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Development of a forced-choice task for the evaluation of affective blindsight in humans and monkeys

Sviluppo di un compito di scelta forzata per la valutazione del blindsight affettivo nell'uomo e nella scimmia

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INDEX

ABSTRACT

Damages confined to the primary visual cortex (V1) abolish visual awareness and lead to chronic blindness in humans. Nonetheless, patients' behaviour can be influenced by the presence as well as some basic properties of visual stimuli, even if they fall in the "blind" visual field and are not consciously perceived. This form of V1-independent vision is present even in nonhuman primates and it is known as "blindsight". The various abilities exhibited by blindsight subjects suggest that the extrastriate pathways carry visual information that can influence voluntary behaviour even in the absence of visual awareness. Subcortical structures, such as the pulvinar and superior colliculus, may play a crucial role in routing visual information by-passing V1. As a first step in the exploration of the neural mechanisms underlying V1-independent vision, here we developed a behavioural paradigm aimed at investigating residual visual discrimination of complex emotional stimuli (affective blindsight) in humans and nonhuman primates, through a visuo-motor forced-choice task. Preliminary results indicate that training and plasticity are necessary for regaining function in blindsight both in humans and monkeys, thereby supporting the possibility to comparatively investigate the neural mechanisms underlying V1-independent vision, paving the way for possible rehabilitative approach to bring unconscious residual visual skills to consciousness.

ABSTRACT IN ITALIANO

Danni confinati alla corteccia visiva primaria (V1) aboliscono la consapevolezza visiva e conducono nell'uomo a una cecità corticale cronica. Nonostante ciò, il comportamento dei pazienti può essere influenzato da proprietà base di stimoli visivi nonostante essi rientrino nel campo visivo cieco e non siano coscientemente percepiti. Questa forma di visione indipendente da V1 appare essere comune anche fra i primati non umani ed è conosciuta come "blindsight". Le varie abilità esibite dai soggetti blindsight suggeriscono che pathway della corteccia extra-striata forniscano le informazioni visive che controllano il comportamento, anche in assenza di consapevolezza. Strutture sottocorticali come il pulvinar e il collicolo superiore potrebbero giocare un ruolo fondamentale nel processo dell'informazione visiva che è indipendente da V1. Come primo passo nell'esplorazione dei meccanismi neurali che sottolineano una visione indipendente da V1, abbiamo sviluppato un paradigma comportamentale che mira ad investigare la discriminazione visiva residua di stimoli emotivi complessi (affective blindsight) nell'uomo e nella scimmia tramite un compito visuo-motorio di scelta forzata. Studi preliminari indicano come addestramento e plasticità corticale siano necessari per ripristinare la funzione sia nell'uomo che nella scimmia, supportando quindi la possibilità di investigare in modo comparato i meccanismi neurali che sottostanno a una visione indipendente da V1, ponendo le basi per un possibile approccio riabilitativo per portare le abilità visive residue da inconsapevoli a consapevoli.

1. INTRODUCTION

The survival and evolutionary success of all animals depend on their ability to process information from their surrounding environment and to generate adaptive behavioural responses to external stimuli. Depending on the stimulus and context, an animal may choose to approach, avoid, or simply ignore something that is occurring around it (Isa et al., 2021). Human and non-human primates' impressions of the world are based on vision more than on any other sensory function. At every moment, the visual system is confronted with the vast amount of information offered by visual scenes, but it did not evolve to treat all this information equally; instead, the visual system is best suited to extract the type of information most useful to the subject in the current context (Squire, 2013). Vision allows animals to navigate in the world, to judge the speed and the distance of objects and to identify food, members of other species, and familiar or unfamiliar members of the same species. This is the result of the processing of various subcortical and cortical areas. Much of our current understanding of higher visual processing comes from studies of the macaque monkey, whose visual system is in many respects identical to that of humans (Van Essen & Gallant, 1994).

Both eyes see most of the visual field, with the exception of a portion of the contralateral peripheral portion of the visual field, known as the monocular temporal crescent. Particularly, the inferior part of the visual field is projected in the superior part of the retina, whereas the superior part in the inferior one. Image is also crossed on the vertical plane: if we divide the visual field in right and left, the visual information deriving from the right is projected on the ipsilateral nasal and the contralateral temporal retina, and vice versa for the left visual field (Kandel, 2013). The part of the visual field corresponding to the fixation point is projected on a specific region of the retina with specific features to optimize visual acuity, called fovea.

Visual information leaves the eye via the optic nerve, which is composed by axons of ganglion cells. The optic nerve begins at the optic disc. At the level of the optic chiasm the axons of

ganglion cells deriving from the nasal retinas cross the midline and join the axons deriving from the contralateral temporal hemiretina, which in turn remain on the ipsilateral side. Thus, after the optic chiasm the axons forming the optic tract carry signals from the complete contralateral visual hemifield to the lateral geniculate nucleus (LGN) of the thalamus. The LGN is a bilateral structure that relays visual information to the visual cortex. The neurons of the LGN are monocular cells because each layer receives from only one eye, with no binocular integration at this stage. This segregation is maintained from the lateral geniculate nucleus to the input layer (IV) of the primary visual cortex (V1), producing the alternating left-eye and right-eye ocular dominance bands (Kandel, 2013).

In case of injuries affecting the visual pathway, the outcome is a partial or total loss of visual function, depending on the level at which the damage occurs (Figure 1).

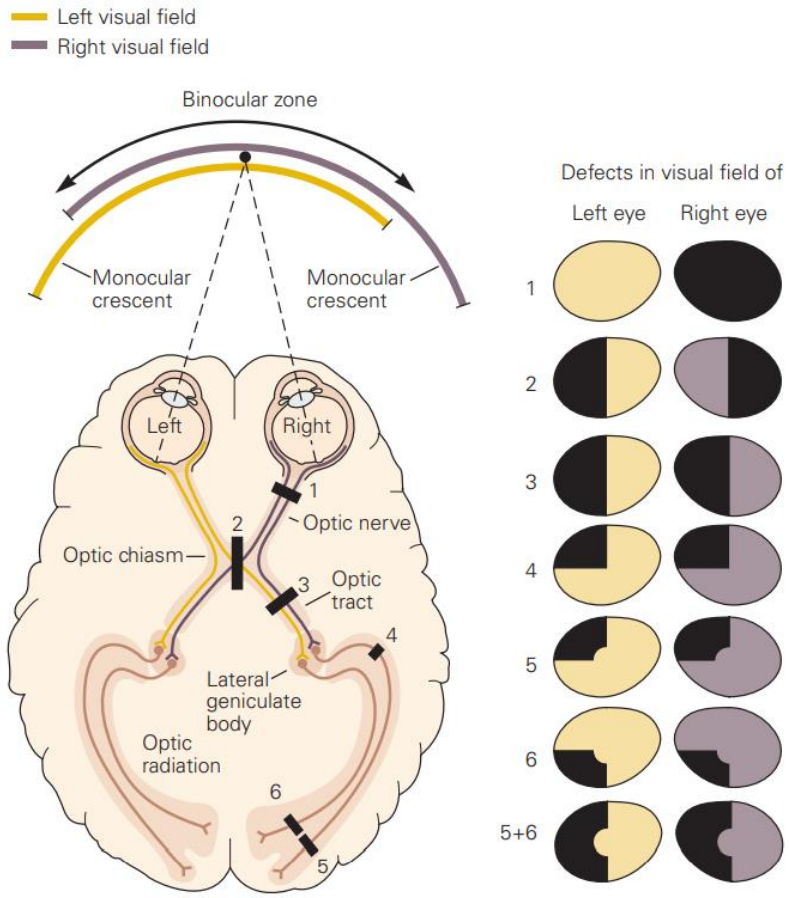


Figure 1. Deficits of the visual field after a lesion. 1. Total loss of vision of the right eye; 2. Bitemporal hemianopia; 3. Homonymous hemianopia; 4. Homonymous superior quadrantanopia; 5. Superior hemianopia; 6. Inferior hemianopia; 5+6. Contralateral hemianopia. Deficits of type 5 and 6 could often associate with macular sparing, consisting in the maintaining of the foveal vision. Figure from (Kandel, 2013).

Because the retinal spatial relationship is maintained in the subsequent stages of the visual pathway up to the central structures, an accurate analysis could reveal the specific site of neurological lesion. Relatively extended visual field loss are called *anopsia*, whereas more confined lesions cause scotomas. A lesion of the retina or of an optic nerve, before it reaches the optic chiasm, causes a total loss of vision limited to the affected eye. A lesion at the level of the central portion of the optic chiasm involves fibres deriving from the nasal sector of the retina of each eye that cross there, preserving the temporal fibres of the two retinas which proceed ipsilaterally. Thus, the portions of the lost visual field correspond to the temporal sectors of each eye (2), and this deficit is called *bitemporal hemianopia*; it is also called *heteronymous hemianopia* to underlie that the lost portions of the visual field in each eye don't overlap. Subjects with this injury can see the left and the right visual when both eyes are open. Instead, all information regarding more peripheral visual fields (which are caught only by the nasal retina) is lost. Lesions involving levels more central than the optic chiasm - such as the lateral geniculate nucleus (LGN), the optic radiation or the visual cortex - cause deficits affecting both eyes' contralateral visual hemifield. For example, the interruption of the right optic tract (3) causes a loss of vison of the left visual field (so, a blindness in the visual field sector projected onto the temporal hemiretina of the left eye and of the nasal hemiretina of the right eye). This type of damage, implying a complete loss of vision in a visual hemifield, is called *homonymous hemianopia*.

It is rare that the central visual structures are damaged in a complete way. This is particularly true for the optic radiation, a system of geniculo-cortical fibres travelling under the temporal and the parietal lobes and connecting the LGN with the striate cortex. Some axons of the optic radiation curve until the level of the temporal lobe, a ramification called Meyer's loop, which conveys information deriving from the superior portion of the contralateral visual hemifield. The medial

portion of the optic radiation, passing under the lobe of the parietal cortex, carries information relating to the inferior portion of the contralateral visual hemifield. Lesions involving the temporal lobe, including the Meyer's loop, could cause a *homonymous superior quadrantanopia* (4). A damage at the level of the cortical territory related with the central visual field is often associated with a phenomenon known as *macular sparing*, a loss of vision regarding large areas of the visual field, except the foveal vision. Partial lesions of the visual cortex lead to localized deficits in specific portions of the contralateral visual hemifield. For example, a lesion in the lower bank of the calcarine sulcus (5) causes a visual deficit in the superior quadrant, while a lesion in the upper bank (6) causes a visual deficit in the inferior quadrant.

Subjects with V1 lesion do not always lose all visual abilities in the so-called "blind" portion of the visual field. Indeed, some patients possess a certain degree of residual sensitivity to motion and/or shape and/or colour (Perenin & Jeannerod, 1975; Zeki & Ffytche, 1998). The patient may be unaware of this preserved sensitivity, which can thus be unconscious: therefore, this form of "blind vision", consisting of preserved visual capacities in the absence of visual awareness, is called *blindsight* (Weiskrantz et al., 1974).

1.1 Blindsight

In the domain of vision, damages confined to the primary visual cortex abolish visual awareness and lead to chronic blindness. This observation, combined with data from electrophysiological and functional magnetic resonance imaging (fMRI) studies in human and nonhuman primates, has raised speculation that neural activity in V1 might have a direct and critical role in the generation of a percept (Leopold, 2012). The blindness following damages to V1 in humans appears to be common even among nonhuman primates, but not in other mammals, probably because they have more relay projections from the thalamus to other cortical areas, bypassing the primary visual cortex (Funk & Rosa, 1998). Despite the loss of vision shared with human patients,

monkeys with surgical lesions of V1 retain some level of visuomotor function as well, suggesting that the phenomenon of blindsight is present in monkeys (Humphrey & Weiskrantz, 1967). A case report of a patient (D.B.) who had his V1 surgically removed at the age of 33 years elegantly illustrated the phenomenon of blindsight. D.B. showed the typical hemianopia resulting from V1 lesion, but he was able to reach for a target presented in the blind field without visual awareness of the target (Weiskrantz et al., 1974). Another famous blindsight patient was G.Y., who experienced damage to the left V1 and optic radiation at the age of 8 years in a traffic accident (Barbur et al., 1980). A third fundamental case was reported by Pegna and colleagues (Pegna et al., 2005). T.N. is a physician who became cortically blind following two consecutive strokes, which destroyed both his right and left visual cortices. These patients lose visual awareness of objects in the blind visual field but retain a certain level of visuo-motor behaviour towards these objects when they are forced to do so (Poppel et al., 1973; Weiskrantz et al., 1974). Since these interesting cases, blindsight has attracted considerable attention not only from clinicians but also from neuroscientists.

1.2 Studies on human subjects

After the first report of residual vision in patients with damage to V1 (Poppel et al., 1973) extensive studies of patients including D.B (Weiskrantz, 2009) and G.Y. (Barbur et al., 1980) were conducted to gain a greater understanding of blindsight.

Concerning low-level visual information processing, the performance in discriminating the orientation of moving stimuli is very high in blindsight patients (Weiskrantz et al., 1995), but it drop down to chance level when tested with static line segments (Morland et al., 1996). In a detection task of grating stimuli, the threshold for luminance contrast is increased in blindsight compared to normal sighted subjects (Sahraie et al., 2006). Concerning colours, there are reports that chromatic information can be detected and discriminated (Cowey & Stoerig, 2001), but conversely there are some others reporting that human blindsight patients with V1 damage or

hemispheric cortical resection are unable to detect stimuli composed of blue-yellow opponent channels (koniocellular pathway) (Sumner et al., 2002; Tamietto et al., 2010).

Concerning facial recognition, blindsight subject G.Y. answered correctly to two-choice tasks more often than chance when discriminating facial expressions (de Gelder et al., 1999), and this ability is known as "affective blindsight". In fact, perception of emotional expressions in the absence of awareness in normal subjects has some similarities with the unconscious recognition of visual stimuli which is well documented in patients with striate cortex lesions. Presumably, in these patients, residual vision engages alternative extra-striate routes such as the superior colliculus and pulvinar. A further study based on functional magnetic resonance imaging showed that the processing of emotional faces can be mediated by an extrageniculo-striate neural pathway (Morris et al., 2001).

Pegna and colleagues studied the blindsight phenomenon on T.N. (Pegna et al., 2005). They first estimated the campimetry of the patient by presenting black squares and circles in a white background and, to exclude any form of visual awareness, they asked him to guess the shape. The patient's performance was not statistically different from chance level. Then, a random series of emotional faces were presented, and they could be angry or happy faces. Again, it was required to the patient to guess which kind of emotion it was expressed; here, the patient answered correctly at a level that was significantly higher than chance. To verify if the patient's performance was strictly due to the emotional expressions, or broadly to more general non-emotional facial characteristics, two tasks were assessed. First male and female face images were presented with a neutral emotion expression and T.N. had to guess the gender on each photograph. The second task was consisted of presenting normal or scrambled faces and the patient had to identify if they were authentic faces. T.N.'s performances were at the chance level in both tasks, in contrast to the performance with emotional faces. To determine which areas were involved in these abilities, an fMRI experiment was conducted, in which happy, angry, neutral and fearful faces were presented to the patient (Figure 2). Only for emotional faces, a right amygdala response was found

(angry, happy and fearful faces compared to the neutral one, Figure 1a,b). Furthermore, right amygdala activations were considered separately, with the strongest effect for fear (Figure 1c).

