Visual information processing, welfare, and cognition in the rhesus macaque

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Authors' contributions

Visual-motion and binocular disparity processing in Macaque Monkeys area MST.

Antonino Calapai (AC), Stefan Treue (ST) and Cheng Xue (CX) designed the experiment; AC and CX performed the experiment and analysed the data; AC implemented the reverse correlation analysis underlying figure 1-5; CX implemented the population decoding analysis underlying figure 6-7; AC, CX and ST interpreted the data and wrote the paper.

A cage-based training, cognitive testing and enrichment system optimized for rhesus macaques in neuroscience research.

AC, Michael Berger (MB), Michael Niessing (MN), ST and Alexander Gail (AG) designed the experiment; AC and MB collected the data; AC analysed the data, AC, MB, ST, and AG interpreted the data and wrote the paper. Klaus Heisig (KH) built the device and Ralf Brockhausen (RB) wrote the software.

Standardized automatic training of rhesus monkeys for neuroscience research in their housing environment.

AC, MB, MN, Valeska Stephan (VS), Leonore Burchardt (LB), ST and AG designed the experiment; AC, MB, VS and LB collected the data, MB analysed the data; AC, MB, ST, VS, MN and AG interpreted the data; AC, MB, ST and AG and wrote the paper.

Sustained spatial attention by itself is sufficient to account for the bias in the direction of human microsaccades.

CX, AC and ST designed the experiment; CX, Kristin Dannhäuser (KD) and Julius Krumbiegel (JK) performed the experiment; CX analysed the data; CX, AC and ST interpreted the data and wrote the paper.

General Introduction

Vision

When electromagnetic radiation leaving the sun, 149.6 millions of kilometres away, hits a tree on our planet, a portion of that radiation is absorbed and transformed into energy, while another portion bounces back. When this rebounded radiation hits the retina of a passing monkey, the tree is seen and its image begins an intricate journey into the animal's mind. Rebounded light is first transduced into electro-chemical information and progressively distributed to the rest of the brain for further computations. It is safe to assume that the transformation of light into information is an essential ability for the vast majority of animal species. Regardless of their complexity, all animals (with only very few exceptions[1]) have indeed evolved mechanisms to detect light. Simple unicellular organisms can be either attracted to light or repelled by it, a phenomenon known as *phototaxis*; multicellular organisms like earthworms have light sensitive cells on their body surface; the *nautilus* (a marine mollusc of the cephalopod family) has all the photoreceptors concentrated into small openings on both sides of its head, a proto-eye; many insects and crustaceans show compound eyes, a collection of repeating and independent visual receptors clustered in large spheres protruding from the head; birds' eyes often have two regions of high density of photoreceptors in within the same eye to simultaneously monitor the ground for foraging and the sky for predators; humans have the highest ratio of exposed sclera among all the primates to presumably

[1] Some moles, a spider, a deep-sea lobster, the blind cave fish, the Texas salamander, the Salem cave crayfish and most of the *troglobites* don't make use of light in any way to survive. Some of these animals either have nonfunctioning eyes, or no eyes at all.

favour the rapidity of eyeball movements relative to the slowness of head and body movements when scanning the environment (Kobayashi & Kohshima, 2001).

Understanding the mechanics underlying the transformation of light into information is only the tip of the iceberg of visual perception. While transforming light into information is an instantaneous event, seeing is rather a continuous and diversified process through which animals can express agency upon their world. The ability to infer others' intentions by observing their behaviour; the possibility of predicting where a certain fruit will fall given the current wind direction; the faculty of discriminating colours, of estimating distances, of clustering objects into categories and remembering them; the ability to share a friend's smile, to recognise a loved one by the way she walks; are all skills made available to us by our brain unceasingly computing the stream of photons hitting the photoreceptors. Some studies have even found that imagery and visual perception share common processing mechanisms in the human brain (O'Craven & Kanwisher, 2000), suggesting that vision occurs even in the absence of light hitting the retina. The object of inquiry for cognitive neuroscientists of vision is to understand how the brain achieves these diverse and complex skills starting from light transduction.

The emphasis of the second chapter of this dissertation is on how *medial superior temporal area* (MST) of macaque brain makes use of visual information to infer the motion energy of an object and its distance from the eyes, with a special focus on whether and how a given neuronal population simultaneously takes these two features into account to infer self-motion.

The visual system

Given their brain's size, one major difference compared with animals from other mammalian taxa, is that primates seem to have more neurons (Herculano-Houzel, Collins, Wong, & Kaas, 2007). This is partially a consequence of the smaller size of primates' neurons (Sherwood & Hof, 2007) and partially because of the glia / other cells proportion in primates and other mammals. As a result, primates' neurons are more densely packed with respect to other mammals. This distinctive neurobiological difference seems more pronounced in the visual system and in particular in its primary visual cortex, V1 (Collins, Airey, & Young, 2010). The proportion of cerebral cortex devoted to vision is 20-30% in humans and 50% in macaque monkeys (Van Essen, 2004), where it accounts for more than 30 distinct areas (Felleman & Van Essen, 1991a). One of the reasons macaques have become the primary animal model in neurophysiology of vision research is the considerably high level of homology between human and macaque brains (Kaas, 2004), especially with regard to the visual system[2].

Retina

The first transduction of light into information occurs at the level of the retina. Like other vertebrate, the primate retina comprises approximately 80 types of cells, subdivided into 5 major groups. Among these, photoreceptors are the group of cells capable of phototransduction[3]. Signals transduced by photoreceptors immediately reach bipolar cells, which in turn dispatch the message to ganglion cells. Ganglion

[2] It is important to note that the quality of the homology of visual areas and their position in the hierarchy for visual processing are anti-correlated. For a review see Orban et al., 2004.

[3] Photoreceptors can either be rods, responsible for night vision, or cones, responsible for colour vision. Both contain one of several proteins tuned to the absorption of light at a particular region of the electromagnetic spectrum. When photons hit the photoreceptors, hyperpolarization of the cell's membrane occurs, which is the first step of the process called visual phototransduction (Ebrey & Koutalos, 2001).

cells' axons form the optic nerve, through which the information is finally transmitted to the rest of the brain for all sorts of computation. It is important to note that at the level of the retina, the circuit is already capable of advanced forms of computation – motion detection and compensation as well as object localization – (Gollisch & Meister, 2010) thanks to a number of different ganglion cells and to two intermediate layers of cells responsible for integrating multiple photoreceptors (horizontal cells), and multiple bipolar cells (amacrine cells).

LGN

Three neuronal populations compose the output from the optic nerve: the magnocellular (M), parvocellular (P) and the koniocellular (K) streams. While the precise roles of M, P and K streams in vision is currently under extensive debate[4], within the lateral geniculate nucleus (LGN) of the thalamus the three streams represent respectively 80%, 10% and 10% of the total number of neurons (Kaplan, 2004). The LGN neurons, as the relay between the optic nerve and the occipital lobe, send their axons through the optic radiation directly to the primary visual cortex. Moreover, the LGN receives numerous feedback connections from the primary visual cortex. While the functions of the LGN are various and diversified, this thalamic structure seems crucial in summing the signals originating from the left and right hemifields captured by the two eyes, as the basis for stereopsis. LGN is indeed the first brain structure to present binocular neurons: cells which are sensitive to the disparity in image position of a stimulus seen by the left and right

[4] Keeping in mind that many substreams have been identified over the years and that in a non-linear system such as the primate brain, it seems rather unlikely to have isolated computational nodes, some agreement can be found around the basic notions that the M system feeds the initial input of the *where?* pathway, the P system feeds the *what?* pathway and the K stream contributes to some aspect of colour vision (for a review see Kaplan, 2004).

eye, due to the horizontal separation of the two eyes – binocular disparity (Parker, 2007).

V1

Most visual information from the LGN reaches the primary visual cortex (V1 – Broadman's area 17) at the very back of the occipital lobe. Here, a thick series of myelinated axons from LGN form the stripes that give this brain region its alternative name of striate cortex. The over 280 million neuronal cells in the left and right portion of adult human V1 (Leuba & Kraftsik, 1994) are thought to code for the orientation of visual objects, their spatial and temporal frequency, the direction of a moving object, its colour and its disparity, a concept explained in detail later in this chapter. V1 contains a very precise representation of the visual field and neighbouring neurons in area V1 are sensitive to visual stimulation of adjacent portions of the visual field. From a neurophysiological perspective, this means that neurons in this area are specialized to respond to stimulation occurring inside a very specific sub-region of the visual field, termed the receptive field of the neuron. The resulting topographic property of a map of the visual field, known also as *retintopy*, is a feature common to most of the visual areas of the primate's brain. What makes V1 unique is that at this processing stage, different mechanisms take place to guarantee the precision of the map. These mechanisms are called into action to battle different sources of distortions: magnification distortions, due to the overrepresentation of the central visual field versus the peripheral one; and geometrical distortions, resulting from the transformation of spherical visual elements into a Cartesian representation with a horizontal and a vertical axis (Daniel & Whitteridge, 1961). V1 takes the signal from the LGN, applies these

compensatory algorithms and sends the transformed signal to the rest of the visual cortex to help the brain recognise any given object regardless of changes in its size, distance and orientation.

The two streams hypothesis

Behavioural evidence from lesion studies in monkeys led Mishkin and Ungerleider (Mishkin & Ungerleider, 1982) to the conclusion that an anatomical as well as functional bifurcation occurs in the visual system after the signal has crossed V1. Such a bifurcation, with roots at the level of the LGN's magnocellular and parvocellular layers, revolves around the idea that information exiting the occipital lobe clusters into two anatomically distinct (Goodale & Milner, 1992; Schenk & McIntosh, 2010), but functionally interconnected pathways for the analysis of the visual scene (for a review see Milner and Goodale, 2008). Both streams are responsible, to different extents, for the processing of the structure and of the location of the objects in a scene and both have proven to be highly influenced by attention. It has been proposed that the ventral stream, reaching the temporal cortex, provides information about the identity of a certain object, while the dorsal stream, reaching the parietal cortex, provides information about size, shape and position of an object, seemingly independently of its identity. In the framework of vision for action (Milner & Goodale, 2008), the areas in the ventral stream pass on the identity of an object of interest in the visual field to motor areas, while dorsal stream areas extract contextual information about size, shape and position to prepare and control the action of reaching it.

Within this framework, the second major visual processing area of the primate brain is area V2, strongly interconnected with area V1 with which it shares many

functional properties like a tuning to orientation, spatial and temporal frequency, and colour of visual stimuli. Unlike V1, area V2 seems to accomplish a more elaborate representation of the visual scene, by responding for example to the orientation of illusory contours[5] (Heyclt, Peterhans, & Baurngartner, 1984), and seems to be involved in the network of areas responsible for object-recognition memory (Bussey & Saksida, 2007).

While the extent of V3, the third major stage along the visual processing, as well as its functionality are still a matter of debate and are not directly relevant to this dissertation, some consensus emerges around the idea that V3 is fundamentally involved in the processing of global motion, defined as the perception of motion coherence in a noisy motion stimulus (Braddick et al., 2001).

As the third processing node in the ventral stream after V2 and V3, V4 is strongly connected to temporal areas, especially PIT, and shows the strongest attentional modulation of all the visual areas mentioned so far (Moran & Desimone, 1985). V4 seems to share analogous tuning with V2 – orientation, spatial frequency and color (Conway, Moeller, & Tsao, 2007) – although the full extent of V4 selectivity and tuning to complex objects is not yet known.

MT, MST and the computation of motion

Along the dorsal stream, motion decoding and perception is highly expressed in visual areas MT and MST. Located on the lower bank of the superior temporal sulcus, these two areas seem to be concerned with several aspects of motion of visual stimuli, among which the direction, the speed and the distance of a moving

[5] Illusory contours are a type of visual illusion that elicit the perception of an object's edge, either two or three dimensional, without any physical edge being present. A very famous example of such visual illusion is Kaniza's triangle, where three black circles with three inward facing triangular openings give the illusion of an occluding superimposed white triangle.

pattern are the most studied features. MT and MST neurons are mostly activated when a certain stimulus, often in the form of a random dot pattern (RDP), moves with a certain direction – for MT, linear motion: horizontal, vertical and all the possible combinations (Maunsell & Van Essen, 1983); for MST, spiral motion: expansion / contraction, rotation and all their possible combinations (Duffy & Wurtz, 1991; Graziano, Andersen, & Snowden, 1994; Orban et al., 1992; Saito et al., 1986). While motion sensitivity of area MT is a direct consequence of projections from V1 and V2 (Felleman & Van Essen, 1991b; Ungerleider & Desimone, 1986), where some rough form of linear motion selectivity can be found, MST receives strong fibre projections only from MT (Ungerleider & Mishkin, 1979). This led to the idea that the spiral sensitivity of a given MST cell can be constructed by putting together the excitatory inputs from many linearly selective MT cells (K. Tanaka & Saito, 1989a). This idea, supported by the consideration that several MT receptive fields can fit into a single MST receptive field, suggests that MT and MST can be viewed as a single network for motion processing in the primate brain. In addition, it has recently become more clear that the motion processing carried out by MT+, the homologue of areas MT and MST in humans (Dukelow et al., 2001), can be selectively altered while early visual functions are still preserved, a phenomena under considerable literature debate, known as dorsal-stream vulnerability (Atkinson & Braddick, 2010; Braddick, Atkinson, & Wattam-Bell, 2003; Grinter, Maybery, & Badcock, 2010). In schizophrenia (Kim, Norton, McBain, Ongur, & Chen, 2013), in autism (Spencer et al., 2000), as well as in Down's syndrome (Del Viva, Tozzi, Bargagna, & Cioni, 2015) and in some developmental disorders (Braddick et al., 2003) there it seems to be a general deficiency in the processing of global motion, as opposed to global form processing, which seems to be unaffected.

The case of binocular disparity

Having more than one eye is crucial to perceive stereoscopic depth, necessary for three-dimensional visual perception. Each eye obtains a slightly different image of the world as they originate from a slightly different viewpoint. Binocular disparity is simply the differences between these images. While the vast majority of visual areas of the macaque brain contain neurons responding selectively to binocular disparity, no brain region nor specific pathway has yet been identified to be exclusively specialized in binocular depth perception (Parker, 2007). From this perspective, *stereopsis* – the perception of depth based on visual information coming from both eyes in combination – is thought to be processed in parallel by the dorsal and ventral pathways of the visual system. Nonetheless, evidence has been found for the dorsal pathway being responsible for what is known as coarse stereopsis and the ventral pathway taking care of its finer aspects (Tyler, 1990).

Although no clear pattern emerges from disparity sensitivities across visual areas, either in the dorsal or the ventral pathway, it seems that – at least in humans – V1's binocular interaction sets a common denominator which later computational nodes use to generate the sense of depth (Backus, Fleet, Parker, & Heeger, 2001; Cumming & Parker, 1999). From V1 to V2 the sensitivity to disparity changes from absolute to relative (Thomas, Cumming, & Parker, 2002). V2 consistently codes the angular separation of two given objects, in the left and in the right retinas, rather than absolute disparity like V1 (Cumming & Parker, 1999). This means that from V2 on, the disparity reference frame moves with any movement of the eyes. In turn, this has led to the hypothesis that disparity can be used to compute *vergence* and *version* eye movements (Takemura, Inoue, Kawano, Quaia, & Miles, 2001; M. K. Ward, Bolding, Schultz, & Gamlin, 2015). Such a preference for relative disparity has also

been observed in areas V3 (Poggio, Gonzalez, & Krause, 1988), V4 (Watanabe, Tanaka, Uka, & Fujita, 2002) and MT (DeAngelis & Newsome, 1999), but always when planar stimuli and a centre-surround configuration is used (Parker, 2007).

While it is rather unclear how the sense of depth emerges from all these areas being sensitive to disparity, or even what the reason is for this signal to be passed on and on into the hierarchy of visual areas, previous literature suggests that disparity is used to infer self-motion at the level of MST (Roy, Komatsu, & Wurtz, 1992; Smolyanskaya, Ruff, & Born, 2013; but see Yang, Liu, Chowdhury, DeAngelis, & Angelaki, 2011). At this stage of visual processing, around 100 ms after the stimulus has been presented (Azzopardi, Fallah, Gross, & Rodman, 2003), a proportion of units show a systematic change of their preferred linear motion direction with changes in disparity. These cells, representing around 40% in the study of Roy and colleagues and ~5% in the study of Yang and colleagues, showed what has been termed direction-dependent disparity tuning (DDD, Roy et al., 1992). DDD is hypothesized to be at the very core of MST's involvement in self-motion computation and is also one of the key aspects of the second chapter of this dissertation.

Research with Non-Human Primates

From basic research to clinical trials, virtually every step of any medical scientific investigation involves research with Non-Human Primates (NHP), either directly or indirectly. While the dichotomy between basic and applied science helps us understand the general nature of a given NHP experiment, it does not account for the fact that all applied medical science is literally based on basic research. The

white paper on "*The critical role of Non-Human Primates in medical research"*[6] published this year reports a list of example scientific advances linked to research in Non-Human Primates, from 1900 to 2000. The picture that emerges from this report seems very clear: NHP research, while contributing to the accumulation of scientific knowledge *per se*, simultaneously leads to medical as well as technological advances of undeniable significance for humankind. At the same time, the white paper stresses that NHP research is highly regulated and that the welfare of the animals is always taken into consideration, not only in terms of monitoring the nutritional and environmental needs of the animals, but also their psychological needs. Overall the report represents a detailed but easy to read complementary document to the *Three Rs* principle for the ethical use of animals in testing (Russell & Burch, 2009). The three Rs proclaims that to reach a more ethical use of animals in testing researchers should take into account putative alternative methods if available (Replacement), should make use of the least number of animals possible (Reduction) and should try to alleviate or minimize pain, suffering and distress of the animal, while enhancing their welfare (Refinement). According to the authors of the white paper and to Russell and Burch, but also to all the almost 4000 signatories of the Basel Declaration[7], regulated animal testing is not only an essential ethical choice, but also helps increase the quality of the scientific output.

[6] The white paper is a collaboration between Foundation for Biomedical Research and eight premier scientific groups: the American Academy of Neurology, the American College of Neuropsychopharmacology, the American Physiological Society, the American Society for Microbiology, the American Transplant Foundation, the Endocrine Society, the Federation of American Societies for Experimental Biology and the Society for Neuroscience. Available in free download at www.monkeyresearch.org

[7] Founded on October 5th 2011, the Basel Declaration aims "to bring the scientific community together to further advance the implementation of ethical principles such as the 3Rs whenever animals are being used and to call for more trust, transparency and communication on the sensitive topic of animals in research."

Replace, Reduce and Refine are the focus of the work described in chapter 3 of this dissertation, detailing a cage-based testing system optimized for rhesus macaques, which was built to allow spontaneous and self-paced training of captive animals on typical cognitive neuroscience tasks, directly from their own social housing environment.

Environmental Enrichment

Taking the welfare of a captive animal into account often means enhancing the quality of its daily life in the animal facility. Periodic, scrupulous physiological and psychological assessments of the animal are of extreme importance to keep track of the animal's wellbeing and to, if needed, allow intervention in case of illness. At the same time, it is crucial to prevent discomfort. In this respect, providing the best quality of life to the animal means providing environmental stimuli with enriching capabilities. Enrichment can here be translated into giving more value to the conditions in which the animal lives, the captivity.

In more practical terms, it is important to avoid the onset of *displacement activities* (McFarland, 1966) and *stereotypies* (Ridley & Baker, 1982). Displacement activity is the performance of an inappropriate act for the stimulus that evoked it, like a chimpanzee rough grooming during times of intense neighbouring vocalization and gentle grooming in situations of low to no neighbouring vocalization (K. C. Baker & Aureli, 1997). Displacement activities have been suggested as a non-invasive measure of acute stress in an animal (Maestripieri, Martel, Nevison, Simpson, & Keverne, 1991; Schino, Perretta, Taglioni, Monaco, & Troisi, 1996; Troisi, 2002) and although undesirable for a lab manager they nonetheless represent the animal's

coping mechanism to a stressful situation, namely an attempt to manage the stress caused by an insurmountable situation (Berridge, Mitton, Clark, & Roth, 1999; Watson, Ward, Davis, & Stavisky, 1999). At the right-most extreme of the spectrum between adaptive and maladaptive behaviours of captive animals, where displacement activity often sits in the middle, lie stereotypies. Stereotypies are chronic (and hard to alleviate) displacement activities that tend to repeat themselves in a pattern that serves no purpose (like an animal running in circle inside of the cage). Those behaviours, which are maladaptive in nature and are often due to mechanical constraints, have been proven to confound behavioural research in rodents (Garner & Mason, 2002). Stereotypies are usually considered an indicator of an animal with an already compromised well-being, and thus require special effort to be alleviated (Coleman & Maier, 2010). For a review see Mason, 1991.

Avoiding aberrant behaviours is one part of the effort needed to truly enrich the animals' environment, but equal consideration and effort needs to be put into increasing the occurrence of desirable species-specific behaviours (like exploring, foraging, grooming, in the case of macaque monkeys). From the "Guidelines for developing and managing and environmental enrichment program for non human primates" by Bloomsmith et al, 1991, it emerges that the five main categories of enrichment are social, physical, nutritional, occupational and sensory. While these categories make use of very different types of enrichments, they are all subject to the same problem: habituation, the decrement in response to the enrichment tool as a result of repeated presentation (Harris, 1943). Habituation can be avoided by giving the animal an apparatus that can be controlled and that responds to the

animal in some way, and by constantly introducing novelty in the environment. For a review on environmental enrichment effectiveness see Tarou & Bashaw, 2007.

Cage-based testing systems

Several devices developed for behavioural data acquisition with different species of primates, have the advantage of being responsive to the animal and of introducing some novelty (Anagnostaras, Josselyn, Frankland, & Silva, 2000; Andrews & Rosenblum, 1994; Fagot & Bonté, 2010; Gazes, Brown, Basile, & Hampton, 2012; Mandell & Sackett, 2008; Miller, Lim, Heidbreder, & Black, 2016; Richardson, Washburn, Hopkins, Savage-Rumbaugh, & Rumbaugh, 1990; Truppa et al., 2010; Washburn, Hopkins, & Rumbaugh, 1991; Weed et al., 1999). While they can all be controlled more or less freely by the monkeys for which were designed, only a few of these systems are actually capable of providing constant novelty to the animal, via adaptive and automatized training schedules (Anagnostaras et al., 2000; Fagot & Bonté, 2010; Miller et al., 2016). As a result, using such a device to give a laboratory animal control over the time and pace of its laboratory-related training schedule can further improve its welfare (Westlund, 2014).

Chapter 3 of this dissertation comprises two manuscripts on this issue. Section 1 describes the experimental behavioural instrument (XBI), a cage-based stand-alone device for the behavioural training and cognitive testing of rhesus macaques, designed for a seamless integration into conventional neuroscience experiments. Section 2 contains a follow-up study on how the same 8 animals performed on an algorithm-based automated training protocol, which also gives insights into how

much the experimenter can learn about different individuals by comparing their learning behavior.

Fixational eye movements and visual spatial attention

In the primate retina, the non-uniform distribution of rods and cones, sensitive to high and low light intensities respectively, results in a degradation of visual acuity going from the centre (*fovea* – the region of highest acuity) to the periphery (Mollon & Bowmaker, 1992). For this reason, an observer who wants to thoroughly inspect an object in the periphery of the visual field needs to bring that object as close as possible to the fovea. By simply moving the eyes, the subject is able to sequentially focus on different objects, shifting her internal attentional focus (James, 1890) from one object to another. Whether it is the subject that deliberately switches her attentional focus around (top-down attention) or it is the environment that catches her attention (bottom-up attention), attention towards a specific location, object or feature can also be directed without moving the eyes, a phenomenon known as covert attention (Posner, Snyder, & Davidson, 1980). The primate brain achieves this by improving the sensory representation of a specific location (as well as a certain feature of an object or the whole object itself) over other locations (or features or objects). Physiologically, the firing rate of those neurons with receptive fields coding for the portion of the visual field to which the subject attend, is increased. For a review see Moore & Zirnsak, 2015. Behavioural studies in humans have also shown that when a subjects is asked to attend to a certain location, reaction times are reduced and performance is enhanced. For a review see Carrasco, 2011.

Oculomotor control and attention

In a very influential experiment, Sheliga and colleagues investigated the perturbation of saccade trajectories by covert attention (Sheliga, Riggio, & Rizzolatti, 1994). The authors found that the trajectory of the saccades systematically deviated towards the attended location. This result and other similar studies have contributed over the course of the last 3 decades to the development of the premotor theory of attention – PMA (Rizzolatti, Riggio, Dascola, & Umiltá, 1987). According to this view, the neuronal mechanism responsible for the enhancement of a particular spatial location in the internal representation of a covertly attending subject, overlaps with the neuronal mechanisms that actively control saccadic eye movements. In a nutshell, this theory postulates a single neuronal control mechanism for both action and attention. In such a network, covert attentional deployment is nothing else than a programmed, but not executed, saccade. While this theory has received some support from experiments in human subjects with fMRI techniques (Corbetta, 1998; Craighero, Nascimben, & Fadiga, 2004) and in monkeys through microstimulation of the Frontal Eye Field[8] (Moore, 2003), its plausibility remains controversial. As shown by Smith and co-authors in 2012, the mandatory coupling between attention and motor plan postulated by the PMA does not account for cases in which attention is deployed covertly. The authors suggest a variation of the PMA in which the neuronal activation of the motor system contributes to the on-going competition between different sensory representations. "Action preparation can increase the probability of the goal of the action being

[8] A region in primate prefrontal cortex involved in the programming and execution of saccadic eye movements and in the deployment of visual attention, as assessed by electrophysiological recordings, electrical stimulation, lesion and inactivation studies. For a review see Noudoost, Chang, Steinmetz, & Moore, 2010.

selected for processing, but it cannot guarantee it, and the absence of motor preparation does not prevent a location from being attended" (Smith & Schenk, 2012) page 1112.

Microsaccades

Microsaccades are small and involuntary miniaturized saccades occurring every few seconds while fixation is maintained. Since their discovery (R. W. Darwin & Darwin, 1786), they have been considered a basic compensatory mechanism for the natural drift of the eyes and a compensatory mechanism for the fading of images on the retina due to fatigue or habituation. Neurophysiological investigations have also found microsaccade-related modulation of several visual areas and LGN – for a review see Martinez-Conde, Macknik, Troncoso, & Hubel, 2009.

Interestingly, over the course of the last decade, thanks in part to the availability of more precise, more powerful and less expensive eye-tracking systems, several behavioural studies have reported that these small fixational eye movements are biased towards the attended location in covert spatial attention tasks (Engbert & Kliegl, 2003; Hafed & Clark, 2002; Rolfs, Engbert, & Kliegl, 2005). At the same time, just as much evidence emerged in support of a completely different hypothesis: microsaccades are simply the manifestation of oculomotor preparation (Horowitz, Fine, Fencsik, Yurgenson, & Wolfe, 2007; Tse, Sheinberg, & Logothetis, 2002; Valsecchi, Betta, & Turatto, 2007). The outcome of this unresolved debate is not only of importance to the understanding of the nature of microsaccades and fixational eye movements in general, but it would also greatly contribute to the fine-tuning of the PMA.

The final chapter of this dissertation, chapter 4 will describe a psychophysical study conducted to determine whether microsaccades reflect motor preparation or attentional allocation.

References

Anagnostaras, S. G., Josselyn, S. A., Frankland, P. W., & Silva, A. J. (2000). Computer-assisted behavioral assessment of Pavlovian fear conditioning in mice. *Learning & Memory (Cold Spring Harbor, N.Y.)*, *7*(1), 58–72.

Andrews, M. W., & Rosenblum, L. A. (1994). Automated recording of individual performance and hand preference during joystick-task acquisition in group-living bonnet macaques (Macaca radiata). *Journal of Comparative Psychology (Washington, D.C. : 1983)*, *108*(4), 358–362.

Atkinson, J., & Braddick, O. (2010). Dorsal Stream Vulnerability: Interaction of Intrinsic Programmes and Acquired Developmental Disorders. *Journal of Vision*, *10*(7), 20–20. http://doi.org/10.1167/10.7.20

Azzopardi, P., Fallah, M., Gross, C. G., & Rodman, H. R. (2003). Response latencies of neurons in visual areas MT and MST of monkeys with striate cortex lesions. *Neuropsychologia*, *41*(13), 1738–1756. http://doi.org/10.1016/S0028-3932(03)00176-3

Backus, B. T., Fleet, D. J., Parker, A. J., & Heeger, D. J. (2001). Human cortical activity correlates with stereoscopic depth perception. *Journal of Neurophysiology*, *86*(4), 2054–2068.

Baker, K. C., & Aureli, F. (1997). Behavioural indicators of anxiety: an empirical test in chimpanzees. *Behaviour*, 1031–1050.

Berridge, C. W., Mitton, E., Clark, W., & Roth, R. H. (1999). Engagement in a non-escape (displacement) behavior elicits a selective and lateralized suppression of frontal cortical dopaminergic utilization in stress. *Synapse*.

Braddick, O. J., O'Brien, J. M., Wattam-Bell, J., Atkinson, J., Hartley, T., & Turner, R. (2001). Brain areas sensitive to coherent visual motion. *Perception*, *30*(1), 61–72.

