial perception.

First of all, I would like to state that the assumption 'multisensory stimulation can enhance perception' is a core assumption in both neuroscience and experimental psychology research. Here we can see that multiple fields of research can run in harmony and can support a core theory on various levels. To test whether multisensory stimulation indeed enhances spatial perception, experiments are designed to compare outcomes generated by unisensory and multisensory stimuli. In the remainder of this article I will focus on the experimental psychology approach.

In the field of experimental psychology, 'enhancement' can be defined as faster and/or more accurate localisation. The methods to gather data are varied: detection tasks as well as localisation tasks are being used, and outcome values range from spatial accuracy to reaction time measures derived from both manual and saccadic responses (Aller, Giani, Conrad, Watanabe, & Noppeney, 2015; Frassinetti, Bolognini & Làdavas, 2002; Frens et al., 1995; Hughes et al., 1994). The use of the different tasks shows that there is no golden standard in method being used. However, it does show that there is a golden standard in operationalisation, since all tasks are designed to gather and compare outcomes generated by unisensory and multisensory stimulation. Assume that all those paradigms indeed find that multisensory stimulation can enhance perception. Now a question arises: how can this enhancement be explained?

Enhancement of perception could have multiple explanations. These hypotheses currently reside in the protective belt of the research program. The first hypothesis states that 'the senses integrate information' (H1). The principle of multisensory integration is that responses to multisensory stimuli overshadow the responses to unimodal input, yielding faster and more accurate localisation. So to say, the whole is more than the sum of its parts.

A second, alternative, hypothesis states that 'the senses do not integrate information, but just facilitate optimal results' (H0). This hypothesis claims that the senses work independently, and that multisensory enhancement arises due to statistical facilitation.

To investigate which hypothesis holds, the race model inequality violation test is used (Miller, 2016). The race model argues that the chance of winning a race with a shorter finishing time is higher when more runners (e.g. vision and hearing) are present. Imagine that the auditory domain holds a personal record of 7 seconds and the visual domain holds a personal record of 8 seconds as their best unisensory processing times. Let's assume that the winning time of the current race is 8 seconds. Both modalities have a chance to win the race, but based on earlier performance, it is more likely for the auditory domain to win it. However, the chance that the race will be won with a finishing time of 8 seconds is higher when there are multiple runners. The finishing time is then based on statistical facilitation. However, if finishing times are observed that cannot be explained by statistical facilitation, it is assumed that integration occurred. In this case, instead of racing against each other, the senses would work together and both contribute their power to the speed, resulting in a shorter finishing time than can be explained by the best unisensory finishing time.

On the one hand, both explanations can lead to the observation of the hard core assumption 'multisensory stimulation can enhance perception'. On the other hand, they can also be modified or falsified by data collection. By interpreting data collection outcomes by means of statistical methods such as the race model, researchers try to judge which protective belt assumption would best predict their observations and would withstand falsifications, and would thus be judged as most reliable. Finally, the reliability of both assumptions can be assessed and the weighted average belief in an assumption can be shifted. Either one of both hypotheses can become reliable enough, thereby discarding the other hypothesis, or an integrated assumption might develop around the weighted average. In sum, by gathering data, interpreting outcomes and assessing the reliability of predictions following from assumptions, we can finetune our knowledge of the truth in a Bayesian optimal fashion.

CONCLUSION

Philosophers of science have paved the way for contemporary scientific practice. Although various accounts of good practice have been introduced by Popper, Kuhn, Lakatos and Feyerabend, there still is no agreed upon conclusion on what perspective one should adopt to perform science, and multiple definitions of 'growth of science' have been formulated. I suggest the Bayesian framework to arrive at scientific progress by assessing the reliability of multiple theories in predicting outcomes generated by paradigms from different research fields.

The Bayesian framework can be applied at multiple levels of research. Between the research programs, the reliability of the hard cores in general can be compared. Proportional to the reliabilities of the hard core assumptions, a weight would be attributed to that program. The weighted average 'belief' in a research program shifts towards the more reliable one. Eventually, either one of both programs can become reliable enough

to dominate the other theory, or an integrated model would develop around the weighted average to yield the most reliable results.

Within the research programs, protective belt assumptions are judged on their predictive value and on whether they would withstand falsifications. Those values can be compared, yielding one more and one less reliable hypothesis. The less reliable hypothesis can be discarded when the other one becomes reliable enough. Otherwise, an integrated assumption might develop around the weighted average. The protective belt is now finetuned.

In conclusion, by applying the Bayesian framework to multiple levels of research, it is possible to assess the reliabilities of research programs, theories and hypotheses. The presence of multiple research fields, and paradigms, can facilitate this assessment. This Bayesian integration of theoretical concepts contributes to scientific finetuning of the truth and, thus, leads to growth of science.

REFERENCES

Alais, D., & Burr, D. (2004). The ventriloquist effect results from near-optimal bimodal integration. Current Biology, 14(3), 257-262. doi 10.1016/j.cub.2004.01.029

Aller, M., Giani, A., Conrad, V., Watanabe, M., & Noppeney, U. (2015). A spatially collocated sound thrusts a flash into awareness. Frontiers in Integrative Neuroscience, 9: 16 doi: 10.3389/fnint.2015.00016

Battaglia, P. W., Jacobs, R. A., & Aslin, R. N. (2003). Bayesian integration of visual and auditory signals for spatial localization. Journal of the Optical Society of America A (JOSA A), 20(7), 1391-1397, doi: 10.1364/JOSAA.20.001391

Bernardo, J. M., & Smith, A. F. (2009). Bayesian Theory (Vol. 405). John Wiley & Sons.

Bertelson, P., & De Gelder, B. (2004). The psychology of multimodal perception. In Spence, C., & Driver, J. (Eds.). Crossmodal space and crossmodal attention (pp. 141-177). Oxford University Press.

Bickle, J. (2003). Philosophy and neuroscience: A ruthlessly reductive account (Vol. 2). Springer Science & Business Media.

Blauert, J. (1997). Spatial hearing: the psychophysics of human sound localization. MIT press.

CBS. (2018). HBO en WO; gediplomeerden, studierichting, leeftijd 1995/'96-2015/'16. Available at: https://opendata.cbs.nl/statline/#/CBS/nl/dataset/70962NED/table?dl=8C03. Accessed: July 3, 2018.

Chalmers, A. F. (1999). What is this thing called science? (3rd ed.). Indianapolis / Cambridge: Hackett Publishing Company, Inc.

Ernst, M. O. (2006). A Bayesian view on multimodal cue integration. In Knoblich, G., Thornton, I.M., Grosjean, M., & Shiffrar, M. (Eds.) Human body perception from the inside out, 131, 105-131. Oxford University Press.

Feyerabend, P. (1970). Consolations for the specialist. In Lakatos, I., & Musgrave, A. (Eds.) Criticism and the Growth of Knowledge, 4, (pp. 197-229). Cambridge: Cambridge University Press.

Feyerabend, P. (1993). Against method. Verso.

Finger, S. (1994). Origins of neuroscience: a history of explorations into brain function. Oxford University Press, USA.

Frank, P. (1957). Philosophy of Science. The Link Between Science and Philosophy. Englewood Cliffs, N.J.: Prentice-Hall, Inc.

Frassinetti, F., Bolognini, N., & Làdavas, E. (2002). Enhancement of visual perception by crossmodal visuo-auditory interaction. Experimental Brain Research, 147(3), 332-343. doi: 10.1007/s00221-002-1262-y

Frens, M. A., Van Opstal, A. J., & Van der Willegen, R. F. (1995). Spatial and temporal factors determine auditory-visual interactions in human saccadic eye movements. Attention, Perception, & Psychophysics, 57(6), 802-816

Ghahramani, Z. (1995). Computation and psychophysics of sensorimotor integration (Doctoral dissertation, Massachusetts Institute of Technology).

Gholson, B., & Barker, P. (1985). Kuhn , Lakatos , and Laudan. Applications in the History of Physics and Psychology. American Psychologist, 40(7), 755–769.

Grill-Spector, K., & Malach, R. (2004). The human visual cortex. Annu. Rev. Neurosci., 27, 649-677. doi: 10.1146/annurev.neuro.27.070203.144220

Howson, C., & Urbach, P. (2006). Scientific reasoning: the Bayesian approach. Open Court Publishing.

Hughes, H. C., Reuter-Lorenz, P. A., Nozawa, G., & Fendrich, R. (1994). Visual-auditory interactions in sensorimotor processing: saccades versus manual responses. Journal of Experimental Psychology: Human Perception and Performance, 20(1), 131 - 153.

Kalat, J. W. (1980). Biological psychology. Cengage Learning.

Kuhn, T. S. (1970). The Structure of Scientific Revolutions (2nd ed., Vol. II). International Encyclopedia of Unified Science. Chicago: University of Chicago Press.

Lakatos, I. (1970). Falsification and the Methodology of Scientific Research Programmes. In Lakatos, I., & Musgrave, A. (Eds.) Criticism and the Growth of Knowledge, 4, (pp. 91-196). Cambridge: Cambridge University Press.

Lee, P. M. (2012). Bayesian statistics: an introduction. Chichester, United Kingdom: John Wiley & Sons Ltd.

Lüthy, C. (2007). De Draad van Ariadne: Een pleidooi voor de wetenschapsgeschiedenis. Available at: http://repository.ubn.ru.nl/bitstream/handle/2066/30944/30944_draavaar.pdf. Accessed: June, 2018.

Mandler, G. (2007). A history of modern experimental psychology: From James and Wundt to cognitive science. Cambridge, MA: MIT press.

Miller, J. (2016). Statistical facilitation and the redundant signals effect: What are race and coactivation models? Attention, Perception, & Psychophysics, 78(2), 516-519. doi: 10.3758/s13414-015-1017-z

Mills, A. W. (1958). On the minimum audible angle. The Journal of the Acoustical Society of America, 30(4), 237-246. doi: 10.1121/1.1909553

Oaksford, M., & Chater, N. (2007). Bayesian rationality: The probabilistic approach to human reasoning. Oxford University Press.

Pick, H. L., Warren, D. H., & Hay, J. C. (1969). Sensory conflict in judgments of spatial direction. Perception & Psychophysics, 6(4), 203-205.

Popper, K. (2005). The logic of scientific discovery. Routledge. Available at: https://archive.org/stream/PopperLogicScientificDiscovery/popper-logic-scientific-discovery_djvu.txt. Accessed: June, 2018

Purves, D., Cabeza, R., Huettel, S. A., LaBar, K. S., Platt, M. L., Woldorff, M. G., & Brannon, E. M. (2013). Cognitive Neuroscience. Sunderland: Sinauer Associates, Inc.

Stein, B. E., & Meredith, M. A. (1993). The merging of the senses. The MIT Press.

Stein, B. E., & Stanford, T. R. (2008). Multisensory integration: Current issues from the perspective of the single neuron. Nature Reviews Neuroscience, 9(4), 255-266. doi: 10.1038/nrn2377

The Logic of Scientific Discovery. (2012). Retrieved from http://www.math.chalmers.se/~ulfp/Review/logicscdis.pdf

VSNU. (2012). The Netherlands Code of Conduct for Scientific Practice. Available at: https://www.vsnu.nl/files/documenten/Domeinen/Onderzoek/The_Netherlands_Code_of_Conduct_for_Scientific_Practice_2012.pdf. Accessed: July 13, 2018.

Walker, T. C. (2010). The Perils of Paradigm Mentalities : Revisiting Kuhn, Lakatos, and Popper. Perspectives on Politics, 8(2), 433–451. doi: 10.1017/S1537592710001180

Welch, R. B., & Warren, D. H. (1980). Immediate perceptual response to intersensory discrepancy. Psychological Bulletin, 88(3), 638-667.

