A Rodent Model for Visual Masking

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Abstract

Visual masking describes the reduction in the perception of a target stimulus by a preceding (forward masking) or succeeding (backward masking) stimulus. It provides a unique opportunity to investigate the neuronal mechanisms associated with visual perception because by manipulating the temporal separation of the target and mask, it is possible to illustrate a disconnect between the physical stimulus, its neuronal representation, and its percept. Yet, few studies have recorded neuronal responses to visual masking stimuli and even fewer have collected perceptual reports in the same species. This is necessary to know that the changes in neuronal processing actually coincide with perceptual deficits, and therefore can potentially offer insight into the development of conscious visual perception. To the best of our knowledge, there have not been any detailed neuronal investigations of visual masking in over a decade. In this time, there have been significant advancements in the technology available to probe neuronal mechanisms, particularly in rodent species. We therefore aimed to determine if the Long Evans rat were a suitable model to investigate visual masking and perception.

In Chapter 2, we describe our investigation of the neuronal correlates of masking in anesthetised rats. Using a 32-electrode linear array we recorded neuronal responses to brief visual masking stimuli from all layers of the primary visual cortex (V1). Target stimuli were sine-wave gratings presented at stimulus onset asynchronies (SOA) of -333 to 333 ms relative to an uninformative mask. Firing rates and orientation selectivity were reduced by the presentation of a mask at short SOAs (>50 ms) for neurons found in all cortical layers.

To determine if these neuronal changes were associated with perceptual deficits we designed behavioural paradigms that enabled perceptual reports to be collected from rats performing complex visual tasks. Using similar target and mask stimuli to that of our neuronal investigation, we trained rats to perform an orientation discrimination task (Chapters 3 and 4). From the neuronal responses reported in chapter 2, we predicted that rodent perception would be impaired at short SOAs in this task. Although, we found this trend to be true of human perception, rodent perception did not systematically change across SOA as is typical of visual masking.

We further investigated the effects of visual masking on rodent and human perception by examining how target contrast affects detectability (Chapter 5). In both humans and rats, target detection performance increased as the contrast of the target was increased. The mask significantly impaired target detectability in humans. Similarly, the mask generally reduced rodent performance, however, the effects were inconsistent at the level of individual animals.

The neuronal data did not accurately predict the perceptual outcome, possibly because of differences in the stimuli and animal's state of consciousness. Our results also suggest that it may be difficult to consistently observe perceptual masking in rodents as the task parameters that permit perceptual deficits to occur, greatly reduce baseline performance in the absence of a mask. This reduces the sensitivity of the testing protocol to any perceptual deficits that may be occurring. In conclusion, if perceptual masking cannot be consistently observed, then the benefits of a rodent model for the study of visual masking is limited.

Student Declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes one original paper published in a peer-reviewed journal and one publication that is under review. The core theme of the thesis surrounds the neuronal and perceptual effects of visual masking. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the student, working within a doctorate of philosophy under the supervision of Dr Nicholas Price. The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

In the case of *Chapter 2 and 4* my contribution to the work involved the following:

Thesis Chapter	Publication Title	Status	Nature and % of student contribution	Co-author name(s) Nature and % of Co-author's contribution*	Co-author(s), Monash student
2	Masking reduces orientation selectivity in rat visual cortex	*Published*	Design: 50% Data Collection: 40% Analysis: 75% Writing: 50%	***1) Dasuni Alwis*** Design: 25% Data Collection: 50% Analysis 25% Writing: 40% ***2) Nicholas Price*** Design: 25% Data Collection: 10% Writing: 10%	*No* *No*
4	Perceptual masking is absent in rats performing an orientation discrimination task	*Submitted*	Design: 50% Data Collection: 100% Analysis: 100% Writing: 80%	***1) Nicholas Price*** Design: 40% Writing: 10% ***2) Ehsan Arabzadeh*** Design: 10% Writing: 10%	*No* *No*

I have renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Student name: Katrina Dell (Richards)

Student signature:  **Date:** 23/7/17

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work. In instances where I am not the responsible author I have consulted with the responsible author to agree on the respective contributions of the authors.

Main Supervisor name: Dr Nicholas Price

Main Supervisor signature:  **Date:** 23/7/17

List of Research Output During Candidature

Meetings and Workshops

Throughout my PhD candidature I have attended 11 meetings and workshops, and some of the works of my PhD have appeared in six abstracts:

- **Australian Neuroscience Society** (4-7/12/16)
  Hobart, Australia
  Poster title: The perceptual and neuronal effects of visual masking.
- **Painting the Big Picture** (3/12/16)
  The Centre of Excellence in Integrative Brain Function**,** Hobart, Australia
- **Society for Neuroscience** (12-16/11/16)
  San Diego, USA
  Poster title: The perceptual and neuronal effects of visual masking.
- **Australian Course in Advanced Neuroscience** (10-30/4/16)
  North Stradbroke Island, Australia
- **Systems Computational Neuroscience Down Under** (15-18/12/15)
  Brisbane, Australia
  Poster title: The perceptual and neuronal effects of visual masking.
- **Monash Brain Function Workshop** (14/12/15)
  The Centre of Excellence for Integrative Brain Function, Melbourne, Australia
  Poster title: The perceptual and neuronal effects of visual masking.
- **Students of Brain Research student symposium** (23/11/15)
  Latrobe University, Melbourne, Australia
  Poster title: The perceptual and neuronal effects of visual masking.
- **Neuroscience Showcase of Early Career Research** (5/12/14)-
  Monash University, Melbourne, Australia
  Poster title: The perceptual and neuronal effects of visual masking in a rodent model.
- **Students of Brain Research student symposium** (30/10/14)
  The Melbourne Brain Centre, Melbourne, Australia
- **Integrative Brain Function Workshop** (30/6/14)
  The Centre of Excellence for Integrative Brain Function, Melbourne Australia
- **Matlab for Neuroscience Workshop** (28-29/4/14)
  Monash Biomedical imaging, Melbourne, Australia

Publications

During my PhD candidature, I was first author on one publication (Chapter 4; under review) and joint first author on another (Chapter 2; published). I also contributed to five publications that do not involve the work reported in this thesis:

Publications of this thesis

- Alwis DS*, **Richards KL*** (* equal contribution) & Price NSC (2016) Masking reduces orientation selectivity in rat visual cortex. *Journal of Neurophysiology* **116:** 2331-2341
- **Richards KL**, Arabzadeh E, Price NSC (2017) Perceptual masking is absent in rodents performing an orientation discrimination task. (under review)

Publications unrelated to this thesis

- Allitt B, Johnstone V, **Richards K**, E Yan & R Rajan (2016) Progesterone sharpens temporal response profiles of sensory cortical neurons in animals exposed to traumatic brain injury. *Cell Transplantation* (in press)
- Allitt B, Johnstone V, **Richards K**, Yan E and Rajan R (2015) Progesterone exacerbates short-term effects of traumatic brain injury on supragranular responses in sensory cortex and over-excites infragranular responses in the long-term. *Journal of Neurotrauma* **32**:1-15
- Straznicky NE, Grima MT, Sari CI, Eikelis N, Lambert GW, Nestel PJ, **Richards K**, Dixon JB, Schlaich MP, Lambert EA (2015) Pioglitazone treatment enhances the sympathetic nervous system response to oral carbohydrate load in obese individuals with metabolic syndrome. *Metabolism* **64**(7): 797-803
- Straznicky NE, Grima M, Sari CI, Eikelis N, Lambert GW, Nestel PJ, Karapanagiotidis S, Wong C, **Richards KL**, Marusic P, Dixon JB, Schlaich MP and Lambert EA (2014) A Randomized Controlled Trial of the Effects of Pioglitazone Treatment on Sympathetic Nervous System Activity and Cardiovascular Function in Obese Subjects with Metabolic Syndrome. *Journal of Clinical Endocrinology and Metabolism* **99**(9): E1701–E1707.
- Straznicky NE, Lambert EA, Grima MT, Eikelis N, **Richards KL**, Nestel PJ, Dawood T, Masuo K, Sari CI, Dixon JB, Esler MD, Paul E, Schlaich MP and Lambert GW (2014) The effects of dietary weight loss on indices of Norepinephrine Turnover: Modulatory influence of hyperinsulinemia. *Obesity* **22**(3) 652- 662

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List of Abbreviations

2AFC- Two Alternative Forced Choice

CFF- Critical Fusion Frequency
CRF- Classical Receptor Field
CSD- Current Source Density

LGN- Lateral Geniculate Nucleus

OSI- Orientation Selectivity Index

PSTH- Peri-Stimulus Time Histogram

ISI- Inter Stimulus Interval
IT- Inferior Temporal Cortex

RFs- Receptor Fields

SOA- Stimulus Onset Asynchrony
STA- Stimulus Termination Asynchrony

t_{resp}-Latency to Response
t_{sel}- Latency to Selectivity

V1- Primary Visual Cortex

1 Introduction to Visual Masking

What is perceived of a visual scene is rarely a direct reflection of the pattern of light falling on the retina. Instead, perception of any stimulus is strongly influenced by other stimuli that occur nearby in space or time (Schwartz et al., 2007). The phenomenon known as visual masking is a prime example of this contextual modulation. In visual masking, the perception of an otherwise visible stimulus, referred to as the target, is impaired by another temporally and/or spatially adjacent visual stimulus, referred to as the mask. Intriguingly, the effect of the mask on target perception can still be observed when the mask is presented tens to hundreds of milliseconds after the target stimulus, which tells us something about the temporal limits of visual processing and perception (Lefton, 1973). Varying the temporal separation between the target and mask stimuli systematically alters the perception and neuronal representation of the target without changing the physical properties of the target stimulus itself. This reveals a disconnect between the actual physical stimulus and its perceptual interpretation. Presumably this disconnect develops throughout the visual processing hierarchy, beginning at the photoreceptors with a relatively faithful representation of the stimulus and eventually evolving into something that more closely represents the perceptual experience. The neuronal mechanisms that contribute to the perceptual effects of masking are likely to include a complex interaction of both peripheral and cortical processes, however the precise neuronal regions and mechanisms involved remain unclear (Breitmeyer et al., 2004). Uncovering the neuronal mechanisms involved in visual masking may offer important insights into how the electrical activity of neurons may lead to conscious visual perception.

In this chapter, we describe the well-accepted psychophysical observations associated with visual masking, which have mostly been acquired in human observers. This is followed by a description of the results from electrophysiological studies of the neuronal responses to standard masking stimuli. In section 3 and 4, we discuss the primary visual cortex as a possible locus for visual masking and the advantages of using a rodent model to investigate the neuronal mechanisms. Last, we describe the numerous mechanistic theories that have been proposed to account for masking and discuss the merits and limitations of the most prominent neuronal theories.

1.1 Perceptual effects of masking

Visual masking involves the presentation of two brief (~10-100 ms) stimuli: a target and a mask. The effects of a mask are typically quantified as the reduction it causes in target detection or discrimination performance across varying stimulus onset asynchronies (SOA; the temporal separation between target and mask stimuli). Since the visual masking illusion was first described by Exner (1868), the phenomenon has been explored using countless variations of the spatial and physical properties of target and mask stimuli. For example, studies have used target stimuli that are alphabetic letters, lines, gratings, coloured or luminance defined discs, textured shapes and faces (Eriksen and Lappin, 1964; Rolls and Tovee, 1994; Lamme, 1995; Macknik and Livingstone, 1998; Herzog et al., 2003). The type of psychophysical trend that is observed with visual masking depends strongly on the relationship between the target and mask stimuli, namely their 1) temporal and 2) spatial configuration (Turvey, 1973; Macknik and Livingstone, 1998; Sayim et al., 2014). For this reason, visual masking is often categorised according to these properties.

The temporal categories of visual masking include forward and backward masking, which describe when target perception is impaired by a mask that is presented before or after the target, respectively (Figure 1.1A). In general, forward masking is indicated by negative SOAs and backward masking, positive SOAs. In particular, backward masking is of interest as the effects of the mask on target perception occur at SOAs that cannot be explained by photochemical depletion in the retina or adaptation in the thalamus (Crawford, 1947). There are two psychophysical trends that may be observed across SOA, termed type A and type B functions (Figure 1.1B-left)(Breitmeyer and Ogmen, 2006). Type A masking describes the case when target perception is most impaired at an SOA of zero (common onset) and then gradually recovers as the SOA lengthens. On the other hand, type B masking describes the case when the greatest impairment in target perception occurs at an intermediate SOA, typically around 30-100 ms, thus creating a U-shaped function (Figure 1.1B-right) (Alpern, 1953; Weisstein, 1972). In most cases, forward masking will result in a type A psychophysical function, whereas the type of trend observed in backward masking depends on other properties of the target and mask stimuli including their spatial configuration (Bachmann, 1994; Breitmeyer and Ogmen, 2006).

The spatial categories of visual masking can be broadly divided according to whether the mask overlaps the target location, or is presented in a non-overlapping nearby location. For the purpose of this review, these categories will be referred to as spatially overlapping and spatially distinct. In backward masking, both spatial configurations of stimuli are capable of producing either a type A or type B psychophysical trend, however, spatially distinct stimuli tend to produce U-shaped functions (type B) more frequently than spatially overlapping stimuli (Breitmeyer, 1984). Below we describe, for both spatial configurations, how the contrast, size, duration, eccentricity, spatial separation between, and viewing conditions (monoptic/dichoptic) of target and mask stimuli, affect the type and the precise shape of the psychophysical trends.

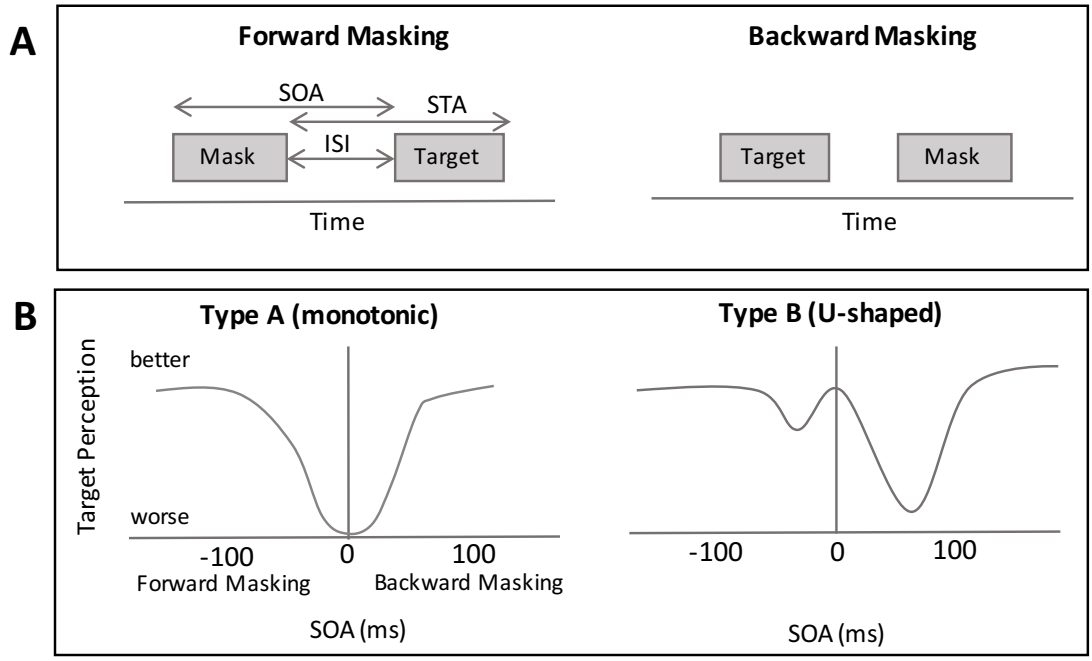

Figure 1.1. Visual masking. (A) temporal order and separation parameters of target and mask stimuli. The stimulus onset asynchrony (SOA) is the most frequently used parameter however in some instances interstimulus interval (ISI) and stimulus termination asynchrony (STA) have also been used. (B) type A and type B psychophysical functions obtained in visual masking. Only example values are provided for SOA, as precise shape and peak location is highly dependent on stimulus properties.

1.1.1 Spatially Overlapping Stimuli

The effects of spatially overlapping masking differ according to the properties of the mask. We therefore describe the psychophysical trends separately, depending on whether the mask is a uniform flash of light (masking by light), or comprises patterns and contours (masking by pattern).

1.1.1.1 Masking by light

Visual masking by light specifically describes the case when the perception of a brief target stimulus (~10-100 ms) is impaired by a flash of light. Regardless of the temporal order of stimuli, masking by light always produces a type A function, where the greatest impairment in perception occurs at an SOA of 0 (Figure 1.2) (Kolers, 1962). However, the function is asymmetrical, as the effects of forward masking persist to longer SOAs than backward masking (Sperling, 1965). The strength of this masking effect is dependent on stimulus energy, where the effect of the mask increases as a function of its total energy, i.e. its contrast, brightness, size and duration (Baxt, 1871; Boynton and Siegfried, 1962; Sperling, 1965; Turvey, 1973).

The effects of masking by light disappear when target and mask stimuli are presented dichoptically; that is when the target is presented to one eye and the mask to the other eye (Schiller, 1965). Given that the first site of binocular combination is the primary visual cortex, this suggests that the effects of masking by light occur through peripheral, most likely retinal, processes (Battersby et al., 1964).

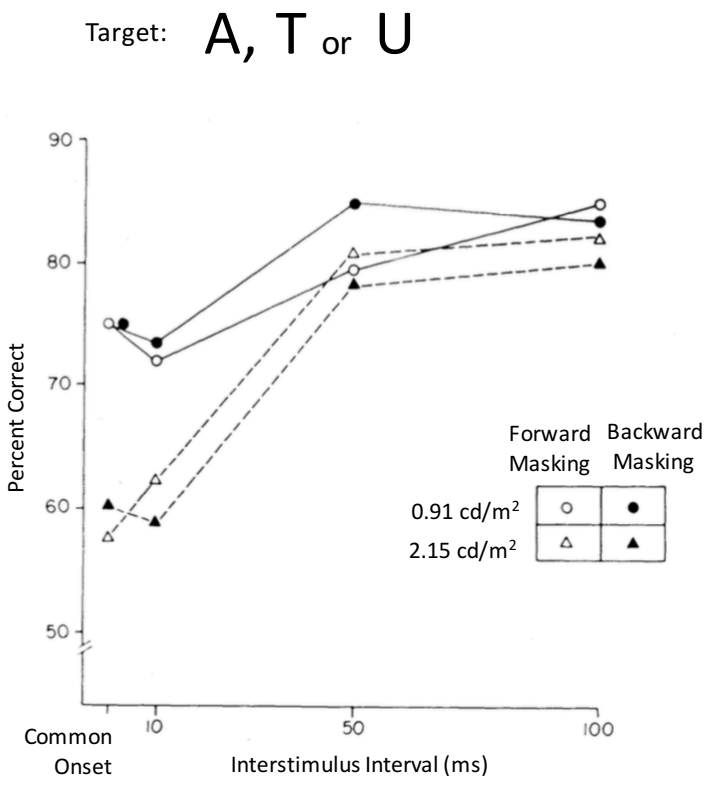

Figure 1.2. **Masking by light always produces a monotonic function.** Target perception was measured as the ability to discriminate the target letter. The duration of the target was adjusted for each subject so that performance was 80-85% correct in the absence of a mask. Under both forward and backward masking conditions, target perception was impaired at short interstimulus intervals (an alternative measure of the temporal separation of stimuli). The effect of the mask increased as a function of the luminance of the masking flash, which was either 0.91 or 2.15 cd/m2. The data presented here represents the psychophysical trends averaged across four human participants. Figure adapted from (Eriksen and Lappin, 1964).

1.1.1.2 Masking by pattern

Experimental paradigms implementing pattern masking use target and mask stimuli with spatially patterned forms and contours (Figure 1.3) (Breitmeyer, 1984). Although, forward pattern masking with spatially overlapping stimuli is always type A (monotonic), backward pattern masking is either type A or type B (U-shaped) depending on the relative energy (contrast, size & duration) of the target and mask stimuli (Bachmann, 1994). When the total energy of the mask exceeds that of the target, type A masking is observed (Schiller, 1966; Kovács et al., 1995). The type A trend is different from masking by light, however, as the effects of backward masking generally persist to longer SOAs than forward masking (Bachmann, 1994). When the energy of the mask is equal to or less than that of the target, then type B masking occurs (Purcell and Stewart, 1970; Spencer and Shuntich, 1970; Weisstein, 1971; Turvey, 1973). Regardless of the type of psychophysical trend, the effects of pattern masking are strongest when the stimuli share similar physical properties such as orientation, shape, and contour, and when the energy of the mask is greater than that of the target (Figure 1.3) (Levelt, 1965; Schiller, 1969; Turvey, 1973; Saarela and Herzog, 2008).

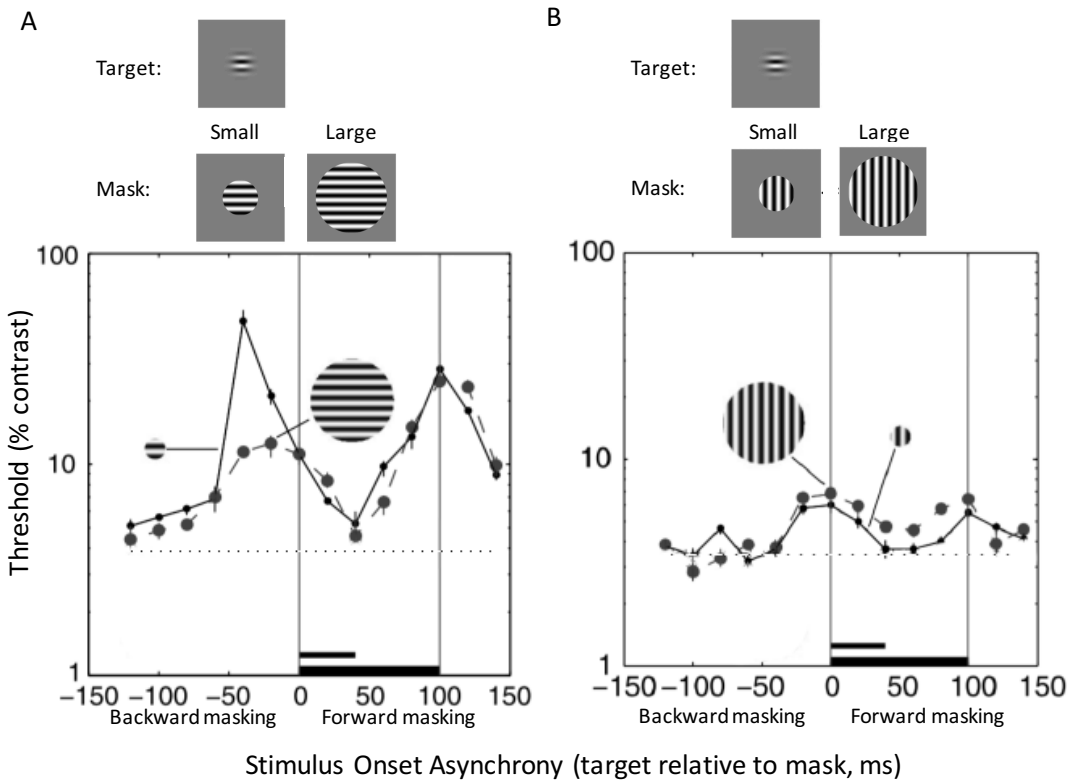

Figure 1.3. Pattern masking increases target contrast detection thresholds at short stimulus onset asynchronies. Target perception was measured as a contrast detection threshold, i.e. the contrast at which the participants' performance reached 75% correct in a 2-interval detection task. Therefore, impairment in target perception is indicated by an increase on the contrast detection threshold. Target stimuli were horizontally oriented Gabors and mask stimuli were either A) iso-oriented or B) cross-oriented circular gratings all with a spatial frequency of 4 cpd. A) The effects of the iso-oriented mask were greater when the size of the mask was similar to that of the target. B) The size of a cross-oriented mask did not impact its effects on target perception. In general, iso-oriented masks had a greater effect on target perception than cross-oriented masks. In this study, the stimulus onset asynchrony was negative when the target preceded the mask (backward masking); this is the reverse of the normal situation where negative values indicate forward masking. The dotted horizontal line indicates control threshold, measured with no mask. Mask presentation (100 ms) is indicated by the black bar on the abscissa. The short horizontal bar indicates target duration (40 ms), but the time of its presentation varied with SOA. Error bars indicate standard error. The data presented here represent the psychophysical trends collected in a single human participant. Figure adapted from (Saarela and Herzog, 2008).

Unlike masking by light, pattern masking may also be observed under dichoptic viewing conditions, which suggests that cortical mechanisms are involved (Kinsbourne and Warrington, 1962a). However, the effects of forward masking are significantly diminished when stimuli are presented dichoptically rather than monoptically, which suggests that forward masking arises predominantly through peripheral mechanisms (Smith and Schiller, 1966). Further to this, the influence of stimulus energy on both forward and backward pattern masking is removed under dichoptic viewing conditions, suggesting that the effects of stimulus energy also manifest in the periphery (Turvey, 1973). In contrast, the effects of backward masking remain similar regardless of the viewing condition (Smith and Schiller, 1966), indicating that backward masking involves predominantly cortical contributions. Altogether, these results indicate that pattern masking involves a complex interaction of peripheral and cortical mechanisms.

1.1.2 Spatially Distinct Stimuli

Forward masking using spatially distinct stimuli is unique compared to other types of forward masking as it can produce either a type A or type B function, depending on the experimental task (Alpern, 1953; Kolers and Rosner, 1960; Lefton and Newman, 1976; Growney et al., 1977; Pulos et al., 1980). This is different from backward masking where the shape of the trend depends on stimulus properties. However, the type B function obtained under forward masking conditions shows weaker masking spanning over shorter SOAs (typically 30-70 ms) than those observed under backward masking (Figure 1.1B) (Breitmeyer, 1984). For this reason, this type of masking has received significantly less attention than its backward masking counterpart (Breitmeyer, 1984).

Spatially distinct backward masking shares many of the same qualities as backward pattern masking; it can also yield either type A or type B functions depending on the energy ratio of the target and mask (Fehrer and Smith, 1962; Kolers, 1962; Spencer and Shuntich, 1970; Lefton, 1974; Breitmeyer, 1978). However spatially distinct stimuli result in non-monotonic (type B) functions more frequently than pattern masking stimuli (Breitmeyer, 1984). Type A masking will occur if the target energy is considerably lower than that of the mask (Schiller and Smith, 1966; Hernandez and Lefton, 1977). Otherwise, a U-shaped function is obtained where target perception decreases, reaching an asymptote at an SOA typically between 30 and 100 ms, before improving towards longer SOAs (Figure 1.4) (Lefton, 1973). The precise SOA for the peak masking effect varies according to factors such as the relative energy of, and spatial separation between, target and mask stimuli (Alpern, 1953; Growney et al., 1977; Macknik and Livingstone,

1998). The strength of the masking effect, like in pattern masking, depends on the energy of the mask and the similarity of stimuli (Figure 1.4) (Ishikawa et al., 2006; Sayim et al., 2014). However, the magnitude of the masking effect also depends on the spatial separation between stimuli and the eccentricity of their presentation. For example, the effect of masking is strongest when the target and mask have contiguous contours and decreases as the distance between stimuli is increased (Alpern, 1953; Kolers and Rosner, 1960; Kolers, 1962; Weisstein and Growney, 1969; Growney et al., 1977; Breitmeyer and Horman, 1981; Saarela and Herzog, 2009). Similarly, the likelihood and strength of masking is greater when stimuli are presented in the periphery of the visual field (Sturr et al., 1965).

Like pattern masking, spatially distinct backward masking may also be observed under dichoptic viewing conditions, indicating cortical mechanisms are involved (Kinsbourne and Warrington, 1962a). However, unlike binocular viewing conditions, the trends are always type B regardless of the target and mask energies (Schiller and Smith, 1968; Weisstein, 1971). This supports the notion that the effects of stimulus energy arise primarily through peripheral mechanisms. Altogether it is clear that both pattern and spatially distinct visual masking involve an amalgamation of peripheral and cortical mechanisms that are capable of interacting in complex ways.

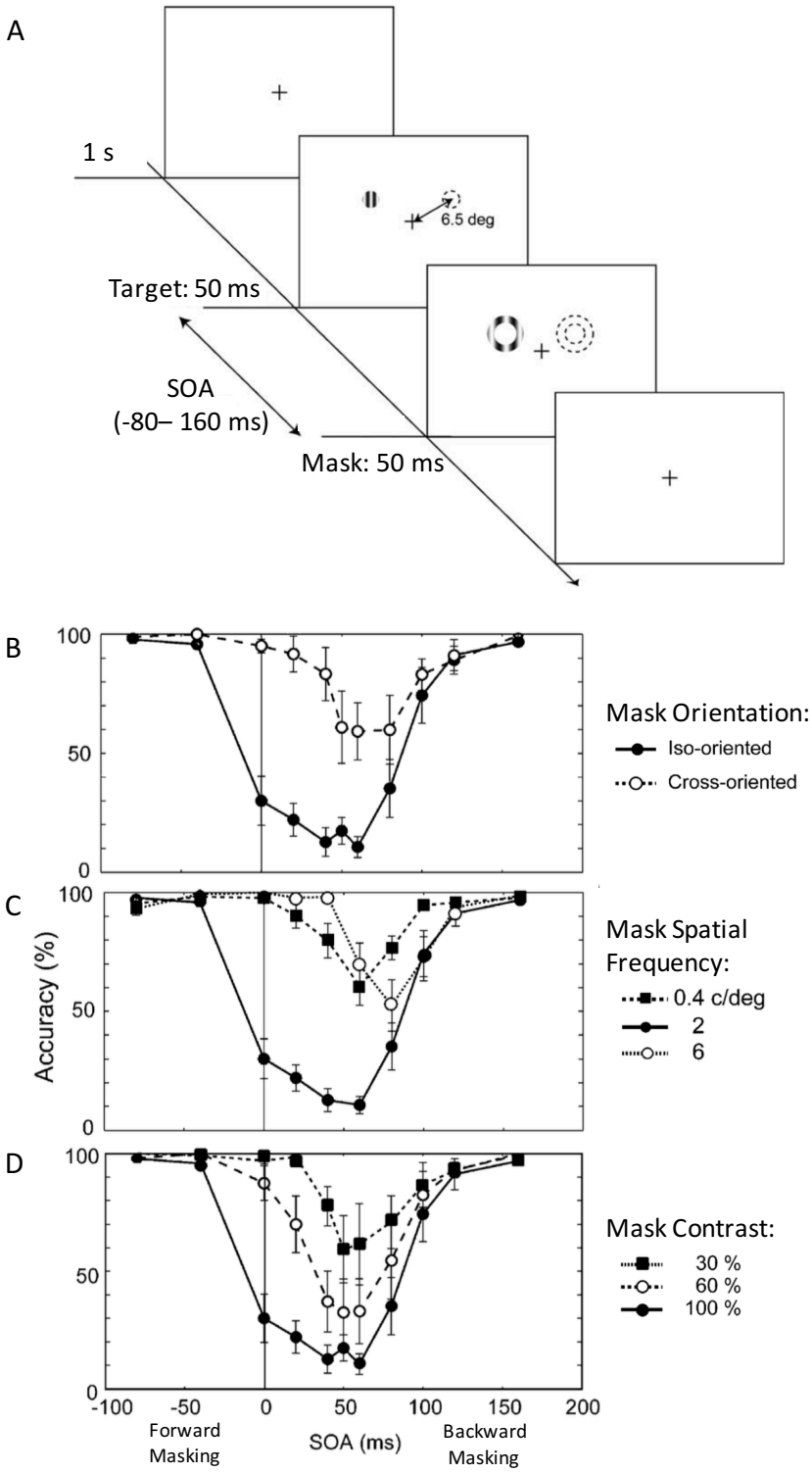

Figure 1.4. A spatially distinct mask reduces target perception across stimulus onset asynchrony (SOA) in a U-shaped function. A) Target perception was quantified as the accuracy of detection. The target was presented in 50% of trials and could appear at one of two locations. The target was a vertical circular grating 2.3° in diameter, and the mask was an annular grating with 3° and 4.7° inner and outer diameters, respectively. Both target and mask durations were 50 ms. The effect of the mask on target perception was greatest when the mask had the same A) orientation and B) spatial frequency as the target. C) The effect of the mask also increased with the contrast of the mask. The data presented represents the average perceptual trends across five human participants. Error bars represent standard error. Figure adapted from (Ishikawa et al., 2006).

1.2 Neuronal responses to masking stimuli

While visual masking has been fairly well described psychophysically, the neuronal mechanisms responsible for the multitude of perceptual effects remain controversial. Currently, only a small number of studies have recorded neuronal responses to common visual masking stimuli (Schiller and Chorover, 1966; Schiller, 1968; Vaughan and Silverstein, 1968; Levick and Zacks, 1970; Coenen and Eijkman, 1972; Bridgeman, 1975, 1980; Schwartz and Pritchard, 1981; Rolls and Tovee, 1994; Kovács et al., 1995; Macknik and Livingstone, 1998; Rolls et al., 1999; Macknik and Martinez-Conde, 2004), and only a handful of these have characterised visual masking both neuronally and psychophysically in the same species (Fehmi et al., 1969; Bridgeman, 1980; Macknik and Livingstone, 1998). This highlights a serious gap in the current literature that will need to be addressed if the neuronal mechanisms of visual masking are to be identified. Moreover, it highlights the speculative nature of the models that are currently used to explain perceptual masking phenomena (see section 1.5).

1.2.1 Spatially Overlapping Stimuli

1.2.1.1 Masking by light

The neuronal effects of visual masking by light have been recorded in single cells of anaesthetised (Schiller, 1968; Levick and Zacks, 1970) and awake (Coenen and Eijkman, 1972) cats and via electroencephalography in awake humans (Donchin and Lindsley, 1965) and monkeys (Fehmi et al., 1969). Collectively, neurons throughout the visual processing hierarchy, including the retina (Levick and Zacks, 1970), optic tract (Fehmi et al., 1969; Coenen and Eijkman, 1972), lateral geniculate nucleus (LGN) (Schiller, 1968; Fehmi et al., 1969; Coenen and Eijkman, 1972) and visual cortex (Donchin and Lindsley, 1965; Fehmi et al., 1969), have shown the effects of masking as a suppression of the target-evoked neuronal activity. At each processing stage, the magnitude of the masking effect remained consistent and closely resembled the psychophysical functions (Levick and Zacks, 1970; Coenen and Eijkman, 1972). This supports the notion that masking by light arises through peripheral mechanisms and that its effects are carried through to cortical regions.

1.2.1.2 Pattern Masking

In pattern masking, single cell recordings in the LGN of anaesthetised cats have shown that the neuronal response to the target decreases monotonically with shorter SOAs (Schiller, 1969). This is likely to be the result of the same peripheral mechanisms responsible for masking by light. However psychophysical results under dichoptic viewing conditions suggest additional cortical mechanisms are involved (Smith and Schiller, 1966). Single and multi-unit recordings in the primary visual cortex (V1) and inferior temporal (IT) cortex of alert monkeys have shown neuronal responses to target stimuli consist of two components, an early transient and a late sustained response (Figure 1.5A) (Rolls and Tovee, 1994; Lamme et al., 2002). Under stimulus conditions that produced perceptual backward masking, the sustained neuronal activity was significantly reduced (Figure 1.5B) (Rolls and Tovee, 1994; Kovács et al., 1995; Rolls et al., 1999; Lamme et al., 2002). This suggests that perception depends on the sustained neuronal activity, not just the transient response. Therefore, determining the origin of the sustained activity and the mechanisms through which it is suppressed is of particular importance to the study of perception.

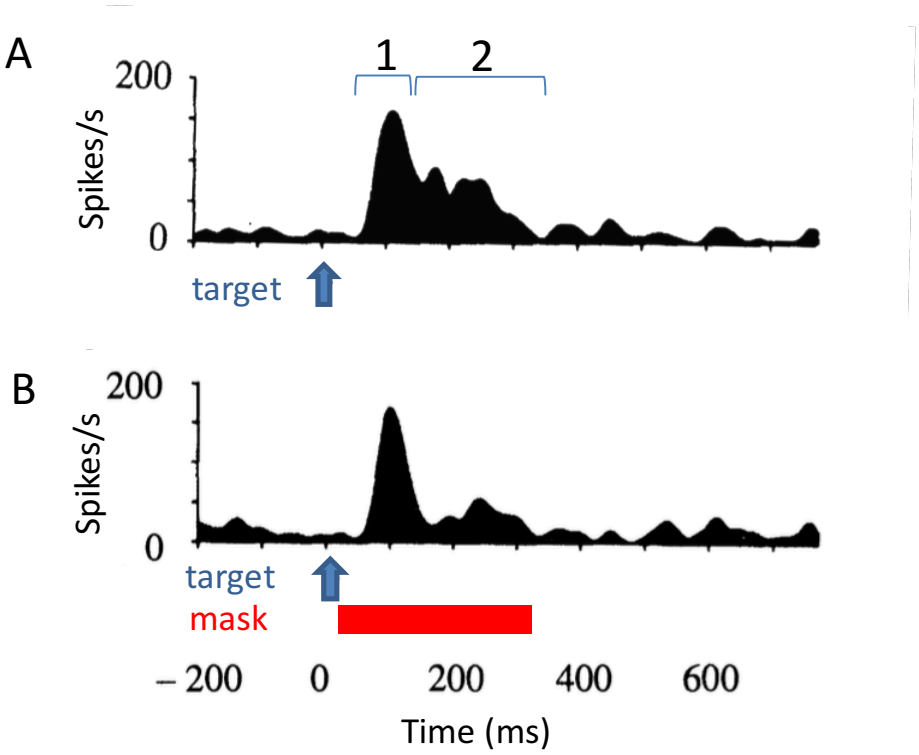

Figure 1.5. Backward pattern masking reduces the late sustained component of the response to the target in the inferior temporal cortex of awake monkeys. Peri-stimulus time histograms show the population firing rate in response to A) target only and B) masked trials. The response to the target stimulus consists of two components, an early transient (1) and a late sustained component (2). The response to the target occurs at roughly a 60 ms delay from the onset of the target. The late component was reduced by the presentation of the mask. Target stimuli were faces presented for 16 ms (blue arrow) and mask stimuli were either a N-O pattern or face presented for 300 ms (red bar). Figure adapted from (Rolls and Tovee, 1994).

1.2.2 Spatially Distinct Stimuli

The electrophysiological results of masking with spatially distinct stimuli have revealed similar findings to those of pattern masking. In the optic tract and LGN of awake, curare-paralysed cats, the neuronal response to the target decreased in a monotonic fashion as SOA decreased, directly reflecting a type A psychophysical function (Bridgeman, 1975). Under dichoptic viewing conditions, neuronal responses in the LGN of alert monkeys revealed an absence of masking at this level, whereas in V1, responses were still affected (Macknik and Martinez-Conde, 2004). However, the reduction in the target response in V1 was weak when compared to the effects of monoptic masking, despite perceptual masking remaining equally as strong (Macknik and Martinez-Conde, 2004). Collectively this suggests that the monotonic trends of visual masking arise predominantly through peripheral and monocular mechanisms, but that additional cortical mechanisms are involved. The weaker cortical effects under dichoptic viewing conditions may reflect a smaller contribution of peripheral masking mechanisms to cortical representations or alternatively that interocular and intracortical inhibitory inputs to binocular cells are relatively weak. It is also possible that cortical mechanisms of visual masking occur in higher order regions and that only feedback effects were observed in V1.

Target representations in V1 of awake and anaesthetised monkeys as well as awake, curare-paralysed cats showed similar two-component responses to that of pattern masking, however, in 25% of the cells sampled, the sustained component was partially separated from the transient response by a 40 ms period of inhibition, thus appearing as a secondary peak at roughly 200 ms post target onset (Figure 1.6A-left) (Bridgeman, 1975, 1980; Macknik and Livingstone, 1998). In this small subset of cells the late peak was suppressed under backward masking conditions in the same U-shaped function that has been observed psychophysically in monkeys and in humans (Figure 1.6A-right & B-left) (Schiller and Chorover, 1966; Bridgeman, 1975, 1980; Macknik and Livingstone, 1998). However, for the majority of cells, which lacked this late-peak of activity, the sustained component of the response was reduced in a monotonic manner, that did not agree with the perceptual trend (Bridgeman, 1975; Schwartz and Pritchard, 1981) (Figure 1.6B-right). The early transient component was rarely affected by the presentation of the mask, regardless of if the target response included a secondary peak. Collectively this suggests that the neuronal correlate of backward masking is a selective suppression of the late sustained component of the neuronal response to the target; but that there are two populations of cortical neurons that are affected at different timescales. Precisely what defines these sub-populations is yet to be determined.