Braddick, O., Atkinson, J., & Wattam-Bell, J. (2003). Normal and anomalous development of visual motion processing: motion coherence and “dorsal-stream vulnerability.” *Neuropsychologia*, *41*(13), 1769–1784. http://doi.org/10.1016/S0028-3932(03)00178-7

Bussey, T. J., & Saksida, L. M. (2007). Memory, perception, and the ventral visual-perirhinal-hippocampal stream: Thinking outside of the boxes. *Hippocampus*, *17*(9), 898–908. http://doi.org/10.1002/hipo.20320

Carrasco, M. (2011). Visual attention: The past 25 years. *Vision Research*, *51*(13), 1484–1525. http://doi.org/10.1016/j.visres.2011.04.012

Coleman, K., & Maier, A. (2010). The use of positive reinforcement training to reduce stereotypic behavior in rhesus macaques. *Applied Animal Behaviour Science*, *124*(3-4), 142–148. http://doi.org/10.1016/j.applanim.2010.02.008

Collins, C. E., Airey, D. C., & Young, N. A. (2010). Neuron densities vary across and within cortical areas in primates. Presented at the Proceedings of the National

Academy of Sciences of the United States of America
Conway, B. R., Moeller, S., & Tsao, D. Y. (2007). Specialized color modules in macaque extrastriate cortex. *Neuron*, *56*(3), 560–573. http://doi.org/10.1016/j.neuron.2007.10.008
Corbetta, M. (1998). Frontoparietal cortical networks for directing attention and the eye to visual locations: Identical, independent, or overlapping neural systems? *Proceedings of the National Academy of Sciences*, *95*(3), 831–838.
Craighero, L., Nascimben, M., & Fadiga, L. (2004). Eye Position Affects Orienting of Visuospatial Attention. *Current Biology*, *14*(4), 331–333. http://doi.org/10.1016/j.cub.2004.01.054
Cumming, B. G., & Parker, A. J. (1999). Binocular neurons in V1 of awake monkeys are selective for absolute, not relative, disparity. *The Journal of Neuroscience*, *19*(13), 5602–5618.
Daniel, P. M., & Whitteridge, D. (1961). The representation of the visual field on the cerebral cortex in monkeys. *The Journal of Physiology*, *159*(2), 203–221. http://doi.org/10.1113/jphysiol.1961.sp006803
Darwin, R. W., & Darwin, E. (1786). New Experiments on the Ocular Spectra of Light and Colours. By Robert Waring Darwin, MD; Communicated by Erasmus Darwin, MDFRS. *Philosophical Transactions of the Royal Society of …*.
DeAngelis, G. C., & Newsome, W. T. (1999). Organization of disparity-selective neurons in macaque area MT. *The Journal of Neuroscience*, *19*(4), 1398–1415.
Del Viva, M. M., Tozzi, A., Bargagna, S., & Cioni, G. (2015). Motion perception deficit in Down Syndrome. *Neuropsychologia*, *75*(C), 214–220. http://doi.org/10.1016/j.neuropsychologia.2015.06.005
Duffy, C. J., & Wurtz, R. H. (1991). Sensitivity of MST neurons to optic flow stimuli. I. A continuum of response selectivity to large-field stimuli. *Journal of Neurophysiology*, *65*(6), 1329–1345. http://doi.org/10.1152/jn.00847.2015
Dukelow, S. P., DeSouza, J. F., Culham, J. C., van den Berg, A. V., Menon, R. S., & Vilis, T. (2001). Distinguishing subregions of the human MT+ complex using visual fields and pursuit eye movements. *Journal of Neurophysiology*, *86*(4), 1991–2000.
Ebrey, T., & Koutalos, Y. (2001). Vertebrate Photoreceptors. *Progress in Retinal and Eye Research*, *20*(1), 49–94.
Engbert, R., & Kliegl, R. (2003). Microsaccades uncover the orientation of covert attention. *Vision Research*, *43*(9), 1035–1045. http://doi.org/10.1016/S0042-6989(03)00084-1
Fagot, J., & Bonté, E. (2010). Automated testing of cognitive performance in monkeys: Use of a battery of computerized test systems by a troop of semi-free-ranging baboons (Papio papio). *Behavior Research Methods*, *42*(2), 507–516. http://doi.org/10.3758/BRM.42.2.507
Felleman, D. J., & Van Essen, D. C. (1991a). Distributed Hierarchical Processing in the Primate Cerebral Cortex. *Cerebral Cortex*, *1*(1), 1–47. http://doi.org/10.1093/cercor/1.1.1
Felleman, D. J., & Van Essen, D. C. (1991b). Probing the Primate Visual Cortex: Pathways and Perspectives. "From Pigments to Perception" pp 227-237, Edited by A. Valberg and B.B. Lee, Plenum Press, New York, 1991.
Garner, J. P., & Mason, G. J. (2002). Evidence for a relationship between cage stereotypies and behavioural disinhibition in laboratory rodents. *Behavioural Brain Research*, *136*(1), 83–92.
Gazes, R. P., Brown, E. K., Basile, B. M., & Hampton, R. R. (2012). Automated cognitive

testing of monkeys in social groups yields results comparable to individual laboratory-based testing. *Animal Cognition*, *16*(3), 445–458. http://doi.org/10.1007/s10071-012-0585-8
Gollisch, T., & Meister, M. (2010). Eye smarter than scientists believed: neural computations in circuits of the retina. *Neuron*, *65*(2), 150–164. http://doi.org/10.1016/j.neuron.2009.12.009
Goodale, M. A., & Milner, A. D. (1992). Separate visual pathways for perception and action. *Trends in Neurosciences*, *15*(1), 20–25. http://doi.org/10.1016/0166-2236(92)90344-8
Graziano, M. S., Andersen, R. A., & Snowden, R. J. (1994). Tuning of MST neurons to spiral motions. *The Journal of Neuroscience*, *14*(1), 54–67.
Grinter, E. J., Maybery, M. T., & Badcock, D. R. (2010). Vision in developmental disorders: Is there a dorsal stream deficit? *Brain Research Bulletin*, *82*(3-4), 147–160. http://doi.org/10.1016/j.brainresbull.2010.02.016
Hafed, Z. M., & Clark, J. J. (2002). Microsaccades as an overt measure of covert attention shifts. *Vision Research*, *42*(22), 2533–2545.
Harris, J. D. (1943). Habituatory response decrement in the intact organism. *Psychological Bulletin*, *40*(6), 385–422. http://doi.org/10.1037/h0053918
Herculano-Houzel, S., Collins, C. E., Wong, P., & Kaas, J. H. (2007). Cellular scaling rules for primate brains. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(9), 3562–3567. http://doi.org/10.1073/pnas.0611396104
Heyclt, Von Der, R., Peterhans, E., & Baurngartner, G. (1984). Illusory contours and cortical neuron responses. *Science*.
Horowitz, T. S., Fine, E. M., Fencsik, D. E., Yurgenson, S., & Wolfe, J. M. (2007). Fixational eye movements are not an index of covert attention. *Psychological Science*, *18*(4), 356–363.
James, W. (1890). The Principles of Psychology. *Psychology*.
Kaas, J. H. (2004). The evolution of the visual system in primates. *The Visual Neurosciences*.
Kaplan, E. (2004). The M, P, and K pathways of the primate visual system. *The Visual Neurosciences*.
Kim, J., Norton, D., McBain, R., Ongur, D., & Chen, Y. (2013). Deficient biological motion perception in schizophrenia: results from a motion noise paradigm. *Frontiers in Psychology*, *4*, 391. http://doi.org/10.3389/fpsyg.2013.00391
Kobayashi, H., & Kohshima, S. (2001). Unique morphology of the human eye and its adaptive meaning: comparative studies on external morphology of the primate eye. *Journal of Human Evolution*, *40*(5), 419–435. http://doi.org/10.1006/jhev.2001.0468
Leuba, G., & Kraftsik, R. (1994). Changes in volume, surface estimate, three-dimensional shape and total number of neurons of the human primary visual cortex from midgestation until old age. *Anatomy and Embryology*, *190*(4), 351–366.
Maestripieri, D., Martel, F. L., Nevison, C. M., Simpson, M., & Keverne, E. B. (1991). Anxiety in rhesus monkey infants in relation to interactions with their mother and other social companions. *Developmental Psychobiology*, *24*(8), 571–581.
Mandell, D. J., & Sackett, G. P. (2008). A computer touch screen system and training procedure for use with primate infants: Results from pigtail monkeys (Macaca nemestrina). *Developmental Psychobiology*, *50*(2), 160–170.

http://doi.org/10.1002/dev.20251
Martinez-Conde, S., Macknik, S. L., Troncoso, X. G., & Hubel, D. H. (2009). Microsaccades: a neurophysiological analysis. *Trends in Neurosciences*, *32*(9), 463–475. http://doi.org/10.1016/j.tins.2009.05.006
Mason, G. J. (1991). Stereotypies: a critical review. *Animal Behaviour*, *41*(6), 1015–1037.
Maunsell, J. H., & Van Essen, D. C. (1983). Functional properties of neurons in middle temporal visual area of the macaque monkey. I. Selectivity for stimulus direction, speed, and orientation. *Journal of Neurophysiology*, *49*(5), 1127–1147.
McFarland, D. J. (1966). On the causal and functional significance of displacement activities. *Zeitschrift Für Tierpsychologie*, *23*(2), 217–235.
Miller, B., Lim, A. N., Heidbreder, A. F., & Black, K. J. (2016). An Automated Motion Detection and Reward System for Animal Training. *Cureus*, *7*(12), 1–15. http://doi.org/10.7759/cureus.397
Milner, A. D., & Goodale, M. A. (2008). Two visual systems re-viewed. *Neuropsychologia*, *46*(3), 774–785. http://doi.org/10.1016/j.neuropsychologia.2007.10.005
Mishkin, M., & Ungerleider, L. G. (1982). Contribution of striate inputs to the visuospatial functions of parieto-preoccipital cortex in monkeys. *Behavioural Brain Research*, *6*(1), 57–77.
Mollon, J. D., & Bowmaker, J. K. (1992). The spatial arrangement of cones in the primate fovea. *Nature*.
Moore, T. (2003). Microstimulation of the Frontal Eye Field and Its Effects on Covert Spatial Attention. *Journal of Neurophysiology*, *91*(1), 152–162. http://doi.org/10.1152/jn.00741.2002
Moore, T., & Zirnsak, M. (2015). Neural Mechanisms of Selective Visual Attention. Annual Review of Psychology 2017 68:1, 47-72
Moran, J., & Desimone, R. (1985). Selective attention gates visual processing in the extrastriate cortex. *Science*, *229*(4715), 782–784.
Noudoost, B., Chang, M. H., Steinmetz, N. A., & Moore, T. (2010). Top-down control of visual attention. *Current Opinion in Neurobiology*, *20*(2), 183–190. http://doi.org/10.1016/j.conb.2010.02.003
O'Craven, K. M., & Kanwisher, N. (2000). Mental imagery of faces and places activates corresponding stimulus-specific brain regions. *Journal of Cognitive Neuroscience*, *12*(6), 1013–1023. http://doi.org/10.1162/08989290051137549
Orban, G. A., Lagae, L., Verri, A., Raiguel, S., Xiao, D., Maes, H., & Torre, V. (1992). First-order analysis of optical flow in monkey brain. *Proceedings of the National Academy of Sciences*, *89*(7), 2595–2599.
Parker, A. J. (2007). Binocular depth perception and the cerebral cortex. *Nature Reviews Neuroscience*, *8*(5), 379–391. http://doi.org/10.1038/nrn2131
Poggio, G. F., Gonzalez, F., & Krause, F. (1988). Stereoscopic mechanisms in monkey visual cortex: binocular correlation and disparity selectivity. *The Journal of Neuroscience*, *8*(12), 4531–4550.
Posner, M. I., Snyder, C. R., & Davidson, B. J. (1980). Attention and the detection of signals. *Journal of Experimental Psychology: General*, *109*(2), 160.
Richardson, W. K., Washburn, D. A., Hopkins, W. D., Savage-Rumbaugh, E. S., & Rumbaugh, D. M. (1990). The NASA/LRC Computerized Test System. *Behavior Research Methods, Instruments, & Computers*, *22*(2), 127–131.
Ridley, R. M., & Baker, H. F. (1982). Stereotypy in monkeys and humans.

Psychological Medicine, *12*(1), 61–72.
Rizzolatti, G., Riggio, L., Dascola, I., & Umiltá, C. (1987). Reorienting attention across the horizontal and vertical meridians: evidence in favor of a premotor theory of attention. *Neuropsychologia*, *25*(1A), 31–40.
Rolfs, M., Engbert, R., & Kliegl, R. (2005). Crossmodal coupling of oculomotor control and spatial attention in vision and audition. *Experimental Brain Research*, *166*(3-4), 427–439. http://doi.org/10.1007/s00221-005-2382-y
Roy, J. P., Komatsu, H., & Wurtz, R. H. (1992). Disparity sensitivity of neurons in monkey extrastriate area MST. *The Journal of Neuroscience*, *12*(7), 2478–2492.
Russell, W., & Burch, R. L. (2009). The Principles of Humane Experimental Technique. London, Methuen, 1959. P-238.
Saito, H.-A., Yukie, M., Tanaka, K., Hikosaka, K., Fukada, Y., & Iwai, E. (1986). Integration of direction signals of image motion in the superior temporal sulcus of the macaque monkey. *The Journal of Neuroscience*, *6*(1), 145–157.
Schenk, T., & McIntosh, R. D. (2010). Do we have independent visual streams for perception and action? *Cognitive Neuroscience*, *1*(1), 52–62. http://doi.org/10.1080/17588920903388950
Schino, G., Perretta, G., Taglioni, A. M., Monaco, V., & Troisi, A. (1996). Primate displacement activities as an ethopharmacological model of anxiety. *Anxiety*, *2*(4), 186–191.
Sheliga, B. M., Riggio, L., & Rizzolatti, G. (1994). Orienting of attention and eye movements. *Experimental Brain Research*, *98*(3), 507–522. http://doi.org/10.1007/BF00233988
Sherwood, C. C., & Hof, P. R. (2007). The evolution of neuron types and cortical histology in apes and humans. *Evolution*.
Smith, D. T., & Schenk, T. (2012). The Premotor theory of attention: Time to move on? *Neuropsychologia*, *50*(6), 1104–1114. http://doi.org/10.1016/j.neuropsychologia.2012.01.025
Smolyanskaya, A., Ruff, D. A., & Born, R. T. (2013). Joint tuning for direction of motion and binocular disparity in macaque MT is largely separable. *Journal of Neurophysiology*, *110*(12), 2806–2816. http://doi.org/10.1152/jn.00573.2013
Spencer, J., O'Brien, J., Riggs, K., Braddick, O., Atkinson, J., & Wattam-Bell, J. (2000). Motion processing in autism. *NeuroReport*, *11*(12), 2765–2767. http://doi.org/10.1097/00001756-200008210-00031
Takemura, A., Inoue, Y., Kawano, K., Quaia, C., & Miles, F. A. (2001). Single-unit activity in cortical area MST associated with disparity-vergence eye movements: evidence for population coding. *Journal of Neurophysiology*, *85*(5), 2245–2266.
Tanaka, K., & Saito, H. (1989a). Analysis of motion of the visual field by direction, expansion/contraction, and rotation cells clustered in the dorsal part of the medial superior temporal area of the macaque monkey. *Journal of Neurophysiology*, *62*(3), 626–641.
Tarou, L. R., & Bashaw, M. J. (2007). Maximizing the effectiveness of environmental enrichment: Suggestions from the experimental analysis of behavior. *Applied Animal Behaviour Science*, *102*(3-4), 189–204. http://doi.org/10.1016/j.applanim.2006.05.026
Thomas, O. M., Cumming, B. G., & Parker, A. J. (2002). A specialization for relative disparity in V2. *Nature Neuroscience*, *5*(5), 472–478. http://doi.org/10.1038/nn837
Troisi, A. (2002). Displacement activities as a behavioral measure of stress in

nonhuman primates and human subjects. *Stress: the International Journal on the Biology of Stress*, *5*(1), 47–54. http://doi.org/10.1080/10253890290012378
Truppa, V., Garofoli, D., Castorina, G., Piano Mortari, E., Natale, F., & Visalberghi, E. (2010). Identity concept learning in matching-to-sample tasks by tufted capuchin monkeys (Cebus apella). *Animal Cognition*, *13*(6), 835–848. http://doi.org/10.1007/s10071-010-0332-y
Tse, P. U., Sheinberg, D. L., & Logothetis, N. K. (2002). Fixational eye movements are not affected by abrupt onsets that capture attention. *Vision Research*.
Tyler, C. W. (1990). A stereoscopic view of visual processing streams. *Vision Research*, *30*(11), 1877–1895.
Ungerleider, L. G., & Desimone, R. (1986). Cortical connections of visual area MT in the macaque. *Journal of Comparative Neurology*, *248*(2), 190–222. http://doi.org/10.1002/cne.902480204
Ungerleider, L. G., & Mishkin, M. (1979). The striate projection zone in the superior temporal sulcus of Macaca mulatta: location and topographic organization. *Journal of Comparative Neurology*, *188*(3), 347–366. http://doi.org/10.1002/cne.901880302
Valsecchi, M., Betta, E., & Turatto, M. (2007). Visual oddballs induce prolonged microsaccadic inhibition. *Experimental Brain Research*, *177*(2), 196–208. http://doi.org/10.1007/s00221-006-0665-6
Van Essen, D. C. (2004). Organization of visual areas in macaque and human cerebral cortex. The visual neurosciences.
Ward, M. K., Bolding, M. S., Schultz, K. P., & Gamlin, P. D. (2015). Mapping the Macaque Superior Temporal Sulcus: Functional Delineation of Vergence and Version Eye-Movement-Related Activity. *The Journal of Neuroscience*, *35*(19), 7428–7442. http://doi.org/10.1523/JNEUROSCI.4203-14.2015
Washburn, D. A., Hopkins, W. D., & Rumbaugh, D. M. (1991). Perceived control in rhesus monkeys (Macaca mulatta): enhanced video-task performance. *Journal of Experimental Psychology: Animal Behavior Processes*, *17*(2), 123–129.
Watanabe, M., Tanaka, H., Uka, T., & Fujita, I. (2002). Disparity-Selective Neurons in Area V4 of Macaque Monkeys. *Journal of Neurophysiology*, *87*(4), 1960–1973. http://doi.org/10.1152/jn.00780.2000
Watson, S. L., Ward, J. P., Davis, K. B., & Stavisky, R. C. (1999). Scent-marking and cortisol response in the small-eared bushbaby (Otolemur garnettii). *Physiology & Behavior*, *66*(4), 695–699.
Weed, M. R., Taffe, M. A., Polis, I., Roberts, A. C., Robbins, T. W., Koob, G. F., et al. (1999). Performance norms for a rhesus monkey neuropsychological testing battery: acquisition and long-term performance. *Brain Research. Cognitive Brain Research*, *8*(3), 185–201.
Westlund, K. (2014). Training is enrichment—And beyond. *Applied Animal Behaviour Science*, *152*, 1–6.
Yang, Y., Liu, S., Chowdhury, S. A., DeAngelis, G. C., & Angelaki, D. E. (2011). Binocular disparity tuning and visual-vestibular congruency of multisensory neurons in macaque parietal cortex. *Journal of Neuroscience*, *31*(49), 17905–17916. http://doi.org/10.1523/JNEUROSCI.4032-11.2011

Motion and disparity in Macaque area MST are independent from one another

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Abstract

Within the visual cortex, information from sensory stimulation is first decomposed into features, represented by neurons in specialized visual areas, and later integrated to form a global percept. It has been suggested that at the processing level of macaque visual cortical area MST, the integration of the direction and the perceived distance of a moving stimulus, occurs; with such integration providing the basic computational input to the network responsible for self-motion perception. While the theory is elegant, the evidence for this process is rather scarce, with only few studies available in literature. Here, we recorded from area MST of gaze fixated awake macaque monkeys, while displaying stereoscopic random dot patch stimuli with various combinations of features. Surprisingly, we found that the interaction of motion direction and disparity did not explain more variance in the neuronal activity. In addition, on the population level, the decoding of motion direction seems to be rather independent from the decoding of disparity, suggesting that the integration of the two domains here considered, as basis for the computation of self-motion, is unlikely to take place in area MST.

Introduction

Amongst the over 30 visual processing areas identified in the macaque's cerebral cortex (Felleman & Van Essen, 1991), extrastriate areas V2, V3, V4, MT and MST (Brodmann areas 18 and 19) can be partitioned into two distinct pathways: the *form-colour* pathway (Zeki, 1978b; 1978a) and the *visual-motion* pathway (Maunsell & Van Essen, 1983c). Both pathways are traditionally thought to follow a serial and hierarchical functional organization, according to which, lower areas serve as computational node to the processing of higher areas, with a certain degree of reciprocity (Felleman & Van Essen, 1991), for a review see Perry & Fallah, 2014. While most of the areas comprising these two pathways seem well defined regarding their respective hierarchical function; along the visual-motion pathway, the medial superior temporal area (MST) shows rather diversified selectivity. In macaque monkeys, MST can be anatomically partitioned into two subareas with distinct functions: a dorsal portion (MSTd), mainly composed of neurons with large receptive fields and selectivity to the basic motion components of optic flow (expansion, contraction, rotation and translation); and a ventral portion (MSTl), composed of neurons with smaller receptive fields and selectivity to linear motion direction, much resembling the properties of MT neurons (Tanaka, Sugita, Moriya, & Saito, 1993). Given its complex architecture and functionality, human and macaque studies suggest MSTd's involvement in a number of processes: heading perception (Britten & van Wezel, 2002); integration of motion information through feature decomposition of optic flow (Duffy & Wurtz, 1991; Graziano, Andersen, & Snowden, 1994; Orban et al., 1992; Saito et al., 1986; Tanaka & Saito, 1989); inertial motion in darkness (Takahashi et al., 2007); perceptual cue integration (Gu, Angelaki, & DeAngelis, 2008); gaze stabilization in smooth pursuit (Kawano, Inoue, Takemura,

Kodaka, & Miles, 1999; Takemura, Inoue, Kawano, Quaia, & Miles, 2001); integration of vestibular and visual cues (Sakata, Shibutani, & Kawano, 1983); visual spatial attention (Treue & Maunsell, 1996); visual working memory (Mendoza-Halliday, Torres, & Martinez-Trujillo, 2014) and integration of colour (Perry & Fallah, 2014; Tchernikov & Fallah, 2010). Moreover, within the most studied domain - the sensitivity to visual motion - MST's neurons located in both anatomical subdivisions MSTl and MSTd encode multiple feature dimensions at once: motion directions in both the spiral space (Graziano et al., 1994; Mineault, Khawaja, & Butts, 2012) and the linear space (Saito et al., 1986); binocular disparities (Roy, Komatsu, & Wurtz, 1992; Takemura et al., 2001; Yang, Liu, Chowdhury, DeAngelis, & Angelaki, 2011); the speed of a given motion pattern (Maunsell & Van Essen, 1983a; Price & Born, 2013). While these tuning preferences are most often considered in isolation, the potential dependence of the encoding of one feature on another is still under considerable debate, and yet may reveal important functions.

Disparity-dependent direction selectivity

MST's sensitivity to binocular disparity - the difference between the right and left retinal projections of an object - has often been an influential factor in this area's motion selectivity, as well as vestibular selectivity. A currently leading hypothesis is that binocular disparity sensitivity and motion selectivity are functionally integrated at the processing level of MST to infer self-motion (Roy et al., 1992; Takemura et al., 2001; Yang et al., 2011). Cells showing direction-dependent disparity tuning (or DDD) in which the tuning for motion depends on the disparity value considered, have been reported in area MST. (Roy et al., 1992; Roy & Wurtz, 1990). However, the

reported proportions of DDD cells in this area vary considerably. Roy et al. observed DDD tuning in around 40% of MST cells, while Yang et al. reported it in around 5% of the cells analysed. Considering also that multiple studies have suggested the DDD cells do not exist in MT (DeAngelis & Newsome, 1999; Maunsell & Van Essen, 1983b; Smolyanskaya, Ruff, & Born, 2013) - an area in close functional and anatomical proximity to MST – it seems that DDD cells might be exclusive to MST.

The present study aims at shedding some light onto the functional relationship between disparity selectivity and motion directionality in macaque area MST, by focussing on two experimental questions. First, to characterize the area contribution in the estimation of self-motion, we determine the proportion of cells showing DDD tuning. Secondly we quantify the involvement of each feature dimension, as well as their joint contribution, in explaining the overall population response to ultimately address the role of area MST in the processing of these two features along the visual-motion pathway.

Materials and Methods

Single unit activity was recorded from two rhesus monkeys (Macaca mulatta, both male; monkey I 10-year-old, weighed 9 kg; monkey N, 16-year-old, weighed 10kg), implanted with custom made titanium headpost and recording chamber (19 mm diameter), over the superior temporal sulcus (monkey I on the left hemisphere, monkey N on the right hemisphere). Surgeries were performed under general anaesthesia and post-surgical care using standard techniques. All procedures were conducted in accordance with German laws governing animal care and approved by the district government of Oldenburg, Lower Saxony, Germany.

Setup

The animals were seated in a primate chair for the duration of the experimental session. The animals were positioned in front of a rear projection screen (dlp Black Bead, Denmark, 171.5 x 107.2 cm) so that the screen laid 104 cm from the animal's eyes. Stereoscopic visual stimulation was achieved by mean of two coupled projectors (Projection Design F22, Norway, 60 Hz refresh rate, 1920 x 1200 pixels) and circular polarization filters (SX42 – HD). Binocular crosstalk, as assessed by a spectroradiometer (SpectraScan PR-650, Photo Research, USA), was below the minimum measurable luminance of 0.2 foot-lambert (or 0.68 candela/meter^2). Eye position was monitored with a binocular eye tracking system (Eyelink 1000, SR-Research, Canada) throughout the course of the experimental session at a sampling rate of 500 Hz.

Behavioral Tasks

Every recording session was comprised of two consecutive behavioural protocols. In the first part, we place a single probe stimulus at various locations to identify the neuron's receptive field (RF). Subsequently, in the second part, we characterized the neuron's response to visual stimuli placed at the centre of the RF, with various combination of motion and disparities. Basic behavioural requirements to the animals in the two protocols were identical: a red dot (2x2 degrees of visual angle - dva) placed at the centre of the projected screen, instructed the animal to engage eye fixation, and initiate the trial (monkey I by depressing a mechanical button, monkey N by touching a lever; both installed inside the primate chairs). The dim fixation point then lit up, signalling the animal that a new trial was about to start.

When, during the trial, the fixation point would dim down again, the animal was required to release the button, or turn the lever, within 500ms, to earn a drop of fluid reward. Breaking eye fixation at any time during a trial, reacting before a fixation dot dim, or fail to react to a fixation dot dim within the 500ms time window, would lead to the abortion of the trial and no reward would be delivered. Regardless of the outcome, after 1.5 seconds a new trial was presented. The mean reaction times were 290ms (sd 27 ms) for monkey I and 366ms (sd 25ms) for monkey N.

In the *mapping of the receptive field* protocol (RF protocol), upon correct initiation of the trial, a single random dot pattern (RDP, 4 dva in diameter, 20 dots, each measured 0.25 dva in diameter moving at speed of 10 dva/s, with zero-coherence in motion directions, at a luminance of 7.07 cd/m^2) would appear for 3 frames (~50 ms) at a random position on the projection screen. The stimulus then disappeared and, after one blank frame (16.67 ms), reappeared at a different and randomized location. At a random point in time during RDPs flashing (between 1500 and 3500 ms from the appearance of the first stimulus), the dimming of the fixation point described above would occur. The behavioural protocol was terminated after reaching 150 successful trials, which resulted in 5850 probes presented, over an x and y space of 41 * 41 dva around the centre (0,0) of the horopter, with positive and negative values around the fixation position (x = from -10 to 30, y = from -20 to 20).

The characterization of the neuronal sensitivity to different visual features (Tuning Protocol), was carried out in direct succession of the receptive field mapping protocol. Upon receptive field identification, a single RDP (with full motion coherence, variable diameter adjusted to the receptive field size determined through

online analysis, 200 dots of 0.25 dva each, with an average luminance of 12.8 cd/m^2), was placed at the centre of a neuron's RF and its x and y position was then kept constant throughout the experiment. The stimulus' motion domain (spiral or linear), motion direction (0, 45, 90, 135, 180, 235, 270, 315 degree, for linear motion, the values refer to the angles between dot velocity and the horizontal line; for spiral motion, the values refer to the angles between dot velocity and the radial line of the RDP aperture, see (Graziano et al., 1994)), binocular disparity (-2, -1.5, -1, -0.5, 0, 1, 1.5 degree) and speed (at 1 dva from RDP's centre), would rapidly and randomly change every 5 frames (83.33 ms). Here as in the RF protocol, the animal was required to depress the lever in within 500 ms after the dimming of the fixation point (between 1500 and 3500 ms from the appearance of the first stimulus). Each session of this experimental protocol requires 500 hit trials to complete, so that a total of ~13000 stimuli would be displayed. Considering the number of possible feature combinations (8 directions * 8 disparities * 8 speeds *2 motion domains = 1024), each stimulus would be displayed for 12 repetitions on average.

Data Collection

The recording electrodes (platinum/tungsten cores, quartz insulated, Thomas Recording, Germany, and FHC, ME), single tip as well as four channels (impedance between 0.8 and 2.5 MΩ) were either loaded into a multi-electrode manipulator (Tetrode Mini Matrix System, Thomas Recording, Germany) or into a custom made guide tube held on a chamber grid. The respective recording device was mounted on the recording chamber of the animal, prior the recording session. Consequent to manual adjustment of the *medio lateral* and *anterior posterior* coordinates on the x-y

table of the manipulator, the guide tubes was manually lowered enough to penetrate the superficial tissue covering the dura. The micro-drive system of the manipulator, by mean of a dedicated motor controller, would then lower the electrodes at ~10 μm/second, upon regular impedance monitoring by the experimenter. Electrical signals were amplified and then recorded with a sampling rate of 40 kHz and 16-bit precision, using an Omniplex acquisition system (Plexon, USA). After recording, the raw signal acquired was filtered with a 6-pole Bessel high pass filter (250 Hz cut-off) using the OfflineSorter V3 software (Plexon, USA). Single units were identified as clusters of similar waveforms, crossing an individually set detection threshold, and separated from the main noise cluster in the space of the first two PCs (for a review see Lewicki, 1998). We thus isolated 229 cells for monkey I and 18 cells from monkey N, with 154 for monkey I and 10 for monkey N showing clear responses to visual stimulation.

Data Analysis

Both protocols, employing a rapid series of stimuli presentations, were optimized for *reverse correlation* analysis (Bair, Cavanaugh, Smith, & Movshon, 2002; Borghuis et al., 2003; Chichilnisky, 2001; de Boer & Kuyper, 1968; Ringach, Hawken, & Shapley, 1997), where any given spike train is probabilistically associated with individual stimulus features. Given a range of latencies, stretching from 300 milliseconds before the spike to 50 ms after the spike, binned in 5 ms steps, we implemented the reverse correlation by first counting the number of total occurrences of a certain stimulus category (for example expansion) at a given

latency relative to the spike and then dividing this sum by the total occurrences of all categories comprising the corresponding feature (for example spiral motion). For directionally selective cells, for example, this procedure outputs a probability value for each motion direction at each latency. Ultimately the results are interpreted as the likelihood of each feature category, at each latency considered, to have preceded each spike in the spike train. It is important to note that in such two dimensional space (latency vs category), the sum of the probability of all categories at any latency is always equal to 1.

Two-dimensional Gaussian for receptive field mapping

To quantitatively estimate the size and the distance of the receptive field from the fixation point, on a cell-by-cell basis, we first identified the latency yielding the highest variance of spike counts for all probe locations, and fit a 2 dimensional Gaussian of the following form:

$$G = B + \left(A * exp\left(-\left(\frac{(x \cdot cos\theta - y \cdot sin\theta - x_0)^2}{2\sigma_x^2} + \frac{(y \cdot cos\theta + x \cdot sin\theta - y_0)^2}{2\sigma_y^2} \right) \right) \right)$$

where B is the baseline probability; A is the amplitude; x_0 and y_0 are the coordinate of the centre of the receptive field in degrees of visual angle; σ_x and σ_y are the standard deviation of the Gaussian in the two dimensions; θ is the orientation of the longer axis of the fitted ellipse. The size of the receptive field is defined as the area obtained considering 2 standard deviations and assuming an elliptical shape.

Piecewise Polynomial Interpolation for disparity tuning estimation

Disparity tuning of each cell was computed in MATLAB through a piecewise polynomial interpolation with a smoothing parameter of 0.99, using the built-in function *fittype* under the mode 'SmoothingSpline'.

Von Mises fit for directionality estimation

The tuning of each neuron to the motion stimuli, for both the linear and the spiral domains, was computed by fitting the probabilities of each motion direction, derived by the reverse correlation of the each neurons' spike train, to a von Mises distribution, a circular approximation of the normal distribution (Berens, 2008; Mineault et al., 2012; Smolyanskaya et al., 2013; Takahashi et al., 2007), of the following form:

$$f(x \mid \mu, \kappa, a, b) = b + a * \frac{e^{\kappa * \cos(x-\mu)}}{2\pi I_0(\kappa)}$$

where μ and $1/\kappa$ represent preferred direction and variance, a and b amplitude and baseline probability and the component $I_0(\kappa)$ is the modified Bessel function of order 0.

Negative Binomial Regression Model

To assess the amount of variability explained by the motion and the disparity, on a cell by cell basis, we built four generalized additive models considering spike count as response variable and disparity, direction and their putative interaction, as

predictors. Model 1 assumes that motion direction does not contribute to the variance of spike count:

$$E_{sc} = \exp(\beta_0 + \beta_1 \cdot disparity)$$

Model 2 assumes that disparity does not contribute to the variance of spike count:

$$E_{sc} = \exp(\beta_0 + \beta_2 \cdot direction)$$

Model 3 assumes both disparity and direction contribute to the variance independently:

$$E_{sc} = \exp(\beta_0 + \beta_1 \cdot disparity + \beta_2 \cdot direction)$$

Model 4 further adds an interaction term between disparity and direction:

$$E_{sc} = \exp(\beta_0 + \beta_1 \cdot disparity + \beta_2 \cdot direction + \beta_3 \cdot dispa\square ity \cdot direction)$$

It is important to note that in models considering the contribution of motion direction (m2, m3 and m4), this circular covariate was linearized with a Von Mises transformation, by adding a squared covariate in the regression models. Note also that the spike count E_{sc} consisted of the total number of spikes occurring within an 80ms time window, shifted according to the latency yielding the highest variability (*optimal latency*) assessed trough reverse correlation.

Principal component analysis for population decoding

In order to achieve the unsupervised clustering of feature domains analysis described in the results section, we first constructed a covariance matrix based on the spike count of the 154 cells we recorded from monkey I as variables, and the 512

stimuli of one stimulus category (linear or spiral) as observations. The covariance matrix is z-scored through observations, so as to normalize the neurons to their general firing rate. A principal component analysis (PCA) is then performed on the covariance matrix, using the build-in *pca* function of MATLAB. Once the clustering of stimuli in the subspace expanded by PCs were obtained, individual dots were marked post hoc according to stimuli features, so as to determine which stimulus feature drives the clustering. Finally, to quantify the performance of the classification between the clusters (as in Fig 3B), first the centroids of each category in the PC subspace were identified and then connected. Stimuli from the two categories were projected on this connecting axis and the area under receiver operative characteristic curve from the two distributions resulted in the performance of classification.

Results

General population statistics.

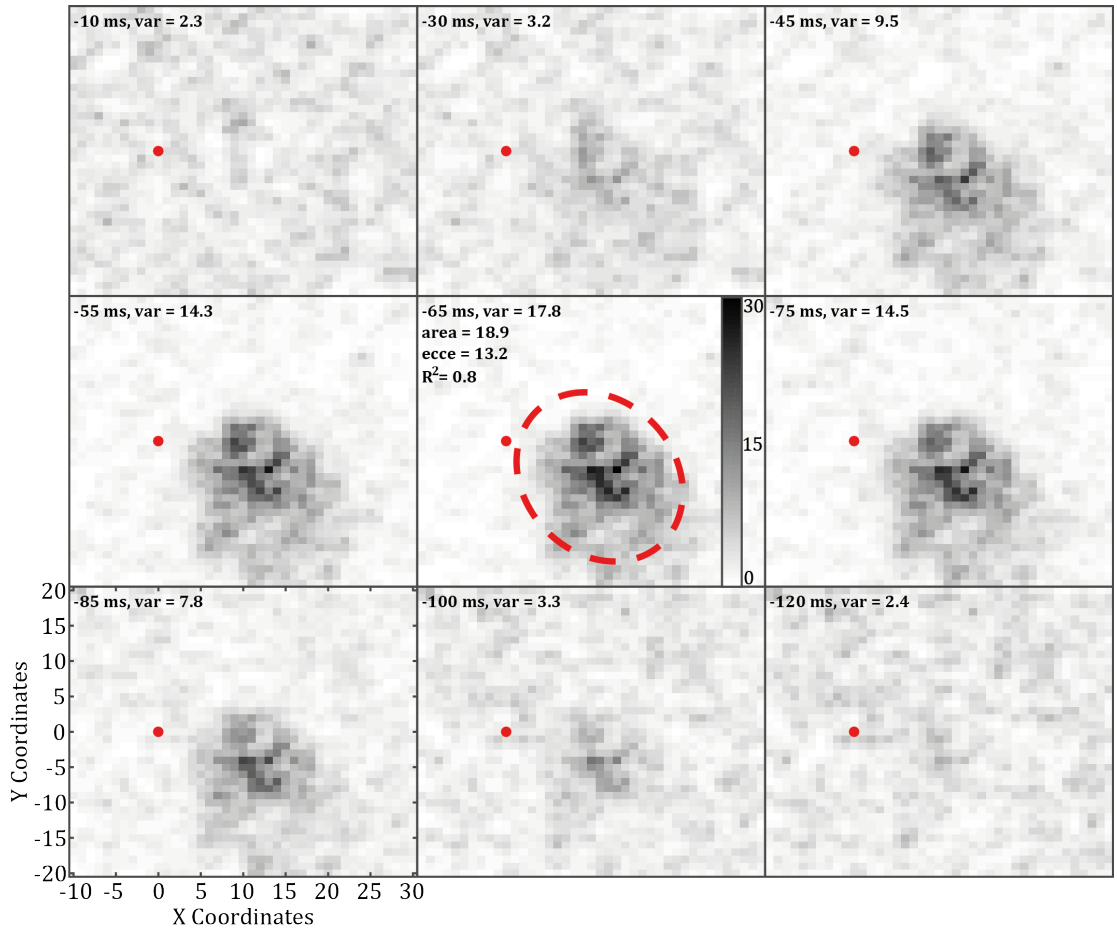

Figure 1 – Receptive field maps describing the dynamics of an example cell as assessed through reverse correlation and fit with a 2 dimensional Gaussian (see Methods – Data Analysis). Each subplot shows the spatial selectivity at incremental latencies. The greyscale map spans from white to black for low probability to high probability respectively. The array of probabilities depicted in the central plot, showing the latency containing the highest variance of the probabilities indicated at the top right of each panel (var), was fit with a 2 dimensional Gaussian to derive size (area) and eccentricity (ecce), in dva, with respect to the fixation point (red dot). Bar on central plot shows absolute count of occurrences of each location, from which probabilities are derived.