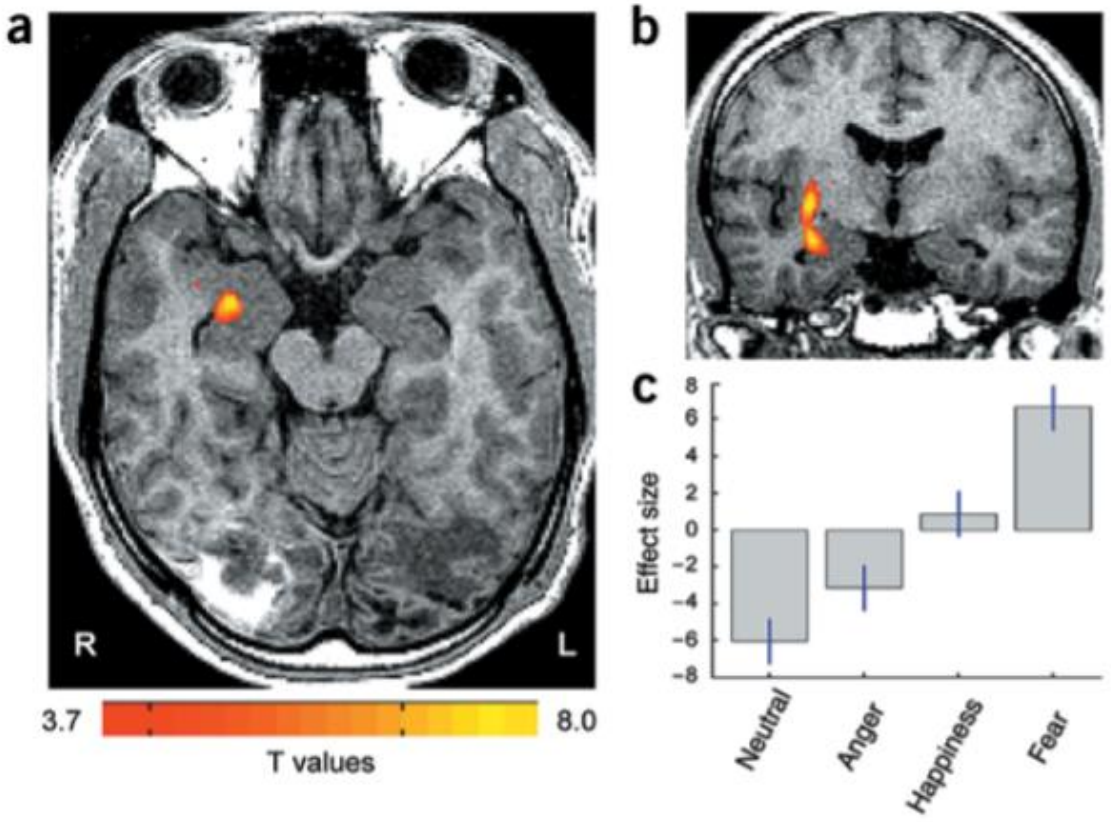


Figure 2. Right amygdala response to emotional faces. (a) Horizontal section evidences the right amygdala activation and also illustrating the bilateral lesions of the patient. **(b)** Amygdala activation viewed from the coronal axis. **(c)** All emotional conditions significantly differ from neutral face, particularly fearful faces. (Figure from Pegna et al., 2005).

Studies with G.Y. indicate that he is able to discriminate different emotional facial expression in his blind hemifield (de Gelder et al., 1999). This residual ability is notable in that it parallels the ability of healthy subjects to discriminate masked ("unseen") emotional expressions, which is associated with skin conductance and brain activation changes (Esteves et al., 1994; Morris et al., 1998). Morris and colleagues reported differential amygdala responses in G.Y. performing a task in which fearful and happy faces were presented in both hemifields, while being scanned with functional MRI (fMRI) (Morris et al., 2001). He should indicate the sex of the face presented. G.Y. denied any perception of faces presented in his right (blind) field. However, he reported non-visual awareness that `something happened' during right hemifield (blind) during

the presentation of the stimuli. Despite the absence of normal vision in his blind hemifield, G.Y. was significantly above chance in identifying the sex of `unseen' faces. Explicitly seen faces both in the left and in the right (blind) hemifield, independently from the emotional expression, evoked enhanced responses in the striate, fusiform and dorsolateral prefrontal cortices in a significantly way.

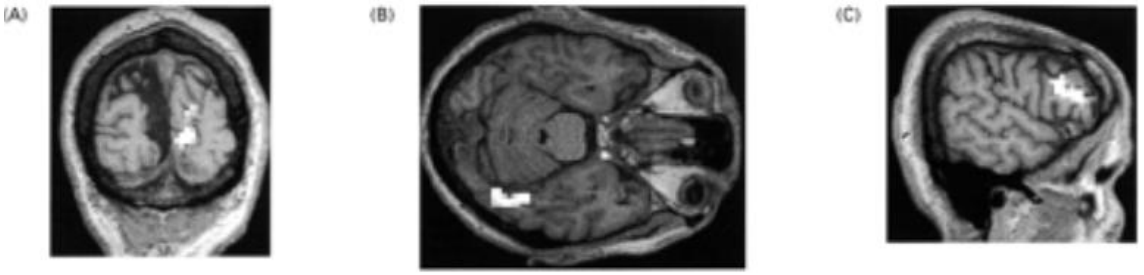


Figure 3. fMRI images of cortical activations in G.Y. Neural responses to "seen" faces presented in the left hemifield. **(A)** Right striate cortex. **(B)** Right fusiform area. **(C)** Right prefrontal cortices. Figure from (Morris et al., 2001).

However, face stimuli presented in the right (blind) hemifield did not evoke increased striate, fusiform or dorsolateral prefrontal responses. Blind hemifield presentation of fearful faces evoked increased responses (compared with happy faces) in bilateral regions of the amygdala. The results of this study also indicate that several subcortical visual structures, such as the superior colliculus and pulvinar, are involved in processing `unseen' emotional faces. The present neuroimaging data obtained in G.Y., shows condition-dependent colliculo-amygdala and thalamo-amygdala response covariation, which was more positive during presentation of `unseen' fearful than `unseen' happy faces.

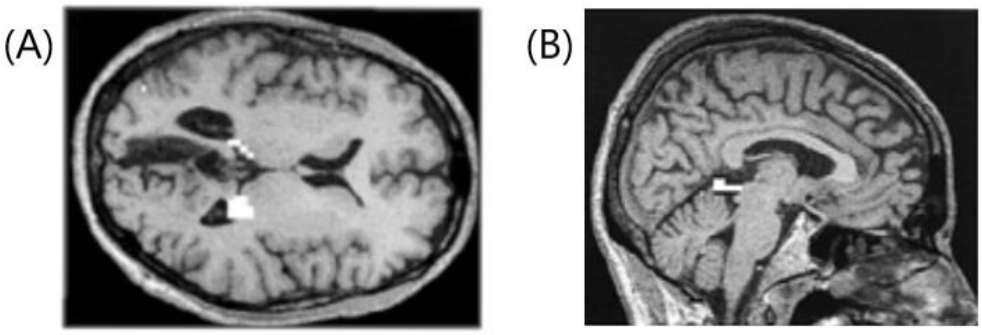


Figure 4. fMRI images of subcortical activations in G.Y. Neural responses to "unseen" fearful than "unseen" happy faces, where the covariation with bilateral amygdala was more positive. **(A)** A bilateral region of the pulvinar. **(B)** Region of the superior colliculus. Figure from (Morris et al., 2001).

Given that the superior colliculus is implicated in non-striatal visual processing in both monkeys (Mohler & Wurtz, 1977) and humans (Sahraie et al., 1997), and that the posterior visual thalamus (pulvinar) is activated by visual stimulation in the blind hemifield of patients with a V1 lesion (Ptito et al., 1999), it has been proposed that the residual visual abilities of blindsight patients depend on an extrageniculate colliculo-thalamic visual pathway (Barbur et al., 1980; Weiskrantz et al., 1974).

Regarding attention, blindsight subject G.Y. showed attentional effects such as a shorter response latency to invisible visual stimuli using information either from a foveal or peripheral cue in an attentional task using the Posner cueing paradigm (Kentridge et al., 2004). This type of blindsight is called "attention blindsight".

Residual visuomotor activity, such as reaching or saccades in hemianopia, is sometimes called ''action blindsight" (Danckert & Rossetti, 2005), and it includes accurate localization by pointing and accurate avoidance of obstacles without the awareness of them.

Although the superior colliculus and posterior thalamus, in addition to the amygdala, have been implicated in mediating differential responses to masked emotional facial expression in healthy subjects, evidence that these subcortical structures can mediate emotional discrimination in blindsight is lacking. About emotional visual processing, one popular theory suggests that emotional signals are processed automatically by a subcortical pathway through which retinal inputs from the superior colliculus are sent to the pulvinar and then relayed directly to the amygdala, bypassing the neocortex (Tamietto & de Gelder, 2010). According to this "standard hypothesis", ecologically important (emotional and social) stimuli are processed initially by a dedicated, modular system that operates rapidly, automatically (without the need to pay attention) and largely independently from conscious awareness. This hypothesis is related to two features.

First, the presumed central role of the amygdala in the automatic and non-conscious processing of emotional and social stimuli. Second, the existence of a "low-road" consisting in a specific subcortical pathway, which culminates in the amygdala via the superior colliculus and the pulvinar. Although significant amygdala activation has been demonstrated in both healthy adults and in patients with affective blindsight (Pegna et al., 2005) during non-conscious emotional processing, it remains unclear the pathway from which it receives information to participate in this function.

This subcortical pathway, through the auditory thalamus and the amygdala, has been demonstrated in rodents, which is sufficient for some forms of auditory Pavlovian fear conditioning. However, critics of the so called "low-road" hypothesis have argued that there is limited anatomical evidence for the existence of this visual pathway in primates (Striemer et al., 2019). Furthermore, a study of Adolphs and colleagues, consisting in detecting fearful faces among distractors on a patient with a complete amygdala lesions, indicates that the reaction time in detecting the fearful stimuli were within the normal range (Tsuchiya et al., 2009). For this reason, Pessoa and colleagues offer a different perspective emphasizing physiological and anatomical data concerning the pulvinar, the "key-link" structure of this subcortical pathway (Pessoa & Adolphs, 2010). The pulvinar is better conceptualized perhaps as a dynamic element of this brain circuitry and not only a passive station relaying visual information from the superior colliculus. Studies in monkeys and humans with pulvinar lesions have suggested that this structure is involved in determining what is salient in a visual scene. A fMRI study in humans of Pessoa and colleagues found that pulvinar responses didn't associate to the affective significance of visual stimuli but to their possible conscious perception (Padmala et al., 2010). Moreover, in another fMRI study, pulvinar responses were associated with the subject's perception of a change (Pessoa & Ungerleider, 2004). Responses were observed during 'false alarm' trials (those in which a stimulus change was reported but did not actually occur) but not during 'miss' trials (those in which a stimulus change occurred but went unnoticed by the participant). As reviewed

by Pessoa and Adolphs, the inferior pulvinar receives inputs from the superior colliculus. However, the inferior pulvinar is strongly connected with visual cortex, not with the amygdala. Instead, the amygdala receives input from the medial pulvinar which is highly interconnected with a number of different cortical structures, including those at various levels of the ventral stream hierarchy.

In conclusion, these studies challenge the single "low-road" pathway hypothesis suggesting that the evaluation of visual emotional signals (both conscious and non-conscious) takes place via processing in a number of parallel pathways that involve input from both cortical and subcortical regions which include the amygdala and pulvinar.

1.2.1 Functional recovery and cortical-subcortical plasticity

Several reports have suggested that training and plasticity are necessary for regaining function in blindsight. In a study by Sahraie and colleagues, patients with damage to the visual cortex were trained to a visual discrimination task, in which stimulus presentation can occur in two periods of the trial separated by beeps. At the end of each trial, the patients had to report in which period the stimulus was presented by pressing one of the two buttons of the mouse, and they should indicate if they had any awareness of the stimulus presentation or not. The subjects continued to perform this type of training at home and their performance improved over several months (Sahraie et al., 2006). Another study reported that subjects trained to discriminate between directions of random dot-motion stimuli improved their sensitivity to near normal levels after 9–18 months (Huxlin et al., 2009).

Subjects considered in these two studies were adults and the rehabilitation training started several months after the injury; this suggests that, even in the adult brain, functional recovery may occur through large-scale structural changes. Diffusion tensor imaging has revealed possible sites of pathway plasticity after brain injury in patients with blindsight: for example, stronger connectivity from the LGN to the middle temporal (MT) area in patient G.Y than the control healthy subjects

(Bridge et al., 2008). Concerning the affective blindsight, connectivity from the superior colliculus (SC) to the amygdala (AMG) via the pulvinar (Pulv) is implicated: these changes consist in the strengthening of several fibers tracts already existing in the intact brain. This analysis evidence that a specific subset of fibers connecting SC-Pulv-AMG passe from the pulvinar and does not extend to other cortical or subcortical structures. Moreover, the same pathway was also reconstructed in both hemispheres of G.Y., but the strength of the connection was higher in his (damaged) left hemisphere compared to controls, therefore strongly suggesting that it conveys visual information independently from V1.

1.3 Studies on non-human primates

In the case of human studies, a limit consists in the variability of the extent of the lesion, which could involve not only V1 but also other regions (like in the case of G.Y.). To complement these human studies, several lines of nonhuman primate research have been conducted. The advantage of the nonhuman primate model is that the extent of the lesion is controllable, and some additional manipulations of circuit function are possible.

The neural mechanisms of blindsight have been intensively studied in the macaque monkey model. It has been shown that visual awareness is impaired in monkeys with V1 lesions, as judged by their behaviour in a "Yes–No choice" task, in which the animals were required to signal their awareness of the visual cue (Stoerig & Cowey, 1989; Yoshida & Isa, 2015). There is a consensus that the SC is critical for the relay of visual inputs because lesion/inactivation of SC has been shown to impair the visually guided behaviours or visual responses in the extrastriate cortex (Mohler & Wurtz, 1977). The role of the thalamic relay has been less clear, with some researchers suggesting a major role for the SC-pulvinar and extrastriate cortical pathway (Kinoshita et al., 2019) and others favouring the SC-dLGN (koniocellular layer)-extrastriate cortex pathway (Schmid et al., 2010).

In a very recent study of Isa and colleagues, both dLGN and pulvinar were inactivates in the same animal. It was found that inactivation of dLGN, but not of the pulvinar, on the contralesional (intact) side impaired visually guided saccade (VGS) performance to the intact field during a detection task in which, after fixating a central point, the monkey had to make a saccade when a peripheral stimulus is presented (in correspondence with the offset of the central fixation). Instead, both inactivations impaired the VGS toward the ipsilesional (affected) side. These results suggest the SC-pulvinar pathway can partially compensate for the damage to the dLGN-striate cortical pathway after the V1 lesion (Takakuwa et al., 2021).

The first observation of blindsight in macaques derives from the studies of Humphrey's group which examined residual visual capacity after bilateral lesion of V1 and the surrounding cortices. The monkey "Helen" was able to reach for moving stimuli and walk around in an open space, avoiding some obstacles without any problem (Humphrey, 1974). Later studies examined residual vision and visuomotor processing in monkeys with unilateral ablation of V1, thus enabling selective lesioning of V1 and comparing residual vision with the normal visual field in the same animal. For example, monkeys with complete or partial unilateral ablation of V1 can make saccades or press a lever to indicate the presence of a target in the visual field corresponding to the injured side (Mohler & Wurtz, 1977). An explicit test of the loss of phenomenal awareness is needed, but this may seem to be impossible for monkeys in the absence of language. Nonetheless, there is evidence for the loss of visual awareness in monkeys provided by studies showing that successful performance in a visually guided saccade task is possible only when it implies a forced choice condition (Moore et al., 1995).

Concerning plasticity, the age at which lesioning occurs seems to be a key factor. Studies from Charlie Gross's lab showed a better performance in the detection of stimuli in the contralateral hemifield in monkeys with surgical ablation of unilateral V1 at 5–6 weeks of age than in monkeys with ablation in adulthood (Moore et al., 1996). In addition, recent studies by Bourne and colleagues have shown that the connections between the pulvinar and area MT are strengthened

in the marmoset with V1 lesion at earlier age than the adult animals (Warner et al., 2015), suggesting that the age at which lesion occurs is a critical factor for plasticity.

Connections of the primate visual system, established initially by retinal waves and molecular cues in utero, provide a minimal operating system at birth where the optimal performance is acquired through changes involving a refinement of retinothalamic, thalamocortical, corticothalamic, and corticocortical projections, which results in the improvement of visual sensitivity and discrimination as the animal matures (Shatz, 1996). During normal development, the competitive restructuring in the postnatal period of the neurons originating from medial portions of inferior pulvinar (PIm) and V1 (terminating in MT) could explain the retraction of retinal input from PIm. However, after a disruption of the course of the normal development caused by a lesion in V1, in the case of blindsight, the PIm-MT projection is integrated into the neuronal circuit during the period of cellular maturation, but not in the adult, enabling stabilization of the retinal input to PIm, which continues to drive the pulvinar relay neurons. The removal of V1 in early life results in the selective sparing of the pulvinar afferent pathway and contemporary in the rapid degeneration of retinal ganglion cells projecting to the magnocellular and parvocellular layers of the LGN (Warner et al., 2015).

Anatomical and physiological studies in monkeys have helped to elucidate the neural mechanisms of V1 independent vision, although it is still unclear to what extent the phenomenology of the residual vision in monkeys parallels that of human blindsight (Moore et al., 1995).