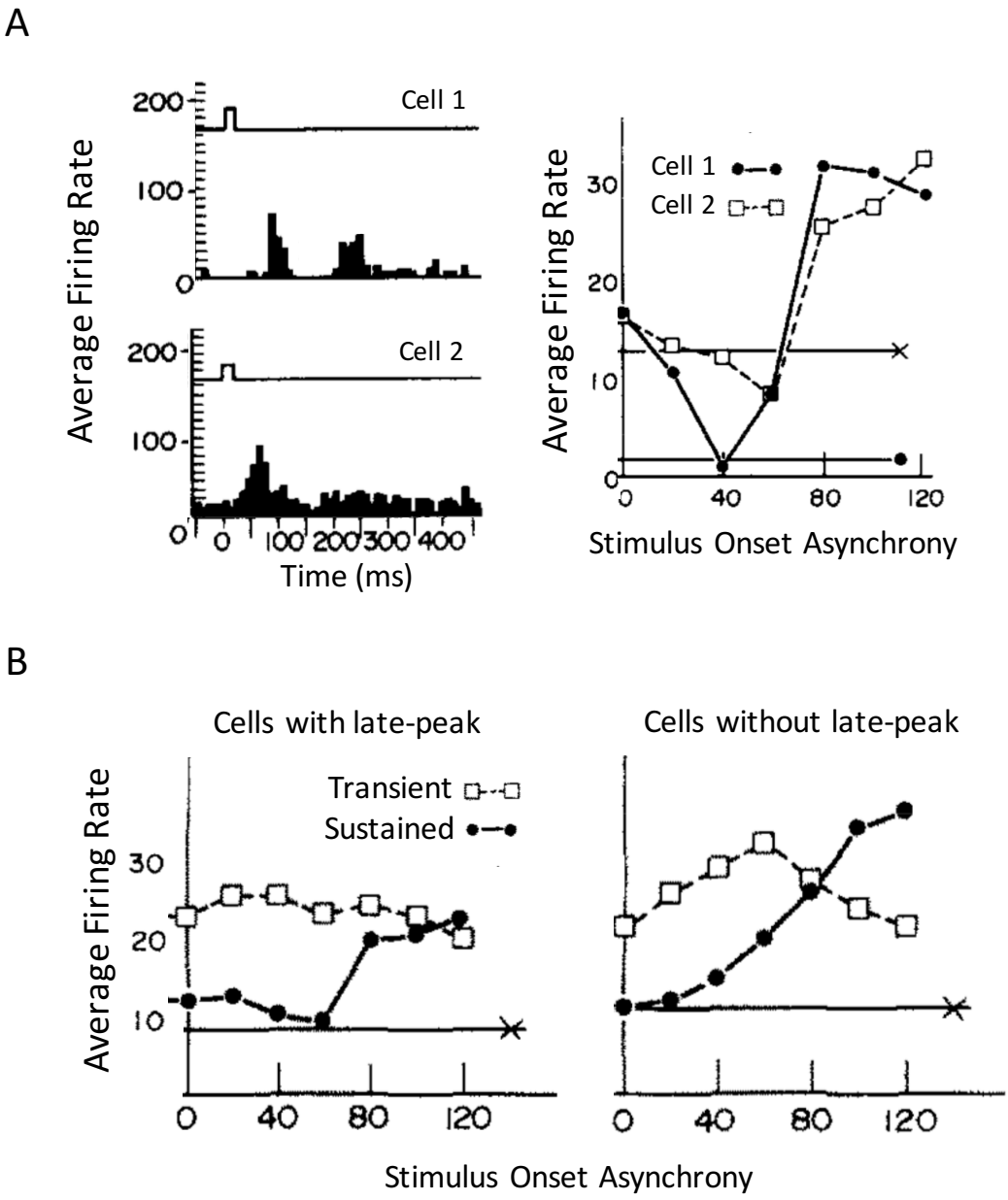

Figure 1.6. The effects of backward masking using spatially distinct stimuli on A) single cell and B) population responses. (A-left) Peri-stimulus time histograms are shown for two example cells in V1 of curarized cats. Cell 1 had the most pronounced late-peak and cell 2 had the least pronounced late-peak of the population that were classified to include a secondary late-peak. (A-right) The effects of the mask on this late-peak are shown across stimulus onset asynchrony for each cell. (B) The effects of the mask on both the transient (0-160 ms) and sustained (160-260 ms) components of the response to the target are shown across stimulus onset asynchrony for cells that included a secondary late-peak (left) and those that did not (right). Cells were classified as including this late-peak of activity if the sustained activation was significantly greater than that which occurred in the 40 ms (120-160 ms post target onset) immediately preceding the sustained window. Target and mask stimuli were vertical bars that were presented symmetrically around the centre of the receptor field. Figure adapted from (Bridgeman, 1975).

The neural correlate of forward masking is also a reduction in the neuronal response to the target; however, the specific response components that are affected have been inconsistent across studies. Using a single line as the target, target responses in area 17 of awake cats were affected by the presence of parallel, flanking lines, with forward masking only reducing the sustained component (Bridgeman, 1975). Despite using similar line stimuli, another investigation found that forward masking only affected the transient component in V1 of anesthetized monkeys but inhibited the entire target response in awake monkeys (Macknik and Livingstone, 1998).

Collectively the neuronal findings suggest that the effects of visual masking can be observed at numerous points throughout the visual processing hierarchy. Type A visual masking occurs predominantly through peripheral mechanisms and the effects are carried through to cortical regions, whereas Type B visual masking arises primarily in the cortex, however perhaps only in a small subset of cells. Thus, it is clear that the effects of visual masking involve a complex interaction of both peripheral and cortical mechanisms. However, the precise mechanisms and neuronal circuits that are involved are yet to be determined.

1.3 Contextual modulation phenomena similar to masking

There are a number of phenomena that are similar to visual masking, whereby the spatial or temporal context alters the neuronal representation and perception of a stimulus. Cross-orientation and surround suppression are examples of contextual modulation that may be particularly relevant to the study of visual masking as they may share some of the same mechanisms.

1.3.1 Cross-orientation suppression

Cross-orientation suppression describes the phenomenon in which the response of a V1 neuron to a target grating at its preferred orientation is suppressed by the superposition of an orthogonal mask (creating a plaid). The result is an impaired ability to detect the target grating. This effect can be observed when the target and mask gratings are drifting or stationary and its magnitude depends on the spatiotemporal properties of the mask; masks with low spatial frequencies and high temporal frequencies impair target perception the most (Meese and Holmes, 2007). The effects of cross orientation suppression were

originally explained through lateral inhibition between cells with different preferred orientations. However, intracellular recordings revealed that cross-oriented masks actually suppress lateral inhibition as well as lateral excitation in V1 (Priebe and Ferster, 2006). It is now thought that the effects arise primarily through a reduction in the feedforward excitatory signal from the LGN, possibly via synaptic depression or normalisation computations such as contrast saturation and rectification (Freeman et al., 2002; Priebe and Ferster, 2006).

These feedforward mechanisms of cross-orientation suppression could play a role in monotonic visual masking. For example, contrast saturation of LGN responses would predict that the magnitude of the masking effect would be strongly dependent on the contrast of the mask, which is certainly the case (Francis, 2003; Ishikawa et al., 2006) However, the spatial extent of the horizontal interactions in the LGN would not be capable of explaining all of the effects observed in visual masking with spatially distinct stimuli (Tapia and Beck, 2014). Furthermore we know that U-shaped masking involves at least some cortical mechanisms (Kinsbourne and Warrington, 1962a). Thus, it is clear that the feedforward mechanisms of cross-orientation suppression are incapable of explaining all of the idiosyncrasies of visual masking.

1.3.2 Surround suppression

Surround suppression describes when a neuron's response to a target stimulus presented within its classical receptor field (CRF), is reduced by another stimulus presented outside of the CRF, thus impairing target perception. This suppression is greatest when the stimuli are iso-oriented. Similar to cross-orientation suppression, surround suppression has often been explained entirely through lateral inhibition (Nelson and Frost, 1978; Knierim and Van Essen, 1992; DeAngelis et al., 1994), however, surround suppression can propagate across 6-8 mm of cortex as fast as ~1m/sec (Bair et al., 2003), which is considerably faster than the expected propagation speed of lateral inhibition (Girard et al., 2001). Furthermore, centre-surround interactions occur over a larger spatial area than can be explained solely by horizontal connections in V1 (Angelucci et al., 2002). It is unlikely that surround suppression occurs through the same feedforward mechanisms as cross-orientation suppression, as they could not account for the spatial scale of surround interactions (Tapia and Beck, 2014). Furthermore the two phenomena possess different relationships between the strength and latency of suppression (Smith et al., 2006). Instead, the short latencies of suppression and the large spatial scale of centre-surround interactions are proposed to

occur primarily through feedback connections from cells with larger CRF sizes (Angelucci and Bullier, 2003; Bair et al., 2003). However, that is not to say that feedforward and horizontal connections are not involved. In fact, the latest view is that the CRF surround consists of two mechanistically distinct regions; a near-surround that mediates response suppression through feedforward and horizontal connections, and a far-surround that exerts suppression exclusively through feedback mechanisms (Angelucci and Bullier, 2003).

It is reasonable to think that cross-orientation suppression and surround-suppression could contribute to some of the trends observed in visual masking with spatially distinct stimuli. In both phenomena the magnitude of response suppression increases as a function of the contrast of the mask, and is greatest when stimuli are presented in the periphery of the retina and when the mask shares the same orientation as the target (Xing and Heeger, 2000; Webb et al., 2005; Ishikawa et al., 2006). However, spatially distinct visual masking cannot be fully explained by the mechanisms involved in surround suppression, as there are some important differences between the phenomena. In particular, the magnitude of surround suppression does not depend on the similarity between the target and mask when it comes to spatial frequency; surround suppression is greatest when the spatial frequency of the mask is low or high (Webb et al., 2005).

1.4 Visual Masking in the Primary Visual Cortex

It is currently unclear what aspects of neuronal activity are necessary for conscious visual perception to arise. Perception may occur through the activation of a specific network of neurons or rather a specific type of neuronal activity. The hallmark of the latter perspective, that visual perception arises through a specific type of neuronal activity, is that perceptual experience is not linked to a particular brain structure but is instead the result of an intrinsic property of neuronal activity that occurs throughout a number of cortical regions (Lamme et al., 2000). For example, coherent gamma oscillations or specific modulations in firing rates have been proposed as neuronal correlates of conscious visual perception (Singer and Gray, 1995). This perspective also implies that the neuronal correlates of visual perception can be observed and studied in multiple regions of the brain. In visual masking, there is evidence to suggest that perception correlates with a reduction in the response to the target, and in backward masking, specifically the late sustained component of the response is important (Macknik and Livingstone, 1998). This response suppression has been observed in areas as early as the primary visual cortex, as well as higher order regions such as IT (Rolls and Tovee, 1994; Kovács et al., 1995; Macknik and Livingstone, 1998; Rolls et al., 1999).

If, instead, we take the viewpoint that a specific network of neurons is responsible for perception, then we are implicitly inferring that there are neurons dedicated to generating conscious visual perception. These neurons could be located within a specific area of the brain or could be limited to a specific type of neuron that is scattered throughout the visual cortex (Lamme et al., 2000). In visual masking, there is evidence to suggest that a specialised subset of neurons exist in the primary visual cortex. Under conditions that result in U-shaped perceptual masking, only 25% of the cells recorded in the primary visual cortex show neuronal response suppression in the same U-shaped trend (Bridgeman, 1975; Macknik and Livingstone, 1998); in the majority of V1 cells the neuronal response was suppressed in a monotonic trend that did not match the psychophysical results (Bridgeman, 1975; Schwartz and Pritchard, 1981). Precisely what defines this sub-population of neurons is unclear. However, given that the neuronal representation of a stimulus is expected to develop throughout visual processing from an accurate reflection of the physical properties of the stimulus to something that more closely reflects perceptual experience, it is possible that the subset of V1 cells, represent neurons that are located in the output layers, at later stages of visual processing.

To address whether specific sub-populations of neurons in V1 are affected by masking stimuli, or show different transient and sustained responses, we will employ recent advances in electrophysiological technology that provide the opportunity to simultaneously record from multiple layers of V1 and assign neurons to input or output layers.

1.5 Theories of Visual Masking

Inspired by psychophysiological findings, three major theories have emerged to explain visual masking; integration, interruption and competition (Figure 1.7). At the foundation of these and other visual masking theories, is the understanding that a neuronal response to a brief visual stimulus is delayed in onset and persists beyond the offset of the stimulus (Figure 1.7A) (Levick and Zacks, 1970). This persistence allows neuronal representations of the target and mask stimuli to interact at some level of visual processing. Below we explore the simple proposal underlying the integration theory, a number of competing theories that employ interruption and, lastly, the concept of competition, which has been incorporated into one of the interruption theories.

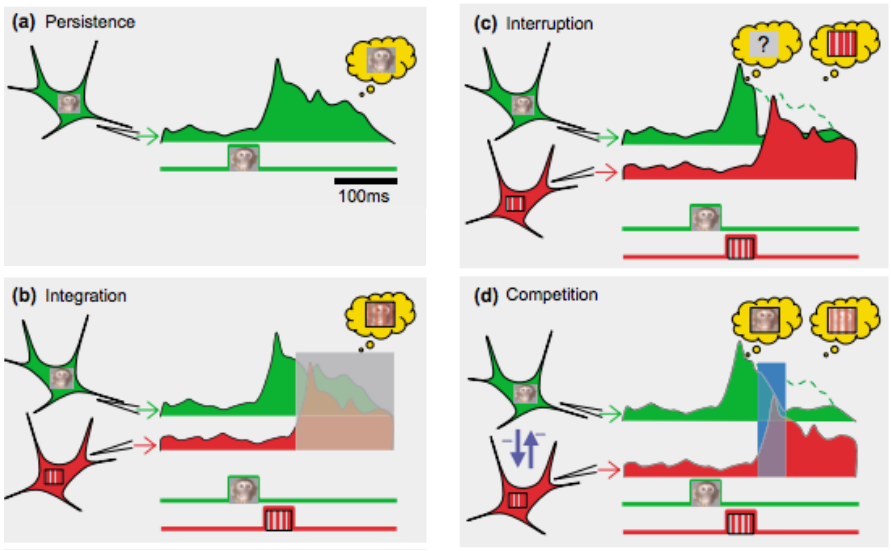

Figure 1.7. Visual persistence and the most prominent theories of visual masking. The activity of two hypothetical neurons, one of which is processing an image of a face (green) and the other a grating (red). The resulting perception at different time-points is indicated in the yellow bubbles. A) Visual persistence describes the case when the neuronal response to a brief stimulus, in this case a face, persists for longer than than the duration of the stimulus itself, allowing continued perception of the stimulus. B) Integration theory suggests that the effects of visual masking arise due to temporal limits of visual processing, resulting in an averaging of information across a particular perceptual window (indicated here by a gray window). C) Interruption predicts that neuronal processing of the target (face) is abandoned when the red neuron is activated by the presentation of the mask (grating). D) Neuronal competition predicts that overlapping periods of activation in the two cells compete and that the neuron with the strongest activation wins the competition. Figure adapted from (Keysers and Perrett, 2002).

1.5.1 Integration

Integration has been a dominant and widely-accepted theory in the history of visual masking (Crawford, 1947; Boynton, 1961; Kinsbourne and Warrington, 1962b; Eriksen and Hoffman, 1963; Eriksen and Lappin, 1964; Thompson, 1966; Stoper and Banffy, 1977). The theory proposes that limits in the temporal resolution of the visual system can cause the neuronal representation of the target stimulus to fuse with that of the mask, resulting in the perception of a singular and combined image (Figure 1.7B) (Eriksen and Collins, 1967, 1968). More specifically, the close temporal presentation of stimuli is suggested to reduce the perceived stimulus contrast obscuring the target and its features within a montage (Kinsbourne and Warrington, 1962b; Eriksen and Hoffman, 1963; Eriksen and Lappin, 1964; Eriksen, 1966; Thompson, 1966; Coltheart and Arthur, 1972; Schultz and Eriksen, 1977). The simplest model of integration proposes linear summation of the neuronal responses to target and mask stimuli, where the response to the stimuli in sequence is the same as that which would occur when stimuli are presented simultaneously. In this way,

the temporal range of masking would correspond to the range of temporal summation. Due to the persistence of the neuronal responses to both target and mask, this is capable of explaining aspects of both forward and backward masking and also predicts that the relative stimulus energy will dictate the degree of masking (Baker, 1953, 1955, 1963). However, it also predicts a relatively symmetrical and monotonic function (Schultz and Eriksen, 1977), and thus cannot explain the occurrence of type B psychophysical functions. This is not necessarily problematic given that dichoptic results indicate the involvement of both peripheral and cortical mechanisms (Macknik and Martinez-Conde, 2004). As it stands, the integration theory offers a robust explanation for type A visual masking and has become widely accepted as a likely peripheral mechanism (Scheerer, 1973; Turvey, 1973; Breitmeyer and Ganz, 1976; Breitmeyer, 1980; Keysers and Perrett, 2002). The occurrence of type B visual masking may also be explained by integration when nonlinear summation is assumed (Stewart and Purcell, 1974). In this model, the system is presumed to be overloaded by a massive response to the mask, thus reducing its capacity to represent the target stimulus and requiring a higher target energy to break through (Kahneman, 1968). Critically, strong evidence against integration as a complete mechanism for explaining perceptual masking came from studies of target recovery where a secondary mask presented after the primary mask resulted in an increase in target perception (Robinson, 1966, 1968; Turvey, 1973; Breitmeyer et al., 1981; Oğmen et al., 2006). This complicated stimulus setup required that the second mask masked the effect of the earlier mask! The argument was that such an effect could not arise if the target and mask stimuli had been integrated into a single response, because the integration of the responses to the target and two masks should even more effectively suppress detection of the target. The interruption theory was put forward to accommodate these surprising results.

1.5.2 Interruption

The interruption theory suggests that neuronal processing of target information is “abandoned” at the arrival of the response to the mask (Figure 1.7C). In this way, target information processing is left unfinished leading to impairments in target perception. It has been particularly important for explaining type B, backward masking results. By itself, the theory would be incapable of explaining forward masking, however for those that maintain peripheral integration mechanisms, this is of little consequence (Breitmeyer and Ganz, 1976). Some versions of the theory incorporate the degradation or erasure of target information, however, the ability to recover target perception via a secondary mask discredits any theory involving the permanent loss of target information (Turvey, 1973). The widespread use of visual masking as a tool to limit neuronal processing of a stimulus suggests the implicit adoption of an interruption theory

(Scheerer, 1973). This seems precarious given the level of debate surrounding the mechanisms of cortical visual masking and only emphasizes the need for additional study in this area. Below we discuss the most prominent variants of the interruption theory.

1.5.2.1 Dual-Channel Theory (Breitmeyer & Ganz)

The psychophysical evidence that sinusoidal gratings with low spatial frequencies (<7.6 cpd) elicit faster responses with less visual persistence than higher spatial frequencies led to the introduction of a sustained-transient model of visual processing (Kulikowski and Tolhurst, 1973; Tolhurst, 1973). The model suggests that there are two parallel channels through which neuronal activity travels from the retina to the visual cortex; one which is sensitive to rapid motion and flicker, contributing to a transient component of the neuronal response and the other that prefers slow moving or stationary stimuli, resulting in a late sustained response component (Breitmeyer and Ganz, 1976). As a result, a number of dual-channel theories using this concept were developed to explain type B visual masking (Matin, 1975; Weisstein et al., 1975b; Breitmeyer and Ganz, 1976; Breitmeyer, 1980; Breitmeyer, 1984). The primary mechanism of visual masking was proposed to be inhibition between these channels. In particular the theory put forward by Breitmeyer and Ganz (1976) predicted inter-channel inhibition would be most effective when the sustained component of the target stimulus coincided with the transient component of the mask stimulus, thus in line with a U-shaped function (Figure 1.8A). They also suggested a plausible mechanism for U-shaped forward masking may be intra-channel inhibition via centre-surround antagonism within the sustained pathway. Breitmeyer (1992) later took the sustained-transient channels to be analogous with magnocellular and parvocellular retino-geniculate pathways because of the differences in their temporal sensitivities (Figure 1.8B), although, it should be noted that there is now evidence to suggest this cannot be the case (see discussion below). However, the neurophysiological and neuroanatomical developments that ensued in the following years highlighted issues with this version of the theory, particularly in the involvement of discrete detectors as functional units, as this meant that the model was incapable of incorporating properties of the nervous system that could not be attributed to single cells. This provided motivation for a shift in theoretical perspective.

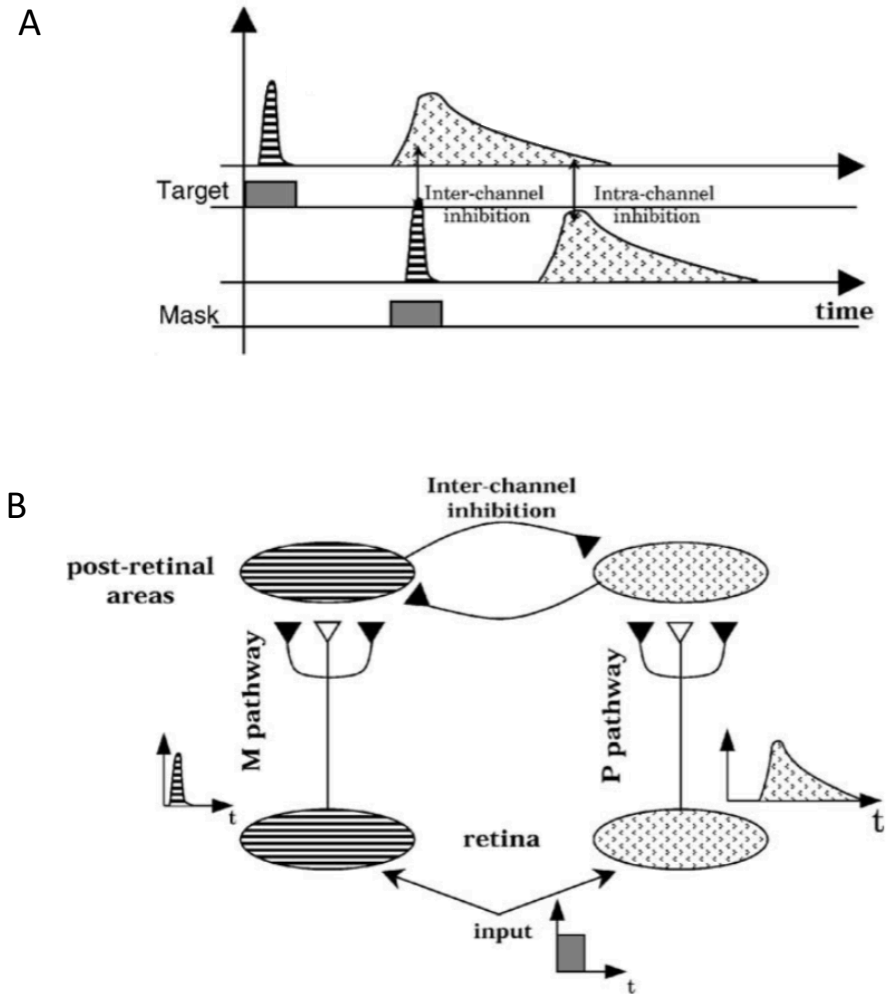

Figure 1.8. Neuronal interactions as proposed by the sustained-transient interruption theory. A) The sustained component of the target is reduced by the transient response (via inter-channel inhibition) and by the sustained response (intra-channel inhibition) to the mask. **B)** The transient and sustained channels were proposed to be synonymous with the magnocellular (M) and parvocellular (P) pathways. The cortical areas receiving inputs from these pathways are represented as lumped networks. The filled and open triangle symbols indicate inhibitory and excitatory synapses, respectively. Figure adapted from (Oğmen et al., 2003).

Inspiration was found in the progression of research highlighting the role of cortico-cortical feedback connections in visual awareness (Lamme, 1995; Lamme and Spekreijse, 2000; Lamme et al., 2000; Pascual-Leone and Walsh, 2001; Supèr et al., 2001; Silvanto et al., 2005; Laycock et al., 2007). It was proposed by some that the late sustained component of neurons in the early visual cortex (i.e. V1) was the result of re-entrant activation from higher cortical areas with larger receptive fields (Lamme, 1995; Lamme and Spekreijse, 2000; Lamme et al., 2000; Supèr et al., 2001; Lamme et al., 2002); therefore it was the suppression of sustained feedback (not feedforward) activity that correlated with target visibility (Lamme et al., 2002). A role for feedback was incorporated into Breitmeyer and Ogmen's (Breitmeyer and Ogmen, 2000) modified dual channel theory featuring the retino-cortical dynamics (RECOD) model originally proposed by Ogmen in 1993 (Ogmen, 1993).

1.5.2.2 Retino-cortical dynamics (RECOD) model (Breitmeyer & Ogmen)

The RECOD theory suggests that short (magnocellular) and long-delay (parvocellular) retinal ganglion cells in two parallel processing channels fire to create a dual-component response. Circuits originating in the long-delay retinal ganglion cells convey information to higher order cortical regions, and as their signal decays into a plateau, the system enters a feedback dominant phase (Breitmeyer and Ogmen, 2006). Unlike the previous model, the role of inter-channel inhibition is instead to curb feedback contributions when the input changes at a given retinotopic location (Breitmeyer and Ogmen, 2006). In this way, the feedforward response to the mask would cause a suppression of the sustained feedback from the target, producing U-shaped visual masking. Evidence against this theory, and any relying on the role of feedback activity, came from Macknik & Livingstone's (1998) study finding that the peak masking magnitude varies as a function of stimulus termination asynchrony and therefore the sustained activity is more likely to represent the offset of the target stimulus (Macknik and Livingstone, 1998; Macknik and Martinez-Conde, 2007). Macknik argued that if the sustained activity were to arise from feedback caused by the target onset, then the regions providing feedback would need to be able to predict the moment of stimulus offset (Macknik and Martinez-Conde, 2007). However, Breitmeyer pointed out that, although their results are theoretically interesting, attempts to predict peak masking magnitude purely through temporal parameters would be disregarding a wealth of evidence (Breitmeyer and Ogmen, 2000). It has already been shown that peak masking magnitude varies as a function of several variables including but not limited to, the ratio of target and mask energies (Fehrer and Smith, 1962; Kolers, 1962; Weisstein, 1972; Stewart and Purcell, 1974; Hellige et al., 1979) and the degree of target and mask feature similarity (Hellige et al., 1979; Michaels and Turvey, 1979). Based on these and other known regularities, Breitmeyer provided a convincing argument that Macknik & Livingstone's (1998) findings were not incompatible with the RECOD model. Regardless of

this, the model still has its problems; it is difficult to obtain empirical support for the model, as it lumps the effects of all post-retinal areas together, and therefore predicts no specific physiological locus for the effects to take place. The relative latency of magnocellular and parvocellular pathways is just a few milliseconds, meaning that it cannot account for the larger delays (10-100 ms) that would be necessary to explain when type B masking is most effective (Lefton, 1973; Schmolesky et al., 1998). Furthermore there is very little physiological evidence to suggest inter-channel inhibition actually exists (Enns and Di Lollo, 2000).

1.5.2.3 Feed-forward lateral inhibition (Macknik)

The models outlined above depend on complex interactions between multiple anatomical pathways with specialised cell types. At the other end of the complexity spectrum, Macknik proposes a simple, feed-forward model of lateral inhibition to explain visual masking (Macknik and Livingstone, 1998; Macknik and Haglund, 1999; Macknik et al., 2000; Macknik and Martinez-Conde, 2004; Macknik, 2006; Macknik and Martinez-Conde, 2007). Whereas the RECOD model achieves latency differences for the transient and sustained components of the neuronal response to the target through different retinal cell types, Macknik's theory simply proposes that the appearance and disappearance of the target drive the two response components. Therefore, his theory works on the assumption that the late sustained component of the response represents the termination of the target stimulus rather than re-entrant activation. He draws this conclusion from evidence that the timing of sustained neuronal activity varies with target duration (Macknik and Livingstone, 1998; Macknik and Martinez-Conde, 2007). Although valid, this finding would be more convincing had they maintained constant stimulus energy by modulating contrast as they varied target duration. Furthermore, the presence of a sustained neuronal component for a stimulus as short as 1 ms in duration suggests that the activity does not simply represent stimulus offset (Bischof and Di Lollo, 1995). In Macknik's model, visual masking occurs through a suppression of the sustained component of target elicited activity by feed-forward lateral inhibition caused by the mask, thus predicting a U-shaped backward masking function with a dependence on the spatial separation of stimuli. The theory aligns well with findings of visual masking in early processing regions such as V1 as well as higher order regions such as IT since the primary mechanism, lateral inhibition, is ubiquitous in neuronal circuitry. While some have argued that feed-forward lateral inhibition is too basic to cause some of the high-level effects such as feature integration and four-dot masking (see below) (Enns, 2002), Macknik and colleagues have defended their model suggesting that lateral inhibition in high-level areas would be likely to have high-level effects (Macknik and Martinez-Conde, 2007). Altogether, the model is a plausible mechanism for explaining U-shaped masking, but cannot account for monotonic or forward masking effects. This need not be a problem, since, as noted earlier, there is little argument against the Integration theory explaining forward

and type-A masking. Thus, neuronal integration and lateral inhibition likely work in parallel to achieve the various different flavours of masking. While it remains controversial whether sustained neuronal activity arises through feedback or feed-forward connections, there is little doubt that lateral inhibition is a possible mechanism of cortical visual masking (Bridgeman, 1971; Weisstein et al., 1975b; Francis, 1997; Herzog et al., 2003).

1.5.2.4 Boundary Contour System (Francis)

The boundary contour system (BCS) was originally developed to explain boundary detection and the segmentation of visual features into coherent visual forms (Grossberg and Mingolla, 1985b, a), however exploration of the model's dynamic properties led Francis to believe it was capable of explaining visual masking (Francis et al., 1994; Francis, 1996b, a; Francis and Grossberg, 1996b, a; Francis, 1997). The boundary contour system (BCS), like the RECOD model, assumes that sustained visually-evoked activity arises from excitatory feedback. However, the suppression of the target response is suggested to result from lateral inhibition within a cortical area rather than inhibition between short- and long-delay retino-geniculate pathways. The neural network model begins with on-centre/off-surround lateral geniculate neurons that innervate pairs of like-oriented simple cells in V1 with opposing contrast polarities. Each pair of simple cells feed their rectified output to a single complex cell that is selective to the same orientation but is insensitive to contrast. The complex cells activate multiple hypercomplex cells with competing on-centre/off-surround connections, where the off-surround connection adds selectivity for end-stopped stimuli. The second stage of competition involves lateral inhibition as multiple end-stopped cells selective for different orientations feed antagonistic input into higher order hypercomplex cells. The resulting output informs cooperative bipolar cells about stimulus edge location and orientation, which is fed back to earlier levels of processing for spatial sharpening. Francis argued that the interaction between excitatory feedback and lateral inhibition is capable of explaining many of the regularities found in type B masking (Francis, 1997). At short SOA's the lateral inhibition generated by the mask is relatively weak compared with the target elicited feedback drive, therefore, there is little effect of the mask on target perception (Figure 1.9A). As the SOA increases the target response decays and lateral inhibition has a proportionally larger effect leading to impaired target perception (Figure 1.9B). However, as the SOA continues to increase the effect of the mask reaches an asymptote and target perception begins to improve. This is because target processing is near completion before mask driven inhibition arrives (Figure 1.9C). The model explains U-shaped masking and predicts a large number of empirical findings including, weaker forward masking, changes in the shape of the psychophysical function with relative stimulus energies, target recovery with a secondary mask and a decrease in masking magnitude with increasing spatial separation (Francis et al.,

1994; Francis, 1996b, a; Francis and Grossberg, 1996b, a; Francis, 1997). However, some have claimed that models based on contour-sensitive mechanisms (interactions between adjacent boundaries of target and mask stimuli) are limited as they do not predict that masking will change as a function of attention (Enns and Di Lollo, 1997). This criticism seems unwarranted, given that the major mechanism of response suppression in the BCS theory is lateral inhibition and that attention is known to modulate lateral inhibition (Kastner et al., 1998; Reynolds et al., 1999; Zenger et al., 2000). The neural network model may also be limited as the mechanism is based in early stages of visual processing which may not be sufficient to explain electrophysiological findings of visual masking in higher order regions such as IT (Rolls and Tovee, 1994). While the BCS has great explanatory power, there are a large number of free parameters, and while it is based on known neuronal properties, it incorporates very specific assumptions about the functional role of many different cell types in the absence of strong evidence to confirm that each cell type (simple, complex, hypercomplex etc.) actually has a distinct function and the required sequential connectivity in the hierarchical processing of information.

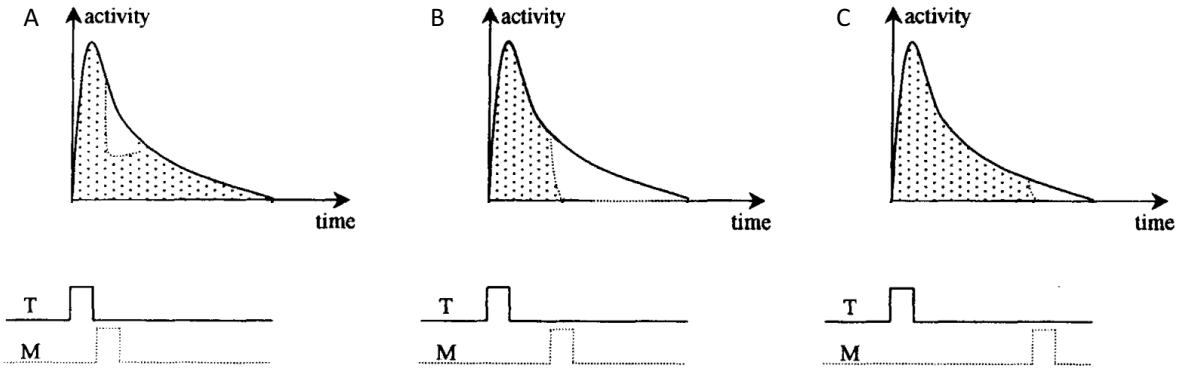

Figure 1.9. The effects of mask inhibition on the target response at A) short, B) intermediate, and C) long stimulus onset asynchronies (SOAs) as predicted by the boundary contour system. The solid line illustrates the target response in the absence of a mask and the dotted line demonstrates the inhibitory influence of the mask. The dotted area represents the net response to the target, taking into account the inhibitory influence of the mask. At short SOAs the mask-elicited inhibition arrives when target driven feedback is strong, leading to little to no effect on target perception. At intermediate SOAs, mask inhibition is proportionally larger as target driven feedback has begun to decay when mask inhibition arrives therefore causing a greater reduction in target visibility. At long SOAs mask inhibition arrives after when target processing is near completion and does not have much of an effect on target visibility. Figure taken from (Breitmeyer and Ogmen, 2000).

1.5.2.5 Higher-level Mechanisms

While many lower level contour-sensitive mechanisms explain a number of the perceptual phenomena found in visual masking, the discovery that a non-spatially overlapping four-dot stimulus (Figure 1.10) is capable of producing type-B visual masking has provided cause to consider the involvement of higher-level mechanisms such as attention (Enns and Di Lollo, 1997). They showed that four surrounding dots with no contiguous contours are capable of reducing target visibility if two conditions are met: 1) the target and mask are presented in the periphery and 2) the stimulus location is unpredictable. Although Enns & Di Lollo have not proposed any specific neuronal mechanisms, they support theories such as Bachmann's perceptual retouch model that requires interactions with higher-level processing (see discussion below).

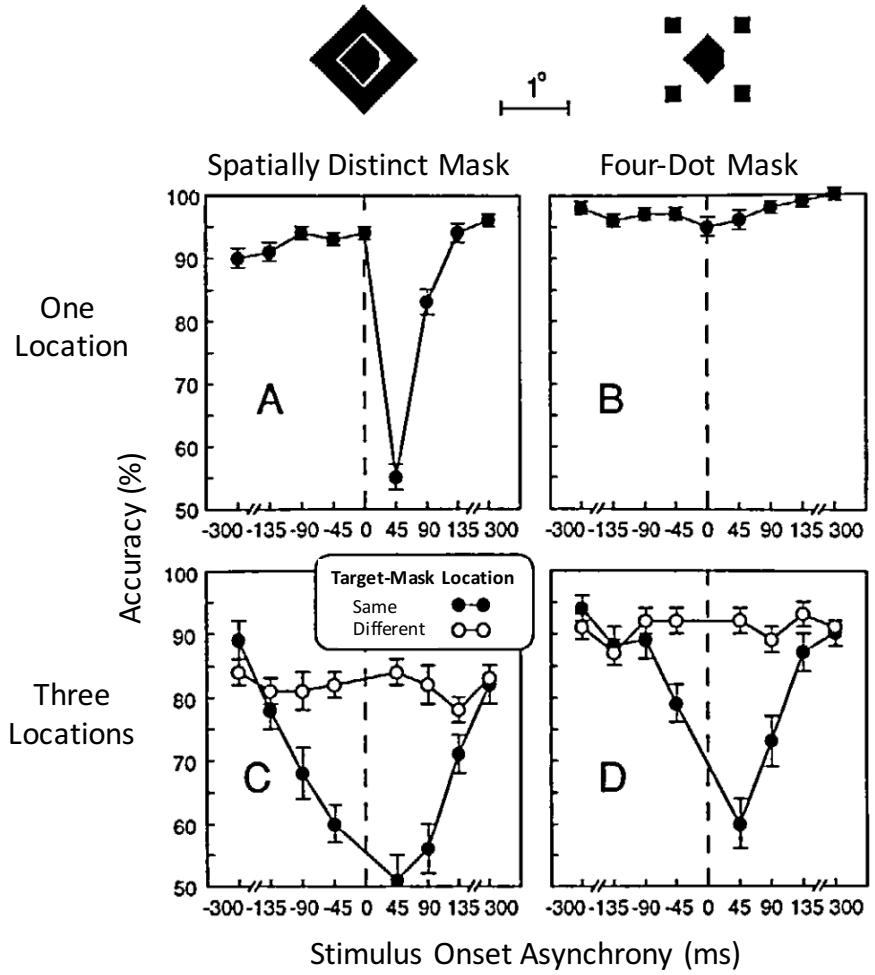

Figure 1.10. The effects of attention in spatially distinct and four-dot masking. Target stimuli were centrally displayed in a single location with a spatially distinct mask (A) or four-dot mask (B). Target stimuli were also presented in one of three locations along a horizontal plane with a spatially distinct mask (C) or four-dot mask (D). In conditions C and D, target and mask stimuli could be presented in the same (filled circle) or different (open circle) locations. The four-dot mask only caused a reduction in target visibility when stimulus location was unpredictable (panel D). Figure taken from (Enns and Di Lollo, 1997).

1.5.3 Competition theory (Keysers)

In place of neural integration and interruption, Keyser and Perrett (2002) proposed a theory of neural competition (Figure 1.7D). The theory suggests that when two stimuli are presented so that their neuronal representations overlap in time, if they cannot be interpreted as a single plausible percept, the activity of the two neuronal populations compete. The theory implies that low-level sensory representations should closely reflect the stimulus properties and that masking arises because of limitations in how this activity can be decoded or interpreted. In this way, any changes that occur in early cortical areas such as V1 would be expected to occur post-decision and therefore be observed in the late components of the response. The theory differs from neural interruption as it makes no assumption about the temporal order of the stimuli. Since neural competition does not rely on degradation of the neuronal representation of the target, the theory aligns with evidence of target recovery (Robinson, 1966, 1968; Turvey, 1973; Breitmeyer et al., 1981; Oğmen et al., 2006). The concept has a lot of flexibility as competition could occur through lateral, feed-forward or feedback interactions that need not be mutually exclusive. However, the theory does require that the target and mask stimuli activate different populations of neurons. Competition could explain a great number of visual masking effects and should be considered along with others as a plausible mechanism. To date, the only specific model that incorporates the concept of competition is the perceptual retouch model.

1.5.3.1 Perceptual retouch theory (Bachman)

The perceptual retouch model proposed by Bachmann (1984) incorporates two known neuronal pathways activated by visual stimuli: a modality specific, retino-geniculo-striate pathway and a non-specific retino-reticulo-cortical pathway (Singer et al., 1976; Singer, 1977; Hassler, 1978; Frizzi, 1979; Schiller, 1986; Steriade and McCarley, 1990; Breitmeyer, 1992; Shapley, 1992; Hartveit et al., 1993). The primary role of the specific pathway is to generate a neuronal representation of the visual stimulus while the non-specific pathway, activated via collaterals, is necessary for the generation of conscious experience. It is assumed that perceptual awareness only occurs when the impulses from both pathways converge and temporally overlap at a common cortical and retinotopic location. However, because of a 40-50 ms delay between the arrival of the specific and non-specific input (Jung, 1958; Hassler, 1978), an array of visual masking effects are possible. The model incorporates neuronal integration within specific and non-specific pathways and is capable of explaining both monotonic and U-shaped masking effects. At short SOAs the integration of stimuli within both pathways would lead to the perception of a target-mask montage, predicting a monotonic function for both forward and backward masking conditions. However, if the target energy was

sufficiently high or the stimuli were spatially distinct, target perception would be unaffected, thus providing an important condition for U-shaped masking. At intermediate SOAs, the awareness-generating activity elicited by the first stimulus would reach the cortex when specific information about the second stimulus had the highest signal-to-noise ratio, and so in backward masking, perception of the mask would prevail over that of the target. At long SOAs both target and mask representations would have sufficient time to form and merge with their respective conscious-experience-generating impulses, therefore leading to the perception of both stimuli. Altogether the theory is capable of explaining most of the regular findings in visual masking only falling short in explaining the effects of U-shaped forward masking. However, the model does not explain why the neuronal representations of the target and mask must compete in order to be perceived. It conveniently involves a retino-reticulo-cortical pathway that produces responses with a 40-50 ms latency difference, despite the lack of anatomical or physiological evidence to support the role of this pathway in perception. Furthermore, the model does not make any predictions about the location of stimulus integration between the two pathways, therefore making it difficult obtain empirical evidence to support the theory.

1.6 A Rodent Model for Vision

In order to conduct a detailed investigation of the neuronal mechanisms of visual masking, an animal model is necessary. Although historically rodents have not been the classical choice for research in vision or perception, they are becoming increasingly popular due to a number of technical and cost advantages. In particular, rodents offer the opportunity to collect data from large cohorts of animals with good options to monitor and control the activity of specific neuronal cell types and circuitries (Andermann et al., 2010; Lee et al., 2012; Juavinett and Callaway, 2015). While it is true that rodent and primate vision differs in several ways, these differences are not necessarily problematic. For example, the fact that rodents lack a high-acuity fovea, have larger receptive field sizes and a smaller binocular zone are inconsequential as long as the spatial properties of the stimulus is scaled to be appropriate for the animal (Shaw et al., 1975; Girman et al., 1999). Furthermore, neurons in V1 of rodents feature the same basic properties as in other species, including orientation tuning, the presence of simple and complex cells and surround suppression (Dräger, 1975; Wiesenfeld and Kornel, 1975; Birch and Jacobs, 1979; Parnavelas et al., 1981; Rosa and Krubitzer, 1999; Prusky et al., 2002). In fact, V1 in rodents may actually present a significant advantage compared to that of non-human primates; rodent V1 performs many computations that have not been demonstrated in

other species, such as memory and reward encoding (Gavornik and Bear, 2014b, a). It is therefore possible that neural correlates of masking that can only be observed in higher order regions of primates may be evident in V1 of rodents. Together, this suggests that rodent V1 may be a good place to begin investigating the neuronal mechanisms of visual masking.