Of the 164 cells comprising the population in analysis, data to estimate the receptive field was available for 147 units. We applied a single inclusion criterion of an adjusted r-squared above 0.15, based on the fit of neuronal responses with a 2

dimensional Gaussian, to include only units for which at least 15% of the variance is explained. This reduced the data to dataset to 85 units, for which the size and location of the receptive field was computed (for monkey I receptive field population average is 20 dva, range 27 dva; average population eccentricity is dva, range 22 dva). Figure 1 illustrates the process of determining the receptive field dynamics for one example unit (cell-074-01+01-137.3), convoluted with a 3-by-3 kernel.

Throughout the 85 cells depicted in figure 2, no simple correlation was observed

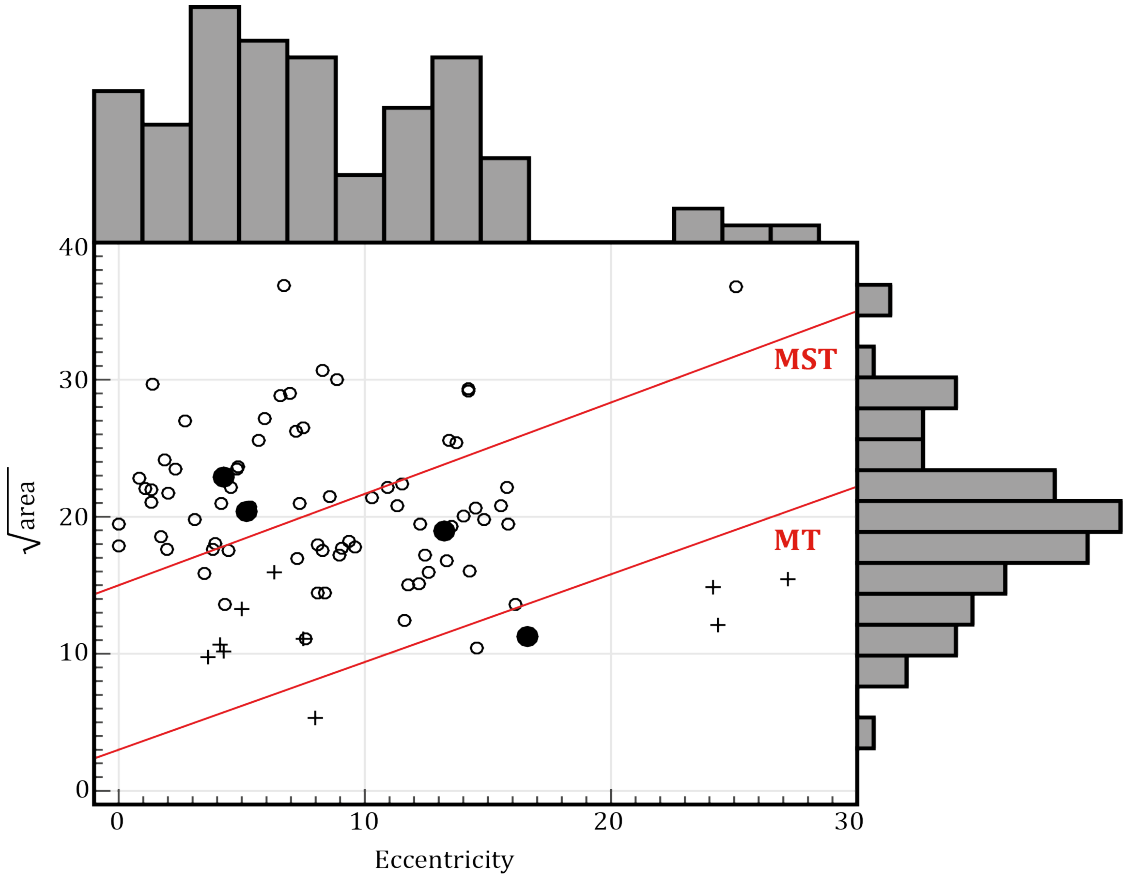

Figure 2 – Scatter plot and distribution histograms of receptive fields' size (square root of the area) and eccentricity for the 85 cells (75 from monkey I – circles, 10 from Monkey N – crosses) satisfying the inclusion criteria of adjusted $r^2 > 0.15$ to a 2 dimensional Gaussian fit. Red lines are derived from existing literature on MST and MT receptive field size and eccentricity (see results) and are here shown as reference for our data set. The filled circle indicates the example units.

between areas and eccentricities (rho = -0.06, p = 0.53; Spearman's rank correlation test – all values are rounded to the next integer). While areas range from 10 to 37 dva, with an average value of 20 dva, eccentricities range from 0 to 22 dva, covering mostly the right hemifield, with several units coding for the foveal region and often crossing the midline, towards the ipsilateral visual field, as expected for MST neurons (Saito et al., 1986; Tanaka & Saito, 1989). In line with existing literature of anesthetized monkeys on single cell activity of area MST and MT (Desimone & Ungerleider, 1986), units described in this study show receptive fields' size and eccentricity spanning all the way from values almost approaching MT's typical ratio, at the low end of the spectrum, to MST's typical ratio and beyond (red lines in figure 2 are extracted from Desimone & Ungerleider, 1986 and represent best fitting regression lines for MT and MST, histologically identified).

Similarly to the example receptive field map shown in figure 1, figure 3 illustrates the process of characterizing motion and disparity selectivity for the same example unit (cell-074-01+01-137.3). For each given cell, upon identification of the latency yielding highest variance, a von Mises distribution was fit to the probability of each motion category for both motion domains (see Methods) to extract preferred direction. To ensure that only directional cells where included in the analysis, inclusion criteria was set to an adjusted r^2 above 0.64, through which were accepted 89 out of 164 cells for spiral motion and 115 out 164 for linear motion (some units satisfied the criteria only for one of the two motion domains). Figure 4 summarizes the distribution of the preferred directions, for the two motion domains. In line with previous literature (Duffy & Wurtz, 1991) there is no evidence for an underlying non-uniform distribution of the preferred directions as assessed by the Rayleigh statistical test for non-uniformity of circular data (for spiral p = 0.059, for linear p =

0.152). Suggesting that neurons were sampled from an area representing all the motion direction with the same likelihood.

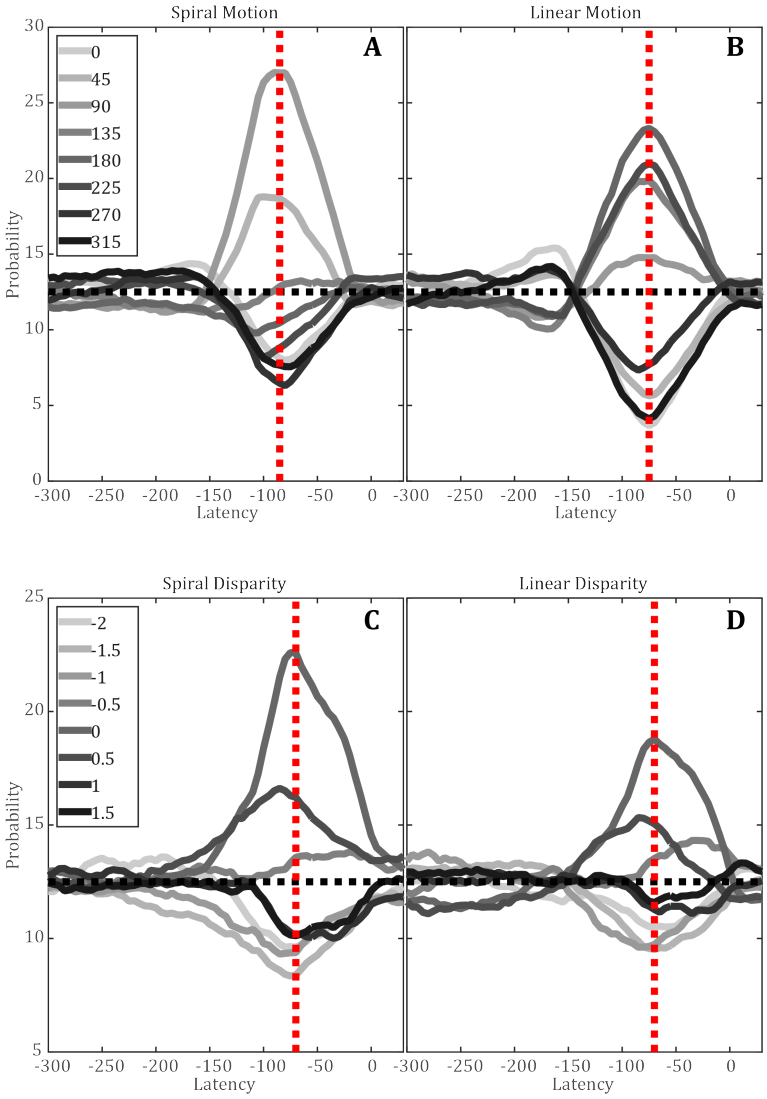

Figure 3 - Time course of motion directions and disparity selectivity for the example unit, based on 36194 spikes. Each subplot shows the probability of each motion category (A and B) or each disparity level (C and D) assessed in the spiral (A and C) and linear (B and D) domains, versus the temporal distance between each spike and each stimulus presentation. Latency 0 indicates simultaneous occurrence of spike and stimulus. Red dashed lines indicate the latency with the largest separation (highest variance), marking the time at which the unit shows optimal selectivity (A= -85 ms; B= -75 ms; C= -70 ms; D= -70 ms).

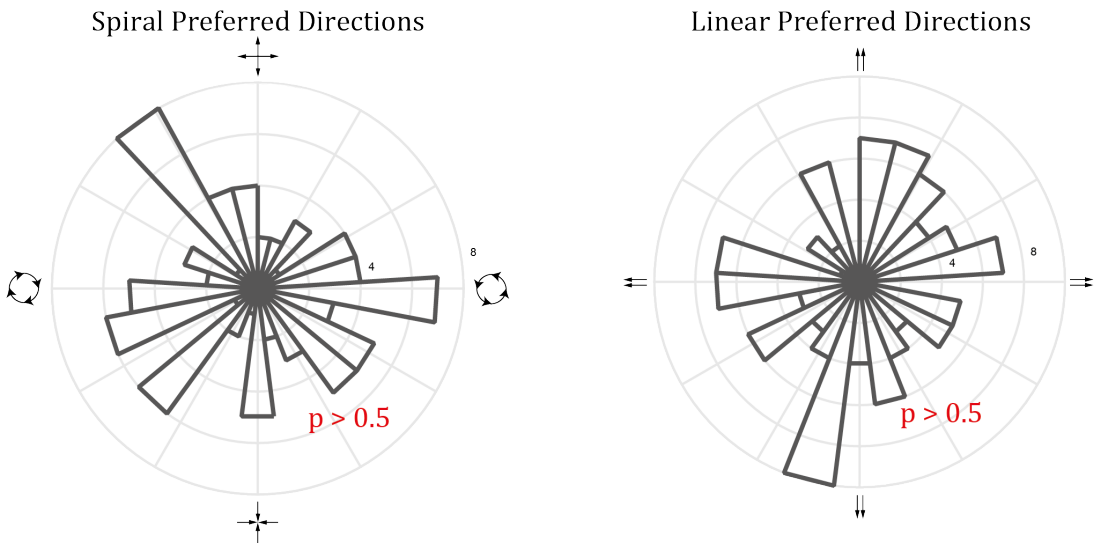

Figure 4 – Distribution of preferred directions for spiral motion (left) and linear motion (right). Only units with an adjusted r^2 above 0.64 are considered. P values, referring to Rayleigh test for non-uniformity of circular data.

Quantitative measurements of disparity selectivity throughout the population were based on two indices previously introduced by (Roy et al., 1992). Figure 5 shows the distribution of the *disparity index* (DI) derived with the formula:

$$DI = 1 - \left|\left(\frac{null - exp}{preferred - exp}\right)\right|$$

where *exp* represents the expected probability 0.125 (1 over 8, the number of disparities tested), *null* the lowest probability and *preferred* the highest. The resulting value indicates the strength of the disparity tuning for each given cell. Considering then the units with a disparity index above or equal to 0.2 (Roy et al., 1992), with the second index, the *zero index* (ZI), is possible to determine whether

the disparity selectivity refers to disparity zero (namely no binocular disparity) or either far or the near disparity, derived with the formula:

$$ZI = \frac{zero - exp}{\max - exp}$$

where the term *zero* indicates the probability for the disparity value 0, *max* is the probability for any non-zero category and *exp* is again the expected probability of 0.125. As a result any cell yielding a ZI above 1 is considered tuned to 0 retinal disparity and conversely, any cell with a ZI equal or below 1 is considered to be tuned to either the far or the near space (Roy et al., 1992).

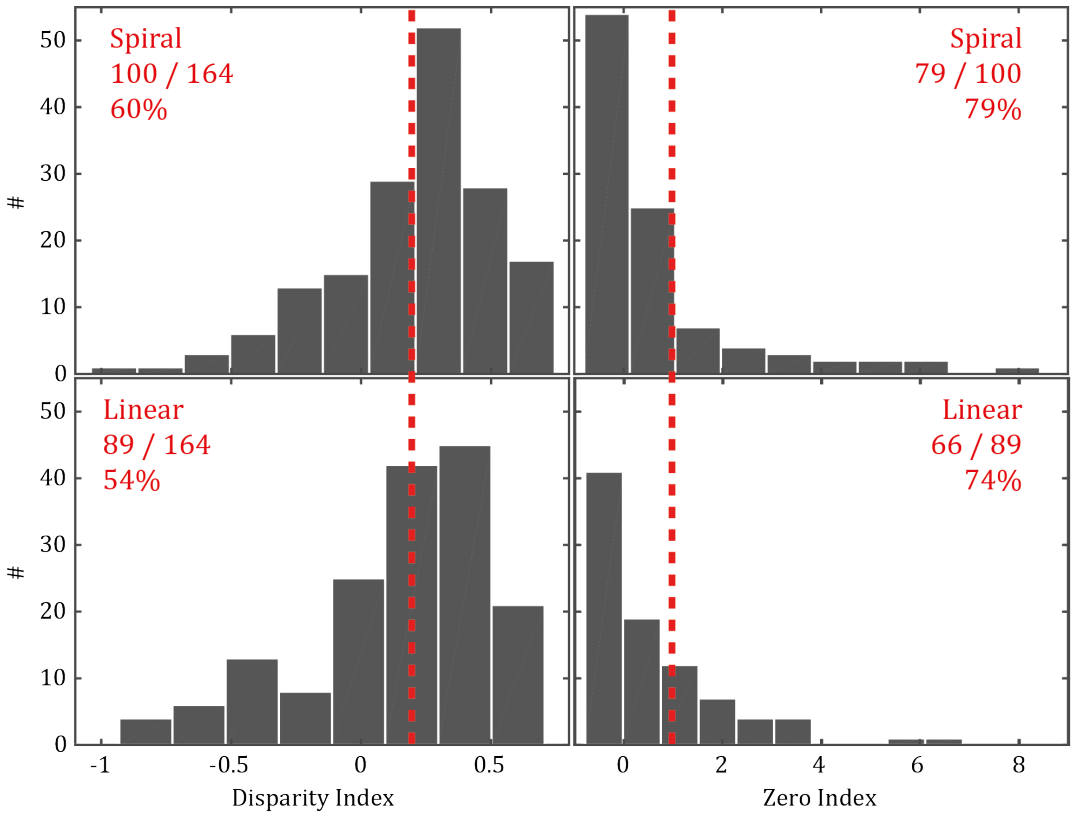

Figure 5 – Distributions of disparity index and zero index values across the population. Disparity index thresholds of 0.2 and zero index thresholds of 1 are indicated by the red dashed lines, while the ratios in red indicate the proportion of units falling respectively above and below the thresholds.

In line with previous reports (Maunsell & Van Essen, 1983a; 1983b; Takemura et al., 2001), but contrasting with other reports (Roy et al., 1992), 61% of the units recorded showed sensitivity to spiral disparity. 78% of these cells showed tuning to either far or near with equal proportion. 53% of the units showed sensitivity to linear disparity, with 74% tuned to either near or far space with equal proportion.

The population encode linear and spiral motion direction

In addition to single cell selectivity profiles, we also investigated: 1) which feature dimension can be decoded from the neuronal activities of the whole population; 2) if such decoders can be constructed for the features here considered; 3) how well can the decoders perform within each feature dimension and 4) how the different decoders relate to each other.

Specifically, we performed a principal component analysis (PCA) based on the spike counts of the 154 neurons recorded in monkey I in response to the 512 linear motion stimuli (see materials and methods). As a result, we obtain 154 principal components (PCs, weighted linear combinations of the 154 neurons), ranked by their contributions to the spike count variance across stimuli. Based on the responses of the first two PCs to the stimuli, we found that the 512 stimuli automatically formed eight clusters, which happened to align with the eight linear motion directions (Fig 6A). This made the combination of the first two PCs a very good decoder for motion direction. Based on the activities of these 154 neurons, it is also possible to reliably decode the direction of linear motion with the first two components (classification performances between neighbouring directions are all

above 93%, Fig 6B). Similarly, a separate PCA on the spiral motion stimuli also yielded a similar outcome. As a result motion directions can be decoded through an unsupervised clustering (Fig 6B).

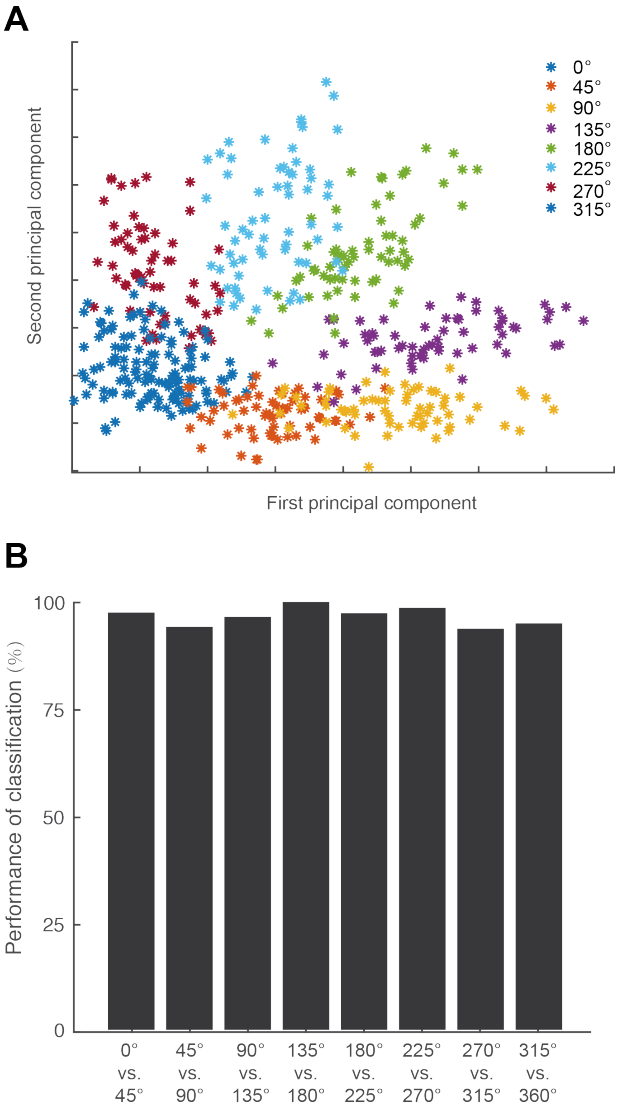

Figure 6 - The first two principal components decodes motion direction. A) the unsupervised clustering of stimuli in the subspace of the first two principal components. Each dot represents the neuronal response to a stimulus, projected on the first two principal components. The dots were coloured according to their spiral motion direction of the stimuli. B) the performance of classification between neighbouring motion directions.

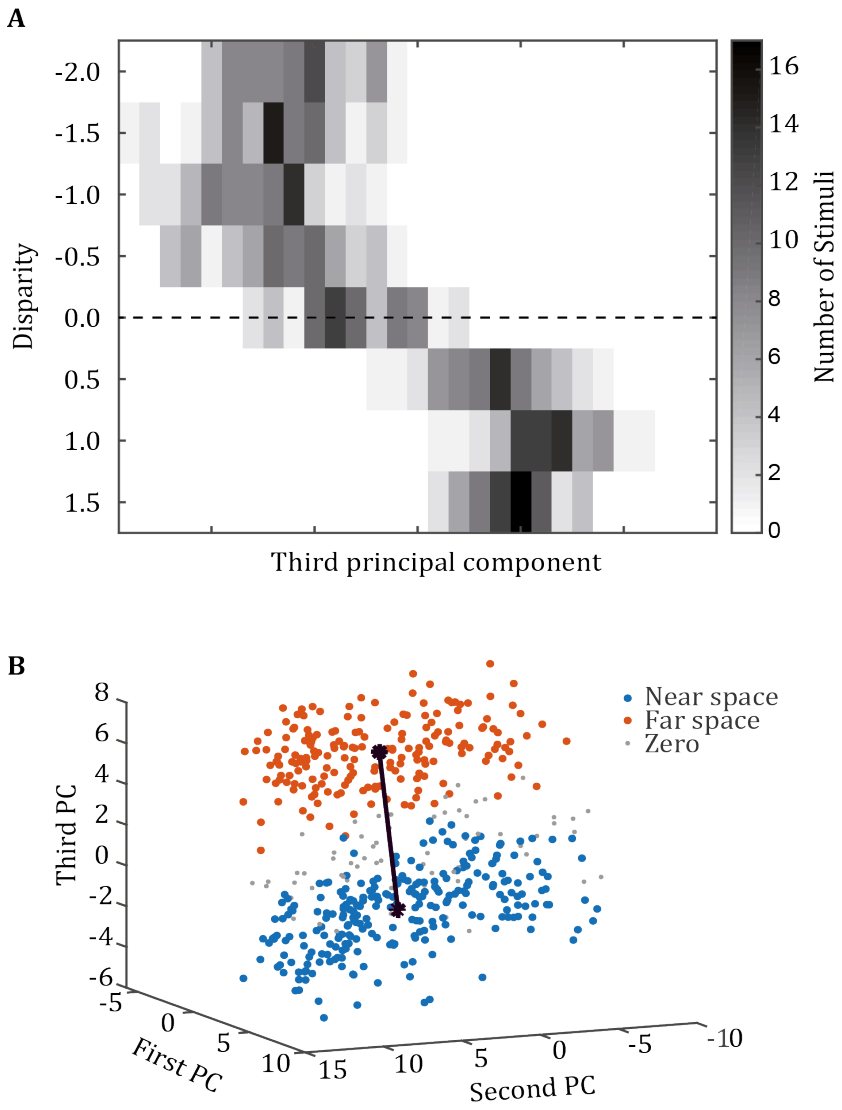

Figure 7 - The categorization of near and far stimuli is independent of the representation of motion direction. A) Near-far categorization with the third principal component. The greyscale map shows the distributions of stimuli with a certain disparity (vertical axis) on the third principal component (horizontal axis). B) The relationship between near-far decoder (third principal component) and motion direction decoder (first two principal components). Each dot represents the neuronal response to a stimulus, projected on the first three principal components. The dots were colored according to their relative depths with the fixation point. The difference between the center of near stimuli and far stimuli (black segment) is almost parallel to the third principal component axis.

The encoding of near-far categorization is independent from the encoding of other stimuli features

While the first two components encode the motion direction of the stimuli, the third component seems to be independently encoding disparity. Stimuli displayed in the near space (disparity smaller than zero) and far space (disparity bigger than zero) have distinct distributions in the third component (Fig 7A).

Furthermore, this representation of near-far categorization and the representation of motion direction are largely independent. As shown in Fig. 7B, In the 3-D space szplained by the first three PCs, we obtained the centroids of dots representing near stimuli (blue) and dots representing far stimuli (red), and create a disparity axis connecting the two centroids (dark black line). The smaller the angle between a given PC and this axis, the larger the PC contribute to the near-far categorization. We found the disparity axis is almost perpendicular to the plane expanded by the first two PCs (88°, Fig. 7B), which contains the representation of motion direction; while the third PC alone contributed 99.8% to the disparity axis.

Such clear independence of disparity encoding from motion direction encoding solicits the question whether it is even possible to categorize near stimuli from far stimuli regardless of what type of motion is displayed (linear or spiral). To test this, we obtained the disparity decoder from a PCA with data from monkey I with linear motion stimuli, and applied it on the spike count data from the same animal but with spiral motion stimuli, so as to guess which of the spiral motion stimuli were displayed in the near space and which in the far space. Compared post hoc with the real disparity values, the guessed near-far categorization is 98.75% correct, which shows that the near-far categorization in area MST is largely agnostic about the motion domain of the stimulus.

Interdependence of features in area MST

In order to probe any putative dependence of motion selectivity upon disparity change, as previously investigated for MST and MT (Roy et al., 1992; Smolyanskaya et al., 2013), the joint probability of motion and disparity, independently for the two motion domains, was calculated at the best latency of the reverse correlation.

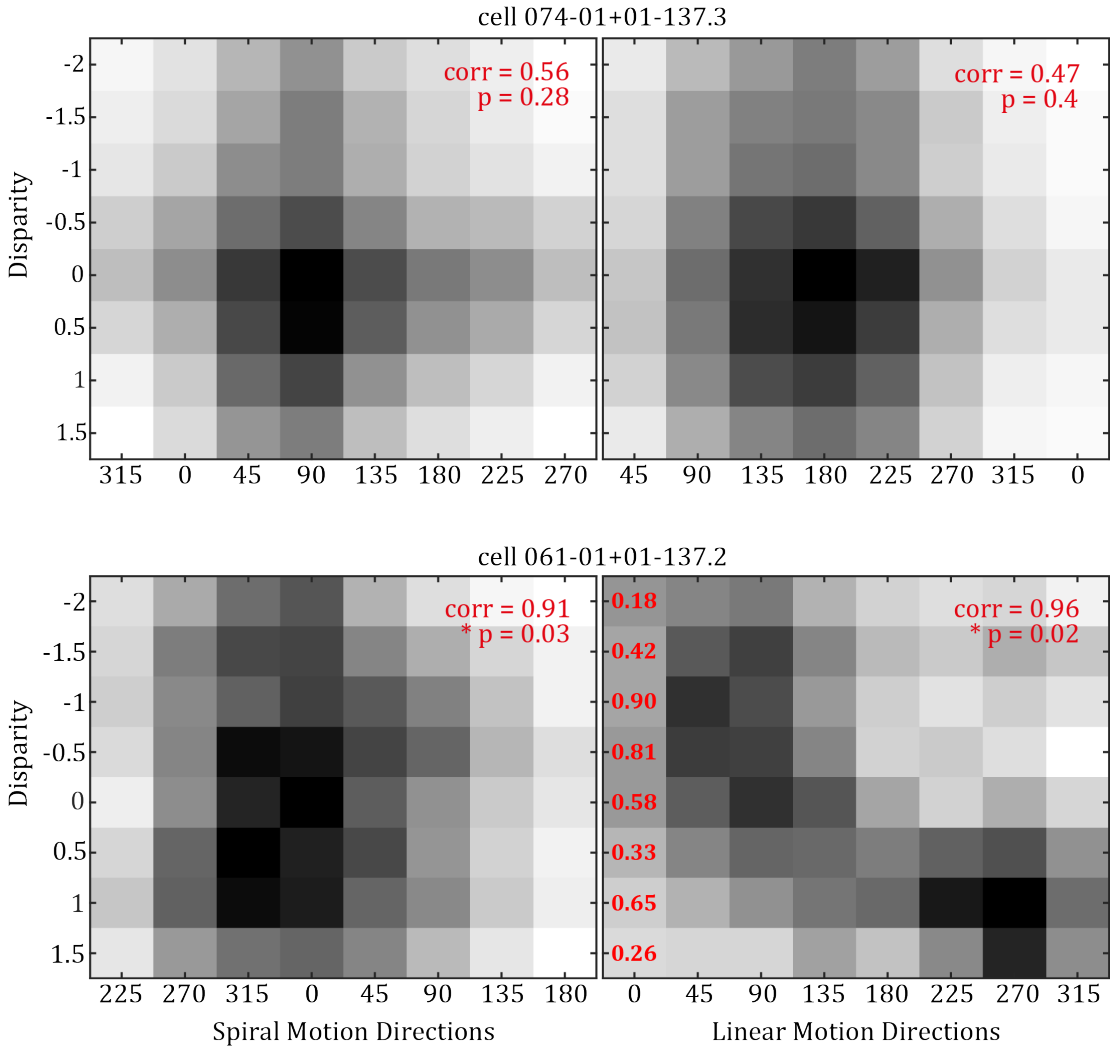

Figure 8 – Directionality and disparity selectivity joint probability heat maps for two example cells. While cell 074-01+01-137.2 (top left and right for spiral and linear motions respectively) shows no correlation between the two feature domains (Spiral p = 0.28, Linear p=0.4), cell 061-01+01-131.2 shows a significant switch of the preferred direction together with shifts in disparity (Spiral p = 0.03, Linear p = 0.02), with linear motion accounting for the greater shift of ~135 degrees.

Figure 6 upper row illustrates one example unit for which there is no change of directionality together with a change in disparity, for neither spiral nor linear motion. Figure 6 lower row illustrates another example unit, displaying a shift of preferred direction for linear motion stimuli depending on the stimulus disparity. The first example summarizes the entirety of the population, making the second example the only unit showing such property. Nonetheless a closer look at the only disparity-dependent direction cell tuning, based on firing rate rather than probability, reveals a rather sudden shift of linear motion selectivity at disparity 0.5 (Figure 9). Although this behaviour is in line with previous reports (Roy et al., 1992; Takemura et al., 2001), the proportion of cells showing this response pattern in our study seems critically discordant with the aforementioned studies.

Variance explained by individual features

In order to assess the second experimental question – how much of the neuronal variability is explained by disparity, direction and their putative interaction – four generalized linear models were tested (see Methods section). To illustrate the quality of each model to describe the variability of the spike count, the resulting distributions of deviances are reported in figure 10 for the whole population of 164 cells here considered (red dots in figure 10 represent median of the population). While *disparity only* (m1) describes around 6% and 4% of the variability for spiral and linear motion respectively; *direction only* (m2) reaches 37% and 43%, making this second feature the most dominant across the population (*t-test* pairwise comparison between m2 and m1 reveals p-value < 0.001, for both spiral and linear).

Figure 9 – Disparity dependent directional cell 061-01+01-137.2. Firing rate based tuning fit (error bars represent 95% confidence interval) at different disparity ordered from far to near and from top to bottom. Red texts indicate the resultant adjusted R^2 of the fit and the gain, as ratio between highest to lowest point of the curve. Red dots represent the preferred direction resulting from the fit. From near (negative values) to far (positive values) the preferred direction switches of ~ 135 degrees. Panels with grey background indicate those disparities at which the von Mises fit returns an adjusted r squared below our inclusion criteria.