Wolfe, J. M., Kluender, K. R., Levi, D. M., Bartoshuk, L., Herz, R., Klatzky, R. L., & Merfeld, D. M. (2015). Sensation & perception (4rd ed). Sunderland, MA: Sinauer Associates.

Wundt, W. M. (1904). Principles of physiological psychology (Vol. 1). London: Swan Sonnenschein & Co. LIM.

Expansion Microscopy

A different take on super-resolution

Al Naqib, V.A.B.[1]

[1] Department of Neuroscience and Pharmacology, UMC Utrecht Brain Center Rudolf Magnus, Utrecht, the Netherlands

In all its forms, light microscopy has relied on the same principle: Improve the apparatus, (de)colour the specimen. But there are other ways to peer deeper into the microscopic. Edward Boyden's lab at the MIT developed a technique with which fixed specimens can be imaged at super-resolution levels with regular fluorescent or confocal microscopes, called Expansion Microscopy (ExM) (Chen, Tillberg, & Boyden, 2015).

The idea is as simple as it is ingenious: Instead of making the microscope better, why not make the specimen bigger? If a mouse brain expands to four times its original size, all structures within become four times as far apart from one another. This expansion in physical size translates to an effective increase in optical resolution because of the underlying physical constraints of microscopy. According to the law that defines the diffraction limit of light microscopy, puncta (e.g. individual fluorophores) can only be resolved as separate points if their distance to one another is more than half the wavelength of the light involved, which is around 250 nm in most applications. For scale: the size of a synapse in the macaque visual system is around 500-1500 nm (Stettler, Yamahachi, Li, Denk, & Gilbert, 2006). Keeping this law in mind, it is easy to see that physically moving away individual proteins or strands of RNA, and thereby the fluorophores that mark them, can drastically increase the amount of detail captured by the optical system.

Although different labs have come up with slightly different approaches to ExM (Chozinski et al., 2016; Ku et al., 2016; Tillberg et al., 2016) the principle is retained in all of them. The structures of interest are linked to a monomeric solution of acrylamide, bis-acrylamide and sodium acrylate, which is then polymerised into a gel (for a schematised overview see Figure 1). Either before or after the gelation treatment, staining of the sample through ordinary staining techniques is possible, or the tissue already contains endogenously expressed fluorescent proteins. To ensure that the tissue can expand evenly and equally in all three spatial dimensions, tis-

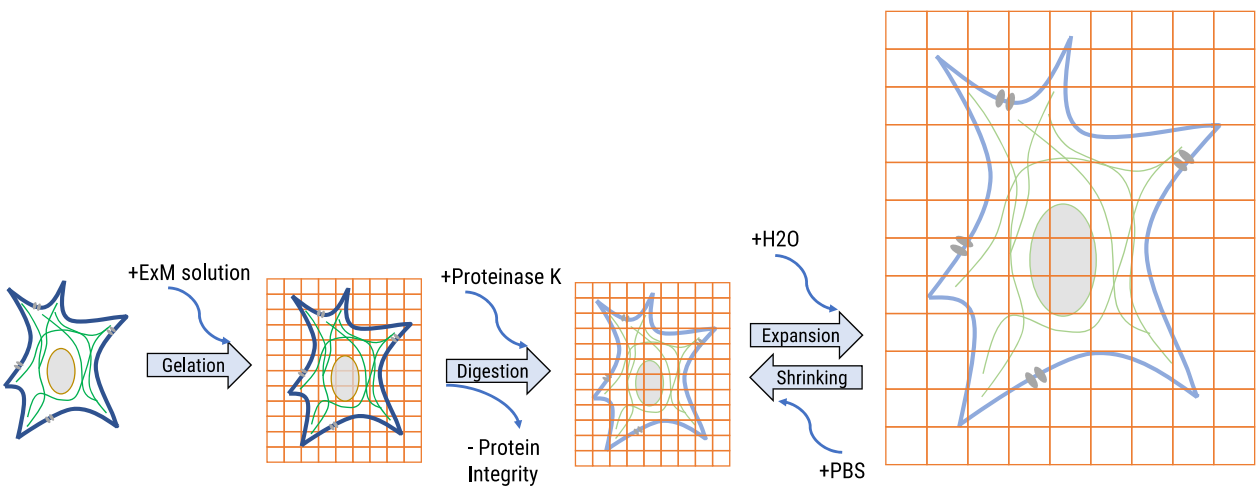


Figure 1 | Schematised overview of an ExM treatment. *A tissue that is either endogenously expressing fluorescent proteins or has been tagged through fluorescent antibodies is subjected to the ExM solution. To avoid breakage upon expansion, the tissue must be digested – in this example through a treatment with proteinsase K. Afterwards, water makes the tissue expand, and a saline (e.g. phosphate-buffered saline, PBS) reverses this process.*

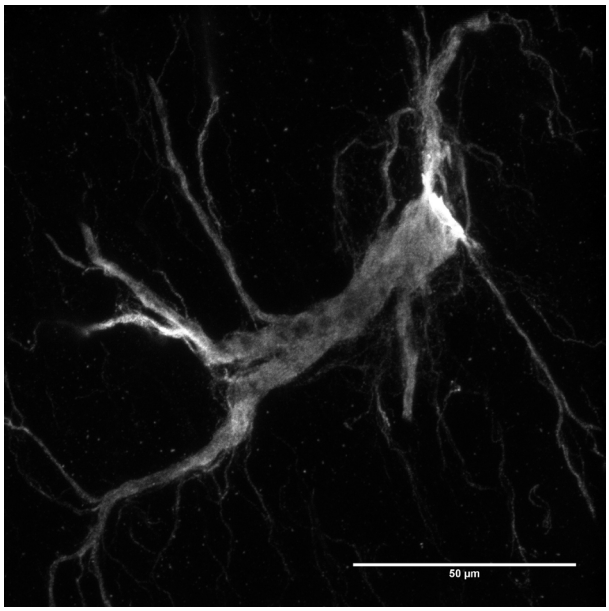


Figure 2 | GFAP-stained mouse astrocyte from an ExM-treated brain slice, 40x magnification.

sue integrity is disrupted via denaturation/digestion treatments (e.g. proteinase K digestion). Expansion then happens through the addition of ultraclean water, which is bound by the sodium acrylate moieties in the polymer chain, leading to a straightening of the chain. This process can be reversed by replacing the water with a saline solution, like PBS. In essence, the tissue – be that whole murine brains, brain slices, organs or organoids (Figure 2) – is interwoven with a polymer matrix, acting like a three-dimensional grid and this grid can extend the lengths of its edges by incorporating water.

Since its development in 2015, ExM has become a helpful tool for labs involved in neuroscience (Freifeld et al., 2017), pathology (Zhao et al., 2017), cell biology (Decarreau et al., 2017) and more. The Boyden lab offers free training courses in ExM, and collaborations between them and other prestigious institutions like Janelia Research Campus (Gao et al., 2018) have pushed light microscopy to levels of detail that were considered science-fiction only ten years ago. It truly is the golden age of light microscopy.

REFERENCES

Chen, F., Tillberg, P. W., & Boyden, E. S. (2015). Expansion microscopy. Science, 347(6221), 543 LP-548. Retrieved from http://science.sciencemag.org/content/347/6221/543.abstract

Chozinski, T. J., Halpern, A. R., Okawa, H., Kim, H.-J., Tremel, G. J., Wong, R. O. L., & Vaughan, J. C. (2016). Expansion microscopy with conventional antibodies and fluorescent proteins. Nature Methods, 13, 485. Retrieved from http://dx.doi.org/10.1038/nmeth.3833

Decarreau, J., Wagenbach, M., Lynch, E., Halpern, A. R., Vaughan, J. C., Kollman, J., & Wordeman, L. (2017). The tetrameric kinesin Kif25 suppresses pre-mitotic centrosome separation to establish proper spindle orientation. Nature Cell Biology, 19, 384. Retrieved from http://dx.doi.org/10.1038/ncb3486

Freifeld, L., Odstrcil, I., Förster, D., Ramirez, A., Gagnon, J. A., Randlett, O., … Boyden, E. S. (2017). Expansion microscopy of zebrafish for neuroscience and developmental biology studies. Proceedings of the National Academy of Sciences, 114(50), E10799 LP-E10808. Retrieved from http://www.pnas.org/content/114/50/E10799.abstract

Gao, R., Asano, S. M., Upadhyayula, S., Pisarev, I., Milkie, D. E., Liu, T.-L., … Betzig, E. (2018). Cortical Column and Whole Brain Imaging of Neural Circuits with Molecular Contrast and Nanoscale Resolution. BioRxiv. Retrieved from http://biorxiv.org/content/early/2018/07/23/374140.abstract

Ku, T., Swaney, J., Park, J.-Y., Albanese, A., Murray, E., Cho, J. H., … Chung, K. (2016). Multiplexed and scalable super-resolution imaging of three-dimensional protein localization in size-adjustable tissues. Nature Biotechnology, 34, 973. Retrieved from http://dx.doi.org/10.1038/nbt.3641

Stettler, D. D., Yamahachi, H., Li, W., Denk, W., & Gilbert, C. D. (2006). Axons and Synaptic Boutons Are Highly Dynamic in Adult Visual Cortex. Neuron, 49(6), 877–887. https://doi.org/https://doi.org/10.1016/j.neuron.2006.02.018

Tillberg, P. W., Chen, F., Piatkevich, K. D., Zhao, Y., Yu, C.-C. (Jay), English, B. P., … Boyden, E. S. (2016). Protein-retention expansion microscopy of cells and tissues labeled using standard fluorescent proteins and antibodies. Nature Biotechnology, 34, 987. Retrieved from http://dx.doi.org/10.1038/nbt.3625

Zhao, Y., Bucur, O., Irshad, H., Chen, F., Weins, A., Stancu, A. L., … Boyden, E. S. (2017). Nanoscale imaging of clinical specimens using pathology-optimized expansion microscopy. Nature Biotechnology, 35, 757. Retrieved from http://dx.doi.org/10.1038/nbt.3892

Electroencephalography

Measuring the human auditory brainstem response using EEG

Vink, E.[1]

[1] Department of Experimental Psychology, Helmholtz Institute, Utrecht University, Utrecht, the Netherlands

Electroencephalography (EEG) is a commonly used technique in the field of Cognitive Neuroscience. It is generally used to measure electrical cortical activity. Responses of subcortical brain regions, like the human brainstem, are rarely investigated. This is due to their submerged position in the brain, making it more difficult to record electrical signals from these areas. This is unfortunate, because the brainstem is a very basal and vital region of the human brain, involved in, for example, cardiovascular and respiratory control and integrating sensory input (Kandel, Schwartz, Jessell, Siegelbaum & Hudspeth, 2012). The brainstem transforms sensory inputs at a primary level and any disruption at this low level of processing could have devastating consequences for higher order processing in the cortex.

Interestingly, Sohmer and Feinmesser (1967) discovered that it might be possible to measure activity of the brainstem using EEG. The authors measured the auditory brainstem response (ABR), which is an electrical brainstem response to the presentation of sounds. The ABR consists of a series of six to seven waves, with wave I representing the beginning of the auditory pathway (cochlear nerve), and the final wave representing activity from the upper part of the brainstem, before it reaches the auditory cortex (Fig. 1; Laumen, Tollin, Beutelmann & Klump, 2016).