To confirm that the changes that are observed in neuronal processing actually coincide with perceptual deficits, it is important to be able to collect perceptual report from the same species, ideally at the same time as neuronal data is collected. Recent behavioural studies have shown that rodents are capable of rapidly learning a variety of tasks including visual detection and discrimination with performance comparable to primates (Andermann et al., 2010; O'Connor et al., 2010; Busse et al., 2011; Histed et al., 2012). Furthermore, rats have been shown to be capable of recognising objects despite variations in the angle, size or position of their view, indicating that their visual processing is reasonably sophisticated (Zoccolan et al., 2009; Tafazoli et al., 2012). In particular, Long Evans rats have an excellent track record for learning and performing reasonably complex visual tasks. For this reason, we sought to develop a rodent model of visual masking in the Long Evans rat. For a more detailed explanation of our motivations to select the Long Evans species see Chapter 3.

1.7 Aims & Hypotheses

The disconnect between stimulus and percept revealed by visual masking provides the opportunity to investigate how neuronal processing changes with different perceptual interpretations of the same stimulus. To date the phenomenon has been well characterized psychophysically in a variety of mammalian species, however the neuronal effects of visual masking have received significantly less attention. This is reflected by the contention that remains concerning the neuronal mechanisms involved in visual masking; theories disagree on the origin of the sustained neuronal activity evoked by the target, how this sustained activity is sometimes suppressed, and the neuronal circuits that are involved. This highlights the necessity of further investigation into the cortical mechanisms of visual masking. Our work is intended to address this need by investigating visual masking psychophysically and neuronally in the Long Evans rat.

Although rats have not been a typical choice in vision research, they provide a number of experimental advantages that are not easily achieved in primates. For example, there is the potential to monitor and control specific neuronal cell types and circuitries in relatively large cohorts of awake and behaving animals. This provides the opportunity to conduct detailed investigations of the neuronal mechanisms associated with perception and behavior. For this reason, we seek to determine if the rat is a suitable model for visual masking research.

In Chapter 2, to determine if the neuronal effects of visual masking are analogous to those that have been observed in other mammalian species, we explore the effects of spatially overlapping and spatially distinct mask stimuli on the neuronal representation of target gratings in V1 of anaesthetized rats. Specifically, we characterize how the firing rate, orientation selectivity and latencies of the target response are affected across a range of positive and negative SOAs. We also investigate whether the effects of visual masking differ between the transient (early) and sustained (late) components of the response to the target.

To determine if the changes observed in neuronal processing are accompanied by perceptual deficits, and perhaps related to perception, it is necessary to collect perceptual reports from the same species. We therefore designed behavioural paradigms that enable perceptual reports to be collected from rats viewing visual masking stimuli. In Chapter 3, we address some important considerations for designing,

and training rodents in, complex visual tasks. These considerations include the choice of species and strain, the type of task, and the apparatus that best enables simultaneous neuronal recordings. Subsequently, we outline the methods that were used to train Long Evans rats for discrimination and detection visual masking tasks. In this section, we place particular emphasis on the problems that are common to rodent behaviour and how we addressed these issues.

In Chapter 4 we discuss the psychophysical effects of visual masking in rodents and humans performing an orientation discrimination task. In the task, we examine the effects of SOA and the spatial configuration of stimuli on target perception.

In Chapter 5 we further investigate the effects of spatially distinct masks on rodent and human perception in a target detection task. In humans we examine how SOA, target and mask separation and target contrast affect target detectability. In rodents, to minimise parameter manipulations, we only explore the effects of target contrast on target detectability at a fixed SOA.

Finally, Chapter 6 summarises the findings of this thesis and discusses the major implications of our results.

2 Masking reduces orientation selectivity in rat visual cortex

2.1 Abstract

In visual masking the perception of a target stimulus is impaired by a preceding (forward) or succeeding (backward) mask stimulus. The illusion is of interest because it allows uncoupling of the physical stimulus, its neuronal representation and its perception. To understand the neuronal correlates of masking, we examined how masks affected the neuronal responses to oriented target stimuli in the primary visual cortex (V1) of anaesthetized rats (n=37). Target stimuli were circular gratings with 12 orientations; mask stimuli were plaids created as a binarised sum of all possible target orientations. Spatially, masks were presented either overlapping or surrounding the target. Temporally, targets and masks were presented for 33 ms, but the stimulus onset asynchrony (SOA) of their relative appearance was varied. For the first time, we examine how spatially overlapping and centre-surround masking affects orientation discriminability (rather than visibility) in V1. Regardless of the spatial or temporal arrangement of stimuli, the greatest reductions in firing rate and orientation selectivity occurred for the shortest SOAs. Interestingly, analyses conducted separately for transient and sustained target response components showed that changes in orientation selectivity do not always coincide with changes in firing rate. Based on the near-instantaneous reductions observed in orientation selectivity even when target and mask do not spatially overlap, we suggest that monotonic visual masking is explained by a combination of neural integration and lateral inhibition.

2.2 Introduction

Visual masking describes a phenomenon in which perception of a target stimulus is reduced or entirely abolished by another stimulus, the mask (Breitmeyer, 2008). By varying the relative presentation times of the target and mask, the perception and neuronal response to the target stimulus may be systematically altered. In this way, masking reveals a disconnect between the physical stimulus, its neuronal representation, and its perception. Uncovering, the precise mechanisms involved in visual masking will provide important insights into how neuronal activity leads to conscious visual perception.

The effects of masking on target perception depend on a range of spatial and temporal stimulus factors, and are likely to involve a diverse family of mechanisms (Macknik and Martinez-Conde, 2007; Breitmeyer, 2008). As such, masking phenomena are commonly categorized according to: (1) the temporal relationship of the target and mask (forward versus backward masking); (2) the temporal dynamics of the influence of the mask on the target (A- and B-type masking); and (3) the spatial configuration of mask and target (spatially overlapping versus centre-surround masking). The stimulus-timing categories of visual masking include forward and backward masking in which the mask either precedes or succeeds the target stimulus, respectively. Backward masking illusions are of particular interest as the perception of the target stimulus is retroactively reduced by mask-evoked neuronal activity; the timing means that, unlike forward masking, this cannot be explained through photochemical depletion in the retina or adaptation in the thalamus (Crawford, 1947). Furthermore, psychophysical studies of backward masking have shown that mask presentation in one eye can reduce the visibility of a target presented to the other eye (Weisstein, 1971; Turvey, 1973). This binocular interaction of target and mask responses suggests that cortical mechanisms are involved (Kinsbourne and Warrington, 1962a).

In perceptual masking studies, two psychophysical trends have been described: A- and B-type masking (Kolers, 1962). In A-type masking, target perception is maximally impaired when target and mask stimuli are presented simultaneously, and monotonically improves with increasing stimulus onset asynchrony (SOA) between the target and mask. In most cases forward masking produces an A-type trend (Bachmann, 1994). On the other hand, in B-type masking the greatest impairment in target perception occurs at SOAs of 30-100 ms (Lefton, 1973). B-type masking is often obtained if the target and mask stimuli do not spatially overlap, however, the same stimuli can cause A-type masking if the energy (i.e. contrast and duration) of the target is considerably lower than that of the mask (Schiller and Smith, 1966; Hernandez and Lefton, 1977).

Two prevailing theories are commonly used to explain psychophysical masking; neural integration and neural interruption (Scheerer, 1973). Neural integration proposes that the reduction in target visibility is due to limits in the temporal resolution of the visual system, therefore causing the neuronal representation of the target to fuse with that of the mask. This has been the most widely accepted mechanism explaining A-type masking for both forward and backward masking conditions (Eriksen and Lappin, 1964; Stoper and Banffy, 1977; Pilz et al., 2013). Neural interruption instead proposes that target processing is disrupted by the arrival of the response to the mask, leading to reductions in target perception. While a prominent explanation for B-type masking, the origins of the different processing delays for target and mask, and the specific means through which interruption occurs remain highly contentious (Breitmeyer and Ganz, 1976; Francis, 1997; Macknik and Livingstone, 1998; Keysers and Perrett, 2002; Breitmeyer and Ogmen, 2006; Macknik and Martinez-Conde, 2007).

In macaque primary visual cortex (V1), target-evoked activity is reduced under stimulus conditions that cause perceptual masking, directly reflecting the psychophysical trends (Schiller and Chorover, 1966; Vaughan and Silverstein, 1968; Schiller, 1969; Bridgeman, 1975, 1980; Macknik and Livingstone, 1998). In the absence of a mask, brief target stimuli evoke biphasic activity, consisting of an early transient component and a late sustained component that can persist for hundreds of milliseconds. Under backward masking conditions, only the late component of the response to the target is reduced at SOAs that cause perceptual deficits (Bridgeman, 1975, 1980; Macknik and Livingstone, 1998). Interestingly, only approximately 25% of cells in V1 show a temporal pattern of response reduction consistent with B-type masking (Bridgeman, 1975; Macknik and Livingstone, 1998); it remains unclear what defines this particular neuronal sub-population.

The majority of physiological studies have focused on how visual masking affects stimulus detectability. However, it is equally important to understand how the ability of neurons to support stimulus discrimination is affected. In the inferior temporal cortex (IT), neuronal discriminability of shapes is impaired by masking (Kovács et al., 1995; Rolls et al., 1999), however, these changes may be inherited from earlier processing regions such as V1. Indeed, in the context of figure-ground textures, orientation selectivity in V1 is weakly impaired at short SOAs (Lamme et al., 2002), but this study was limited to backward masking conditions and only used stimuli with two orientations.

In order to evaluate the effects of visual masking on neuronal discriminability in V1, we recorded responses to brief, oriented gratings presented before or after plaid mask stimuli. Firing rates and orientation selectivity were reduced at short SOAs, reflecting an A-type trend, for both spatially overlapping and spatially distinct stimuli under forward and backward masking conditions. This demonstrates that visual masking affects stimulus visibility and also discriminability. We also observed biphasic responses to our target stimuli and comparisons between transient and sustained response components revealed separate effects of masking on firing rate and selectivity. Notably, we demonstrate that the effects of backward masking are not limited to the sustained component; orientation selectivity is affected throughout the entire response to the target, often in the absence of significant changes in responsivity. When responses of neurons in supragranular, granular and infragranular layers were analysed separately, no laminar-specific differences were revealed. We propose that A-type visual masking cannot be explained by neural integration alone, other mechanisms such as lateral inhibition are necessary to account for near instantaneous reductions that occur in orientation selectivity.

2.3 Materials & Methods

Experiments were conducted in accordance with the Code of Practice for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council, Australia) and received approval from the Monash Animal Research Platform Animal Ethics Committee (MARP/2013/081). Adult, male Long-Evans rats (n=37; 320-350 g) were obtained from the Monash University Animal Research Precinct (MARP) and housed in 12h light/dark cycles with food and water provided *ad libitum*.

2.3.1 Surgery and extracellular recordings

Animals were placed in an induction chamber and anesthetized with 5% halothane (in 1 L/min O_2). Once surgical anaesthesia was established (confirmed by the absence of a hindpaw withdrawal reflex), animals were intubated with a 16G polymer tube to allow mechanical ventilation (75-80 breaths/min) with a constant maintenance of anaesthetic (1-2.5% halothane in 0.3 L/min O_2). A thermostatically-controlled heating pad and rectal probe were used to maintain body temperature at 37-38°C throughout the duration of the experiment. Depth of anaesthesia was regularly monitored via the withdrawal reflex, palpebral reflex, and via ECG and EMG recordings taken from the upper forelimbs.

Animals were placed in a stereotaxic frame and a scalp incision was made to expose the skull overlying the known binocular zone in V1 (approximately 1.8 mm rostral from lambda and 4.5 mm lateral to the midline suture). A craniotomy of approximately 4 mm in diameter was drilled over V1 and a durotomy performed to allow electrode penetration. Neuronal activity was recorded using a single-shank linear electrode array with 32 contact points (<1.2 MΩ, 50 µm contact spacing; A1x32-6mm-50-177-A32, NeuroNexus Technologies, USA). Electrodes were inserted up to a depth of 2000 µm to span all cortical layers. Neuronal signals were amplified, filtered between 0-250 Hz (for LFPs) and 0.75-5 kHz (for spikes), and recorded at a sampling rate of 30 kHz using a Cereplex Direct data acquisition system (Blackrock Microsystems, USA). Raw signals were spike sorted offline (Plexon Offline Sorter, USA) to separate multi- and single-unit activity.

2.3.2 Visual stimuli

Stimuli were generated using Psychtoolbox in MATLAB (Brainard, 1997; Pelli, 1997) and presented on a 120 Hz refresh rate VIEWPixx/3D LCD monitor (VPixx Technologies Inc., Canada; Ghodrati et al., 2015) at a viewing distance of 30 cm.

Receptive fields (RFs) were mapped for each of the array's 32 channels using a stimulus consisting of 5° white dots presented at random positions on a 9x17 grid across the monitor. Dots were presented on a black background (50 ms flash on, 50 ms flash off). Once RF locations and sizes were characterized, flashed static square-wave gratings were used to probe orientation selectivity. Orientation tuning stimuli

were optimized to the location and size of the RFs of the majority of the units on the array, and consisted of gratings randomly presented at 6 orientations (0-150° degrees, 30° increments; 50 ms flash on, 500 ms inter-stimulus interval) and 2 phases (0 and 180°) on a gray background.

Responses to spatially overlapping and spatially distinct (centre-surround) forward and backward masking stimuli were recorded using square-wave gratings as the target stimuli. These were visible within a circular aperture matching the size and shape of the RFs of the majority of units on the array. The target grating had 100% contrast and was randomly presented at 1 of 12 different orientations (0-165°, 15° spacing) and 4 different phases (90° spacing), for 33 ms. The mask stimulus was also presented for 33 ms and consisted of a black and white hyperplaid generated randomly for each trial, by binarising the sum of 12 gratings with each possible target orientation, and randomized phase (see Fig. 2A, inset). Mask stimuli were either presented at the same spatial location and dimensions as the target stimulus, or were presented with a centre-surround arrangement, where the masks were full-screen with an aperture matching the target size and location. As the surround masks did not overlap the classical RF, we expected them to evoke little or no response. The relative time of the target and mask stimuli, referred to here as stimulus onset asynchrony (SOA), was measured from the onset of the first stimulus to the onset of the second stimulus. To examine the effect of spatially overlapping forward and backward masking, target and mask stimuli were presented at SOAs between ±33.3 and ±333.3 ms; forward and backward masking are associated with negative and positive SOAs, respectively. For centre-surround forward and backward masking, SOAs ranged from ±8.3 to ±333.3 ms, including SOAs with temporal overlap of target and mask presentation. In both masking paradigms, an SOA of ±333.3 ms was used as a control. Note that forward and backward masking form a continuum – an SOA of 0 ms simply indicates that target and mask are presented simultaneously. However, to ensure stability of recordings during a single type of masking, we presented forward and backward masking in separate blocks. When no target or mask was visible, the screen displayed a blank gray background (luminance = 53.2 cd/m^2) during non-overlapping SOAs. The inter-trial interval was set to 500 ms and each unique stimulus condition was presented 8-10 times.

2.3.3 Determination of cortical depth

Current source density (CSD) was calculated as the second spatial derivative of the local field potential collected in response to full screen flashes alternating between black on white and white on black (flash duration = 8.3 ms; blank duration = 408.3 ms). The CSD traces were examined in order to identify the boundary between layers 4 and 5 as indicated by a reversal from current source to current sink (Mitzdorf, 1985). The current sinks identified at the boundary, in combination with depth-from-cortical-surface measurements, were used to define the granular layer. Units were then assigned to one of three depth categories; supragranular, granular or infragranular, according to their location relative to the granular layer.

2.3.4 Masking analysis

Initially, to check the orientation tuning of multi- and single-units, spikes were counted in a 50-150 ms window from target onset and a von Mises function fitted to the mean spike rates at each SOA in response to every orientation. Comparing the distribution of responses to preferred and anti-preferred orientations, units with d' values above 0.3 at the control SOA (±333.3 ms) were classified as tuned and were included in our analysis, with single and multi-unit responses pooled together. These selection criteria yielded 73 and 95 tuned units in the spatially overlapping forward and backward masking conditions, respectively, and a total of 42 and 63 tuned units in the centre-surround forward and backward masking conditions, respectively. Note that relatively few units are strongly selective as we used brief flashes of static gratings. Forward and backward masking recordings for both paradigms were taken from the same penetrations as far as possible, however the differences in numbers of responsive units reflects that forward or backward conditions, and spatially overlapping or centre-surround conditions, were tested in separate blocks.

Transient and sustained target responses were found in the time windows 50-100 ms and 100-300 ms after target onset, respectively. To examine the effect of response integration on orientation selectivity, 3 spike counting windows (80-100, 80-120 and 50-150 ms, relative to target onset) were used to determine orientation tuning for each masking condition. These windows were chosen as they centred around the average peak latency for orientation selectivity, and so could be used to probe the effect of integration window size on our ability to discriminate target orientation using the response of a single neuron.

Responses to the preferred (θ_{pref}) and orthogonal (θ_{null}) orientations were used to calculate an orientation selectivity index (OSI) across time for each SOA:

$$OSI = \frac{R_{pref} - R_{null}}{R_{max}}$$

Where R_{pref} refers to the mean response to the preferred orientation, R_{null} refers to the mean response to the orientation orthogonal to the preferred, and R_{max} refers to the maximum preferred response across the entire stimulus presentation window (-500 to 400 ms relative to target onset in the forward masking condition, and -100 and 700 ms relative to target onset in the backward masking condition).

2.3.5 Latencies

Response latencies (t_{resp}) were calculated as the time taken to reach a response that was greater than the mean +3SD of the spontaneous firing rate, over 10 ms. Latency to selectivity (t_{sel}) values were calculated as the time taken to reach an OSI that was greater than the mean OSI prior to stimulus presentation +3SD of the mean pre-stimulus OSI, over 20 ms. Units were only included in latency analyses if t_{resp} or t_{sel} were under 250 ms, which was the maximum length for a target response in the control (±333.3 ms) SOA condition.

2.4 Results

2.4.1 Neuronal firing rates are reduced at low stimulus onset asynchronies

To determine the effect of our experimental masking paradigms on neuronal responses, we obtained extracellular recordings from populations of neurons in V1 in response to spatially overlapping and centre-surround visual masking stimuli under both forward and backward masking conditions. As the target stimulus was an oriented grating, we used changes in firing rates and orientation selectivity as measures of the strength of masking. Across the population of recorded neurons, we found that orientation tuning was sharper at long stimulus onset asynchronies (SOAs), and that tuning became broader as SOAs decreased (see Figure 2.1 for single unit example).

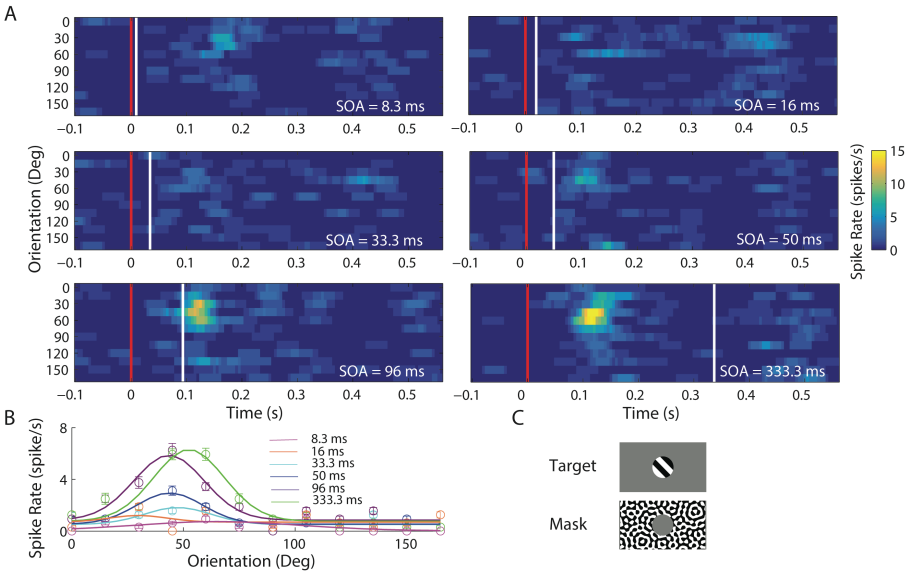

Figure 2.1 Orientation tuning is reduced at short stimulus onset asynchronies (SOA). A) Responses of a single unit to 12 target orientations with a *centre-surround* backward masking stimulus (examples of stimulus configuration shown below). Responses are averaged in sliding 40 ms time windows. Vertical red and white lines indicate the time of target and mask onset, respectively. Note that the mask in isolation (control; SOA = 333.3 ms) evokes little response. **B)** Tuning curves for the unit shown in (A), based on responses in a time window 50-150 ms relative to target onset. **C)** Example target and mask configuration in the centre-surround paradigm.

Figure 2.2 illustrates the population-average peristimulus time histograms (PSTH) for centre-surround masking stimuli. Before averaging, firing rates for each unit were normalized relative to the peak response to the target with the longest SOA, measured across 50-150 ms relative to the target onset. With this stimulus configuration, the mask is outside the classical receptive field and evokes little response; however, the target stimulus elicited a significant response regardless of orientation. Previous studies have characterised transient and sustained components of the response to the target, and explored how they are affected by the presence of a preceding or succeeding mask. In the case of forward masking, we found that both the transient and sustained components of the target response are greatly reduced when SOAs are less than 100 ms (Figure 2.2A). In backward masking conditions, the transient target response is unaffected even at the shortest SOAs (Figure 2.2B); however, the sustained target response is suppressed at short SOAs, and recovers with increasing SOAs. For each analysis in the following sections, we will first report our results for spatially overlapping configurations, and then for centre-surround configurations.

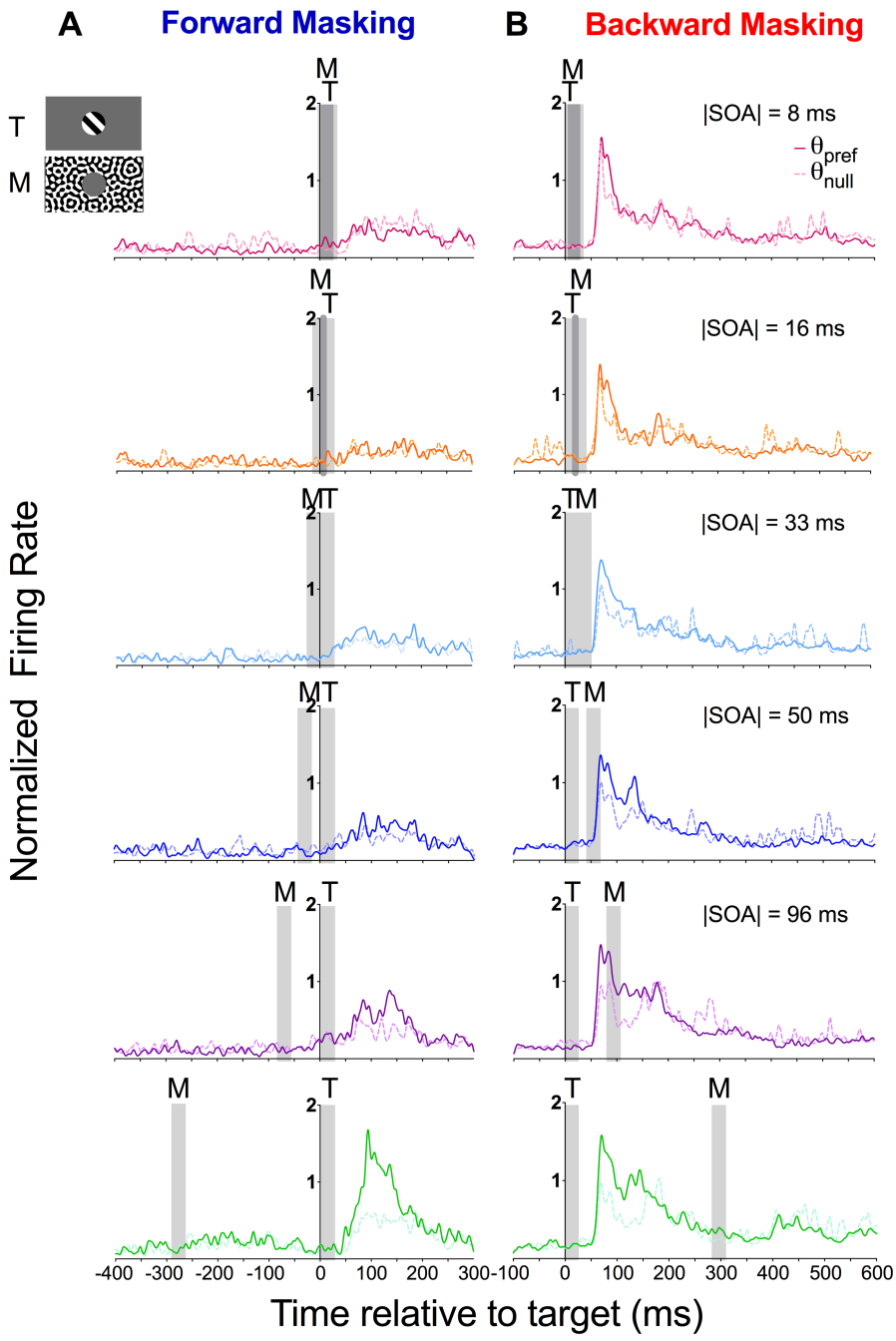

Figure 2.2 Population responses in centre-surround forward (A) and backward (B) masking conditions. Peristimulus time histograms for preferred and null orientations were aligned to target onset and averaged across units ($n_{forward}$=42; $n_{backward}$=63). Firing rates were normalized to the maximum response to the target at the longest stimulus onset asynchrony, measured over a window of 50-150 ms. Gray bars indicate the periods in which target (T) and mask (M) are visible. Inset figure shows example target and mask stimuli for the centre-surround configuration.

Given that the transient and sustained target responses appear differently affected under forward and backward masking conditions, we separately quantified how masking affected transient (50-100 ms) and sustained (100-300 ms) responses. Recordings were initially segregated according to cortical layers based on CSD analyses, however, we found no significant differences between supragranular, granular and infragranular layers, and the data were henceforth combined.

In the *spatially overlapping* paradigm, we found higher firing rates in response to the preferred versus the null target orientation in both transient (Figure 2.3A; $p_{forward}<0.001$, $F_{1,720}=45.7$; $p_{backward}<0.001$, $F_{1,884}=91.0$; Two-way ANOVA) and sustained time windows ($p_{forward}<0.001$, $F_{1,720}=40.8$; $p_{backward}<0.001$, $F_{1,884}=53.0$; Two-way ANOVA). Critically, target-evoked firing rates were lower with shorter SOAs, and SOA significantly affected firing rates for both transient ($p_{forward}<0.001$, $F_{4,360}=8.1$; $p_{backward}=0.04$, $F_{4,443}=2.5$; One-way ANOVA) and sustained time windows ($p_{forward}=0.02$, $F_{4,360}=3.1$; $p_{backward}=0.002$, $F_{4,443}=4.3$; One-way ANOVA). This demonstrates that the firing rates of both transient and sustained components of the target response are affected by changing SOAs in spatially overlapping forward and backward masking.

In the *centre-surround* paradigm, transient firing rates were significantly higher in response to preferred target orientations for both forward and backward masking (Figure 2.3B; $p_{forward}<0.001$, $F_{1,738}=21.3$; $p_{backward}<0.01$, $F_{1,1053}=59.1$; Two-way ANOVA), but sustained firing rates were only significantly higher in the forward masking condition ($p_{forward}<0.001$, $F_{1,738} = 24.8$; $p_{backward}=0.23$, $F_{1,\ 1053} = 1.5$). Transient firing rates significantly decreased with SOA for forward ($p<0.001$, $F_{8,369}=5.6$; One-way ANOVA), but not backward masking ($p=0.18$, $F_{8,531}=1.4$). However, sustained firing rates were significantly affected by SOA for both forward and backward masking ($p_{forward}<0.001$, $F_{8,369}=9.4$; $p_{backward}<0.001$, $F_{8,\ 531}=5.5$; One-way ANOVA). These results demonstrate that the presence of a surround mask *before* a target affects both transient and sustained components of the target response. In centre-surround backward masking, where presentation of the mask *follows* target presentation, only the firing rates of sustained target response components are affected. Therefore, in the centre-surround masking paradigm, firing rates for the transient and sustained components are affected differently according to the temporal position of the mask.

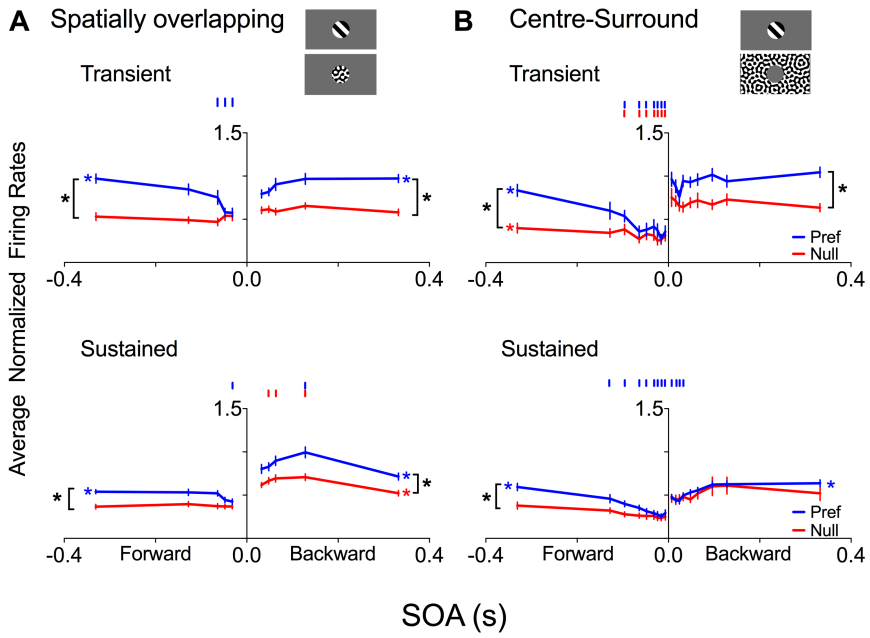

Figure 2.3 Comparison of transient and sustained responses. Firing rates were calculated for preferred and null target orientations using transient (50-100 ms relative to target onset) and sustained (100-300 ms relative to target onset) time windows. Responses were calculated for spatially overlapping (A) and centre-surround (B) paradigms. Vertical coloured bars indicate specific SOAs where OSIs are significantly different from controls (SOA = -0.333 or 0.333 s; forward and backward masking, respectively; $p<0.05$), in response to either the pref or null target orientation. Black asterisks indicate a significant main effect for differences in the responses to preferred versus null orientations ($p<0.05$). Colored asterisks indicate significant main effects of stimulus onset asynchrony ($p<0.05$). Errorbars show SE.

2.4.2 Masking differently alters transient and sustained orientation selectivity

As the target stimuli used in the present study were oriented gratings, we calculated orientation selectivity indices (OSI) to determine whether the masking-induced changes in spiking rate also affected orientation selectivity. In *spatially-overlapping* masking, OSIs calculated using a transient window were significantly higher than those using a sustained window under forward, but not backward masking (Figure 2.4A, $p_{forward}=0.03$, $F_{1,720}=8.8$; $p_{backward}=0.07$, $F_{1,896}=3.4$; Two-way ANOVA). Further, in forward masking, OSIs were significantly reduced in the shortest SOAs for both transient and sustained windows (Figure 2.4A; $p_{transient}<0.001$, $F_{4,360}=13.1$; $p_{sustained}=0.006$, $F_{4,360}=3.6$; One-way ANOVA). In backward masking, there was only a significant decrease in OSI at the shortest SOA, and this was found using only the transient window ($p_{transient}<0.001$, $F_{4,448}=5.9$; $p_{sustained}=0.08$, $F_{4,448}=2.1$; One-way ANOVA).

In *centre-surround* masking, OSIs calculated using the transient response were significantly higher than those using the sustained component, but only in the backward masking condition (Figure 2.4B; $p_{backward}<0.001$, $F_{1,1116}=72.4$; $p_{forward}=0.86$, $F_{1,738}=0.03$; Two-way ANOVA). OSIs in the forward masking condition were only significantly lower than control values at the five shortest SOAs, using a transient window ($p<0.001$, $F_{8,369}=3.7$; One-way ANOVA), with no differences when using a sustained window ($p=0.34$, $F_{8,369}=1.1$; One-way ANOVA). In backward masking, OSIs in the transient window were significantly lower than the control at only the shortest SOA, and at the three shortest SOAs in the sustained window ($p_{transient}=0.02$, $F_{8,558}=2.3$; $p_{sustained}=0.001$, $F_{8,558}=3.2$; One-way ANOVA). In general, while we routinely found changes in firing rate as a result of masking, these did not always guarantee a change in OSI in either the spatially overlapping, or centre-surround masking paradigms.

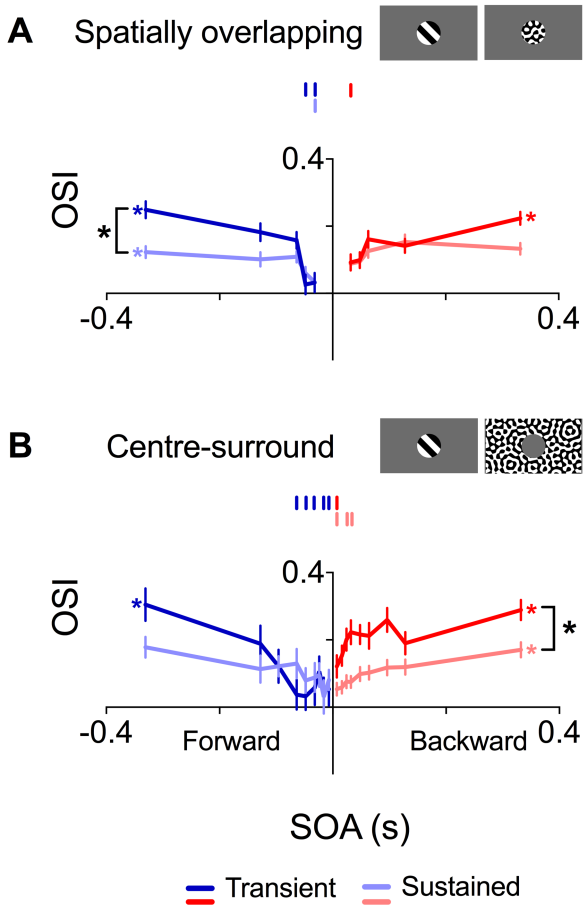

Figure 2.4 Orientation selectivity is higher in the transient response. OSI calculated using transient (50-100 ms) and sustained (100-300 ms) integration windows is shown for spatially overlapping (A) and centre-surround conditions (B). Vertical coloured bars indicate specific SOAs where OSIs are significantly different from controls (SOA = -0.333 or 0.333 s; forward and backward masking, respectively; $p<0.05$), in either the transient or sustained windows. Black asterisks indicate a significant main effect for differences in the OSIs between transient and sustained windows ($p<0.05$). Colored asterisks indicate significant main effects of stimulus onset asynchrony ($p<0.05$). Errorbars show SE.

2.4.3 Orientation selectivity is reduced at short SOAs, regardless of spike counting window

In order to assess the effect of the mask on the evolution of orientation selectivity, we averaged OSIs, calculated in sliding 40 ms windows, across units (see Figure 2.5). In centre-surround masking, selectivity is clearly highest with the longer SOAs, and the effects of forward masking are more profound than those of backward masking (Figure 2.5A & C). We also observed that the duration over which neurons were selective appeared shorter with short SOAs, especially for backward masking. Similarly, under spatially overlapping masking conditions, OSIs decreased as SOAs approached zero, for both forward and backward masking (Figure 2.5B & D). As the interesting temporal dynamics of selectivity mostly overlap with the transient window used in previous analyses, for each masking condition, we quantified orientation selectivity in three new integration windows: 80-100, 80-120 and 50-150 ms relative to target onset. This allows us to manipulate the proportion of mask-evoked activity that was included in calculations of target orientation selectivity. These windows were chosen as they centred around the average peak latency for orientation selectivity, but the exact choice of window has little effect on the results reported below.

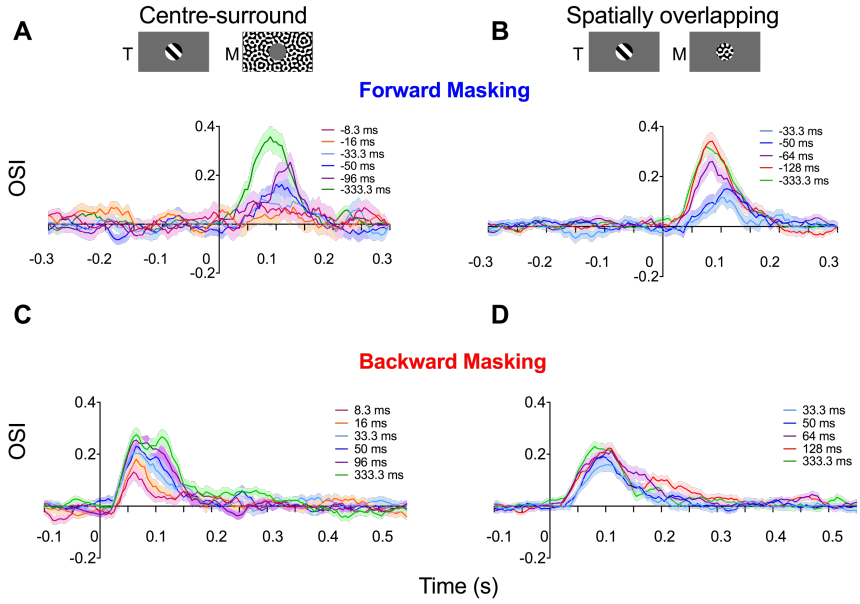

Figure 2.5 Population orientation selectivity indices (OSI) decrease at shorter stimulus onset asynchronies (SOA). Effect of stimulus onset asynchrony on orientation selectivity, calculated using a sliding 40 ms window for centre-surround masking paradigms (A, $n_{Forward}$= 42; C, $n_{Backward}$= 63) and spatially overlapping (B, $n_{Forward}$= 73; D, $n_{Backward}$= 95). Solid lines and filled regions indicate mean and SE, respectively.

The following analyses were performed to determine whether the masking effects seen in the firing rates and OSIs are consistent with the theory of neural integration. We first investigated whether changing the size of the integration window size affected OSIs, but found no effect for *spatially overlapping* conditions in forward or backward masking (Figure 2.6A; $p_{forward}$=0.85, $F_{2,1080}$=0.16; $p_{backward}$=0.29, $F_{2,1344}$=1.2; Two-way ANOVA). To determine whether selectivity was affected by SOA in specific integration windows, we calculated OSIs separately at all SOAs, for each integration window. OSIs were compared with those at their respective control SOAs (±333.3ms), as in these conditions, neuronal responses to target and mask stimuli are temporally well separated. In the forward masking condition, OSIs measured at the three shortest SOAs (-33.3, -50 and -66.7 ms) were significantly lower than at the control using the 100 ms integration window, and the two shortest SOAs were significantly lower than the control using the 40 and 20 ms integration windows (Figure 2.6A; p_{100}<0.001; p_{40}<0.001; p_{20}<0.001; One-way ANOVA), with no differences between OSIs at other SOAs (p>0.05, ANOVA). In the backward masking condition, for all integration windows, OSIs were significantly lower at only the shortest SOA (33.3 ms) compared to the control (Figure 2.6A; p_{100}<0.001; p_{40}<0.001; p_{20}<0.001; One-way ANOVA).

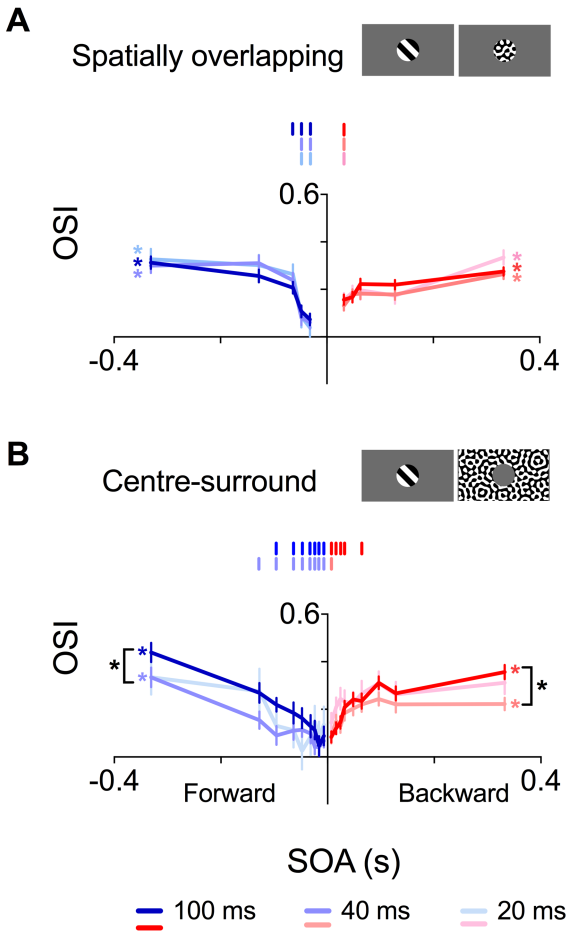

Figure 2.6 Integration window has little influence on orientation selectivity. OSIs for spatially overlapping (A) and centre-surround (B) conditions, calculated using 3 integration windows: 80-100 ms, 80-120 ms and 50-150 ms relative to target onset. Vertical coloured bars above plots indicate where OSIs are significantly different from control (SOA = -0.333 or 0.333 s; forward and backward masking, respectively; $p<0.05$), at each of the integration windows. Black asterisks indicate a significant main effect for differences in the OSIs between integration windows ($p<0.05$). Colored asterisks indicate significant main effects of stimulus onset asynchrony ($p<0.05$). Error bars show SE.