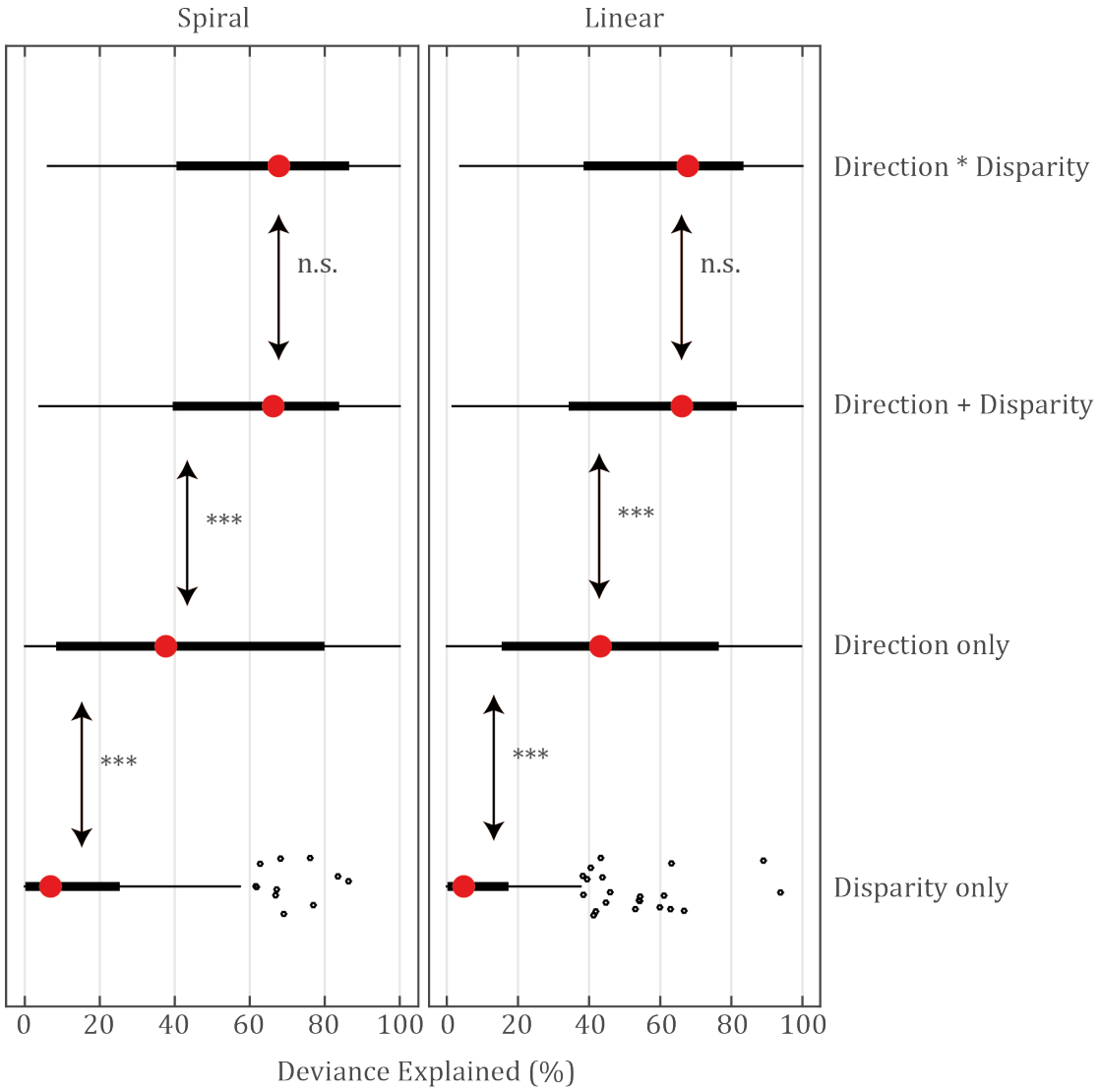

Figure 10 – Distributions of deviance explained by the four GLM models across 154 cells comprising the population in analysis. From bottom to top, each row indicates the distribution of deviances for a given model, for both spiral (left) and linear (right) motions, on the right. Red dots indicate the median of the distribution, while thick black lines indicate the 1st to 3rd quartile range, thin black lines the minimum and the maximum values of the distribution and circles the outliers. Three stars indicate p value below 0.001 to a t-test pairwise comparison and n.s. stands for non-significant difference.

Moreover, best describing the spike counts across the population are the models accounting for both feature dimensions at the same time. Model 3, considering an additive effect of direction and disparity, explains a median of 66% of the variance in both motion domains, significantly diverging from the explanatory power of both previous models (*t-test* pairwise comparison between m3 and m2, m3 and m1,

reveals p-value < 0.001, for both spiral and linear). Finally, on top of model 3, despite an interaction parameter is added in model 4, the resulting deviance of 67% for both motion domains, does not improve the explanatory power of model 3 (*t-test* pairwise comparison between m4 and m3 reveals p-value > 0.05, for both spiral and linear). This suggests that interaction between motion direction selectivity and disparity selectivity is not necessary to explain more variability in activity of single cells, which echoes with the PCA result, that disparity representation in the population is independent from the other features of the stimulus.

Discussion

After decades of research exploring MST's selectivity in the motion domain, two considerations seem to find ample agreement. The first one wants the middle superior temporal area to be responsible for the decomposition of optic flow information into the two major axis constituting it, namely the rotation and expansion/contraction (Duffy & Wurtz, 1991; Graziano et al., 1994; Orban et al., 1992; Saito et al., 1986; Tanaka & Saito, 1989). The second one relates to the proximity of this area to a variety of other anatomical as well as functional networks which, as a consequence, puts the area at the centre of a very diversified computational node for heading direction estimation and self-motion computation (Gu et al., 2008; Roy et al., 1992; Sakata et al., 1983; Yang et al., 2011). While this study is in substantial agreement with the first consideration, at the same time it fails to provide supporting evidences for the second. This section will first summarize the two main observations behind such dissonance and secondly will elaborate on its motives as well as consequences.

First, while almost half of the neurons here considered show tuning to binocular disparity either in the linear or in the spiral domain, in line with existing literature (Roy et al., 1992; Takemura et al., 2001; Yang et al., 2011); only 1 cell out of 164 showed disparity-dependent-direction selectivity, in striking contrast with the original 1992 study of Roy *et al* in which 40% of units there considered reversed their directionality with changes in disparity. Our proportion also fails at the comparison with a more conservative proportion of 5% DDD cells in the experiment of Yang and collaborators, 2011. The first factor one must consider when searching for potential explanations of differences in neuronal responses in higher order

visual areas, situated at the top of the *superior temporal sulcus* of the macaque brain, is the proximity with adjacent areas with similar but not identical functional properties. Additionally, in the case of MST one must also take into consideration that such area has been partitioned in several sub regions with rather diversified properties (Desimone & Ungerleider, 1986). For example, while showing very clear sensitivity to binocular disparity, no DDD cells were found in area MT (Smolyanskaya et al., 2013) and yet a very different sensitivity to disparity was described for area MSTl (Eifuku & Wurtz, 1999) compared to MSTd. Moreover, when comparing studies in which area localization was done histologically, with studies whose localization was MRT based (like in our case), different cellular responses can simply be due to having probed unidentified sub portions of the target area. Finally, while the discretisation of brain regions (Brodmann, 1909) sits at the very core of most neurophysiology works, one must consider that in reality, brain areas gradually fade into one another and that boarders strongly depend on the methodology and the statistics employed (Coalson et al., 2016).

The second major observation resulting from this study relates to the explanatory power of the two visual features here considered, motion direction and binocular disparity. Under the hypothesis that MST is indeed the node in which joint selectivity of depth and motion is computed and passed to next hierarchical processing stages, one would expect to find that complex interaction of the aforementioned features would significantly better explain the spiking behaviour of the population. On the contrary, considering that the *interaction* model did not better explain the distribution of spikes count than the *additive* model, our generalized linear model approach suggests independence of the two features at the

MST spiking level. Making disparity and direction independently accessible to later computational stages, brings MST's functionality closer to MT (DeAngelis & Newsome, 1999) and at the same time moves some of the numerous functional responsibilities of this area to later computational nodes (Raffi, Persiani, Piras, & Squatrito, 2014).

A number of studies has found disparity sensitive neurons in MT (DeAngelis & Newsome, 1999; Maunsell & Van Essen, 1983b; Smolyanskaya et al., 2013) and MST (Roy et al., 1992; Smolyanskaya et al., 2013; but see Yang et al., 2011). However, with our principal component analysis we found that the population decoder for disparity is more related to the coarse categorization of near space and far space rather than a continuous representation of depths. While some studies have found that MST neurons selectively respond to stimuli at different disparities, there has been no decisive conclusion on the role of the area MST in the perception of depth. As our analysis suggest, the contribution from MST to an accurate and fine perception of depth is probably very limited.

In conclusion, we confirmed that motion direction and binocular disparity are both represented in MST. However, individual cells and linear population decoding analyses both showed that the encoding of binocular disparity is independent from the other features of the stimuli. Therefore, while it seems reasonable that cells with disparity dependent direction tuning could be crucial for self-motion perception, such a integration may not happen directly in MST. Further investigations in areas higher in the visual hierarchy are necessary to finally reveal the underlying mechanism for self-motion perception.

References

Bair, W., Cavanaugh, J. R., Smith, M. A., & Movshon, J. A. (2002). The timing of response onset and offset in macaque visual neurons. *Journal of Neuroscience*, *22*(8), 3189–3205.

Berens, P. (2008). Comparing the feature selectivity of the gamma-band of the local field potential and the underlying spiking activity in primate visual cortex. *Frontiers in Systems Neuroscience*, *2*, 1–11. http://doi.org/10.3389/neuro.06.002.2008

Borghuis, B. G., Perge, J. A., Vajda, I., van Wezel, R. J. A., van de Grind, W. A., & Lankheet, M. J. M. (2003). The motion reverse correlation (MRC) method:. *Journal of Neuroscience Methods*, *123*(2), 153–166. http://doi.org/10.1016/S0165-0270(02)00347-3

Britten, K. H., & van Wezel, R. J. A. (2002). Area MST and Heading Perception in Macaque Monkeys. *Cerebral Cortex*, *12*(7), 692–701. http://doi.org/10.1093/cercor/12.7.692

Brodmann, K. (1909). Vergleichende Lokalisationslehre der Grosshirnrinde in ihren Prinzipien dargestellt auf Grund des Zellenbaues.

Chichilnisky, E. J. (2001). A simple white noise analysis of neuronal light responses. *Network (Bristol, England)*, *12*(2), 199–213.

Coalson, T. S., Robinson, E. C., Hacker, C. D., Harwell, J., Yacoub, E., Ugurbil, K., et al. (2016). A multi-modal parcellation of human cerebral cortex. *Nature*, 1–11. http://doi.org/10.1038/nature18933

de Boer, R., & Kuyper, P. (1968). Triggered correlation. *IEEE Transactions on Bio-Medical Engineering*, *15*(3), 169–179.

DeAngelis, G. C., & Newsome, W. T. (1999). Organization of disparity-selective neurons in macaque area MT. *The Journal of Neuroscience*, *19*(4), 1398–1415.

Desimone, R., & Ungerleider, L. G. (1986). Multiple visual areas in the caudal superior temporal sulcus of the macaque. *Journal of Comparative Neurology*, *248*(2), 164–189. http://doi.org/10.1002/cne.902480203

Duffy, C. J., & Wurtz, R. H. (1991). Sensitivity of MST neurons to optic flow stimuli. I. A continuum of response selectivity to large-field stimuli. *Journal of Neurophysiology*, *65*(6), 1329–1345. http://doi.org/10.1152/jn.00847.2015

Eifuku, S., & Wurtz, R. H. (1999). Response to motion in extrastriate area MSTl: disparity sensitivity. *Journal of Neurophysiology*, *82*(5), 2462–2475. http://doi.org/10.1146/annurev.ne.08.030185.002203

Felleman, D. J., & Van Essen, D. C. (1991). Distributed Hierarchical Processing in the Primate Cerebral Cortex. *Cerebral Cortex*, *1*(1), 1–47. http://doi.org/10.1093/cercor/1.1.1

Graziano, M. S., Andersen, R. A., & Snowden, R. J. (1994). Tuning of MST neurons to spiral motions. *The Journal of Neuroscience*, *14*(1), 54–67.

Gu, Y., Angelaki, D. E., & DeAngelis, G. C. (2008). Neural correlates of multisensory cue integration in macaque MSTd. *Nature Neuroscience*, *11*(10), 1201–1210. http://doi.org/10.1038/nn.2191

Kawano, K., Inoue, Y., Takemura, A., Kodaka, Y., & Miles, F. A. (1999). The role of MST neurons during ocular tracking in 3D space. *International Review of Neurobiology*, *44*, 49–63.

Lewicki, M. S. (1998). A review of methods for spike sorting: the detection and classification of neural action potentials. *Network (Bristol, England)*, *9*(4), R53–

78.
Maunsell, J. H., & Van Essen, D. C. (1983a). Functional properties of neurons in middle temporal visual area of the macaque monkey. I. Selectivity for stimulus direction, speed, and orientation. *Journal of Neurophysiology*, *49*(5), 1127–1147.
Maunsell, J. H., & Van Essen, D. C. (1983b). Functional properties of neurons in middle temporal visual area of the macaque monkey. II. Binocular interactions and sensitivity to binocular disparity. *Journal of Neurophysiology*, *49*(5), 1148–1167.
Maunsell, J. H., & Van Essen, D. C. (1983c). The connections of the middle temporal visual area (MT) and their relationship to a cortical hierarchy in the macaque monkey. *The Journal of Neuroscience*, *3*(12), 2563–2586.
Mendoza-Halliday, D., Torres, S., & Martinez-Trujillo, J. C. (2014). Sharp emergence of feature-selective sustained activity along the dorsal visual pathway. *Nature Publishing Group*, *17*(9), 1255–1262. http://doi.org/10.1038/nn.3785
Mineault, P. J., Khawaja, F. A., & Butts, D. A. (2012). Hierarchical processing of complex motion along the primate dorsal visual pathway. *Proceedings of the National Academy of Sciences of the United States of America* (Vol. 109, pp. E972–E980). Proceedings of the …. http://doi.org/10.1073/pnas.1115685109/-/DCSupplemental
Orban, G. A., Lagae, L., Verri, A., Raiguel, S., Xiao, D., Maes, H., & Torre, V. (1992). First-order analysis of optical flow in monkey brain. *Proceedings of the National Academy of Sciences*, *89*(7), 2595–2599.
Perry, C. J., & Fallah, M. (2014). Feature integration and object representations along the dorsal stream visual hierarchy. *Front Comput Neurosci*. http://doi.org/10.3389/fncom.2014.00084/abstract
Price, N. S. C., & Born, R. T. (2013). Adaptation to Speed in Macaque Middle Temporal and Medial Superior Temporal Areas. *The Journal of Neuroscience*, *33*(10), 4359–4368. http://doi.org/10.1523/JNEUROSCI.3165-12.2013
Raffi, M., Persiani, M., Piras, A., & Squatrito, S. (2014). Optic flow neurons in area PEc integrate eye and head position signals. *Neuroscience Letters*, *568*, 23–28. http://doi.org/10.1016/j.neulet.2014.03.042
Ringach, D. L., Hawken, M. J., & Shapley, R. (1997). Dynamics of orientation tuning in macaque primary visual cortex. *Nature*, *387*(6630), 281–284. http://doi.org/10.1038/387281a0
Roy, J. P., & Wurtz, R. H. (1990). The role of disparity-sensitive cortical neurons in signalling the direction of self-motion. *Nature*, *348*(6297), 160–162. http://doi.org/10.1038/348160a0
Roy, J. P., Komatsu, H., & Wurtz, R. H. (1992). Disparity sensitivity of neurons in monkey extrastriate area MST. *The Journal of Neuroscience*, *12*(7), 2478–2492.
Saito, H.-A., Yukie, M., Tanaka, K., Hikosaka, K., Fukada, Y., & Iwai, E. (1986). Integration of direction signals of image motion in the superior temporal sulcus of the macaque monkey. *The Journal of Neuroscience*, *6*(1), 145–157.
Sakata, H., Shibutani, H., & Kawano, K. (1983). Functional properties of visual tracking neurons in posterior parietal association cortex of the monkey. *Journal of Neurophysiology*, *49*(6), 1364–1380. http://doi.org/10.1152/jn.00847.2015
Smolyanskaya, A., Ruff, D. A., & Born, R. T. (2013). Joint tuning for direction of motion and binocular disparity in macaque MT is largely separable. *Journal of Neurophysiology*, *110*(12), 2806–2816. http://doi.org/10.1152/jn.00573.2013
Takahashi, K., Gu, Y., May, P. J., Newlands, S. D., DeAngelis, G. C., & Angelaki, D. E.

(2007). Multimodal coding of three-dimensional rotation and translation in area MSTd: comparison of visual and vestibular selectivity. *Journal of Neuroscience*, *27*(36), 9742–9756. http://doi.org/10.1523/JNEUROSCI.0817-07.2007

Takemura, A., Inoue, Y., Kawano, K., Quaia, C., & Miles, F. A. (2001). Single-unit activity in cortical area MST associated with disparity-vergence eye movements: evidence for population coding. *Journal of Neurophysiology*, *85*(5), 2245–2266.

Tanaka, K., & Saito, H.-A. (1989). Analysis of motion of the visual field by direction, expansion/contraction, and rotation cells clustered in the dorsal part of the medial superior temporal area of the macaque monkey. *Journal of Neurophysiology*, *62*(3), 626–641.

Tanaka, K., Sugita, Y., Moriya, M., & Saito, H. (1993). Analysis of object motion in the ventral part of the medial superior temporal area of the macaque visual cortex. *Journal of Neurophysiology*, *69*(1), 128–142.

Tchernikov, I., & Fallah, M. (2010). A Color Hierarchy for Automatic Target Selection. *PLoS ONE*, *5*(2), e9338–4. http://doi.org/10.1371/journal.pone.0009338

Treue, S., & Maunsell, J. H. (1996). Attentional modulation of visual motion processing in cortical areas MT and MST. *Nature*, *382*(6591), 539–541. http://doi.org/10.1038/382539a0

Yang, Y., Liu, S., Chowdhury, S. A., DeAngelis, G. C., & Angelaki, D. E. (2011). Binocular disparity tuning and visual-vestibular congruency of multisensory neurons in macaque parietal cortex. *Journal of Neuroscience*, *31*(49), 17905–17916. http://doi.org/10.1523/JNEUROSCI.4032-11.2011

Zeki, S. M. (1978a). Functional specialisation in the visual cortex of the rhesus monkey. *Nature,* Vol. 274, pp 423-428

Zeki, S. M. (1978b). Uniformity and diversity of structure and function in rhesus monkey prestriate visual cortex. *The Journal of Physiology*, *277*, 273–290. http://doi.org/10.1111/(ISSN)1469-7793

Supplementary material

List of cells included in the analysis, collected through a recording chamber placed on the left hemisphere of monkey Igg (-3.1 mm AP and -19.5 mm ML), and a recording chamber placed on the right hemisphere of monkey Nic (-2.6mm AP and 14.0mm ML)

Cell ID	date	x	y	z (micron)
'igg-002-01+01-129.1'	150407	1	-4	4323
'igg-002-01+01-129.2'	150407	1	-4	4323
'igg-004-01+01-129.1'	150410	-3	-2	12391
'igg-004-01+01-129.2'	150410	-3	-2	12391
'igg-006-01+01-129.1'	150415	-3	-5	10750
'igg-011-01+01-129.1'	150423	-4	0	7086
'igg-011-01+01-129.2'	150423	-4	0	7086
'igg-013-01+01-129.1'	150428	-5	1	10727
'igg-013-01+01-129.3'	150428	-5	1	10727
'igg-014-01+01-129.1'	150429	-3	-1	6149
'igg-014-01+01-129.2'	150429	-3	-1	6149
'igg-015-01+01-129.1'	150504	-4	1	10293
'igg-015-01+01-129.2'	150504	-4	1	10293
'igg-016-01+01-129.1'	150505	-4	1	9374
'igg-018-01+01-129.1'	150513	-4	1	11169
'igg-019-01+01-129.1'	150519	-4	1	8500
'igg-019-01+01-129.2'	150519	-4	1	8500
'igg-020-01+01-129.1'	150520	-4	1	8400
'igg-021-01+01-129.1'	150525	-4	1	8750
'igg-023-01+01-129.1'	150528	-4	1	7930
'igg-024-01+01-129.1'	150529	-4	1	9187
'igg-025-01+01-129.1'	150608	-4	1	6844
'igg-025-01+01-129.2'	150608	-4	1	6844
'igg-026-01+01-129.1'	150609	-4	1	9834
'igg-027-01+01-130.1'	150618	-4	1	11782
'igg-028-01+01-129.1'	150622	-4	1	12666
'igg-028-01+01-129.2'	150622	-4	1	12666
'igg-028-01+01-130.2'	150622	-4	1	12666
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'igg-029-01+01-129.2'	150623	-4	1	12500
'igg-029-01+01-129.3'	150623	-4	1	12500
'igg-032-01+01-129.1'	150728	-5	0	8311
'igg-032-01+01-129.2'	150728	-5	0	8311
'igg-033-01+01-129.1'	150729	-5	0	8256
'igg-033-01+01-129.2'	150729	-5	0	8256
'igg-033-01+01-129.3'	150729	-5	0	8256
'igg-034-01+01-129.1'	150730	-5	0	8250
'igg-034-01+01-129.4'	150730	-5	0	8250
'igg-035-01+01-129.1'	150731	-5	0	8550
'igg-035-01+01-129.2'	150731	-5	0	8550
'igg-035-01+01-129.3'	150731	-5	0	8550
'igg-036-01+01-129.1'	150803	-5	0	8550
'igg-037-01+01-129.1'	150804	-5	-1	7530
'igg-037-01+01-129.2'	150804	-5	-1	7530
'igg-037-01+01-129.3'	150804	-5	-1	7530
'igg-037-01+01-129.4'	150804	-5	-1	7530
'igg-037-01+01-130.1'	150804	-5	-1	7680
'igg-037-01+01-130.5'	150804	-5	-1	7680
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'igg-039-01+01-130.1'	150806	-5	-1	8005,00
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'igg-062-01+01-137.3'	151216	-5	-2	4500,00
'igg-062-01+01-137.4'	151216	-5	-2	4500,00
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'igg-065-01+01-129.3'	151230	-5	-2	8966,00
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'igg-065-01+01-129.5'	151230	-5	-2	8966,00
'igg-065-01+01-129.6'	151230	-5	-2	8966,00
'igg-065-01+01-133.1'	151230	-5	-2	9509,00
'igg-065-01+01-133.2'	151230	-5	-2	9509,00
'igg-066-01+01-129.1'	160104	-5	-2	3742,00
'igg-066-01+01-129.2'	160104	-5	-2	3742,00
'igg-066-01+01-129.3'	160104	-5	-2	3742,00
'igg-066-01+01-129.6'	160104	-5	-2	3742,00
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'igg-066-01+01-133.3'	160104	-5	-2	6889,00
'igg-067-01+01-129.1'	160105	-5	-1	8512,00
'igg-067-01+01-129.2'	160105	-5	-1	8512,00
'igg-067-01+01-129.3'	160105	-5	-1	8512,00
'igg-067-01+01-129.4'	160105	-5	-1	8512,00
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Excluded cells due to insufficient number of repetitions or no response to visual stimulation:

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BRIEF COMMUNICATION

A cage-based training, cognitive testing and enrichment system optimized for rhesus macaques in neuroscience research

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Abstract In neurophysiological studies with awake non-human primates (NHP), it is typically necessary to train the animals over a prolonged period of time on a behavioral paradigm before the actual data collection takes place. Rhesus monkeys (*Macaca mulatta*) are the most widely used primate animal models in system neuroscience. Inspired by existing joystick- or touch-screen-based systems designed for a variety of monkey species, we built and successfully employed a stand-alone cage-based training and testing system for rhesus monkeys (eXperimental Behavioral Intrument, XBI). The XBI is mobile and easy to handle by both experts and non-experts; animals can work with only minimal physical restraints, yet the ergonomic design successfully encourages stereotypical postures with a consistent positioning of the head relative to the screen. The XBI allows computer-controlled training of the monkeys with a large variety of behavioral tasks and reward protocols typically used in systems and cognitive neuroscience research.

Keywords Cognitive neuroscience · Non-human primates · Automated testing · Animal housing · Animal welfare · Environmental enrichment · Behavioral management

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Introduction

In conventional neurophysiological experimental settings, macaque monkeys normally are required to temporarily leave the housing facility to be trained in dedicated experimental settings outside their cage environment. Animals are therefore moved, by means of a *primate chair,* into a dedicated room or area (here referred to as a *setup*) equipped with the apparatuses needed to run the experiment. In the setup the animals are trained to solve behavioral and cognitive tasks, usually by operating levers, sensors, or touch-screens, while their behavior, for example eye and hand movements, is monitored and, once the training has been completed, their brain activity can be recorded. This classic procedure has been widely used for decades to bring animals to the expertise level required for a given experiment in cognitive neuroscience. However, such a procedure limits the scope of research questions in terms of social and motor behavior, limits self-paced engagement of the animal in the behavioral task, and may give rise to animal welfare concerns due to movement constraints during the sessions in the setup. Overcoming these limitations by providing a cage-based training and testing system opens opportunities to investigate a broader range of activities, such as social behavior, by keeping the animal in its housing environment, together with its social group members (for a review see: Drea, 2006; Fagot & Paleressompoulle, 2009), or motor tasks, by removing body movement constraints (McCluskey & Cullen, 2007). From a training perspective, the potentially more self-paced interaction of the animal with the device, rather than an experimentally imposed training schedule, might create a motivational advantage, with a corresponding learning benefit (Andrews & Rosenblum, 1994; Evans et al., 2008; Gazes et al., 2012; Washburn et al., 1989). From an animal welfare perspective, physical constraints and periods of separation from the peer group in the setup should be

refined, reduced, and replaced where possible (3R principle; Russell & Burch, 1959). Even though positive reinforcement training (Fernström et al., 2009; Perlman et al., 2012; Schapiro et al., 2003) is routinely used in neuroscience research to accustom animals to physical movement restraints step-by-step over extended periods, one cannot fully rule out a detrimental effect of movement restraints and setup isolation on well-being. Even for experiments that require physical constraints for scientific reasons, there can be early phases of behavioral training where movement restraints are not yet necessary. Such testing and training therefore could be conducted in the animal's housing environment, perhaps even while maintaining the monkey's social situation.

With the XBI (eXperimental Behavioral Intrument) we developed a cage-based, yet mobile and remotely controllable behavioral testing system for rhesus macaques in research-typical housing environments (for similar devices see Andrews & Rosenblum, 1994; Fagot & Bonté, 2010; Fagot & Paleressompoulle, 2009; Gazes et al., 2012; Mandell & Sackett, 2008; Rumbaugh, Hopkins, Washburn, & Savage-Rumbaugh, 1989; Richardson et al., 1990; Truppa et al., 2010; Washburn et al., 1989; Washburn & Rumbaugh, 1992; Weed et al., 1999). To minimize management requirements, the system is very robust and spray-water resistant. For maximal comparability, the XBI mimics conventional neuroscience settings in that it uses a precise fluid reward system. Also, the view of the visual display and physical access to the touch-screen is only minimally constrained, as is desirable for most cognitive neuroscience studies, while maintaining a uniform screen-eye distance. Finally, to allow behavioral assessment beyond the immediate task performance as registered by the touch screen, e.g., analyzing facial expressions of the animal, the XBI includes video surveillance with a full-body frontal view of the animals during task performance.

Here, we provide a technical description of the XBI and preliminary behavioral tests as proof-of-concept, including data on the initial experiences of naïve animals with the XBI. We also provide an account of our experience with the device in the daily routines of an animal housing facility.

Methods

The XBI is designed as a device for training and behavioral testing of rhesus macaques in their housing environment, and can also be used for environmental enrichment. It has been developed with five design requirements in mind. First, the device needs to be cage-mountable to allow easy access for the animals without human interference (Gazes et al., 2012; Richardson et al., 1990; Truppa et al., 2010; Weed et al., 1999) or having to restrain the animals during transportation to the setup. Second, the electronics and other internal parts need to be protected against dirt and spray water typically present in such environments. Third, the XBI must be robust to resist potential forces applied by the animals. Fourth, operating the device should be easy enough to be handled by different people, including non-scientific personnel. Finally, the XBI's hard- and software should be flexible enough to allow for a wide variety of training procedures and experimental task designs. This includes complex visually instructed cognitive tasks with well-defined stimulus viewing conditions and a high degree of flexibility in how the animal interacts with the device.

To address these needs the XBI's hardware is divided into two parts: the animal Interface (AI) and the control interface (CI) (Fig. 1). In the following, we will describe the main design features and technical specifications. More detailed information on custom-built parts or purchased equipment are available upon request from the corresponding author.

Animal interface (AI)

The AI, used inside the animal facility, is the part of the XBI to which the animal has access (Fig. 2). It consists of mechanical and electronic components. For handling and safety reasons, the mechanical parts are lightweight and, where possible, built from aluminum. The dimensions of the whole device are 106 cm × 93 cm × 30 cm (W × H × D) and it weighs approximately 23 kg. By reducing the size of the outer frame and using lighter panels, we expect to substantially reduce the weight of future versions. The AI can be stored or transported using a custom-built wheeled frame (Fig. 1A), providing comfortable access to the front and rear for cleaning and maintenance. The XBI can be used either with the cart (no lifting required) or by directly attaching it to the animal's enclosure (freeing the cart). For safety reasons all electronics of the AI run on low-voltage (maximum 12 V). Parts close to the animal that have to be powered include the touch-screen as the interaction device, a peristaltic pump for delivering reward, a loudspeaker to provide feedback or instructions, a surveillance camera for remote observation, and a cable connector box to minimize the number of cables between both interfaces. The rest of the XBI electronics reside remotely in the CI.

All animals had access to the AI in their home enclosures. These consisted of a room-sized group compartment and a smaller front compartment, physically separable by a dividing gate. The AI is attached to the front compartment with an aluminum-mounting frame, replacing one side panel of the compartment (Fig. 2B). For nine out of 11 animals the front compartment was connected to the group compartment such that the tested animal could be seated on-sight with peer animals. For two out of 11 animals the arrangement of the front compartment with respect to the group compartment did not allow visual contact.

The middle part of the XBI-AI is shaped as a funnel that narrows to the dimensions of a touch-screen (ELO 1537L),

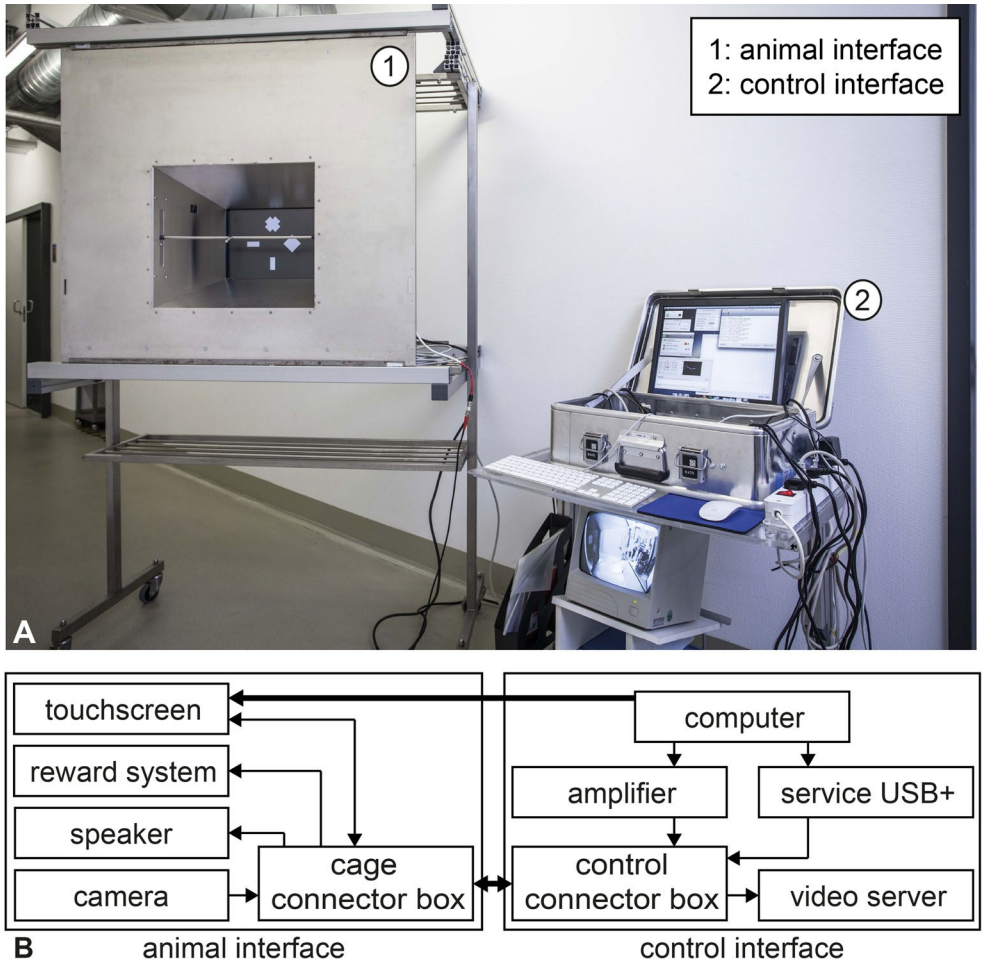

Fig. 1 A Image of the XBI. (1) Animal interface (AI) in the wheeled frame. A modified version of this frame is used to mount the AI on the front compartment in cases where it could not be anchored directly. (2) Control interface (CI) on a custom-made cart designed for easy relocation and accessibility. B Schematics of the XBI. Thick arrows represent connections between the two interfaces and thin arrows represent internal connections between elements of the same interface. The direction of an arrow represents the direction of the signal

such that only the 15-in. LCD display is accessible for the animal. The dimensions of the front opening of the funnel are 48.6 cm × 41 cm (W × H) and the distance to the screen is 26.2 cm. This distance was chosen based on prior experience with rhesus macaques interacting with a touch-screen in neurophysiology experiments in our laboratory (Gail et al., 2009; Westendorff et al., 2010). The display is operated at a resolution of 1024 × 768 at 75 Hz. The touch panel in front of the display utilizes ultrasonic waves in combination with piezoelectric transducers for the sensing of the touch signal with a positional accuracy of 2.5 mm or better. The touch-screen is designed to be resistant against mechanical forces. A stainless steel tube with 8-mm inner and 12-mm outer diameter reaches across the funnel, at a fixed distance of 24 cm from the touch-screen. Fluid reward is delivered through a 1-mm opening in a 30-mm spout in the middle of this tube, precisely controlled via a peristaltic pump (see below). The stainless steel tube with the spout can be rotated and adjusted horizontally and vertically in position. In this way it is possible to set it to comfortable positions for individual monkeys of different size. Given that the animals usually operate the device with the reward tube as close as possible to their mouths (Fig. S1), the eye-to-screen distance is around 28–32 cm, depending on an individual's head orientation and size. The screen size of 30.4 cm horizontal and 22.8 cm vertical provides 54° of visual angle along the horizontal and 42° along the vertical axis.

The AI's backside contains a reward unit consisting of a fluid container (2.5-L plastic bottle), connected to the metal reward tube using flexible PVC tubes with 6-mm inner diameter. These tubes are exchanged after every 2 weeks of use. A peristaltic pump (Verderflex OEM M025 DC) allows electronic control of the reward flow. This reward unit can be placed at either the left or right outer side of the funnel to adapt to different cage structures. The pump delivers 1.8 ml/s of activation time, with a precision of approximately 0.01 ml. The reward was precisely timed and dosed via the experimental control software, which is crucial for cognitive neuroscience testing.