Nowadays, the ABR technique is commonly used to measure hearing abilities in clinical settings. It has not often been used in research settings, possibly due to the complexity of the required set-up and the sensitivity needed to measure the ABR. The ABR has low amplitudes

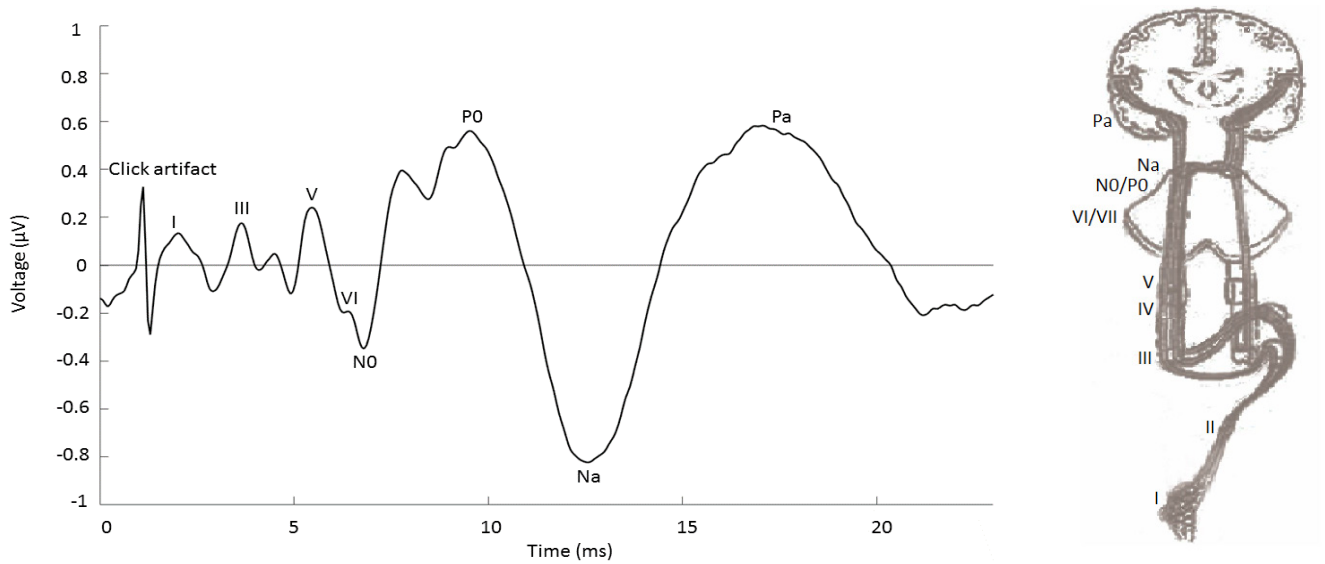


Figure 1 | The ABR waveform and the subsequent processing at cortex level.
The graph displays the ABR and the signal that is recorded after the ABR. The x-axis contains the time in ms while the y-axis represents the voltage in µV. The ABR consists of waves I-VI. After wave VI, the signal is transferred from the midbrain to the cortex (wave N0 – Pa). Each wave represents a different area of the auditory pathway. Wave III-VI are known to originate from the brainstem, after which the signal is passed to the cortex. As can be seen in the figure, the ABR has a relatively small response amplitude when compared to cortical potentials.

(below 1 μV) and a short response time (within 10 ms after sound onset). Thus, an average of a large number of trials is needed to actually derive scientific conclusions from the ABR. Despite the complexity of the technique, it would be very interesting to study perceptual and cognitive influences on the ABR more thoroughly. For example, the ABR is measured in current studies of brainstem plasticity in patients with hearing loss who undergo a multisensory recalibration therapy, changing their auditory spatial representation based on input of their intact visual system.

Over the last decades, the equipment used in measuring ABRs has improved, making it easier to also apply ABR measurements in research settings. The Experimental Psychology lab of Utrecht University uses BioSemi equipment to measure ABRs. Instead of using 32 or 64 pintype electrodes and an EEG cap, only 5 AgCl flattype electrodes are needed to measure a reliable signal. Two active electrodes are placed behind the left and right ear (mastoids), close to the brainstem, and three electrodes are placed on the forehead; one reference electrode at Fpz and one ground electrode at either side of the reference electrode. The EEG signal is amplified by 100-150K, with a bandwidth of 0-3.3 kHz and it records at a sampling rate of 16-kHz (Paulraj, Subramaniam, Yaccob, Bin Adom & Hema, 2015). A sample size of around 2000 trials is needed to attain a solid ABR signal for one sound stimulus. Furthermore, the presentation of the sound stimuli and its corresponding EEG markers needs to be very precise in order to accurately analyse EEG data from sound onset. The ABR data is acquired using ActiABR software and markers are received via a 16-bit coding system. The acquired data needs to be filtered offline (100-3000 Hz) to improve the signal-to-noise ratio.

Although the set-up of an ABR measurement is somewhat complex, it is possible to record brainstem responses using EEG in both clinical and research settings. The ABR has low amplitudes and a short response time and therefore requires a conscientious procedure to reliably record activity from the brainstem. Research on the ABR could provide key insights into the early processing of auditory stimuli and any plasticity of the auditory brainstem, for example in people with hearing loss.

REFERENCES

Kandel, E.R., Schwartz, J.H., Jessell, T.M., Siegelbaum, S.A., & Hudspeth, A.J., (2012). Principles of Neural Science (5 th ed.). Colombus, OH: McGraw-Hill Education

Laumen, G., Tollin, D. J., Beutelmann, R., & Klump, G. M. (2016). Aging effects on the binaural interaction component of the auditory brainstem response in the Mongolian gerbil: Effects of interaural time and level differences. Hearing Research, 337, 46–58. https://doi.org/10.1016/j.heares.2016.04.009

Paulraj, M. P., Subramaniam, K., Yaccob, S. Bin, Bin Adom, A. H., & Hema, C. R. (2015). Auditory evoked potential response and hearing loss: A review. Open Biomedical Engineering Journal, 9, 17–24. https://doi.org/10.2174/1874120701509010017

Sohmer, H. and Feinmesser, M. (1967) Cochlear Action Potentials Recorded from the External Ear in Man. Annals of Otology, Rhinology & Laryngology, 76, 427-435. https://doi.org/10.1177/000348946707600211

Fibre photometry

Shedding light on neuronal populations

van den Heuvel, M.W.[1]

[1] Department of Translational Neuroscience, UMC Utrecht Brain Center Rudolf Magnus, Utrecht, the Netherlands

Our understanding of brain function has traditionally been linked to anatomically defined brain areas – for example, when we listen to someone speak, Wernicke's area is active. This notion has been put into theory by Fodor's *modularity of mind*: the idea that the mind consists of innate neural structures with specialised functions (Fodor, 1983). The rise of more advanced techniques allowed for investigation of such neural structures, leading to more nuanced understanding of brain functioning. Many novel tools used in neuroscientific research have unraveled how billions of neurons function and interact with each other in order to process information and rapidly produce complex behaviours. This article discusses one of the techniques in the field of *in vivo* calcium imaging: fibre photometry.

GCaMPs: visualisation of calcium activity

Calcium imaging refers to the visualization of intracellular calcium (Ca^{2+}) concentrations via fluorescent proteins. During an action potential, voltage-gated Ca^{2+} channels open through which extracellular Ca^{2+} flows into the axon terminal, resulting in neurotransmission (Südhof, 2012). It is this function of Ca^{2+} that forms the core upon which *in vivo imaging techniques* are developed, as they measure changes in intracellular Ca^{2+} by utilising GCaMPs. A genetically encoded Ca^{2+} indicator, GCaMP, consists of two halves of green fluorescent protein (GFP), calmodulin and myosin light chain kinase (MLCK). As intracellular Ca^{2+} increases it will bind to calmodulin. This Ca^{2+}-calmodulin complex then binds with MLCK, a Ca^{2+}-calmodulin binding domain. The binding of these domains brings the two halves of GFP in close proximity of each other, resulting in functional GFP responsive to light. These GCaMPs alter their fluorescence in response to changes in intracellular Ca^{2+} concentrations (Akerboom et al., 2013). The three most widely used types of *in vivo* calcium imaging are 2-photon calcium imaging, single photon imaging using miniature microscopes, and fibre photometry (Girven & Sparta, 2017).

In vivo calcium imaging techniques

In 2-photon calcium imaging, GCaMP molecules are excited by a coherent laser beam focused through the objective of a microscope. At the focus point of the laser, fluorescence is greatest and allows for visualization of calcium fluctuations at the axon terminals. Traditionally, 2-photon calcium imaging could only measure superficial layers of the brain. By using gradient-refractive-index (GRIN) lenses, structures deeper in the brain can also be visualised. Although this technique offers superior resolution, a major constraint is that animals must be head-fixed. A second *in vivo* calcium imaging technique using GRIN lenses is single photon imaging. A GRIN lens is implanted in the brain and during measurements a microscope, small and light enough not to hinder movement, is attached to the lens. Single photon imaging suffers from more light scattering, increasing background fluorescence and resulting in a lower spatial resolution. A second drawback are the GRIN lenses; their diameter ranges from 0.5 ≥ 1 mm, inevitably resulting in substantial invasion of brain tissue (Akerboom et al., 2013).

Fibre photometry

The third *in vivo* calcium imaging technique is fibre photometry where fluorescent changes are detected via an optic fibre which is attached to the ferrule of the animal; a small fibre implanted above the brain region of interest. The fibre is sensitive enough to detect the fluorescent changes over neuronal populations. However, because a fibre is used instead of a lens there is loss of spatial resolution; fibre photometry does not offer information on single cells. Just as with the other forms of calcium imaging, this technique offers high cell-type specificity by using GCaMPs to target cells. The temporal precision offered by fibre photometry is excellent as well, in the order of milliseconds (Akerboom et al., 2013). These characteristics allow insight into the relations of activity patterns evoked by natural behavior (Gunaydin et al., 2014). The light weight and small size of the ferrule and optic fibre attached to the animal make fibre photometry applicable to a

wide range of behavioural paradigms, such as the elevated plus maze, the tail suspension test and operant behaviours. Data acquired via fibre photometry can complement chemo- and optogenetic data, providing extra insight into the functions of the targeted circuit. Furthermore, fibre photometry can be combined with other techniques such as fMRI (Schulz et al., 2012; Li et al., 2017) and optogenetics (Liang, Watson, & Zhang, 2017). Costs for setting up a fibre-photometry set-up are relatively affordable with prices starting at $10K for custom-built set-ups and going up to $20K for ready-made set-ups. The operations necessary for fibre photometry are easier to perform than procedures for single and two-photon calcium imaging (Li et al., 2017). As such, fibre photometry is a powerful technique allowing for the observation of specific elements or projections of neural circuits.

That said, fibre photometry also has a major drawback compared to other forms of calcium imaging, since it measures the activity of a neuronal population, rather than single cells. Since this technique does not offer the cellular resolution to differentiate between activities of individual neurons, any potential heterogeneity in these populations is ignored. Despite this drawback, fibre photometry has given new insights into various topics. For example, Gunaydin et al. (2014) gained a new understanding of the dynamics of the mesolimbic dopamine system during social interaction in mice. In another study, fibre photometry was used to show that subregions of the striatum receive fundamentally different signals, giving a new perspective on the diversity of midbrain dopamine neurons (Lerner et al., 2015). Muir and colleagues (2018) used the technique to demonstrate that D1-type medium spiny neuron activity is a predictive marker of depression in mice. Furthermore, fibre photometry unveiled how specific cell types in the arcuate nucleus of the hypothalamus control appetitive aspects of feeding behavior (Chen, Lin, Kuo, & Knight, 2018).

Fibre photometry is an ideal tool to get insight into how various parts of a hypothesized circuit interact, providing data on relatively large areas compared to the single cell data acquired by two- and single photon imaging. In conclusion, fibre photometry has proved to be a quick, user-friendly addition to current imaging methods measuring cell-type specific and projection-specific signals that can improve our understanding of how neural circuits process information and produce complex behaviours.