Moore and colleagues trained two monkeys with a striate cortex damage to fixate on a central point and to make saccadic eye movements to some visual targets (light spots) presented on the hemifield contralateral to the lesion, appearing at variable time after the presentation of the fixation point. There was a non-forced choice task (detection task), where the fixation point remained with the appearing of peripheral stimuli presented in the blind spot, and a forced choice task (with the offset of the central fixation point) in which peripheral stimuli were presented, after

the offset of the central fixation point, in the contralateral hemifield where peripheral stimulus in the detection phase appeared. All trials were tested in monocular conditions to have more control of all variables. Monkeys were unable to detect visual stimuli in the non-forced choice condition, in fact monkeys continued to fixate the central fixation point, ignoring the peripheral stimulus. In the forced choice condition, the offset of the fixation point represented a cue for the monkeys to make a saccade on the target; in this case, both animals did so with precision and accuracy. These results suggested that in the first condition monkeys didn't detect the stimulus within the blind spot, or better, they could not make a conscious saccade on the stimulus; when they are forced to make a saccade by the cue, they unconsciously correctly directed eye movements on the target. Probably, it depended on the fact that the presence of the fixation point, and the active fixation by the animal in the standard condition may have prevented or reduced the number of responses to contralateral visual targets. Moreover, the offset of the central fixation could disinhibit weaker signals from targets within the scotoma (Moore et al., 1995). In fact, these signals elicited by the presentation of stimuli in the scotoma may weren't sufficient to evoke an oculomotor response away from the fixation, if it remains on screen contemporary to the peripheral stimulation.

The experiments of Yoshida and colleagues on macaques consisted in a forced choice task and a Yes-No task (Yoshida & Isa, 2015). They introduced a revised version of the ''Yes–No" task, with the presence of "catch trials" in which the target did not appear and trials in which the monkeys had to maintain fixation and to respond with saccades in the remaining trials to report that they had detected the target. In the forced choice task, the target appeared in 100% of trials either in the upper or lower part of the visual field. In this experiment, the performance in the forced choice task was nearly 100% in the intact and affected visual hemifields; the performance in the ''Yes–No" task dropped close to the chance level when the target was in the affected visual field, while performance was still >90% successful when the target was in the intact visual field. To remove the influence of the decision bias, they introduced signal detection theory and compared the sensitivity to estimate awareness: the sensitivity dropped significantly when the

target was in the affected visual field compared to the intact field. These results suggested them that visual awareness was impaired in the affected visual field. However, the value was still not zero, which suggests the existence of some conscious experience.

The fact that the animals produced a saccade on the target stimulus in the forced choice task doesn't mean necessarily that they had a kind of conscious experience of the stimulus, because those saccades could be merely the result of subcortical activity (e.g., superior colliculus neuronal activity). Moreover, both the studies by Moore and Yoshida considered only the oculomotor response of the animal to determine the awareness map. It should be necessary to assess another type of detection response to be sure to have an indication of a residual implicit visual experience

1.4 Neural pathways for residual vision in blindsight

1.4.1 The colliculo-pulvinar pathway

The superior colliculus (SC), also called tectum in non-mammals, is a subcortical structure situated on the dorsal surface of the midbrain, just beneath the thalamus. A prerequisite for navigating the visual world is the ability to track objects as they move or to stabilize vision as strong visual flow takes place, and the tectum/SC plays an important role in producing the eye saccades and head movements that allow this tracking and stabilization (Isa et al., 2021). Like the LGN, the left superior colliculus receives inputs from the right visual field, while the right superior colliculus receives from the left visual field. The intrinsic neural circuitry of tectum/SC, including its visual and other sensory inputs and its output connectivity, is conserved throughout vertebrate phylogeny, but the details of the sensory processing have shifted through evolution and with the varying demands of its diverse owners (Drager & Hubel, 1976; Jones et al., 2009). In mammals, anatomically distinct superficial and deep SC layers have developed (the sSC and dSC), with information flowing from the retina through the sSC, to the dSC, and passing from LGN and pulvinar, it reaches extrastriate, parietal and premotor areas (Isa et al., 2021). The computations performed by these circuits culminate in the delivery of processed visual

information, carried by sSC projection neurons, principally to the dSC neurons and, in parallel, to the visual thalamus including the pulvinar and LGN.

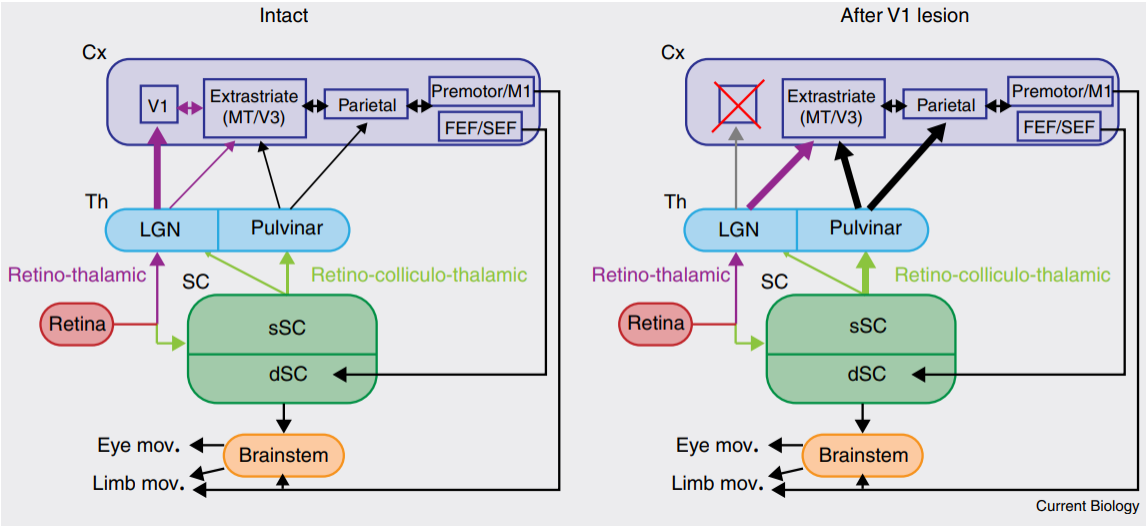


Figure 5. Schematic colliculus-pulvinar pathway. Visuo-motor pathway for the control of eye or limb movements with the intact V1 (left) and following damage to the V1 (right). The thickness of the arrows indicates the strength of connectivity. Figure from (Isa et al., 2021).

The main function of SC in mammals is the control of rapid eye movements. In classical saccade tasks in which the target is presented simultaneously with the fixation point offset, the saccadic reaction times are distributed within the 150–250 ms interval from stimulus onset in macaques ('regular saccades'). In contrast, when a short time gap (for example 200 ms) is inserted between the fixation offset and the target onset ('gap saccade' task), the reaction times are markedly shortened and form a distinct peak around 80– 120 ms (Fischer & Boch, 1983). SC is part of a largely nonconscious system that helps us directing the attention toward new or approaching objects. Approximately 90% of retinal ganglion cells project to the LGN, and about 10% go to the SC. It receives projections from cortical areas and, in turn, influences the visual cortex, also beyond the primary visual cortex, via its projection to the thalamus. As in the LGN, also the SC includes a retinotopic map of the visual field. The main pathway derives from retinal ganglion cells projecting to SC and carrying information about the stimulus' position, thereby allowing it to control quick eye movements (Schwartz & Krantz, 2016). This organization also enables the

SC to rapidly guide movements of various body parts towards target objects and determining orienting or defensive responses (Isa et al., 2021).

An interesting feature of the superior colliculus is that it receives inputs from other sensory systems, particularly the auditory and the somatosensory systems. This allows the eyes to be directed quickly to the location of a sound or a stimulus touching the body surface. Furthermore, if something is visually and auditorily detected at the same time from the same source, the SC response will be larger than for either stimulus alone (Stein et al., 1993).

The SC is much more than a simple visuomotor relay. The sSC implements intrinsic processing of visual signals to extract their critical features and sends outputs to the pulvinar and then to the extrastriate cortex. This is the **retino-colliculo-thalamic pathway** which appears to be involved in the integrated processing of visual information about the position and motion of the targets. A study using the trans-synaptic retrograde tracing technique showed that there exists a pathway from SC to MT (medio-temporal area) or parietal cortex via the Pulvinar (Lyon et al., 2010).

The **visual pulvinar** is especially well developed and differentiated into distinct nuclei in primates. The primate visual pulvinar consists in several divisions of the inferior and lateral pulvinar, while the medial and anterior pulvinar have mainly multisensory and somatosensory functions. The visual pulvinar receives inputs from subdivisions of visual cortex and projects back to them. Some inputs to selective parts of the visual pulvinar are from the superior colliculus.

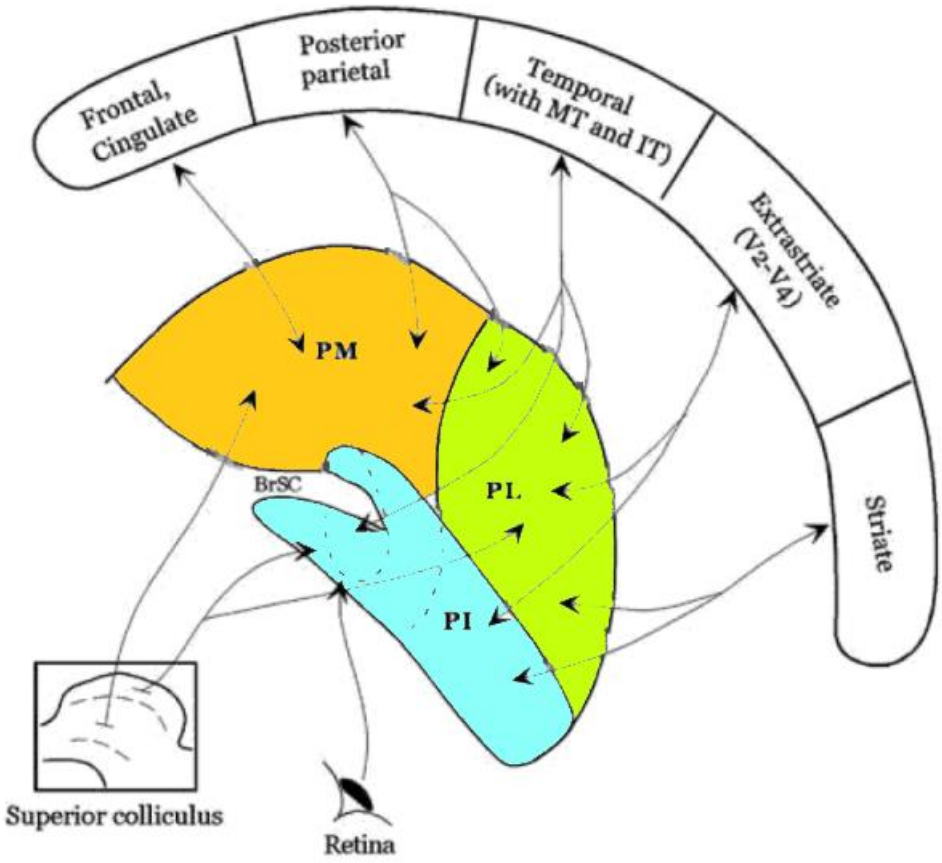


Figure 6. Pulvinar connections. The major cortical and subcortical connections of the medial, lateral, and inferior pulvinar. Cortical connections are reciprocal (double-headed arrows). Inferior pulvinar (PI) and ventral lateral (PL) are mostly connected to striate and near extrastriate cortices, while the dorsal PL and the medial pulvinar (PM) are connected to higher cortices (parietal, frontal and cingulate). Figure from (Stepniewska, 2004).

There is an overall trend in the organization of the connections between pulvinar and cortex, so that ventrolateral portions of the pulvinar are connected to striate and near extrastriate cortex, and more dorsomedial portions are connected to more associative cortical areas (such as, posterior parietal and frontal cortices). All pulvinar subdivisions receive inputs from the superior colliculus, with PI and PL receiving inputs from the superficial layers and PM from the deeper layers (Stepniewska, 2004). Because the terminations in PI from the neurons of the SC express a neurotransmitter, called substance P, it may be a marker of one class of superior colliculus inputs to the pulvinar across mammals. In cats, the substance P terminals in the pulvinar are large and terminate on cells that project to the cortex, suggesting that these superior colliculus inputs drive pulvinar neurons that could in turn drive cortical neurons (Kelly et al., 2003). To examine whether

the pulvinar is essential for visually guided saccade (VSG) in blindsight monkeys, Kinoshita's group performed a reversible blockade of the pulvinar using microinjection of muscimol, a GABA-A receptor agonist (Kinoshita et al., 2019). After the muscimol injection, performance of VGS task to the contralesional visual field, affected by V1 lesion, was severely impaired.

Thus, the retino-thalamic pathway is generally regarded as necessary for conscious and high acuity vision, while the retino-colliculo-thalamic pathway has been shown to regulate non-conscious and reflexive visuo-motor processing (Isa et al., 2021).

1.5 The theme of visual processing and awareness in blindsight

Base on the studies so far considered, the blindsight suggests that the primary visual cortex (V1) plays a unique role in visual awareness, and extrastriate activation needs to be fed back to V1 to be consciously processed. Is blindsight truly a dissociation between visual detection and awareness? Is there sufficient evidence that the loss of awareness after a V1 lesion reflects a unique role of V1 in conscious experience rather than a reduced extrastriate activation?

When assessed through forced choice paradigms, some patients with V1 damage are able to detect stimuli presented in their blind field, despite reporting a complete lack of conscious visual experience. Subsequently, it was shown that patients with V1 damage can localize unseen stimuli also by pointing, even more accurately than by eye movements (Cowey & Stoerig, 2004). The failure of extrastriate activation to reach awareness when V1 is lesioned, despite the ability of this activation to guide visual detection, appeared to suggest a unique role for V1 in visual awareness.

The primates' cortical visual system is generally conceived of as a parallel hierarchical system because visual information enters the visual cortex mainly via the LGN, and from there it is passed on to V1, from there to the extra-striate areas, and then to more and more high order areas (Felleman & Van Essen, 1991). It also has the characteristics of a parallel system, because different pathways (magno-, parvo-, and koniocellular) can be distinguished based on different kinds of information travelling from the LGN to the cortex (DeYoe & Van Essen, 1988). In this

way, the retinal parallel pathways are recombined into two main cortical pathways. A dorsal, magno-dominated pathway flows to the parietal cortex, which has been suggested to be involved in information about space, movement, and action. A ventral, parvo-dominated pathway flows into temporal areas, and itis suggested to be devoted to object identification and perception (Mishkin et al., 1983).

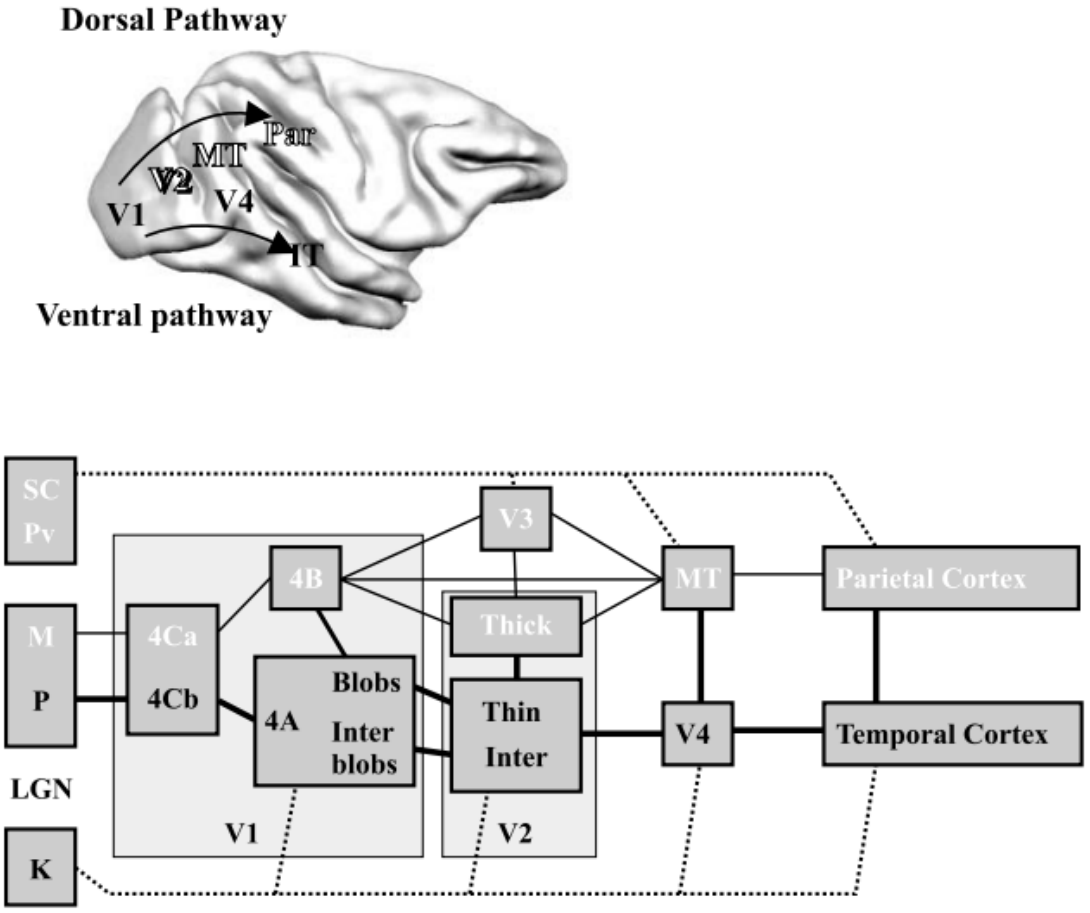


Figure 7. Cortical streams of visual information processing. The upper panel shows the areas that feed into the dorsal (white) and ventral (black) cortical pathways. The lower panel shows the interareal connections of the early visual areas and the segregation of these connections. Figure from (Lamme, 2001).