In *centre-surround* masking conditions (Figure 2.6B), we found significant overall differences in OSI between integration windows and OSIs significantly decreased as the SOA was shortened for both forward (integration window - $p=0.03$, $F_{2,575}=5.60$; SOA - $p<0.001$, $F_{8,575}=7.12$) and backward masking (integration window - $p=0.03$, $F_{2,774}=3.53$; SOA - $p<0.001$, $F_{8,774}=6.12$). We also investigated the effect of integration window size on the effect of masking (reduced OSI with shorter SOAs). There was no effect of SOA on OSI in the 20 ms integration window. However, using both the 100 ms and 40 ms integration windows, we found that forward masking OSIs were significantly lower in the shortest 3 SOAs (-8.3, -16.7 and -25 ms) compared with the control SOA (-333.3 ms; $p_{40}<0.001$; $p_{100}<0.001$; One-way ANOVA). Similarly, in the backward masking condition, using a 100 ms integration window we found a significant reduction in OSIs at the 5 shortest SOAs (8.3-50 ms), when compared with the control ($p<0.001$; One-way ANOVA), with no differences in OSI at any of the other SOAs. OSIs were only significantly reduced at the shortest SOA using the 40 ms integration window, with no effects of SOA on OSI in the shortest 20 ms window.

Finally, we examined whether spatially overlapping and centre-surround masking affect OSI differently. This is important because isolated masks (control; SOA = ±333.3 ms) induce a response in the spatially overlapping, but not the centre-surround conditions. Focussing on the 100 ms integration window, we found no differences in OSIs between the two masking types in either the forward or backward masking condition ($p_{forward}=0.36$, $F_{1,545}=0.83$; $p_{backward}=0.12$, $F_{1,713}=2.45$; Two-way ANOVA).

The neural integration theory of masking predicts that the effect of SOA on OSI will be smaller with shorter integration windows, reflected by a flattening of the OSI curves. This was not observed for the spatially overlapping masking condition. Regardless of the integration window size the orientation selectivity was significantly affected by the mask at short SOAs. In the centre-surround condition, the effect of SOA was removed in the 20 ms integration window, however this finding is likely due to the increase in variance rather than neural integration. This suggests that neural integration occurring solely in V1 is not a plausible mechanism for explaining how target discriminability decreases with decreasing SOA.

2.4.4 Effect of masking on latencies to response and selectivity

As response amplitude, selectivity and latency often interact, we examined whether masking affected the latency of responses to the target (t_{resp}) or the latency until orientation selectivity emerged (t_{sel}). Again, we found little to no layer-specific effects of SOA on latency in either spatially overlapping (t_{resp} - $p_{forward}$=0.17, $p_{backward}$=0.60; t_{sel} - $p_{forward}$=0.80, $p_{backward}$=0.68) or centre-surround masking (t_{resp} - $p_{forward}$=0.39, $p_{backward}$=0.07; t_{sel} - $p_{forward}$=0.04, $p_{backward}$=0.49; Two-way ANOVA) paradigms.

In the *spatially overlapping* paradigm, response latencies were shorter in forward than backward masking, but the magnitude of these latency differences was small ($p<0.001$; Two-way ANOVA). Between SOAs, t_{resp} was not significantly different in the backward masking condition, and approached significance for the forward masking condition (Figure 2.7A; $p_{forward}$=0.05; $p_{backward}$=0.72; One-way ANOVA). Due to the response evoked by a spatially overlapping mask, it is difficult to interpret the changes in target response latency when considering forward masking. The response to the mask is likely to interfere with measures of target response latencies, particularly at short SOAs, creating the appearance of a shorter latency. In fact, the delay observed in the latency to selectivity suggests that the response to the target may in fact be delayed under these conditions, similar to what is observed in centre-surround masking.

In the *centre-surround* paradigm, response latencies were shorter in backward than forward masking ($p<0.001$; Two-way ANOVA), and in the forward masking condition, there was a weak, but significant main effect of SOA on t_{resp} (Figure 2.7B; $p_{forward}$=0.04; $p_{backward}$=0.74; One-way ANOVA). Again, these latency differences were small. Finally, we found no influence of SOA on t_{sel}, in either forward or backward masking conditions ($p_{forward}$=0.51; $p_{backward}$=0.49; Two-way ANOVA). However, similar to the response latencies, there was a significant main effect of latencies to selectivity between the forward and backward masking conditions, where latencies were significantly shorter in backward masking than in forward masking ($p<0.001$; Two-way ANOVA). Collectively, our latency data suggest that the presentation of a mask immediately prior to the target causes inhibition that delays the onset of target processing. This agrees with response patterns observed in monkey V1, in which the response to a briefly presented preferred orientation sinusoidal grating is delayed if the grating is preceded by the anti-preferred orientation rather than a blank screen (Bair et al. 2002).

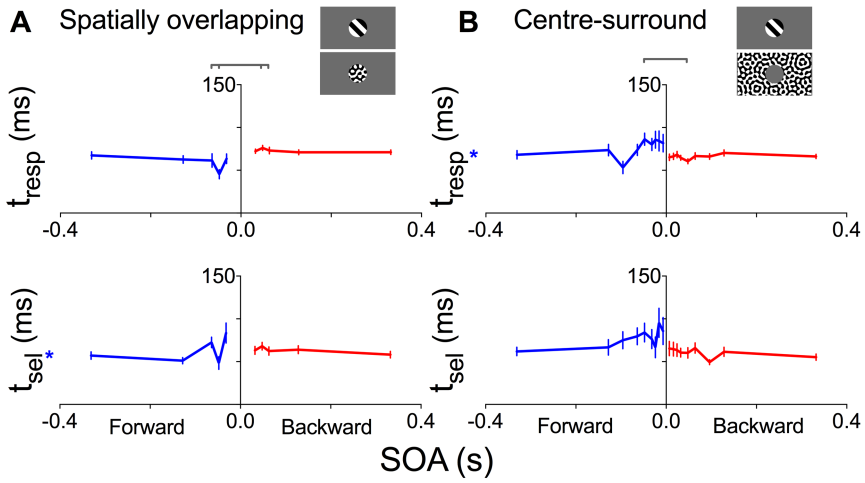

Figure 2.7 Latencies to selectivity and responsivity in spatially overlapping (A) and centre-suround (B) masking conditions. Top and bottom panels illustrate average latencies to responsivity and orientation selectivity, respectively. Gray brackets above plots indicate specific SOAs where average latencies between forward and backward masking conditions are significantly different ($p<0.05$). Colored asterisks indicate significant effects of stimulus onset asynchrony ($p<0.05$). Errorbars show SE.

2.5 Discussion

We examined how the responsivity and selectivity of V1 neurons were affected by masking stimuli presented either before or after flashed target gratings. Neurons responded to 33 ms target stimuli with a two-component response; an initial transient component lasting 50-100 ms, followed by a sustained component extending up to 300 ms. For all conditions, our data followed an A-type visual masking trend; responsivity and selectivity were lowest with short SOAs. For spatially overlapping masks, the neuronal responses to target and mask stimuli began to merge and eventually became indistinguishable as the SOA approached zero. Using centre-surround masks that did not evoke a neuronal response in isolation, the entire response to the target was reduced when using forward masks, while in backward masking only the sustained component was reduced at short SOAs. Below, we explore the different effects of masking on transient and sustained components, the difference between detection and discrimination tasks, and how our data are consistent with theories of neural integration and lateral inhibition.

Previous studies have suggested that the neural correlate of backward masking is a reduction in the sustained component of the response to the target (Bridgeman, 1975; Rolls and Tovee, 1994; Macknik and Livingstone, 1998; Lamme et al., 2002). Therefore, we specifically examined how masking affected both transient and sustained responses, and orientation selectivity during these periods. The long-lasting activation (100-300 ms) that we observed in response to a brief, 33 ms target grating in the absence of a mask is frequently reported in studies of V1 and the inferior temporal cortex (IT) (Schiller, 1969; Bridgeman, 1975; Rolls and Tovee, 1994; Macknik and Livingstone, 1998; Rolls et al., 1999; Lamme et al., 2002). With centre-surround stimuli, primarily the sustained response was reduced under backward masking conditions. With spatially overlapping stimuli, it is impossible to determine whether the target response was similarly affected as the responses to target and mask merge and become indistinguishable at short SOAs. However, analogous response reductions have been observed in IT using spatially overlapping masks that, in isolation, did not elicit a response, such as faces or a pattern formed by 'N' and 'O' letters (Rolls and Tovee, 1994). Interestingly, the trends in neuronal responsivity and selectivity were different. Orientation selectivity was affected by visual masking in the transient component for the spatially overlapping condition and in both the transient and sustained components for the centre-surround condition. This is important because it demonstrates that the effects of backward masking are not limited to a reduction in the sustained firing rate; feature selectivity is also affected throughout the entire response to the target.

Similar to backward masking, the neural correlate of forward masking is a reduction in the response to the target, however, the precise response components affected vary between studies. Using a single line as the target, Bridgeman (1975) showed that target responses in area 17 of anaesthetized cats were affected by the presence of parallel, flanking lines, with forward masking reducing only the sustained component. Despite using similar line stimuli, Macknik & Livingstone (1998) found that forward masking affected only the transient component in V1 of anesthetized monkeys, and in awake monkeys inhibited the entire target response. Our forward masking data, although collected in anaesthetized rodents, agree with the latter finding, showing a reduction in the entire response to the target. Yet, for the centre-surround condition, orientation selectivity was significantly reduced only in the transient component. Thus, response rate and selectivity follow an A-type masking trend regardless of the timing and spatial arrangement of stimuli. However, a reduced firing rate does not predict an impairment in selectivity; changes in selectivity may occur in isolation, or accompany firing rate reductions.

Previous studies using spatially distinct stimuli have often yielded a B-type (U-shaped) backward masking trend (Bridgeman, 1975; Macknik and Livingstone, 1998), and this is commonly expected if the energy of the target is greater than or equal to that of the mask (Fehrer and Smith, 1962; Kolers, 1962; Spencer and Shuntich, 1970; Lefton, 1974; Breitmeyer, 1978). This does not preclude observing B-type masking with high contrast targets (Macknik and Livingstone, 1998; Bruchmann et al., 2010; Agaoglu et al., 2015). In the past, stimulus energy has been defined as a function of the contrast and duration of a stimulus (Tapia et al., 2011). By this definition, our target and mask stimuli would be considered to have equal energy, predicting B-type masking. However, since our surround stimuli were larger than traditionally used annuli, we believe that the size of our mask is an important factor determining our observed A-type trend. While it is possible that B-type masking trends occur under differing circumstances in rodents, this seems unlikely as human psychophysical data using the same stimuli show similar A-type trends (in preparation).

Macknik & Livingstone (1998) and Bridgeman (1975) found that only units with distinct (rather than continuous) transient and sustained peaks showed a B-type trend in the late activity. As our neurons were not separated according to anatomical, functional or response properties, we may not have been able to observe B-type masking. That said, when our neurons were separated according to their laminar location, we found no important differences. Furthermore, a study using electroencephalography, a technique that groups neuronal activity more broadly than our own approach, has also shown that the late target evoked activity can reflect a B-type trend (Vaughan and Silverstein, 1968). Thus, it seems unlikely that our pooled analyses of the sustained activation were insufficient to show B-type masking if it were present.

Most psychophysical and electrophysiological studies of masking have focused on changes in visibility, or how masks affect *detection* performance (Bridgeman, 1975, 1980; Mitov et al., 1981; Macknik and Livingstone, 1998; Snowden, 2001). Electrophysiologically, this requires observing a large mask-induced reduction in the target response. This is problematic, as a large reduction in firing rate need not correlate with changes in visibility, as evident if stimulus contrast is reduced. As V1 responds strongly to oriented stimuli, we examined how masking affects orientation *discrimination* performance. Orientation selectivity indices were lower than commonly reported, because our target stimuli were static and only briefly presented. The flashes mean that target stimuli elicited significant responses regardless of their orientation, but selectivity persisted throughout both transient and sustained periods, contrasting previous results (Lamme et al. 2002). Generally, firing rates in response to preferred orientations decreased with

shorter SOAs, while the firing rates induced by null orientations were less affected. As a consequence, the orientation selectivity decreased, and persisted for less time, with shorter SOAs. This is consistent with results in area IT where the difference in the response to the 'best' compared with the 'worst' stimulus, and the amount of information about the target stimulus decreased with SOA (Kovács et al., 1995; Rolls et al., 1999). Thus, it is clear that cell selectivity also follows an A-type trend, suggesting that discriminability may be impaired, even when using high-contrast stimuli where the visibility of the target should not have been significantly reduced.

While the SOAs affected by masking in our study might be explained purely through spatial and temporal summation in the retina, our results with large centre-surround stimuli make it likely that thalamic and cortical processes further contribute. Recordings from retinal ganglion cells have shown that forward masking reduces the subsequent target response for SOAs of -80 up to -160 ms (Coenen & Eijkman, 1972). In backward masking, retinal interactions can account for reductions in the target response for SOAs of up to 50 ms; after this point, the target information will have already entered cortical regions before the mask impinges on the retina (Battersby et al., 1964). However, the spatial relationship and size of our centre-surround stimuli make it unlikely that retinal interactions alone could account for our results. Furthermore, to discount the involvement of any cortical contributions would be to disregard a wealth of information; numerous psychophysical studies have shown that visual masking occurs even under dichoptic stimulus presentation, which, given that the first sight of binocular combination is V1, implies some cortical involvement (Smith & Schiller, 1966; Schiller & Smith, 1968; Weisstein, 1971; Turvey, 1973).

One cortical-based theory that is frequently used to explain perceptual masking is neural interruption, where neuronal processing of the target is abandoned at the arrival of the response to the mask (Breitmeyer & Ogmen 2006). In this way, target processing is left unfinished resulting in impaired perception. However, this requires presentation of the target to precede the mask, and is therefore incapable of explaining forward masking. Furthermore, in backwards masking, most neural interruption theories predict a B-type visual masking trend, therefore, we suggest that neural interruption is unlikely to contribute to the visual masking observed in this study.

Neural integration, where the neuronal representation of the target and mask stimuli are grouped together in a relatively long 'perceptual window', is frequently used to explain A-type masking (Eriksen and Collins, 1967, 1968). Integration predicts the perception of a fused image therefore reducing target perception. In order to evaluate whether integration was contributing to the masking effect in our data, we restricted the duration of our spike counting windows to avoid the window including responses to both target and mask, in a sense, artificially shortening the 'perceptual window'. If cortical integration were sufficient to explain perception, we would expect orientation information to be unaffected by masking when using a short 'perceptual window'. In our data, this would be seen as the slope of the OSI curves flattening with shorter spike counting windows (Figure 2.5B), however this was not observed. Thus, neural integration alone is not capable of explaining our A-type masking trends, something further is needed to explain the near-instantaneous reduction in selectivity that we observe even under centre-surround conditions.

One likely explanation is lateral inhibition, a mechanism that is ubiquitous in neuronal circuitry and that has already been incorporated into a number of visual masking theories (Bridgeman, 1971; Weisstein et al., 1975a; Francis, 1997; Herzog et al., 2003). To explain our data, it is not necessary for lateral inhibition to operate faster than the response to the target, completely abolish the target response (Figure 2.2), or even affect the entire transient response (50 ms). It is only necessary for lateral inhibition to influence orientation selectivity in short time windows (20 ms; Figure 2.5). Given that surround suppression via lateral connections travels at conduction speeds of 0.1-0.3 m/s (Bringuier et al., 1999; Girard et al., 2001; Angelucci and Bressloff, 2006), and that our surround masks were immediately adjacent to the target, it is entirely plausible that orientation selectivity might be affected by masking in time windows as short as 20 ms.

Our results demonstrate that visual masking influences discriminability in V1. Regardless of the spatial and temporal arrangement of the target and mask, the firing rate and neuronal selectivity are reduced at short SOAs, reflecting an A-type trend. However, the impairment in stimulus discriminability occurs in the transient and/or sustained component of the response to the target in a manner that is not always predicted by changes in firing rate. We suggest that the effects of visual masking may be explained through a combination of neural integration and lateral inhibition occurring throughout the early visual processing hierarchy.

3 Training complex visual behaviour in rats

3.1 Introduction

In order to understand the neural bases of visual perception we need to be able to relate neuronal responses to sensory stimuli and behavioural outcomes. This necessitates a tightly controlled visual task that facilitates the collection of reliable perceptual reports. To date, the majority of visual neuroscience studies involving alert animals have been conducted in non-human primates, in part because they possess a similar visual system to that of humans (Newsome et al., 1989; Britten et al., 1992; Read and Cumming, 2003; Williams and Shapley, 2007; Nienborg and Cumming, 2010). However, the use of rodents is becoming increasingly popular due to a number of technical advantages, in particular, the ability to genetically manipulate specific neuronal cell types and circuits during behaviour (Andermann et al., 2010; Marshel et al., 2011; Lee et al., 2012; Froudarakis et al., 2014; Juavinett and Callaway, 2015). Although rodents lack a high-acuity fovea and their visual system is altogether less complex (Shaw et al., 1975; Girman et al., 1999), it is clear that rodents can perform simple visual discrimination and detection tasks with performance comparable to that of primates (Birch and Jacobs, 1979; Keller et al., 2000; Busse et al., 2011; Histed et al., 2012; Lee et al., 2012; Reinagel, 2013; Soma et al., 2014). Rats, in particular, have also been used in some complex visual tasks such as Gabor detection in the presence of distractors and object discrimination (Clark et al., 2011; Meier et al., 2011; Meier and Reinagel, 2011; Tafazoli et al., 2012; Vermaercke and Op de Beeck, 2012; Alemi-Neissi et al., 2013; Rosselli et al., 2015; Bossens and Op de Beeck, 2016). These complex tasks can be difficult to train and thus require careful planning.

This chapter first discusses some important methodological considerations for developing a robust paradigm for behavioural testing of visual perception in rodents including: 1) the choice of species and strain; 2) the type of task; and 3) the apparatus that will best enable the measurement of well-controlled visually guided behaviour along with neuronal responses. Second, we describe the phases of training that were used to shape rodent behaviour for our discrimination (Chapter 4) and detection (Chapter 5) visual masking tasks along with the associated behavioural data. The aim of this section is therefore to highlight techniques that can improve training and motivation as well as some inherent characteristics of rodent behaviour that can make it difficult to measure the desired outcome.

3.1.1 What makes a task complex?

While there is no formal definition of a "complex" behavioural task, there are many factors that contribute to task difficulty, and therefore limit the ease with which a behaviour can be trained through operant conditioning. For example: the task may require linking together multiple actions; there may be multiple response options; there may be multiple timing contingencies for the animal to monitor; the stimulus presentation may be brief and thus require focused attention; the stimuli may be close to the animals' detection or discrimination threshold; or a single informative stimulus may be presented among distractors. All of these factors can complicate the task and training process, but here we reserve the term complex for visual tasks that combine several of these factors. Ultimately, this means that training of a single animal takes many weeks or months, it is not practicable to train more than a handful of animals, and training progression towards the final task design must be carefully considered in advance.

An excellent example of a complex behavioural task for a rodent is the study by Meier et al. 2011, in which rats were required to detect a target Gabor that was presented for 200 ms at varying contrasts (25, 50, 75 & 100%), and flanked by two distractor Gabors (Figure 3.1)(Meier and Reinagel, 2011). In this task, the presence of distractors made the task particularly difficult for the animals to perform, as they had to learn to respond to one visual stimulus while ignoring others of similar appearance. On average, it took 121 days of training across 9 behavioural shaping steps for the rats to learn the task. Despite this lengthy and thorough training, the presentation of flankers still biased the rats to report the presence of a target regardless of if it were actually present. While longer periods of training might be employed in non-human primates (Britten et al., 1992), the short lifespan of rodents' present significant limitations for training and thus make it difficult to know what the precise limits of rodent learning might be.

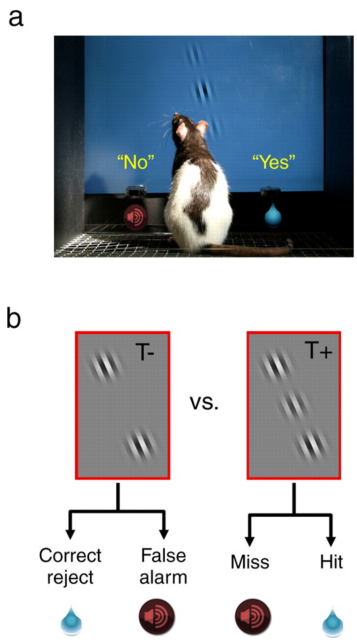

Figure 3.1. Gabor detection in the presence of flanking Gabor distractors: A) The rats are rewarded for going to the right sensor when the central target is presented and for going to the left sensor when the central target is absent. Panel B illustrates the response outcomes for target present (T+) and target absent (T-) trials. Correct responses received a liquid reward, incorrect responses were punished with a timeout during which a tone sounded and the screen flickered. Figure adapted from Meier et al 2011.

3.1.2 Our visual masking task requirements

For our study of visual masking, we aimed to develop a robust behavioural paradigm that would enable us to: 1) measure the effect of a visual mask on the animal's ability to detect or discriminate stimuli; 2) minimise the effect of response bias and impulsivity; and 3) simultaneously collect behavioural and electrophysiological data. Our intention to study visual masking, a phenomenon that is most likely to occur when stimuli are small and brief (Hernandez and Lefton, 1977), meant that it was important for us to select a species with reasonably good visual acuity. Our intention to combine behaviour with electrophysiology necessitated a paradigm that would enable the collection of a large number of trials per session, with multiple repetitions of each unique stimulus condition. Furthermore, it was important for the testing apparatus to provide stability of the animal's head and eye position so that the stimuli remained at a relatively constant location in the animal's visual field. These factors all ultimately shaped our animal selection, task design, and apparatus design.

3.1.3 Species and strain

When selecting an animal species for a complex visual task, there are a number of factors to consider. These include the experimental techniques and infrastructure, the characteristics of the animals' visual system, their cognitive capacity and the practicality of using the animal (Reinagel, 2014). The relative importance of each of these factors will depend on the specific question to be addressed. For our investigation of visual masking, we selected the Long Evans rat. Below we discuss the factors that shaped this decision.

3.1.3.1 Experimental techniques and infrastructure

Non-human primates possess a very similar visual system to that of humans, and are therefore useful for research in visual neuroscience (Newsome et al., 1989; Britten et al., 1992; Read and Cumming, 2003; Williams and Shapley, 2007; Nienborg and Cumming, 2010). However, they are large and costly to maintain, which places significant restraints on population size. We searched for a cheaper alternative with good experimental options and training prospects for complex behaviour. Besides non-human primates, other common animal models for investigations of visual neuroscience include cats, ferrets, tree shrews, squirrels and degus, which have highly-developed and often cone-dominant, visual systems (Petry et al., 1984; Gilbert and Wiesel, 1990; Chapman et al., 1991; Van Hooser et al., 2005; Tolhurst et al., 2009; Reinagel, 2014; Zaltsman et al., 2015). However, many of these species are difficult to train, and lack the tools for genetic manipulations. Although rats and mice have a comparatively impoverished visual system (Uhlrich et al., 1981; Prusky et al., 2002; Busse et al., 2011; Niell, 2011; Histed et al., 2012; Katzner and Weigelt, 2013), they provide the greatest range of experimental options and the most established experimental infrastructure (e.g. breeding colonies, genetic libraries and anatomical atlases) (Carandini and Churchland, 2013; Reinagel, 2014). Apart from their cost, the primary advantage of rats and mice has been their suitability for large-scale genetic manipulations, with relatively simple tools for labelling and identifying specific neuron types, exerting optogenetic control over neuronal activity (Andermann et al., 2010; Marshel et al., 2011; Lee et al., 2012; Froudarakis et al., 2014; Juavinett and Callaway, 2015), and mimicking various disease models (Bourne et al., 1938; Chambers et al., 1996; Umeda, 2010). More importantly, rats and mice have been shown to be capable of learning and performing a variety of visually-guided tasks in a relatively short period of time (Andermann et al., 2010; Busse et al., 2011; Meier et al., 2011; Histed et al., 2012; Tafazoli et al., 2012; Vermaercke and Op de Beeck, 2012; Vinken et al., 2014). Due to the considerable experimental opportunities and proven capabilities for behavioural training, we place particular focus on the advantages and disadvantages of rats and mice in the following sections.

In many cases, it is useful to be able to head-fix the animal. This allows visual stimuli to be presented in known locations relative to the animal's eyes, but also limits motion artefacts and ensures physical stability for experimental techniques such as optical imaging and electrophysiological recordings. Although head-fixation has been successfully achieved in both rats and mice (Hadlock et al., 2007; Mayrhofer et al., 2013; Guo et al., 2014; Roh et al., 2014), head-fixation training is substantially easier in mice as they have a smaller body weight and are weaker than rats, meaning that they cannot exert as much mechanical stress on the head-bar (Schwarz et al., 2010). In rats, successful head-fixation requires a large number of cortical screws (up to 13), with roughly half located very laterally, as well as the use of strong adhesives such as metabond and dental cement (Schwarz et al., 2010). Despite these measures, the longevity of these implants is limited with many studies indicating an expected lifespan of up to three months (Parry and McElligott, 1993; Bermejo et al., 1996; Schwarz et al., 2010; Chaniary et al., 2011). From our own experience of head-bar implantation across 12 male Long Evans rats, we found that 11 animals removed their head-bar during head-fixation training within a month of implantation, a timeframe that did not enable the completion of behavioural training, let alone data collection in the final task. We attempted head-fixation using three different surgical strategies that included up to 10 cortical screws (GVP cortical bone screw, 1.5 x 6 mm) combined with vetbond and dental acrylic. The implant failures were not likely the result of a bad surgical strategy, as we found no evidence of infection, and part of the skull was often removed along with the head-bar. We also took many measures to reduce the animal's anxiety during head-fixation training, including gradual habituation to the equipment and handler, delivery of rewards during head-fixation and the use of a sand blanket and snug enclosure for their body. Based on the limited lifespan of head-fixation implants in rats, we suggest it is worth considering alternative head stabilization strategies, especially for any long-term behavioural study where training and data collection are expected to extend across several months.

3.1.3.2 Rodent visual system

The structural organisation, specializations and acuity of the visual system vary greatly between rodent species (Prusky et al., 2002; Van Hooser et al., 2005; Wong and Brown, 2006; Busse et al., 2011; Histed et al., 2012; Reinagel, 2014). Given that visual masking requires the use of very brief stimuli (<100 ms), it was essential that we selected a species with adequately high temporal acuity. On the other hand, the contrast sensitivity and spatial acuity of the animal can be accommodated by selecting appropriate stimulus parameters, although ideally, we want the animals to have high contrast sensitivity and spatial acuity, allowing a larger range of stimuli to be used in testing.

Unfortunately, there has been no investigation into the temporal acuity of rodent vision. However, Long Evans rats are capable of detecting Gabors presented for 33 ms (Kurylo et al., 2015), suggesting that their temporal acuity is adequate to investigate visual masking. An additional benefit of Long Evans rats is that they possess higher spatial acuity than most rat strains, detecting gratings of up to 1 cpd (at 97% contrast and 43 cd/m^2 mean luminance) in at least 70% of trials compared with 0.5 cpd for albino rats and pigmented mice (Prusky et al., 2000; Prusky et al., 2002; Wong and Brown, 2006). This means that a wider range of spatial frequencies can be used to test Long Evans rats. To our knowledge, the only strain of rat that offers higher spatial acuity is the Fischer-Norway strain (threshold 1.5 cpd), however, these are difficult to obtain and their temporal acuity is also unclear. A further advantage of the rat visual system compared to that of mice, is that the median preferred spatial frequency of V1 neurons lies around 0.1 cpd, which is more than double that of mice at 0.035 cpd (Girman et al., 1999; Niell and Stryker, 2010). Thus, it would be challenging to investigate spatial context in mice, as a single cycle of a grating would need to span a large portion of the visual field (~30°). To present multiple cycles at these low spatial frequencies would require stimuli to be presented on wide-screen monitors positioned close to the animal. Given that most monitors are flat, this would introduce an undesirable stretching of the image towards the periphery where luminance and contrast can vary significantly (Ghodrati et al., 2015).

3.1.3.3 Comparing the cognitive abilities of rats and mice

There are a number of factors that have led us to believe that rats may be better suited to performing complex visual tasks. Between species, cognitive ability has been positively correlated with encephalisation, that is, brain size that is larger than expected for the animal's body size (Rushton and Ankney, 2009). Given that the degree of encephalisation (measured as neuronal index: total number of excess neurons) is larger in rats ($13.51 \pm 33.80 \times 10^6$) than in mice ($-1.68 \pm 10.83 \times 10^6$), it might be expected that the cognitive ability of rats is superior to that of mice (Herculano-Houzel, 2007). Supporting this notion, some reports have specifically stated that, compared to rats, mice were difficult to train for a simple visual task (Reinagel, 2014). Although mice have been proven capable of learning tasks such as contrast detection (Busse et al., 2011; Histed et al., 2012), orientation discrimination (Lee et al., 2012) and object position discrimination (Bussey et al., 2012), mice have never before been trained in tasks as complex as those of rats, for example, where they are required to detect small and brief stimuli, presented among of distractors of similar appearance. Thus, it is unknown whether mice are capable of learning similar tasks. Furthermore, even for simple grating detection tasks, training mice often requires strict water scheduling to maintain the animals at 85-90% of their normal body weight. Despite these measures, as many as half of the cohort may still be unable to reach criterion for the final task (Histed et al., 2012). While this particular study may have had a

high failure rate because the methods for animal training were quite exploratory, the high animal rejection rate is cause for significant concerns about bias in subsequent behavioural reports; such failure rates would probably be deemed unacceptable in behavioural studies with humans, or non-human primates. Investigations of visual behaviour in rats have mostly elected to use Long Evans rats (Meier et al., 2011; Meier and Reinagel, 2011; Tafazoli et al., 2012). This is most likely because along with their relatively high visual acuity, they have a good temperament and are naturally more inquisitive than other strains of rat, thus making them easier to train (Schwarz et al., 2010). Altogether, the proven capability of Long Evans rats in complex visual tasks makes them an attractive choice for any behavioural study in rodents.

3.1.4 Task Design

A key consideration when designing a behavioural experiment is the structure of individual trials of the psychophysical task. An ideal task will allow the researcher to collect a large number of trials per session, maintain rodent motivation, minimize response bias and ultimately quantify the limits of psychophysical performance. Below we discuss the advantages and disadvantages of three common psychophysical paradigms, the Go/No-Go, Yes/No, and 2-Alternative Forced-Choice (2AFC) task designs.

3.1.4.1 Go/No-Go

The simplest psychophysical task design is the Go/No-Go design, where subjects report the presence of a stimulus (or a specific stimulus attribute) by making an operantly conditioned response, (e.g. an eye movement, nose-poke, lever press or lick) and report the absence of the stimulus by withholding the response. The structure results in four possible response outcomes: hit, miss, false alarm and correct reject (Figure 3.2). The simplicity of the task structure makes for fast and easy training which is likely the major motivation for its use in a number of rodent studies (Andermann et al., 2010; Histed et al., 2012; Lee et al., 2012).

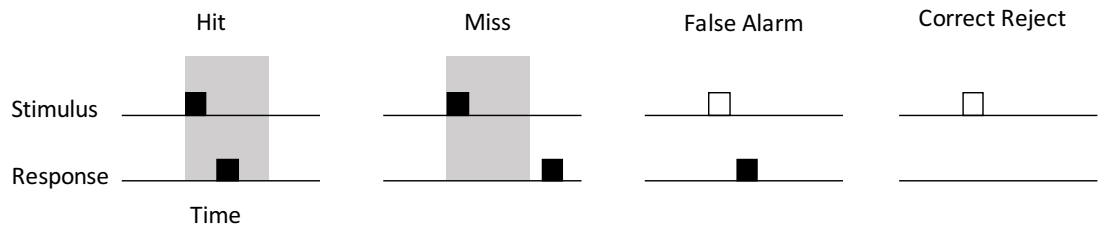

Figure 3.2. Schematic representation of the possible response outcomes in a Go-No-Go task. The gray window indicates the response window. Note that the unfilled boxes for false alarm and correct reject represent the absence of the stimulus attribute for which the go response is rewarded.

The key limitation of the Go/No-Go task is its high susceptibility to changes in motivation and response bias, which have the potential to alter responses in misleading ways (Carandini and Churchland, 2013). If an animal has a high tendency to respond, then it will be biased towards hits rather than misses, and false alarms rather than correct rejects. This in itself is not a problem, as analyses such as d' explicitly compare these metrics. However, d' analyses cannot account for changes in bias throughout a single session. Thus, if the animal become less motivated to respond as the session progressed, it would appear as though the animal was getting worse at detecting the stimulus. This is particularly problematic for Go/No-Go tasks where the animals have no control over when the trials are initiated, where trials are simply presented at regular intervals and the animals are then required to respond in the appropriate time window. Obviously as the animals become satiated they become less likely to respond appropriately. Therefore, the inclusion of a trial initiation step, for example where the animals must press a lever to start the next trial, can help to reduce the issue of low motivation, as, in theory, they will be less likely to initiate a trial if they are not interested in performing the task. In addition, there are a few methods that can allow for these unwanted influences to be monitored throughout the task. The motivation of the animal can be monitored via the inclusion of 'easy trials', or stimulus conditions that elicit a hit at a reliably high rate (Schwarz et al., 2010). Therefore, consecutive misses in these trials can be interpreted as the animal having insufficient motivation to reliably perform the task. It is also possible to monitor an animals' bias to respond by including catch trials, which are trials that do not include any stimulus at all. In these trials, it is possible to measure the rate at which the animal spontaneously produces the operantly conditioned response as part of a random-guessing strategy or because they are impatient to respond (false alarm rate). It should be noted, that by monitoring these unwanted behaviours, the contribution of these influences in the remaining trials can only be inferred. The true extent to which correct hits are confounded by a bias to respond, or misses are confounded by a loss of motivation, cannot be determined.

Another disadvantage of the Go/No-Go task is that there is some uncertainty surrounding the ideal strategy for providing feedback (Schwarz et al., 2010). It is common to reinforce any desired behaviours with food or liquid reward and, sometimes to discourage inappropriate behaviour by punishing the animals with a time-delay until the next trial, an air puff or an aversive-tasting stimulus (Meier et al., 2011; Lee et al., 2012). However, in a Go/No-Go task, rewarding correct rejects and punishing misses runs the risk of confusing the animal by providing contradictory feedback between trials where their perceptual experience (no stimulus presented) was the same, even though the physical stimulus differed. Alternatively, an asymmetric approach of rewarding hits but not correct rejects, and punishing false alarms but not misses, means that there are many trials that do not receive feedback, thus slowing the training process.

Finally, the Go-No-Go task may not be an ideal behavioural task for studies that wish to simultaneously record neuronal activity, as producing a response and withholding a response present confounding differences in motor planning and likely activate different circuits (Carandini and Churchland, 2013). Some of these issues are resolved in the Yes/No and 2-alternative-forced choice design.

3.1.4.2 Yes/No

The Yes/No task, or one-interval task, requires that animals report the presence or absence of a stimulus via the same type of operantly conditioned response (Meier et al., 2011; Meier and Reinagel, 2011; Meier and Reinagel, 2013). Ideally, to minimize any differences in response times or motor planning, these responses would be carried out in a symmetrical manner, for example, lick at the left waterspout if the stimulus were present and at the right waterspout if it were absent. The Yes/No task can also be used as a discrimination task, where the animal must report the presence of a particular stimulus attribute among other options. In the case that only two stimulus attributes are possible, this may sometimes be referred to as a 2-alternative forced choice task. In general, the advantages and disadvantages of these two task designs are the same (see discussion below).

3.1.4.3 2-alternative forced choice task

Although the terminology can be overlapping, the 2-alternative forced choice task (2AFC) is sometimes distinguished from the Yes/No task by the number of stimuli presented per trial. Traditionally in the 2AFC design, the trials include two stimuli that are presented either simultaneously at different positions or in quick succession (i.e. two intervals) at the same position. Thus, the 2AFC design is well suited to discrimination tasks (Tafazoli et al., 2012; Vermaercke and Op de Beeck, 2012; Alemi-Neissi et al., 2013; Vinken et al., 2014; Bossens and Op de Beeck, 2016).

The greatest advantage of the 2AFC task design over the Yes/No task design is that the animals cannot have a bias for the presence or absence of a stimulus, as in each trial they are assigning the presence of a stimulus to a particular location or interval, while assigning the absence of a stimulus to the other location/interval. However, in both tasks, a bias to respond in a particular way (e.g. go to the left waterspout) is easily detected. In theory, these tasks are also robust to changes in motivation, as the animal is always required to respond (Schwarz et al., 2010). Thus, if the animal lacked motivation to perform the task, they would not respond and the trial would be excluded from analyses. However, it is not necessarily so simple, there are still a number of ways that the data may be affected by changes in motivation and impulsive behaviour. For example, the animals may be sufficiently motivated to initiate a trial and respond, but not to pay attention to the stimulus that informs the decision. In this way, some trials reflect an informed decision while others reflect a random-guessing strategy, thus adding noise to the data and flattening the slope of the psychometric curve. For discrimination tasks, there is no simple way to estimate the proportion of these guesses, however in a detection task, catch trials like those described for the Go/No-Go task can serve to monitor this behaviour. It is also common for animals to adopt a selection strategy where they will initiate a trial but only respond if it was easy, thus aborting a higher proportion of trials that are difficult. To some extent, this behaviour can be discouraged by replacing the aborted trials into the pool of trials to be randomly re-selected later within that session, however this strategy relies on the animal performing a predictable number of trials. Ultimately, any aborted trials, although easily excluded from analyses, may still influence the animal's behaviour in the following trials (Gold et al., 2008; Busse et al., 2011; Abrahamyan et al., 2016).

3.1.5 Apparatus design

The final consideration for a behavioural experiment is the design of the apparatus and the mode of response. This will depend largely on whether the animals will be head-fixed or able to move freely around the chamber. Regardless, the size of the testing chamber should be small: in head free tasks this limits the time animals spend exploring and yields a greater number of trials per session (Gharaei, 2015); in head-fixed studies, a snug chamber helps to reduce anxiety (Schwarz et al., 2010).

There are many methods that have been used to obtain perceptual reports from rodents, the most popular include licking at a waterspout or pressing a lever. Ideally the mode of response should be easy to train, which will be largely governed by the animal's natural inclination to produce the response. In our experience, training animals to lick at a waterspout is faster than training them to push a lever, presumably because a thirsty animal is naturally inclined to lick at a spout that delivers a liquid reward. However, it can be helpful to distinguish actions of perceptual report from actions of reward acquisition (Carandini and Churchland, 2013). In head-fixed animals, this is best achieved by using actions that they can carry out with their front paws, such as pressing a lever, pulling a spout-lever, or moving a ball or wheel in a particular direction (Histed et al., 2012; Kimura et al., 2012; Sanders and Kepecs, 2012). Locomotion can also be used for perceptual report in head-fixed studies if the animal is placed over a treadmill or trackball (Youngstrom and Strowbridge, 2012). In addition to training an action for perceptual report and reward acquisition, if the animal is also required to initiate the trials, then this will require yet another type of action. In a simple Go/No-Go detection task, this can be achieved by training the animal to press a lever for trial initiation, release the lever for perceptual report and lick a spout for reward acquisition (Histed et al., 2012). However, in a Yes/No or 2AFC task, the animal must be trained to perform multiple motor responses (e.g. pressing multiple levers) and as such the inclusion of an additional action for trial initiation can be difficult to accommodate in a head-fixed paradigm without further complicating and lengthening the training.

In head-free behaviour, a common apparatus design includes a chamber with two or three ports located along the viewing wall, a central port for trial initiation and flanking ports for perceptual report. In this design, the animal usually responds by licking a spout, touching a screen, or blocking a photo-interrupter sensor with its nose (Meier et al., 2011; Alemi-Neissi et al., 2013; Petruno et al., 2013). While this design does not offer the same stability for viewing stimuli as a head-fixed paradigm, it is still possible to obtain reasonable head-stability during stimulus presentation. Using a photo-interrupter sensor, animals

can be easily trained to maintain a steady nose-poke during stimulus presentation (Lee et al., 2016). Under these conditions, any small head movements are compensated by pupil movements, thus the image remains stable in the visual field (Gharaei, 2015). Another option is to stabilize the head position with a surgically implanted structure that the rodents are trained to lock into place during stimulus presentation (Scott et al., 2013). Either way, it is possible to combine head-free behaviour with electrophysiological and imaging techniques.

3.2 Methods and Results

Visual masking describes a phenomenon in which the perception of a target stimulus is impaired by a closely preceding or succeeding mask stimulus. The magnitude of this masking effect can be modulated by systematically altering the stimulus onset asynchrony (SOA) or contrast of the target and mask stimuli. Thus, it provides a powerful tool to investigate the neuronal mechanisms of perception. In order to determine if rodents provide an appropriate model for the investigation of perceptual masking, we designed two tasks: a 2-alternative discrimination task, and a 2-alternative detection task. Although rats have been proven adequate for some complex visual tasks, the precise limitations of rodent behaviour are unclear. The study of visual masking necessitates the use of brief target and mask stimuli presented in quick succession. If perceptual masking occurs similarly in rodents to that of humans, on some trials the rats may perceive a single fused image containing elements of both the target and mask while on other trials they may perceive two stimuli, one of which they are expected to ignore. Thus, the rules of response for our visual masking tasks are arguably more complex than any other that has been employed in a rodent model. Below we describe for both tasks, the final behavioural paradigm, the sequence of training steps used to shape rodent behaviour, and the animals' performance at each stage of training.

3.2.1 Ethics

All experimental procedures involving animals were approved by the Monash University Committee for Ethics in Animal Experimentation (MARP/2013/81; MARP/2013/130) and were conducted in accordance with the National Health and Medical Research Council guidelines for the care and welfare of experimental animals.

3.2.2 Subjects

Twenty Long Evans rats were trained to perform a discrimination (n=10) or detection (n=10) task. Rats were obtained at 6-8 weeks of age and were group-housed with a 12:12 hr reverse light-dark cycle. Rats had access to food *ad libitum,* but water consumption was restricted to rewards obtained during training as well as a period of 2-hours *ad libitum* access following the last training session for the day. Training sessions were run twice daily, five days a week. On non-testing days rats had *ad libitum* access to water.