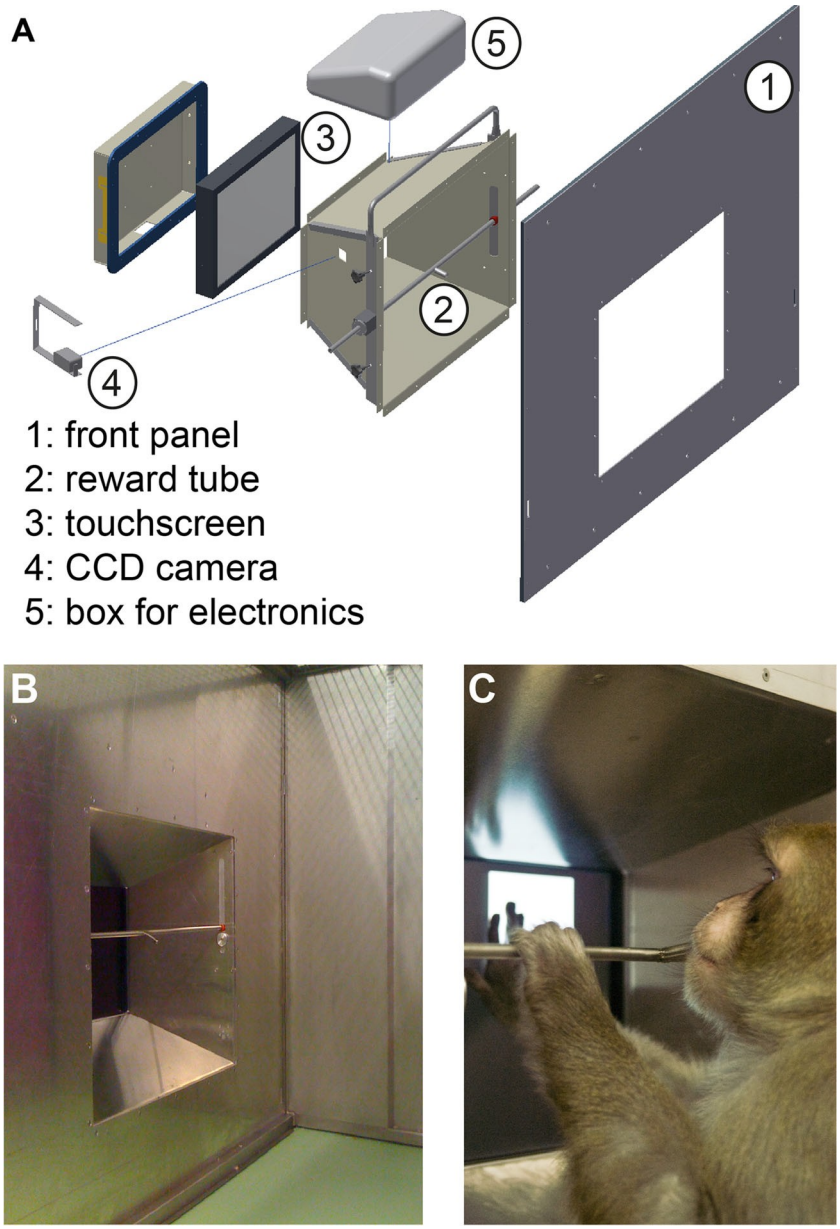

Fig. 2 A Exploded-view drawing of the XBI's front, facing the animal. From left to right: the protective frame for the touch-screen, the touch-screen, the funnel, and the reward tube, the mounting frame for cage anchoring. B XBI front from the animals' perspective. C One animal working at the XBI, in a trial of the touch-hold-release task

A mono sound transducer (Visaton, SpeaKa 130 mm) is glued on the outside of one of the funnel walls, using the wall as resonator for sound amplification. A compact 160° wide-angle CCD camera (ABUS TV7512) with 480 TV lines (438 kPixel) resolution is attached to a small opening in the metal funnel, protected by a clear polycarbonate window. The wide-angle view enables monitoring of the monkey and of the video screen at the same time.

Except for the VGA video cable, all connections (including power and signal lines) are routed to the CI via a custom-made connector box and a standard parallel D-SUB 25 connector cable (up to 15 m). Thus, only these two cables have to be routed to the outside of the animal facility. Within the connector cable we used multiple leads for power and ground lines to increase the amount of current that can be delivered through the cable.

The overall maximal nominal power consumption for the AI is 37.6 W (touch-screen 22 W, camera 0.6 W, active peristaltic pump 15 W). With an operating DC voltage of 12 V the XBI draws a maximum nominal current of 3.13 A. In practice we measured a total current of 1.5 A.

The AI is build to be operated for years, even in a dirty and humid work environment such as an animal facility. The front side facing the monkey cage is resistant against feces, urine

and direct water impact during cage cleaning procedures. On the backside of the AI all components are protected against spray water and particles larger than 2.5 mm. According to IEC 60529, the international protection marking level of the whole XBI is IP 33, with a substantially higher protection from the inside of the monkey cage.

Control interface

The CI consists of all the hardware and software needed for controlling the AI. It usually operates from outside the animal facility, weighs 12.2 kg and fits into a transportable box (W: 59 cm, H: 12 cm, D: 38 cm) for easy transport. The CI receives and sends signals from the AI through the VGA and connector cables. A second custom-made connector box distributes all connections from the connector cable to the individual components. The VGA cable as well as the serial RS232 connection from the touch-screen is connected to a computer that controls the XBI (Fig. 1). To control various devices from the computer, we integrated a USB interface (Service USB plus, Böning und Kallenbach). This platform provides multiple analogue and digital GPIOs (General Purpose Inputs/Outputs) which can deliver currents of up to 1.3 A. One of the digital outputs is used for operating the peristaltic pump, while the others have not been used in the context of the experiments described here. In addition, the computer's audio output is connected to a custom-built sound amplifier, which provides the audio signal for the sound transducer. The camera signal is routed to a video server (TRENDnet TV-VS1P) and from the video server to an analogue screen for on-site observation. The video server and the XBI computer are connected to the Local Area Network (LAN). In this way any computer on the LAN can be used for remotely controlling the XBI as well as recording videos and downloading data.

As long as the necessary interfaces are available, hardware requirements for the CI computer to run the XBI do not exceed those of standard desktop or laptop computers. We used VGA and USB connections with a RS232 adapter for the touch-screen in the AI, another USB port for the Service USB plus device, DVI-D for the CI's screen, and the headphone audio out for the audio amplifier. Although LAN connectivity is not necessary for the XBI to operate, it provides useful remote control capability. The video server is not directly connected to the computer but can be accessed via LAN. For the computer we either used an Apple Mac mini (2.5 GHz Intel i5, 8 GB RAM) or an Apple MacBook (2.4 GHz Intel Core 2 Duo, 2 GB RAM). The Mac OS is used since it interfaces optimally with MWorks (http://mworks-project.org/). This open-source software is a highly flexible C^{++}-based package for designing and real-time controlling behavioral tasks for neurophysiological and psychophysical experiments. MWorks can be expanded by dedicated software plug-ins to serve a wide range of experimental needs. Behavioral tasks are coded as XML files. A custom-made XML editor makes programming and modifying task files easy even for users without programming experience. MWorks runs in a client-server structure. The XBI can be run either as a standalone system or be operated via LAN. Data files are generated on the CI-computer that runs the server software.

Animals, grouping and fluid control

Overall, a total of 11 male rhesus monkeys (*Macaca mulatta*) were trained on the XBI within their housing facility. Three animals (Gro, Chi, and Zep) had access to the XBI as a group directly from the group compartment of their home cage. We report their behavioral data as group performance. We confirmed that an off-line analysis of the video footage allows for determining which animal was responsible for each of the XBI interactions. Since performance comparisons between individual animals are not the purpose of this report and since future ID tagging will render manual performance assignment to individuals unnecessary, we did not extend our pilot off-line analysis to the full data set.

The other eight animals had individual access to the XBI from within the smaller front compartment of their home enclosures. These eight animals were physically separated from their social group by a dividing wall separating the front compartment from the group compartment during the XBI sessions. Animals Fla, Alw, Nor, Odo, and Pru were in sight with their social group, while animals Han, Toa, and Zor were in sight only with members of other groups in the housing facility.

Most of the 11 animals had at least 2 h of unlimited access to water and fruits before and after each XBI session (Monday to Friday) and 24 h on all other days (see Table 1 for details). Two animals (Pru and Zor) were trained on the XBI under fluid control, in which the XBI provided the only access to fluid on working days (Monday to Friday). Animal Pru, in the early phases of the training, received plain water as reward. The other animals were rewarded with fruit-flavored sweetened water (active O2, Adelholzener) diluted with plain water at a ratio of 1:3.

Note that monkey Zor, a 12-year-old animal, was tested only during the development phase of the device.

Behavioral paradigms

To date four units of the XBI are in ongoing use and have been tested in various experiments. All experiments complied with institutional guidelines on Animal Care and Use of the German Primate Center and with European (Directive 2010/63/EU) and German national law and regulations, and were approved by regional authorities where necessary. Two experimental paradigms shall serve as examples of the

Table 1 For each of the 11 animals (rows) that took part in the two experiments the table lists the fluid access scheme (before and/or after the XBI session), which, if any, of the social group members was undergoing XBI training, which experiment or experiments were used, and the animals' age at the time of their first encounter with the device

Animal	Fluid access	XBI mates	Experiment	Age (years)
Alw	Before/After	-	AS	4
Chi	Before/After	Gro, Zep	AS	4
Fla	Before/After	-	AS	3
Gro	Before/After	Chi, Zep	AS	4
Han	Before/After	-	AS	3
Nor	Before/After	-	AS	3
Odo	Before/After	-	AS	7
Pru	XBI only, Before/After	Zor	FTS, THR, MS	7
Toa	After	-	AS	3
Zep	Before/After	Chi, Gro	AS	4
Zor	XBI only	Pru	THR	12

AS accommodation study, *FTS* free-task selection, *THR* touch-hold-release task, *MS* delayed match-to-sample

functionality of the system and acceptance by the animals. The first paradigm, the *accommodation study*, probed the ability of naïve animals to autonomously learn how to successfully operate a touch-screen on a basic level with no formal training (e.g., training to human handling). The second experiment, the *free-task selection* tested the XBI as a cognitive testing system and as an enrichment tool.

Accommodation study

Nine animals (age: 4–7 years) participated in the accommodation study (AS). They were naïve with respect to the XBI, and the accommodation study marked their first encounter with the device. Each animal had 90 min of daily access (typically from Monday to Friday) to the XBI over a period of 2 weeks excluding the weekend. None of the animals had previously participated in any type of cognitive training.

In the accommodation study the monkeys had to perform a simple touch task. At the beginning of each trial a steady blue (white for monkey Fla) square target stimulus 20 × 20 cm^2, was displayed on the screen on a black background. Touching the target for at least 100 ms triggered a fluid reward (successful trial). Touching the background terminated the trial without a reward (unsuccessful trial). Each trial was followed by an inter-trial interval during which the screen remained black. After 1 s without touching the screen the next trial started. This requirement of releasing the touch of the screen prevented the animals from successfully completing a series of tasks by simply keeping a finger (or any other body parts) on the screen. In addition to the delivery of the fluid reward, two different sounds indicated whether a trial was a success or not.

Free-task selection

One animal (Pru, 7 years old) participated in the *Free-Task Selection* (FTS). Note that before entering the free-task selection, the monkey underwent 4 months of positive reinforcement training to enter and exit the primate chair and 12 months of training on the XBI (see below for details).

In the free-task selection, at the beginning of each trial, four symbols were displayed on the screen (see Fig. 3), each one permanently associated with one subtask (Washburn et al., 1991):

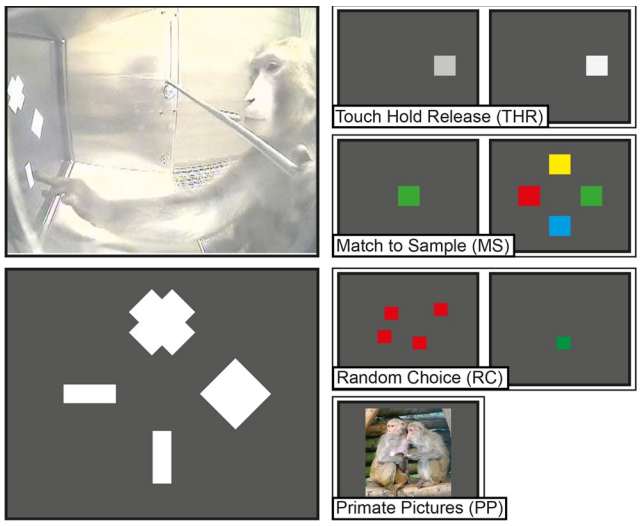

Fig. 3 Left column, top: view of the internal XBI camera while animal Pru chooses which task to execute next. Bottom: representation of the first frame of each trial of the four-choices tasks. Each white symbol is associated with one of the four tasks depicted in the right column, from top to bottom: cross for Touch Hold Release (THR), rhombus for Match to Sample (MS), vertical bar for Random Choice (RC), horizontal bar for Picture Presentation (PP, representative picture)

- *The cross* was associated with a simple *touch-hold-release* (THR) task, an extension of the touch task in the accommodation study. After the animal selected the cross symbol and after a 500-ms delay the four symbols were replaced by a gray square (5 × 5 cm). The animal had 4, 000 ms to reach for the target, which once touched, it brightened. After 500–2,500 ms of maintaining the touch the square dimmed. Now the animal had to release the touch within 500 ms to successfully complete the trial. The position of the stimulus on the screen and the required hold-time were randomized trial-by-trial. For this subtask the average duration of a successful trial was 4.8 s from when the animal selected the cross symbol.
- *The rhombus* was associated with a color-based *delayed match-to-sample* (MS) task. In MS trials the animal had to first touch a colored square (8 × 8 cm) at the center of the screen and after a randomized delay (1.5–3 s), touch the square with the same color amongst four differently colored squares of the same size displayed left, right, above, and below the screen center. The colors of the squares were randomly assigned trial-by-trial. The animal had to select the target within 4 s for correct performance, otherwise the trial would terminate without a reward. The same outcome would occur if the wrong stimulus was selected. For this subtask, the average length of a successful trial was 2.7 s.
- *The horizontal bar* was associated with a *random choice* (RC) task in which the animal had to touch one of four identical 3 × 3 cm red squares that were randomly positioned on the screen. Only one randomly determined stimulus would trigger a reward. By setting the amount of reward to four times the reward in the touch-hold-release and match-to-sample tasks the average reward was equated across these task types. For this subtask the average length of a successful trial was 3.6 s.
- *The vertical bar* was associated with a *primate picture* (PP) task in which one out of 20 photographs of non-human primates were shown on the screen for 5 s. After selection, no additional touch was necessary and no fluid reward was given in this task. For this subtask the average length of a trial was 5.6 s.

The animal was trained on the touch-hold-release task for over 6 months while technical aspects of the XBI prototype were under development and the match-to-sample task for 3 months. Once the monkey had reached a consistent performance above 80 % over 10 sessions (2 weeks) in these two tasks he was introduced to the free-task selection task. It included the two known tasks and the two novel tasks each associated with its corresponding symbol (see above). To determine the influence of relative reward amounts on relative choice probabilities, the first 31 sessions (3 months) of the free-task selection have been collected in two experimental conditions: lower reward RC task (20 sessions) versus higher reward RC task (11 sessions). We statistically verified the influence of relative reward amount on relative choice probabilities by the mean of the Multinomial Logit Model with estimated p-values using pairs cluster bootstrapped t-statistics (Cameron, Gelbach, & Miller, 2008).

Results

The XBI is designed for behavioral training, cognitive testing, and enrichment of physically unrestrained rhesus monkeys in an animal facility. Both of its components (the AI and the CI) are safely useable for the experimenter and the monkeys in this environment. Below, we will describe the usability of the XBI from the experimenter's perspective as well as behavioral example data recorded with the XBI as a proof-of-concept for cognitive testing and environmental enrichment.

Handling by the experimenters

A single person can handle the XBI safely. The use of a wheeled frame for storage and transport allows the XBI to be directly transferred to the sides of a cage avoiding the need to lift the AI. The mesh grid of the cage can be conveniently removed after the XBI has been mounted in front of it.

The XBI can be set up quickly. Given some experience, aligning the device to the cage and preparing a given experiment takes less than 10 min. In this time: the device is mounted to the cage replacing one of the cage's walls, is connected to permanently installed cables for the electronic communication between the two interfaces, the reward system is filled up, and the task and the video recording are initiated. From this point on the system is able to run autonomously, and without supervision, until it is manually stopped. If needed, the touch-screen as well as the cage are briefly cleaned before starting a new XBI session. This takes less than 10 min. To prevent technical malfunction by accumulating dirt the AI is thoroughly cleaned after about five sessions and the plastic tubes for reward delivery are replaced when needed.

The XBI is robust enough to endure repeated mounting and dismounting. In our setting one of the devices was used daily in three different rooms. Despite the substantial amount of mechanical stress of changing the location of the device multiple times per day over many months, malfunctions that delayed the starting procedure or prevented the system from running altogether were very rare. Most of these malfunctions resulted from cables not properly connected or partially damaged by the frequent use. Switching to more resistant cables eliminated such problems. Other technical issues were not observed. Across four separate XBI devices operated for more

than 1 year, only one bent reward tube and one broken peristaltic pump had to be exchanged.

The XBI requires little regular maintenance. The electronic devices attached to the AI are protected against spray water and dirt by their encapsulation. However, water and dirt on the touch surface can interfere with the assessment of behavioral performance by creating false triggers. To reduce dirt accumulation, the floor of the cage in which the XBI was placed was either a mesh or covered with dry wood-chip bedding. Accordingly, regular maintenance is inexpensive in terms of parts and materials. For hygienic reasons, we replaced the silicon tube (1 m) of the reward system after 2 weeks of use.

The XBI is easy to handle. Daily setup routines were performed not only by the experimenters, but also by students and technical assistants. It required only 2–3 sessions under supervision until a person was experienced enough to independently operate the XBI.

The XBI approach is scalable to a larger number of devices. Given the remote control and video surveillance options, we were able to simultaneously control our three XBI devices, even when they were located in different buildings. This allowed one single experimenter to remotely manage the training of several animals.

Monkey interactions

In the following section we will report behavioral data collected to probe (1) the XBI's attractiveness to naïve animals and (2) its suitability for cognitive tests.

Accommodation experiment: Unsupervised training of naïve animals in minimally restrained conditions

With the accommodation experiment we determined that naïve animals learn to operate the XBI without human instruction, supervision, or intervention. The animals were naïve in the sense that while they had received positive reinforcement training for their handling in the housing environment (moving into and out of the front compartment, holding still, etc.), they had never experienced a touch-screen before and never had been part of experimental procedures or computer-controlled training in a cognitive task. During each of the ten sessions of the accommodation experiment, the animal had the opportunity to freely explore the device. Presumably driven by both their curiosity and the odor of the fruit-flavored water at the tip of the reward spout, eight out of nine monkeys approached first the reward tube and subsequently the shiny aluminum frame of the XBI. For eight out of nine animals, the first successful interaction with the touch-screen occurred during the very first 20 min.

During XBI sessions most of the animals were in the front compartment by themselves (with visual contact to their social group, see Methods), except for three (Chi, Zep, and Gro) that had access to the XBI as a group. As shown in Fig. 4A, animals Chi, Zep, and Gro, after gaining some experience with the touch-screen in the first two sessions, substantially increased both their number of interactions with the XBI and the proportion of successful trials in the following days. Although with high variability and different success proportions, animals Alw, Fla, Nor, and Odo showed a substantial interest in the XBI, generating hundreds of successful trials each day and progressively improving their ability to trigger a successful trial (Fig. 4B). Only animal Han showed no interest in the XBI.

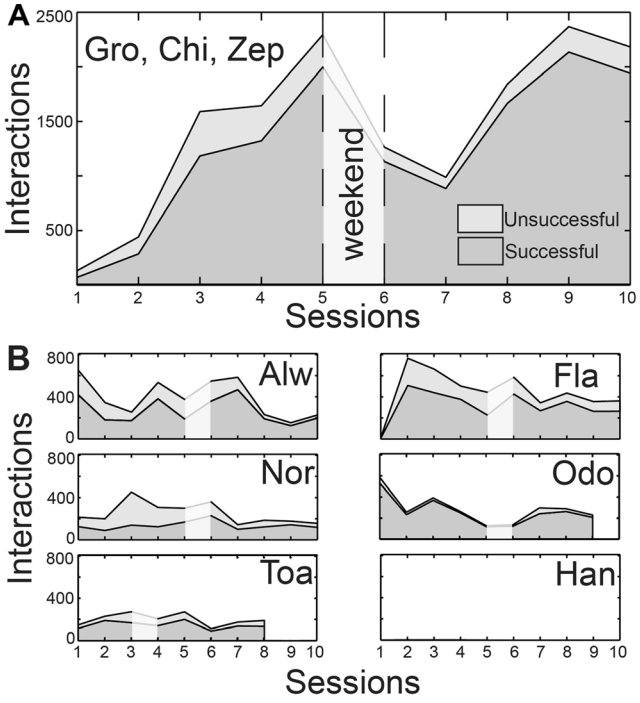

Fig. 4 A Number of interactions with the XBI system pooled across the monkeys Zep, Gro, and Chi. Successful trials (dark gray area), unsuccessful trials (light gray area), and total trials (top line) are plotted for up to 10 consecutive working days during the first 2 weeks, interrupted by 2 days off (weekend) between the fifth and sixth sessions. B Interactions for monkeys Alw, Fla, Nor, Odo, Toa, and Han. Note that animals Odo and Toa underwent respectively nine sessions (for technical reasons) and eight sessions (for unrelated reasons). Animal Toa started his first week on a Wednesday and the break lasted a whole week instead of a weekend. Animal Han did not interact with the XBI's touchscreen at all during these sessions

Free-task selection experiment

The choice proportions of monkey Pru across the four tasks stabilized within the first two sessions. To determine the influence of relative reward amounts on relative choice probabilities, the reward associated with a successful random choice trial was set to three times the reward associated with the touch-hold-release (THR) and the match-to-sample (MS)

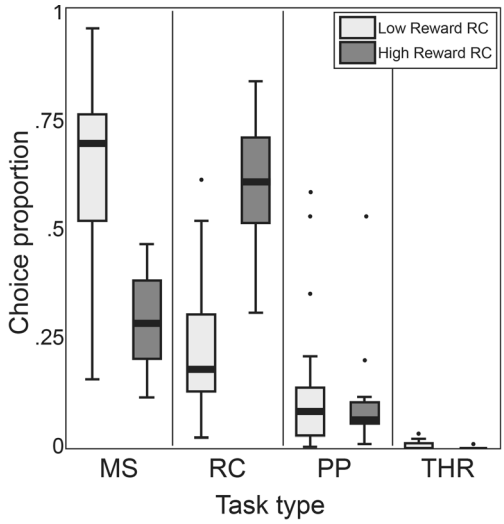

Fig. 5 Box-and-whisker plot of the distribution of choices of task type during the free-task selection, in two conditions for monkey Pru. White boxes represent the experimental condition (20 sessions) in which the reward in the random choice task (RC) was three times the amount of reward in the match-to-sample (MS) and touch-hold-release (THR) tasks. Gray boxes represent the experimental condition (11 sessions) in which the RC reward was increased to four times the amount in the MS and THR tasks. The distribution of the difference between higher reward and lower reward was estimated for each task and compared with the other tasks. To achieve such comparison the data set was repeatedly re-sampled by cluster; a model estimated and inferences were made on the sampling distribution of the pivotal (t) statistic. For each comparison the confidence interval for the significance level was weighted by the number of comparisons (confidence interval's significance level: 1–(0.005/6)) and the confidence interval for each task comparison was determined (MS to RC 0.0566–0.8195; MS to PP 0.3595–3.9314, MS to THR 0.3760–14.7022; RC to PP 1.2448–24.4346; RC to THR 0.8994–132.1582; PP to THR 0.5127–7.6293). P-values for the six comparisons, corrected with the Bonferroni method for multiple testing, are: MS to RC 0.012; MS to PP 1.00; MS to THR 0.78; RC to PP 0.036; RC to THR 0.60; PP to THR 1.00

tasks (PP did not deliver a fluid reward). For the next 11 sessions it was increased to four times.

We statistically verified the influence of relative reward amount on relative choice probabilities (see Methods and Fig. 5 legend for details). We found that MS to RC is the only comparison that yields moderate evidences for a statistical difference ($p = 0.012$), while RC to THR comparison shows a trend ($p = 0.036$) and all the other comparisons show no significant influence by the relative reward amount. This suggests that when the RC task was highly rewarded, the animal selected the RC task more often, at the expense of the MS and THR tasks but not the PP task. As can be seen in Fig. 5, the distribution of MS and RC choice proportion are reversed in the two conditions; the distribution of the THR choices, already very low in the low reward condition, approach zero, while the frequency of PP choices is unaffected. This demonstrates that the fluid reward amounts in the XBI can be used to flexibly and precisely change the animal's preferences as needed, for example, in decision-making experiments.

Discussion

We developed the XBI as a cage-based stand-alone device for behavioral training and cognitive testing of rhesus macaques and designed for a seamless integration into conventional neuroscience experiments. We tested the XBI for over a year and found it robust and flexible enough for use in different animal facilities. It is easy to handle such that one non-expert person is able to operate it on a daily basis with short setup times and without the need to remove it during wet cage cleaning procedures. Animals do not have to leave their housing environment and naïve animals learn to interact with the device in an unsupervised fashion, at a self-paced rate within the time window of device access. As a proof of concept, we presented training examples matching neuroscience research questions, e.g., training visually instructed goal-directed movements, but a much broader spectrum of behavioral testing is possible. Despite lacking physical constraints, the animals adopted stereotyped postures, adapted to the ergonomic design of the XBI, creating a well-defined perspective and distance from the visual stimuli and the reach goals on the monitor. The close-up full-body video surveillance embedded in the system allows further behavioral assessments.

Devices similar to the XBI have proved to be highly useful in cognitive assessments of non-human primates (Andrews & Rosenblum, 1994; Fagot & Bonté, 2010; Fagot & Paleressompoulle, 2009; Fagot & Parron, 2010; Gazes et al., 2012; Mandell & Sackett, 2008; Rumbaugh et al., 1989; Richardson et al., 1990; Truppa et al., 2010; Washburn et al., 1989; Weed et al., 1999). In systems and cognitive neuroscience research additional features of such devices are desirable, which we implemented to increase the range of possible uses for the XBI.

First, most existing systems use solid rewards (Andrews & Rosenblum, 1994; Fagot & Bonté, 2010; Gazes et al., 2012; Truppa et al., 2010; Weed et al., 1999), with the exception of Mandell and Sackett (2008). We use fluid rewards for the XBI, since in typical neuroscience behavioral protocols, rewards need to be precisely dosed and timed, e.g., for decision-making studies with fine-grained reward schedules (for example: Klaes et al., 2011; Platt, 2002; Sugrue et al., 2004) and as reinforcers in eye-position contingent, complex visual, and sensorimotor tasks (for example: Gail et al., 2000; Gail & Andersen, 2006; Katzner et al., 2009; Niebergall et al., 2011; Patzwahl & Treue, 2009).

Second, to be suited for a large range of neuroscience questions, the monitor and interactive touch surface should be easily accessible. In most of the touch-screen-based systems using radio-frequency identification (RFID) the monkeys need to reach through ports equipped with antenna coils, to reliably read the RFID tags (Andrews & Rosenblum, 1994; Fagot & Bonté, 2010; Gazes et al., 2012). We do not use view and reach ports to not constrain reaching movements toward

and across the touch-screen and because preliminary technical tests indicate that our design is suitable for hand-specific RFID tagging without such ports. A further advantage of not having ports or physical shielding of the touch-screen is the unobstructed full-body frontal video image of the animal in the XBI, which can be used for various forms of behavioral assessments, e.g., more complex video-based motion tracking, analysis of emotional facial expressions, etc. On the other hand, we want to encourage an ergonomic posture of the animals with a defined viewing distance from the screen. In systems without reach or view ports the screen was placed in the same plane or close to the wall of the cage, allowing the animals more freedom in choice of the posture and screen-eye distance (Gazes et al., 2012; Truppa et al., 2010; Weed et al., 1999). Since many studies in the neurosciences use visually guided tasks, it is critical to provide a controlled visual stimulus, including a well-defined retinal size. We achieved this by positioning the reward tube and touch-screen at opposite ends of a funnel, with the funnel depth adjusted to the arm lengths of rhesus monkeys and the reward tube position optimized for their sitting posture. With the aid of the integrated full-body video recordings, we verified that the animals quickly adopted a desirable and stereotypical posture in front of the screen, with the face in front of the screen and the mouth at the opening of the reward tube (see Supplementary Fig. 1 and supplementary videos). In future, this will presumably allow for an easy integration of video-based eye-tracking and face-recognition systems. Moreover, given the central placement of the reward spout, animals were free to use either hand for interacting with the device (see monkey Nor and Fla in Supplementary Fig. S1 and video).

Third, we designed the XBI to be compact and mobile, including remote control via LAN (Mandell & Sackett, 2008, 2009). This makes individual devices easily transferable between rooms, floors, or even buildings, and adaptable to different enclosures. Using one server we simultaneously operated our three devices in two buildings, switching them amongst six social groups.

Finally, we believe that the spontaneous and continued engagement of the naïve animals that we observed during early exposure to the XBI, despite no restrictions on fluid intake, shows that cage-based devices, beyond showing great potential as an alternative to some conventional setup training for neuroscience research, can also serve as valuable tools for environmental enrichment, in compliance with the 3Rs principle (Evans et al., 2008; Fagot et al., 2014; Richardson et al., 1990; Russell & Burch, 1959; Washburn et al., 1991; Washburn & Rumbaugh, 1992). It is important to note that the XBI does not trigger the same level of interest in all naïve animals (Evans et al., 2008). We are currently expanding these observations in a separate study to address the need for more systematic behavioral profiling of such inter-individual differences.

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References

Andrews, M. W., & Rosenblum, L. A. (1994). Automated recording of individual performance and hand preference during joystick-task acquisition in group-living bonnet macaques (Macaca radiata). *Journal of Comparative Psychology, 108,* 358–362.

Cameron, A. C., Gelbach, J. B., & Miller, D. L. (2008). Bootstrap-based improvements for inference with clustered errors. *The Review of Economics and Statistics, 90*(3), 414–427.

Drea, C. M. (2006). Studying primate learning in group contexts: Tests of social foraging, response to novelty, and cooperative problem-solving. *Methods, 38,* 162–177.

Evans, T. A., Beran, M. J., Chan, B., Klein, E. D., & Menzel, C. R. (2008). An efficient computerized testing method for the capuchin monkey (Cebus apella): Adaptation of the LRC-CTS to a socially housed nonhuman primate species. *Behavior Research Methods, 40*(2), 590–596.

Fagot, J., & Bonté, E. (2010). Automated testing of cognitive performance in monkeys: Use of a battery of computerized test systems by a troop of semi-free-ranging baboons (Papio papio). *Behavior Research Methods, 42*(2), 507–516. doi:10.3758/BRM.42.2.507

Fagot, J., Gullstrand, J., Kemp, C., Defilles, C., & Mekaouche, M. (2014). Effects of freely accessible computerized test systems on the spontaneous behaviors and stress level of Guinea baboons (Papio papio). *American Journal of Primatology, 76,* 56–64.

Fagot, J., & Paleressompoulle, D. (2009). Automatic testing of cognitive performance in baboons maintained in social groups. *Behavior Research Methods, 41*(2), 396–404. doi:10.3758/BRM.41.2.396

Fagot, J., & Parron, C. (2010). Relational matching in baboons (Papio papio) with reduced grouping requirements. *Journal of Experimental Psychology: Animal Behavior Processes, 36*(2), 184–193. doi:10.1037/a0017169

Fernström, A. L., Fredlund, H., Spångberg, M., & Westlund, K. (2009). Positive reinforcement training in rhesus macaques-training progress as a result of training frequency. *American Journal of Primatology, 71*(5), 373–379. doi:10.1002/ajp.20659

Gail, A., & Andersen, R. A. (2006). Neural dynamics in monkey parietal reach region reflect context-specific sensorimotor transformations. *Journal of Neuroscience, 26*(37), 9376–9384.

Gail, A., Brinksmeyer, H. J., & Eckhorn, R. (2000). Contour decouples gamma activity across texture representation in monkey striate cortex. *Cerebral Cortex, 10*(9), 840–850.

Gail, A., Klaes, C., & Westendorff, S. (2009). Implementation of spatial transformation rules for goal-directed reaching via gain modulation in monkey parietal and premotor cortex. *Journal of Neuroscience, 29*(30), 9490–9499. doi:10.1523/JNEUROSCI.1095-09.2009

Gazes, R. P., Brown, E. K., Basile, B. M., & Hampton, R. R. (2012). Automated cognitive testing of monkeys in social groups yields results comparable to individual laboratory-based testing. *Animal Cognition, 16,* 445–458.

Katzner, S., Busse, L., & Treue, S. (2009). Attention to the color of a moving stimulus modulates motion-processing in macaque area MT: Evidence for a unified attentional system. *Frontiers in Systems Neuroscience, 3*(12), 11–18.

Klaes, C., Westendorff, S., Chakrabarti, S., & Gail, A. (2011). Choosing goals, not rules: Deciding among rule-based action plans. *Neuron, 70*(3), 536–548.

Mandell, D. J., & Sackett, G. P. (2008). A computer touch screen system and training procedure for use with primate infants: Results from pigtail monkeys (Macaca nemestrina). *Developmental Psychobiology, 50*(2), 160–170. doi:10.1002/dev.20251

Mandell, D. J., & Sackett, G. P. (2009). Comparability of developmental cognitive assessments between standard and computer testing methods. *Developmental Psychobiology, 51*(1), 1–13. doi:10.1002/dev.20329

McCluskey, M. K., & Cullen, K. E. (2007). Eye, head, and body coordination during large gaze shifts in rhesus monkeys: Movement kinematics and the influence of posture. *Journal of Neurophysiology, 97*(4), 2976–2991. doi:10.1152/jn.00822.2006

Niebergall, R., Khayat, P. S., Treue, S., & Martinez-Trujillo, J. (2011). Multifocal attention filters out distracter stimuli within and beyond receptive field boundaries of primate MT neurons. *Neuron, 72,* 1067–1079.