The feedforward model conventionally considers that conscious perception is fundamentally hierarchical in nature (Crick & Koch, 1995). One of the features this model is based on is the anatomical hierarchy, the principle that connections between visual areas are naturally reciprocal (Maunsell & van Essen, 1983a, 1983b). So, there is one type of *feedforward* projection, which transit from the retina and LGN to V1 toward V4, IT, and prefrontal cortex, until it affects motor

areas that control a potential action. There is also a second type of descending or *feedback* projection arising from frontal regions of the neocortex and travelling back to lower sensory cortical areas (Kandel, 2013).

1.5.1 The hierarchical models

The model of cortical hierarchy was constructed such that each area was located just below the higher area to which it provides ascending input; visual areas sharing intermediate projections were placed on the same level of the hierarchy. In this model, V1 is at the bottom, and the parietal, temporal and frontal regions at the top of the hierarchy. Complex visual processing (such as detecting animals in natural, cluttered scenes) can be accomplished by the cortex within 130–150 msec from stimulus onset (VanRullen & Koch, 2003), far too slow for conscious perception to be involved in these tasks. It is quite plausible that such behaviours are mediated by a purely feed-forward moving wave of spiking activity; the hypothesis that the basic processing of information is feedforward is supported most directly by the short times required for a selective response to appear in IT cells. Coupled with a suitable motor output, such a feed-forward network implements a rapid and efficient behaviour, which during a task distinguishes between animal and non-animal pictures, in the absence of any conscious experience (Kandel, 2013). Conversely, conscious perception is believed to require more sustained, reverberatory neural activity, most likely via global feedback from frontal regions of the neocortex back to sensory cortical areas (Crick & Koch, 1995). The reverberatory activity builds up over time until it exceeds a critical threshold. In this view, the sustained neural activity rapidly propagates to parietal, prefrontal, and anterior cingulate cortical regions, thalamus, claustrum, and related structures that support short term memory, multimodality integration, planning, speech, and other processes intimately related to consciousness. This is the hypothesis at the heart of the *global workspace* model of consciousness (Dehaene & Changeux, 2005). Sending visual information to more frontal structures would allow the associated visual events to be decoded and placed into context (for instance, by accessing various memory banks) and to have this interpretation fed back to the sensory representation in

visual cortex (Jazayeri & Movshon, 2007). In brief, while rapid but transient neural activity in the thalamo-cortical system can mediate complex behaviour without conscious sensation, it is assumed that conscious perception requires sustained but well-organized neural activity dependent on long-range cortico-cortical feedback.

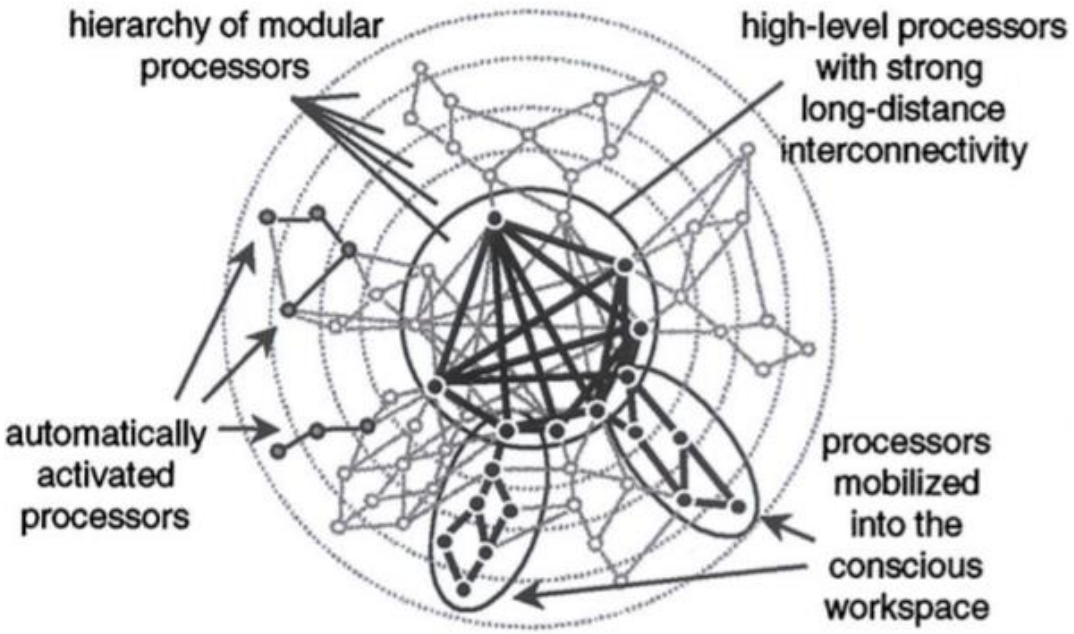


Figure 8. The global neuronal network workspace model. Symbolic representation of the hierarchy of connections between brain processors (each symbolized by a circle). Higher levels of this hierarchy are assumed to be widely interconnected by long-distance interconnections, thus forming a global neuronal workspace. Figure from (Dehaene & Naccache, 2001).

One prediction of the feedforward model is that response latencies of a given visual area can be predicted from its level in the hierarchy; areas at high levels should have longer latencies than those at lower ones, as a result of the time required for the transfer of information from one level to the next. The other problem relates to the phenomenon that neuronal tuning evolves during the visually evoked response. A hierarchical model would predict that the functional role of a lower-level area is concluded once it has fed information forward to the higher level. However, this is difficult to reconcile with the findings that ‘‘early’’ and ‘‘late’’ components of evoked responses in V1 can be functionally distinct. In V1, 50 ms after the presentation of a textured figure

overlying a textured background, neurons show selectivity for the local orientation of the line segments that make up the figure; at 80 ms, the figure ground boundary selectively evokes a larger response than the rest of the scene, and at 100 ms the elements of the interior of the figure evoke a stronger response than the background elements (Lamme, 1995). Importantly, it is the late stage of V1 activity that correlates with the monkey's behavioural report; but in a strict feedforward model, these ''late'' responses would have no relevance on the information processing at higher levels. A third phenomenon inconsistent with the feedforward models is that normal responsiveness of neurons at the bottom of the cortical hierarchy (V1 and V2) is dependent on feedback from higher-level regions: inactivation of V5/MT leads to a significant decrease in neuronal responses in early visual areas, an effect already present in the earliest stages of the V1 response (Hupe et al., 1998).

1.5.2 The recurrent models

The *recurrent* models of visual processing propose recursive or adaptive resonance networks to link the visual system through a series of ascending and descending connections. The underlying principle of these models is that sensory data activate a *feedback* process wherein a learned template modulates the sensory data until a consensus is reached between what the data are (provided by bottom-up, feedforward input) and what we ''expect'' them to be (via top-down, feedback modulation) (Grossberg, 1976). One of the models motivated by the new neurophysiological evidence was the *Integrated Model of Visual Processing*, which was not concerned with how conscious perception arises, but rather, attempted to explain how information across the visual scene can be integrated so that ''global'' properties such as shadows and lighting artifacts can be taken into account when the ''local'' aspects of the visual image are computed (Bullier, 2001). In theory, this could be achieved with local horizontal connections within a single cortical area. However, a V1 axon can reach a distance of only 0.6 degrees of visual angle and as a result, transmission of information over a distance of one degree visual angle through horizontal connections would take 100 ms, the time necessary to transmit the output of the V1 neuron; so, it

is unlikely that the integration of the information takes place through horizontal connections in V1 (Heller et al., 1995). Higher level areas, with their larger receptive fields in the dorsal and ventral processing streams, are more capable of integrating information across long distances in the visual field. However, as higher visual areas are also more selective, this integration can only involve a particular stimulus attribute. In the Integrated Model of Visual Processing (Bullier, 2001), the problem of long-distance integration of various stimulus parameters is solved by projecting back again the global computations carried out by higher-level areas through feedback connections into V1 and V2, where they guide the fine-detail analysis. The convergent nature of feedback connections means that they can carry information from long distances in the visual field and are therefore perfectly suited for guiding the fine detail analysis in V1 (Angelucci et al., 2002).

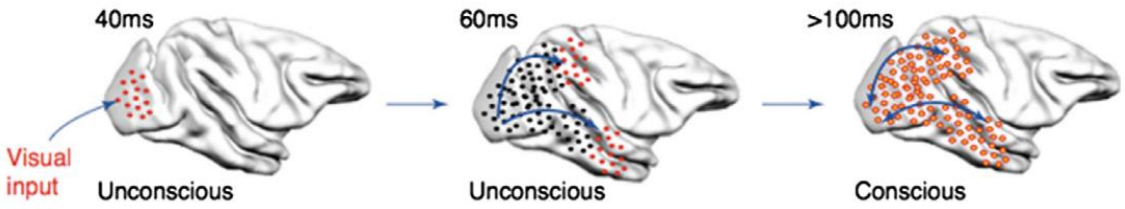


Figure 9. Lamme's model. Conscious visual experience requires recurrent processing. Visual input reaches the early visual areas (V1) at 40 ms after stimulus onset. Visual information is then rapidly fed forward to the extrastriate areas and parietal and temporal cortex (60 ms). At this level the information processing is still unconscious. At around 100 ms, early visual areas and higher areas engage in recurrent interactions, which are necessary for visual awareness. Specifically, extrastriate activation is fed back to V1 to consciously perceive (Lamme, 2001). Figure from (Silvanto, 2015).

While V1 appears to be indispensable to visual awareness, its activation is insufficient to generate a percept if the integrity of regions such as the parietal cortex is disrupted. In the Pollen's model, feedforward and feedback pathways link visual areas together into recursive loops (Pollen, 1999).

Blindsight has played a key role in the study of visual awareness because it appears to demonstrate a direct link between V1 and conscious experience of visual information. This because firstly, unconscious detection and discrimination performance in blindsight has been taken to indicate that V1 lesions selectively impair conscious perception while leaving unconscious visual functions intact. The second argument involves the cortical basis of blindsight. Residual

unconscious vision in the absence of V1 could be explained in terms of subcortical regions which continue to process visual information, activating extrastriate areas in the absence of V1 (Goebel et al., 2001). Thus, in the absence of V1, extrastriate regions can be activated and this activation can guide visual functions but not reach conscious experience. The dissociation between conscious and unconscious visual processing, together with demonstrations of the cortical basis of blindsight, gave rise to the view that conscious experience of all visual attributes relies on V1 and that all extrastriate activation needs to be fed back to V1 for its content to be consciously perceived (Silvanto, 2015). The model formalized by Lamme starts from the premise that *unconscious visuo-motor transformations* (as in blindsight) *may be executed in an entirely feedforward processing cycle, while visual awareness is critically dependent on feedback connections to V1* (Lamme, 2001), as shown in Figure 4. As soon as a region has been activated by the feedforward sweep, recurrent interactions between neurons within that area and neurons that have been activated earlier at lower levels can begin. These interactions are mediated by horizontal connections and feedforward/feedback circuits between and within areas. They are expressed in neuronal responses as modulatory influences from beyond the classical, feedforward, receptive field (Lamme & Spekreijse, 2000).

2. AIMS

The main purpose of the present study is to develop and test a behavioural paradigm to assess affective blindsight through a visuo-motor forced-choice task, in both humans and non-human primates. To this end, we developed a complex visuomotor forced-choice task that would be able to monitor the perceptual state of the tested subject without receiving any verbal instruction of feedback. In order to have the most comparable experimental training pipeline in both humans and non-human subjects, we trained human subjects to the performance of this novel behavioural paradigm without any explicit verbal instruction in any phase of the task, mimicking the type of training and the stages that will have to be applied in non-human primates. In this way, we aim to develop a suitable animal model for the discriminative capacities that are found in human blindsight patients, which will enable us to identify the unknown subcortical routes and hopefully how to leverage and boost them to promote some recovery of visual awareness following V1 lesions.

3. MATERIALS AND METHODS

3.1 Subjects

In the first phase of the study, the task was administered to five human subjects (3 males and 2 females), unaware of the rules and purposes of the task. Next, two males *Macaca mulatta* (Mk1, 13 Kg and Mk2, 10 Kg) were recruited to be trained in the same task. Monkeys were pair-housed with no water restriction and controlled daily access to a variety of food, including pellet and seeds (rice, sunflower and others), fruits, vegetables and a special mesh of pellet flour with fruit juice, varied depending on the training and nutritional needs. Environmental enrichment was provided and rotated on a daily basis. Night and day cycle was ensured by natural light through large windows and an automatic artificial lighting system. Temperature and humidity were controlled within an optimal range for the species.

All experimental protocols complied with the European (Directive 2010/63/EU) and national (D.lgs 26/2014) laws on the protection of animals used for scientific purposes, they were approved by the Veterinarian Animal Care and Use Committee of the University of Parma and authorized by the Italian Ministry of Health.

3.2 Behavioural tasks and apparatus

A MATLAB-based software for behavioural control and data acquisition (MonkeyLogic) was used to design the task (Asaad et al., 2013). The timing of task events can be synchronized with external devices via event code exchanges. Specifically, an eye-tracking software (*Oculomatic*) was used to convey, via a DAQ board, eye movement analog signals to the MonkeyLogic behavioural control software, which receives these signals and control task unfolding accordingly. Wrong answers or a specific stimulus presentation could generate a transistor-transistor logic (TTL) pulse within MonkeyLogic, aligned with the neuronal activity, so that it will be possible to relate neuron firing with a specific task phase or event.

3.2.2 Human paradigm and training task steps

The human subjects were positioned in front of a screen at 57 cm of viewpoint distance, lying on a chinrest to fix the position of the head, making possible the calibration of the eye; no instruction was given. The only feedback the subjects received for their performance was a green thumb up indicating a correct trial, and a red thumb down indicating an incorrect trial. This was done to simulate the liquid reward (consisting in drops of fruit juice) for macaques that is the only feedback they can get following correct responses.

The stimuli presented had dimensions of 7 x 9.5 degrees (70 mm width x 95 mm height) and were downloaded from the Karolinska Institute database. In particular, the database includes a subset of stimuli, that is the Karolinska Directed Emotional Faces (KDEF), with a set of 4900 pictures of human facial expressions, and a subset called The Averaged KDEF (AKDEF) that is a set of averaged pictures created from the original KDEF images. For our purposes, we used the KDEF subset.

Figure 10. Exemplars of facial expressions presented during the task. On the left, six different stimuli presented during the task that are organized as follows: female stimuli on the top, male stimuli on the bottom. The columns represent the emotional content of the stimuli. First column: neutral emotion; second column: positive emotion; third column: negative emotion. On the right, white points illustrate the spatial location where the stimuli have been presented: 10 and 15 degrees of eccentricity with a polar angle of 45°.

Stimuli were presented in four different positions in the right hemifield at 10 and 15 degrees (Fig. 10). The factorial design of the final task is 3 x 2 x 2 x 2 x 2 (Emotion: Neutral, Happy, Disgusted; Actor: male, female; Condition: Go (sample present), no-Go (sample not present); Target position: up, down; Distractor: one among the two remaining stimuli not chosen as target.

The visuo-motor forced-choice task (VMFCT) is composed by two different types of trials (Fig 11): Go trials, in which a sample is presented, and no-Go trials or *blank trials* where no sample is presented, which correspond to two different conditions. These two different trial conditions are randomly presented to the subjects within the same session with a probability of 80% (Go trials) and 20% (no-Go trials). They are defined Go trials because in this condition the subjects have to press a button during the presentation of a peripheral stimulus (sample); whereas no-Go trials or *blank trials* because no peripheral stimulus is presented, and the subjects have to remain still and avoid to press the button.