REFERENCES

Fodor, J. A. (1983). Précis on the modularity of mind. The Behavioural and Brain Sciences, 8 (1), 1 – 5.

Südhof, T. C. (2012). Calcium control of neurotransmitter release. Cold Spring Harb perspect Biol, 4 (1). doi: 10.1101/cshperspect.a011353

Akerboom, J. Calderón, N. C., Tian, L., Wabnig, S., Prigge, M., Tolo, j., Gordus, A., Orger, M. B., Severi, K. E., Macklin, J. J., Patel, R., Pulver, S. R., Wardill, T. J., Fischer, E., Schüler, C., Chen, T., Sarkisyan, K. S., Marvin, J. S., Bargmann, C. I., Kim, S. D., Kügler, S., Lagnado, L., Hegemann, P., Gottschalk, A., Schreiter, E. R., & Looger, L. L. (2013). Genetically encoded calcium indicators for multi-color neural activity and combination with optogenetics.

Girven, K. S., & Sparta, D. R. (2017). Probing deep brain circuitry: New advances in in vivo calcium measurement strategies. ACS Chemical Neuroscience, 8, 243 – 251. doi: 10.1021/acschemneuro.6b00307

Gunaydin, L. A., Grosenick, L., Finkelstein, J. C., Kauvar, I. V., Fenno, L. E., Adhikari, A., Lammel, S., Mirzabekov, J. J., Airan, R. D., Zalocusky, K. A., Tye, K. M., Anikeeva, P., Malenka, R. C., & Deisseroth, K. (2014). Natural neural projection dynamics underlying social behavior. Cell, 157 (7), 1535 – 1551. doi: 10.1016/j.cell.2014.05.017

Schulz, K. Sydekum, E., Krueppel, R., Engelbrecht, C. J., Schlegel, F., Schröter, A., Rudin, M., Helmchen, F. (2012). Simultaneous BOLD fMRI and fibre-optic calcium recording in rat neocortex. Nature Methods, 9, 597 – 602. doi: 10.1038/nmeth.2013

Li, L., Tang, Y., Sun, L., Rahman, K., Huang, K., Xu, W., Yu, J., Dai, J., & Cao, G. (2017). In vivo fibre photometry of neural activity in response to optogenetically manipulated inputs in freely moving mice. Journal of Innovative Optical Health Sciences, 10 (5). doi: 10.1142/S1793545817430015

Liang, Z., Ma, Y., Watson, G. D. R., & Zhang, N. (2017). Simultaneous GCaMP6-based fibre photometry and fMRI in rats. Journal of Neuroscience Methods, 289, 31 – 38. doi: 10.1016/j.jneumeth.2017.07.002

Lerner, T.N., shilyansky, C., Davidson, T. J., Evans, K. E., Beier, K. T., Zalocusky, K. A., Crow, A. K., Malenka, R. C., Luo, L., Tomer, R., & Deisseroth, K. (2015). Intact-brain analyses reveal distinct information carried by SNc dopamine subcircuits. Cell, 162, 635 – 647. doi: 10.1016/j.cell.2015.07.014

Muir, J., Lorsch, Z. S., Ramakrishnan, C., Deisseroth, K., Nestler, E. J., Calipari, E. S., & Bagot, R. C. (2018). Signature of future stress susceptibility in nucleus accumbens. Neuropsychopharmacology, 43, 255 – 263. doi: 10.1038/npp.2017.122

Chen, Y., Lin, Y., Kuo, T., & Knight, Z. A. (2015). Sensory detection of food rapidly modulates arcuate feeding circuits. Cell, 160 (5), 805 – 806. doi: 10.1016/j.cell.2015.01.033

Epi(c)genetics

Peter Bos

Dr. Peter Bos is a researcher at the Department of Experimental Psychology of Utrecht University. His research focusses on neuroendocrinology underlying variations in caregiving and social emotional behaviour.

What is your background?

"I studied Psychology, with an interest in biological psychology. In high school, I wanted to be a biologist, but I was scared to do a lot of chemistry. One way or another, I ended up in biological psychology, which had the best of both worlds for me. During my studies, I came into contact with Jack van Honk, who was a professor here and still is. By doing my internship with one of his PhD students on testosterone administration, I enrolled in social endocrinology. Later, when I was a research assistant, Jack got a grand. While I was at a party, somebody said 'You are the new PhD student of Jack van Honk'- a fact that I was unaware of. I had a job which I never officially applied for."

What was your PhD about?

"This PhD position focussed on the role of vasopressin and oxytocin in human behaviour. Unfortunately, when I started my PhD, the vasopressin substance that I was going to study was taken off the market. Therefore, we moved to testosterone, since there was still a protocol for testosterone research. Furthermore, we went into oxytocin administration studies and cortisol studies. I was, and still am, always focussing on human social emotional behaviour like empathy and emotional processing."

What did you do after your PhD?

"After my PhD I got to set up oxytocin experiments. I moved on doing the same kind of experiments, but slowly incorporating new measures. At this moment, I am focussing on human caregiving behaviour. We are relating social emotional behaviour and the role of endocrinology to human caregiving. One of the very fundamental hormones, important for caregiving, is assumed to be oxytocin. It is involved in social bonding, but its role is much more complex than that."

How did you measure the influence of testosterone on social behaviour?

"We were quite unique, especially at that time. There were only a few groups in the world that were doing testosterone administration paradigms in humans. We developed paradigms, or used paradigms from other people, to measure for example empathy. We collaborated with other people or we built our own experiments to measure any kind of behaviour we thought was relevant for testosterone."

How do you measure parenting/caregiving behaviour is the lab?

"We have different approaches. The best, more naturalistic approach, is having people in the lab or in a home situation and observing their behaviour. You are a bit restricted because you measure for a short period and during an interaction with a child, there is not a lot you can control. We also try to create experiments which capture fundamental aspects of parenting. We have been stripping parenting down to its bare essentials and creating experiments for these. One of them, for example, is sensitivity towards infant faces, which is a core mechanism of parenting. Irresponsiveness to an infant's face or a cry sound, might say something about how some would act as a parent. Another example is exposing people in a scanner to crying sounds or infant faces, and see how their neural responses changes after drug administrations. We are also doing paradigms with EEG to measure temporal aspects and use EMG to measure facial responses towards an infant. Interestingly, we have related sensitivity of an EEG response towards an infant face to sensitivity observed in the lab from parents interacting with their own child."

Can you observe maltreatment in a research setting?

"Even within a stratified population of white, highly educated people, you will see a lot of variation in how people respond to children. It is actually surprising sometimes. That is a nice thing about video observations."

How long do you tape them for these video observations?

"Generally, it is a fixed period of 8 minutes. It is relatively short, but you can see a lot of variation within this time frame. For example, restrictiveness, and whether and how people structure the play of the child. There is a video of a father interacting with his child. It is a very young child, and the father is constantly actively playing with the kid. There is no connection, because the kid does not understand what is going on. The father is much too intrusive, much too quick. He is probably doing his best to be a great dad, but he does not level with the child. This is a very subtle example and it does not relate to child abuse. However, you can already see that there is a lot of mismatch between the interest of the child and the interest of the father. Even within this short time period."

Do you also tape behaviour that is less subtle, or more related to maltreatment?

"There is a task which I haven't used, but would like to, in which you instruct the parents that the toddler should put all his toys in a box. You see lot of variation in parents. Some get very annoyed that the child does not do what they say, whereas others are much more relaxed. You will even see that in a lab or in a home visit, with people watching. Some parents become very intrusive towards their child, even harsh. If that happens with a camera on top, then what can happen without the camera..."

> ***"It is actually possible to already have some markers for parental behaviour, even before the child is born."***

Are the participants in these experiments all parents or also non-parents?

"This depends on if we let them interact with a real child. In this case, it is always their own child. However, most work with hormone administration is in non-parents, since we test that in students. This is partially based on that it is practical. At the moment that someone becomes a parent, a lot of changes happen in their neuronal endocrinology. If we observe endocrine changes in parents towards their child, we do not know if they are actually representative for what happens in all people in caregiving situations or reflect changes that occur because they became a parent. We now look if we can see changes in behaviour towards infants after hormone administration before someone becomes a parent. We can then test if this predicts parenting behaviour once these participants become parents themselves. In one of our studies I collaborated with the group of prof. Carolina de Weerth from the University of

Nijmegen. We measured the endocrine response of participants interacting with a crying baby doll before they became parents. Later, when their child was born, we measured their sensitivity towards their own child. In fathers, for example, the sensitivity towards their own child is related to cortisol responses towards the infant doll. This shows that it is actually possible to already have some markers for parental behaviour, even before the child is born."

Do you also combine clinical work with your research?

"At this moment, we are looking if we can combine studies. It is very expensive to do this, but we would like to combine fundamental research with intervention studies to see whether we can use fundamental knowledge on endocrine mechanisms to change behaviour."

Do environmental influences have an impact on hormones?

"Definitely, on a lot of levels. Already during conception, some SNPs of the oxytocin and testosterone gene make you more or less sensitive towards these hormones. From there on, it becomes more complex. The endocrines that you are exposed to in the womb can have an effect later on in your life. Your childhood environment can also have an influence on your stress system."

What influence does the stress system have on hormones?

"Testosterone and oxytocin are strongly related to the stress system. It changes your sensitivity towards these hormones, which can then influence your own social behaviour and parenting skills. The environment interacts with the hormones and the hormones also interact among each other. It is very complex. Manipulating one hormonal level can affect other hormone levels. Therefore, you always have to realise the timing of your administration and it is going to get converted into all kind of other substances, which affect the outcome of your studies. We can control for this by acute administration: after administration, we directly measure behaviour. The problem with long term administration, which has also been done a lot, is that after a couple of administrations you do not know which feedback mechanism is causing the outcome. And that, in humans, is very problematic to tear apart. That is why we do acute administration of the hormone, and causally manipulate the hormone."

Can hormones also have a negative impact on parenting behaviour?

"Yes. There is quite some indication that in some cases testosterone can have a negative effect on parenting. The same holds for cortisol. One of my studies showed that higher levels of cortisol and testosterone made fathers more insensitive towards their child, which in general is not a good thing. However, generally it is never the case that a hormone either has a positive or negative effect because the effects of these hormones are very context depended. Good parenting is very dependent on the context. It is not very nice if someone grabs a child very roughly by the arm, but if you do this to save the child from getting run over by a bus, it becomes protective caregiving."

> ***"We want to find predictors for core aspects of human caregiving, and epigenetics is one step closer to behaviour than genes."***

Do you also use epigenetics in your field of research?

"One of the studies where we look at epigenetics, is where we investigate women who are not mothers yet. The reason that we started to look at epigenetics is that we knew, from meta analyses of genetic literature, that SNPs are poor predictors of behaviour. We knew that we could manipulate behaviour with drug administration, but we also wanted to fill in the black boxes in between. Two or three studies came out that showed that oxytocin receptor methylation predicted, for example, neural responses in a scanner and social behaviour. If oxytocin is indeed this bonding hormone, then this methylation of the oxytocin receptor would be relevant for caregiving behaviour

and investigated in a group of young women whether oxytocin methylation changes their responses towards infants before they became a parent. We are now running the first studies; it confirms that methylation biases you for being more socially sensitive towards children. We want to find predictors for core aspects of human caregiving, and epigenetics, or methylation, is one step closer to behaviour than genes. Epigenetics does predict these women's responses towards infants. But we are currently still analysing this data."

How would you define epigenetics?