3.2.3 Apparatus

Our experimental apparatus consisted of a custom-made Plexiglas chamber (20x30x30 cm) with three small holes located in the front viewing wall of the chamber through which we placed photo-interrupter detectors (Little Bird Electronics, GP1A57HRJ00F) to monitor animal behaviour. This design enabled the number of detectors to be easily manipulated throughout training and between different task designs (Figure 3.3). In both our detection and discrimination tasks, the central sensor was used for trial initiation and the flanking sensor/s for perceptual report. The flanking sensors also incorporated a 16-guage stainless steel tube connected to a computer-controlled syringe pump (New Era Pump Systems, NE-500) for reward delivery. Stimuli were presented on a 120 Hz LCD monitor (Samsung 2232RZ or Eizo FG2421) presented 25 cm from the viewing wall of the chamber. Photointerrupter outputs were sampled at 120 Hz (Measurement Computing, USB 1208FS) by custom MATLAB scripts, which also registered rat behaviour, controlled stimulus presentation and administered rewards or timeouts.

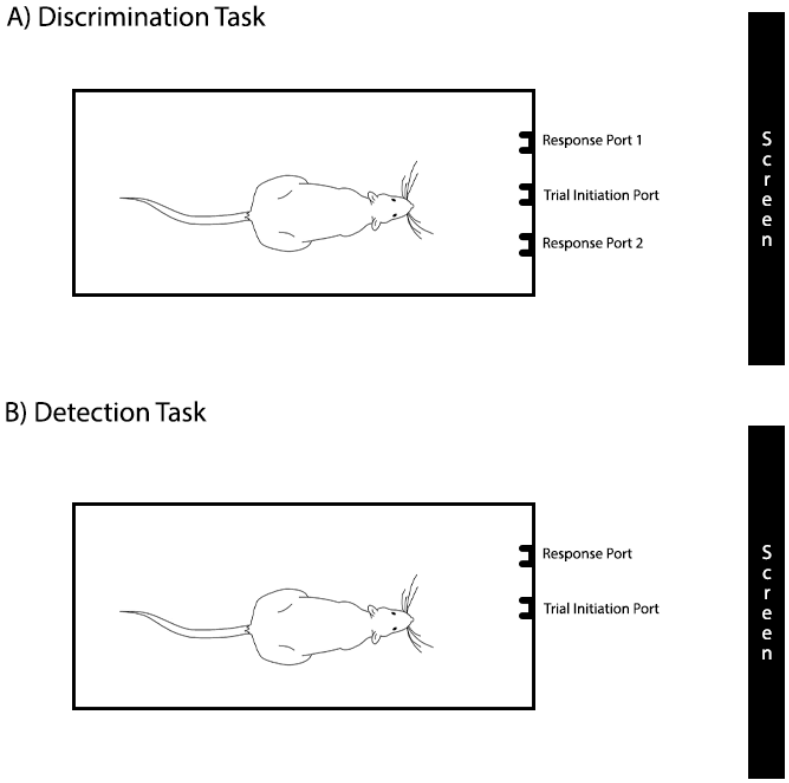

Figure 3.3. Apparatus Design for our Visual Masking A) Discrimination and B) Detection tasks. For both tasks, the central sensor was used to initiate trials and view stimuli; the flanking sensors were used for perceptual report. In the discrimination task the flanking sensors were used to report the target orientation, which was either horizontal or vertical. These orientations were arbitrarily allocated to each of the flanking sensors. In the detection task the response port was used to indicate the presence of the target, which either appeared early (400 ms delay from trial onset) or late (1200-1300 ms delay) in the trial.

3.2.4 Discrimination Task

In order to determine if visual masking affected the ability of rodents to discriminate target orientation, we designed a 2AFC discrimination task in which rats were required to report the orientation of horizontal and vertical target gratings/Gabors by going to the appropriate flanking report sensor. Rats were trained in two cohorts: in the first, we used a version of the task that allowed us to examine reaction times; in the second, trials had fixed durations to remove the confounding influence of impulsivity that was observed in the reaction time version. The second cohort was also trained to perform the task with two spatial configurations of the target and mask stimuli, spatially overlapping and then centre-surround. As both cohorts were trained with spatially overlapping stimuli the details of the training steps focus on this spatial configuration in particular. For all versions of the task, the target and mask contrast were held constant across trials and the SOA was varied. Below we describe the details of the stimuli, the task design and the

training protocols used to shape the animal's behaviour for this task. In some instances, the training procedures reflect the outcome of multiple rounds of trial and error, as we attempted to optimise training with each animal. On these occasions, we specifically highlight the advantages associated with the described methods over the alternative strategies that were trialled.

3.2.4.1 Stimuli

Spatially Overlapping: Target stimuli were Gabors with contrast 100%, orientation 0 or 90°, spatial frequency 0.1 cycles/degree and random phase (mean luminance: Samsung – 113 cd/m^2; Eizo - 79 cd/m^2). The space constant, defined as the standard deviation of the Gaussian applied to the contrast envelope, was adjusted between 6-18° according to the abilities of each rodent. Mask stimuli were a plaid created by summing the two target orientations and were either 20 or 40 percent contrast, held constant a block of training sessions.

Centre-surround: Target stimuli were circular gratings with contrast 100%, orientation 0 or 90°, spatial frequency 0.1 cycles/degree and random phase (Figure 3.4). Mask stimuli were 100% contrast, full screen plaids created by the sum of both target orientations, with an aperture matching the size and location of the target stimulus.

Regardless of the spatial configuration, target and mask stimuli were presented for 42 ms, and when no stimuli were being presented a blank gray screen was displayed.

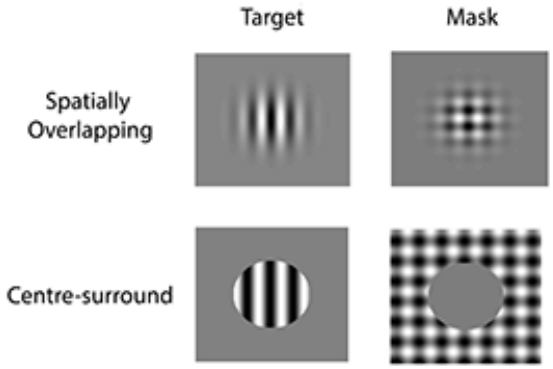

Figure 3.4. Example stimuli for our final orientation discrimination task. Target and Mask stimuli were presented with either spatially overlapping (top) or centre-surround spatial configurations.

3.2.4.2 Final task design:

Rodents were trained to perform a two-alternative forced-choice discrimination between horizontally and vertically oriented Gabors or gratings. A nosepoke at the central sensor initiated a trial and, following a brief pre-stimulus delay, a target and mask were presented at one of 13 stimulus onset asynchronies: -333 to 333 ms (Figure 3.5). For the trial to be considered valid the nosepoke had to be maintained until the end of the trial, which was indicated by a 3.3kHz tone, and the rodent's response had to occur within the allowed response window (16.6 s from the time that the central sensor was exited). Note that in the fixed-duration version of the task, trials included a post-stimulus delay in which only a blank screen was visible. For the reaction time version of the task, the trial ended at the completion of the final stimulus.

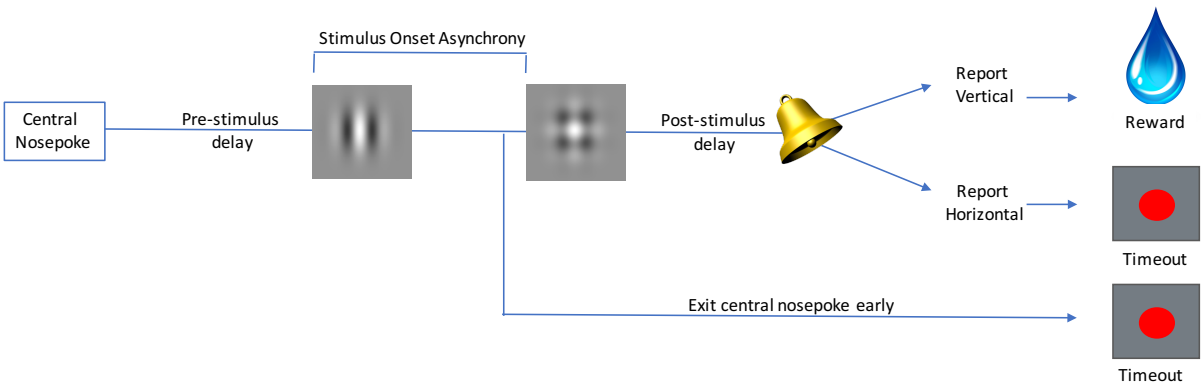

Figure 3.5. Schematic of a backward masking trial for the orientation discrimination task.
A nosepoke at the central sensor triggers the onset of a trial. After a pre-stimulus delay the target and mask are presented at one of 13 stimulus onset asynchronies. A 3.3 kHz tone indicates the end of the trial. Rats are then able to exit the central sensor and report their perceived orientation at one of the flanking sensors. Target orientation was arbitrarily assigned to the flanking sensors for the duration of the study. Correct responses were rewarded with sucrose solution. Incorrect responses were not rewarded and received a timeout, delaying the possible onset of the next trial. If the central nosepoke was aborted prior to the end of the trial, stimulus presentation was the abandoned and a timeout ensued.

3.2.4.3 Training Phases

Five phases of training were used to shape behaviour: 1) Nosepoke-reward association; 2) Two alternative training; 3) Patience and Orientation association; 4) Shorter target and 5) Mask introduction.

1: Nosepoke-Reward Association

Aim: In the first stage of training, we wanted animals to develop an association between performing a nosepoke at the central sensor and reward acquisition.

Apparatus: The experimental chamber was fit with a central sensor and waterspout. The flanking sensors were not introduced into the chamber to avoid confusion.

Training Process: The animal was placed into the chamber and several rewards were manually delivered so that the waterspout had a drip of sucrose water at the tip. We found that this increased the rats' interest in the central sensor and thus the rate at which they learned the association. During this phase of training, the rats only needed to block the central sensor for 10 ms to obtain a 50 µl reward, which was inevitable if they tried to lick the spout (Figure 3.6). If they maintained the nosepoke, then rewards were delivered constantly at 100 ms intervals. The session ended if the rats obtained 200 rewards, or if they had been in the chamber for 30 minutes. If the rats demonstrated a clear association between the central sensor and reward acquisition, then in the next session the delay between rewards was increased to 1 s.

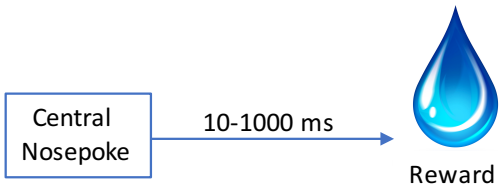

Figure 3.6. Schematic of a trial during the Nosepoke-reward association phase of training. A nosepoke at the central sensor triggered reward delivery after a fixed time delay.

Criteria to progress: Animals progressed to the next phase of training if they were able to obtain 200 rewards within a 30-minute window for two consecutive sessions.

Required training duration: ~2 days (4 sessions).

2: Two-Alternative Training

Aim: Once the rats demonstrated an association between the central sensor and the reward acquisition, they were trained to perform two nosepokes in order to achieve a reward: one nosepoke at the central sensor followed by a nosepoke at one of the flanking sensors.

Apparatus: Both flanking sensors were introduced into the front viewing wall of the chamber. All sensors incorporated a spout for reward delivery, however only the flanking sensors were connected to the

computer-controlled syringe pumps. We found that the novelty of the newly introduced sensors increased the animals' exploration time at the sensors and thus the rate at which the rats learned this phase of training. If the animals lost interest in the central sensor, manual rewards could be delivered via handheld syringe.

Training Process: The animal was placed into the chamber and allowed to explore the sensors. The central sensor was activated by a brief 10 ms nosepoke, which triggered the presentation of a large target Gabor or grating of horizontal or vertical orientation. Orientation was arbitrarily assigned to the report sensors so that if the stimulus was vertical a nosepoke at the left report sensor was rewarded and vice versa. The target stimulus remained on screen until a nosepoke was performed at the correct report sensor, which triggered the offset of the stimulus and reward delivery (Figure 3.7). Once the central sensor had been activated, any subsequent nosepokes at the central or incorrect report sensors were ignored and received no penalty. In our experience, it was important for this training phase to have orientation already assigned to the report sensors, because this avoided the animals developing a strong response bias, for example, where they abandoned going to the left report sensor altogether.

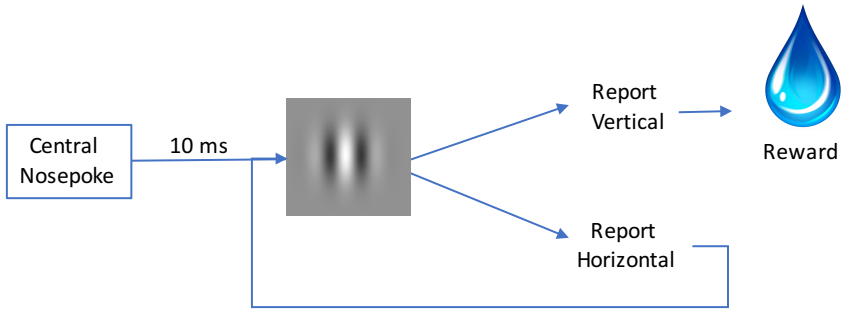

Figure 3.7. Schematic of a trial in the two-alternative phase of training. A nosepoke at the central sensor triggered the onset of a target stimulus. The target remained on screen until a nosepoke at the correct flanking sensor, which triggered the delivery of a sucrose reward. Target orientation was arbitrarily assigned to a flanking report sensor for the duration of the study.

Criteria to progress: Animals progressed to the next phase of training when they were able to obtain 200 rewards within a 30-minute period across two consecutive sessions.

Required training duration: ~2 days (4 sessions).

3: Patience and Orientation Association

Aim: In the third phase of training our aim was twofold: 1) to train the animals to steadily hold the central nosepoke for the trial duration that would be used in the final task and 2) for the rats to learn the association between target orientation and the report sensor direction (vertical=go left; horizontal= go right).

Apparatus: The reward spout was removed from the central sensor, meaning rewards could only be obtained at the perceptual report sensors. This was the final alteration of the apparatus during training.

Training Process: During this phase of training the duration that the animals were required to hold a nosepoke at the central sensor was gradually increased to 850 ms (Figure 3.8). Once performing a central nosepoke, the target appeared after a pre-stimulus delay, and remained on screen until a response was made. When the central nosepoke had been held for the required duration, a 3.3kHz tone sounded in order to indicate that the animal was allowed to exit the central sensor and report their decision. A nosepoke at the correct report sensor received a liquid reward while a nosepoke at the incorrect report sensor received a 2 s timeout, delaying the possible onset of the next trial. In the case that the rat exited the central sensor before the required length of time had passed, target presentation was abandoned (screen returned to gray) and no reward was received.

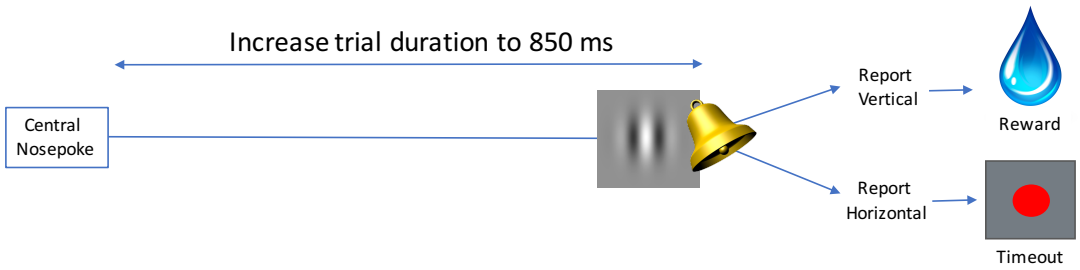

Figure 3.8. Schematic of a trial in the patience and orientation association phase of training. A nosepoke at the central sensor triggered the onset of a trial and after a pre-stimulus delay (which was increased throughout this phase of training) a target stimulus was presented. At the completion of stimulus presentation, a 3.3kHz tone indicated the end of the trial. A nosepoke at the correct report sensor received a sucrose solution reward while a nosepoke at the incorrect report sensor incurred a 2 s timeout, delaying the possible onset of the next trial.

Criteria to progress: The rats progressed to the next phase of training when they were able to achieve a threshold criterion of 70% correct discrimination of target orientation at trial durations of 850 ms, in two consecutive sessions.

Required training duration: 8-14 days (16-28 sessions).

Rodent exclusion: Three rats of the initial cohort of ten never learned to discriminate target orientation, failing to perform above chance after 21-31 days of training and thus training was abandoned for these animals.

4: Shorter Target

Aim: In order to improve the likelihood of visual masking occurring in the final task, we aimed to decrease the duration of the target.

Training Process: During this phase of training, the duration of the target was gradually reduced to 42 ms (Figure 3.9). In our fixed duration task, to maintain the trial duration, a post-stimulus hold was introduced after the target whereby the screen returned to gray for a period of time, before a 3.3 kHz tone indicated the end of the trial. Both the pre- and post-stimulus hold times varied by up to 100 ms between trials so that the timing of target onset was unpredictable. In our reaction time task, there was no post-stimulus period, the trial duration was consequently shortened as the target duration was decreased, and the trial ended at the completion of target presentation, which was also indicated by a 3.3 kHz tone. We found it was important that we did not start the training process with short duration (42 ms) stimuli because of the low likelihood of the animals noticing the stimuli and developing an association between orientation and report sensor.

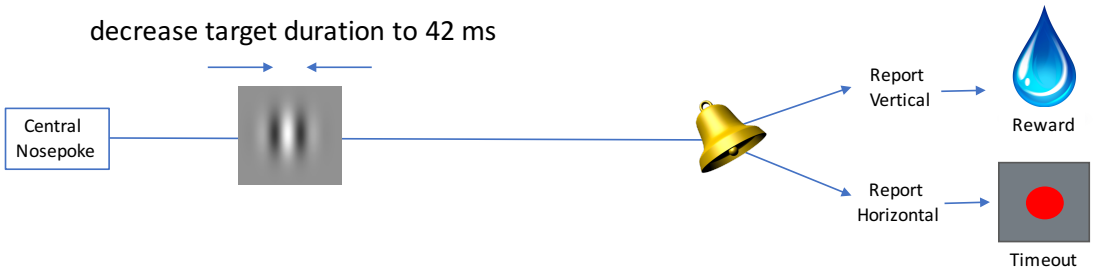

Figure 3.9. Schematic of a trial in the shorter target phase of training. A central nosepoke triggered the onset of a trial. After a pre-stimulus delay a target stimulus was presented. The duration of the target stimulus was decreased throughout this phase of training. Note that the post-stimulus hold illustrated here was only present in the task with fixed trial duration. The trials in the reaction time version of the task ended when target presentation was complete. Following the 3.3kHz tone that indicated the end of the trial, a nosepoke at the correct report sensor received sucrose solution reward while a nosepoke at the incorrect report sensor incurred a 2 s timeout, delaying the next possible onset of a trial.

Criteria to progress: In order to progress to the next phase of training the rats were required to achieve 70% correct discrimination of 42 ms target stimuli, across two consecutive sessions.

Required training duration: 8-20 days (16-40 sessions)

5: Final Task- Mask Introduction

Aim: Once the rodents were reliably discriminating the orientation of a brief target stimulus, we introduced a plaid mask into the trials.

Training Process: Across sessions, the contrast of the mask was gradually increased from 0 to 20, 40 or 100% depending on the intended test contrast (Figure 3.10 & Figure 3.11). We found that introducing the mask for all stimulus onset asynchronies simultaneously worked better than gradually introducing the different timing conditions, as it prevented the animals from learning to ignore the mask based on a specific timing cue. The presentation of the mask made the task significantly more difficult for the animals to perform, thus for the 100% contrast mask condition, we also introduced some target-only trials to prevent the animals from becoming too discouraged and ceasing to perform the task. The size of the target was adjusted according to each animal's capabilities. The specific challenges we faced here were that the rats might begin to respond to the mask in forward masking (even though it was uninformative) or respond immediately after the target (so that they have seen the target, but their initial motor behaviour was not stimulus driven). Thus, even though they may ultimately make the correct decision, the trial is unrewarded because they abandoned the central nosepoke prematurely.

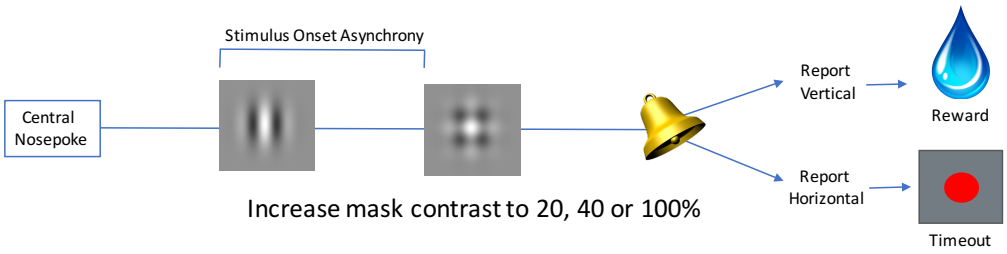

Figure 3.10. Schematic of a trial during the mask introduction phase of training. A central nosepoke triggered the onset of a trial. After a pre-stimulus delay, the target and mask were presented at one of 13 stimulus onset asynchronies. The contrast of the mask was gradually increased throughout this phase of training. Note the post-stimulus delay illustrated here was only present in the fixed duration version of this task. Trials in the reaction time version ended at the completion of the last stimulus presentation, the mask in this example trial. After the 3.3kHz tone indicating the end of the trial, a nosepoke at the correct report sensor received sucrose solution reward while a nosepoke at the incorrect report sensor incurred a 2 s timeout, delaying the possible onset of the next trial.

Criteria to progress: When the animals performed at or above our 70% threshold criterion at the required mask contrast, the animals were deemed ready for data collection in the final task.

Required training duration: 15-30 days (30-60 sessions)

Rodent exclusion: One rat never learned to discriminate target orientation in the presence of a mask, failing to perform above chance after 39 days of training. Thus, data was collected from only six rats for the final task.

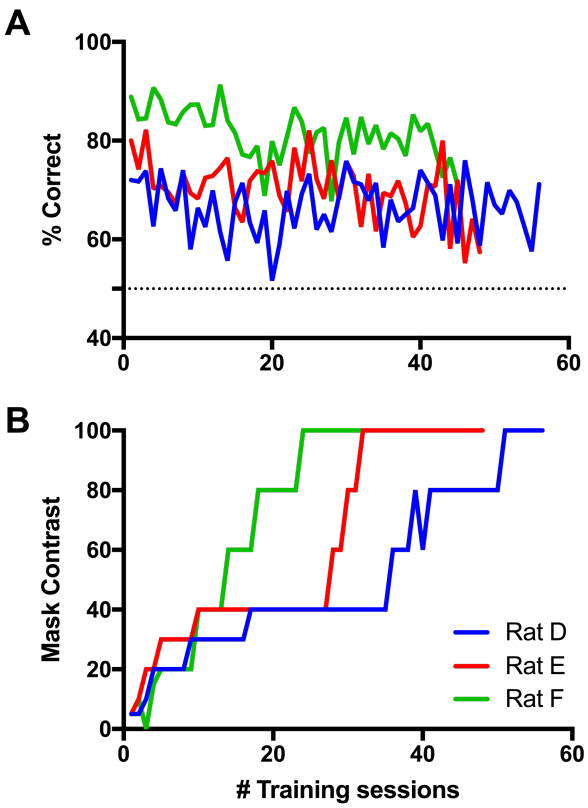

Figure 3.11. Discrimination performance throughout the mask introduction phase of training. (A) Discrimination performance (B) and mask contrast across sessions. Performance is shown only for the animals that learned to discriminate target orientation in the presence of a 100% contrast mask.

3.2.4.4 Timeline of Training

Altogether, we found that it took between 45-101 days for the animals' behaviour to be ready for data collection in the final task (Figure 3.12).

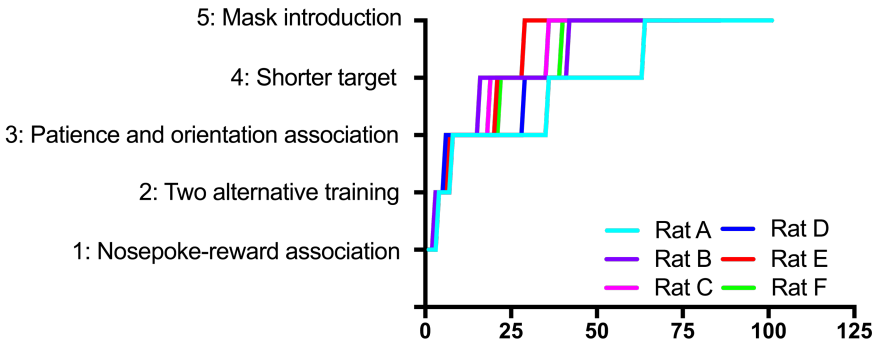

Figure 3.12. Progression of discrimination training across days. Animals underwent five phases of training to shape their behaviour for the final task. Generally, one day of training consisted of two training sessions, one in the morning and one in the afternoon.

3.2.4.5 Training for centre-surround stimulus configuration

Following data collection with spatially overlapping stimuli, the second cohort of rats (D, E & F) were trained to undertake the same discrimination task but with a centre-surround stimulus configuration. For this transition, the spatially overlapping mask was removed from all trials, the target Gabor was replaced with a circular grating and the surround mask was gradually introduced according to the same procedure outlined in phase 5.

3.2.5 Detection Task

In our discrimination task, we aimed to determine if the animals' ability to discriminate target orientation was impaired by a mask presented at varying stimulus onset asynchronies. Here we sought to determine if the animals' ability to detect target stimuli, regardless of their orientation, was impaired by a mask. To remove any timing cues, in this task we held the SOA between the target and mask at 50 ms and manipulated the contrast of the target between trials. Below we describe the details of the stimuli, task design and training protocols used to shape the animal's behaviour for this task.

3.2.5.1 Stimuli

In the final task, target stimuli were 51° diameter circular gratings with 0 or 90° orientation, spatial frequency of 0.1 cpd and random phase (Figure 3.13). Mask stimuli were full screen plaids (0 + 90°) with a 56° aperture positioned in the centre of the screen. Thus, there was a 5° separation between the outer edge of the target and the inner edge of the mask. Target stimuli were presented at screen centre for 48 ms and mask stimuli for 68 ms, with an SOA of 50 ms relative to the target. Between trials, the contrast of the mask was held constant at either 50 (rats 1, 4, 6 & 7) or 100% (rats 2, 3 & 5) while the target contrast varied between 6.25-100%.

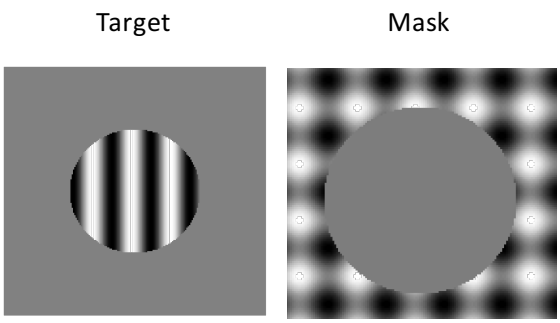

Figure 3.13. Example stimuli for our visual masking detection task.

3.2.5.2 Final task design:

Animals were trained to perform a two-interval forced-choice detection of a circular grating. Trials were initiated by a nosepoke at the central sensor. A 48 ms target stimulus was presented on every trial at either a 400 ms delay (early target) or 1200 (rats 1, 4 & 6) / 1300 ms (rats 2, 3, 5 & 7) delay (late target) from trial initiation. The time of the late target onset was determined by the duration of the response window, so that the early and late response windows were always separated by 100 ms. Two thirds of trials also included the presentation of a 68 ms mask stimulus at a 450 ms delay from the onset of the trial. Following the target onset, the animals had a 700 (rats 1, 4 & 6) or 800 ms (rats 2, 3, 5 & 7) response window to exit the central sensor followed by a 2 second window to report their detection at the flanking report sensor. Exits from the central sensor that were not followed by a nosepoke at the report sensor were ignored and excluded from analyses. If a target had been presented and the animal reported their detection in the allowed response window a reward was delivered. Incorrect detections, when the animal exited the central sensor outside of the allowed period and performed a nosepoke at the report sensor, received no reward and triggered a brief 3.3kHz error tone. The inter-trial interval was held constant at 2.9-3.1 seconds to discourage the animals from abandoning trials early. The trial structure and outcome are illustrated in Figure 3.14.

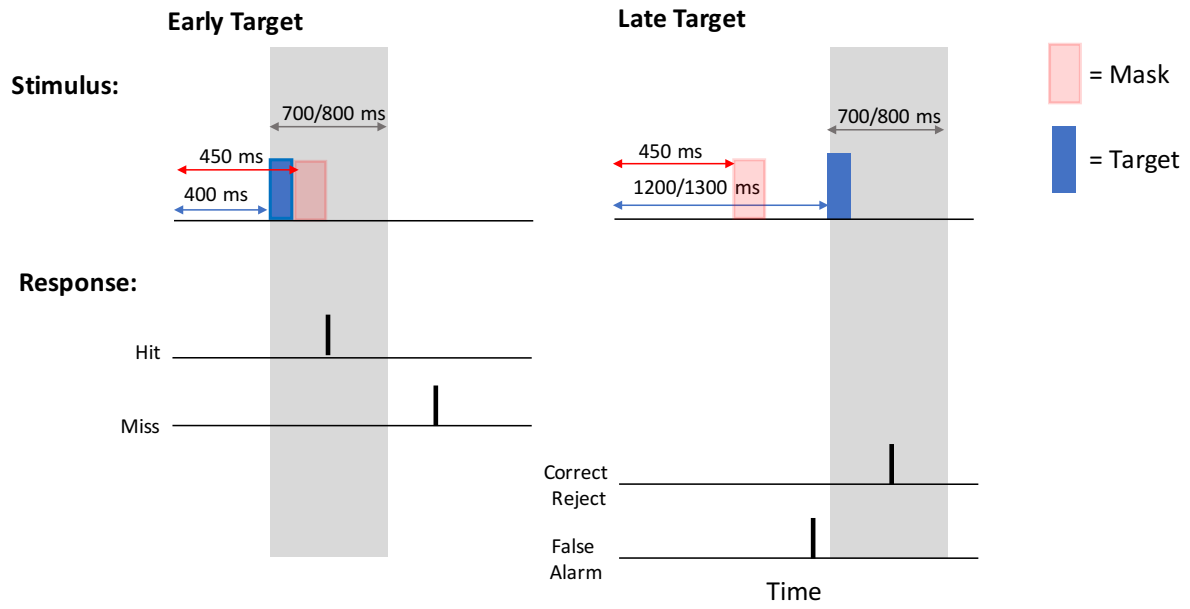

Figure 3.14. Structure of trials in the visual masking detection task and example response categorisation. Target stimuli (blue rectangles) are presented for 48 ms on every trial in either the early interval at a 400 ms delay from trial onset, or in the late interval at 1200 or 1300 ms from trial onset. Mask stimuli (red rectangles), are presented in two thirds of the trials for 68 ms at a 450 ms delay from trial onset. The gray shaded window indicates the allowed response window (either 700 or 800 ms). The black vertical lines indicate the time the central nosepoke was exited. Note that if the animal did not also activate the report sensor within a 2 s window after exiting the central sensor, the trial was not included in analyses.

3.2.5.3 Training Phases

Seven phases of training were used to shape behaviour: 1) Nosepoke-reward association; 2) Two nosepokes for reward; 3) Two Interval training; 4) Patience training; 5) Smaller and shorter targets; 6) Mask introduction; and 7) Variable target contrast.

1: Nosepoke-reward association

The initial step of training was the same as that outlined above for the discrimination task.

2: Two Nosepokes for Reward

Aim: Once the rats demonstrated an association between a central nosepoke and reward acquisition, they were trained to perform one nosepoke at the central sensor followed by a nosepoke at the flanking sensor to receive a reward.

Apparatus: The left flanking sensor was introduced into the front viewing wall of the chamber. Both the central and flanking sensor incorporated a spout for reward delivery, however only the flanking sensor was

connected to the computer-controlled syringe pumps. Manual rewards could be delivered via handheld syringe to the central sensor if the animals stopped performing central nosepokes. We found that the novelty of the newly introduced sensor increased the animals' exploration time at the sensor and thus the rate at which the rats learned this phase of training.

Training Process: In this phase of training the central nosepoke was activated by a brief 10-50 ms nosepoke, which triggered a 50 µl reward to be delivered at the flanking report sensor. When a reward was dispensed the delivery mechanism made a distinctive noise and rats exited the central sensor to go in search of the reward. Once the animals were regularly going between the central and report sensors, the contingency was changed so that a reward was only delivered if the perceptual report sensor was activated within 6 seconds of activating the central sensor (Figure 3.15). In the case that the rat maintained the central nosepoke, rewards were constantly delivered at the flanking sensor at 100 ms intervals. If the rats were able to achieve 200 rewards within a 30-minute period, the duration required to hold the central nosepoke was increased to 100 ms in the following session.

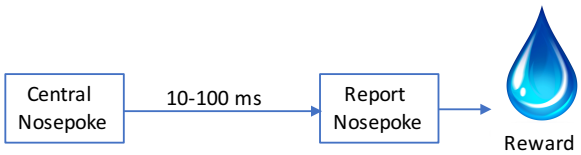

Figure 3.15. Schematic of a trial during the two nosepokes for reward phase of training. A nosepoke at the central sensor followed by a nosepoke at the flanking report sensor received a sucrose solution reward.

Criteria to progress: Animals progressed to the next phase of training when they were able to obtain 200 rewards within a 30-minute period across two consecutive sessions.

Required training duration: ~3 days (4 sessions). Note that due to the large number of animals, it wasn't always possible to train animals twice/day.

3: Patience Training

Aim: Once rats were successfully completing two nosepokes to receive a reward, we trained them to hold the central nosepoke for 1350 ms (the trial duration in the final task). In our experience, rats tend to respond impulsively, so it was important to train them to be patient as early as possible to expedite the later stages of training.

Apparatus: The waterspout was removed from the central sensor meaning rewards could only be obtained at the flanking sensor. This was the final alteration to the apparatus.

Training Process: The time that the animal was required to hold a nosepoke at the central sensor was gradually increased up to 1250 (rats 1,2 & 5) or 1350 ms (rats 3,4,6 & 7) (Figure 3.16 & Figure 3.17). In the final task design the target stimulus acted as a go-for-reward trigger. Therefore, we flashed a brief (100 ms) but full screen grating at the end of each trial to signal that the animal was allowed to exit the central sensor and go to the report sensor. To obtain a reward, the animals were required to exit the central sensor within 2 seconds of the target onset and perform a nosepoke at the report sensor within 6 seconds of exiting the central sensor. Note that at this stage of the task, it is impossible to distinguish whether the animals were responding using a timing cue (i.e. wait 1250/1350 ms) or a visual cue (i.e. wait for the grating).

In the case that the animal exited the central sensor prior to target onset (early abort), no reward was delivered. Early in this phase of training, when the total trial duration was very short, these early aborts did not incur a penalty and another trial could be initiated immediately. However, later in the training, a constant inter-trial interval of 2.9-3.1 seconds was introduced, meaning that the duration of the time delay penalty was inversely proportional to the time the trial was abandoned. The introduction of this constant intertrial period also allowed us to monitor nosepokes at the report sensor following early aborted trials, which enabled the distinction between accidental slips in the central nosepoke hold, and intentional exits.

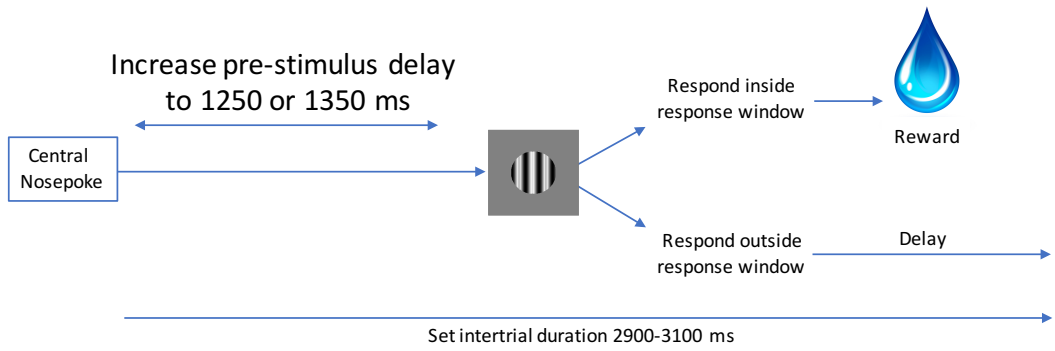

Figure 3.16. Schematic of a trial during the patience phase of training. A nosepoke at the central sensor triggered the onset of a trial. After a pre-stimulus delay, a brief but large target stimulus was presented, acting as a go-for-reward signal. The duration of the pre-stimulus delay was gradually increased during this phase of training. If the rats exited the central sensor within 2 seconds of the target onset and then activated the flanking report sensor within 6 seconds of exiting the central sensor, a sucrose solution reward was delivered. To discourage animals from exiting the central sensor prematurely the intertrial duration was held constant.

Criteria to progress: Rats progressed to the next training phase when they achieved the threshold criterion of 70% correct in two consecutive sessions. Only trials that included a nosepoke at the report sensor were included in this calculation.

Required training duration: 15-37 days (30-74 sessions)

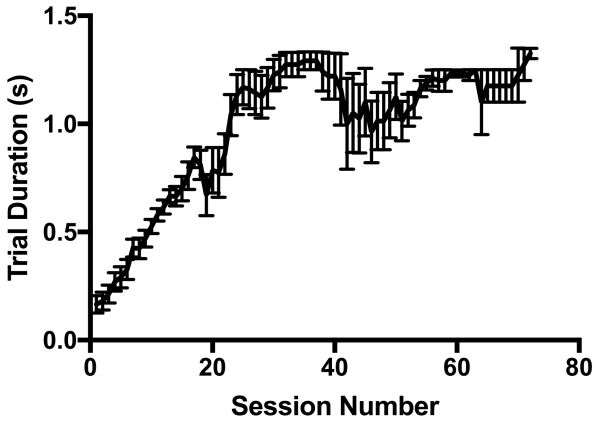

Figure 3.17. Progression of patience training for our visual masking detection task. The data represent the mean ± SE across ten animals, three of which were excluded from the final task due to time constraints that prevented the completion of their training.

4: Smaller and Shorter Target

Aim: In order to improve the likelihood of perceptual masking occurring in the final task design, we wanted target stimuli to be quite small and brief, allowing the presentation of a spatially surrounding mask. Thus, we next aimed to maintain the rodents' detection performance, while decreasing the size and duration of the target.

Training Process: During this phase of training, the duration of the target was reduced from 100 ms to 50 ms and the size from full screen to circular gratings of 51° diameter (Figure 3.18). In preparation for two-interval training, we also reduced the response window from 2 seconds to 700/800 ms. During this phase, the performance of rats 3 and 5 regressed and did not recover until the trial duration was decreased and then gradually increased up to the required duration. As such this phase of training was significantly longer for these two rats (**Figure 3.19**).

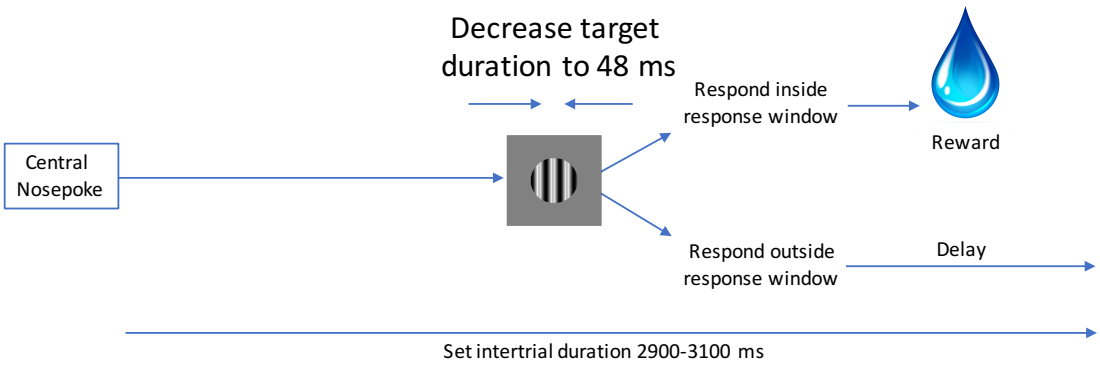

Figure 3.18. Schematic of a trial from the smaller and shorter target phase of training. A nosepoke at the central sensor triggered the onset of a trial. After a pre-stimulus delay, the target stimulus was presented. The size and duration of the target stimulus was reduced during this phase of training. From the onset of the target, if the rats exited the central sensor within 700/800 ms and then activated the flanking report sensor, a sucrose solution reward was delivered.

Criteria to progress: In order to progress to the next phase of training the rats were required to obtain a threshold criterion of 70% correct detection of 48 ms target stimuli of 51° diameter, across two consecutive sessions. Only trials that included a nosepoke at the report sensor were included in this calculation.

Required training duration: 2-6 days (4-12 sessions). Rat 3=21 days, Rat 5=13 days.