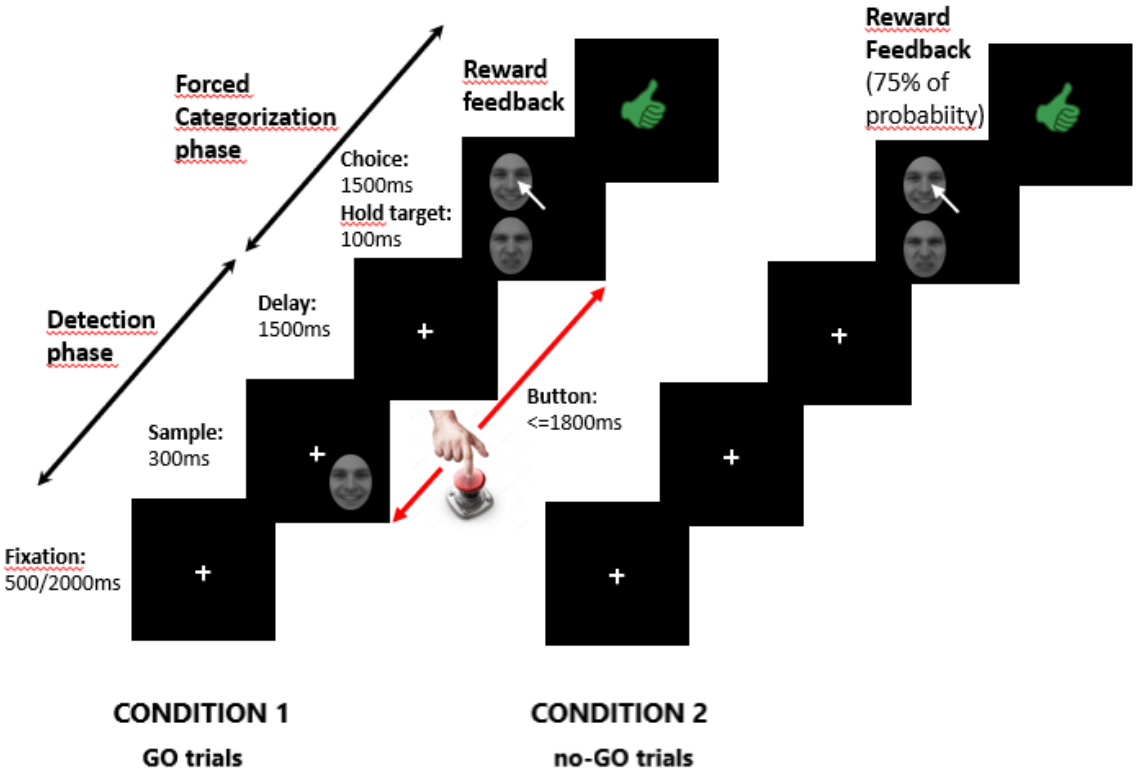


Figure 11. Sequence of events that describe the behavioural paradigm (VMFCT): Condition 1 refers to the GO trials in which the sample is present. Condition 2 refers to the no-Go trials or blank trials in which no sample is presented. The entire duration of each type of trial is the same. Both conditions are organized in a first phase (detection phase) where the subject must press or refrain from doing it depending on the presence (Condition 1) or not (Condition 2) of the sample, and in a second phase (forced-choice phase) where the subject is presented with two alternatives (target and distractor) at random positions (up and down). The target matches with the sample previously presented during the detection phase.

Condition 1 starts with the presentation of a fixation point. The subject must engage fixation as soon as possible within a period of 2000 ms. Once the subject reaches the fixation through a saccade, it must be maintained for a random period ranging between 500/2000 ms. After this period, a sample (one among those illustrated in Fig. 10) appears at random eccentricity (one among those described in Fig. 10) for 300 ms. This sample presentation phase is followed by a delay period of 1500 ms, in which only the fixation cross is presented, and it represents an extra-time available to the subject for pressing or not the button during the detection phase, as the sample presentation period is too short for allowing subject to perceive and detect the visual stimulus. From the beginning of the fixation period to the end of the delay period, including the sample phase, the subject must hold the fixation at the central position and shift the attention toward the peripheral part of the visual field in order to: 1) monitor the presence of the sample, 2) press the button as soon as the sample is detected. The button press moves the subject in the next phase of the trial, that is the forced-choice phase or categorization phase, in which the target and one among two possible distractors (neutral or remaining emotional face) are presented in the opposite hemifield relative to that where the sample was presented; subject has to perform a saccade toward the target, which matches to the stimulus (sample) previously presented during the detection phase within a period of 1500 ms. The subject has to maintain the fixation on the target for 100 ms in order to receive as feedback the green thumb up.

Condition 2 is characterized by having the same duration of each Condition 1's event or epoch, except that no sample is presented during the detection phase. Thus, the subject has to refrain from pressing the button (no-Go trials). If the subject maintains the fixation on the cross, the software moves the performer directly to the subsequent categorization phase during which he/she has to perform a saccade toward one among two alternatives. In this case there is not a correct match to perform, and the positive feedback or correct response is returned with a probability of 50%.

To train human subjects as a proof of concept for the training stages to be performed in monkeys, the whole training pipeline has been subdivided into 10 consecutive steps and subjects have been trained to the execution of each step.

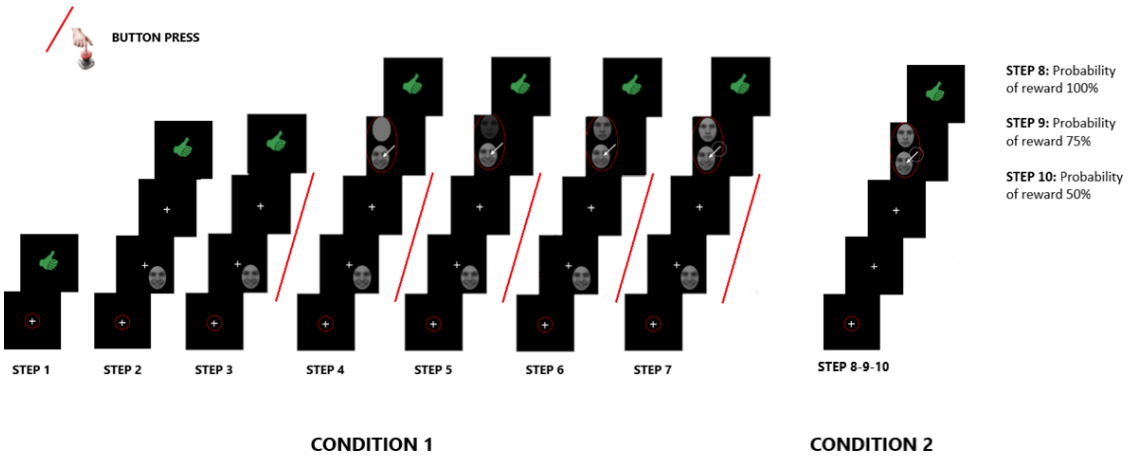


Figure 12. Organization of the training steps. Condition 1 has been subdived into 7 steps (from STEP 1 to 7), while Condition 2 into 3 consecutive steps (from STEP 8 to 10). **STEP 1:** The subject has to maintain fixation. **STEP 2:** The subject has to maintain the fixation on the white cross and ignore the peripheral sample. **STEP 3:** The subject has to maintain the fixation on the white cross and press a button as soon as a sample is detected in the periphery. **STEP 4:** The subject has to maintain the fixation on the white cross, press the button as soon as a sample is detected in the periphery, and perform a saccade toward the target corresponding to a facial stimulus. **STEP 5.** The subject has to follow the same rules of STEP 4 taking in to account the luminance of the target. **STEP 6.** The subject has to follow the same rules of STEP 5. **STEP 7:** The subject has to follow the same rules of STEP 4 taking in to account in this case the visual features of the sample. **STEP 8:** The subject has to maintain the fixation on the white cross and remain still (not button pressure) during the sample phase. Finally, he/she has to perform a saccade toward one of the two alternatives with a probability to receive positive feedback of 100%. **STEP 9.** Same rules of STEP 8 except for the probability of positive feedback equals to 75%. **STEP 10.** Same rules of STEP 8 except for the probability of positive feedback equals to 50%

STEP 1: The first step requires to the subject to maintain fixation on the central position for a random period of 500/3000 ms. In case of fixation break or missed fixation, a red down thumb appears on the screen. Each correct trial is signaled by a green thumb up.

STEP 2: Once the fixation on the central cross exceed the required fixation period, a peripheral stimulus (sample) appears in a random position periferally to the cross, lasting for a random time interval of 1500/2300 ms; the sample presentation is longer than final sample time (300 ms) because we want that subjects actively neglect the peripheral stimulus. The sample presentation phase is followed by a short delay period (100 ms) as occurs during the final version of the

behavioral task. A correct trial requires to maintain the gaze on the fixation cross along all the unfolding period from the beginning of the fixation to the end of the delay interval.

STEP 3: A this stage a button is introduced for the first time in the working space of the subject. Again, subjects had to hold the fixation on the primary position for a time of 500/2300 ms. Then, the peripheral sample is presented for a random interval of 1500/2300 ms and followed by a delay interval of 100 ms. Subjects have to press the button as soon as possible within a maximum time of 2400 ms (2300 + 100ms) in order to receive a positive feedback. Any other performed behaviors is considerd an error.

STEP 4: At this step, for the first time, the pressure of the button moves immediately the subjects in the next phase of the trial, that is the *categorization* phase. Two different alternatives are presented on the monitor: the target and the distractor (a grey oval stimulus). They are always presented on the opposite side of the screen relative to that where the single peripheral sample has been presented. Trial begins with holding fixation on the central cross for the required time; subjects have to press button as soon as they detect a peripheral sample. The pressure of the button actives for the first time the chategorization phase, during which the subject has to perform a saccade on the target (face) that matches with the peripheral sample previously presented. The phase of choice time lasts 1500 ms, while the holding fixation target 100 ms.

STEP 5: This step has the same rules of STEP 4, except that the distractor is a one of the remaining stimuli with a luminance reduced at 60% of its maximum. The target maches with the sample previously presented. This step has been thought to induce subject to be aware of the presence of a second facial stimulus and then increase the probability that he/she focuses its attention on the emotional content of the stimulus during the detection phase. However, the subject can still correctly perform the trial by just discriminating the difference of luminance between the target and distractor.

STEP 6: This step has the same rules of STEP 5 except that the distractor luminance is reduced at 80% of its maximum.

STEP 7: This step has the same rules of STEP 5 and 6 except that both stimuli have the same luminance (100%) that is not reduced at all. In this case to perform a correct trial, the subjects have to change strategy, following the emotional content of the sample stimulus, in order to receive positive feedback.

STEP 8: During this step, the Condition 2 is introduced for the first time. Both conditions are randomly presented from the beginning. The Condition 2 consisting in no-Go trials or *blank* trials differs from Condition 1 just because no peripheral sample is presented during the detection phase. Therefore, subjects don't have to press the button in this phase. The rest of the trial' phases are the same for both conditions. Because no sample is presented in the visual field, no correct target is available in this phase. To induce subjects to move the gaze on one of the two presented alternatives, positive feedback with 100% of probability is given, whatever performed choice. This allows to learn the correct sequences of visuomotor behaviours. So, subjects must move the gaze by chance on the target. Any other performed behaviours are considered as an error.

STEP 9: At this step, the only difference relative to the previous one, is that choices in the categorization phase are rewarded with a probability of 75%. This is made to encourage the human subjects to keep high attention and try to find a possible rule.

STEP 10: The final step has the same sequence of events of the previous step except for the fact that the probability to receive positive feedback is reduced at 50%.

1.3 Monkey behavioural paradigms and training steps

With the aim of investigating the neural bases of blindsight phenomena in non-human primates and developing valid rehabilitation protocols, two monkeys will be trained to the execution of the VMFCT described above. However, referring to the previous literature (Moore et al., 1995;

Yoshida & Isa, 2015) in order to validate the new behavioural paradigm, the same subjects were trained to the execution of two slightly different types of saccadic tasks.

3.3.1 Chair training session: from the home-cage to the chair

The first steps of the monkeys training need to acclimate them into their transport device. We habituated them to enter the primate chair that has been carried out leveraging positive reinforcement training (PRT) and equipment (Mason et al., 2019). PRT for these protocol phases involved the presentation of treats (fruits, dates, raisins, peanuts, or juice liquid reward) for desired behaviours when produced at the instructional signal (visual or vocal stimulus). Commands such as 'head' or 'up' were used to encourage monkeys to present their head through the primate chair aperture and were followed by a clicker sound after a correct behaviour to anticipate the reward delivery. It is important to emphasize that clear definitions and training goals must be carefully planned by the trainer to determine what is the specific desired behaviour, so that the reward schedule can be reproducible but also flexible enough to promote the most rapid progresses in the training.

During the training session in the laboratory, the monkey receives liquid reward (fruit juice) by a tube mounted on the chair, which rests on its mouth once it is head fixed. This protocol phase is necessary to teach the various phases of the behavioural tasks, record eye movements during the fixation and saccade tasks, and correctly test the visual field.

3.3.1 Training task procedures

The training to the behavioural tasks was divided in different steps and started with the introduction for the first time of the calibration of the eye movements through the eye tracker. Once calibrated, the eye tracker provided online information about gaze position, which was used by the software to manage the behavioural paradigm and deliver the reward when correct trials were performed.

Fixation task. During this task monkeys were required to fixate a central white cross on a 55" monitor for a random period of 400/1200 ms.

Saccade-off task. A trial started with a fixation point (white cross) that appeared on the center of the monitor, and the monkey had to reach it with a saccade within a fixed time of 2000 ms and a fixation window radius of 2 degrees. Monkey had to maintain fixation for a random period of 400/1200 ms. Then, a target on the periphery (from 5 to 20 degrees of eccentricity, in steps of 5°) appeared in one among several different available positions with a polar angle of 30° while the fixation point was turned off. The animal had to perform a saccade toward the peripheral target and fixate the target for a period of 200 ms in order to receive a juice reward.

Saccade-on task. A trial started with a fixation point (white cross) that appeared on the center of the monitor. The monkey had to reach through a saccade the fixation point within a fixed period of 2000 ms and a fixation window radius of 2 degrees. Monkey had to maintain the fixation for a period ranging from 400 to 1200 ms. After the fixation phase, a peripheral stimulus appeared on a random position among those available while the white cross in the central position remained turned on the screen. The monkey had to perform a saccade on the peripheral stimulus and maintain the fixation on it for a period of 200 ms; then, the reward was delivered. Any other behaviour (e.g., late response, break of fixation) was not rewarded. Differently from what occurred for the saccade-off task, this task included also 20% of blank trials, in which no peripheral stimulus was presented, and then prevented monkeys to perform a saccade, maintaining conversely the fixation on the white central cross. This task will be crucial to measure the extension and of the blind field (scotoma) and have detailed information on perimetry.

3.4 Eye tracking

Oculomatic, an open-source software solution for eye tracking in human and non-human primates *(Zimmermann et al., 2016)*, was used in this study. Most eye tracking solutions for non-human primates require the integration of eye signals within the electrophysiological signal chain. The primary output of the X and Y pupil position is normalized to the image sensor size, and the gain is adjusted by the experimenter *(Zimmermann et al., 2016)*. Voltages are sent to a National Instruments DAQ board which carries the signal in a PC. The *Oculomatic* software tracks eye position within the acquired image frame, at the frame rate dictated by the image sensor. Eye position image coordinates are then transformed into voltages according to user-defined parameters and sent to the behavioural control software (Monkeylogic) via the National Instrument DAQ board.

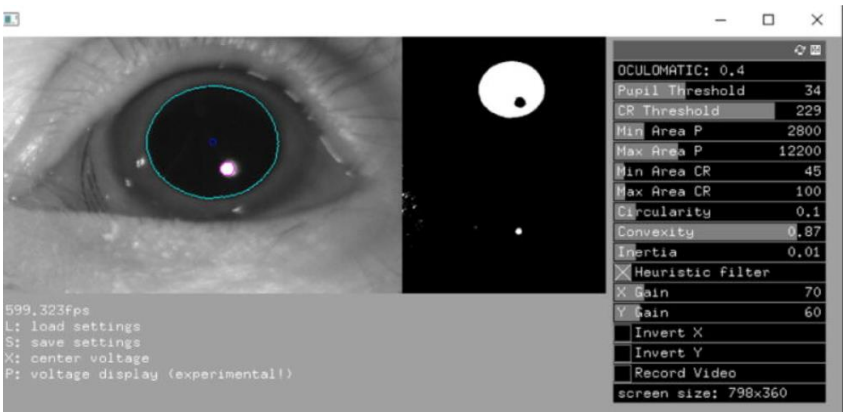


Figure 13. *Oculomatic* graphical user interface. The original camera image overlaid with the estimated pupil location of a non-human primate. Figure from (Zimmermann et al., 2016).