"It is a very interesting, intermediate step. But epigenetics is only one of the predictors for diversity in social emotional behaviour. We want to use the measure and that is fine, but also problematic to ignore the complexities. For example, it is very interesting to look at the methylation of the oxytocin receptor, but we do not know what part or location of the oxytocin receptor is responsible, and what the exact neurobiological effects of this methylation are. I am a psychologist, so my interest is to look at human behaviour, but I still want to use biology and genetics if that can help me explain it better. We cannot fully ignore those complexities, that is why collaborations are nice."

How do you think that epigenetics fits in the nature/nurture debate?

"It fits nicely in finally getting over this debate. Epigenetics is the final death stroke in killing this debate and shows you that it is completely irrelevant to talk about these different aspects in isolation."

What do you think the role is of epigenetics will be in future research?

"We have to temper our expectations. When genetics became big, we thought it could explain everything. It was a big dissolution. Some SNPs explained this behaviour, but the meta-analyses showed zero effect. I think we have to reduce our expectations for epigenetics immediately from the beginning. Epigenetics is still far from human behaviour. It is a very important step from our genetic make-up to our behaviour, but there are still a lot of in-between steps that we have to discover. Epigenetics, the discovery of these mechanisms, is definitely going to help, but it is going to take years before we have established these pathways. There is also a lot of discussion within the epigenetic field on: 'What is still unknown about the whole process of methylation? How definite is methylation? Is methylation passed on to the next generation?' Every publication that comes out makes the story a bit more complex. Which is, in the end, a good thing. If we want to understand it, it should not be a simple story. Simple stories are never true."

> ***"Epigenetics is the final death stroke in killing the nature/ nurture debate."***

Do you have any general advice for neuroscience students?

"That is a difficult one. I was just discussing with a colleage last week that I am very lucky that I am not a Master Neuroscience and Cognition student at the moment. Because I think I would not have made it - the competition has gotten worse. Research budget is generally a bit declining in the Netherlands. It can be the best thing in the world to do research, but you have to have this very strong inherent interest in what you are doing, you should not do it because of the career perspective. In this moment, you enter science in a difficult phase, because we have the replication crisis going on. I think that has not fully reached neuroscience, but that is about to come. I think it is a difficult moment and a lot is happening. The future should define whether that is a good or bad thing. Individual competition is also not doing science good. We all know that science is a communal thing, but grants are all given to individuals. The grand system motivates people in the wrong direction. In that essence, it is very important that we have a new generation of people who can change this system for good and make it into a better one. I think this generation is much more aware of these problems and that can really make a difference. That is my hope. Time for new people." ■

Epi(c)genetics

Marco Boks

Dr. Marco Boks is a renowned researcher in the epigenetic field. He is currently investigating how trauma influences psychiatric disorders and how Gene and Environment interplay. By understanding how epigenetic mechanisms influence DNA transcription, he aims to find potential pharmaceutical manipulations in order to help patients.

What is your background?

"I studied medicine at Groningen and found that psychiatry was the most interesting speciality. The thought that we live in our brains, that our whole perception of the world, our whole perception of happiness and sadness happens in our brains intrigued me. You can have people with terrible circumstances that are happy and enjoy their lives, and people that have everything and they are miserable. So, what is happening in our brains? In these 1.5 kilos up there, largely consisting of cholesterol? Feelings, emotions and social functions are the most interesting function of the brain, so I started doing psychiatry."

Did you ever have a personal experience that led you to psychiatry?

"One of my friends developed schizophrenia when we were 18, which made an impression on me. I wanted to understand why this wonderful normal intelligent guy became confused and started to talk about the Russians. I still think this is one of the main outrages of this horrible disorder, it takes young people in their early 20's and is a chronic disease. It motivated me to help these young human beings and find a cure for schizophrenia."

What does your work as a psychiatrist comprise of?

"We work on the treatment of resistant acoustic hallucinations. About 80% of our patients respond to antipsychotics but 20% do not. The patients that are irresponsive are managed with the proper combination of medications and trained to be more resilient to the voices; we encourage our patients to realise that the acoustic hallucinations are an odd brain function and that they can ignore them. I work three days as a clinical consultant and two days in research."

What is your research based on?

"My research is focussed on schizophrenia, bipolar disorder and post-traumatic stress disorder (PTSD). Right now, I am moving away from epidemiological cohorts and focusing on research with brain organoids, because I find that a wonderful model for the brain and slightly better than mice."

What made you go into research?

"At the end of my medicine training I started to do some research. I entered numbers in the computer and I did the statistics. There were some interesting results coming out and everybody was really enthusiastic. I realised that I could use my computer skills to do research. That is when my interest in research started."

What is your experience in combining both clinical and research settings?

"What I like most about the combination is that it keeps me motivated and asking clinical questions. One of the problems with the research trajectory is that it is very competitive; after your master's you find a PhD position, which is competitive; then you find a post-doc position that is also competitive and often temporary, and that is a real burden for scientists. The fact that I am a clinician and can always work means that I am in a fortunate position. The drawback is that I always see patients while I need to do research. It is tough to find that balance, so it has advantages and disadvantages."

You are recognised in the epigenetic research field, since you focus on the interaction between environmental risk and genetic background. How did you get interested in this specific topic?

"Fifteen years ago, the genomic age commenced. Scientists where very keen to find key genes for schizophrenia and bipolar disorder in order to understand the aetiology; that turned out to be very complicated. One of the reasons is that some of the genes may not get involved in the disease trajectory if there is no environmental exposure. If you have genes that cope for example with cannabis and you never use cannabis, then you may be vulnerable but it will never show. I started to do gene-environment interactions and as a logical follow up, I thought there are other ways in which the environment can influence genes that may be of tremendous interest. The first experiment that we did in epigenetics is looking at discordance twins. Schizophrenia has a heritability of 80%, which is a strong genetic predictor of schizophrenia. However, the concordance between genetically identical twins is much lower, only 50%. If it is 80% genetically determined and they are genetically identical, then 80% of the twins should be concordant. The first thing that we started to do is to look if differences between the healthy and non-healthy identical twin could be explained by differences in DNA methylation. This kind of interaction really intrigues me."

How do you measure epigenetic changes?

"There are many epigenetic marks. DNA methylation is one of the easier ones, because DNA methylation is very stable. DNA methylation measurements are from samples that have been stored for human studies years ago. There are very convenient arrays that you can use to measure it. Other epigenetic measures are for example histones marks."

> ***"We are now starting to think that epigenetic changes are adaptive."***

What does the data look like?

"We use DNA chips. If we would run a simple experiment where we are going to look at whether smokers have different DNA methylation compared to non-smokers, we would run an array of heavy smokers and non-smokers, measuring DNA methylation in the two groups. It is like doing GWAS, but instead of alleles, you measure the methylation levels at many positions."

What statistics do you use?

"The statistics are complicated due to many technical and methodological challenges but generally based on linear regression. We do everything in R; there are many packages for analysing and you can get an answer on the help line within hours."

What kind of research are you currently involved in?

"I am interested in the way our environment influences our DNA by means of epigenetics. I am currently involved in researching how trauma in-

"One of the things that we are pursuing is to find out if this DNA methylation is important for staying well."

fluences our DNA, since trauma is very relevant for psychiatric disorders. Half of the psychiatric disorders in childhood and about one third of the disorders in adulthood are related to childhood trauma. Currently we are looking at soldiers that had been sent to Afghanistan. We profiled them on DNA methylation before they went to Afghanistan where they were exposed to combat trauma. Some of them developed post-traumatic stress disorder (PTSD) once they returned, whereas others also exposed to trauma did not develop any mental health disease. We were able to find DNA methylation differences related to trauma and to their outgoing PTSD.

We also discovered that there are trauma related changes in DNA methylation related to the way people responded to stress, by performing a Trier Social Stress Test (TSST) and measuring their cortisol response. We were able to show that childhood trauma was related to the cortisol response. From both studies we hypothesized that trauma related DNA methylation changes are the scars of trauma: they make you sick. However, the opposite turned out to be true: participants that did not have the methylation changes were the ones that were at risk. We are now starting to think that these epigenetic changes are adaptive. It is good to trigger your stress system."

If you find the epigenetic locus, could it be that, in the future, medication would be personalised in order to change the responsiveness of the sites?

"One of the things that we are pursuing is to find out if this DNA methylation is important for staying well. If you need to adapt your DNA to childhood trauma in order to stay well, we can perhaps facilitate that by medication. DNA methylation is not entirely stable, there are many things that impact DNA methylation. If smoking impacts DNA methylation, why not other compounds? And there are in fact nutritional compounds that impact DNA methylation, like folic acid. This is taken during pregnancy, even before conception. It reduces the risk of spina bifida in the baby. This is epigenetic therapy because the folic acid is in the biological pathway towards the methylation mechanism.

The compound that we are interested in is S-Adenosil Methionine (SAMe) which is a methyl donor for studying DNA methylation in bipolar disorder. 70% of the persistent symptoms in bipolar disorder are of a depressive nature. We give bipolar patients trauma therapy Eye Movement Desensitization Reprocessing (EMDR) and randomise them to a placebo or to a SAMe treatment condition. We mildly re-expose them to trauma, by reliving the memories. We hope that patients that receive SAMe the methylome can be pushed in a way that they can adapt to their childhood trauma. We think we can enhance the effects of epigenetic treatment specific by combining it with trauma therapy."

Are you doing epigenetic research with other researchers in Utrecht?

"Yes, I cooperate with Mark Timmer, Menno Creyghton, Elly Hol and many others. Originally, oncology was driving the epigenetic field since epigenetic changes the oncology-genes. Oncologists are not particularly interested in an environmental perspective; they find tumours in which the epigenetic control is just gone. They are typing these tumours by their genetic profile and finding the genes that are out of control. These are targeted for a treatment. The first epigenetic drugs are developed for oncology."

How would you define epigenetics?

"Epigenetics are heritable mitotic changes in DNA other than in the sequence.

One question that is still out there is whether these epigenetics marks of the environment can be inherited. The X chromosome for instance has a lot to do with epigenetics, because the silencing process of one of the maternal X chromosomes is mainly by DNA methylation."

Are there some cases in which the epigenetic changes of a mother where given to the child?

"There is a beginning of evidence that some of the anxieties of the mothers can be inherited in the offspring. But it is really doubtful, whether this mechanism is really epigenetic or has something to do with microRNAs. The speculation is that you may inherit the alcoholism, or the stress of your mother or the stress of your father, but I am far from convinced as for now.

One argument that I find very strong against epigenetic inheritance is the thought about the sheep Dolly. It was a perfect clone, but she was an epigenetic nightmare since she had to change all the functions of the cells. However, the offspring was completely normal. We know that all the epigenetic reprogramming is sort of wiped and reprogrammed on sixth or seventh day of the embryogenesis."

What do you think is the biggest breakthrough in epigenetics?

"If you look at biology books from 50 years ago it will tell that DNA methylation was brought on in the sixth day of the embryogenesis and it defined tissue specification. When people started to learn that DNA methylation was variable, that it changed with age and environmental exposures, and that it was related to cancer and other diseases; theses discoveries started the field. This was about ten to fifteen years ago, so it is a very young field."

Do you think in the future people will only investigate nurture or only genes?

"One of the intriguing things of this field is that it has become more complicated. At the beginning, all we measured was DNA methylation. Later we knew that there is a lot of genetic variation that controls the number of variations in methylation. We combine both GWAS and EWAS. We also have data that showed a cross-chromosome interaction, in which the chromosomes influence each other. We need to look at gene expression and RNA sequence and move towards a system biology approach."

Do you have any advice for neuroscience students in their future career?

"My general advice would be to look around and find something that really intrigues you and try to find the path that best suits your goal. You have to find something that you want to figure out and then find the best laboratory that you can think of and do your PhD there.