Patzwahl, D., & Treue, S. (2009). Combining spatial and feature-based attention within the receptive field of MT neurons. *Vision Research, 49,* 1188–1193.

Perlman, J. E., Bloomsmith, M. A., Whittaker, M. A., McMillan, J. L., Minier, D. E., & McCowan, B. (2012). Implementing positive reinforcement animal training programs at primate laboratories. *Applied Animal Behaviour Science, 137*(3-4), 114–126. doi:10.1016/j.applanim.2011.11.003

Platt, M. L. (2002). Caudate clues to rewarding cues. *Neuron, 33,* 316–318.

Richardson, W. K., Washburn, D. A., Hopkins, W. D., Savage-Rumbaugh, E. S., & Rumbaugh, D. M. (1990). The NASA/LRC computerized test system. *Behavior Research Methods, Instruments, & Computers, 22*(2), 127–131.

Rumbaugh, D. M., Hopkins, W. D., Washburn, D. A., & Savage-Rumbaugh, E. S. (1989). Lana chimpanzee learns to count by BNUMATH^: A summary of a videotaped experimental report. *Psychological Record, 39*(4), 459–470.

Russell, W. M. S., & Burch, R. L. (1959). *The principles of humane experimental technique*. Methuen, London. ISBN 0900767782.

Schapiro, S. J., Bloomsmith, M. A., & Laule, G. E. (2003). Positive reinforcement training as a technique to alter nonhuman primate behavior: Quantitative assessments of effectiveness. *Journal of Applied Animal Welfare Science: JAAWS, 6*(3), 175–187. doi:10.1207/S15327604JAWS0603_03

Sugrue, L. P., Corrado, G. S., & Newsome, W. T. (2004). Matching behavior and the representation of value in the parietal cortex. *Science, 304*(5678), 1782–1787.

Truppa, V., Garofoli, D., Castorina, G., Piano Mortari, E., Natale, F., & Visalberghi, E. (2010). Identity concept learning in matching-to-sample tasks by tufted capuchin monkeys (Cebus apella). *Animal Cognition, 13*(6), 835–848. doi:10.1007/s10071-010-0332-y

Washburn, D. A., Hopkins, W. D., & Rumbaugh, D. M. (1989). Video-task assessment of learning and memory in macaques (Macaca mulatta): Effects of stimulus movement on performance. *Journal of Experimental Psychology: Animal Behavior Processes, 15*(4), 393–400.

Washburn, D. A., Hopkins, W. D., & Rumbaugh, D. M. (1991). Perceived control in rhesus monkeys (Macaca mulatta): Enhanced video-task performance. *Journal of Experimental Psychology: Animal Behavior Processes, 17*(2), 123–129.

Washburn, D. A., & Rumbaugh, D. M. (1992). Testing primates with joystick-based automated apparatus: Lessons from the language research center's computerized test system. *Behavior Research Methods, Instruments, & Computers, 24*(2), 157–164. doi:10.3758/BF03203490

Weed, M. R., Taffe, M. A., Pollis, I., Roberts, A. C., Robbins, T. W., Koob, G. F., … Gold, L. H. (1999). Performance norms for a rhesus monkey neuropsychological testing battery: Acquisition and long-term performance. *Cognitive Brain Research, 25;8*(3), 185–201.

Westendorff, S., Klaes, C., & Gail, A. (2010). The cortical timeline for deciding on reach motor goals. *Journal of Neuroscience, 30*(15), 5426–5436. doi:10.1523/JNEUROSCI.4628-09.2010

Standardized automatic training of rhesus monkeys for neuroscience research in their housing environment

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Abstract

Teaching non-human primates, the complex cognitive behavioural tasks that are central to cognitive neuroscience research is an essential and challenging endeavour. Training animals to properly interpret the often complex task rules, and reliably and enduringly act according to these rules, is crucial for the scientific success. To achieve consistent behaviour and comparable learning histories across animals, it is desirable to standardize training protocols. Automatizing the training can also significantly reduce the time that has to be invested by the person training the animal. And self-paced training schedules with individualized learning speeds and continuous updating of task conditions could enhance the animals' motivation and welfare. Using the XBI, a housing-based and computerized interactive system for rhesus monkeys in neuroscience research (Calapai et al., 2016), we developed a paradigm for standardized and automated behavioural training of a memory-guided center-out reach task in the animals' cage environment.

The automated training revealed inter-individual differences in the animals' learning behaviour, and helped to identify easier and more difficult learning steps in behavioural task designs. Learning progress primarily reflected the number of interactions with the system, rather than the total time exposed to it. Our results demonstrate that rhesus monkeys stay engaged with the XBI over months and learn cognitive tasks of sufficient complexity for state-of-the art systems and cognitive neuroscience in a housing environment without human supervision.

Introduction

Cognitive neuroscience research involving non-human primates (NHPs) often requires extensive animal training using positive reinforcement training (PRT). Animals have to learn to accurately operate devices such as a touchscreen or a joystick, interpret sensory cues and react in a required manner. Training of an animal from a naïve state to expertise in a complex cognitive task can last more than a year and the success is dependent on the animal's motivation and cognitive abilities but also the training strategy chosen by the trainer's intuition.

Standardizing animal training protocols avoids variability in training history and should thereby help to improve data quality. The better an animal's behaviour is determined by the design of the cognitive task and understood by the experimenter, the lower is the risk of confounding interpretations of the behavioural data and the neurophysiological data collected for understanding the neural basis of cognitive behaviour. When multiple animals have to be trained to the same experimental protocol, animals should solve the task with the same cognitive strategies so that behavioural and neural results stay comparable between animals. Any

unpredictable and probably even unconscious influence of the experimenter's training strategy might in fact bias the strategy the animal employs to solve the task, resulting in mismatching outcomes for the different animals. Additionally, a systematic comparison of different animals' potential to learn a certain cognitive task seems unreasonable as long as the influence of the experimenter cannot be ruled-out.

Automatizing training also reduces the trainer's work load (Anagnostaras, 2014; Miller, Lim, Heidbreder, & Black, 2015) and allows for self-paced training schedules (Fagot & Bonté, 2010). In conventional settings the training schedule is typically determined by the experimenter and not by the animal, the training period for complex cognitive neuroscience projects can often last several months, and fluid control schedules are typically used to create incentives for the animals' engagement in the experiment and faster learning (Prescott et al., 2010). Automated cage-based training provides the animals the possibility of choosing the time of their engagement with the training protocol. Such choice permits the animal more control about the experimental environment which benefits the animals welfare (Westlund, 2014).

Also, individualizing the difficulty and speed of training might be motivating for animals. The standardization of training described above does not necessarily imply that the same task demands should be imposed on each animal. The idea behind of our approach is rather to standardize the rules according to which animals progress through the learning steps of a new task. We believe this approach helps reaching an optimization of each animal's learning rate by keeping a stable medium

performance. Frequent availability of cognitively demanding interaction tools can serve as environmental enrichment which can have a strong impact on welfare (Newberry, 1995). Monkeys might lose interest very quickly in enrichment tools such as invariable objects. Maintaining the animal's interest in a device can only be achieved by using intrinsic *reinforcers* such as food, or by constantly introducing novelty into the environment (Tarou & Bashaw, 2007). Cognitive training by an automated protocol, which dynamically adjusts the difficulty to the animal's current skill level, might represent a very powerful enrichment strategy to enhance the animal's well-being.

We developed and implemented an automated, algorithm-based training protocol, optimized for cage-based touchscreen interactions (Calapai et al., 2016) which was inspired by existent cage-based testing systems (Andrews & Rosenblum, 1994; Fagot & Bonté, 2010; Fagot & Paleressompoulle, 2009; Gazes, Brown, Basile, & Hampton, 2013; Truppa et al., 2010; Weed et al., 1999). Eight animals were gradually and autonomously trained, starting from basic touchscreen interactions up to a cognitive task which required spatial working memory and visuomotor coordination. We here report evidence supporting the idea that automated training, based on a computerized training algorithm, allows: 1) standardized and autonomous training of naïve animals to tasks typical for cognitive neuroscience research; 2) several months of training with maintained animal's engagement; 3) a systematic analysis of training performance for animal selection and task optimization. We will show how naïve rhesus macaques can successfully learn a typical sensorimotor and working memory task without supervision by an experimenter and, although not the whole day, with free access to water outside of

the training. The variability of training progress across monkeys can be significantly better explained by the amount of interaction that animals performed rather than the time they spent with training.

Materials and Methods

All the experiments complied with institutional guidelines on Animal Care and Use of the German Primate Centre and with European (Directive 2010/63/EU) and national law, and were approved by regional authorities were necessary.

Animals

A total of eight male rhesus monkeys (*Macaca mulatta*, age range 4 to 7 years) had 90 minutes daily individual access to the XBI (hereafter referred to as "session") from Monday to Friday with free fluid access for at least two hours prior and at least two hours after every session and 24 h during both days of the weekend (one exception: during working days, animal Toa did not receive fluid prior to the experiment but immediately afterwards for at least two hours). During experimental sessions, the participating animal was separated from its peer group into a smaller (approx. 0.8 qm, 1qm or 1.8 qm) cage compartment, having auditory and visual contact with the members of its housing group and of other groups belonging to the same animal facility. All eight animals were accustomed to the XBI with at least 8 days of prior access and showed interest in repeatedly interacting with it, as described elsewhere (Calapai et al., 2016). We excluded a ninth animal, who participated in the previous study, since the animal did not interact with the XBI. None of the animals received specific prior training towards the behavioural tasks introduced in the current study. All animals received fruit-flavoured sweetened

water (Active O2 Orange, Adelholzer Alpenquellen GmbH, Germany) diluted with plain water as reward for correct performance on the XBI.

Apparatus

The XBI is a touch screen based training and testing system for rhesus monkeys, optimized for use in an animal facility (Figure 1a) and for cognitive behavioural experiments in a neuroscientific context described in a previous study (Calapai et al., 2016). Animals have access to a 15-inch touchscreen (ELO 1537L; 1024 x 768 resolution, 75 Hz refresh, 2.5 mm touch accuracy) mounted in an aluminium frame replacing one side panel of the cage compartment. Three devices have been used to simultaneously test animals belonging to three different groups and housed in two different facilities.

Automated training protocol

In order to automatize the training of the animals and gradually adjust the complexity of the task, the training starts with a very easy task to then become more and more difficult at a speed which depends on the individual animal's performance. Within each training stage individual task parameters might vary randomly but such that the practical or conceptual difficulty of the task remained constant. For example, within a stage the position of a reach target on the screen might be selected randomly, but the spatial and temporal precision of the requested behavioural response (the reach) does not vary. Between stages the task difficulty was increased. For example, the reach target might decrease in size, thereby requesting higher reach accuracy, without changing other parameters of the task.

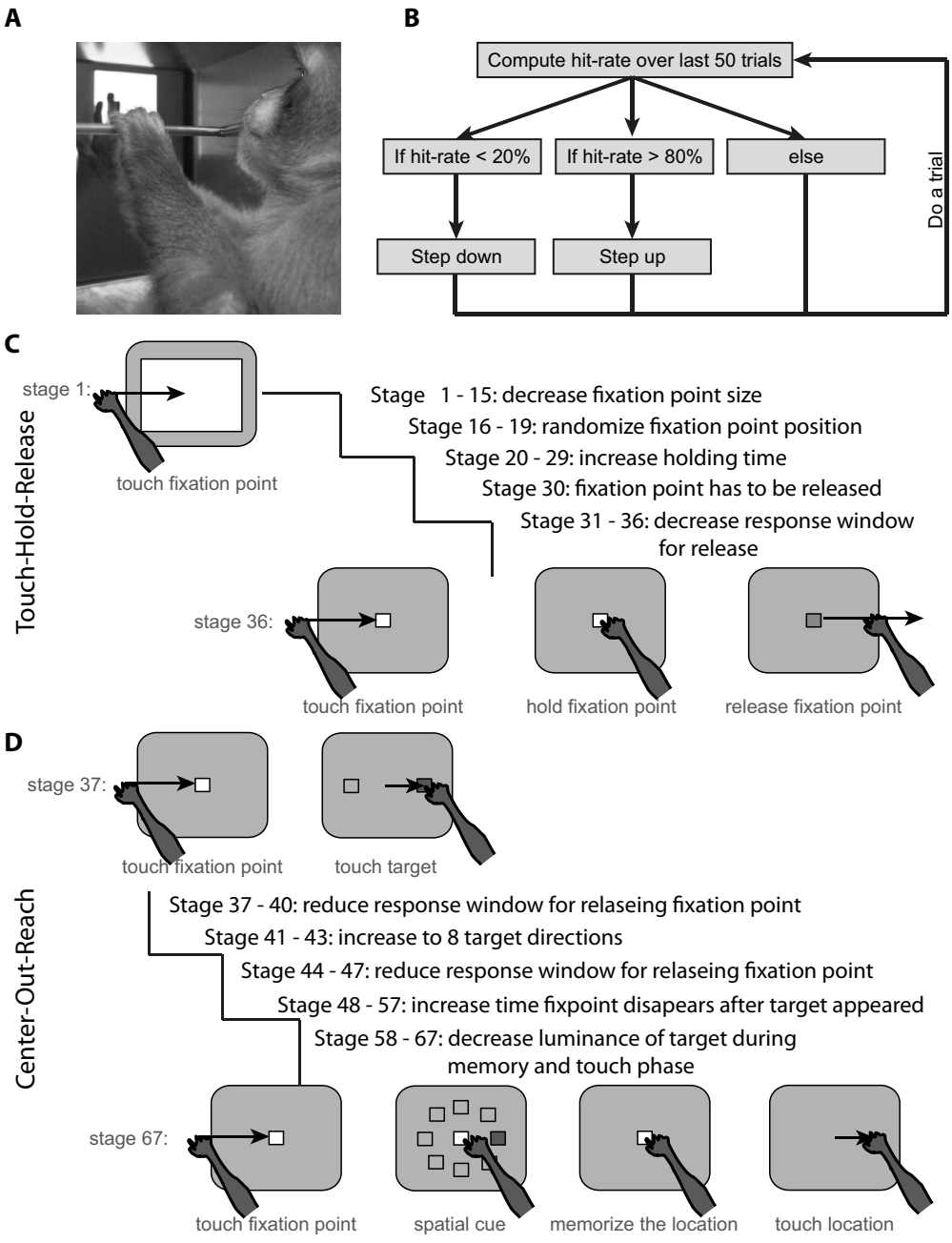

Figure 1 - Automated training protocol. A) Image of a monkey working on the cage-based touch-screen device. B) Staircase algorithm to determine the trial-by-trial training stage based on the performance in the preceding 50 trials. C) Automated Touch-Hold-Release (THR) training protocol. Over a total of 36 different stages the animals learn to touch a small blue square on the screen (fixation point), keep their hand on the square as long as it is visible, and release the screen within a certain response time window once the square disappears. B) Automated memory guided Center-Out-Reach (COR) training protocol; following the Touch-Hold-Release training. Within a total of 30 stages, the animals learn to touch and hold a small blue square in the middle of the screen (fixation point), remember the location of a flashing white square (target) in one out of 8 peripheral locations, wait for a certain instructed-delay period, release the fixation point within a certain period of time (response window) after the fixation stimulus disappears, and reach to the remembered (now invisible) target location.

A simple staircase algorithm is responsible for selecting the appropriate training stage from which the following trials are drawn from (figure 1b) depending on the animal's performance. If during a given experimental session the proportion of correctly executed trials over the previous 50 trials on the current stage is more than 80% then the algorithm steps up to the next stage (the difficulty increases). If performance is less than 20% the algorithm steps down to the previous stage (the difficulty decreases). If performance is between 20% and 80%, the algorithm keeps drawing the trials from the current stage (the difficulty stays the same while individual task parameters might still vary). After every stage change, the counter on which the performance is calculated is reset and no difficulty change occurs before the next 50 trials, then the performance is re-calculated after each trial.

Note that the length of the time window for the instantaneous performance computation limits the maximal speed of progression through the training stages (max. 1 stage of progression per 50 successive trials). Note also that the initial definition of the successive training stages is based on an a priori assumption of the experimenters about task difficulty and about manageable transitions from one precursor of the final task to the next. The definition of the individual precursor tasks and the assumption about their difficulty resulted from our previous experience with conventional training of rhesus monkeys on these tasks (Gail & Andersen, 2006; Klaes, Westendorff, Chakrabarti, & Gail, 2011; Westendorff, Klaes, & Gail, 2010). As a result of training larger numbers of animals in an automated fashion with this predefined set of training stages, as attempted here, such a priori definition of difficulty might have to be adapted later (see Discussion).

To prepare for the case that an animal does not succeed in progressing through the whole set of training stages without modifying the training approach, an animal's automated training was stopped if no training progress was observed for a prolonged amount of time. We defined two criteria for stagnation in learning: 1) after reaching a certain stage *n*, an animal did not reach the next stage *n+1* within 40 sessions; 2) after reaching a certain stage *n*, an animal did not reach the next stage *n+1* within 25 sessions and made no progress in performance even within the current stage(s) of level *n* or lower. Within-stage performance progress was estimated by computing for each trial the proportion of correct trials of the preceding 50 trials. This value, lying between 0 and 1, was added to the stage number of this trial, thereby converting the discrete stages plus the instantaneous within-stage performance into a single continuous numerical value (pseudo-stage) as a function of total trial number. We then regressed the pseudo-stage with the trial number, starting from the trial at which the so far highest discrete stage was reached for the first time. The slope of this regression was the estimate of progress in performance. If the slope was zero or negative, we interpreted it as no progress in performance and the criterion was met if this happened for 25 successive sessions. If one of the two criteria were met, the automated training was aborted for the animal.

Touch, hold and release task (THR)

The THR task is a basic task for goal-directed reaching towards visual targets on a touch screen. Over the 36 stages of the THR training protocol, the animal is expected to reach for a blue square on the screen, keep holding the position until the square

dims and release the square within time to receive the reward (figure 1C). This is achieved by (1) progressively reducing the stimulus size and hence the required reach accuracy from 13 cm to 3 cm – stages 1 to 16; (2) randomizing the target position on the screen (left-center-right, up-center-down) within 6 cm eccentricity – stages 17 to 19; (3) increasing the hold time from 150 ms to random times between 700 and 1500 ms – stages 20 to 29; (4) reinforcing the release rather than the hold (stage 30), and finally by gradually decreasing the response window for releasing the stimulus from 1000 ms to 500 ms – stages 31 to 36).

All eight animals participated in an automated training of the THR task. One of the eight animals (Fla) aborted this first phase of the experiment without meeting one of the two abortion criteria since it was needed for a different project. We still kept this animal's data for analysis, since our quantification of the results does not depend on reaching the final stage.

Memory-guided center-out reach task (COR)

The COR task is a widely used task in sensorimotor neuroscience for goal-directed motor planning based on spatial working memory content (e.g. Kuang, Morel, & Gail, 2016; Snyder, Batista, & Andersen, 1997; Wise & Mauritz, 1985) (figure1D). The 31 stages (stages 37 – 67) that comprise the COR training protocol are intended for animals that are already accustomed to the use of the touch screen and learned to reach for a visual target. In the COR training animal had to learn to reach for a central blue stimulus (the same blue square as used in THR), observe another stimulus (cue) briefly flashed at one of eight discrete peripheral locations on the

screen, remember the position of the cue, and finally reach for the previous cue location as soon as the central hand-fixation stimulus disappears. This is achieved by (1) displaying the cue as consequence to the touch of the central hand-fixation stimulus from 5000 ms to 3000ms – steps 37 to 40; (2) randomizing the position of the cue (up/down, 4 cardinal directions, all 8 directions – steps 41 to 43; (3) reducing the response window again from 2500 ms to 800 ms – steps 44 to 47; (4) delaying the disappearance of the central hand fixation stimulus (= "go" instruction) from 100 ms to 1300 ms after appearance of the peripheral cue – steps 48 – to 57 (instructed-delay task); and finally, (5) reducing the cue luminance from 50% to 0 during the instructed delay and reaching phase so that the visual cue is rendered less and less visible and finally has to be remembered for proper reach performance – steps 58 to 67).

Five animals that had completed the final stage of the THR task participated in automated training of the COR task.

Memory-guided center-out pro-anti reach task (PAR)

The pro-anti reach task is an extension of the COR task in which proper selection of the reach goal is contingent upon choosing the correct visual-to-motor transformation rule instructed by a coloured context cue (Crammond & Kalaska, 1994; Gail & Andersen, 2006). The colour of the peripheral cue instructs the animal either to perform a direct (pro) reach (magenta) or to reach the opposite location of the cue, i.e. to perform an anti-reach (cyan). The PAR task was not part of the original experimental design and the according training protocol was adapted

during the course of the experiment. As a consequence, not all animals admitted to this third training phase experienced the exact same protocol and we will therefore only report anecdotal results. We consider the report of this training data still noteworthy, since the PAR task marks an advanced level of task difficulty relevant for cognitive neuroscience, particular the analysis of context-dependent goal-directed behaviour (Gail & Andersen, 2006; Klaes et al., 2011; Westendorff et al., 2010). Three of the five animals that had completed the final stage of the COR task (Chi, Gro, Zep) participated in automated training of the PAR task.

Results

Table 1 shows an overview of the general performance of all monkeys that took part in this experiment, indicating the amount of sessions and trials, successful or not, animals spent on the THR and COR tasks. Five out of seven animals learned the full THR task successfully. These animals needed between 12 and 117 sessions to accomplish the 36 training stages of the THR task, and between 4787 and 11372 trials. While the number of trials needed partially scales with the number of sessions needed, the amount of trials and of sessions were not directly related. Animals Odo and Toa stagnated at stage 30, which means they successfully accomplished the touching and holding of a target stimulus, but they did not learn to release the target stimulus in response to its visual dimming.

Four out of five animals accomplished the final stage of the COR task. Again, the numbers of sessions and trials needed varied substantially (56-125 sessions, 13935–24295 trials) even if considering only the successful animals. Given that the training was standardized across animals, this large inconsistency in number of

sessions and trials needed to learn the task might reflect an interindividual variability of the learning progress, which we will analyse below. Animal Nor, stagnating at stage 63, learned to wait for the go cue before reaching to the target but did not learn to memorize the target position.

Three out of the four animals that had been successful in the COR task, were also admitted to the PAR task. For two of the animals, we modified the task in response to performance difficulties that both animals encountered at the same stage of the PAR task. Since the stagnation added extra sessions to the training, the learning is not equally comparable anymore and, thus, not included in table 1 and corresponding analysis.

	Touch-Hold-Release		**Center-Out-Reach**		
Monkey	Trials	Sessions	Trials	Sessions	Final stage
Alw	5958	21	13935	70	67
Chi	11372	31	20272	56	67
Gro	6797	30	24295	125	67
Zep	4787	12	18639	58	67
Nor	7891	117	28640	159	63
Odo	4973	74	-	-	30
Toa	10909	96	-	-	30
Fla	8178	44	-	-	30*

Table 1: Overview of participating animals. The table shows the number of trials/session the animals performed in each task. The column "Final stage" denotes the maximally reached stage, where THR covers stage 1-36 and COR 37 – 66. That means Alw, Chi, Gro and Zep finished both tasks; Nor finished THR but not COR; Odo, Toa and Fla did not finish THR and thus not participated in COR. The '' denotes that animal Fla where taken out of the experiment for reasons unrelated to the current study. "Trials" denotes the total number of trials needed to reach the highest achieved stage within the task, "Sessions" the corresponding number of training sessions.*

Motivated by the observed variability in training progresses, we analysed the learning progress across and within animals for the THR and COR training protocols. We used the performance data from the automated training, on the one hand, to

quantify inter-individual differences between animals and tried to identify whether time spent in training or experience with the task better explains the average training progress of animals. On the other hand, we used the performance data to characterize different phases of a training protocol in terms of their difficulty.

Performance in THR and COR task

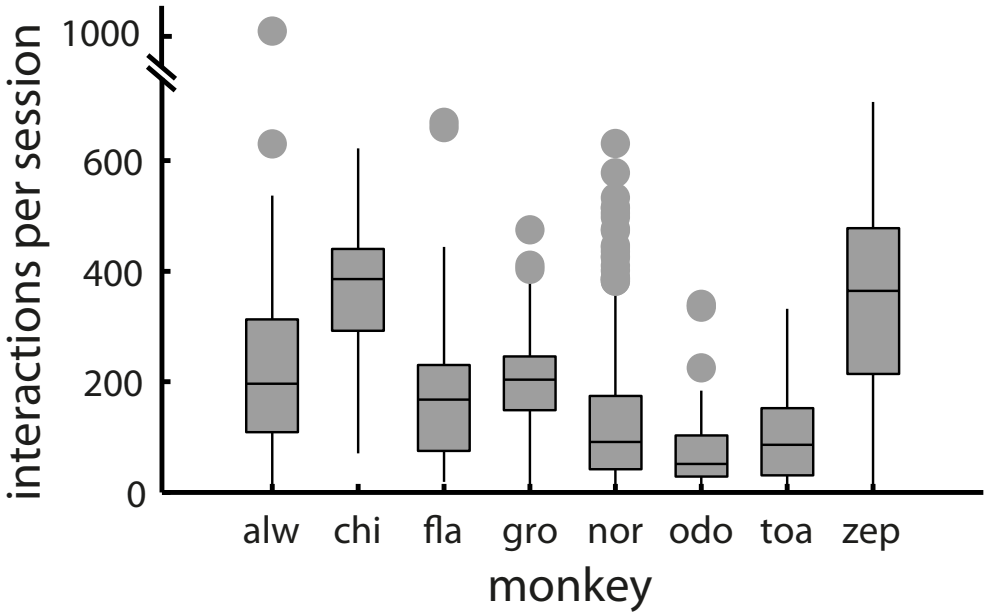

Figure 2 - Number of touchscreen interactions per session for each monkey during the THR and COR task. The boxplot indicates median (middle line) and 25th to 75th percentile (box). The whiskers correspond to q_75+1.5 (q_75-q_25).

Over the course of two years, we collected 897 training sessions (17 sessions excluded due to technical malfunctions). The animals interacted with the XBI with different rates. Figure 2 shows interactions per session (one session per working day) for each animal. The median number of interactions varied from 51 trials (Odo)

to 386 trials (Chi). Also, the spread was different across animals. The difference between the 25th and 75th percentile varied from 75 trials (Odo) to 264 trials (Zep). While the amount of interactions per session partly varied over the course of the study, none of the animals stopped interacting completely with the device.

All animals had been habituated to the XBI prior to study begin (Calapai et al., 2016), so that they knew that a successful interaction with the touchscreen would result in a drop of flavoured water. The progress for stepwise learning of the two new tasks (THR, COR) is shown in figure 3 for each animal. By and large, the achieved level of difficulty increased monotonically for all animals, with slower speed of progression at higher training stages in both tasks. When plotted as function of session number (right panels), the achieved level of difficulty after a certain time could be different between animals by a factor of 2-3. When the same performance data is analysed as a function of number of trials performed in each training protocol (left panels), the spread between animals seems less. This suggests that progress in learning does not depend on the absolute time of exposure to the task, but rather the experienced gained through individual interactions with the task (see below). Note that the first and last steps of the THR task are excluded from analysis (stage 1 and stage 36): stage 1 was used to accustom the animals to the XBI and was reported previously; the transition to COR (stage 36 to 37) was not automated. By excluding those stages our analysis only incorporates stages where the animals entered and left a stage chosen by the automated algorithm.

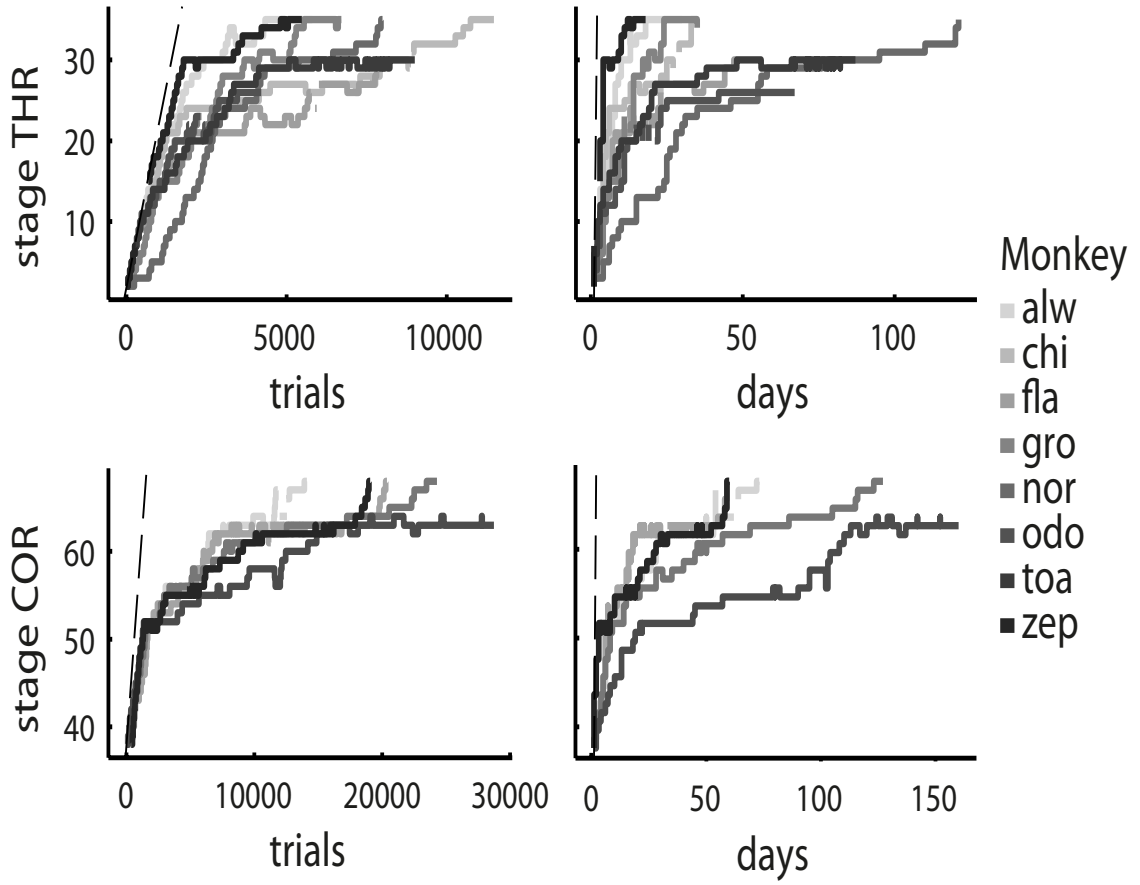

Figure 3 - Training progress of individual animals over time during the THR (lower plots) and the COR training (upper plots). Training progress is plotted against number of trials (left) and sessions (right) conducted on the XBI. Plot is corrected when da data was missing by shifting the following data points about the minimal number of trials needed for accomplishing the change in steps. The dashed line represents the fastest possible training progress, which was 50 trials per stage.

To test for the effect of time versus experience in the learning progress, we estimated the time and the trials needed by each animal for reaching a certain stage for the first time, after they reached the preceding stage for the first time (figure 4 inset). In the analysis so far, we had characterized the learning of the animals by their performance across the different training stages. We now characterize the training stages by means of the learning behaviour across animals. In the first step, we will compare training stages with this approach (figure 4), in the second step, we will compare the influence of time versus experience on learning (figure 5). By

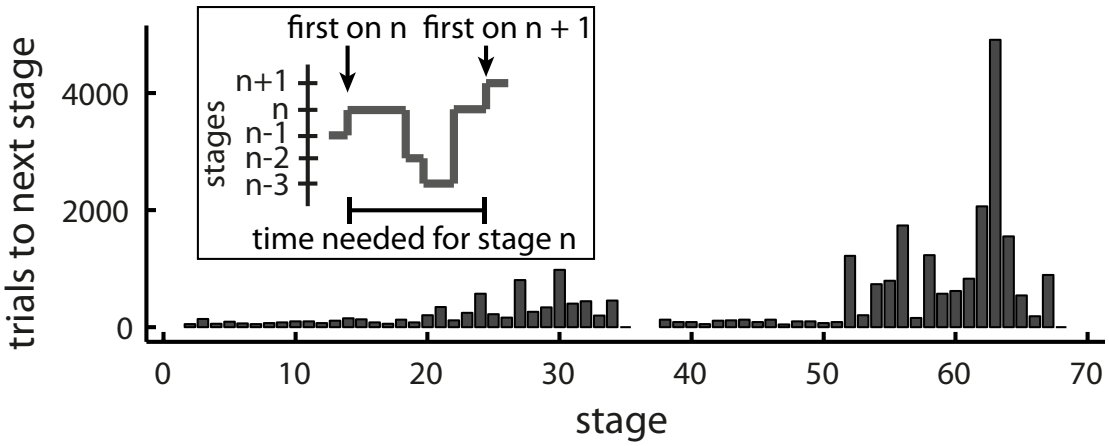

Figure 4 - Average amount of trials animals needed to complete a certain stage. Horizontal axis indicates all stages within the THR task (1-35) and COR task (37-67). The inset shows an example for computing this amount of trials.

comparing the average amount of trials the animals spent on individual stages (figure 4), we can identify stages or phases for which the animals needed more attempts to complete. As an example, between stage 58 and 67 the luminance of the touch target decreased stepwise until it vanished completely. Around stage 63, the touch target was not visible anymore for the animals so that they needed to memorize the visual cue shown at the beginning of the trial to know the correct touch position. Since most of the animals spent more trials on this stage compared to the average of the other stages, we can infer from the monkeys' perspective an elevated difficulty level of this stage. In this way, we can use the automated training approach to validate a given training strategy and thus identify the difficulty of certain phases within this strategy.