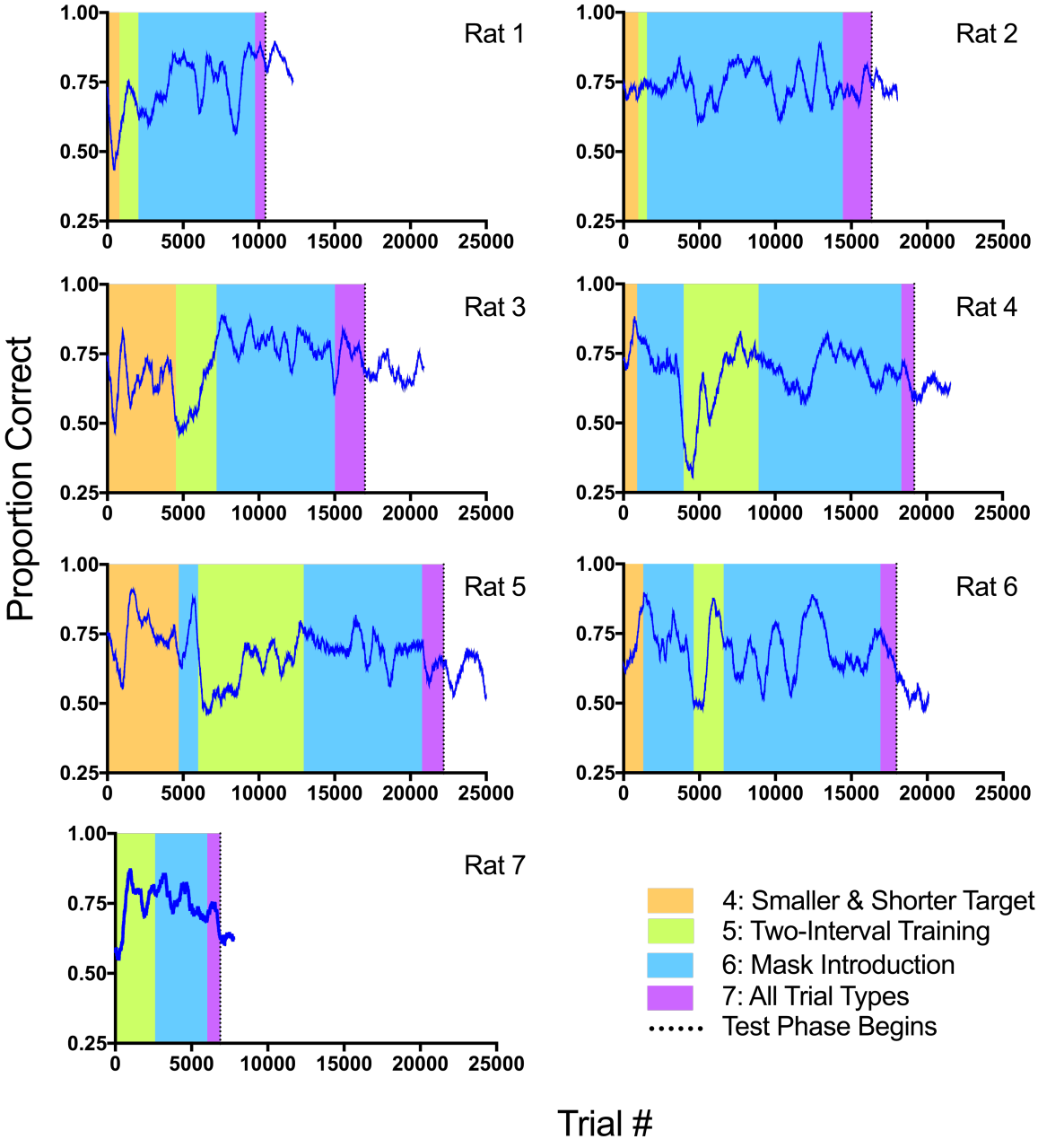

Figure 3.19. Rodent performance throughout the late phases of training. Performance for each rat is plotted as a 500-trial running average beginning at phase 4 of training and extending to show the beginning of the test phase. The first 3 phases of training did not allow performance to be calculated as percent correct out of trials that included a nosepoke at the report sensor. Rats were trained in two cohorts, however, due to time constraints rat 7 was the only rat from the second cohort to reach the final testing phase.

3.2.5.4 5: Two Interval Training

Aim: Once the animals were capable of responding to a stimulus presented at a constant onset (1250/1350 ms) we aimed to train animals to respond to target stimuli presented at variable onset times within a brief (700/800 ms) response window. The targets were presented either early (400 ms delay from trial onset) or late (1200/1300 ms delay) in the trial.

Training Process: Entering this phase of training rodents were reliably responding to a stimulus presented with a 1250/1350 ms delay. When we introduced an early (400 ms delay) target onset into 50% of the trials, we found that the rodents' detection performance was greatly impaired in all except one rat, suggesting that most of the rats were responding in a time dependent and not visually-driven manner (Figure 3.19). With the introduction of the early target (Figure 3.20), rodent response times shifted to earlier times across training sessions, and in some cases, the rats stopped waiting long enough to view the target on late-onset trials. To counter the animals' tendency to respond in the early interval, we took three measures: 1) the target was presented in the late interval on 67% of trials, so the reward rate would be 33% if they only ever responded early; 2) correction trials were introduced so that if an animal responded incorrectly twice in a row for the same target timing condition (i.e. early or late), regardless of if they performed correctly for a different timing condition in-between these errors, the target timing was fixed to the timing condition that elicited the errors until a correct response was made; and 3) a 3.3 kHz error tone was sounded for 5 ms if the rats exited the central nosepoke prior to target onset. We were reluctant to keep this error tone in place for long, however, as the animals may have learned not to go to the report sensor after hearing the tone. This would have confounded our performance measure, as it was only calculated in trials where the rodent had activated the report sensor. Thus, once the rats' performance had recovered to a minimum of 70% detection for both target intervals the contingency was altered so that an error tone only occurred after the incorrect detection had been reported at the flanking sensor.

Note that for rats 4,5 and 6, we attempted to introduce the mask stimulus into the late onset target trials prior to introducing an early target. We did this in the hope that the rats would learn to ignore the mask. In part this worked, as the rats quickly learned to ignore the mask, however, this strategy also encouraged the animals to respond in a time-dependent manner. Thus, when early targets were introduced the rats became confused and began responding to the mask.

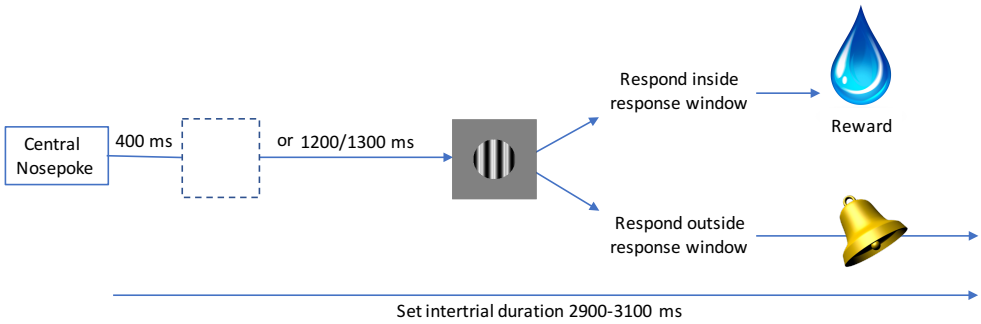

Figure 3.20. Schematic of a trial during the two-interval phase of training. A nosepoke at the central sensor triggered the onset of a trial. The target was either presented at a 400 ms or 1200/1300 ms delay from the onset of the trial. The allowed response window began at the onset of the target. If rats responded within the allowed period, a sucrose solution reward was delivered. If the rats exited the central sensor outside the allowed period, and activated the flanking report sensor, a 3.3kHz error tone sounded.

Criteria to progress: The rats were progressed to the next phase of training when they achieved the threshold criterion of 70% correct for both trial types in two consecutive sessions. This percent correct was calculated out of the trials that included a nosepoke at the report sensor.

Required training duration: 2-13 days (4-26 sessions)

Rodent exclusion: Due to timing constraints, we abandoned the training of three animals during this phase, thus only seven of the original cohort of ten proceeded to phase 6 of training.

6: Mask Introduction

Aim: The aim of this phase was to introduce an uninformative, high contrast mask stimulus that the rats were able to ignore. The mask stimulus was a full screen plaid with an aperture that matched the location of the target. The size of the mask aperture was manipulated throughout training (see details below).

Training Process: The introduction of the mask stimulus proved to be the most difficult phase of training, presumably because animals had just been trained to respond to a visual stimulus, but now had to learn to ignore a second type of visual stimulus. This phase therefore included several rounds of trial and error. As previously mentioned, we found that it was important to introduce variability in the target onset before introducing the mask so that the animals did not learn to ignore early visual stimuli and respond in a time dependent fashion.

We next attempted to gradually introduce a mask by increasing its contrast in 10% increments (Figure 3.21). The mask stimulus was presented in half of the trials at a 450 ms delay from trial onset. In the trials that included a mask, the target was presented in the early interval 33% of the time and the late interval 66% of the time. Despite this strategy, we found that all animals were unable to achieve a satisfactory level of performance to progress all the way up to a 100% contrast mask, as they began responding to the mask.

In an attempt to improve the rodents' ability to discriminate between the target and mask and thus respond only to the target, we spatially separated the stimuli by increasing the size of the mask aperture to 1.6 x the target diameter. We returned mask contrast to 0 and then began slowly incrementing contrast. Doing this, we found that the animals were able to achieve a reasonably high overall performance, but still performed badly (~50%) in the mask + late target trials.

To increase the incentive to perform above our 70% threshold criterion in the mask + late target trials, we removed all target-only trials (i.e. the mask was visible on every trial). We then gradually reduced the separation between the target and mask so that the aperture of the mask was 1.3 x the diameter of the target. With this method, three of our rats were able to reach criterion to progress with a 100% contrast mask. The remaining four rats were unable to perform above criterion at high mask contrasts so for these rats we elected to proceed at a contrast with which they were all capable of performing above criterion, a 50% contrast mask.

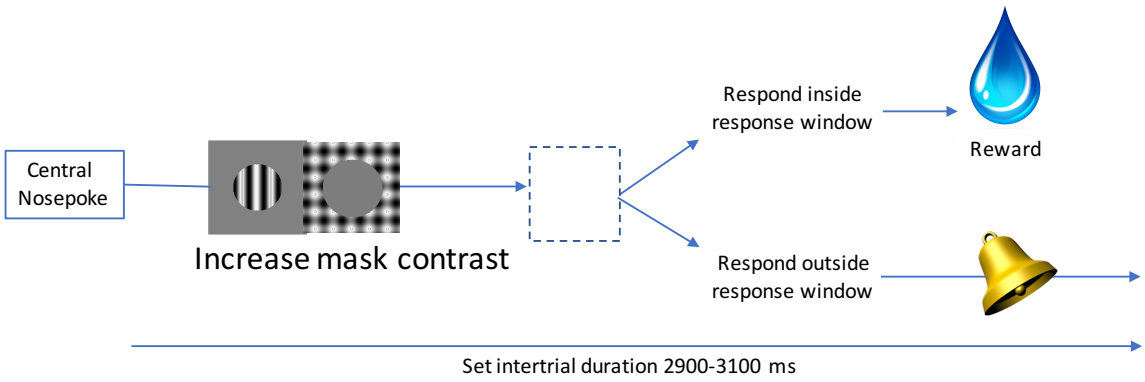

Figure 3.21. Schematic of a trial during the mask introduction phase of training. A nosepoke at the central sensor triggered the onset of a trial. The target stimulus was presented on every trial either 400 ms or 1200/1300 ms after trial onset. The mask stimulus was always presented at a 450 ms delay from trial onset, regardless of when in the trial the target stimulus was presented. In the case that the rats responded within the allowed period, a sucrose solution reward was delivered. If the rats responded outside the allowed period, a 3.3kHz error tone sounded.

Criteria to progress: To progress we required rodents to achieve a 70% threshold criterion in at least two sessions on consecutive days with either the 100% or 50% mask contrast.

Required training duration: 24-35 days (48-70 sessions).

7: Final Task- Variable Target Contrast

Aim: To prepare for the final task parameters we aimed to introduce variable contrast early targets. Note that the late targets were always 100% contrast, as these constituted a "Catch" trial, or a Correct reject, in which the animal did not respond to the absence of a target. However, we still required a behavioural response to ensure that the animals were attending to the visual stimulus.

Training Process: In the final stage of training we reintroduced the target-only trials, as they act as an important control for the effects of the mask. The target-only trials accounted for 33% of trials, the remaining 66% of trials included a mask stimulus. Rats completed sessions with all four trial types for at least 3 days before the low contrast targets were introduced. The contrast of the early target was randomly selected each trial from four contrasts; 12.5%, 25%, 50% and 100% which were introduced simultaneously. Two animals reliably detected the 12.5% contrast target, thus we later introduced an additional 6.25% target contrast for these animals.

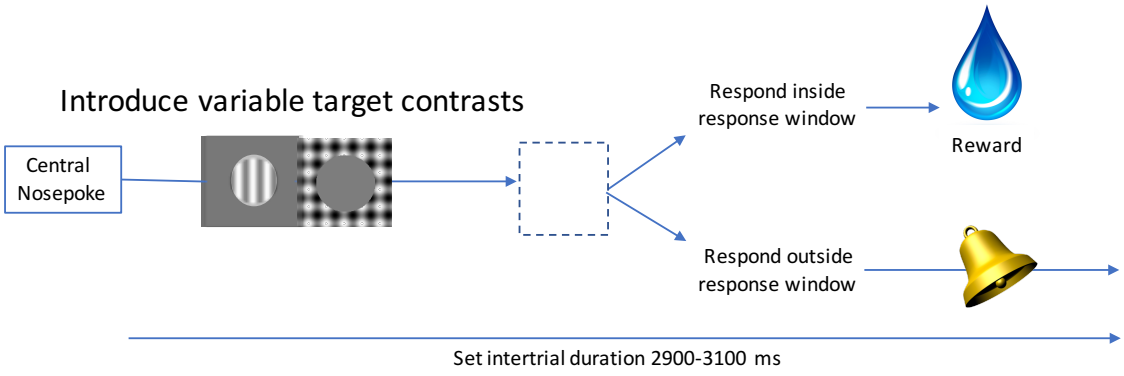

Figure 3.22. Schematic of a trial for the variable target contrast phase of training. A nosepoke at the central sensor triggered the onset of a trial. A target stimulus was presented on every trial at either a 400 ms or 1200/1300 ms delay from the onset of the trial. We introduced variable contrast (6.25, 12.5, 25, 50 & 100%) for the early target stimulus. The mask and late target stimulus remained at a constant contrast between trials. The mask stimulus was presented in 67% of trials at a 450 ms delay from trial onset. Responses within the allowed period were rewarded with sucrose solution while responses outside the allowed window triggered a 3.3kHz error tone.

Criteria to progress: Data collection commenced from the introduction of the low contrast target stimuli.

Required training duration: 3-6 days (6-12 sessions)

3.2.5.5 Training Timeline

Altogether, we found that it took between 52-106 days to shape the animal's behaviour for the final task. The specific timeline of progression through the phases of training is illustrated in Figure 3.23 for each animal.

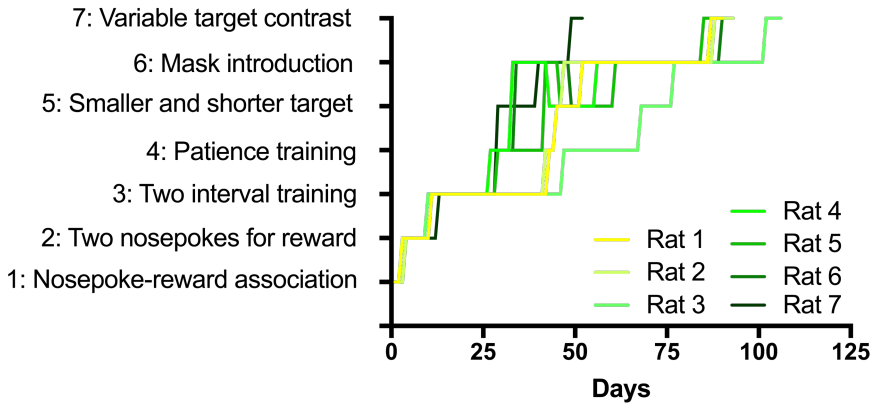

Figure 3.23. Progression of detection training across days. Animals were trained across 7 phases of training before data collection in the final task. Generally, rats were trained in two sessions per day, one in the morning and one in the afternoon.

3.2.5.6 Motivation throughout the day and week

There have been reports that a 5:2 schedule of water access can influence the motivation of animals across weekdays, with animals performing significantly more trials on Friday compared with Monday (Carandini and Churchland, 2013). As such, we specifically analysed rodent performance, as percent correct and the number of trials per session, across the time of day and day of week. The analyses were conducted across training sessions beginning in phase 4 and extending to the final task. We found no consistent trends in rodent behaviour across the time of day or week, thus suggesting that our water schedule did not introduce undesirable variations in performance (Figure 3.24.

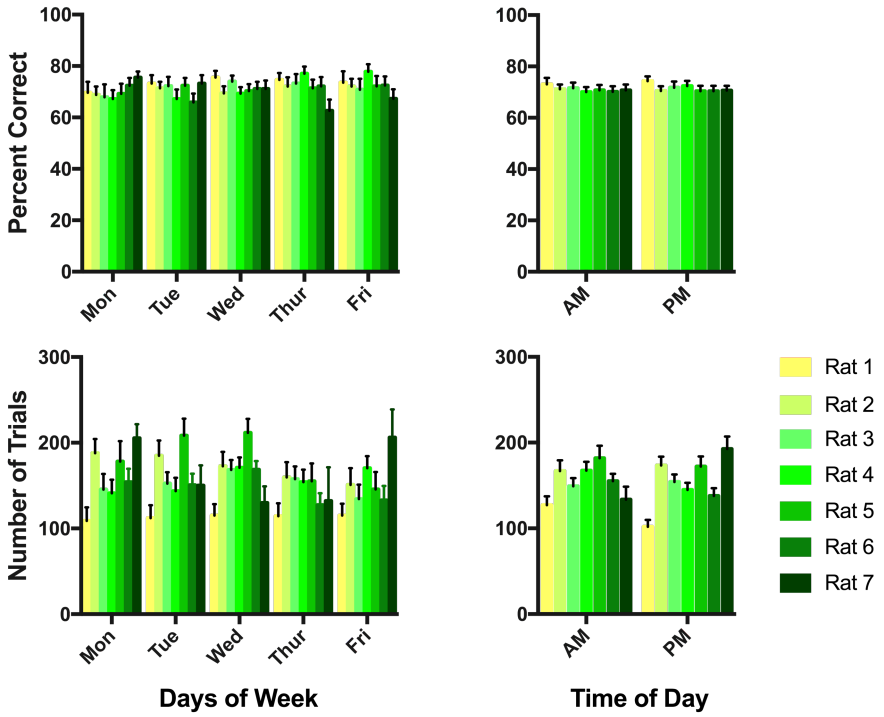

Figure 3.24. Rodent behaviour, measured as percent correct (top panels) and number of trials per session (bottom panels), was unaffected by the time of day (right panels) or day of week (left panels) in our detection task. The data displayed represents an average ± SE across all training sessions.

3.2.6 Electrophysiology Feasibility

Above we demonstrate that rats are capable of learning and performing complex visual masking tasks. Here we show that these tasks may be coupled with simultaneous electrophysiological recordings via chronic electrode implants. For our detection task, before training commenced, rats 1 and 2 were surgically implanted with chronic 32- electrode linear arrays (Neuronexus dDrive XL) into the binocular zone of V1 (1.8 mm rostral from lambda and 4.5 mm lateral to the midline suture). During the patience training period, approximately 3 months after implantation, we recorded neuronal activity in response to full screen flashes alternating between black on white and white on black (flash duration: 8.3 ms; blank duration: 408.3 ms). The animals were not required to perform a task during stimulus presentation, only to hold a steady nosepoke while intermittent rewards were delivered. These recordings revealed no spiking activity, however local field potentials confirmed the electrode was correctly placed in a visual processing region (Figure 3.25). Due to the lack of action potentials, we did not record neuronal activity during the final task. It was our intention to implant the remaining rats with electrode arrays later in the training so to improve the likelihood of recording spiking activity during task performance. Unfortunately, due to unexpected time constraints we were unable to implant the electrodes and record spiking activity, thus awake-

electrophysiological data is not discussed in this thesis. Nevertheless, our successful implantation and recordings of local field potentials indicate that our behavioural setup is suitable to be coupled with chronic electrophysiological recordings.

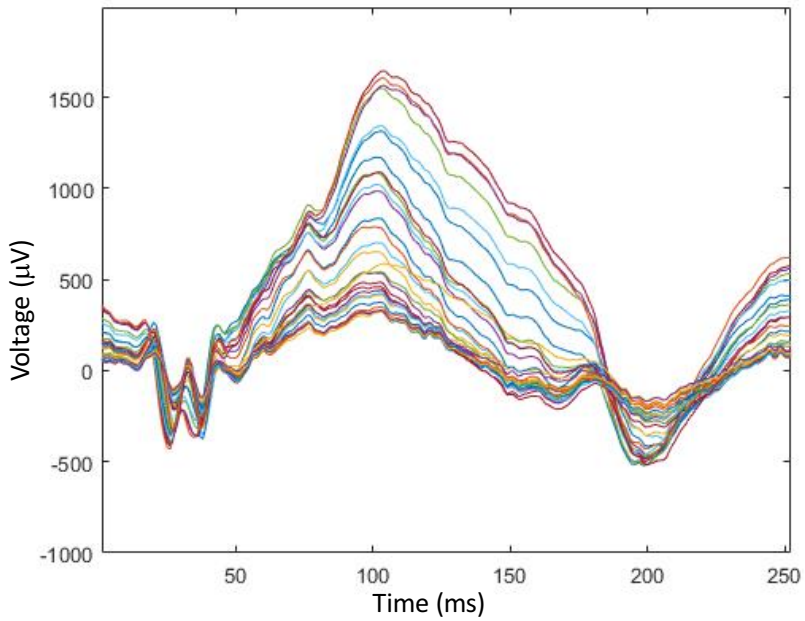

Figure 3.25. Local field potentials recorded from V1 of rat 1. Each trace represents the average electrical response to black on white or white on black full screen flashes for one of the 32 electrodes. The figure shows a simultaneous increase in electrical activity across all electrodes beginning at roughly 50 ms from the onset of the full screen flash, which is a typical delay for visually evoked activity to reach the primary visual cortex (Battersby et al., 1964).

3.3 Discussion

We sought to design an experimental paradigm that would enable perceptual reports to be collected from Long Evans rats in complex visual masking tasks. In this chapter, we show that our apparatus allows rodent performance to be measured in multiple task designs and can be easily coupled with chronic electrophysiological recordings. Using this apparatus, within 45-106 days, we successfully trained rats to perform visual masking tasks of two designs, a two-alternative discrimination task and a two-alternative detection task. Below we discuss some characteristics of rodent behaviour that complicated training and data collection and several methods that we used to counteract these challenges and improve rodent performance and motivation. Finally, we consider how behavioural training might be optimized in future endeavours.

3.3.1 Methods to improve motivation to perform the task

3.3.1.1 Rewards and punishment

There are many strategies to motivate and shape animal behaviour. Some tasks, such as the Morris water maze, condition animal behaviour through negative reinforcement, using imminent danger or discomfort to motivate animals to learn an association between geometric shapes and the location of a platform (Golob and Taube, 2002). Although this strategy allows for rapid learning of a simple task, it is not ideal for training complex behaviour as each trial is relatively time-consuming and causes significant stress in the animal (Carandini and Churchland, 2013). Alternatively, animals can be trained through positive reinforcement, where a desired behaviour, such as a lever press, is encouraged with food, liquid or other rewards. More recently, studies such as ours have tended to combine elements of both positive and negative reinforcement (Lee et al., 2012). In our case, animals received a liquid reward for desired behaviours, and a timeout, for unwanted behaviour. We specifically elected to use time delays rather than other aversive stimuli such as air puffs or electric shocks, because we did not want to cause our animals distress. Similarly, we selected liquid rewards rather than food rewards because: 1) liquid rewards are easier to titrate; 2) allow for greater head-stability; 3) are easier to couple with neuronal recordings and; 4) do not satiate the animals as quickly, thus leading to a greater number of trials per session and faster training progression.

In order for liquid rewards to effectively motivate animals to perform, it is useful to implement a water schedule, where the experimenters limit the animal's intake of water according to a specific regime. For example, with a 5:2 schedule where animals are allowed free access to water on weekends but are restricted to rewards obtained in training from Monday through Friday. In some instances, researchers have found this schedule can lead to variations in performance throughout the week, where animals perform significantly more trials on Friday compared to Monday (Carandini and Churchland, 2013). To counteract this effect, it is possible to continue water restriction and training across all 7 days of the week (Histed et al., 2012). However, in our study we implemented a 5:2 schedule with daily 2-hour periods of *ad libitum* access and found that the schedule neither affected the number of trials completed nor the animals' performance across the time of day or week.

During training, the reward volume can be manipulated so to improve both the number of trials completed and the overall performance. On average, the daily water intake for rats is 20-30 ml (Schwarz et

al., 2010), which, for a reward volume of 50 μL, could be obtained in 400-600 correct trials. However, during training we found it useful to manipulate the reward volume according to the animal's abilities. For example, we found an increase in the reward volume to 75 μL helped motivate animals that were easily discouraged in our discrimination training to perform more trials. It is also possible to manipulate the reward volume in a way that encourages animals to perform the trials correctly (Meier and Reinagel, 2011). In our detection task, we instigated a jackpot reward system where the reward volume increased with consecutive correct responses, where one correct answer received 50 μL, two consecutive correct answers received 100 μL and 3 or more consecutive correct answers received 175 μL. In theory, this ramped reward system provides additional incentive for the rats to perform the task correctly. However, it is important to acknowledge that it is impossible to determine if the rats ever learn the association between consecutive correct answers and a larger reward volume. Furthermore, this strategy runs the risk of the animals performing fewer trials, as they may be satiated more rapidly.

3.3.2 The challenges of rodent behaviour and how to overcome them

One of the greatest challenges that we encountered when training rodents was that the animals were always impatient to respond. In both our discrimination and detection tasks, the animals showed a strong bias to respond early and always performed better in shorter duration trials, despite lengthy and careful patience training. In our detection task, we found that the most effective method to counteract this problem was to manipulate the proportion of trials so that the majority of trials (2/3) included a late target (presented at 1200/1300 ms delay from trial onset) and thus a bias to respond early was penalized more severely. Similarly, when the animals showed a bias to respond in the presence of a mask, we manipulated the proportion of trials so that the majority (2/3) of trials included a mask. Altogether, although the response bias was never fully abolished, the trial proportion manipulations were highly effective and enabled the animals to reach threshold criterion for the progression of training,

Another problem we encountered in our behavioural tasks was that rodents would sometimes be content to use an incorrect rule of response that enabled reward acquisition 50% of the time. In our detection task this overlaps with the animal's tendency to respond early, thus performing appropriately for early target trials but not for late target trials. In our discrimination task this was instead a tendency to respond correctly for one target orientation but not the other. To aid the animals learning process we found that correction trials were particularly effective as it forced the animals to continue to perform the

'problem' trial type until a correct response was made. However, the risk of implementing correction trials is that the animals may learn to use the trials as part of an error-switch-response strategy in order to perform better. That said, the unwanted influences of correction trials could be easily avoided by removing correction trials from the final task or by excluding correction trials from analyses.

3.3.3 How to optimise behavioural training

Training animals to perform a reasonably complex task is a time-consuming process. Even with four functional training rigs that were semi-automated, training and monitoring seven animals completing two daily sessions required the full-time attention of an experimenter across a minimum of four months. One important optimisation was the ability to remotely monitor each animal's performance by sending network text messages via our custom Matlab scripts that registered rat behaviour. This remote monitoring freed the experimenter's time for other tasks, and removed a potential source of distraction for the animals. Below we comment on how behavioural training may be improved.

In order to minimize the time spent training, it is useful to start with a large cohort of animals and then progressively reject animals that do not meet the performance thresholds within a certain period of time. However, ideally, very few of the initial cohort should be rejected, as this will help to maintain the population size and to minimize selection bias. We believe that this rejection rate can be minimized through constant monitoring of animal behaviour so that the task parameters can be adjusted according to the individual animal's needs. This is because each phase of training can affect animals differently, and as such, one training strategy may work for one animal but not another. It is also possible that the efficiency of training may be improved by automating the animals' access to the testing chamber or by allowing the animal unrestricted access to the testing chamber, thus minimizing the need for human intervention. This has been achieved in a previous study by connecting the testing chamber directly to the housing chamber so that the animals could freely move between chambers as desired (Meier and Reinagel, 2013). This provides the potential to collect a large number of trials per day, although it may also result in larger changes in motivation across trials.

In order to increase the number of rats that can be simultaneously trained, it would be necessary to increase the number of testing chambers. While this might be easily achieved for a purely behavioural

study, for combined electrophysiology it may be difficult as the equipment necessary for recording is costly. Thus, even if a larger cohort of animals were trained for the final task, if there were only one rig equipped for electrophysiological recordings, there would not be sufficient time within a single day to collect combined behavioural and electrophysiological data in more than 7 rats.

3.3.4 Summary

We successfully trained Long Evans rats to perform discrimination and detection visual masking tasks. Three rats were trained for our reaction time discrimination task, reaching psychophysical threshold within 45-101 days. Similarly, three rats were trained for our fixed-duration discrimination task within 69-87 days. Finally, for our detection task, we trained seven rats which reached psychophysical threshold within 52-106 days. Our results thus indicate that Long Evans rats are capable of learning complex visual tasks.

4 Perceptual masking is difficult to observe in rodents performing an orientation discrimination task

4.1 Abstract

Visual masking occurs when the perception of a brief target stimulus is affected by a preceding or succeeding mask. The uncoupling of the target and its perception allows an opportunity to investigate the neuronal mechanisms involved in sensory representation and visual perception. To determine whether rats are a suitable model for subsequent studies of the neuronal basis of visual masking, we first demonstrated that decoding of neuronal responses recorded in the primary visual cortex (V1) of anaesthetized rats predicted that orientation discrimination performance should decline when masking stimuli are presented immediately before or after oriented target stimuli. We then trained Long-Evans rats (n=7) to discriminate between horizontal and vertical target Gabors or gratings. In some trials, a plaid mask was presented at varying stimulus onset asynchronies (SOAs) relative to the target. Spatially, the masks were presented either overlapping or surrounding the target location. In the absence of a mask, all animals could reliably discriminate orientation when stimulus durations were 16 ms or longer. In the presence of a mask, discrimination performance was impaired, but did not systematically vary with SOA as is typical of visual masking. In humans performing a similar task, we found visual masking impaired perception of the target at short SOAs regardless of the spatial or temporal configuration of stimuli. Our findings indicate that it may be difficult to observe visual masking in rodents as requisite aspects of the task push the boundaries of rodent visual and behavioural abilities. Therefore, rodents may not be an ideal model to investigate the effects of visual masking on perception.

4.2 Introduction

The perception of a stimulus is altered by the context in which it is presented. In visual masking, the perception of a brief target stimulus is impaired by a mask presented in close spatial and temporal proximity (Breitmeyer, 2008). The neuronal mechanisms responsible for this phenomenon remain unclear, but are likely to involve interactions throughout the visual processing hierarchy starting as early as the retina (Alwis et al., 2016). Varying the temporal separation between target and mask stimuli by just a few milliseconds systematically alters the perception and neuronal representation of the target, providing the opportunity to investigate the neuronal mechanisms involved in the development of conscious visual perception.

Masking effects depend strongly on the temporal and spatial configuration of the target and mask stimuli (Schiller, 1969; Macknik and Martinez-Conde, 2007). The temporal categories of visual masking include forward and backward masking, which describe when target perception is impaired by a mask that precedes or succeeds the target stimulus in time, respectively (Kahneman, 1968). Backward masking is of particular interest as target perception is retroactively reduced by mask-evoked neuronal activity and therefore cannot be explained by adaptation in the early sensory processing pathway (Crawford, 1947). The spatial categories of visual masking are broadly divided according to whether the mask overlaps the target's location or is presented in a non-overlapping location, typically as a surround with contiguous contours (Kahneman, 1968). Centre-surround masking is commonly used because the size and relative position of the stimuli means that any neural interaction between them must occur primarily in the cortex (Tapia and Beck, 2014). Psychophysically, visibility of a target presented to one eye may be reduced by a mask presented to the other eye (Weisstein, 1971; Turvey, 1973), suggesting that perceptual masking involves binocular interactions which do not occur until the primary visual cortex (V1) (Kinsbourne and Warrington, 1962b).

Although masking is widely used in human perceptual studies, investigating the associated neuronal mechanisms necessitates an animal model (Breitmeyer, 2008). Of the studies that have recorded neuronal responses to visual masking stimuli (Schiller and Chorover, 1966; Schiller, 1968; Vaughan and Silverstein, 1968; Levick and Zacks, 1970; Coenen and Eijkman, 1972; Bridgeman, 1975, 1980; Schwartz and Pritchard, 1981; Rolls and Tovee, 1994; Kovács et al., 1995; Macknik and Livingstone, 1998; Rolls et al.,

1999; Macknik and Martinez-Conde, 2004), only a handful have characterized both the neuronal and psychophysical effects within the same species, and only two studies collected neuronal and perceptual data simultaneously (Bridgeman, 1980; Kovács et al., 1995; Macknik and Livingstone, 1998). In order to address this gap, we aim to develop a model that will allow alert rodents to report what they perceive, simultaneous to neuronal data collection.

Despite their impoverished spatial acuity and contrast sensitivity (Prusky et al., 2002; Busse et al., 2011; Histed et al., 2012), rodents are increasingly popular in vision research because they offer the capacity to collect data from large cohorts coupled with good options for genetic manipulations (Lee et al., 2012; Juavinett and Callaway, 2015). Furthermore, rodent detection and discrimination performance on a variety of tasks is comparable to primates (Busse et al., 2011; Meier et al., 2011; Histed et al., 2012; Tafazoli et al., 2012; Zoccolan, 2015; Bossens and Op de Beeck, 2016). Our electrophysiological studies of masking in anesthetized rats showed that neuronal responses in V1 to oriented targets were altered by spatially overlapping and surround masks (Alwis et al., 2016). However, it remains to be seen whether these changes are accompanied by perceptual deficits, or if visual masking in rodents and humans follow similar trends. If perceptual masking is not observed in rodents, the benefits of a combined perceptual and neuronal study would be limited.

We first predicted rodent perceptual performance on an orientation discrimination task by linearly decoding neuronal responses to masking stimuli. Subsequently, we tested rats in three orientation discrimination tasks. In experiment 1, we varied target duration to assess the temporal acuity of rats. In experiment 2, we varied the SOA between target and mask to ascertain the influence of a spatially overlapping mask on discrimination performance and reaction times. In experiment 3, we further assessed the effects of backward masking on discrimination performance using both spatially overlapping and surround masks. Finally, we collected perceptual data from humans using similar stimuli to those used in the rodent tasks. While our neuronal decoding predicts that perceptual performance should decline as SOA is reduced, and human perception was strongly affected by masking stimuli, we found little evidence of perceptual masking in rodents. Altogether, our results suggest that essential aspects of visual masking, such as the inclusion of multiple, briefly presented stimuli, limit the performance of rodents and thus our ability to observe perceptual masking in rodents.

4.3 Methods

All experimental procedures involving animals were approved by the Monash University Committee for Ethics in Animal Experimentation (MARP/2013/81; MARP/2013/130) and were conducted in accordance with the National Health and Medical Research Council guidelines for the care and welfare of experimental animals. All experimental procedures involving humans were approved by the Monash University Human Research Ethics Committee (CF16/392 - 2016000178) and were conducted in accordance with the National Statement on Ethical Conduct in Human Research.

4.3.1 Neuronal Decoding

Predictions of rodents' ability to discriminate vertical and horizontal oriented targets were generated by decoding previously published neuronal data, collected from extracellular recordings in V1 of 37 halothane-anaesthetised Long-Evans rats (Alwis et al., 2016). Neuronal responses to spatially overlapping and centre-surround visual masking stimuli were recorded. Target stimuli were square-wave gratings (12 orientations; 4 phases) presented in a circular aperture matched to the receptive field of the neurons. The masks were black and white hyperplaids generated randomly on each trial by binarising the sum of gratings with all 12 possible target orientations and random phase. Mask stimuli were either presented at the same spatial location and dimensions as the target (spatially overlapping) or were presented full-screen with an aperture matching the target size and location (centre-surround). All stimuli were presented for 33.3 ms, with either 10 SOAs (±33.3 - 333.3) for the spatially overlapping condition or 16 SOAs (±8.3 - 333.3) for the centre-surround condition. When no target or mask was visible, a blank gray screen was displayed (luminance= 53.2 cd/m^2). Only neurons that were tuned to orientation ($d'_{pref\ vs\ null} > 0.3$) were included in the decoding analyses ($n_{SO\_Forward}$ =73; $n_{SO\_Backward}$=95; $n_{CS\_Forward}$=42; $n_{CS\_Backward}$=63). Here, we focus only on responses to horizontal and vertical target gratings, and the responses to gratings with the preferred and null orientation of the neuron.

We used spike counting windows from 50-100, 50-150 and 50-300 ms after target appearance. While the overall level of decoding performance differed depending on time window, the effects of SOA on performance were qualitatively similar. We therefore focussed on the 50-300 ms window, as it encompassed the entire neuronal response to the target. As neurons were not recorded simultaneously,

we generated 200 pseudo-populations each containing 20 neurons sampled without replacement from our database of neurons. For each pseudo-population, we simulated 10,000 trials of horizontal and vertical target gratings, by drawing spiking responses from each neuron, with replacement. We then used Fisher's linear discriminant analysis to predict whether the presented stimulus was horizontal or vertical. Separate trials were used for training and testing, and decoding performance was 10-fold cross-validated. Decoders were also trained and tested separately for each SOA, temporal and spatial category of masking.

4.3.2 Rodent Perception

Data were collected from seven adult male rats weighing 300-400g. Long-Evans rats were selected for their high visual acuity (~1.0 cycle/degree) (Prusky et al., 2002). Rats were group-housed in environmentally enriched enclosures with a 12:12 hr reversed light-dark cycle. Animals had *ad libitum* access to food, but daily water consumption was restricted to rewards obtained during experimentation as well as a two-hour period of access following the last test session in a day. Test sessions were run once or twice daily, five days/week. On non-testing days, animals had *ad libitum* access to water.

Three rats were excluded from a cohort of ten as their performance remained at chance during the initial phase of orientation discrimination training.

4.3.2.1 Testing Apparatus

Training was conducted in a custom Plexiglas testing chamber (20W x 30L x 40H cm) with three infrared photo-interrupters (Little Bird Electronics, GP1A57HRJ00F) embedded in the front 'viewing' wall of the enclosure (Figure 2A). Rats activated the sensors by performing a 'nose-poke', blocking the infrared photo-interrupter beam with their nose. Animals used the central sensor to initiate stimulus presentation and the two flanking sensors to indicate their perceptual response. The flanking sensors incorporated a 16-gauge stainless steel tube for reward delivery from a computer-controlled syringe pump (New Era Pump Systems, NE-500). Visual stimuli were presented to rats on 120 Hz LCD monitors (Samsung 2232RZ or Eizo FG2421)(Ghodrati et al., 2015) positioned 25 cm from the viewing wall. All stimuli were generated in MATLAB, using the Psychophysics Toolbox extensions (Brainard, 1997; Pelli, 1997; Kleiner M, 2007). Photo-interrupter outputs were sampled at 120 Hz (Measurement Computing, USB 1208FS) by custom MATLAB scripts, which also registered rat behaviour, controlled stimulus presentation and administered rewards or timeouts.

4.3.2.2 Rodent Discrimination Task

Rats performed a two-alternative forced choice (2AFC) orientation discrimination task, with visual stimuli presented in one of three trial structures: 1) target only; 2) variable-duration; or 3) fixed-duration. Each trial structure was tested in a discrete block over a number of consecutive sessions. All stimuli were presented against a gray background (mean luminance: Samsung – 113 cd/m2; Eizo - 79 cd/m2). At the end of stimulus presentation on each trial a 3.3 kHz 'trial complete' tone signalled to the rats that they could leave the central port and indicate their perceived target orientation at a flanking port. Each target orientation was assigned to a single flanking response sensor for the duration of the study. Correct responses were rewarded with 0.05-0.075 ml of 5% sucrose solution. Incorrect responses received no reward and incurred a 3-6 second timeout period, delaying the possible onset of the next trial. Trials in which rats left the central sensor before the tone were not rewarded.

To prevent reward port bias, if 3 consecutive incorrect choices for the same orientation were made, a 'correction trial' was implemented, in which the target was fixed to that orientation until a correct response was obtained. Correction trials were excluded from analyses. In practice, after ~21 days of performing the task, correction trials were rarely needed.

4.3.2.2.1 Experiment 1- Target Only Trials

The influence of target duration on orientation discrimination performance was examined for all rats (n=7). Target stimuli were full contrast (100%) Gabors with orientation 0 or 90°, spatial frequency 0.1 cycles/degree and random phase. The space constant, defined as the standard deviation of the Gaussian applied to the contrast envelope, was adjusted between 6-18° according to the performance level of each rodent. Target stimuli were presented for 8.3-100 ms within a fixed trial duration of 1000 ms.

4.3.2.2.2 Experiment 2- Variable-Duration Trials

Three animals (A, B and C) were trained to complete sessions with forward and backward masking trials randomly interspersed. This ensured that the motivational state of the rodents remained consistent across both forward and backward masking conditions. Target stimuli were identical to those used in target-only trials and the mask was a spatially overlapping plaid created by summing both target stimuli. Target stimuli had 100% contrast while mask stimuli had either 20 or 40% contrast. In each trial, the target and mask

were presented at one of 13 stimulus onset asynchronies (SOA; -250 to 250 ms), where negative SOAs indicate forward, and positive indicate backward masking trials. We selected to use both a 16 ms and a 42 ms target duration, which was in trade-off between maintaining overall performance levels, while retaining a target duration that would enable masking to occur. In the 42 ms target contition trials with short SOAs (±16, 33 ms) the presentation of the target was cut-off by the presentation of the mask, while for SOA of 0, only the mask was visible. The mask was always presented for 42 ms. Trial duration varied between 316-650 ms, with the trial ending at the completion of the second stimulus; the mask in backward masking trials and the target in forward masking trials. Thus, trial duration was correlated with SOA. To investigate how trial duration variability might influence rodent behaviour we measured response times from the onset of the target and mask. Animals had 8.3 s to leave the central nosepoke and a further 16.6 s to choose a response port, as such, there was no penalty for long response times and no explicit incentive for short response times. Aborted trials, where the animal left the central nosepoke before the tone, were not rewarded. Trials where response times were implausibly short (<100 ms) were excluded from percent correct analyses.