I would recommend doing something that motivates you and use technologies that are fun, rather than choosing a nice city." ■

For further questions or internship positions, contact Dr Marco Boks

M.p.m.Boks@umcutrecht.nl

Recommended readings

Rutten, B., et al. (2018). Longitudinal analyses of the DNA methylome in deployed military servicemen identify susceptibility loci for post-traumatic stress disorder. ***Molecular Psychiatry***, 23, 1145–1156.

Houtepen, L. C., et al. (2016). Genome-wide DNA methylation levels and altered cortisol reactivity following childhood trauma in humans. ***Nature Communications***, 7, 1-10.

Smith, A. R., Smith, R. G., Condliffe, D., Hannon, E., Schalkwyk, L., Mill, J., & Lunnon, K. (2016). Increased DNA methylation near TREM2 is consistently seen in the superior temporal gyrus in Alzheimer's disease brain. ***Neurobiology of aging***, 47, 35-40

Utrecht University

UMC Utrecht

Looking for a PhD position?

Graduate School of Life Sciences

Our PhD programmes offer research ranging from molecules to population

- Biomembranes
- Toxicology & Environmental Health
- Cardiovascular Research
- Environmental Biology
- Clinical & Translational Oncology
- Computational Life Sciences
- Medical Imaging
- Clinical & Experimental Neuroscience
- Epidemiology
- Infection & Immunity
- Drug Innovation
- Molecular Life Sciences
- Regenerative Medicine
- Cancer, Stem Cells & Developmental Biology

The 11th Federation of European Neuroscience Societies (FENS)

Forum of Neuroscience congress

Nina van Bruggen

WHERE *Berlin, Germany*

WHEN *7-11 July 2018*

At the beginning of 2018 I received an email that the Network of European Neuroscience Schools (NENS) was organising a *best poster competition* for MSc and PhD students. I was just rounding off my project in Toronto (Canada), so I decided to give it a shot and I entered the competition. Soon I received a confirmation by Geert that I was chosen to represent our graduate programme. Some time later it was also confirmed that I was selected as one of the 10 finalists. This was a big honour as NENS represents over 160 graduate schools and programmes across approximately 30 European countries that offer MSc, PhD and MD-PhD degrees in neuroscience.

When I arrived at the venue, I was increasingly surprised by the massive scale of the congress. In halls comparable to the Amsterdam RAI or Utrecht Jaarbeurs, endless boards with research posters stretched before me, as well as numerous booths of smaller and larger technical companies. Going to a talk was as if you were going to a large concert, with three massive screens. Luckily all the posters were listed in the FENS app, with which you could check in which section you could find the posters of your field of interest. After a quick scan through the cognitive posters (CNtrack), I arrived at the small corner that was dedicated to the posters of the seven PhD and three MSc finalists.

I hung up my poster amidst the other finalists and took a step back. It was immediately clear that the other finalists had used the maximum measurements that had been emailed to us previously. Unlike me, as I had decided to print my poster one size smaller to be sure it fit. So, ***Tip #1: always print a poster with the (exact) recommended measurements***. Also, pay attention to portrait- vs. landscape view, as someone else had printed in landscape view.

At 3 pm, lots of people had arrived and started asking questions about our research. There was no plenary start, so at first, I hadn't noticed that about four jury members were circling around us as well. During my internship I analysed structural and functional MRI scans of adolescents with gender dysphoria; people who experience a discontent with their assigned gender at birth. With our functional analyses we found that functional connectivity between the medial prefrontal cortex and posterior cingulate cortex might reflect this experience. Our results suggest that people who are born with a female body but experience being male have a weaker functional connection between those two areas compared to girls, as do boys who are cisgender[1].

I think about ten people asked me questions about my research, and I also got to talk a bit with the other MSc finalists. Someone else won the 1000 euro prize, but it was an experience well worth the trip to Berlin. I would definitely recommend everyone who is able to, to enter the competition next year. For me, it was also a perfect last stage of MSc degree. October 1st I will start a traineeship in data science & business analytics, which I am very excited about. ■

[1]Someone who experiences their natal sex to be in line with their experienced gender.

Dutch Neuroscience Meeting (DNM)

Marleen van den Munkhof

WHERE *Lunteren, the Netherlands*

WHEN *7-8 June 2018*

On the rainy morning of the 8th of June, a car with aspiring neuroscientists was driving through the green fields of Gelderland. Far away from the comfort of their lab coats and cell cultures, they arrived at what seemed the most rural village of our country: Lunteren. Here, they would attend the second day of the Dutch Neuroscience Meeting (DNM) and leave their supervisors desperately alone at the lab.

The DNM is a two-day annual congress that provides the opportunity for scientists from different career stages and disciplines in the field of neuroscience to meet and share their research. The programme offers presentations in various settings: parallel seminar sessions, a poster session and a keynote lecture to top off. One of the pronounced aims of this meeting is to enable interaction between junior scientists and well-established senior scientists. To stimulate such interaction, Master students are allowed to contribute to the meeting by means of a poster presentation. A number of Neuroscience and Cognition students gladly grabbed that opportunity this year. For many of us this was the first time we got to present our own data outside of the lab. Exciting! Although, the fact that such a contribution gave us free entrance to the DNM might have also played a role – we are still students after all.

The day started with the first round of parallel seminar sessions. These sessions touched upon a diverse range of subjects within the field of neuroscience. I realised just how broad this field actually is, when I encountered a seminar titled: *"Is the penis part of the affective touch system?"*. Other seminars later in the day covered subjects such as Alzheimer's disease, gut-brain interaction, stress, and energy homeostasis. This wide diversity in topics enabled everyone to find a session of their interest throughout the programme, or discover new subjects of interest (such as the affective touch system…).

In between the parallel seminar sessions, it was time for us to stand in the spotlight: the poster presentations. Luckily it was only a small spotlight, as around one hundred posters were presented at the same time and the session took place during the lunch break. As we can all imagine, it is impossible to show interest in such a high number of posters while also trying to ingest some highly needed nutrients. Nonetheless, it was very motivational to talk to the people who took the time to read your poster and showed interest in the project. While discussing my research with them, it became clear to me how much I have learned about my own topic these past few months.

Nearing the end of the meeting, everyone came together for the key lecture and the official closure. There had been too much information to soak up that day, but it was very informative to gain insight in how research is shared and – even more so – how to present your own research to others. One way or another, this will be an important aspect for many of us in the future. Knowing this, the young scientists left fulfilled, their brains fully satiated with knowledge about the brain. ■

Mind the Brain Symposium 2018

BrainTech - Advances in Neurotherapy

Mind the Brain is a two-day annual symposium organised by students from the Utrecht University Master's program "Neuroscience & Cognition". The symposium offers its visitors a platform to connect and mutually explore cutting edge research. This year's theme, ***"BrainTech - Advances in Neurotherapy"***, reflected the fast-paced developing field of neurotechnology used within therapeutics. It took place on June 13th and 14th in the heart of Utrecht Science Park at the inspiring Marinus Ruppert building.

In the morning, visitors were greeted upon registration with a refreshing cup of coffee and a custom-made goodie-bag, fully stocked with various practicalities for the mind and brain (pun intended), such as IQ chocolates, Aspire drinks and a Quest magazine. The symposium kicked-off with a keynote lecture on *visual-cortical prosthesis* by prof. dr. Pieter Roelfsema, director of the Netherlands Institute for Neuroscience and professor

at the Vrije Universiteit Amsterdam. During the discussion, visitor questions delightfully streamed in through the engaging use of the throwable microphone. The day continued with contributions by Neuroscience and Cognition Master's students giving 1 minute-pitches on their research and a poster presentation in the main hallway during lunch, where stands by Mind Affect and the Journal of Neuroscience & Cognition peaked through bushes of grey and turquoise balloons. After lunch, endowed prof. dr. Willem Verbeke from Erasmus University Rotterdam gave a uniquely inspiring keynote lecture on *neuromarketing*, which was followed by some truly exceptional N&C student presentations. The day was finalised with four practical workshops, which were held by independent contributors and UU Career Services.

The second day was internally called the "BCI-day", as it not only contained great pitch and poster sessions by N&C students, but also a thun-

derous keynote lecture on *deep-brain stimulation* by dr. Rob Rouhl from the UMC Maastricht, an illuminating keynote lecture on *brain-computer interfaces* by dr. Mariska van Steensel from UMC Utrecht, and as the final cherry on top, a BCI demonstration by MindAffect. Enthusiasm ensued as demonstrator Cristiano Micheli, wearing an EEG headset, managed to type "Mind the Brain" by solely using the electrical activity of his brain. CEO Ivo de la Rive Box ended the session with an interesting and interactive discussion, on the futuristic potential of a BCI and its ethical implications.

Finally, a moment was taken during the grand finale of the drinks to announce the award winners of the best abstract (Lisa Bauer), presentation (Lance Bosch), pitch (Sanne Böing) and poster (Khaterah Kohneshin), which were partially determined by contributed votes of the audience. This marked the end of the Mind the Brain symposium 2018. We are incredibly delighted by the involvement and enthusiasm of all attendees, as well as the significant amount of external visitors. In total, we happily welcomed 130 visitors on the 13th and 121 on the 14th. We are proud that our determination, problem-solving efforts and teamwork during the past half year of organizing these two days resulted in feedback that indicated a successful symposium! However, this outcome would have not been possible without the collective contributions of students, lecturers, judges and sponsors.

Therefore, on behalf of the Mind the Brain Committee 2018, with sincere gratitude, honour and pleasure, thank you all! We look forward to your presence during next year's symposium and extend our best wishes to the next committee.

Clarissa, Coco, Joosje, Katerina, Laura, Laurens, Lisa, Merel and Vida

Mind the Brain Symposium 2018 Awards

Best Presentation: Lance Bosch

Best Pitch: Sanne Böing

Best Abstract: Lisa Bauer

Effect of early social experience on GABAergic synapses in the prefrontal cortex of adult rats

Lisa Bauer[1], Rene van Dorland[1], Else Bijvank[1], Louk Vanderschuren[2], Corette J. Wierenga[1]

[1] Biology Department, Faculty of Science, Utrecht University
[2]Department of Animals in Science and Society, Faculty of Veterinary Medicine, Utrecht University

Sensory input during early life is critical for the proper development of the sensory cortex. It has been proposed that experience-dependent development of neuronal connectivity also occurs in brain areas involved in complex cognitive functions, including the prefrontal cortex (PFC). By analogy, we hypothesize that optimal PFC development requires complex input, such as social interaction. Here, we examine how early social experiences shape PFC circuitry and function. To that aim, we deprived young rats from social interaction with peers for 3 weeks during early adolescence and tested how PFC circuitry is affected in adulthood. We previously found that inhibitory input to L5 pyramidal cells was reduced in adult PFC brain slices of socially deprived rats compared to controls, while excitatory input appeared unaffected. We will present our immunohistochemistry data, in which we quantified the number and type of GABA neurons and the density of perisomatic inhibitory synapses in adult PFC from socially deprived and control rats. Our study provides new insight into the development of the circuitry involved in higher cognitive functions under the influence of the social environment.