Some animals needed longer than others to complete the tasks or certain phases of the task. We were interested on the overall magnitude of such variability and on whether there is a difference in the variability if progress is measured over time

rather than over number of attempted trials. For each stage we thus computed the coefficient of variation of the time (in minutes) spent in front of the XBI (CV_{time}) and trials (CV_{trial}) the animals needed to reach a certain stage for the first time after reaching the previous stage for the first time. $CV = \frac{\sigma}{\mu}$, where σ is the standard deviation and μ the mean. CV_{time} was 1.14 and higher than CV_{trial}, which was 0.74 (figure 5; Wilcoxon signed rank test, $p < 0.001$). This indicates that experience with the task rather than time spent on the task is a better predictor for learning progress.

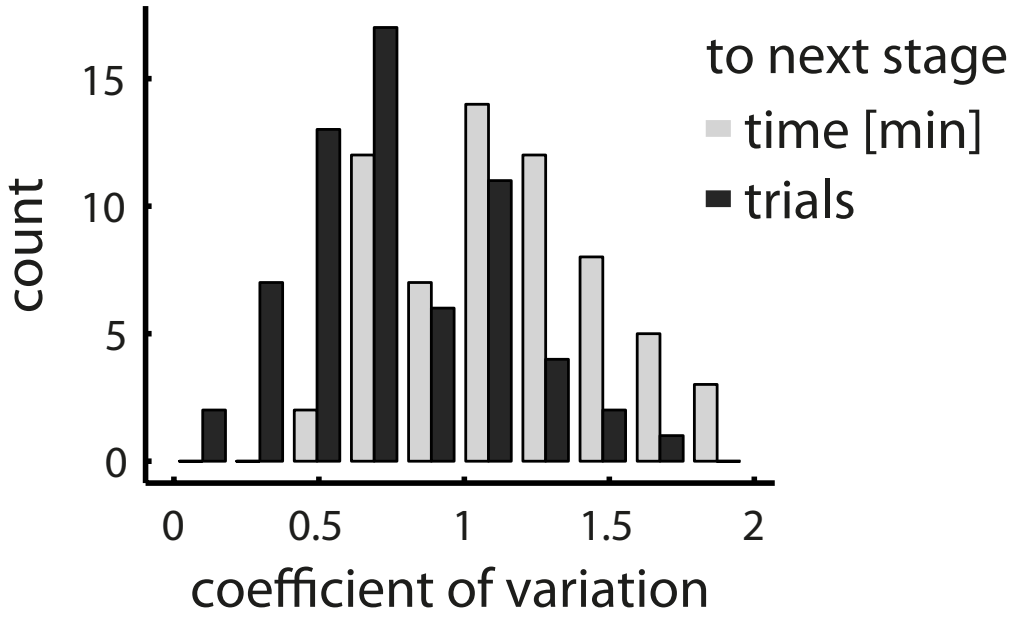

Figure 5 - Distributions of the coefficient of variation (CV) of the time animals needed to reach a stage after they reached the previous stage for the first time. CVs where computed for trials (dark) or time in minutes (light).

Pro-anti reach task

The idea behind this additional task, which was run only with few animals and was not as strictly predefined as the other training protocols, was to provide a proof of

concept that the animals could also be trained on more advanced rule-based cognitive tasks with our standardized algorithm-based training protocol. In contrast to COR, the visual cue in PAR is presented in either of two colours instructing to touch the location of the cue (as in COR) or opposite to it starting from the position in the middle of the screen (see Methods). In our experience, such rule-based task can pose some challenges even when trained to rhesus monkeys by experienced trainers.

After two animals stagnated at the same training stage (dimming of an auxiliary target stimulus at the anti-position to render it invisible), we modified this training stage (by delaying the disappearance of the salient auxiliary stimulus until after reach onset but before reach termination). Using this new approach both animals succeeded to learn the memory guided anti rule, although one of the two did not generalize the task to all reach directions. The third animal that arrived at this stage later did not manage to pass the stage with the new strategy. One of the first animals, monkey Chi, learned the final stage of the PAR task and performed it with a success rate of 71%.

Discussion

Eight rhesus macaques underwent cognitive training on a touchscreen device within their housing environment with an algorithm-based automated training protocol. Five of the eight animals succeeded in learning a simple touchscreen interaction task (touch-hold-release, THR) and continued training in a standard task for sensorimotor research, the memory-guided center-out reach (COR). Four of these

five animals were able to complete this protocol and three of them continued to an extension of the COR, the pro-anti reach task (PAR), the last stage of which was reached and completed by one animal only. By comparing the learning behaviour between animals, we found that the learning progress was better predicted by the amount of trials rather than by the time spent training. Additionally, the standardization of the training protocols allowed us to identify easy as well as difficult steps of the tasks, which in turn helped in the evaluation of the effectiveness of our training approach. Finally, while all animals continued to use the device over several months and despite the fact that fluid and food intake was not restricted outside the training sessions, our results suggest that automated training to cognitive tasks is a valuable tool also for environmental enrichment.

In cognitive neuroscience research with non-human-primates, monkeys are often required to solve complex cognitive tasks, for which the learning process requires extensive training. Some factors that influence training duration are task difficulty, motivation level of the animal as well as training strategy. The latter, set by the trainer, might be highly influenced by their subjective decisions and conscious but also unconscious behaviour. Our results suggest that by employing an automated and standardized approach to animal training it is possible to eliminate the experimenter bias from the list of possible confounds. Moreover, we believe that a direct and unbiased comparison of the strategies employed by different animals to the same learning protocol is particularly useful to identify animals for specific research projects (Capitanio, Kyes, & Fairbanks, 2006) or quantify the spectrum of cognitive skills within a group of animals (REF).

Inter-individual variability of learning progress

In designing the automated tasks, we aimed for a slow but steady increase in difficulty as way of minimizing the risk of animals encountering insurmountable conceptual shifts of task rules. Yet, one animal (Nor) did not succeed in completing the center-out reach task while two (Odo and Toa) did not complete the touch-hold-release task, the most basic task we designed. Interestingly, these three animals performed the least number of interactions per day on the device (Figure 1). Furthermore, we observed that the amount variability in training progress among animals is lower when progress is measured across number of interactions rather than time spent on the device.

Designing the automated training protocols

By measuring the number of trials different animals needed to master a certain stage, we learned about the inherent difficulty of that stage. This measure can be used to evaluate the training approach implemented by the predefined set of stages. For instance, the first 20 steps of the touch-hold-release task seem to be very easy for all the animals. Thus, by omitting several of those stages it might be possible to speed up the learning process. On the other hand, stage 30, having the highest amount of trials across all animals in this task, seems to be the most difficult. In fact, it is the stage where two animals dropped out due to lack of learning progress. Here, it would be useful to introduce easier intermediate steps to reduce the risk of animals stagnating. By omitting easy stages and adjusting difficult stages, it would be possible to optimize the training strategy towards a constant moderate task difficulty over the whole training.

Such an optimized task would be beneficial for identifying animals which are particularly suited for studies requiring overlapping cognitive demands. Training animals on a moderate difficulty would reveal the highest variability across animals, since the task would be easy enough for most animals to succeed but too difficult for most to master it trivially. By fanning out the performance across animals, inter-individual differences become particularly apparent and one can identify the best performers. In our training protocols this was the case approx. between stages 24-32 and between stages 53-65.

On the other hand, moderate training procedure with a large spectrum of task difficulties could be of interest for inter-species comparisons, since a larger spread in cognitive capabilities has to be expected. By choosing a multifaceted training procedure, it will be possible to identify the difficulty of certain cognitive aspects. For example, we saw in Figure 4b that that the animals needed more trials to accomplish stage 58 – 67 (waiting for the cue to respond) than 48 – 57 (memorizing the target location). This could indicate that rhesus monkeys find it easier to withhold an action for a few hundred milliseconds than to memorize a spatial position for this time period. The pattern in Figure 4 could mark a species-specific profile useful for characterization of cognitive skills.

Environmental Enrichment

Our automated and standardized approach to cognitive training resembles some of the key features of what make a good environmental enrichment tool (for review see Murphy, McSweeney, Smith, & McComas, 2003). The goal of environmental enrichment is to enhance the well-being of the animals by modifying their

environment (Newberry, 1995). A useful tool needs to trigger the interest of animals. While monkeys explore new devices for a short period due to curiosity, primary reinforcers, such as food, seem to prolong the interest of an animal into a certain activity. However, even with primary reinforcers, the risk of within-session reduction in the number of interactions an animal performs towards the device, decreases with time, due to an effect known as habituation (McSweeney, Hatfield, & Allen, 1991). We observed that across sessions none of the animals stopped working on the task (figure S1), even though they were not subject to fluid or caloric control schedules. Our experiment was not built to test the habituation hypothesis. Yet, our results could indicate that a dynamic device that changes gradually but constantly will less likely lead to habituation (Tarou & Bashaw, 2007). It should be noted, though, that in the current phase of the project the animals were in a compartment connected but separated from their housing compartment for the purpose of the automated training. There they encountered fewer stimuli than they would normally in their home cage during the rest of the day. The lack of other opportunities might have triggered some of the interactions with the device. On the other hand, occasional access to other objects or peers located in the adjacent compartment did not seem to have a negative effect on the motivation to interact with the device.

Automated cage-based training vs. conventional neuroscience training

With our approach, we were able to train four of the eight animals to a standard task used in cognitive neuroscience research without using fluid or caloric control schedules. Nonetheless, there are several disadvantages in comparison to conventional neuroscience training where the animal sits in a primate chair and water control is typically employed to enhance motivation. First, even the four best

animals, which finished the COR task, still needed on average 77.3 sessions and 19285 trials to learn the task, not considering THR training before. Five animals trained in the conventional way with fluid control, learned the almost identical task on average in 17.6 sessions and 9191 trials. This means, not surprisingly, that water control schedules for increasing the value of reward decreased the total training period in our example on average by a factor of 4.4. Second, most cognitive neuroscience tasks require other or additional devices as a touchscreen, such as eye tracking, joysticks or 3D-vision. Especially scientific constraints or technical devices which require steady head position or body posture, are obviously much harder if not impossible to implement in a cage-based training device. Third, training within the housing environment introduces additional distracting stimuli, which cannot be controlled for such as various noise sources, personnel entering the room, and other monkeys in view. Forth, the conventional training is already performed inside the experimental setup, which the monkey needs to be accustomed to before invasive experimental procedures start. It is not clear yet, how well monkeys will generalize the same task across different setups. Finally, a well-experienced trainer should be able to adapt a training protocol to an individual animal in a way that is beneficial for a fast training progress. Part of the reported difference in the speed of learning between the automated training and the conventional training could be explained by the fact that the automated algorithm was not optimized for speed and animals spent unnecessary long time on easy task stages, which can be prevented in supervised training. On the other hand, deviating from a pre-defined protocol bears the risk of introducing variable learning histories, potentially confounding later results of cognitive testing.

Conclusion

Despite slow training progress, we believe that our cage-based automated training approach has a high potential to aid cognitive neuroscience training. Using our XBI device (Calapai et al., 2016), which can easily be attached to the home cage of the animal, we demonstrated that it is possible to train animals on cognitive tasks without applying fluid control and without intervention by personnel Such cage-based automated training can be used for pre-training animals on cognitive tasks, even in facilities which otherwise do not have experimental setups such as breeding facilities. In addition, our cage-based training approach provides a potentially less stressful training environment for the animal. By allowing the animal to choose how much it wants to interact with the device and at which time, a certain level of control over their own situation is given back to the animal which also benefits their welfare. A less stressful environment might be beneficial for training difficult steps before introducing it in the conventional training to reduce the possibility of frustration. Furthermore, our automated training, which increases in difficulty according to the animal's abilities, shows to keep the animal engaged and cognitively alert when interacting with the device. We observed low habituation effects to the device. Such qualities are demanded of items to enrich the animal's environment, suggesting that the XBI might be used as an enrichment item for animals in their home cage, in particular for animals housed separately from other animals.

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References

Anagnostaras, S. G. (2014). Computer-Assisted Behavioural Assessmentof Pavlovian Fear Conditioning in Mice. *Learning & Memory*, *7*(1), 1–15.

Andrews, M. W., & Rosenblum, L. A. (1994). Automated Recording of Individual Performance and Hand Preference during Joystick-Task Acquisition in Group-Living Bonnet Macaques (Macaca radiata). *Journal of Comparative Psychology*, *108*(4), 358–362. http://doi.org/10.1037/0735-7036.108.4.358

Calapai, A., Berger, M., Niessing, M., Heisig, K., Brockhausen, R., Treue, S., & Gail, A. (2016). A cage-based training, cognitive testing and enrichment system optimized for rhesus macaques in neuroscience research. *Behaviour Research Methods*. http://doi.org/10.3758/s13428-016-0707-3

Capitanio, J. P., Kyes, R. C., & Fairbanks, L. a. (2006). Considerations in the selection and conditioning of Old World monkeys for laboratory research: animals from domestic sources. *ILAR Journal / National Research Council, Institute of Laboratory Animal Resources*, *47*(4), 294–306.

Crammond, D. J., & Kalaska, J. F. (1994). Modulation of preparatory neuronal activity in dorsal premotor cortex due to stimulus-response compatibility. *J Neurophysiol*, *71*(3), 1281–1284.

Fagot, J., & Bonté, E. (2010). Automated testing of cognitive performance in monkeys: use of a battery of computerized test systems by a troop of semi-free-ranging baboons (Papio papio). *Behaviour Research Methods*, *42*(2), 507–16. http://doi.org/10.3758/BRM.42.2.507

Fagot, J., & Paleressompoulle, D. (2009). Automatic testing of cognitive performance in baboons maintained in social groups. *Behaviour Research Methods*, *41*(2), 396–404. http://doi.org/10.3758/BRM.41.2.396

Gail, A., & Andersen, R. A. (2006). Neural Dynamics in Monkey Parietal Reach Region Reflect Context-Specific Sensorimotor Transformations. *Journal of Neuroscience*, *26*(37), 9376–9384. http://doi.org/10.1523/JNEUROSCI.1570-06.2006

Gazes, R. P., Brown, E. K., Basile, B. M., & Hampton, R. R. (2013). Automated cognitive testing of monkeys in social groups yields results comparable to individual laboratory-based testing. *Animal Cognition*, *16*(3), 445–458. http://doi.org/10.1007/s10071-012-0585-8

Klaes, C., Westendorff, S., Chakrabarti, S., & Gail, A. (2011). Choosing Goals, Not Rules: Deciding among Rule-Based Action Plans. *Neuron*, *70*(3), 536–548. http://doi.org/10.1016/j.neuron.2011.02.053

Kuang, S., Morel, P., & Gail, A. (2016). Planning Movements in Visual and Physical Space in Monkey Posterior Parietal Cortex. *Cerebral Cortex*, *26*(2), 731–747. http://doi.org/10.1093/cercor/bhu312

McSweeney, F. K., Hatfield, J., & Allen, T. M. (1991). Within-session responding as a function of post-session feedings. *Behavioural Processes*, *22*(3), 177–86. http://doi.org/10.1016/0376-6357(91)90092-E

Miller, B., Lim, A. N., Heidbreder, A. F., & Black, K. J. (2015). An Automated Motion Detection and Reward System for Animal Training. *Cureus*, *7*(12), e397. http://doi.org/10.7759/cureus.397

Murphy, E. S., McSweeney, F. K., Smith, R. G., & McComas, J. J. (2003). Dynamic changes in reinforcer effectiveness: theoretical, methodological, and practical implications for applied research. *Journal of Applied Behaviour Analysis*, *36*(4), 421–438. http://doi.org/10.1901/jaba.2003.36-421

Newberry, R. C. (1995). Environmental enrichment: Increasing the biological relevance of captive environments. *Applied Animal Behaviour Science*, *44*(2-4), 229–243. http://doi.org/10.1016/0168-1591(95)00616-Z

Prescott, M. J., Brown, V. J., Flecknell, P. A., Gaffan, D., Garrod, K., Lemon, R. N., … Whitfield, L. (2010). Refinement of the use of food and fluid control as motivational tools for macaques used in behavioural neuroscience research: Report of a Working Group of the NC3Rs. *Journal of Neuroscience Methods*, *193*(2), 167–188. http://doi.org/10.1016/j.jneumeth.2010.09.003

Snyder, L. H., Batista, a P., & Andersen, R. a. (1997). Coding of intention in the posterior parietal cortex. *Nature*, *386*(6621), 167–170. http://doi.org/10.1038/386167a0

Tarou, L. R., & Bashaw, M. J. (2007). Maximizing the effectiveness of environmental enrichment: Suggestions from the experimental analysis of behaviour. *Applied Animal Behaviour Science*, *102*(3-4), 189–204. http://doi.org/10.1016/j.applanim.2006.05.026

Truppa, V., Garofoli, D., Castorina, G., Mortari, E. P., Natale, F., & Visalberghi, E. (2010). Identity concept learning in matching-to-sample tasks by tufted capuchin monkeys (Cebus apella). *Animal Cognition*, *13*(6), 835–848. http://doi.org/10.1007/s10071-010-0332-y

Weed, M. R., Taffe, M. A., Polis, I., Roberts, A. C., Robbins, T. W., Koob, G. F., … Gold, L. H. (1999). Performance norms for a rhesus monkey neuropsychological testing battery: Acquisition and long-term performance. *Cognitive Brain Research*, *8*(3), 185–201. http://doi.org/10.1016/S0926-6410(99)00020-8

Westendorff, S., Klaes, C., & Gail, A. (2010). The cortical timeline for deciding on reach motor goals. *Journal of Neuroscience*, *30*(15), 5426–5436. http://doi.org/10.1523/JNEUROSCI.4628-09.2010

Westlund, K. (2014). Training is enrichment—And beyond. *Applied Animal Behaviour Science,* Volume 152, 1-6.

Wise, S. P., & Mauritz, K. H. (1985). Set-related neuronal activity in the premotor cortex of rhesus monkeys: effects of changes in motor set. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, *223*(1232), 331–354. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2858101

Sustained spatial attention accounts for the direction bias of human microsaccades

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Abstract

Microsaccades are involuntary small eye movements that happen while we maintain our gaze on a stationary point. Previous studies have shown that shortly after a symbolic spatial cue, indicating a behaviorally relevant location, microsaccades tend to be directed toward the cued region. This has led to the theory that microsaccades can be seen as an index for the covert orientation of spatial attention. However, this hypothesis faces two major issues. First, physiological effects of visual spatial attention are entangled with those of saccade planning. In this respect a systematic investigation is needed to assess to which extent saccade planning can influence microsaccade directions. Second, it is unclear whether the observed microsaccade direction effect is attention-specific or rather cue-specific. To address the first issue, we investigated the direction of microsaccades in human subjects when they attend to a behaviorally relevant location, while preparing a response eye movement either toward or away from this location. We find that directions of microsaccades are in fact biased toward the attended location rather than towards the

saccade target. To tackle the second issue, we verbally instructed the subjects about the location to attend, before the start of each block, so as to exclude potential visual cue-specific effects on microsaccades. Results indicate that despite the absence of visual cues during the experiment, sustained spatial attention alone reliably produces the microsaccade direction effect. Overall, our findings demonstrate that sustained spatial attention, without influences from saccade planning or the spatial cue per se, is sufficient to explain the direction bias observed in microsaccades.

Introduction

Microsaccades are involuntary, small ballistic eye movements that occur during gaze fixation[1]. They have long been considered as noise in the eye movement system[2] until research within 15 years revealed some non-trivial feature about their frequency and direction. It has been reported in several human psychophysical studies, that around 300 ms after subjects are instructed by a symbolic spatial cue (e.g. an arrow-head at gaze location, a pre-assigned color or a sound source) to attend to a certain location, the directions of microsaccades were biased toward the location indicated by the cue, suggesting microsaccade's role as an index for covert spatial attention[3-5]. However, such an attention-specific interpretation of the post-cue microsaccade direction bias faces two challenges. First, while visual spatial attention is known to be closely entangled with saccade planning[6,7], such planning is known to interfere with the dynamics of microsaccades[5]. Thus, to truly attribute the microsaccade direction effect to attention, it is necessary to remove any effect of saccade planning. Secondly, it is unclear whether the

microsaccade direction effect is a reliable index of sustained attention, or, alternatively, merely a transient effect[8]. Specifically, previous studies that report the post-cue microsaccade direction bias have focused on a very specific time window, around 300 ms after the cue onset[3,5,8,9]**.** and little to no evidence is available regarding whether this effect would last as long as spatial attention is maintained, or if it is only triggered by an immediately preceding spatial cue. It is important to note that for exogenous cues (i.e. a visual stimulus at the cued location) directions of microsaccades are directed away from the cued location[5,8], in contrast to the effect induced by an endogenous one.

To address these challenges, we recorded human eye movements during periods of fixation while the subjects performed a spatial attention guided *match to sample* task (Figure 1A). Our results demonstrate a consistent spatial attention effect on microsaccade directions, that is not directly triggered by a spatial cue, free from influences of saccade planning. These findings not only show the tight correlation between microsaccade direction and the subjects' internal attentional state, but also challenge the notion that spatial attention is functionally equivalent to a planning process of unexecuted movement (i.e. the premotor theory of attention[6]).

Methods

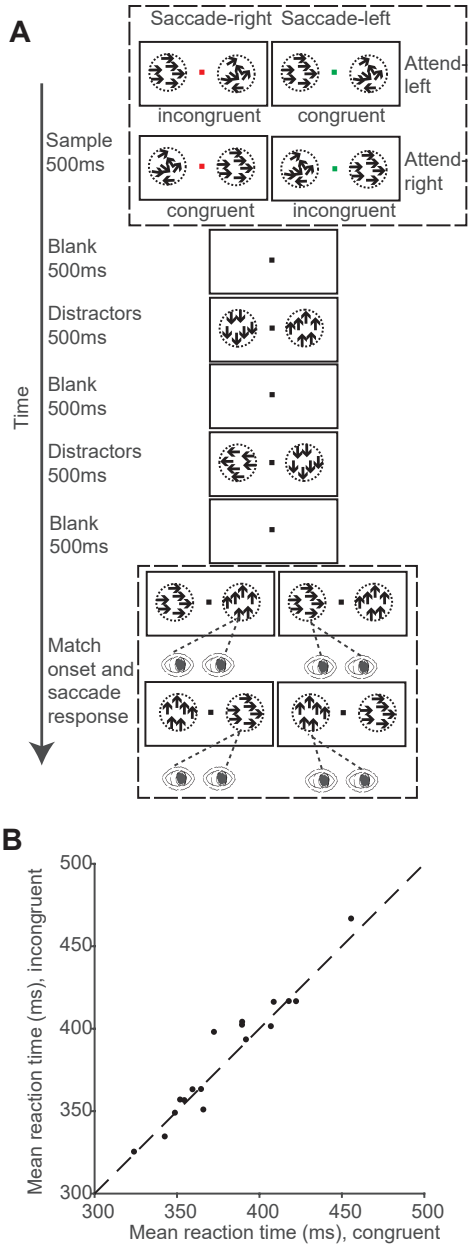

Figure 1. Match to sample task to dissociate attention and saccade planning. A) Task flow. Once the subject pressed a button and foveated the central fixation point. One fully coherent RDP and one non-coherent RDP were displayed. The coherent RDP is the sample stimulus. After a brief blank interval, a series of stimuli-pairs followed, and the subjects needed to respond when they found a match with the sample. and otherwise maintain fixation. The match can occur in any stimuli-pair at the same location as the sample, or in a small fraction of trials, does not appear at all. When the subjects found the match, they have to respond by making a saccade to one of the stimulus locations, which was instructed by the color of the fixation dot during the sample phase (red for rightward saccade, green for leftward saccade). B) Mean reaction times of incongruent hit trials (when the match appeared and the subjects correctly responded) plotted against that of congruent hit trials. Each dot represents one human subject. The dashed diagonal line indicates unity line.

Experiment setup

For both experiments, participants were seated at 57 cm distance from a 22" Samsung SyncMaster 2233RZ monitor, operating at a resolution of 1680 x 1050 pixels, with 120 Hz refresh rate. Eye Movements were acquired with an Eyelink 1000 (Version 4.56) while each subject's chin rested on a platform to maintain head position throughout the experimental sessions. The open-source software MWorks (Version 0.5) was used to run the tasks and to record the subjects' behavioral data.

Human subjects

This study recruited 35 naïve subjects (16 for experiment 1, 19 for experiment 2), whose gender, age, handedness, and vision profiles were listed in supplementary table 1. The study was approved by the Ethics Committee for experiments with humans of the Georg-Elias-Müller-Institute of Psychology, University of Göttingen, and followed the principles of the Declaration of Helsinki. Each subject received verbal and written information about the task, and gave written consent before the experiment started, and received monetary compensations after the experiment.

Experiment 1

In experiment 1 (Figure 1A), subjects depressed a button on a game pad (Logitech Inc., Precision) to start a trial. During the trial, subjects were required to maintain eye fixation at a central dot (size = 1 degree of visual angle – *dva* – in diameter, luminance = 5.65 cd/m^2, fixation window 2 dva in radius) until they decided to make a saccade to the required goals

as a response. Other fixation breaks would terminate the trial, which would be repeated later. Upon trial start, the fixation dot took on a color (either red or green) that informed the subjects about the way of response at the end of the trial (by making a rightward or a leftward saccade). During a sample phase, one random dot motion pattern (RDP, size = 8 dva; luminance = 30.09 cd/m^2, number of dots = 100, dot size = 0.25 dva, speed 5 dva/second) were displayed in each visual hemifield (RDPs centered 15 dva from the fixation point). One RDP had dots moving in random directions with zero coherence, and was irrelevant for the behavioral task. The other RDP (the sample) had coherently moving dots in one of four cardinal directions (up, right, down, left). The sample was followed by up to three alternating blank periods and displays of fully-coherent RDP-pairs. Subjects were required to detect a RDP with the same motion direction with the sample (a *match* stimulus), which might appear in any stimulus display period. In 10% of the trials, none of the three stimulus display periods contained a match, in which case the subjects just needed to maintain fixation till the end of the trial. The match, if it appeared, would always be at the same location as the sample. To report a match-detection, the subjects needed to make a saccade (either leftward or rightward) according to the color of fixation dot during the sample phase (green or red). Therefore, the response-saccade can be directed towards the same side as the match (a pro-saccade in a congruent trial), or to its opposite side (an anti-saccade in an incongruent trial). After match appearance, the subjects was required to respond within a time window individually determined for each subject through a staircase procedure prior the experiment started. The subjects performed the trials (with auditory feedback about the trial outcome at the end of each trial) as the response time

window adapted, until their performance stabilized at 80%; and the corresponding response time window was used throughout the following experiment. Each subject needed to correctly perform 480 trial to complete the experiment.

Experiment 2

A total of 19 subjects took part in Experiment 2. The task for the subjects was similar to experiment 1 except two major distinctions: (1) the trials were performed in blocks (80 correctly performed incongruent trials each block); within each block, all trials had a fixed location of attention (left or right), and a fixed goal for response-saccades (always on the other visual hemifield of the location of attention); (2) the sample phase contained only one fully-coherent sample stimulus located at the center. The location of attention (left or right) was instead given by a verbal instruction before each trial-block; while the goal for response-saccade in that block was inferred since all trials were incongruent trials. In other words, no stimulus during a trial block was spatially informative in any way. Stimuli used in experiment 2 were similar to that of experiment 1: fixation dot, 0.5 dva in diameter, luminance = 59.91 cd/m^2; RDPs, size = 8 dva; luminance = 30.09 cd/m^2, number of dots = 100, dot size = 0.15 dva, speed 4 dva/second.

Microsaccade detection

We adopted the commonly used velocity threshold method described in Engbert & Kliegl, 2003 for microsaccade detection. We calculated the velocity for each eye at each millisecond based on the measured eye positions within a shifting time window of 8

milliseconds. The velocity threshold for each eye is then set at six times the standard deviation of all velocity magnitudes. All threshold crossing events are then compared between the two eyes, and only those with binocular threshold crossings are marked as microsaccades[3]. We detected 10426 microsaccades in experiment 1, and 6790 microsaccades in experiment 2. The algorithm-detected microsaccades were also visually inspected, and the start or endpoint of 643 microsaccades were manually corrected. This operation does not affect the directions of those microsaccades, which were defined as the direction of the peak velocity of the microsaccade.

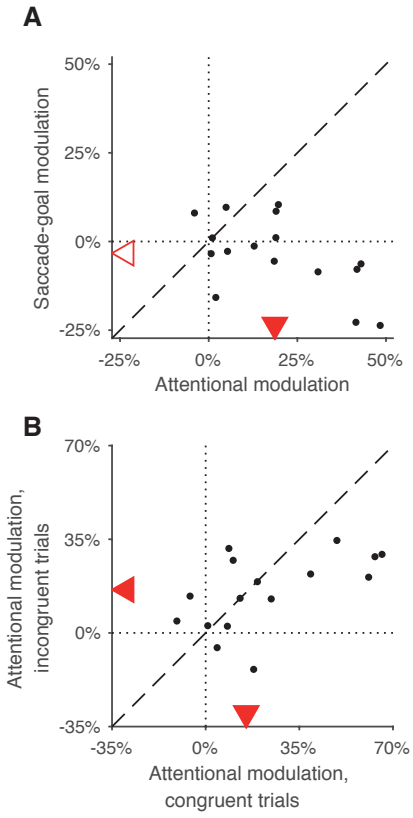

Figure 2. Overall microsaccade-directional modulation (see Material and Methods). A) The microsaccade-directional modulations by attended location (abscissae) plotted against microsaccade-directional modulations by saccade goal (ordinates). Each dot represents one subject. For both attended location and saccade goal, a positive modulation indicates a microsaccade-directional bias towards the respective location. Dotted vertical and horizontal lines indicate the zero line of abscissa and ordinates, respectively. Dashed line shows the unity line. Red arrows on horizontal and vertical axes indecate the median of abscissa and ordinates among all subjects, respectively. Filled symbols indicate the median is significantly different from zero; while open symbols, not. B) The microsaccade-directional modulation by attention for congruent-cue trials (abscissa) plotted against that for incongruent-cue trials (ordinates). Lines and symbols are similarly defined as in A).

This detection procedure clearly distinguished microsaccade from other smaller fixational eye movements and potential noise in the measurement (Figure S1, example microsaccade traces). The detected microsaccades showed a linear relationship (Pearson's correlation coefficient = 0.94, $p<0.0001$) between amplitude and maximal speed (also known as the main sequence[10], see Figure S2). We also observe that after a change in visual stimuli (e.g. the offset of stimuli), the rate of detected microsaccades temporarily drops, and rises to a peak at around 250-300ms after the stimulus change (Figure S3): a similar observation to what had been reported in many other studies[3-5].

To investigate the microsaccade-directional profile while the subjects were expecting a potential upcoming match, most results were based on microsaccades that occurred during the blank periods (except in Figure 3, where direction profile were compared during stimuli with that during blank).

Results

Subjects were required to respond to the onset of a certain stimulus at one of two locations. We looked into the effect of the behaviorally relevant location on the distribution of microsaccade-directions, and whether such an effect is contingent on saccade planning or spatial cuing.

Simultaneous attentional deployment and saccade preparation

To disentangle the effects of spatial attention and saccade planning on microsaccade-direction, two independent spatial cues were given at the beginning of each

trial in experiment 1: the attention cue, indicating the location (left or right side of the screen) of the match if it appears, and the saccade cue, instructing the goal of the response-saccade (towards left or right). The locations of both cues were randomized for each trial, indicating either the same location (congruent trials) or opposite locations (incongruent trials). By dividing the trials either according to the location of spatial attention or the goal of response saccade, the influences of spatial attention and saccade planning on microsaccade direction can be separately evaluated.

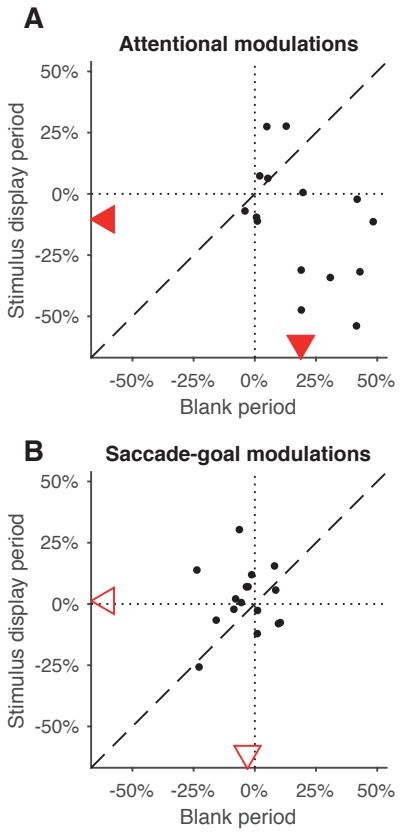

Figure 3. Microsaccade-directional modulations during blank periods versus stimulus display periods. A) shows the modulations by attended location, B) shows the modulations by saccade goal. In both A) and B), each dot represents one subject; its abscissa and ordinate represent the microsaccade-directional modulations during blank periods and during stimulus display periods, respectively. Dotted vertical and horizontal lines indicate the zero line of abscissa and ordinates. Dashed line shows the unity line. Red arrows on horizontal and vertical axes indecate the median of abscissa and ordinates among all subjects, respectively. Filled symbols indicate the median is significantly different from zero; while open symbols, not.

One critical objective of the experimental design is to encourage the subjects to plan a saccade to a given location already before the match appears (while also attending to an

independent location), rather than to plan a saccade only after match detection (when the attended location is no longer relevant). Given that the subjects are under time pressure to respond as quickly as possible (see methods), the latter strategy would likely lead to a longer reaction time in incongruent trials than in congruent trials. However, none of our 16 subjects showed significantly different reaction times between the two trial types (Bonferroni-Holm corrected rank sum test, $p>0.05$ for all subjects) Figure 1B shows the subjects' mean reaction times for congruent trials (abscissa of the scattered dots), and for incongruent trials (ordinates of the scattered dots), respectively. There is no significant pair-wise difference across subjects ($p=0.8$, Wilcoxon signed rank test), either.