4.3.2.2.3 Experiment 3- Fixed-Duration Trials

Three animals (D, E and F) were trained to complete masking sessions with randomly interspersed backward masking and control trials, which only included a target stimulus. We implemented two spatial configurations of stimuli in separate data collection blocks. In the spatially overlapping block, stimuli were the same as in the reaction time trials but with a 100% contrast mask. In control trials, the target was presented for the same duration that would have occurred at each SOA under masked conditions (16, 24, 33, & 42 ms). This allowed us to separate the effects of masking from stimulus duration at short SOAs under spatially overlapping conditions. In addition, we collected masking data using stimuli that did not spatially overlap. In the spatially distinct block, we used a centre-surround arrangement allowing us to examine the effects of a mask on target discrimination at short SOAs, without any changes in target duration. For the centre-surround condition, target stimuli were circular sine-wave gratings 22° in diameter with a spatial frequency of 0.1 cycles/degree. Mask stimuli were full screen plaids with an aperture matching the target size and location.

On each trial, the pre-stimulus delay, target orientation and SOA (in masked trials) were randomly selected. In masked trials, a mask stimulus was presented at one of 8 SOAs (16-250 ms) following the target. Regardless of SOA, all trials were 750 ms to prevent impulsive behaviour. After 750 ms, a 'trial complete' tone sounded and animals were allowed to leave the central nosepoke and make their choice.

4.3.3 Human Discrimination Task

Two authors and four naïve subjects took part in the experiments. All participants had normal or corrected to normal vision. Each subject performed a training session for both spatially overlapping and centre-surround stimulus types.

Visual stimuli were generated using Psychtoolbox in MATLAB and were presented on an 85 Hz refresh rate CRT monitor positioned at a viewing distance of 50 cm. Target and mask stimuli were presented in both spatially overlapping and centre-surround spatial arrangements. For the spatially overlapping condition, the fixation point and visual stimuli were presented in the centre of the monitor. Target stimuli were Gabors with orientation 0 or 90°, spatial frequency 0.2 cpd and space constant 3°. Mask stimuli were a plaid generated by the sum of both target stimuli. For the centre-surround condition, target stimuli were circular sine-wave gratings with a 7° diameter. Relative to the centre of the screen, the fixation point was positioned 10° to the left, while target stimuli were centred 10° to the right. Mask stimuli were a full-screen grating with an aperture matching the size and location of the target. In both spatial conditions, mask stimuli were 100% contrast and 47 ms in duration, with SOAs of ±11-165 ms relative to the target. Target stimuli were presented at 10, 20, 30 and 100% contrast with duration 47 ms (spatial overlapping) or 12 ms (centre-surround). Under spatially overlapping conditions, six SOAs (± 11, 24, 35 ms) were temporally overlapping meaning that presentation of the target was cut off by the mask.

Head position was stabilized with a chin rest. A total of ~2400 trials/subject were collected over four data collection sessions (2 spatially overlapping and 2 centre-surround sessions). Within a session, trials were presented in blocks of 50, allowing participants to take frequent breaks if needed. On each trial, participants fixated on a small cross before a target and mask were presented and then indicated their perceived target orientation by button press. Correct discriminations were indicated by a brief tone. Reaction times were measured from the onset of the target stimulus until the key press response. Participants were allowed to respond at any time after the target onset but were not explicitly requested to respond as quickly as possible for the task. Trials extended until a response was made, so the only incentive to respond quickly was to begin another trial.

4.4 Results

4.4.1 Neuronal decoding predicts impaired discrimination performance at short stimulus onset asynchronies

We have previously demonstrated that single V1 neurons in anaesthetized rats are orientation tuned for static grating stimuli presented for just 33 ms, but that the presence of mask stimuli at short SOAs decreases neuronal orientation selectivity (Alwis et al., 2016). Here we apply linear decoding methods to populations of neurons in order to predict how masks with varying SOA might affect the animal's ability to perceptually discriminate horizontal and vertical orientations. We focus on spikes counted in the window 50-300 ms after target appearance, but shorter spike counting windows (50-100 ms; 50-150 ms) produced similar trends.

As our neurons were not recorded simultaneously, we generated 200 pseudo-populations of 20 neurons, drawing neurons without replacement from our neuronal database. For each SOA and masking condition, we then decoded the responses to 10,000 trials to predict whether the stimulus was a vertical or horizontal grating. We followed the same procedures for neuronal responses to the preferred and null orientations. We restricted our populations to include only 20 neurons because we are interested in how performance changes as SOA is manipulated – with too many neurons, decoding performance simply saturates as each neuron is able to carry independent information about orientation. In general, the neuronal data predicted a monotonic drop in performance towards shorter SOAs regardless of the temporal (forward versus backward masking) or spatial layout of stimuli (spatially overlapping versus centre-surround; Figure 4.1).

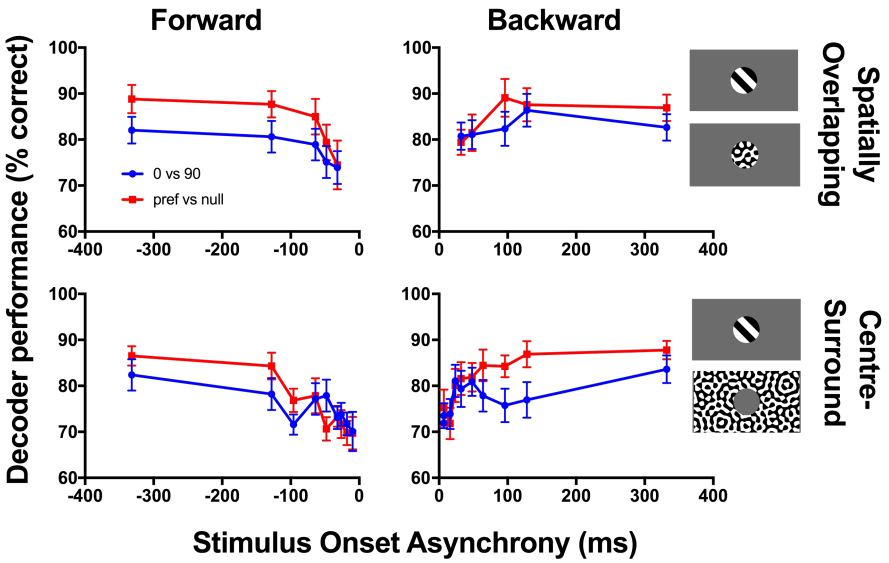

Figure 4.1. Decoding of V1 neuronal responses predicts that perceptual discrimination of orientation will decrease as stimulus onset asynchrony (SOA) approaches zero. We generated 200 pseudopopulations of 20 neurons, selected randomly without replacement. For each pseudopopulation we simulated 10,000 trials of horizontal and vertical target gratings (blue) or gratings with preferred and null orientations (red). We used Fischer's linear discriminant to predict the stimulus orientation. The decoder was trained and tested separately for each spatial condition and for each SOA. Error bars indicate standard deviation across decoders.

4.4.2 Experiment 1: Rodents can discriminate the orientation of brief target stimuli

Visual stimuli are more easily masked if they are presented briefly, but even in the absence of a mask, a stimulus that is presented for too short a time will be difficult to detect. Therefore, studying masking requires finding a target duration that is well-perceived on its own, but still able to be masked. As no studies have previously examined the temporal limits of rodent vision, we first examined how stimulus duration affected orientation discrimination of high contrast targets. Seven male Long-Evans rats were trained to perform a two-alternative forced choice discrimination between vertical and horizontal target Gabors presented for 8.3-100 ms. To prevent impulsive behaviour in the animals, each trial included randomized pre-target (350-650 ms) and post-target (183-633 ms) hold periods. For four rats (C, E, F & G) performance was significantly above chance for all target durations; the remaining rats (A, B & D) performed significantly above chance for target durations of 16 ms (2 frames) and longer ($p<0.05$, binomial cumulative distribution function; Figure 4.2C). Individual performance never reached ceiling; the highest-performing animal achieved a maximum of 95% correct at the 100 ms target duration. We suspect that performance would not substantially improve given larger sampling times, as in many studies, rodents tend

to have relatively high error rates even on the easiest trials of detection or discrimination tasks (Meier et al., 2011; Meier and Reinagel, 2011). For all rats, performance significantly increased with target duration (Figure 4.2C; $p_A<0.0001$, χ^2 (7, N = 1243) = 41.81; $p_B<0.05$, χ^2 (3, N = 829) = 10.40; $p_C<0.0001$, χ^2 (7, N = 2380) = 112.68; $p_D<0.0001$, χ^2 (7, N = 2619) = 101.18; $p_E<0.0001$, χ^2 (7, N = 1856) = 57.13; $p_F<0.0001$, χ^2 (7, N = 1529) = 93.74; $p_G<0.0001$, χ^2 (8, N = 1943) = 40.98; Chi-square goodness of fit).

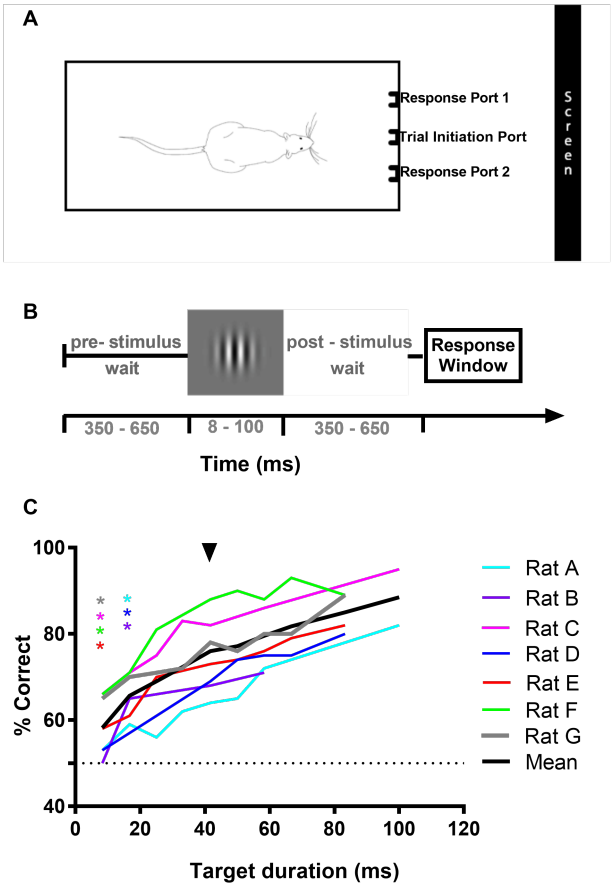

Figure 4.2. Rodent orientation discrimination performance increases with target stimulus duration. (A) The testing chamber is fitted with three photo-interrupter detectors; a central sensor for trial initiation and two flanking sensors equipped with drinking spouts for perceptual report. The rat activates each sensor by breaking the infrared beam with its nose. Trial initiation results in the presentation of visual stimuli on an LCD monitor placed at a 25cm viewing distance. When the rat selects the correct response port, a liquid reward is delivered at the centre of the corresponding flanking sensor. (B) Trial structure. Rodent performance was measured in the absence of a mask across varying target durations. Following a central nose-poke, a blank gray screen was shown for a random period of 350-650 ms. Subsequently, the target grating was visible for 8.3-100 ms, followed by another random period with blank screen. After this period, a tone sounded, signalling that animals could leave the central port and move to a side port to indicate their response. Correct responses were only rewarded if they were made in the allocated response window. (C) Individual and average performance across 7 animals. The dotted line indicates chance performance. For each rat, the target duration where performance became significantly above chance is indicated by an asterisk (*, $p<0.05$). The black arrow indicates the target duration (42 ms) selected for subsequent masking experiments. For rat A, data represents a summary of performance across 1243 trials and 17 sessions (Rat B – 855 trials; 9 sessions. Rat C – 2380 trials; 24 sessions. Rat D – 2619 trials; 23 sessions. Rat E – 3051 trials; 25 sessions. Rat F – 1529 trials; 9 sessions. Rat G – 1805 trials; 29 sessions).

4.4.3 Experiment 2: Visual masking does not reduce orientation discrimination performance

In Experiment 2, we examined how a spatially overlapping mask affected rodent discrimination performance for a 42 ms (

Figure **4.3**B-D) and 16 ms target (

Figure **4.3**E-G) in both forward and backward masking trials (

Figure **4.3**A). In each trial, a mask was presented at one of 13 stimulus onset asynchronies (SOA) relative to the target. Below, we separately describe the results for forward, and backward masking trials. Note that at an SOA of 0, only a mask stimulus was presented, allowing us to check for response bias. On these trials, animals showed no significant bias in how often they selected the left response port ($\%left_A$=54.3; $\%left_B$=50.7; $\%left_C$=50.0. Binomial cumulative distribution tests, $p>0.05$).

4.4.3.1 Forward Masking

Under forward masking conditions, our neuronal decoder predicted that rodent performance would decrease towards the shorter SOAs.

In the 42 ms target condition we found that discrimination performance for SOAs from -33 to +33 ms was strongly impaired compared to when longer SOAs were used (

Figure **4.3**B). While reminiscent of commonly reported masking phenomena, unfortunately, this finding was complicated by the fact that at these SOAs, the duration of the target was reduced by the presence of the mask, and in experiment 1, we found that performance was impacted by the target duration. Therefore, it was impossible to determine if the lower discrimination performance was the result of changing target duration alone or if the presence of a mask immediately adjacent to the target was additionally impairing target perception. We therefore only analysed the effect of SOA on discrimination performance over SOAs where the target duration was consistently 42 ms ($|SOA|\geq 50$ ms): We found rodent performance was not altered across SOA (

Figure **4.3**B-left; p_A=0.310, X^2 (3, N = 1440) = 3.582; p_B=0.890, X^2 (3, N = 1541) = 0.628; p_C=0.477, X^2 (3, N = 969) = 2.492; Chi-square goodness of fit).

To better determine if perceptual masking was present at the short SOAs (<50 ms), we ran 22-32 sessions using a 16 ms target, so that target duration was consistent across all SOAs. We found that rodent performance significantly decreased towards the shorter SOAs for one animal; the rat with the highest overall performance and that had completed the largest number of trials (

Figure **4.3**E-left; p_A=0.117, X^2 (5, N =500) = 8.802; p_B=0.326, X^2 (5, N = 472) = 5.806; p_C<0.0001, X^2 (5, N =998) = 27.383; Chi-square goodness of fit). The two animals that did not display any significant effects of masking also showed a higher target duration threshold in experiment 1, meaning they were performing at the threshold of their capabilities, which was determined in the absence of a mask. It is possible that the introduction of a mask reduced overall performance and therefore compromised our ability to observe perceptual masking in these animals. Altogether this may suggest that perceptual masking was occurring, but that performance levels were too close to chance to reveal any significant effects across SOA in two of the animals.

Given that rodents tend to respond impulsively on some trials, we also considered how the proportion of aborted trials varied across SOA. As the duration of the trial covaried with SOA, we expected that there might be a greater proportion of aborted trials at the longer SOAs. This was true for both target duration conditions (16 ms: p_A<0.0001, X^2 (5, N =1130) = 70.224; p_B<0.0001, X^2 (5, N = 974) = 299.534; p_C<0.0001, X^2 (5, N =2603) = 269.178; 42 ms: p_A<0.0001, X^2 (5, N =4963) = 215.598; p_B<0.0001, X^2 (5, N = 5357) = 379.025; p_C<0.0001, X^2 (5, N =4555) = 597.35; Chi-square goodness of fit).

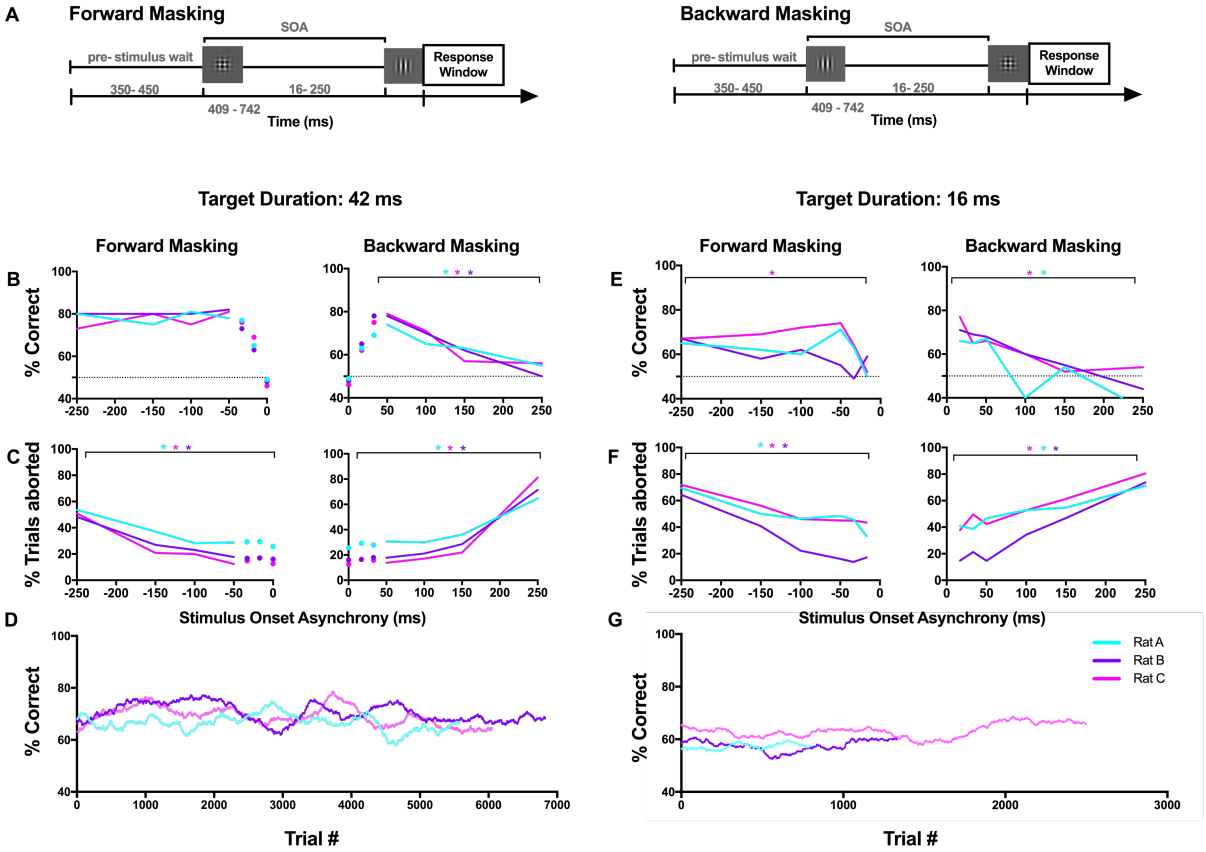

Figure 4.3. Spatially overlapping masks do not affect rodent orientation discrimination performance. (A) Structure of forward (left) and backward (right) masking trials. (B) Orientation discrimination performance and (C) percentage of aborted trials for three animals is shown for the 42 ms target duration. (D) Overall task performance for each rat plotted as a 500-trial running average during data collection. Rats performed roughly 500 trials per day. (E-G) same as (B-D) for the 16 ms target duration. At an SOA of 0 only the mask was presented and rewards were given randomly; therefore, these values reflect how often the animal was rewarded. The dotted line indicates chance performance. In (E) the temporally overlapping stimulus onset asynchronies (SOA) where target duration was reduced, are presented separately from the remainder of the curve. For rat A, the 42 ms target duration data represents a summary of performance across 3167 trials and 85 sessions, the 16 ms data 1247 trials and 21 sessions (Rat B: 42ms - 3975 trials, 66 sessions; 16ms - 1845 trials; 16 sessions. Rat C: 42ms - 3442 trials, 65 sessions; 16 ms - 2744 trials, 32 sessions).

4.4.3.2 Backward Masking

Under spatially overlapping backward masking conditions, the neuronal decoder predicted that rodent performance would decrease with SOA.

In the 42 ms target condition, across the the SOAs where target duration was consistent (SOA: 50-250 ms), we found that performance increased significantly towards the short SOAs for all rats (

Figure **4.3**B-right; $p_A<0.0001$, X^2 (3, N = 1380) = 30.244; $p_B<0.0001$, X^2 (3, N = 1329) = 60.142; $p_C<0.0001$, X^2 (3, N =819) = 41.314; Chi-square goodness of fit). Similarly, in the 16 ms target condition, performance significantly increased towards the short SOAs for two of three rats (

Figure **4.3**E-right; $p_A<0.0001$, X^2 (5, N =484) = 28.697; $p_B=0.069$, X^2 (5, N = 364) = 10.234; $p_C<0.0001$, X^2 (5, N =1881) = 27.185; Chi-square goodness of fit). Given that this trend is atypical of visual masking (Kolers, 1962), it is likely that these changes in performance across SOA reflect behavioural aspects other than perceptual masking, such as changes in attention or impulsivity. When we considered aborted trials, we found there was an increased proportion at the SOAs where discrimination performance was impaired (

Figure **4.3**C-right; 42 ms: $p_A<0.0001$, X^2 (5, N =5048) = 319.671; $p_B<0.0001$, X^2 (5, N = 5568) = 646.612; $p_C<0.0001$, X^2 (5, N =5045) = 951.228;

Figure **4.3**F-right; 16 ms: $p_A<0.0001$, X^2 (5, N =1203) = 108.467; $p_B<0.0001$, X^2 (5, N = 1078) = 379.522; $p_C<0.0001$, X^2 (5, N =2842) = 415.1; Chi-square goodness of fit). This may suggest there is an important interaction between impulsive behaviour and discrimination performance in this task. Although, it is difficult to explain why similar increases in the proportion of aborted trials did not have a detrimental effect on discrimination performance at long forward masking SOAs, that is, unless the order of stimuli was also important.

Throughout the data collection phase, we found that performance levels were stable for all rats (

Figure **4.3**D & G). Thus, it is unlikely that the unusual backward masking trends were the result of insufficient time to learn the task. However, as the forward and backward masking trials were collected simultaneously, it is possible that the animals learnt to respond in a way that favoured forward masking trials; ie. always ignore the first stimulus and respond to the second. This problem could be easily avoided if forward and backward maskng data were collected in discrete sessions.

4.4.4 Experiment 2: Rodent response times change with trial duration

To better understand how rodent behaviour was affected by various task parameters, we calculated response times for the 42 ms target condition based on when the rats exited the central port relative to both target and mask onset. Given the possibility of rats making decisions that were not influenced by the stimulus orientation, we were particularly interested to determine whether the rats responded at a fixed time following the appearance of the informative target stimulus, or whether their responses were simply locked to the appearance of the first stimulus. Note that in all trials, rats had 16.6 seconds to initiate their response, so there was no penalty for long reaction times and no explicit incentive for short response times. Repsonse times were measured in all trials, including aborted trials and correction trials.

In forward masking trials, if the rodents responded at a fixed duration following the target stimulus then response times measured from target onset should be unaffected by SOA, i.e. a flat line in Figure 4A. Instead response times significantly increased as the SOA shortened ($p_A<0.0001$, $F_{3,1823} = 65.53$; $p_B<0.0001$, $F_{3,2535} =32.95$; $p_C<0.0001$, $F_{3,1259} =42.98$; one-way ANOVA) and the response times were significantly different for all SOA comparisons ($p_A<0.001$, $p_B<0.01$, $p_C<0.01$; Tukey's multiple comparisons test).

On the other hand, if the rodents responded at a fixed duration following the mask, then we would expect response times measured from the onset of the mask to remain constant across varying SOAs (Figure 4.4C). Again, this was not the case, the response times significantly decreased with SOA for all animals ($p_A<0.001$, $F_{3,2314} = 6.14$; $p_B<0.0001$, $F_{3,3008} =83.21$; $p_C<0.0001$, $F_{3,2634} = 63.11$; one-way ANOVA). Post hoc analyses revealed that response times were significantly different for all SOA comparisons in Rat B, for all except -100 vs -150 ms in Rat C and for only the longest SOA (-250 ms) in Rat A ($p_A<0.05$, $p_B<0.001$, $p_C<0.001$; Tukey's multiple comparisons test).

Similarly, in backward masking we found that rodents did not respond at a fixed duration following the target or mask stimulus. Response times measured from the target onset decreased with SOA for two rats (Figure 4.4B; $p_B<0.0001$, $F_{3,3366} =55.26$; $p_C<0.0001$, $F_{3,1797} =25.19$; one-way ANOVA), with reaction times being significantly different for all SOA comparisons in rat B and all except two (100 vs 150 ms and 100 vs

250 ms) in rat C (p_B<0.01, p_C<0.001; Tukey's multiple comparisons test). The response times for rat A remained consistent across SOA (p_A=0.8345, $F_{3,2110}$ = 0.287; one-way ANOVA).

Finally, when measuring the response times from the mask onset, we saw a significant increase towards the shorter SOAs for all rats (Figure 4.4D; p_A<0.0001, $F_{3,2074}$ = 35.91; p_B<0.0001, $F_{3,2605}$ =34.94; p_C<0.0001, $F_{3,2169}$ =10.86; one-way ANOVA). The response times were significantly different for most SOA comparisons (p_A<0.01, p_B<0.01, p_C<0.001; Tukey's multiple comparisons test), with a few non-systematic exceptions (rat A - 100 vs. 150 ms; rat B - 150 vs. 250; rat C - 50 vs 100 and 150 vs 250).

Collectively, our results indicate that the rats did not respond at a fixed duration following the onset of either the target or mask stimulus. Interestingly the trends in reaction times were consistent between forward and backward masking conditions when considering the order of stimuli; the response times increased towards longer SOAs when measured from the first stimulus onset and decreased towards the longer SOAs when measured from the last stimulus onset. This suggests that the rodents began planning their response as soon as the trial began regardless of when the target appeared and therefore response times were most affected by the duration of the trial. In line with this idea, when response times were separated into two groups according to the duration of the pre-stimulus hold, we found that trials with longer pre-stimulus durations were associated with shorter response times for all rats (Figure 4.4E). This was found to be significant across all rats (p_A<0.0001, t_{2864} = 4.675; p_B<0.0001, t_{4310} =5.275; p_C<0.0001, t_{2403} =6.398; paired t-test).

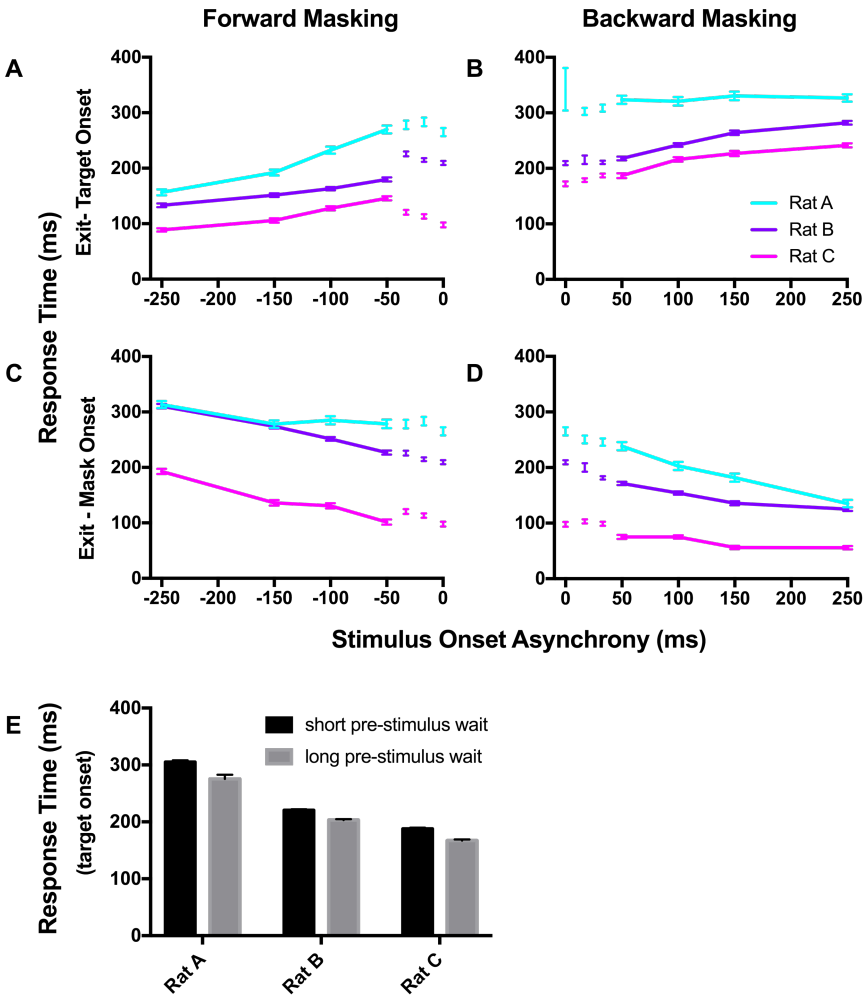

Figure 4.4. Rodent response times are influenced by trial duration but not by visual masking. Response times were measured for the 42 ms target condition from the (A, B) target onset and (C, D) mask onset to the time that the rat exited the central sensor. For each rat, all response times are represented as mean (SE). Response times measured at the temporally overlapping SOAs, where target duration was truncated, are presented separate from the remainder of the curve. At a SOA of 0, only a mask stimulus was presented. (E) rodent response times measured from the target onset divided according to the duration of the pre-stimulus hold, collapsed across SOA (pre-stimulus hold: 350-400 & 400-450 ms). For rat A, data measured from the target onset represents a summary of performance across 3168/3471 trials (forward/backward) and 85 sessions, mask onset data 3860/3619 trials and 85 sessions (Rat B – target onset: 4340/5204 trials; 66 sessions, mask onset: 4830/4427 trials; 66 sessions. Rat C – target onset: 2384/2934 trials; 65 sessions, mask onset: 4489/4007 trials; 65 sessions.).

4.4.5 Experiment 3: Backward masking does not impair rodent orientation discrimination

In experiment 2 we did not find convincing evidence of backward masking in rodents, but our experimental design was limited as it appeared to be subject to the influence of impulsive behaviour, which may have impaired our ability to observe perceptual masking. To further investigate whether visual masking might affect rodent perception, in experiment 3 we used 100% contrast masks that were presented either overlapping or surrounding the target location. Three new rats were trained to complete sessions with control and masked trials randomly interspersed (Figure 4.5A). Importantly, we focused on backward masking and introduced a fixed 750 ms trial duration by adding a post-stimulus waiting period, which removed the possible problems associated with impulsivity that may have affected the long SOA trials in Experiment 2. We also introduced control trials in which no mask was presented and target duration was matched to that in masked trials; in the spatially overlapping condition, these required truncating the target duration to 16 or 33 ms.

In experiment 2, we were concerned that the variation in trial duration across SOA was interacting with impulsivity resulting in a larger proportion of aborted trials and possibly impaired performance at the long SOAs. This idea is supported by our findings in fixed duration trials where the proportion of aborted trials was stable across SOA for both spatially overlapping (Figure 4.5D-left; p_D =0.558, X^2 (7, N=7328) = 5.842; p_E =0.327, X^2 (7, N = 5706) = 75.45; p_F =0.398, X^2 (8, N = 15352) =8.378; Chi-square) and centre-surround configurations (Figure 4.5D-right; p_D =0.032, X^2 (7, N=3793) = 16.787; p_E =0.068, X^2 (7, N = 2933) = 14.56; p_F =0.059, X^2 (9, N = 25486) =8.378; Chi-square). Furthermore, instead of increasing towards the longer SOAs, discrimination performance reached a plateau at an SOA of 100-150 ms. We therefore restricted the figures axes and our analyses to SOAs up to 150 ms. Throughout data collection, performance levels were stable (Figure 4.5E).

In the *spatially overlapping* condition, we expected that if rodents were affected by visual masking, their performance in masked trials would be similar to that of control trials at long SOAs, but would be impaired relative to control performance at shorter SOAs. Instead, for all rats, performance was consistently lower in the masked trials across all SOAs (Figure 4.5B-C-left. p_D<0.0001, X^2 (5, N =1810) = 57.154; p_E<0.0001, X^2 (5, N =1868) = 109.720; p_F<0.0001, X^2 (6, N = 3380) = 721.9209; Chi-square). Our results indicate that the presence of a mask reduces orientation discrimination performance even at long

SOAs, but that the reduction occurs in a manner that is independent of SOA and is thus uncharacteristic of visual masking.

In the *centre-surround* configuration, the mask was a full screen plaid with an aperture matching the size and location of the target. Therefore, the target remained visible for 42 ms for all SOAs, allowing us to assess the effects of a mask at short SOAs without confounding changes in target duration. The results from our neuronal decoder predicted that rodent performance would be impaired at the short SOAs when using a surround mask. Again, we found that discrimination performance was significantly different between control and masked conditions for all SOAs, not just the short SOAs (Figure 4.5B-C-right; $p_D<0.01$, X^2 (6, N =1207) = 20.50; $p_E<0.0001$, X^2 (6, N =1285) = 59.68; $p_F<0.0001$, X^2 (7, N = 4341) = 522.19; Chi-square). Although performance tended to decrease towards an SOA of 0 for all animals in the masked trials, strangely masked performance was sometimes better than control performance in two of the animals. However, the performance of Rat F agreed with our broad predictions for visual masking in rodents, with the difference between masked and control performance systematically lower at short SOAs.

Together, our results suggest that a mask stimulus impairs rodent performance regardless of SOA. The effect of SOA on rodent performance only followed the trends predicted by our neuronal decoder in some animals. This may be because the overall performance levels were too low to consistently reveal perceptual masking across animals, or because our stimuli were not actually capable of producing perceptual deficits.

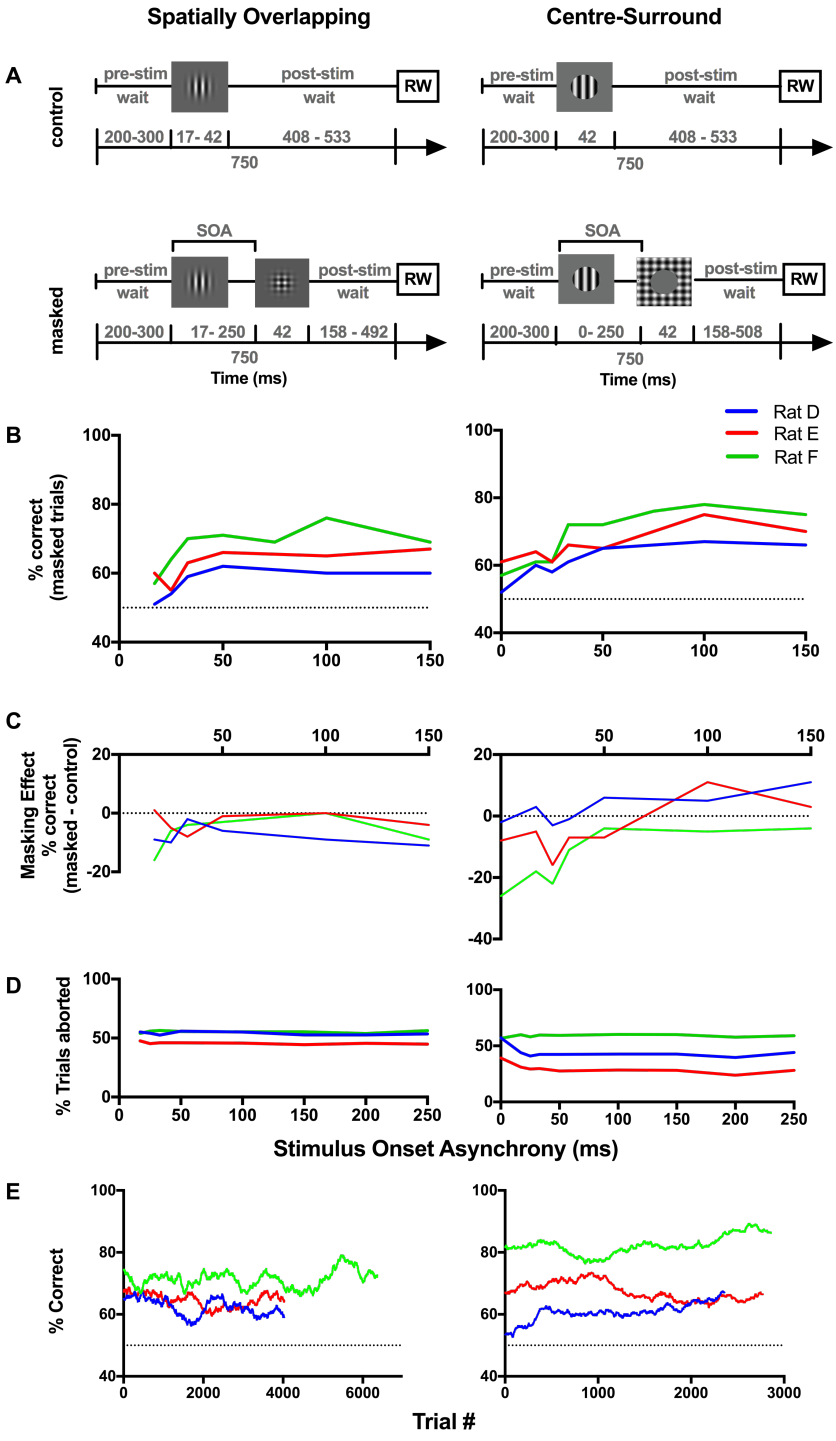

Figure 4.5. Spatially overlapping and centre-surround backward masking does not impair discrimination performance in rodents. (A) Schematic of trial structure for control (top) and masked (bottom) trials using spatially overlapping (left) and centre-surround (right) stimuli. RW indicates the response window. (B) Orientation discrimination performance of three animals. The dotted line at 50% correct represents the chance performance level. (C) The effect of the mask, measured as the difference in performance between control and masked trials. The dotted line at 0% indicates when performance is equal across control and masked trials. (D) Percent aborted trials. (E) Overall task performance for each rat plotted as a 500-trial running average during data collection. Rats performed roughly 500 trials per day. For rat D, spatially overlapping data represents a summary of performance across 3024 trials and 63 sessions, centre-surround data 2400 trials and 52 sessions (Rat E – SO: 2959 trials; 77 sessions, CS: 2557 trials; 52 sessions. Rat F – SO: 4816 trials; 64 sessions, CS: 8661 trials; 112 sessions).

4.4.6 Our visual masking stimuli impairs human orientation discrimination at short stimulus onset asynchronies

We did not find consistent evidence of perceptual masking in rodents, a phenomenon that has been repeatedly observed in humans and non-human primates (Enns and Di Lollo, 2000; Breitmeyer, 2008). To determine how our masking stimuli affected human perception, we measured the effects of masking on human orientation discrimination performance, using stimuli similar to those used in our rodent experiments (Figure 4.6A, D, G). In separate testing sessions, we presented stimuli with spatially overlapping or centre-surround configurations.

For *spatially overlapping* stimuli, we collected both forward and backward masking data across separate testing blocks. All temporally overlapping SOAs, where the target duration was shortened, were excluded from statistical analyses. Under forward masking conditions, performance increased with longer SOAs, saturating near 100% correct for SOAs above 100 ms, regardless of target contrast (Figure 4.6B). Using a two-way ANOVA of arcsine transformed performance (SOA x target contrast), we found performance averaged across participants was significantly affected by SOA ($p_{SOA}<0.0001$, $F_{5, 25} = 8.75$) and contrast ($p_{Contrast}<0.0001$, $F_{2, 10} = 27.36$), and that there was a significant interaction between the factors ($p_{SOA \times Contrast}<0.0001$, $F_{10, 50} = 7.62$).

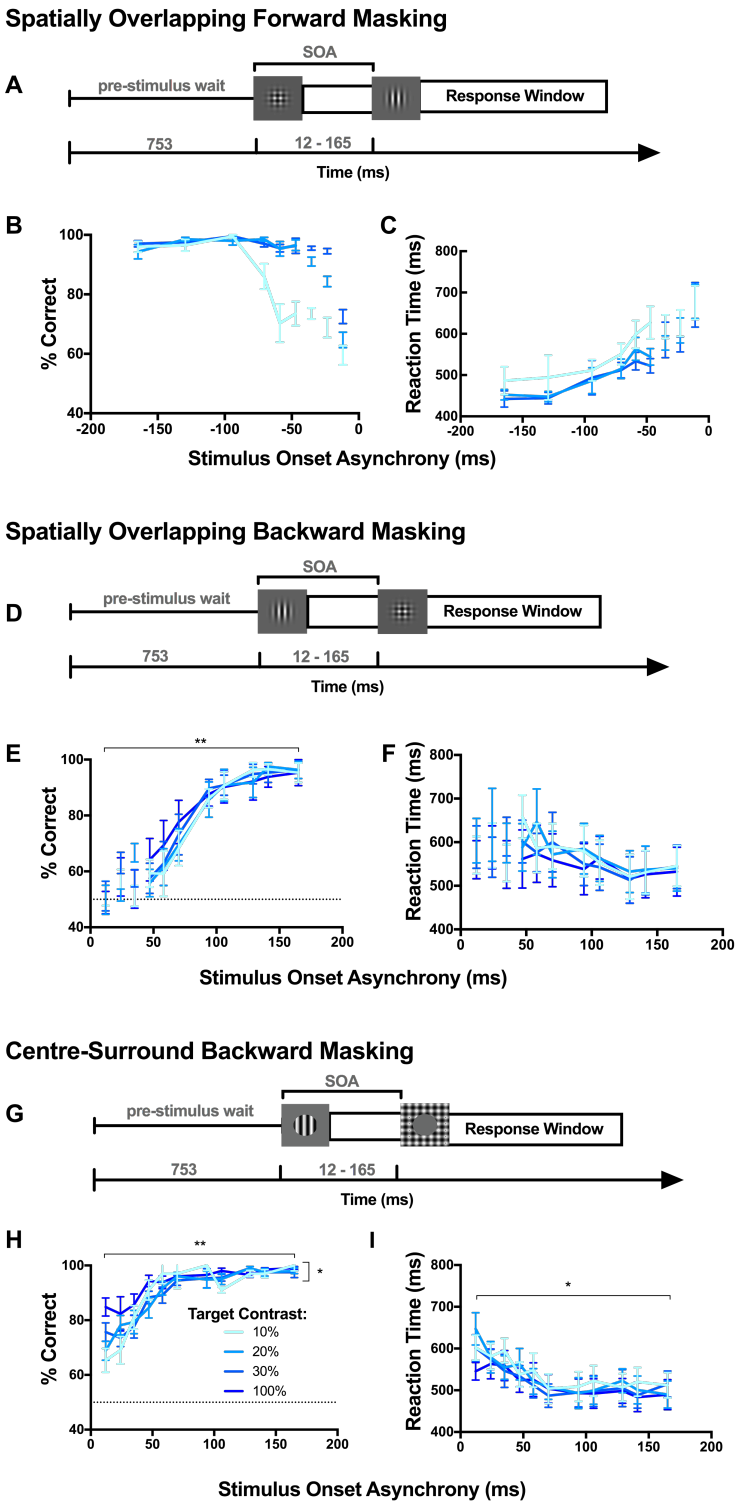

Figure 4.6. Human orientation discrimination and reaction times are influenced by mask stimuli at short stimulus onset asynchronies (SOAs). (A) Schematic of trial structure for spatially overlapping forward masking. (B) Orientation discrimination performance across six participants (mean, SE) for three target contrasts (10,20 and 30%). The dotted line represents chance performance. (C) Mean (SE) reaction times, measured from target onset until keypress response. Performance and reaction times for temporally overlapping SOAs, where the target duration was truncated, are presented separately from the remainder of the curve. (D-F) same as (A-C) for spatially overlapping backward masking. (G-I) Same as for (A-C) for centre-surround backward masking. (**) $p<0.0001$; (*) $p<0.001$.