Best Poster: Khaterah Kohneshin

Correcting for errors in a brain-computer interface (BCI) using neural correlates of error perception

Brain Center Rudolf Magnus

UTRECHT NEUROPROSTHESIS

Khaterah Kohneshin[1], Z.V. Freudenburg[1], E.J. Aarnoutse[1], M.J. Vansteensel[1], M.P. Branco[1], S. Leinders[1], M.A. Van Den Boom[1], E. Pels[1], N.F. Ramsey[1]

1) Brain Center Rudolf Magnus, Department of Neurology and Neurosurgery, University Medical Center Utrecht, The Netherlands

1 Background

- The Utrecht Neural Prosthesis **(UNP)** is a fully implanted communication BCI in a person with locked-in syndrome (LIS) due to late stage **ALS**.
- Based on bipolar electrocorticography (**ECoG**) signals recorded from primary motor cortex (**M1**) hand area and dorsolateral prefrontal cortex (**dLPFC**) [1].
- Activity translated into a 'click' used to select letters on a screen.
  - M1 → Attempted hand movement [2]
  - dLPFC → Counting backwards [1]
- BCIs are prone to **errors** (unintended clicks) [3]
- Error-related potentials (**ErrPs**) are neural correlates of error awareness [3]
- Hypothesized neural basis of ErrPs is the **anterior cingulate cortex** (ACC) [4]
  - regulating emotional responses
- The brain is always active and may never be fully functionally mapped. Thus, unintended actions (FPs) need to be corrected for **BCI usability**.

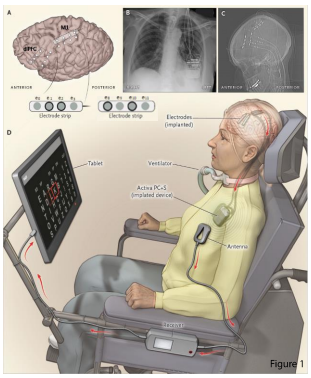


Figure 1

Purpose

To look for detectable brain signals related to perceived UNP system errors and to show that such signals could be reliably used to **correct** for system errors and **improve** system performance.

2 Methods

Whack-a-Mole (WAM) task
Data was collected during **40 sessions** (1467 trials) of a BCI game (WAM). **Goal**: Locate the cell in which a mole is presented by making clicks using the UNP system.

- Selection box (red rectangle) scans at a rate of one step per 2s.
- First, select the row then the column.
- Four types of *feedback moments* (see figure 3).

Signal processing
ECoG data was recorded at a sampling rate of 200Hz (1s) and time locked to the WAM feedback (refresh rate of 40Hz). Data was smoothed with 10 samples.

Z-score calculation
$z = \frac{x - \mu}{\sigma}$
Baseline range and task range used for calculating the Canolty z-scores. Nonparametric testing to find significant differences using 5000 random shifts.

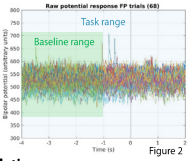


Figure 2

Area under the ROC calculation
0-1.5s after each feedback (TN, TP, FN, and FP) is *correlated* to the FP mean. The area under ROC for FPs vs. the rest is used as a detectability measure. 1000 AROCs are computed using shuffled labels to determine significance.

3 Results

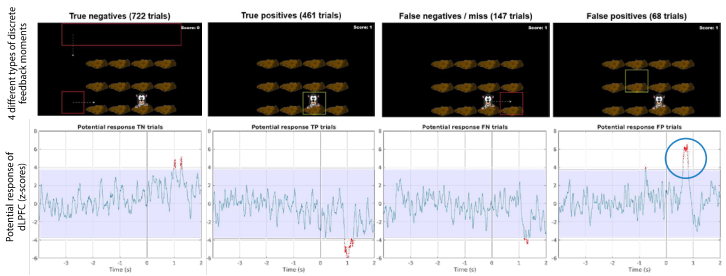


Figure 3. Four feedback moments and potential responses. The red dots mark significant points (p<.002) and the blue shaded area indicates the region of non-significance. The blue circle shows a positive significant peak in the FP trials.

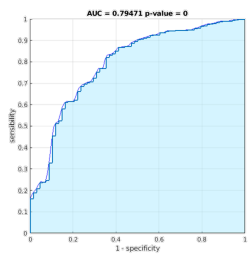


Figure 4. Area under the curve (correlation)

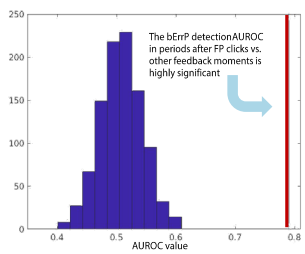


Figure 5. Distribution of random area under the curves

(4) Conclusion

- There is a **significant potential peak at** ~500-800ms after the feedback of the dLPFC signal related exclusively to feedback indicating system errors (UNP bErrP).
- It is possible to **reliably detect** a bipolar error-related potential (bErrP) on a single trial basis.
- It is not (yet) fully possible to use it to correct for system errors.

5 Discussion

- The local nature of the ECoG signal makes it unlikely that the bErrP is a direct measurement of the ACC ErrP.
- However, dLPFC and ACC are closely related (both part of the **corticolimbic system)** [5]
  - ACC → regulating emotional responses
  - dLPFC → regulating motivational responses
- We believe that this finding motivates future work aimed at real-time error detection and correction using the bipolar dLPFC signal in the UNP system.
- Any suggestions?

References

[1] Vansteensel M. J., Pels, E. G., Bleichner, M. G., Branco, M. P., Denison, T., Freudenburg, Z. V., ... & Van Rijen, P. C. (2016). Fully implanted brain–computer interface in a locked-in patient with ALS. New England Journal of Medicine, 375(21), 2060-2066.

[2] Leuthardt, E. C., Schalk, G., Wolpaw, J. R., Ojemann, J. G., & Moran, D. W. (2004). A brain–computer interface using electrocorticographic signals in humans. Journal of neural engineering, 1(2), 63.

[3] Ferrez, P. W., & Del R. Millan, J. (2008). Error-related EEG potentials generated during simulated brain-computer interaction. IEEE Transactions on Biomedical Engineering, 55(3), 923-929.

[4] Holroyd, C. B., Coles, M. G. H., & Program, N. (2002). The Neural Basis of Human Error Processing: Reinforcement Learning Dopamine, and the Error-Related Negativity, 109(4), 679-709.

[5] Benes, F. M. (2010). Amygdalocortical circuitry in schizophrenia: from circuits to molecules. Neuropsychopharmacology, 35(1), 239.

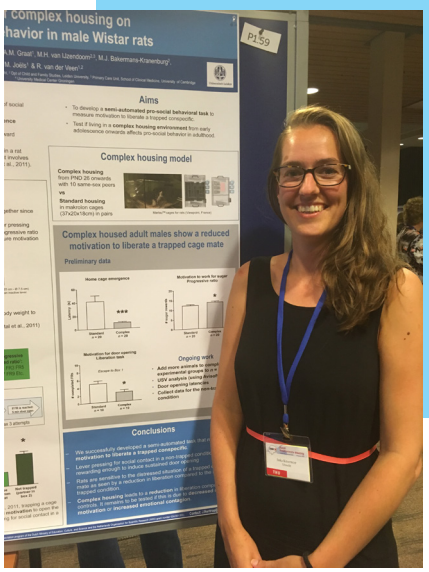
University Medical Center Utrecht

Contact: Khaterah Kohneshin
E-mail: k.kohneshin@students.uu.nl

PhD Experience

"I learned that you need a lot of perseverance, dedication and an eye for detail."

Jiska Kentrop



I am writing this while sitting in the animal facility of the BCRM where my rats, in the room next to mine, are busy liberating (or not liberating?) a trapped cage mate in a pro-social liberation task. This is the last experiment I will perform for my PhD and it is the perfect moment to look back on four years of PhD life. I work in the lab of prof. Marian Joëls on a project focused on the impact of early life environmental factors on social behaviour in rats. More specifically, we want to know what the effects of early life stress and environmental enrichment are on adolescent and adult social and pro-social behaviour. The last two years I worked on the development of two behavioural tasks, aimed to measure pro-social behaviour, and the current experiment will be the pay-off of endless amounts of pilot experiments, re-designing equipment and fine-tuning behavioural protocols.

Before I started my PhD, I studied Bio-Pharmaceutical Sciences in Leiden and did internships at the department of Medical Pharmacology in the lab of prof. Ron de Kloet at the LACDR in Leiden and department of Experimental Psychology of Cambridge University in the lab of prof. Jeffrey Dalley. Both internships were focused on the behavioural effects of stress in animal models. After my first research internship of nine months, I felt I just scratched the surface of things that could be learned and experimentally tested and I knew I wanted at least four more years. Based on the topics of my internships, I applied for a PhD in prof. Joëls lab and got the position. That is not to say I didn't apply for other positions on different neuroscience topics. However, I noticed that the internship topics play a large role whether or not I was invited on a job interview. I would strongly recommend Master students planning their second internship to make sure it is different from the first to ensure you have multiple options.

Looking back, I really enjoyed my time in Utrecht. I especially enjoyed the inspiring atmosphere of people that are passionate about research, learning something new every day, the group effort of explaining unexpected experimental outcomes with colleagues and the sense of freedom in designing and conducting my own experiments. I learned that you need a lot of perseverance, dedication and an eye for detail, because those unexpected experimental outcomes send you right back to the lab to repeat experiments. Sometimes with only minor changes, but potentially major consequences.

After finishing my PhD, I'm not continuing my career as an academic researcher. I do love science, but I mostly enjoyed working with other people on shared projects and even though academia is all about sharing data and collaborating, in the end it is a rather personal success driven career path. I also would like to work (as part of a team) on something that is less fundamental and closer to practical implementation. So, if anyone reading this knows someone working at TNO or the Ministry of Volksgezondheid; please forward this piece and tell them this is my open application. Or if you are a Master student and are not sure about doing a PhD, feel free to contact me for a cup of coffee. ■

From academia to industry

NeuroReality

Faviola Dadis

"I have gained skills from each endeavour I pursued, and I believe this has led to my success as an entrepreneur."

My name is Faviola Dadis and I am the Founder and CEO of a medtech startup company, NeuroReality. I completed my Master's in Cognitive Neuropsychology at the VU University Amsterdam and the University of Oxford. I am currently doing my PhD in Clinical Neuropsychology at the VU University Amsterdam under the supervision of Prof. Dr. Erik Scherder.

While I have had a passion for neuroscience and the brain as long as I remember, I also have a passion for entrepreneurship. I modelled internationally, worked in marketing and PR, and briefly in geopolitics — all the while going to school and finishing my Bachelor's degree in biological psychology.

It may not seem like these worlds collide; however, both academia and modelling are highly competitive and involve a great deal of criticism. You have to build a thick skin and continue to persevere no matter what feedback you get. During my time in marketing and PR, I made amazing connections — many of which have been valuable to the creation of my startup — and learned a lot about branding and how to navigate my way through the business world. I have gained skills from each endeavour I pursued, and I believe this has led to my success as an entrepreneur.

Back to my life as a neuroscientist and my startup company NeuroReality. I specialise in neurodegenerative diseases and traumatic brain injury, but stroke research is where my true passion lies. Strokes are the leading cause of long-term disability, with 15 million people worldwide suffering a stroke each year. Depending upon the neuroanatomical location of lesions caused by the stroke, individuals may experience cognitive problems, for example with attention, memory, visuospatial skills, language, executive functions, and numerical manipulation. The prevalence of post-stroke cognitive impairment ranges from 20% to 80%, varying between demographic factors and diagnostic criteria. Many patients also develop dementia as a result of these deficits.

One of the things I noticed early in my career was that there is a large disparity between research and practice, e.g. how research is applied in the medical field or translated into accessible solutions for patients. My research has always focused on translational projects in the hope that it can have a high impact factor and societal benefit outside of solely publications of significant results.

Therefore, in founding NeuroReality, my aim was to develop medical software using virtual reality that creates an immersive and fun gamified neurorehabilitation program for individuals who experience cognitive deficits following a stroke, and eventually for other clinical populations. Our

software is based upon scientific research, and will be available to patients both in clinical settings and post-discharge. NeuroReality focuses on the gamification of three neurobiological principles: neuroplasticity, engagement of the mirror neuron system, and activation of the reward system.