As shown in Figure 13, the procedure starts with the raw image output from the image sensor, which is then thresholded by a user-defined value (i.e., pupil threshold) to yield a binary image in which the pupil is a white circle within a black surround. To quantify the gaze position, an algorithm matches the contour of a thresholded input image and then computes the image moments to find the centroid. To reduce errors in centroid estimation, *Oculomatic* provides the following user-defined parameters to filter the contour estimate: area, circularity, convexity, and

inertia. Extracted contours are limited to a user-defined area between a minimum (inclusive) and a maximum (exclusive) value (Zimmermann et al., 2016).

3.4.1 Kinematics of the monkey saccades

Movements were recorded continuously during the experimental session and kinematic features were analyzed offline using custom MATLAB programs. We plotted the x and y eye components bi-dimensionally, and the eye movements were included for analysis if the peak eye velocity was higher than 30°/s. Following the methods adopted in a study of Lanzilotto and colleagues, eye onset and offset were then defined as the last points on either side of the peak velocity before which the tangential velocity fell below 30°/s (Lanzilotto et al., 2015). For each eye, we determined the amplitude of the movement (°), the maximal velocity (°/s), and the mean velocity (°/s).

3.5 MRI reconstruction of the monkey brain and cranial implants

A head fixation device was implanted on the monkeys' skull. First, a 3D model customized on monkeys' skull was made using 3D slicer, an open-source software for brain visualization and image analysis. Through this software it was possible to obtain the reconstruction of the skull of each monkey, starting from 7T magnetic resonance images (Fig. 14). The different tissues in their main axis, such as sagittal and coronal sections, were differentiated and automatically marked for each frame.

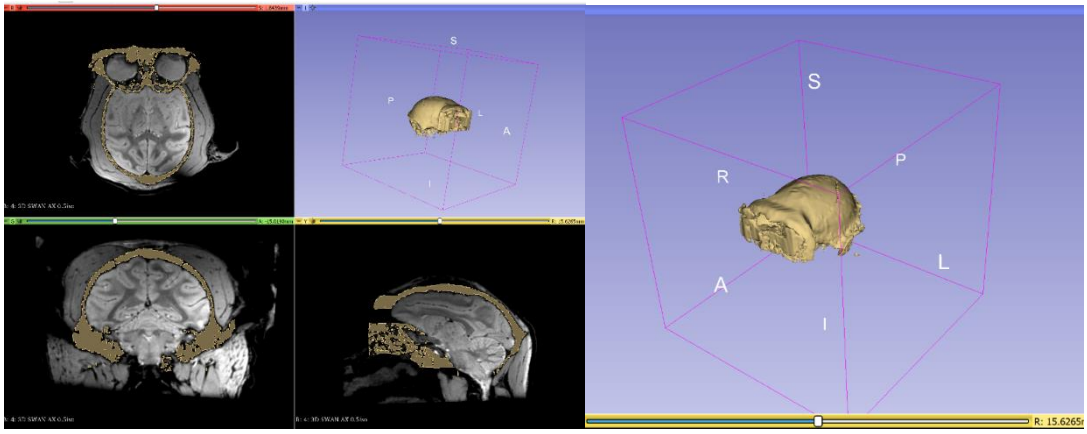


Figure 14. 3D Slicer workspace. On the left, differentiation and marking of bone tissue in two-dimensional MRI images in the axial (upper left), sagittal (lower left) and coronal (lower right) section. On the right, the upper right part of 3Dslicer workspace is represented; it results from the intersection of the three portions.

Therefore, for each frame it was possible to mark the bone while in the upper right of the workspace the three sections simultaneously were intersected, creating the 3D image. Then, using the median method the final image was smoothed.

3.5.1 Surgeries

Anaesthesia was induced with ketamine hydrochloride (5 mg/kg intramuscular) and medetomidine hydrochloride (0.05 mg/kg i.m.) and maintained with 2% isoflurane vaporized in 100% oxygen. Surgery was performed in aseptic and stereotaxic conditions. During all surgeries, hydration of the monkey was maintained with continuous infusion of saline solution and eye hydration was ensured through vitamin A eye gel. A heating pad stabilized the monkey's body temperature throughout the surgical procedure. Heart rate, respiratory depth, and body temperature were continuously monitored. Analgesics were administered intra- and postoperatively. Upon recovery from anaesthesia, each animal was returned to its home cage and closely monitored until complete recovery. Dexamethasone and prophylactic broad-spectrum antibiotics were administered pre- and postoperatively.

4. RESULTS

4.1 Human training results

Five human volunteers underwent training sessions as described in Material and Methods. The entire training procedure to learn the whole behavioural task (VMFCT) required about 3 hours for each subject (subdivided in two days, 1.5 h/d).

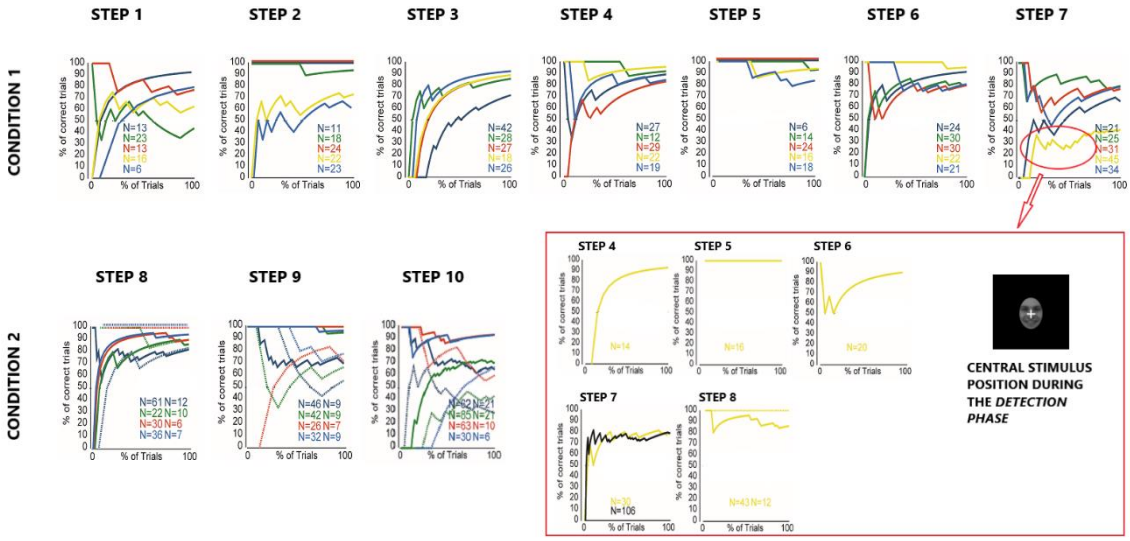


Figure 15. The figure is organized in two rows (Condition 1, upper; Condition 2, bottom). Values on the y-axis for each plot represent the percentage of the cumulative correct responses calculated trial by trial for each subject (five different colour lines). Values on the x-axis represent the percentage of total number of trials (N) performed by each subject in that specific training step. On the right bottom of the figure within the red panel, the additional training for the subject 4 (yellow line) is represented (from the step 4 to the step 8).

To ensure that, in spite of the absence of any verbal instruction, human subjects correctly learned the rule of each step, we asked at the end of each training phase what was the required rule. When subjects did not fully understand the correct rule, their performance was unstable across trials, and they typically performed correctly approximately no more than half of the trials.

All but one human subject learned STEP 1 after a few trials (about 20%) (Fig. 15, STEP 1). Once the subjects were presented for the first time with STEP 2 (a peripheral stimulus is presented), three subjects immediately performed correctly the trials with a percentage of correct responses

higher than 90%. They maintained the fixation on the primary position and neglected the peripheral stimulus, whereas the remaining two subjects made immediately some errors, making an automatic saccade toward the stimulus. However, the performance of both of them increased exponentially. During STEP 3, the button was presented for the first time in the subjects' working space. According to the principles of the operant conditioning, after some negative feedbacks, they tried to push the button placed in front of them, despite no explicit explanation. Some subjects pressed the button just after only a few omissions (Fig. 15, STEP 3), while others needed more trials. However, in all cases the percentage of correct responses exponentially increase after few trials, enabling to move to the next training step. During STEP 4, the pressure of the button moved for the first time the subjects in the next phase of the VMFCT, that is the categorization phase. In this phase, the subjects had to perform a saccade toward the face stimulus and ignore the distractor (grey oval stimulus). Most of the subjects performed the first trial correctly, except for one (Fig. 15, red line). However, after a few mistakes, all subjects reached performances higher than 80% of correct trials. In STEP 5 the only variation relative to the previous one was related to the distractor stimulus, which was an emotional face, although it was reduced at 60% of luminance with respect to the maximum value. Subjects 1 and 2 correctly performed all the trials, whereas the third subject (green line) made mistakes in the latest trials. The remaining two subjects correctly responded to the first trials, but they made mistakes in the subsequent trials; however, all subject reached a high percentage of correct responses, demonstrating to be able to apply the required rules. In the next step (STEP 6), the distractor face stimulus luminance was increased relative to the previous step, from 60% to 80% of the maximum value. Subjects 1 and 2 (dark blue and green lines) made a saccade toward the distractor in the first trial, but their performance increased in the subsequent trials, ending the session with the 75% of correct responses. The remaining subjects correctly performed the first trial, particularly subject 4 (yellow line) maintained high percentage of correct responses for the entire duration of the training phase. Once, all subjects learned the first six steps and they reached a good performance in terms of

visuomotor coordination (hand, eyes), we moved to the STEP 7, where the distractor stimulus was presented with the 100% of maximal luminance. In this case, subjects had to eventually change again their strategy, because no difference in terms of luminance was present between target and distractor. In order to correctly perform the trial, the subject had to take into account the emotional content of the previously presented sample that matched with the target during the choice phase. Interestingly, all subjects after a few of trials reached good levels of performance except for one subject (yellow curve) that was not able to perform correctly the trials (Fig. 15, STEP 7). This subject's performance never went beyond 40% of correct trials.

Since this is an occurrence that may also help when the training will be applied to the monkeys, we devised a way to facilitate this subject's learning of the task. Figure 15 (STEP 8- 10) shows a dedicated training sessions for this subject (plots included in the red box). As previously stated, one among 5 subjects was not able to learn the correct rule relative to the step 7 by following the normal training plan. For this reason, we adopted an alternative training that consisted of presenting, during the detection phase, the sample at the center of the fovea (primary position) rather than at the periphery. This was made re-testing some previous steps as it is illustrated in Figure 15. In particular, we manipulated the sample position from STEP 4) to STEP 8. As shown by the performance of the subject, we were able to increase the salience to emotional component of the sample during the detection phase. To be sure that the subject correctly understood the rule of STEP 7, we repeated step 7 two runs (yellow and black lines) revealing that the rule was consolidated.

At this point, the other 4 subjects were trained according to the original training plan. During STEP 8, for the first time, trials relative to Condition 2 in which no sample was presented during the detection phase were randomly presented altogether with trials of Condition 1. Because no sample was presented in the visual field, no correct target was available in the forced-choice phase (categorization phase), and to induce subjects to move the gaze on one of the two presented alternatives, positive feedbacks with 100% of probability was given, whatever performed choice.

This allowed to learn the correct sequences of visuomotor behaviours. Indeed, all but one subjects correctly performed the trials of Condition 2 since the first presentation (Fig. 15, STEP8). We plotted the performance of each single subject by representing it with a continuous line for trials of Condition 1 and with a dashed line for trials of Condition 2. Two of them had a percentage of correct response during Condition 2 equal to 100% for the entire duration of the training step. It is worth to note that all subjects performed the step with percentage of correct responses beyond 80% during both conditions. In order to induce subjects to find a possible target during trials of Condition 2, we reduce during STEP 9 the probability of receiving a positive feedback from 100% to 75% (Fig. 15, STEP 9). This allowed us to increase the effort of subjects. Therefore, during STEP 9 all subjects performed with very few errors the trials of Condition 1, by reaching percentages of correct responses higher than 70%. As predicted, the percentage of correct responses during trials of Condition 2 became lower than in the previous step. Finally, to reach the final version of VMFCT, during STEP 10 (Fig 15, STEP 10) we further reduced during Condition 2 the probability of receiving a positive feedback from 75% to 50%. Two subjects performed with high level of performance almost all the trials of Condition 1.

Once the training sessions were concluded and all subject learned to perform the whole task, we presented the final version of VMFCT, and we recorded data from all subjects (Fig. 16).

We plotted the percentages of correct trials, button press reaction times relative to each training phase, and differences in terms of performance based on position and emotional content of each presented stimulus.

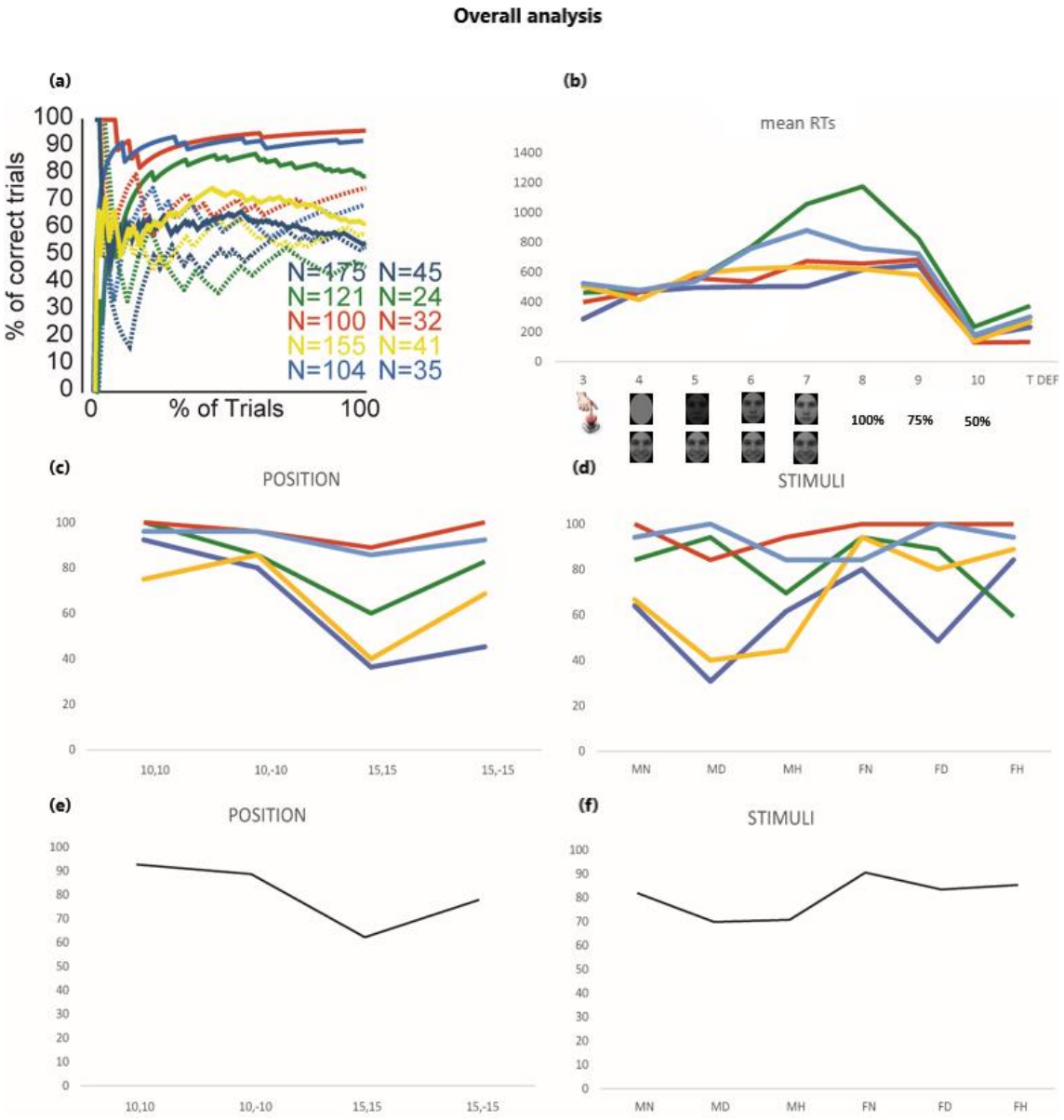


Figure 16. In the first row the percentages of the correct responses trial by trial of the final task **(a)** and the mean of the reaction times **(b)** for each step are represented (with X axis indicating from step 3 to step 10). In the panel **(a)**, N indicates the number of trials which each subject did (the first column refers to the condition 1 and the second column to the condition 2, presented in a random way). The second and the third rows illustrate the average percentage of correct responses for each subject relative to the position **(c, e)** and the type of the stimuli **(d, f)**.