Microsaccade-direction is biased toward the attended location, not the saccade goal. For each subject, we compare the distributions of microsaccade-directions during *attend left* trials against *attend right* trials. Taking the difference of leftward-microsaccade proportions of the two trials types provides a quantitative measure for the magnitude of attentional modulation of microsaccade directions: a positive attentional modulation indicates a bias of microsaccade direction towards the attended location, while a negative indicates a bias away from it. The abscissa of the scatter plot Figure 2A show the microsaccade-directional modulations by spatial attention for the 16 subjects tested in this experiment,. The directions of microsaccades were significantly biased towards the attended location (, median 18.71%, $p=0.0009$, Wilcoxon signed-rank test).

Similarly, by taking the difference of leftward-microsaccade proportions of trials in which

the response saccades are directed towards the left or the right hemifield, we determined the microsaccade-directional modulation by saccade-goal locations. The ordinates of the scatter plot Figure 2A show the microsaccade-directional modulations by saccade-goal. We did not observe any significant effect of the planned saccade goal on the direction of microsaccades (median -3%, p=0.3, Wilcoxon signed-rank test). A pair-wise signed-rank test also confirms that the attentional modulations are significantly larger than saccade-goal modulations, both toward the saccade-goal (comparing attentional modulations and saccade-goal modulations, p=0.003, Wilcoxon signed-rank test), and away from the saccade goal (comparing attentional modulations and the reversed saccade-goal modulations, p=0.003, Wilcoxon signed-rank test). There is also no significant correlation between attentional modulations and saccade goal modulations (Kendall's rank correlation = -0.283, p=0.1).

Attentional effect on microsaccade direction is consistent for congruent and incongruent cue trials

Although the saccade-goal is not a significant modulatory factor of microsaccade directions, it could still have a significant interaction with attention. We therefore looked at the attentional modulations in congruent-cue trials (attention cue and saccade cue at the same location, shown with abscissa of the scatter plot Figure 3B) and incongruent-cue trials (attention cue and saccade cue at opposite locations, shown with ordinates of the scatter plot Figure 3B), respectively. Similar attentional modulations were observed in both trial types (congruent-cue trials, median 15.4%, p=0.003; incongruent-cue trials,

median 16.0%, p=0.003; Wilcoxon signed-rank test). There is also no significant difference between the sizes of attentional modulations (p=0.2, Wilcoxon signed-rank test). We also did a two-way ANOVA on the left microsaccade proportions in all four combinations of attention and saccade-goal locations, which also confirmed the above conclusions: attended location is a significant factor (p<0.0001), while saccade-goal is not (p=0.8), neither is the interaction between attention and saccade goal (p=0.4).

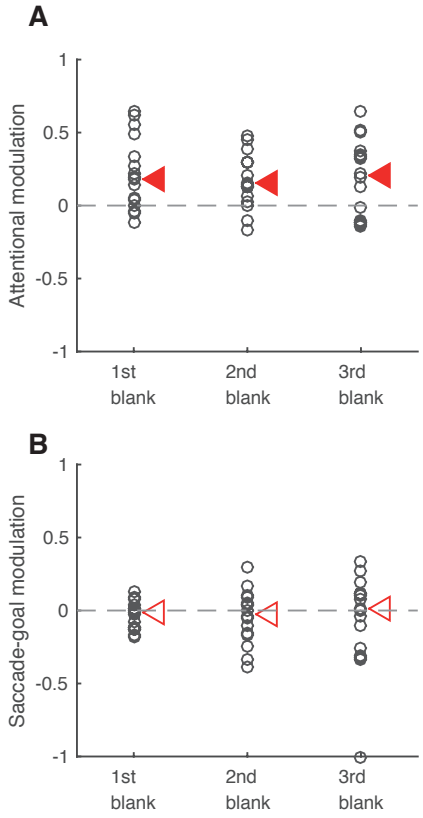

Figure 4. Microsaccade-directional modulations over the course of a trial. A) shows the modulations by attended location, B) shows the modulations by saccade goal. In both A) and B), directional modulations for microsaccades that occured during the first, second, and third blank periods are shown separately for all subjects (denoted by circles). Dashed horizontal line indicates zero modulation. Red arrows indicate the median modulation during each blank period. Filled symbols indicate the median is significantly different from zero (Bonferroni-Holm corrected); while open symbols, not.

Attention modulates microsaccade-directional modulations during blank period and stimulus display period differently.

We have reported that attention biased microsaccade-direction towards the attended

location when the subjects were expecting the onset of a potential match, as shown by the abscissa of the scatter plots Figure 2A and Figure 3A.. Interestingly, however, when we look into microsaccade-directions during the display of RDP-pairs (i.e. Figure 1A distractor periods, during which the subjects correctly maintained fixation), as shown by the ordinates of the scatter plot Figure 3A, microsaccade-directions were biased away from the attended location median -10.32%, p=0.04, Wilcoxon signed rank test). A pair-wise comparison between attentional modulations of microsaccade-direction during stimulus display periods and those during blank periods also showed significant difference (p=0.01, Wilcoxon signed rank test). Similarly, Figure 3B shows the saccade-goal modulations of microsaccade-directions during blank periods (abscissa) and during stimulus display period (ordinates).Saccade-goal does not have a significant effect on microsaccade-direction during stimulus display periods (median 1.3%, p=0.6, Wilcoxon signed rank test), not significantly different from its microsaccade-directional effects during the blank periods(p=0.2, Wilcoxon signed rank test). .

Sustained attention, not spatial cue, modulates microsaccade-direction.

Previous studies have primarily reported a microsaccade direction effect around 300 ms after the spatial cue offset, when the microsaccade rate peaks. This makes it difficult to disentangle the role of sustained attention, and the role of the cue itself. In our design, the first blank period of each trial was preceded by the spatial cue (location of the sample), but the second and third blank periods were preceded with space-neutral distracting stimuli, which masked the direct visual influence from the attention cue. As shown in Figure 3A,

the attentional modulations for the three blank intervals were all positively shifted (first blank period, median 18.56%, p=0.016; second blank period, median 15.05%, p=0.016; third blank period, median 20.59%, p=0.017; all p values were calculated with Bonferroni-Holm corrected Wilcoxon signed rank test). This indicates that microsaccade-directions exhibit a significant bias toward the attended location, even if it is not immediately preceded by a spatial cue. Meanwhile, also consistent with the conclusions based on all blank period microsaccades, saccade-goal also does not significantly influence microsaccade-directions in any of the three periods alone (first blank period, median -1.93%, p=0.7; second blank period, median -2.23%, p>0.9; third blank period, median 0.77%, p>0.9; all p values were calculated with Bonferroni-Holm corrected Wilcoxon signed rank test).

To further isolate the microsaccade-directional modulation by sustained attention from potential confounds due to the visual stimulus used as a spatial cue, we conducted experiment 2, in which we tested the subjects with blocks of incongruent trials. All trials within each block had the same behavioral relevant location, and the same response saccade goal on the opposite side of the behavioral relevant location. Before each block started, we verbally gave the spatial cue, so that the trials within each block did not include a visual stimulus as spatial cue. We again calculated the attentional modulation of microsaccade directions by taking the difference between left microsaccade proportions in attend-left trial block and in attend-right trial block. The distribution of attentional modulations on microsaccade-directions is plotted in the histogram Figure 5. Despite the

absence of a spatial cue, we still found similar attention effects on microsaccade direction as in experiment 1 (median 8.9%, p=0.003), induced only by the subjects' prior knowledge of the location of the potential match. This strongly supports the hypothesis that sustained attention alone is enough to explain the bias in microsaccade direction.

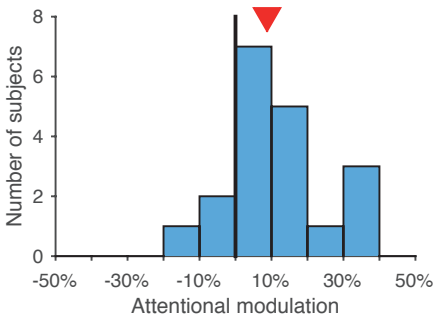

Figure 5. Microsaccade-directional modulations by endogenous attention, without preceding visual cue. Black vertical lines indicates no modulation. The red arrow over the histogram shows the median modulation. As in previously plots, filled symbol indicates the median is significantly different from zero.

Discussion

The exact interpretation over the nature of the microsaccade direction effect has been controversial. While many suggest that microsaccades can be considered as overt indicator of covert attention[3-5,8,9,11-14], others believe microsaccade-directions are dependent on other factors than spatial attention [15-20]. Our study attempted to disentangle microsaccade-direction effect induced by attentional allocation from confounding factors, such as oculomotor preparation, and direct effect from visual cue. Our results showed that microsaccade-direction was biased toward attended location when the subject is expecting an upcoming target. Such an effect can be induced by sustained attention alone, and is not

contingent on an immediately-preceding spatial cue. However, we found no evidence for either a direct influence on microsaccades' direction from oculomotor planning, or an interaction between attention and oculomotor planning [14].

Spatial attention and oculomotor planning at the same time

Many studies have revealed the entanglement between visual spatial attention and oculomotor planning[6,7,21]. Our way to separate the effects of the two is to encourage subjects to sustain spatial attention to one location, while at the same time also be ready to release a saccade towards an independent location. However, the pre-motor theory of attention, which posits that visual spatial attention is a result of oculomotor planning towards the attended location, would necessarily mean that subjects could only plan a saccade to a different location after their spatial attention is disengaged from a previous location, i.e. after the match is detected. Given that we ensure all subjects were under similar time pressure to respond as soon as possible (see staircase procedure in Methods), stimulus-response compatibility would predict longer reaction times for incongruent trials than for congruent trials [22], if oculomotor planning did not take place before the saccade go signal (the match onset). The fact that our subjects do not show such difference (either between reaction time distrubutions within each individual subject, or between mean reaction times across subjects) strongly suggests that oculomotor planning occurred before match onset, while the subjects were also expecting a potential match at an independent location.

Exogenous cue and inhibition of return

Rolfs and his colleagues reported that when using an exogenous cue to indicate a behavioral relevant location, microsaccades tend to be directed away from the cued locations[5], an opposite effect than when an endogenous cue is used[3], bringing questions upon whether the microsaccade-directional modulation is a visual cue-sensitive effect, rather than an attention effect. Meanwhile, it is hypothesized that this opposite effect of an exogenous cue could be attributed to the inhibition of return (IOR) caused by the onset of a salient peripheral visual stimulus [23]. Indeed, Galfano and colleagues showed that microsaccades were directed away from a peripheral stimulus that was not even behaviourally relevant [15]. Besides Rolfs et al 2005, in other exogenously cued attention tasks, it seems such opposing effects on microsaccade-direction can also reach various equilibria, depending on stimulus-parameters: microsaccade-direction showed neither an IOR effect nor an attention effect, when the peripheral cue was merely a flash of small while dot within the context of a large symmetric stimulus-array[18]; or, microsaccade-direction can also show a net effect towards the cued location, when the peripheral cue was very close to the fixation point [4]. In this study, we showed that when the peripheral attention cue was offset by a equiluminant stimulus at a symmetric location (experiment 1), or when attention was not instructed by a visual cue (experiment 2), microsaccade-direction was biased toward the attended location. In this context, our results provided further and stronger evidence that the microsaccade-directional modulation is a reliable attentional effect, not a cue-dependent effect. Meanwhile, the countereffect of IOR needs to be carefully controlled when studying

microsaccade-directional effect.

Different microsaccade-directional modulations during blank periods and stimulus display periods

Microsaccades are notoriously rare events[3-5]. By having alternating blank periods and stimulus display periods, our experiment is designed to induce many more microsaccades, to boost the statistical power of our analyses. On the other hand, one might wonder, with the relatively long trials in our experiment (up to 3.5 seconds) and microsaccades that are biased towards the attended location, how did the subjects ensure proper eye fixation? Our control analysis in Figure 3A showed that microsaccade-direction during stimulus-display periods was biased the opposite way to that during blank periods. This implies that microsaccades occurred during peripheral stimulus-display could have a distinct role: to correct eye position displacements[11,24]. Since a match is not expected during the time a pair of distractor RDPs are still on display, It's conceivable that microsaccades during these periods reflect the oculomotor system's effort to countermand a fixation break after the sudden onset of peripheral RDP-pairs[25]. However, to best address the role of microsaccades during stimulus display, future studies need to introduce stimulus-onset asynchrony to dissociate effects of stumulus-onset and potential counfounding effects from expectation of future events.

The Premotor Theory of Attention: not the complete story?

There is much dispute whether saccade preparation and visual spatial attention share the

same neuronal mechanism, based on findings from various experiments, from human psychophysics to monkey neurophysiology[6,26-28]. Our results shows: 1) incongruent trials do not take longer to complete than congruent trials; 2) when subjects are instructed to maintain spatial attention and also preparing for a saccade, microsaccadic directions are consistently biased towards the attended location, regardless of the saccade goal location. The former suggests that maintaining spatial attention and oculomotor planning can be executed at the same time toward different spatial locations. And the latter suggests that deploying spatial attention, but not preparing for saccades, has an effect on microsaccade directions. Given these findings, it is highly possible that there might be independent neuronal circuitries to implement visual spatial attention and oculomotor planning. Future monkey electrophysiology studies with a similar task might yield interesting insight into the neuronal basis of such mechanism.

Acknowledgements

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Author contributions:

CX, AC and ST designed the experiment; CX, KD and JK performed the experiment; CX analyzed the data; CX, AC and ST wrote the paper.

Conflict of Interest

The authors declare no competing financial interests.

References

1. Martinez-Conde, S., Macknik, S. L., Troncoso, X. G. & Hubel, D. H. Microsaccades: a neurophysiological analysis. *Trends in Neurosciences* **32,** 463–475 (2009).
2. Kowler, E. & Steinman, R. M. Small saccades serve no useful purpose: reply to a letter by R. W. Ditchburn. *Vision Research* **20,** 273–276 (1980).
3. Engbert, R. & Kliegl, R. Microsaccades uncover the orientation of covert attention. *Vision Research* **43,** 1035–1045 (2003).
4. Hafed, Z. M. & Clark, J. J. Microsaccades as an overt measure of covert attention shifts. *Vision Research* **42,** 2533–2545 (2002).
5. Rolfs, M., Engbert, R. & Kliegl, R. Crossmodal coupling of oculomotor control and spatial attention in vision and audition. *Exp Brain Res* **166,** 427–439 (2005).
6. Rizzolatti, G., Riggio, L., Dascola, I. & Umiltá, C. Reorienting attention across the horizontal and vertical meridians: evidence in favor of a premotor theory of attention. *Neuropsychologia* **25,** 31–40 (1987).
7. Smith, D. T. & Schenk, T. The Premotor theory of attention: Time to move on? *Neuropsychologia* **50,** 1104–1114 (2012).
8. Laubrock, J., Engbert, R. & Kliegl, R. Microsaccade dynamics during covert attention. *Vision Research* **45,** 721–730 (2005).
9. Laubrock, J., Kliegl, R., Rolfs, M. & Engbert, R. When do microsaccades follow spatial attention? *Attention, Perception, & Psychophysics* **72,** 683–694 (2010).
10. Bahill, A. T., Clark, M. R. & Stark, L. The main sequence, a tool for studying human eye movements. *Mathematical Biosciences* (1975).
11. Engbert, R. & Kliegl, R. Microsaccades keep the eyes' balance during fixation. *Psychological Science* **15,** 431–436 (2004).
12. Hermens, F. & Walker, R. What determines the direction of microsaccades? *Journal of Eye Movement Research* (2010).
13. Rolfs, M., Engbert, R. & Kliegl, R. Microsaccade orientation supports attentional enhancement opposite a peripheral cue: commentary on Tse, Sheinberg, and Logothetis (2003). *Psychological Science* **15,** 705–7– author reply 708–10 (2004).

14. Hermens, F., Zanker, J. M. & Walker, R. Microsaccades and preparatory set: a comparison between delayed and immediate, exogenous and endogenous pro- and anti-saccades. *Exp Brain Res* **201,** 489–498 (2009).
15. Galfano, G., Betta, E. & Turatto, M. Inhibition of return in microsaccades. *Exp Brain Res* **159,** 400–404 (2004).
16. Horowitz, T. S., Fine, E. M., Fencsik, D. E., Yurgenson, S. & Wolfe, J. M. Fixational eye movements are not an index of covert attention. *Psychological Science* **18,** 356–363 (2007).
17. Horowitz, T. S., Fencsik, D. E., Fine, E. M., Yurgenson, S. & Wolfe, J. M. Microsaccades and Attention: Does a Weak Correlation Make an Index?: Reply to Laubrock, Engbert, Rolfs, and Kliegl (2007). *Psychological Science* **18,** 367–368 (2007).
18. Tse, P. U., Sheinberg, D. L. & Logothetis, N. K. Fixational eye movements are not affected by abrupt onsets that capture attention. *Vision Research* (2002).
19. Tse, P. U., Sheinberg, D. S. & Logothetis, N. K. The Distribution of Microsaccade Directions Need Not Reveal the Location of Attention: Reply to Rolfs, Engbert, and Kliegl. *Psychological Science* **15,** 708–710 (2004).
20. Valsecchi, M., Betta, E. & Turatto, M. Visual oddballs induce prolonged microsaccadic inhibition. *Exp Brain Res* **177,** 196–208 (2007).
21. Moore, T. Microstimulation of the Frontal Eye Field and Its Effects on Covert Spatial Attention. *Journal of Neurophysiology* **91,** 152–162 (2003).
22. Munoz, D. P. & Everling, S. Look away: the anti-saccade task and the voluntary control of eye movement. *Nature Reviews Neuroscience* **5,** 218–228 (2004).
23. Hunt, A. R. & Kingstone, A. Inhibition of return: Dissociating attentional and oculomotor components. *Journal of Experimental Psychology: Human Perception and Performance* **29,** 1068–1074 (2003).
24. CORNSWEET, T. N. Determination of the stimuli for involuntary drifts and saccadic eye movements. *J Opt Soc Am* **46,** 987–993 (1956).
25. Tian, X., Yoshida, M. & Hafed, Z. M. A Microsaccadic Account of Attentional Capture and Inhibition of Return in Posner Cueing. *Front. Syst. Neurosci.* **10,** 428–23 (2016).
26. Corbetta, M. Frontoparietal cortical networks for directing attention and the eye to visual locations: Identical, independent, or overlapping neural systems? *Proceedings of the National Academy of Sciences* **95,** 831–838 (1998).
27. Craighero, L., Nascimben, M. & Fadiga, L. Eye Position Affects Orienting of Visuospatial Attention. *Current Biology* **14,** 331–333 (2004).
28. Moore, T. & Armstrong, K. M. Selective gating of visual signals by microstimulation of frontal cortex. *Nature* **421,** 370–373 (2003).

Supplementary material

Subject list, experiment 1. Note that handedness has been assessed verbally.

Subject	Age	Gender	Handedness	Vision
ANH	22	f	right	normal
CAM	20	f	right	normal
INB	21	f	right	contact lenses
INN	22	f	right	normal
JOD	23	m	right	normal
LEI	22	f	right	normal
MAL	26	m	right	normal
MIS	23	f	right	normal
AGN	23	f	right	glasses
SVE	23	f	right	normal
ANM	24	m	right	glasses
GAE	22	f	right	normal
ALN	20	f	right	normal
THR	23	f	right	normal
LUK	20	m	right	normal
MAG	27	f	right	normal

Subject list, experiment 2. Note that handedness has been assessed verbally.

Subject	Age	Gender	Handedness	Vision
FHA	23	m	right	normal
JKI	20	m	right	normal
MAS	25	m	left	contact lenses
JAK	24	f	right	contact lenses
PAK	24	f	right	contact lenses
LUB	25	f	right	normal
REP	23	m	right	glasses
KES	25	f	right	normal
SIL	33	f	right	normal
TEF	25	f	right	normal
RIW	23	f	right	normal
JUG	23	f	left	normal
DIT	22	f	left	contact lenses
CAR	21	f	right	contact lenses
SGO	21	f	right	normal
ANV	23	f	right	glasses
JMA	31	m	right	glasses

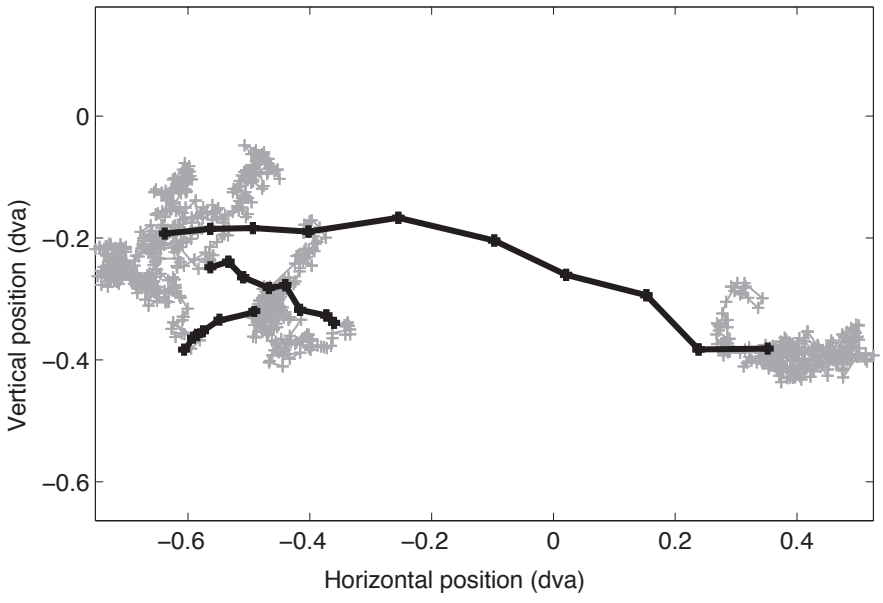

Figure S1. Example binocular eye trace. Black traces indicate detected microsaccades. Detected microsaccades clearly separates itself from other types of eye movement and system noise (grey traces.)

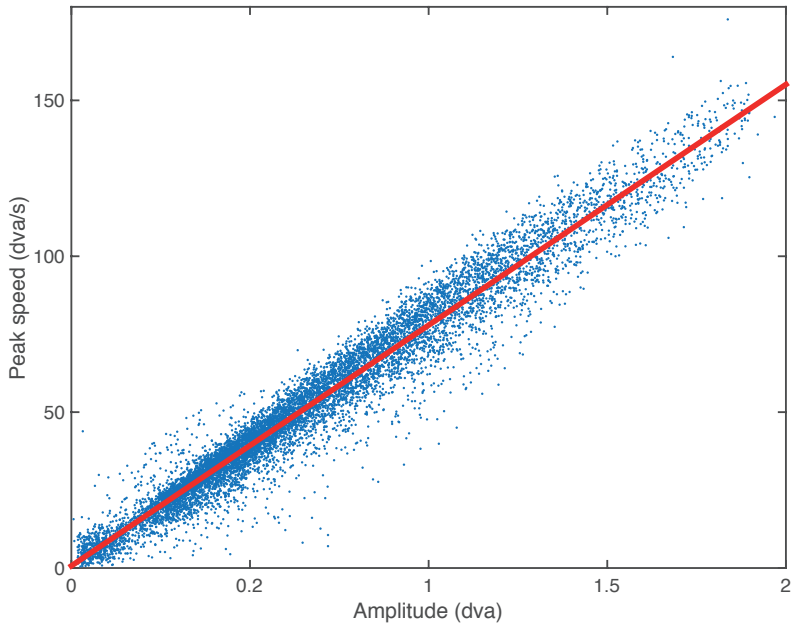

Figure S2. The main sequence. Abscissa and ordinate of the dots indicate the amplitude and peak speed of all the microsaccades across all subjects. black line indicate the linear fit of the data points.

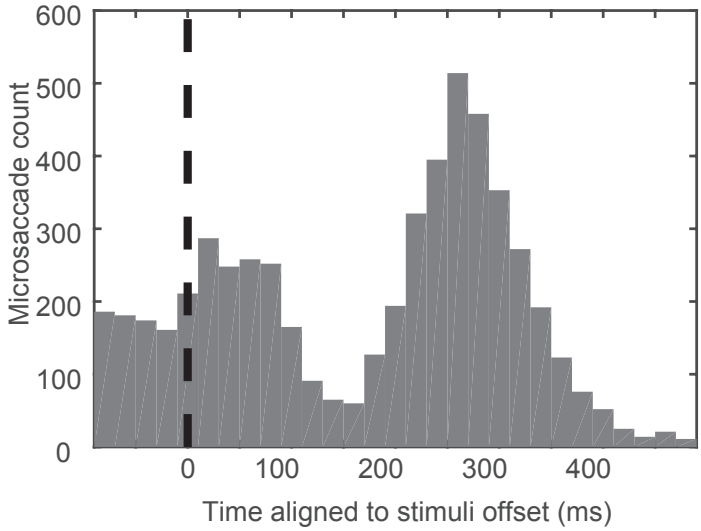

Figure S3. The distribution of microsaccades with respect to the stimulus onset, pooled across all subjects.

General Discussion

Visual-motion and binocular disparity in area MST

From the experiment described in chapter 2, an interesting picture emerges on the role of high order visual area MST in the computation of motion and depth. This area is considered by some to be a crucial node in a diversified computational network for heading-direction estimation and self-motion computation. However, our results suggest that not only are the two features coded independently at the level of this area (in critical disagreement with previous literature where the opposite effect is reported, namely motion and disparity interdependence), but from the population point of view they also have a significantly different weight (motion accounts for ~40% of the variability, disparity accounts for ~5%). We found no evidence supporting the elegant and intriguing idea of integration of motion signals and disparity in area MST, as seen in MST's direction-dependent disparity units. Possible reasons for the discrepancy of our results with respect to existing literature are extensively discussed in the related chapter.

The contribution of our results is relevant for at least three reasons. First of all, they help increasing the understanding of how the brain area MST operates and solves the problem of self-motion, suggesting alternatives to published results and thereby generating hypotheses for future research. Second, redefining the functions of a certain brain region as we have done here may be important when considering the diagnosis and treatment of specific neurological, cognitive and psychiatric disorders anatomically related with the area at issue (e.g. dorsal stream vulnerability). Finally, understanding how a complex biological system behaves when faced with a certain computational problem (e.g.

inferring self-motion) is particularly helpful for the field of robotics and machine learning, where algorithms can be refined on the basis of analogous biological processes established by evolutionary means.

A cage-based training, cognitive, testing and enriching system for Rhesus Macaques

It seems fair to argue that the contribution of macaque monkeys to the advances in science has been proven to be of significant relevance. Refining the way captive animals are trained in certain tasks for use in later neurophysiological recordings is the cornerstone of both section of chapter three. The first section describes a cage-based touch screen device (called XBI) optimized for rhesus macaques and built to be attached to the animals' own enclosure. The second section follows the same eight animals described in section one and compares their performance on an algorithm-based, automatized training procedure. The results described in this chapter suggest that animals can be trained from naïve to expertise in a complex task without direct human interaction during the process.

By means of the algorithmic performance-based program we developed, *ad hoc* standardization of an animal training protocol has been achieved. Importantly, by minimizing training differences among animals, such standardization helps improve data quality by reducing the risk of confounds such as the influence of the experimenter. Moreover, none of the animals involved in the two manuscripts were subjected to any form of fluid intake control and didn't need to leave their own housing, remaining either in physical or visual contact with the rest of the group. Leaving group contact is a factor that can induce significant stress in the animals.

Together with these methodological as well as scientific contributions, we can also consider further factors involved in the ethical debates surrounding NHP testing. While the legitimacy of such testing is beyond the scope of this thesis, the aim of enriching the animals' lives by following the 3R principles permeates both sections. Most of the ethical contribution comes from giving the animals more freedom regarding their typically experimenter-imposed training. Having control helps reduce the frustration related with captivity, improving the animals' psychological as well as physiological wellness, and thus the quality and reliability of any related data. Given its great versatility, the extent of the XBI's contribution in this regard is not easy to estimate, but this is perhaps one of most important contributions of this device.

Spatial Attention and Microsaccades

Psychophysics allows us to non-invasively infer processing mechanisms put in place by the brain, and is in my opinion a great example of the power of the scientific method. When advanced technologies accompany original experimental designs, such power is further increased, and put to good use. The manuscript contained in chapter four tries to combine these three aspects (psychophysics, advanced eye-tracking system, novel experimental design) to explore whether oculomotor and attentional networks share the same neuronal substrate, a debated but still very influential idea called the premotor theory of attention (PMA). The results show that the direction of microsaccadic eye movements of human subjects point towards the attended location rather than the saccade end point, suggesting independence of the attentional system from the oculomotor system.

This contributes to the scientific debate in at least two ways. It first refines some aspects of the PMA, helping the theory to better account for the relationship between attention and oculomotor control. Second, given that microsaccades seem to be tuned to the attended location, future experiments could make use of this information and treat the direction of microsaccades as an online index of subjects' attentional deployment, both in a qualitative way ("is the subject paying attention?") and a quantitative way ("where is the subject attending?").

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A number of primates made this work possible, both humans and non-humans.

I would like to thank, first of all, the 13 non-human primates whose contribution is of great importance to the scientific community and of immeasurable significance to me. I would like them to know that I did my best to make their precious involvement as beneficial as possible to the scientific cause. From them I have learned more than I could possibly ever express with words.

I would also like to thank the many human primates involved, in a way or another, into this work. I would like to start by acknowledging my supervisor, Stefan Treue, and thank him for giving me the possibility of exploring my own ideas throughout the course of the PhD. His passion and dedication for the fields of visual processing, as well as primate welfare, are simply inspiring. The kind, punctual, and helpful criticisms of thesis committee members Hansjörg Scherberger and Julia Fisher have also had a significant impact in shaping this work. Beyond their scientific inputs, throughout the meetings, during email exchanges, and institutional parties as well, they always provided insightful and friendly words. I have been also very lucky in finding my place at the Welfare and Cognition group. Working side by side with Alexander Gail and Michael Berger towards the refinement of animal training was not only helpful in delivering what constitutes half of this work, but made every single effort pleasant and worthy.

Half of this dissertation wouldn't have been possible without the contribution of Cheng Xue, skilled colleague and invaluable friend. I also would like to sincerely thank the TAs of the lab. Beatrix, Ralf, Sina, Leo, Dirk. Great people, with great professional knowledge and inspiring working attitude.

Pierre Morel, Philipp Schwedhelm, Cliodhna Quigley, Valeska Stephan are only some of the friends and colleagues that made my time at the CNL lab delightful and entertaining. I would like to thank them, and with it, to thank all the people from the CNL, SMG and DAG labs of the German Primate Center. From all these people I learned, once again, the value

of friendship and the comfort of living with pluralism of languages, ethnicities, ideas, and opinions.

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doi: 10.3758/s13428-016-0707-3

PRESENTATIONS AND POSTERS

A. Calapai, C. Xue, S. Treue (2016). *The characterization of macaque MT and MST through multidimensional mapping*. **Poster presented at the Primate Neurobiology Meeting (9th), Tübingen, DE.**

M. Berger, A. Calapai,M. Niessing, K. Heisig, R. Brockhausen, L. Burchardt, V. Stephan, S. Treue, A. Gail (2016). *Standardized automated training of rhesus macaques for neuroscience research*. **Poster presented at the Primate Neurobiology Meeting (9th), Tübingen, DE.**

A. Calapai, M. Niessing, M. Berger, L. Burchardt, R. Brockhausen, K. Heisig, S. Treue, A. Gail (2013). *Monkey Home Office*. **Poster presented at the Primate Neurobiology Meeting (6th), Göttingen, DE.**

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Primate Neurobiology Meeting (10th), Göttingen, DE	**March 2017**
Primate Neurobiology Meeting (9th), Tübingen, DE	**March 2016**
Primate Neurobiology Meeting (8th), Göttingen, DE	**March 2015**
Primate Neurobiology Meeting (7th), Tübingen, DE	**March 2014**
Primate Neurobiology Meeting (6th), Göttingen, DE	**March 2013**
Göttingen Meeting of the German Neuroscience Society (10th), DE	**March 2013**
Orienting of Attention, Tübingen, DE	**November 2012**
Primate Neurobiology Meeting (5th), Göttingen, DE	**March 2012**

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LANGUAGES

Italian – native speaker

English –fluent speaker; proficient reader/writer

English course – Level Intermediate (B1) to Advanced (B2) **May 2011**
CELT – Centre for English Language Teaching - www.celt.co.uk
Cardiff, Wales, United Kingdom

German – basic to intermediate competence

German course – Level A2 **Oct / Dec 2014**
Georg August University Göttingen, DE

German course – Level A1 **Oct / Dec 2011**
Institut für Interkulturelle Kommunikation (IIK)
Göttingen, DE

RESEARCH SKILLS AND COMPETENCES

Thought my PhD at the German Primate Center I had the opportunity to learn about Primate Biology, Primate Behavior, Primate Welfare, Human Psychophysics, Non-Human Primates handling, training and cognitive testing. During the same period, I learned my way into Matlab and R programming for online and offline DATA analysis and basic signal processing.

I am part of the Welfare and Cognition group of the Cognitive Neuroscience Laboratory of the German Primate Center, which operates mainly in the context of Non-Human Primate's welfare and cognitive testing. With this group we developed a stand-alone cage based testing system for non-human primates called XBI which will serve as pre-training and selection device, long term and large scale cognitive skills assessment as well as enrichment tool for captive animals.

OTHER SKILLS AND COMPETENCES

Father of one and amateur musician. I have experience in composing electronic music (theantscolony.bandcamp.com) and I am an amateur DJ (mixcloud.com/theantscolony).