As a medtech startup, NeuroReality's team is comprised of individuals from many different backgrounds ranging from neuroscience, to software development, game design, data analytics, industrial engineering, and marketing. As our product is research based, we are always looking for motivated students, and have a number of opportunities for both undergraduate and graduate students to conduct their internship with our company. We are currently located within the Game Cella Lab at the VU University Amsterdam. Please feel free to send me an email if you are interested in knowing more about how you can join our team! ■

WEBSITE

www.neurovirtualreality.com

EMAIL

faviola@neurovirtualreality.com

Danone Nutricia

Danielle Counotte

"I enjoy using my extensive neuroscience knowledge in a setting where I can make a difference to patients' lives."

My name is Danielle Counotte, and I was an MSc student in the Neuroscience and Cognition Master program at Utrecht University between 2003 and 2005. After getting my PhD in Neuroscience from the VU University Amsterdam and spending time abroad as a post-doc in Baltimore (USA), I decided to leave academia and joined Danone Nutricia Research as a scientist.

The company I work for

Danone Nutricia Research, located in the Utrecht Science Park, is the global research and development centre for two of Danone's divisions: early life nutrition (ELN) and advanced medical nutrition (AMN). I am part of the neuroscience platform, and my colleagues and I focus on the development of new nutritional concepts

that support the brain, and on generating scientific evidence to support medical nutrition concepts that are already on the market, for example the ketogenic diet for children with intractable epilepsy. I no longer do hands-on work in the lab myself, because we often work in public-private partnerships. This means that my average workweek consists of managing the collaborations that we have, including looking for new partners, and discussing ideas, experiments and data with our collaborators. In addition, I often meet with colleagues within the company to discuss the progress of projects, and I spend time on my computer doing literature research, for example to better define the neurological deficits of a particular patient population, that need better therapeutic management.

Academia versus industry

Before joining Danone, I pursued a career in academia and spent time as a post-doc and in a non-tenure track faculty position. Because I did not manage to secure research funding to start my own research group at a university, I decided to look outside academia and discovered Danone as a potential employer. For me, the main difference between academia and industry is that in academia both success and failure are much more personal: if a paper was rejected, I found it hard not to feel it as a personal rejection. In industry, your performance is judged on what you do, but also on how you do things, while in academia the outcome (i.e. the funded grant or the high impact factor paper) is the most important measure of success. That said, in industry the research we do needs to be commercially relevant one way or the other, while academia allows you to explore more fundamental concepts in science. Personally, I really enjoy using my extensive neuroscience knowledge in a setting where I can make a difference to patients' lives.

Working at Danone/Nutricia

There are quite a few colleagues at Danone Nutricia Research who have a background in neuroscience. They work in a variety of roles, for example as scientist, clinical study manager, project manager or medical liaison, depending on their personal interests and the company's needs. This is another aspect that I personally value about working at Danone Nutricia Research: there are many different career paths and you are encouraged to grow. In addition, the company values diversity and encourages people from different backgrounds to work together. The company is always looking for new talents, and you can find internships and job opportunities at www.workatdanone.com and through our LinkedIn group Nutricia Research. ■

WEBSITES

www.nutriciaresearch.com

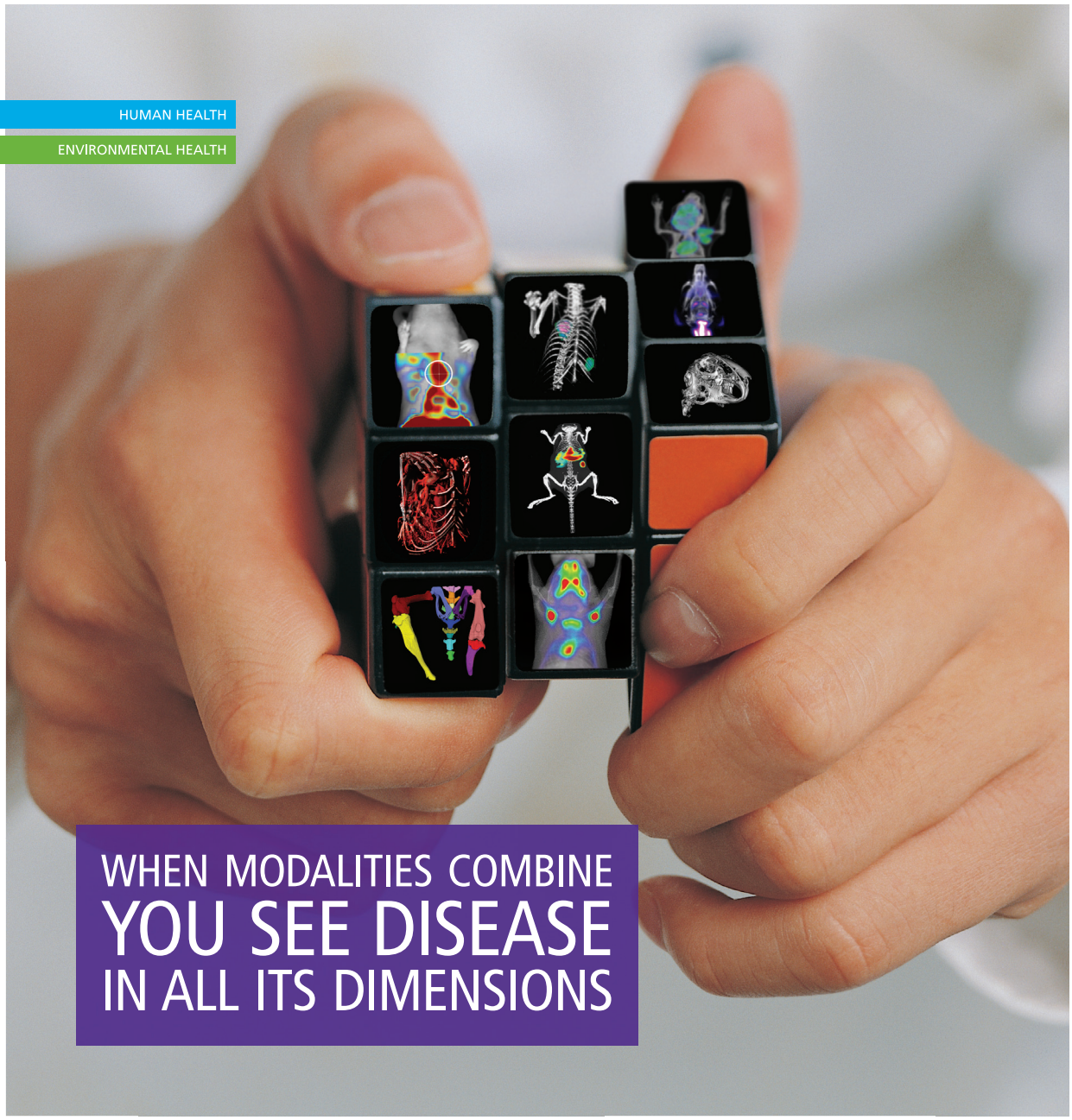
www.workatdanone.com

LINKEDIN

https://www.linkedin.com/company/nutricia-research/

HUMAN HEALTH

ENVIRONMENTAL HEALTH



WHEN MODALITIES COMBINE YOU SEE DISEASE IN ALL ITS DIMENSIONS

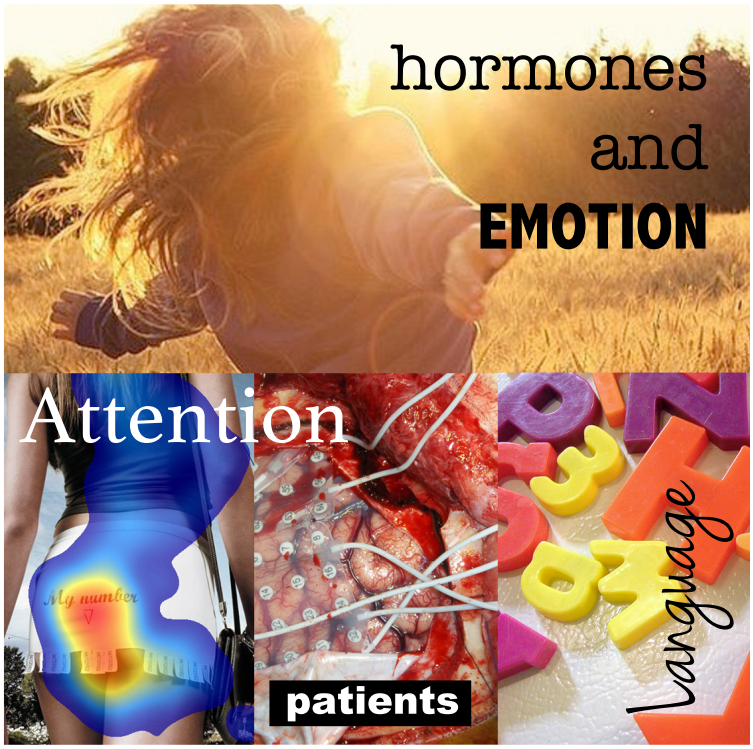
It's simple: You need to glean more information from every *in vivo* experiment, giving you maximum data to enhance and broaden your biological understanding. And that's what's behind our multimodality imaging solutions. Coregistration is enabled through a combination of comprehensive optical, PET, and microCT platforms, plus a unique portfolio of bioluminescent cell lines and fluorescent agents. Together, these technologies offer a more complete visual assessment and understanding of disease treatment and progression over time, along with improved outcomes and treatment efficacy. Because when modalities combine, you see disease from *every* angle.

For research use only. Not for use in diagnostic procedures.

www.perkinelmer.com/Multimodality-Imaging

PerkinElmer®
For the Better


Helmholtz Lectures 2018-2019
14 September 2018 - Janneke Jehee
Visual Computation Lab, Center for Cognitive Neuroimaging, Donders Institute for Brain, Cognition, and Behavior, Nijmegen, The Netherlands
Neural mechanisms of perceptual decision-making
5 October 2018 - David Burr
Department of Neuroscience, University of Florence, Italy
A visual sense of number
19 October 2018 - Anna Cox
UCL Interaction Centre, Division of Psychology and Language Sciences, University College London, UK
TBA
9 November 2018 - Edward Vogel
Department of Psychology and Institute of Mind and Biology, University of Chicago, USA
TBA
25 January 2019 - Holly Bridge
Nuffield Department of Clinical Neurosciences, University of Oxford, UK
Investigating the pathways underlying residual vision in hemianopia
15 February 2019 - Matthew Longo
Department of Psychological Sciences, Birkbeck College, University of London, UK
Distorted body representation in healthy cognition
Time: 16.00 - 17.00
Please check http://www.helmholtzschool.nl for location
Helmholtz Instituut
NCUtrecht



INTERESTED IN NEUROCOGNITIVE RESEARCH?
THE HELMHOLTZ INSTITUTE IS THE PLACE TO BE!

Helmholtz Instituut

www.fss.uu.nl/psn/Helmholtz/mainsite

Contribute to the next issue of the journal!

More information on:

www.journal.neuroscience-cognition.org

Thank you to our contributors

Authors:
Docky Duncan
Corinna Börner
Wouter Droogers
Sanne Böing
Viktor Al Naqib
Emily Vink
Nina van Bruggen
Marleen van den Munkhof
Vida Kašanin
Jet Termorshuizen
Jiska Kentrop
Faviola Dadis
Danielle Counotte

Reviewers:
Dorien Huijser
Jeshua Tromp
Minke Nota
Lisa Nieland
Mats van der Heide
Marieke ter Hoeven
Gözdem Arikan

Interview:
Marco Boks
Peter Bos