The total number of trials was 96 for trials of Condition 1 and 24 for trials of Condition 2, and each error trial was randomly presented with replacement. All subjects performed the VMFCT with high levels of correct responses. Since in the final version of the VMFCT the probability of receiving a positive feedback during the Condition 2 was established at 50% the performance relative to the trials of Condition 2 was definitively lower than Condition 1. In order to test if

there is a correlation between learning phases and button reaction times, we averaged reaction times for each subject at each training step as illustrated in Figure 16. The reaction times remained relatively stable until STEP 5. They significantly increased once the distractor was presented as another facial image (STEP 6 – STEP 7). The lowest reaction times were relative to the STEP 10 for all subjects. In order to verify if there was a correlation between performance and stimulus position or emotional content, we illustrated the average of correct responses for each subject (Fig 16C) on the four tested positions as well as the average across subjects (Fig 16E). We found highest level of performance for the stimuli with 10 degrees of eccentricity, with percentage of correct responses between 90% and 100%. The accuracy of all subjects decreased systematically for peripheral stimuli presented at 15 degrees of eccentricity, particularly for those presented in the upper quadrant of the screen. Interestingly, the two subjects (yellow and dark blue) that showed lowest levels of performance in the VMFCT, had lower performance in correspondence of male facial stimuli, particularly for happy and disgusted faces (MH and MD).

4.2 Monkey results

To prepare monkey experiment, which requires the head fixed, we first reconstructed the skull of the two monkeys based on the 3D MRI images (see *Materials and methods*). We 3D-printed each animal's skull as shown in Figure 17 in order to model the base of the headposts prior to each surgical implantation.

Figure 17. Example of 3D print of the skull. 1:1 model of the Mk2 skull. The holes specify stereotaxic coordinates of interest to be considered for the positioning of the headpost and, subsequently, of the recording probes.

However, on the same 3D-skull model we also simulated the curvatures of each headpost in order to more precisely configure the base of the headpost and its orientation, as shown in Figure 18. This allowed us to refine the surgery procedures because each headpost well-fitted on the specific monkey's skull and no further manipulation was needed during the implantation. This enabled us to autoclave it before the surgery and place them on the skulls in a fully sterilized surgical field.

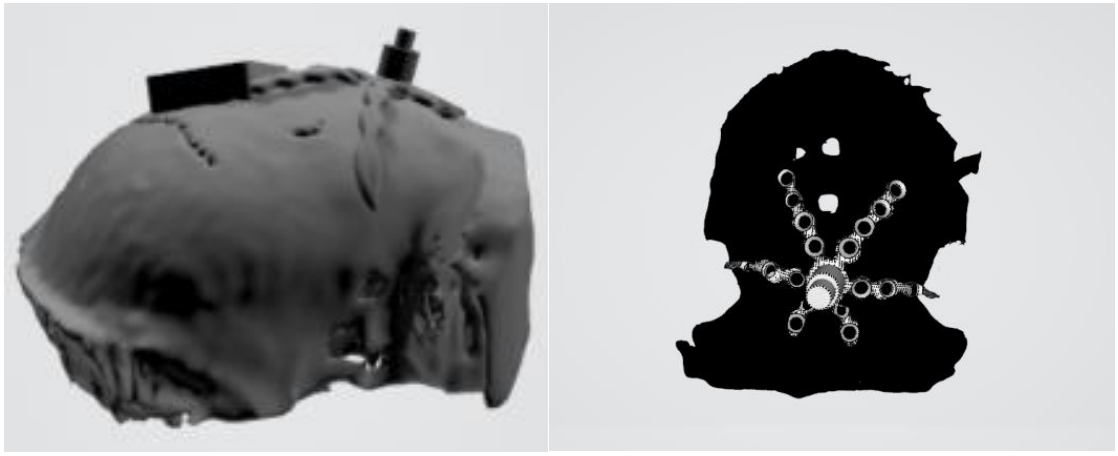


Figure 18. 3D reconstruction of the headpost and its curvature. The headpost fitted on the skull is represented (on the left, Mk1; on the right Mk2). In the first image there is a raw representation of the future position of the chamber.

Finally, by using the same methodologies and the same softwares we also designed a 3D model of the recording chamber to be implanted for the recordings (Fig. 19). By means of Autodesk Fusion 360, the various parts of the chamber perfectly fitted on the 3D-printed skulls.

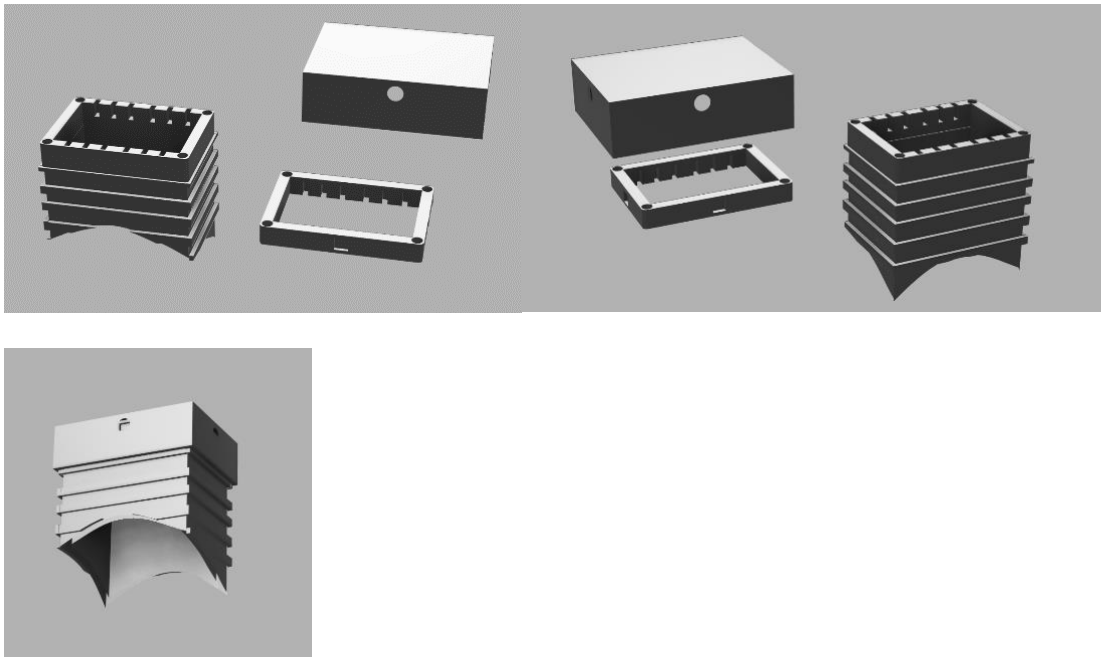


Figure 19. 3D chamber model. Reconstruction of the various parts of the chamber. Respectevely from left to right, the three images represent: front view, back view, and the final result of an implanted assembled chamber.

As stated in Material and Methods, two monkeys were trained to perform a visual fixation task that corresponds to STEP 1 of the training procedures used for human subjects. Moreover, the monkeys were trained also to perform two saccade tasks called “Saccade-off” and “Saccade-on”. For each animal, the behavioural performance was evaluated considering the percentage of correct responses.

Figure 20 shows the performance of each animal to these two latter behavioral tasks. At this stage, the first monkey (Mk1) has been already trained to perform both Saccade tasks, while the second monkey (Mk2) has been trained to perform only the Saccade-off task. It is worth to note that both monkeys show high levels of performance during the Saccade-off task (Fig. 20A, B) higher than 60% of correct response on average. However, the first monkey (Mk1) shows lower values of

standard deviations when compared to the second monkey (Mk2), suggesting a more constant performance across sessions within the same training day and across days.

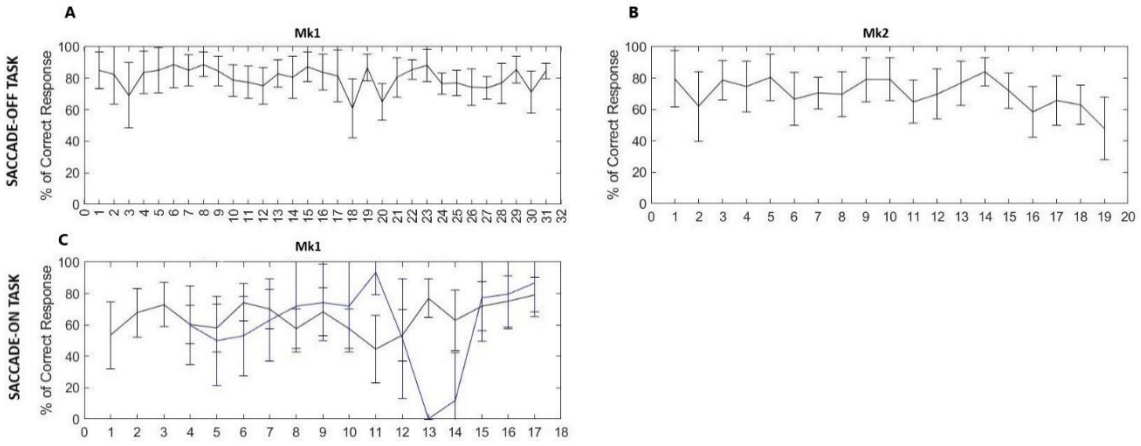
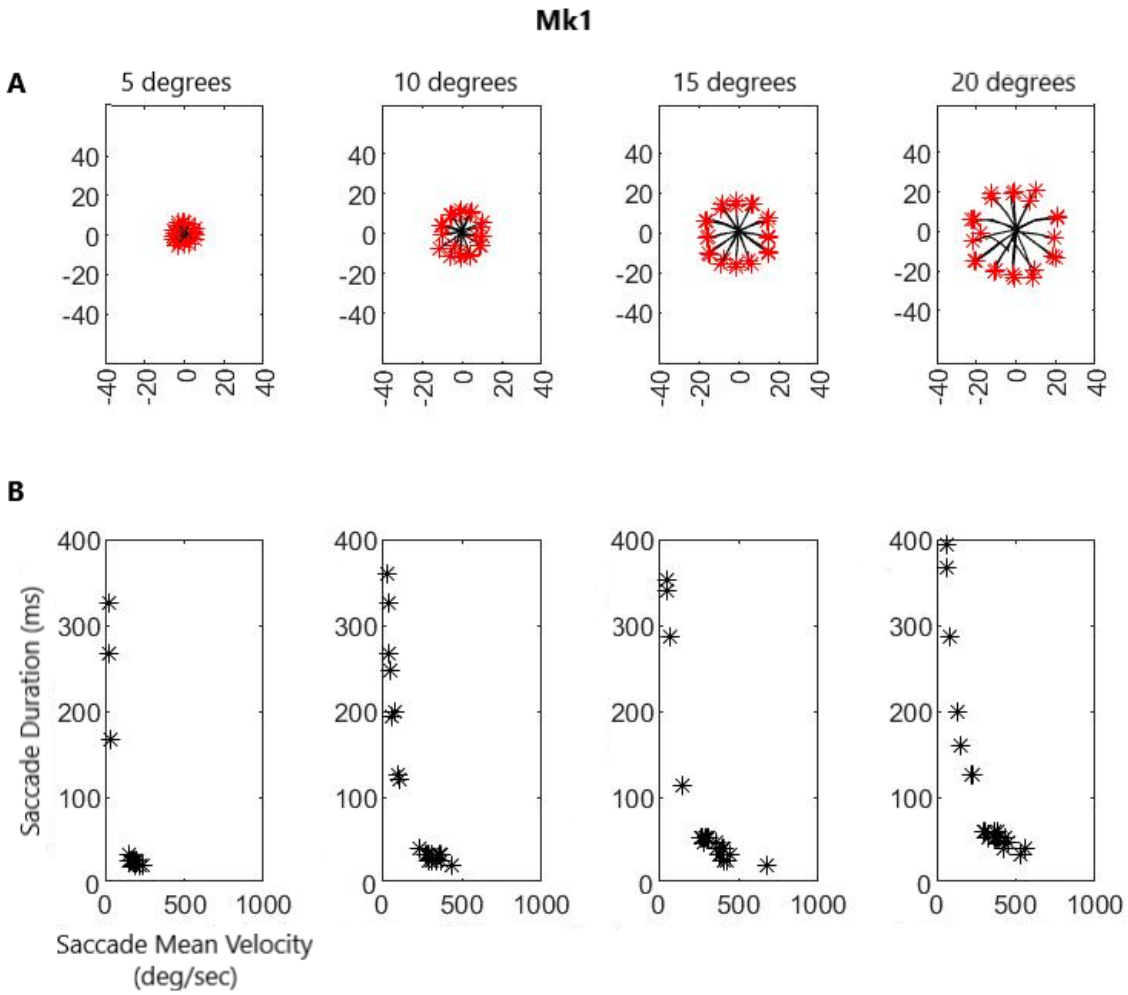


Figure 20. Percentage of correct responses of two monkeys to the behavioural tasks. The percentage of correct responses of Mk1 **(A, C)** and Mk2 **(B)** are represented during the saccade-off and -on tasks. The average percentage of correct responses (X-axis) across days (Y-axis) with their standard deviations are represented. The average percentage of correct responses of one day derives from the mean of the percentage of correct trials of all the sessions performed during a certain training day. In the saccade-on task, two different colour lines discriminate the two different conditions of the task: the black line represents the mean of correct responses of the target trials, whereas the blue line refers to the mean of correct blank trials.

These findings highlight a generally better performance of Mk1 relative to Mk2 from the first day of training. Indeed, Mk1 performed the Saccade-off task with a percentage of correct responses higher than 80%. Moreover, its performance constantly increased along the training days (Fig. 20A), and it was quite stable without any significant variation, except for the day 18 and 20, in which the performance slightly decreased, reaching 60% of accuracy. In general terms, the performance of Mk2 in the execution of the Saccade-off task was poorer and not equally stable across days relative to Mk1 (Fig. 20B). Indeed, the performance of the last days analysed since now exhibited a significative reduction with respect to the previous days, particularly after the 14th day, because of some variations introduced in the experimental setup (changes in the screen background). It is worth to note, however, that Mk1 has been trained to perform the Saccade-off task for a major number of days relative to Mk2. However, because the performance of Mk1 was

higher and stable across days, it was introduced to also perform the Saccade-on task, maintaining, however, the daily training sessions to the Saccade-off task.

Figure 20C shows that Mk1 increased exponentially its performance from 50% to 75% of correct responses. For this reason, in correspondence of day 4, we introduced the second set of trials, that is blank trials, during which no saccade was required. It is worth to note that the performance changed, indicating that the animal was not so confident with the rules of the new task. In particular, when the percentage of correct trials regarding the saccade trials (Fig. 20C, black line) increased, conversely the performance regarding the blank trials (Fig. 20C, blue line) decreased and vice versa. This indicated that monkey likely changes strategy in its performance, and it tried to apply the same rule for both set of trials. With some variations introduced from day 15th, such as the presentation of stimuli only at 20 degrees of eccentricity, it is possible to note that the general performance of the animal increased exponentially for both set of trials until up to the 80% of correct responses.


Mk1
A
5 degrees
10 degrees
15 degrees
20 degrees
B
Saccade Duration (ms)
Saccade Mean Velocity
(deg/sec)

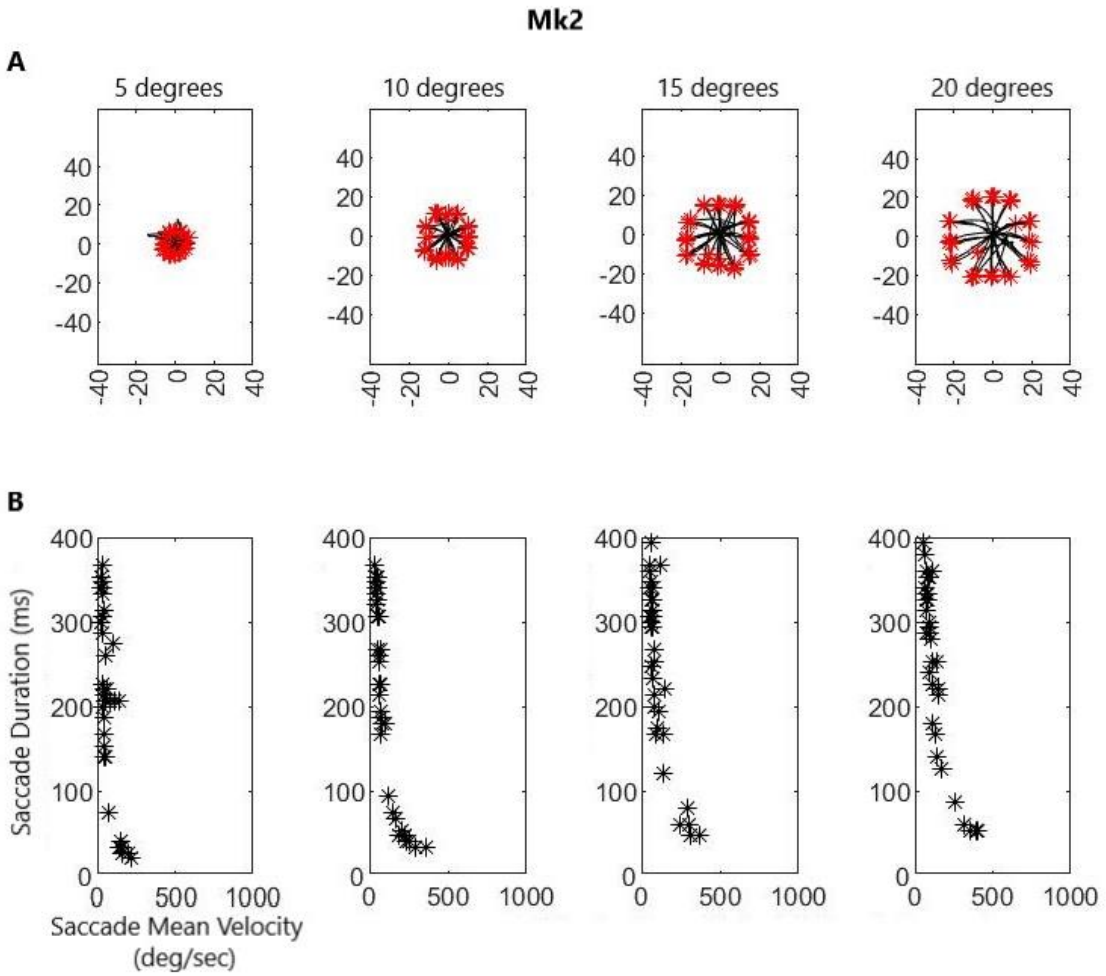


Figure 21. Ocular behaviour and kinematics of each monkey during the execution of the Saccade-off task. A Bidimensional plot of the saccades toward the peripheral stimuli. The black lines represent the trajectory of the eye movement from the central fixation point (0° of eccentricity) to the end point (red symbol) in correspondence to the stimulus. **B** Correlation plot between the average velocity (x-axis) and the duration of the saccades (y-axis).

Next, we analysed the kinematic features of the eye movements. Figure 21A show the ocular behaviour of each monkey during the performance of the Saccade-off task. We analyzed the eye movements for each animal by plotting the trajectories of the eyes during the presentation of specific locations of the visual target. The saccades of the two monkeys differ in accuracy and precision, particularly the saccades of Mk2 are characterized by some deviations and online corrections of the trajectory of the movement. We have also examined the mean of the saccade velocity (deg/sec) as a function of the duration (ms) of the saccades (Fig 21B). The plots show a negative correlation between the average velocity and the duration of the eye movements.

Accordingly, in both monkeys, there are shorter range of the average velocities (0-200 deg/s) for lower degrees of eccentricities than those at higher eccentricities (0-500 deg/s).

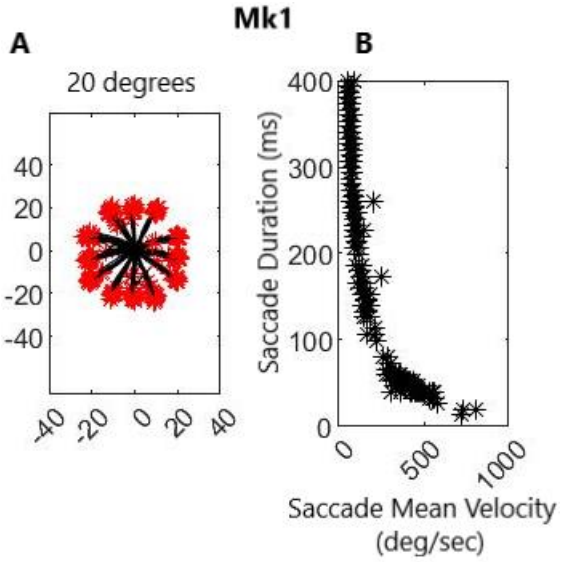


Figure 22. Ocular behaviour and kinematic of Mk1 during the execution of the Saccade-on task. A Bidimensional plot of the saccades toward the peripheral stimuli presented only at 20 degrees of eccentricity. **B** Correlation plot between the average velocity (x-axis) and the duration of the saccades (y-axis).

Figure 22 shows the ocular behaviour of Mk1 during the performance of the Saccade-on task. During the training of saccade-on task for Mk1, it is evident that the eye movements are less precise than those made during the saccade-off task, likewise in Mk2 (Fig 21). In fact, the central fixation is not well defined as the trajectory of the saccade toward the target. It is possible also to see some saccades out of the direction of the target which are modified and corrected online, reaching the target with a deviation from the initial trajectory. The correlation plot between the mean of the saccade velocity and the duration in ms reveals the same trend of the Saccade-off task (Fig 21).

Furthermore, we evaluated whether the peak of eye movement velocity was correlated to the amplitude of the saccades.

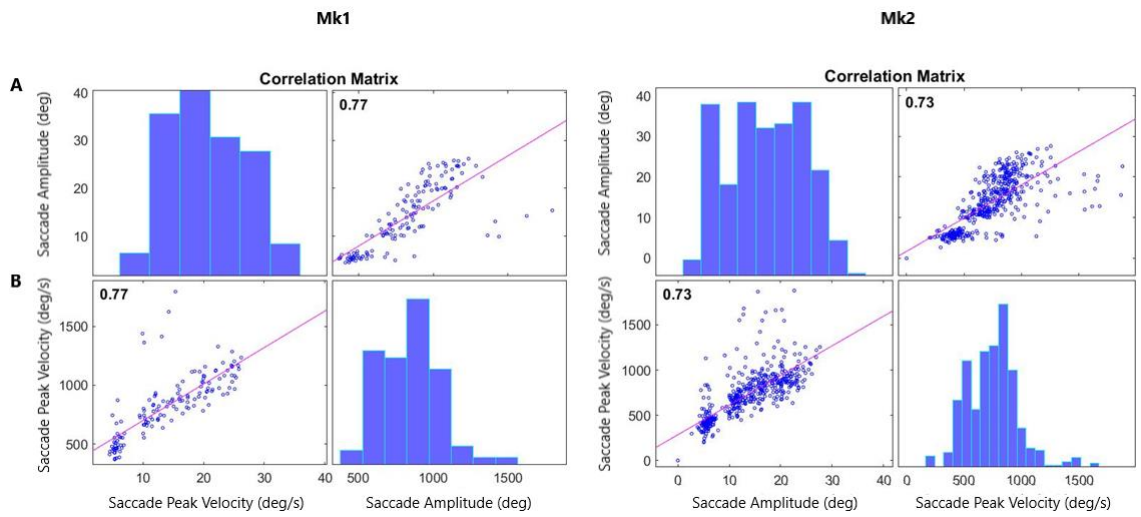


Figure 23. Correlational matrix of the saccade peak velocity (deg/s) and the distance (deg) of Mk1 and Mk2 during the Saccade-off task. **A** Distribution of the saccade amplitude in function of the peak of the velocity. **B** Distribution of the peak of velocity related to the amplitude of the saccades. On the top left of the plots the Pearson correlation coefficient is reported, and the red line represents the Pearson correlation.

To have more information on the kinematics of the eye, it was interesting to consider the peak of the velocity for evaluating the maximum of the velocity reached by each monkey, considering the amplitude (the distance from the central fixation point to the endpoint) of the saccade in degrees of eccentricity. The Pearson coefficient (Mk1, r=0.77; Mk2, r=0.73) revealed a strong positive correlation between the amplitude of the saccade and the peak of velocity. For both monkeys, the saccades made on stimuli at 5 degrees of eccentricity have a peak of velocity of 500 deg/s, reaching a velocity over 1000-1500 deg/s for saccades made at 20 degrees (Fig 23).

5. DISCUSSION AND CONCLUSIONS

Several previous studies aimed to investigate the blindsight phenomenon by presenting simple target stimuli, such as light spots (Moore et al., 1995; Yoshida & Isa, 2015), stimuli of different colours (Cowey & Stoerig, 2001) or shapes (Trevethan et al., 2007), in the scotoma. Other evidences (Celeghin et al., 2015) demonstrated that subjects with blindsight are able to discriminate even between complex images with various emotional valence. This branch of the blindsight literature is usually known with the general term of "affective blindsight" (Tamietto & de Gelder, 2010). However, one of the priority of neuroscientists is to leverage these residual visual abilities to develop complex protocols of rehabilitation that are not still available. The first step to reach this goal is to develop a behavioural paradigm capable to investigate this complex and fascinating phenomenon in a comparative way and that could be generalized across different animal species. Therefore, we developed and tested in human subjects a novel behavioural paradigm called VMFCT potentially applicable even to macaques, in order to investigate subcortical and cortical visual pathways and leverage residual visual abilities to possibly restore awareness.

Differently from previous behavioural tasks used for investigating the blindsight phenomenon (Moore et al., 1995; Yoshida & Isa, 2015), our task involved two different visuomotor phases and two effectors, respectively: a detection phase, during which a motor response with the hand is required to indicate the presence or absence of a visual stimulus, and a forced-choice phase, during which subjects are forced to choose among two alternatives through saccadic movements.

The findings obtained from the training steps performed on the human subjects show an important and interesting result that may be extremely useful during the training of monkeys at VMFCT. Indeed, one among 5 tested human subjects was not able to learn the rules of the STEP 7 by following the planned training procedure. In particular, this subject had difficulties to match the sample presented during the detection phase with the target presented during the forced-choice

task. This could be due to the fact that the subject performed the previous steps just based on luminance differences available between the two presented stimuli (target and distractor), and not by focusing on the emotional features of the sample presented during the detection phase. For this reason, he/she had good performances until STEP 6. When the target and distractor luminance reached the same value (STEP 7), the criterion followed to make the choice no longer applies. Therefore, we needed to present the facial stimuli in correspondence of the central position to retrain the subject. In this way, we induced the subject to focus its attention on a stimulus presented in the fovea where he/she could monitor and extract detailed information that, otherwise, with a peripheral vision was not able to use. This particular result found in the human subject could be more frequent during the training of the monkeys. Therefore, it could be a good strategy to introduce the facial stimuli in the central position when monkeys will be trained to this training stages.

However, our results from human subjects indicated that the percentage of correct trials decreased during STEP 7 for all the tested subjects, and it was possible to observe even an increasing of the reaction times. These results are critically important for the subsequent training stages on the monkeys because we will need to adopt the best training strategies to help animals learning faster this critical step.

Another important issue that emerged from the analysis performed on the spatial locations of the stimuli is that all subjects had systematically lower levels of performance during the presentation of stimuli at 15 degrees of eccentricity when compared to the other positions, particularly for the upper part of the visual field. This is in accord in general with the low-resolution vision that primates have in the periphery (Kandel, 2013).

Another important aspect to be considered emerged from the analysis carried out to investigate the detection abilities of subjects depending on the visual features of the stimuli. Some of them, indeed, had lower levels of performance than others during the execution of the VMFCT. We

found worst performance during the presentation of two particular stimuli: male disgusted and happy faces. These could be due to differences in the contrast of some of the employed images. This result is important because it demonstrates the need to prepare before the experimental session visual stimuli that do not show significant differences in the low-level visual features. Accordingly, with the training plans organized and tested in the human subjects, we will move forward by training two monkeys to the VMFCT by presenting naturalistic, ecological and emotional facial expressions. In order to proceed in this direction, we first trained two monkeys to the execution of a visual fixation task that corresponds to the STEP 1 used for training human subject. Moreover, as claimed before, the forced-choice phase of the VMFCT requires also the ability to perform visually-guided saccades toward peripheral targets. Finally, previous studies (Moore et al., 1995) performed on cortically blind monkeys tested blindsight abilities with two variants of the saccade task. The blindsight monkeys were tested under conditions where the onset of the peripheral target either was unpredictable (in a "standard or non-forced-choice condition") or occurred simultaneously with the offset of the fixation spot (forced-choice condition). In the standard condition the monkeys were not provided with any signals of the target appearance and had to detect with a saccade the target stimulus. Instead, in the "forced-choice" condition the offset of the fixation spot provided a signal for the animal to saccade to the target. So, by removing the fixation spot simultaneously with target onset, was intended to signal or "force" the animal to initiate the eye movement and attempt to localize the target. Taking into account all these considerations, we also proceed to train two monkeys to the performance of two saccade tasks called as: the Saccade-off and Saccade-on task, corresponding respectively with the forced-choice condition and the standard condition of Moore and colleagues' study. The training of both monkeys started with the saccade-off task. The targets were presented in several positions, and we were able to train both monkeys by testing the entire visual field with eccentricities from 5 to 20 degrees (in steps of 5 degrees). We started introducing first the stimuli at 5 degrees of

eccentricity and then added the other positions when the performance of the animals reached high levels (>80%) and appeared constant across sessions and days.

As a consequence of the biological variability, we found that each monkey learned the Saccade-off task with different timing. Particularly, Mk1 learned the phases of the task faster than Mk2 and it was more responsive than the other animal to the variations introduced during the training sessions. Moreover, this prepositive behaviour allowed us to move forward in the next saccade task, that is the Saccade-on task. We started this second task by presenting only trials where a saccade was required (target trials). Once the second set of trials, called blank trials were randomly presented the general performance of the animal decreased. This could be because the animal in some cases (blank trials) started to receive reward just for fixating the central fixation stimulus. The simultaneous presence of the central and the peripheral stimulus during the Saccade-on and the fact that in some cases monkeys could receive reward by just looking to the central position induced the monkey to often break fixation of the peripheral target and to change continuously strategies. To extinguish this undesired behaviour, we presented only peripheral stimuli at 20 degrees of eccentricity, reducing the probability that the monkey had the time to do it. To differentiate the target and the blank trials and giving more salience to the peripheral stimuli, we modified the delivering reward schedule. As soon as the animal made a saccade on the peripheral target it received immediately a drop of juice, and whether it maintained the fixation on it for 200 ms, it received a burst of rewards. Instead, the blank trials had the usual standard reward after a correct central fixation. By adopting this strategy, the monkey was facilitated to learn the new task, with an exponential increase of the percentages of correct responses along the successive days.

The kinematic results obtained through the Pearson correlation analyses revealed an association between the maximal velocity of the eye movement and the amplitude of the saccade. According to the data also obtained by previous studies (Corrigan et al., 2017), in both monkeys the highest peaks of velocity could reach over 1500 deg/s, and this happened particularly for greater saccade

amplitudes. Accordingly, as shown in previous studies (CORRIGAN) we found a negative correlation between the mean of the eye movement velocity and the saccade duration. Particularly, in the case of Mk1 there was a difference in the range of the mean of velocity, which lied on higher values than those of the Saccade-off task. This could be explained by the fact that the monkey has not already consolidated the rule of the task. Higher values of the saccades velocity could be explained by deviations and corrections of the saccades trajectory which were not visible in the Saccade-off task.

Our next purpose consists in consolidating the behavioural performance of both tasks for each monkey and for what concerns the saccade-on task adding the other stimuli with closer eccentricities. Once reached this goal, the next step of the training will be to present a button in the workspace of the animals introducing them to a new phase of the VMFC task.

To conclude, we described and tested a behavioural task that, with a good approximation, will allow us to evaluate the affective blindsight phenomenon in both humans and monkeys. We will develop an animal model in order to explore the relevant mechanisms of residual vision to be exploited in a protocol aiming at restoring consciousness in human subjects. Differently from previous studies (Moore et al., 1995; Yoshida & Isa, 2015), we will compare residual visual abilities by using the hand and ocular movements that are necessarily a good control for testing blindsight subjects. Indeed, the human subjects can easily report of the presence or absence of a stimulus by involving the language system, which is impossible for monkeys. By means of this behavioural paradigm, it will be possible to test in macaques whether several visual maps measured with different effectors may overlap or show differences depending on the neural network involved for processing the visual information. This behavioural paradigm is versatile and adaptable even for investigating additional aspects of consciousness such as the multisensorial integration.

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