Similar to forward masking conditions, backward masking performance increased with SOA, saturating near 100% correct for the majority of participants at SOAs of 130 ms and longer (Figure 4.6E). This trend remained relatively robust regardless of the target contrast. Using two-way ANOVA (SOA x target contrast), we found performance averaged across participants was affected by SOA (p_{SOA}<0.0001, $F_{7, 35}$ =23.29). However, there was no significant effect of target contrast ($p_{Contrast}$=0.20, $F_{3, 15}$ = 1.73), nor was there any significant interaction between target contrast and SOA ($p_{SOA\ x\ Contrast}$=0.87, $F_{21, 105}$ = 0.65). Post hoc analyses revealed performance was significantly lower at the four shortest SOAs (47-94 ms) for all target contrasts when compared to the longest SOA (p<0.05; ANOVA; Dunnett's multiple comparison *post hoc*).

Despite the lack of explicit instructions regarding response timing, under *spatially overlapping* conditions, forward masking reaction times were significantly affected by both SOA and target contrast, but there was no significant interaction between factors (Figure 4.6C; p_{SOA}<0.0001, $F_{5, 90}$ = 8.23; $p_{Contrast}$<0.01, $F_{2, 90}$ = 5.92; $p_{SOA\ x\ Contrast}$=0.98, $F_{10, 90}$ = 0.30; two-way ANOVA). Post hoc analyses revealed that at 10% target contrast reaction times were longer for two SOAs (-47 & -58 ms), and at 20% contrast for one SOA (-58 ms) when compared to the longest SOA (p<0.05; ANOVA; Dunnett's multiple comparison *post hoc*). On the other hand, reaction times for spatially overlapping backward masking were not significantly affected by SOA or target contrast, and there was no significant interaction between the two factors (Figure 4.6F; p_{SOA}=0.33, $F_{7, 160}$ = 1.16; $p_{Contrast}$=0.78, $F_{3, 160}$ = 0.36; $p_{SOA\ x\ Contrast}$>0.99, $F_{21, 160}$ = 0.13; two-way ANOVA).

Using *centre-surround* backward masking stimuli*,* discrimination performance for each participant increased with SOA and saturated at SOAs of 58 ms and longer (Figure 4.6H). Performance averaged across participants was found to significantly increase with SOA (p_{SOA}<0.0001, $F_{10, 50}$ = 29.67; two-way ANOVA) with performance significantly lower at the five shortest SOAs (12-58 ms) for target contrasts 20, 30 and 100%, and at the six shortest SOAs (12-70 ms) for a 10% target contrast when compared with the longest SOA (165 ms; p<0.05; ANOVA; Dunnett's multiple comparison *post hoc*). There was also a significant main effect of target contrast on orientation discrimination, where orientation discrimination performance decreased with target contrast ($p_{Contrast}$<0.01, $F_{3, 15}$ = 9.36; two-way ANOVA). Significant interaction effects revealed this effect of target contrast was largest at the short SOAs ($p_{SOA\ x\ Contrast}$<0.05, $F_{30, 150}$ = 1.96; two-way ANOVA). At 10% contrast, discrimination performance was lower than that of 100% contrast for the five shortest SOAs (12-58 ms). At 20% contrast the drop in performance was only significant for two short SOAs

(12 and 47 ms) and at 30% contrast only for a single SOA (24 ms; $p<0.05$; ANOVA; Tukey's multiple comparison *post hoc*).

Under *centre-surround* conditions, the effect of SOA on reaction times averaged across participants was significant (Figure 4.6I; $p<0.0001$, $F_{10, 220} = 4.60$; two-way ANOVA), but there were no significant effects of target contrast or any interaction ($p_{Contrast}=0.34$, $F_{3, 220} = 1.13$; $p_{SOA \times Contrast}>0.99$, $F_{30, 220} = 0.21$; two-way ANOVA).

Our data demonstrate that, regardless of the spatial configuration of visual masking stimuli, human orientation discrimination performance is impaired at short stimulus onset asynchronies. Although this was similar to the predictions of our neuronal decoder, we did not consistently observe these trends in rodent behaviour. We also found that at the SOAs where human performance was impaired, the reaction times were longer. Collectively this suggests that human reaction times increase when there is uncertainty of the target orientation, rather than relating to the trial duration as was the case in rodents.

4.5 Discussion

We did not find any consistent evidence of visual masking in rodents, even at SOAs where perception is known to be impaired in humans. All rats could reliably discriminate the orientation of a Gabor target in a discrimination task. However, in masked trials, the rats' discrimination performance was reduced and only exhibited the temporal profile characteristic of visual masking in some animals. That is despite the fact that our classification of neuronal activity from visual cortex of anaesthetized rats indicated the presence of masking at short SOAs and that the same stimuli were capable of producing perceptual deficits at these SOAs in humans. Altogether, our results indicate that perceptual masking may occur in rodents, but that it is difficult to observe due to behavioural and visual limitations. Below we discuss: 1) the discrepancy between neuronal and perceptual results in rodents; 2) the importance of task design; 3) the differences in rodent and human behaviour; 4) our results in relation to the most prominent theories of visual masking; and 5) the temporal acuity of rodent vision.

Our perceptual findings in rodents were not always consistent with the pattern of reduction in orientation discriminability observed in V1 neurons recorded in anaesthetized rats (Alwis et al., 2016). Our neuronal data revealed that firing rates and single/multi-unit discriminability, measured as the orientation selectivity index, were impaired at short SOAs (|SOA|<100 ms) (Alwis et al., 2016). For all stimulus conditions, our neuronal population decoder predicted that the ability of rodents to discriminate target orientation would decrease towards a SOA of zero, a typical trend observed in visual masking (Kolers, 1962). However, rodent perceptual performance only followed these trends in a limited number of animals and experimental circumstances. It is possible that perceptual masking is absent in rodents, despite the fact that similar stimuli can alter neuronal processing in V1. It could be that activity in V1 was not sufficiently altered to disrupt rodent perception or alternatively that V1 does not play an important role in informing rodent behaviour. It is also possible that the small differences in the stimuli used for our neuronal and perceptual study (square wave versus sine wave) were sufficient to remove the effects of visual masking in our behavioural task. However, given that two animals did show behavioural trends that were congruent with perceptual masking, and that these animals performed more trials with a higher overall performance, it is more likely that perceptual masking does occur in rodents, but that the effect size is too small to be revealed consistently across rodents performing a task with a relatively high lapse rate. Unfortunately, the stimulus manipulations that increase the strength of visual masking (shorter target duration, lower target contrast), also lead to lower discrimination performance overall. Thus, the manipulations necessary to quantify masking, if it occurs, are a trade-off working directly against the manipulations that allow us to measure psychophysical performance in the first place. Ultimately, if perceptual masking cannot be reliably observed in rodent behaviour then a rodent model may not be suitable for investigating the effects of visual masking in a discrimination task.

Task design can have a large impact on animal behaviour, and our resulting models of animal perception (Carandini and Churchland, 2013). The results of our study suggest an orientation discrimination task may not be ideal for visual masking research in rodents, however we cannot rule out the possibility that perceptual masking might be observed in a different type of task. A number of visual masking studies have implemented a detection task (Crawford, 1947; Kahneman, 1968), which is arguably a simpler task to perform. In a detection task, it may be possible to maintain higher performance using shorter target durations, therefore both improving the sensitivity of the task for measuring the effects of masking and increasing the likelihood of visual masking occurring. However, a visual masking detection task would come with its own difficulties, as the rodents would need to be able to respond to one type of visual stimulus

while ignoring another of similar appearance. In a study investigating the effects of flanking Gabors on target Gabor detection, Long-Evans rats were required to respond to a target while ignoring synchronously presented flankers (similar to our centre-surround task with an SOA of 0 ms) (Meier and Reinagel, 2011). In this task, the presence of flankers impaired target detection, but also biased the animals to respond, even in the absence of a target (Meier and Reinagel, 2011). It is therefore likely that rodent performance would be similarly biased by the presence of a mask. Altogether this implies that essential aspects of visual masking, such as the inclusion of multiple, briefly presented stimuli, limit the performance of rodents and thus our ability to observe perceptual masking in rodents.

In a similar task to that used in rodents, we found that human perception was impaired in a monotonic trend that is typical of visual masking. In the spatially overlapping condition, perception was impaired at short SOAs for both forward and backward masking conditions (|SOA| < 100 ms). This was consistent for all target contrasts in backward masking, but only for the 10% contrast in forward masking, with performance at the higher contrasts reaching ceiling performance earlier (SOA>-50 ms). In rodents, where target contrast was always 100%, we only saw evidence of perceptual masking under forward masking conditions and for only one rat. Similarly, for centre-surround backward masking, human performance was impaired up to SOAs of 70 ms, a trend that was similar to the performance of one rat. These inconsistencies between species could reflect differences in task performance capabilities, in the strength of the masking effect or in the temporal dynamics of visual masking. In an orientation discrimination task, human performance is both high, and stable. Therefore, any factor that yields a small, reliable change in performance will be easily observable. In contrast, rodent performance is lower, and inherently more variable, so any factor yielding a change in performance would need to produce a large change to be observed. In addition to this, it is possible that the strength of masking was different between species. Although we attempted to keep stimuli as similar as possible between human and rodent experiments, the large differences in human and rodent visual abilities, for example in spatial acuity and receptive field size, meant that it was necessary to alter some aspects of the stimuli, namely the size of the target stimulus (Dräger, 1975; Wiesenfeld and Kornel, 1975; Birch and Jacobs, 1979; Parnavelas et al., 1981; Prusky et al., 2002). Given that the strength of the mask is highly dependent on stimulus properties, such as size, contrast and duration, it is possible that the strength of the mask was weaker in the rodent experiments and therefore only occasionally evident in the animal behaviour (Weisstein, 1972; Breitmeyer and Ogmen, 2006). Finally, if the temporal dynamics of visual masking were different between species, it could be that we failed to observe consistent effects of visual masking in rodents because we did not

sample the relevant SOAs. However, this seems unlikely given that the predictions using rodent neuronal responses indicate similar trends over similar SOAs to that of the human perceptual data.

The perceptual effects of visual masking arise through a combination of neuronal mechanisms acting across multiple regions throughout the visual processing hierarchy (Schiller, 1968; Fehmi et al., 1969; Levick and Zacks, 1970; Coenen and Eijkman, 1972; Alwis et al., 2016). The specific combination of these mechanisms is thought to vary depending on the properties of the stimuli, in particular the spatial configuration of the target and mask (Breitmeyer, 2008). Although there are many proposed mechanisms, the majority of neuronal theories explaining perceptual masking can be grouped into two broad categories; neural interruption and neural integration (Breitmeyer and Ogmen, 2000). The first of these theories proposes that neuronal processing of the target is abandoned at the arrival of activity evoked by the mask. This is a favoured explanation for U-shaped visual masking, where the greatest impairment in perception occurs at an intermediate SOA (~ 50-100 ms). However, neural interruption is contingent on the mask being presented after the target in time and is therefore incapable of explaining forward or common-onset (SOA=0) masking (Breitmeyer and Ganz, 1976). Given that the impairments in human perception, and the predictions of our neuronal decoder, always decreased monotonically towards a SOA of 0, it seems unlikely that neural interruption was contributing to our results.

On the other hand, neural integration proposes that, due to temporal processing limits of the visual system, at short SOAs the target and mask are combined in one 'perceptual window' resulting in the perception of a single, fused image (Kinsbourne and Warrington, 1962b; Eriksen and Collins, 1967). As a result, the visibility and perception of the target is reduced. Most researchers agree that neural integration is involved in monotonic visual masking trends, which we observed in our human participants, our neuronal population decoding, and in some of the rodent behaviour (Crawford, 1947; Eriksen and Lappin, 1964; Breitmeyer and Ogmen, 2000). If the size of this 'perceptual window' over which information is integrated were smaller in rodents than in humans, then it might explain why perceptual masking was so rarely observed. To the best of our knowledge, the temporal acuity of rodent vision has only been defined through critical fusion frequency (CFF), the frequency at which a flickering light appears to become continuous (Healy et al., 2013). In hooded-rats, CFF lies between 15-20 Hz (5.5 cd/m^2 luminance), suggesting that their visual perception may be integrated across windows of 50-66 ms (Legg, 1986). This is worse than the temporal acuity of humans, where the CFF lies around 50-90 Hz, predicting integration windows of 11-20 ms (Legg, 1986; Davis et al., 2015). Although this would suggest that the effects of

perceptual masking in rodents should extend to longer SOAs than in humans, it should be noted that if neural integration were the principle mechanism acting in monotonic visual masking, the CFF could not be a good measure of the 'perceptual window' size, as a 50-90 Hz CFF would suggest that visual masking in humans would only ever occur at SOAs shorter than 20 ms, which we have shown to be false.

Numerous rodent studies have stressed the importance of acuity considerations in visual task design (Prusky et al., 2000; Prusky et al., 2002; Wong and Brown, 2006), however, these studies have only addressed spatial and not temporal aspects of visual acuity. It is critical to consider the temporal acuity of animals in any behavioural study to ensure that the duration of the stimulus is appropriate for the animals to perform the task correctly. While the CFF provides some information about temporal acuity, it does not directly address the minimum duration of a stimulus that can be detected or discriminated. In Long Evans rats, the ability to discriminate the direction of motion in moving dots with 85% coherence was shown to increase with stimulus duration and plateau at 75% correct for durations of 200 ms and longer (Reinagel, 2013). However, the effect of stimulus duration has never been addressed for static stimuli. Here we identified a threshold stimulus duration at which rats can reliably discriminate stimulus features such as orientation: all rats performed significantly above chance for durations of 16 ms or longer. However, for a task that does not necessitate constraints on stimulus duration, our data suggests durations of 60 ms or greater would be ideal to maintain high performance levels. Here, we used 16 and 42 ms because we anticipated that longer target durations would be more difficult to mask.

In spite of controls in experimental design and stimulus properties, we found that visual masking did not consistently affect rodent performance in the same way that it affected humans, nor did rodent performance follow the patterns predicted from neuronal responses collected in V1 of anaesthetized rats. The introduction of a mask stimulus reduced overall task performance and compromised the sensitivity of the data to reveal any effects of perceptual masking. Unfortunately, the parameter manipulations that would increase the size of the masking effect, would also reduce rodent performance. Thus, it may be difficult to consistently observe perceptual masking in rodents. Our results indicate there may be limitations for the applications of rodent behaviour in the study of visual masking and perception. While rodents may still provide some significant advantages for investigating aspects of visual processing, there may be some significant limitations in the types of tasks that they can perform with adequate performance levels.

5 Inconsistent perceptual masking across rodents performing a detection task

5.1 Abstract

The perception of a target stimulus may be impaired if it is immediately followed by a mask. This phenomenon, known as backward masking, allows the perception of a target stimulus to be altered, without changing any of the physical properties of the stimulus itself. Therefore, visual masking provides a powerful tool to investigate the neuronal mechanisms of perception using masking. We sought to determine if rodents provide a suitable model to investigate the neuronal basis of perception. Masking requires brief targets, often with low contrasts, which makes detection difficult even in the absence of a mask. It is unknown if rodents are capable of reliably responding to these target stimuli, and therefore if the effects of a mask on target perception can be quantified. To address this, in humans (n=5) and Long Evans rats (n=7), we characterised how target contrast and the presence of a surrounding mask affected the detection of a target sine-wave grating. In humans and rats, target detectability improved with increasing target contrast and was generally reduced by the presence of a mask. However, the mask biased all rats (but not humans) to respond more frequently, even in the absence of a target stimulus. After controlling for this response bias, the reduction in target detectability caused by the mask was only significant in three of seven animals. Our results suggest that perceptual masking may be difficult to observe consistently in rats because the complexity of the requisite tasks pushes the limits of their perceptual and behavioural capabilities.

5.2 Introduction

In backward masking, the perception of a brief target stimulus is impaired when it is closely followed by another stimulus, the mask (Breitmeyer, 2008). In this way, perception of the target is reduced, without altering the physical properties of the target stimulus itself. Visual masking therefore offers a powerful tool to investigate the neuronal correlates of perception. To date, the precise neuronal mechanisms responsible for visual masking are unclear, but likely involve a complex interaction of mechanisms occurring throughout the retina, thalamus and cortex (Fehmi et al., 1969; Levick and Zacks, 1970; Rolls et al., 1999; Alwis et al., 2016). Understanding the mechanisms involved in visual masking may offer important insights into the neural underpinnings of visual perception.

The effect of a mask on target perception is commonly systematically manipulated by varying stimulus onset asynchrony (SOA). In humans, two possible psychophysical trends occur as SOA is varied, referred to as type A and type B functions (Kolers, 1962). In Type A masking, target perception (usually quantified in terms of detection performance) is maximally impaired when the target and mask are presented simultaneously (SOA=0) and then monotonically improves as the SOA is increased. Alternatively, in Type B masking, the greatest impairment in target perception occurs at an intermediate SOA, typically around 30-100 ms. This creates a U-shaped function relating detectability and SOA (Lefton, 1973). Whether Type A or B masking is observed depends on the properties of the target and mask stimuli, in particular their spatial overlap and relative energy (i.e. size, contrast & duration) (Schiller and Smith, 1966; Hernandez and Lefton, 1977; Alwis et al., 2016). For example, type B masking is more likely to occur when the target and mask stimuli are presented in spatially distinct locations, and the mask-to-target energy ratio is less than one (Tapia et al., 2011).

In some studies, rather than varying SOA, the target contrast is varied to manipulate its detectability. In order to increase the likelihood of perceptual deficits in this "contrast masking" paradigm, the SOA is usually selected so that the mask is presented immediately following the target (Saarela and Herzog, 2008). In general, the presence of a mask impairs target detection, however, the magnitude of this effect depends on the size and spatial separation of the mask (Saarela and Herzog, 2009). Although backward contrast masking has been clearly defined in humans s(Saarela and Herzog, 2008, 2009), it has never before been characterized in an animal model.

In order to conduct a detailed investigation of the neuronal mechanisms of visual masking, an animal model is necessary. Yet, few studies have collected neuronal responses to visual masking stimuli (Schiller and Chorover, 1966; Schiller, 1968; Vaughan and Silverstein, 1968; Fehmi et al., 1969; Levick and Zacks, 1970; Coenen and Eijkman, 1972; Bridgeman, 1975, 1980; Schwartz and Pritchard, 1981; Rolls and Tovee, 1994; Kovács et al., 1995; Macknik and Livingstone, 1998; Rolls et al., 1999; Macknik and Martinez-Conde, 2004), and even fewer have collected perceptual data in the same species (Fehmi et al., 1969; Bridgeman, 1980; Kovács et al., 1995; Macknik and Livingstone, 1998). This is necessary to confirm that the changes observed in neuronal activity actually coincide with perceptual deficits. Recently, rodents have become a popular choice for visual research due to a number of cost and experimental advantages (Lee et al., 2012; Reinagel, 2014; Juavinett and Callaway, 2015). In particular, they provide an expansive range of sophisticated tools to monitor and manipulate specific neuronal subsets and circuits (Huberman and Niell, 2011; Lee et al., 2012; Juavinett and Callaway, 2015). Although rodents possess a visual system that is less developed, with lower spatial acuity and contrast sensitivity than non-human primates (Prusky et al., 2002; Busse et al., 2011; Histed et al., 2012), it is clear that they are capable of learning and performing visual tasks with performance levels comparable to that of non-human primates (Busse et al., 2011; Meier et al., 2011; Histed et al., 2012; Tafazoli et al., 2012; Zoccolan, 2015; Bossens and Op de Beeck, 2016). For these reasons, we sought to determine if the rat was a suitable model for research in visual masking and perception.

In Chapter 2, we used an electrophysiological investigation of visual masking in anaesthetised Long Evans rats to show that neuronal responses to oriented circular gratings were altered by the presentation of a mask with analogous trends to those observed in other mammalian species (Bridgeman, 1975, 1980; Kovács et al., 1995; Rolls et al., 1999; Alwis et al., 2016). When we applied a neuronal decoder to the data (Chapter 4), we predicted that performance should decrease at short SOAs in an orientation discrimination task. However, when we trained rats to discriminate the orientation of target stimuli, we found that visual masking rarely altered performance in the way that the neuronal decoder predicted. There were no consistent effects of SOA on discrimination performance. That is despite the fact that human perception was impaired in a similar task design. These results suggest that an orientation discrimination task may not be appropriate for visual masking research in rodents, however we cannot rule out the possibility that perceptual masking might be observed in a different type of task.

The effects of visual masking on target perception are typically quantified as the ability to detect or discriminate the target stimulus. In theory, detection tasks might be easier for animals to learn than discrimination tasks as they involve a more natural rule of response, i.e. respond if you see something. In contrast, discrimination tasks require mapping two different stimulus properties to two different, and arbitrary, behavioural responses. Detection tasks may therefore provide the potential for rats to achieve higher baseline performance levels in the absence of a mask, thus increasing the sensitivity of their behaviour to perceptual deficits. We sought to determine if perceptual masking was present in rats performing a detection task. Ideally, the task would explore how multiple stimulus manipulations affect visual masking (e.g. target contrast, mask contrast, SOA, size, spatial overlap, spatial separation), however in an animal model, training on multiple parameter manipulations is difficult and extremely time consuming. For this reason, we elected to vary only one stimulus parameter. Although SOA is the most common manipulation in visual masking research, from the results in Chapter 4, we were concerned that manipulating SOA interacted with the animals' impulsiveness. We therefore elected to manipulate target contrast, allowing us to use fixed trial durations.

We first characterized the effects of visual masking in humans performing a target detection task where we varied target contrast, SOA and the spatial separation between stimuli. The mask impaired target detection across all target contrasts with the greatest effects of the mask occurring at a SOA of 50 ms. We subsequently trained rats to perform a two-interval forced choice detection task, in which they were rewarded for reporting the presence of a target grating. Targets varied in contrast and were followed by a mask (SOA = 50 ms) on some trials. As in the human subjects, target detectability decreased with target contrast. However, the presentation of a mask biased all animals to respond more frequently, regardless of whether the target was low contrast or even absent. When we controlled for this response bias, the effect of the mask on target detectability was only significant for three of seven animals. Altogether our results suggest that perceptual masking may be difficult to consistently observe in rodents because the complexity of the requisite tasks pushes the boundaries of their perceptual and behavioural capabilities.

5.3 Methods

5.3.1 Ethics

All experimental procedures involving animals were approved by the Monash University Committee for Ethics in Animal Experimentation (MARP2015/003) and were conducted in accordance with the National Health and Medical Research Council guidelines for the care and welfare of experimental animals. All experimental procedures involving humans were approved by the Monash University Human Research Ethics Committee (CF16/392 - 2016000178) and were conducted in accordance with the National Statement on Ethical Conduct in Human Research.

5.3.2 Human Perception

Two authors and three naïve subjects took part in the experiment. All subjects had normal or corrected to normal vision. Each subject performed a training session prior to data collection.

Target stimuli were sine-wave gratings with orientation 0 or 90°, spatial frequency 3 cpd, and limited to a circular annulus with diameter 8°. Mask stimuli were plaids (0+90°) presented in annuli with an outer diameter of 15.5° and an inner diameter that either matched the size of the target, or that was 14.5°, meaning there was a 3.25° separation between the contours of the target and mask. Target stimuli were presented for 23.5 ms in 50% of the trials. The mask stimuli were presented for 100 ms at three stimulus onset asynchronies (0, 50 & 100 ms) relative to the target. A mask stimulus was presented in 66% of trials, with equal probability of the 8 or 14.5° inner diameter mask being shown. Target contrast was varied between 1 and 32% while mask contrast was always 100%. All stimuli were generated using Psychtoolbox in MATLAB and were presented on an 85 Hz refresh rate CRT monitor positioned at a viewing distance of 50 cm.

Head position was stabilized with a chin rest. A total of 1728 trials/subject were collected across two sessions. Within a session, trials were presented in blocks of 50 allowing participants to take frequent breaks. At the beginning of each trial, participants fixated on a small cross, located 5° to the left of screen centre. The target and mask stimuli were presented 5° to the right of the screen centre. Following stimulus presentation, the participants were required to indicate whether they had perceived a target stimulus by button press. Correct detections were indicated by a brief tone.

5.3.3 Rodent Perception

Data were collected from 7 adult male rats weighing 300-400g. Long Evans rats were selected for their high visual acuity (~1.0 cycle/degree) (Prusky et al., 2002). Rats were group-housed in environmentally enriched enclosures with a 12:12 hr reversed light-dark cycle. Animals had *ad libitum* access to food, but daily water consumption was restricted to rewards obtained during experimentation as well as a two-hour period of ad libitum access following the last test session in a day. Test sessions were run once or twice daily, five days/week. On non-testing days, animals had *ad libitum* access to water. The training period ranged from 52-106 testing days.

Three rats of an initial cohort of 10 were excluded due to unavoidable time constraints that prevented the completion of their training.

5.3.3.1 Testing Apparatus

Rodents were trained and tested in a custom Plexiglas testing chamber (20W x 30L x 40H cm) with two beam-break detectors (Little Bird Electronics, GP1A57HRJ00F) embedded in the front 'viewing' wall of the enclosure. To activate the sensors, rats blocked the infrared beam with their nose. The rats initiated stimulus presentation by activating the central sensor and reported their percept by leaving the central sensor and activating the flanking sensor, which incorporated a 16-gauge stainless steel tube for reward delivery from a computer-controlled syringe pump (New Era Pump Systems, NE-500). Visual stimuli were presented to rats on 120 Hz LCD monitors (Samsung 2232RZ or Eizo FG2421)(Ghodrati et al., 2015) positioned 25 cm from the viewing wall. All stimuli were generated in MATLAB, using the Psychophysics Toolbox extensions (Brainard, 1997; Pelli, 1997; Kleiner M, 2007).

Custom MATLAB scripts were used to sample the photo-interrupter outputs at 120 Hz (Measurement Computing, USB 1208FS), register rat behaviour, control stimulus presentation and administer rewards or timeouts.

5.3.3.2 Stimulus Details

Target stimuli were sine-wave gratings with orientation 0 or 90°, spatial frequency 0.1 cpd, and presented in a circular aperture with diameter 51°. Mask stimuli were a full screen plaid (91 by 58.5°) created by the sum of both target orientations, but with a 56° aperture centred over the target location. Thus, there was a 2.5° separation between the outside edge of the target and the inside edge of the mask aperture. Target stimuli were presented for 48 ms at one of four contrasts (12.5, 25, 50 and 100%). Two animals (rats 2 and 3) reliably detected the 12.5% contrast and thus an additional 6.25% condition was also included for these animals. The duration of the mask was 72 ms and the contrast was held constant throughout the testing period at either 50% (rats 1, 4, 6 and 7) or 100% (rats 2, 3 and 5), depending on the capability of the animal (Table 5.1). If rats were unable to reach our threshold criterion of 70% correct detection of high contrast targets in the presence of a 100% contrast mask, they proceeded into the task with the maximum mask contrast at which they could reasonably perform the task (50%).

Table 5.1. Task parameters were adjusted for each rat according to their performance and reaction time capabilities.

Rat #	Mask Contrast	Response Window (ms)	Late Target Onset Delay (ms)
1	50%	700	1200
2	100%	700	1200
3	100%	800	1300
4	50%	700	1200
5	100%	700	1200
6	50%	800	1300
7	50%	800	1300

5.3.3.3 Rodent Task Design

Rodents were trained to perform a two-interval detection task, in which they were rewarded for correctly detecting a target grating that varied in contrast and that was sometimes followed by a mask stimulus. Rats initiated a trial by blocking the infrared beam of the central sensor with their nose and reported target

detections by leaving the central sensor and activating the flanking report sensor. Once a trial was initiated, if the rat maintained the central nose-poke, a target stimulus was presented at either an early (400 ms), or late (1200/1300 ms) delay from the onset of the trial (Figure 5.1). The allowed response window always began at the onset of the target stimulus. In order to determine if the rats were reporting the presence of an early target, the early and late response windows could not overlap in time. We also added a 100 ms gap between the two possible response windows. Initially, we trained animals with a 700 ms response window, meaning that the late target could not be presented earlier than 1200 ms after the initiating nose-poke (400+700+100 ms). However, some animals had slower reaction times during the early training phase, so we allowed an 800 ms response window and a corresponding 1300 ms delay.

Two thirds of trials included a mask stimulus, which was presented at a delay of 450 ms from the onset of the trial. Thus, there was a 50 ms SOA between the early target (if it appeared) and the mask. Early targets had variable contrast (6.25-100%) but targets presented in the late interval always had 100% contrast. In this way, there were four trial categories enabling us to monitor numerous aspects of the rodent behaviour: 1) early target-only trials, which enabled us to observe the rodents' ability to detect each target contrast in the absence of a mask; 2) early target + mask trials which enabled us to investigate the effect of a mask on target detection; 3) late target-only trials which enabled us to monitor the proportion of false detection trials, in which the rats responded in the early window, regardless of stimulus presentation and; 4) early mask + late target trials, which allowed us to determine the rate of incorrect responses to the mask.

On each trial, the rats had 700/800 ms from the onset of the target to exit the central sensor and then 2 seconds to activate the flanking report sensor to indicate their detection. Any exits from the central sensor that were not followed by a nosepoke at the report sensor were ignored and excluded from analyses. Correct responses were rewarded with 75-175 μl of 5% sucrose solution. To encourage rats to perform the task correctly, we implemented a ramped reward system, in which the reward volume increased with each consecutively correct trial: the first correct response following an error received 75 μl; two consecutive correct responses received 100 μl; and three or more consecutive correct answers received 175 μl. Incorrect nose-pokes at the flanking sensor received no reward and triggered a brief 3.3 kHz error tone. To discourage rats from exiting the central sensor prematurely, trial duration was fixed at 2.9-3.1 seconds, regardless of their behaviour.

To prevent rats from developing a time-dependent response strategy, if rats made 2 consecutive incorrect choices for the same target delay, a 'correction trial' was implemented, in which the target delay was fixed until a correct response was obtained. All correction trials were excluded from analyses. On average, for the final task design, correction trials represented less than 10 percent of the trials completed.

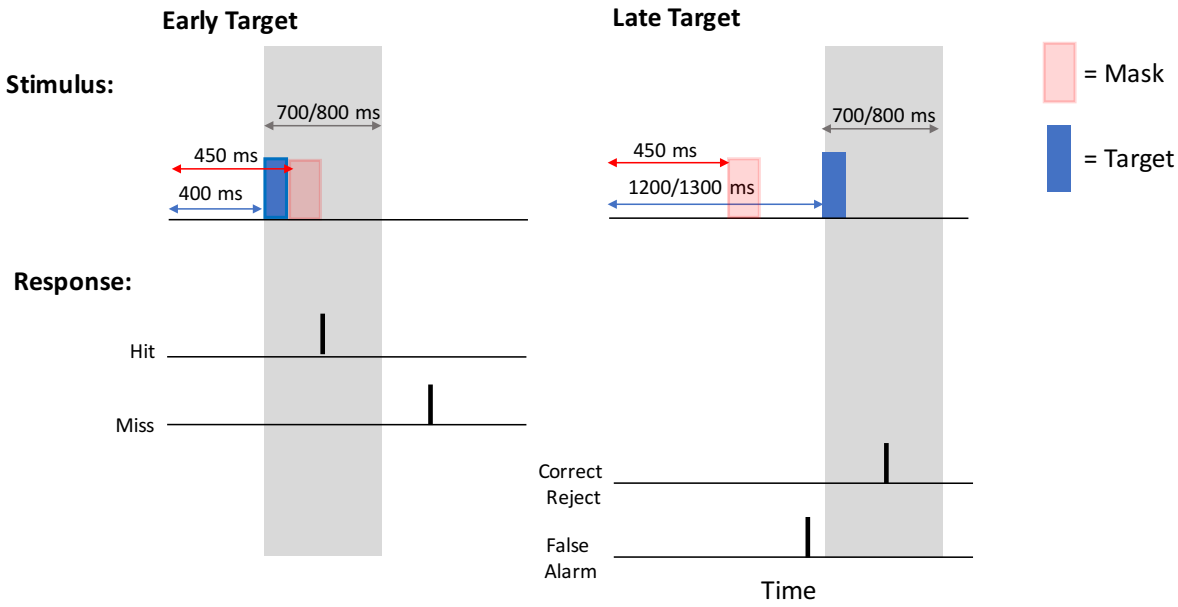

Figure 5.1. Schematic of the different trial types and the categorization of responses. Target stimuli were presented on every trial at either a 400 ms or 1200/1300 ms delay from trial onset. From the onset of the target, the rats had 700 (rats 1,2,4 & 5) or 800 (rats 3,6 & 7) ms to exit the central sensor and then a further 2 seconds to enter the flanking sensor to report their detection. Mask stimuli were presented in 67% of trials at a 450 ms delay from the onset of the trial. Target stimuli presented in the early interval varied in contrast between 6.25-100%. Trials with a late target onset served as catch trials, but included a 100% contrast target to monitor the animal's motivation. The hit and miss rates were calculated from trials with an early target onset. Correct reject and false alarm rates were calculated from trials with a late target onset. Correct rejects required animals to withhold a response during the early period, and then respond to the high contrast target in the late period.

5.3.3.4 Analyses

5.3.3.4.1 Trial exclusion

After animals had reached threshold performance, test sessions were excluded from analyses if the rats incorrectly responded to the mask in the absence of a target at a rate more than two standard deviations above their average performance across all sessions. This resulted in the exclusion of a maximum of 2 data sessions (~400-600 trials) per animal. Correction trials and trials where the rats did not activate the flanking sensor were excluded from all analyses. Altogether we collected 3340-8768 valid trials across 25-38 sessions per animal.

5.3.3.4.2 Detection and response bias calculations

We quantified target detectability for each contrast in both target only and masked trials using the sensitivity index (d'), a statistic used in signal detection theory to measure the separation between noise and signal distributions:

d'= z(Hit rate) - z(False alarm rate).

Where z(X) indicates the z-score of the proportion X.

5.4 Results

5.4.1 Human detection performance is impaired by the presence of a mask

We examined how the detection of a target grating was affected by target contrast, target-mask separation and SOA. As expected, regardless of SOA and target-mask separation, target detectability, quantified using the dimensionless statistic d', increased significantly with contrast (Figure 5.2A; p_{SOA0} <0.0001, $F_{5,20}$ = 56.17; p_{SOA50} <0.0001, $F_{5,20}$ = 30.33; p_{SOA100} <0.0001, $F_{5,20}$ = 26.4; two-way ANOVA).

The presence of a mask reduced target detectability relative to the performance in target-only trials with the contiguous mask producing larger reductions in d' than the spatially-separated mask. The effect of the mask, regardless of its separation from the target, was greatest for the 50 ms SOA condition, indicating a U-shaped psychometric curve (Type B masking). This was clearest for 8% contrast targets, but was evident for a range of contrasts (Figure 5.2B). To determine if the effect of the mask varied across contrast, we calculated the difference in target detectability between target only and masked trials. In general, the effect of the mask, regardless of its separation, was greatest when the contrast of the target was closer to threshold. This is shown for the 50 ms SOA where masking was most effective, but was also evident at other SOAs (Figure 5.2C). For each SOA, detectability was significantly affected by the mask condition (p_{SOA0} <0.001, $F_{2,8}$ = 33.59; p_{SOA50} <0.001, $F_{2,8}$ = 32.12; p_{SOA100} =0.0015, $F_{2,8}$ = 16.4; two-way ANOVA). Post-Hoc analyses revealed that the presence of a contiguous mask reduced detection performance relative to the target only trials across all SOAs (p_{SOA0} <0.001, p_{SOA50} <0.001, p_{SOA100} =0.0012; Tukey's multiple comparisons test). The effects of the spatially separated mask were only significant for the 50 ms and 100 ms SOA (p_{SOA0} =0.109, p_{SOA50} =0.0264, p_{SOA100} =0.0216; Tukey's multiple comparisons test).

In order to determine if the presence of a mask stimulus influenced the participants' bias to respond, we calculated false alarm rates across each masked condition. In general, there was little bias to respond, however, the false alarm rate did increase in the presence of a mask, in particular for the contiguous mask condition (Figure 5.2D). The effect of the mask condition on the false alarm rate was not significant ($p = 0.0587$, $F_{2,8} = 5.365$; one-way ANOVA).

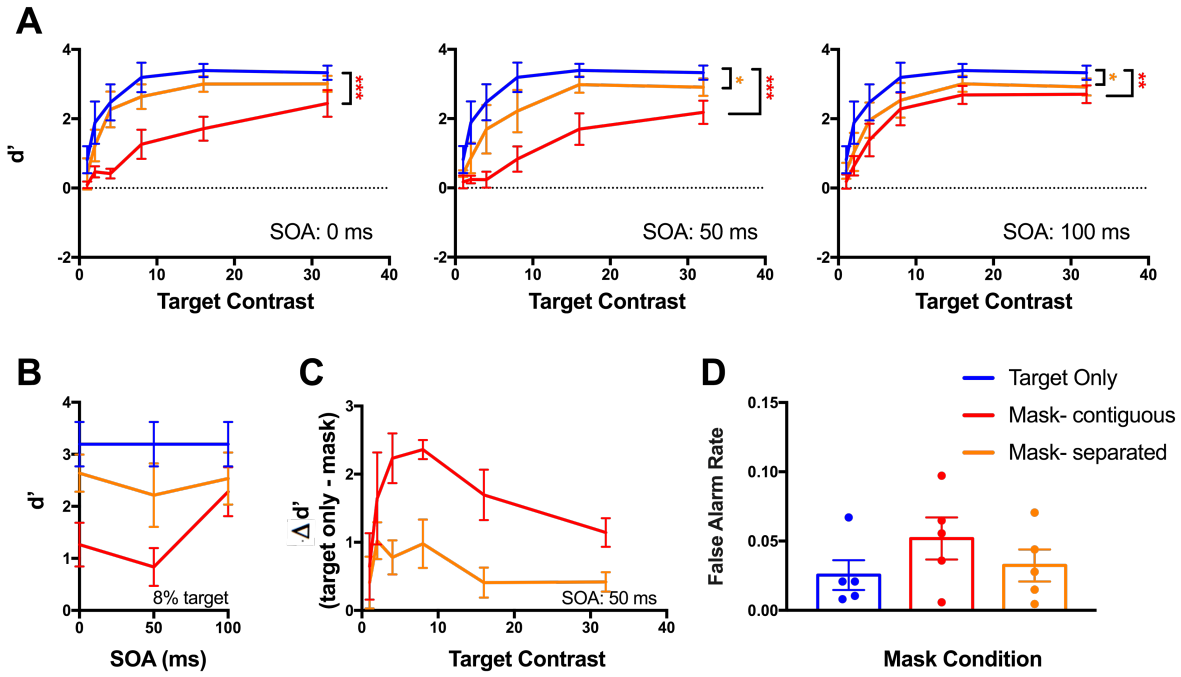

Figure 5.2. Human detection performance is impaired by the presentation of a mask. A) Detection performance (d') was measured for a target only, and two masked conditions, across a range of target contrasts. Results were averaged across participants (n=5). The masks were presented at three SOAs: 0, 50 and 100 ms. The target only condition (blue) is the same dataset for each SOA. B) Detection performance for the 8% target contrast plotted across SOA demonstrates a U-shape function for both mask conditions. C) The difference in target detection accuracy (d') between target only and masked trials is represented for the 50 ms SOA condition. The effect of the mask was greatest when the target was closer to the contrast detection threshold. D) False alarm rates were altogether low and were unaffected by the mask condition. (***) $p<0.0001$;(**) $p<0.01$; (*) $p<0.05$.

5.4.2 Rats reliably detected targets, but were biased by masks

In order to determine if visual masking produced similar perceptual deficits in rodents to those in humans, we trained rats to perform a target detection task. To avoid confusing the rats with multiple parameter manipulations, we only varied the contrast of the target and presented the mask at a fixed 50 ms SOA, as this produced the largest masking effect in humans. For each animal we calculated hit, false alarm and lapse rates across the different contrast and mask conditions (Figure 5.3). Hit rates were calculated from

trials with an early target while false alarm and lapse rates were calculated from late target trials. Across all trials we also calculated the proportion of trials on which the rats were rewarded, as a measure of overall performance. Although overall performance (correct rate) ranged from just 56-82% across animals, lapse rates were consistently low (<0.03) for all animals, indicating that they had sufficient motivation to perform the task correctly. Hit rates increased with the contrast of the target, regardless of if the mask were present. However, false alarm rates were reasonably high, and increased with the presence of a mask. The effects of the mask on response bias are addressed in more detail below. Given that the hit rate is subject to bias, it is not a suitable indicator of target detectability in our study, thus below, we use the metric d'.

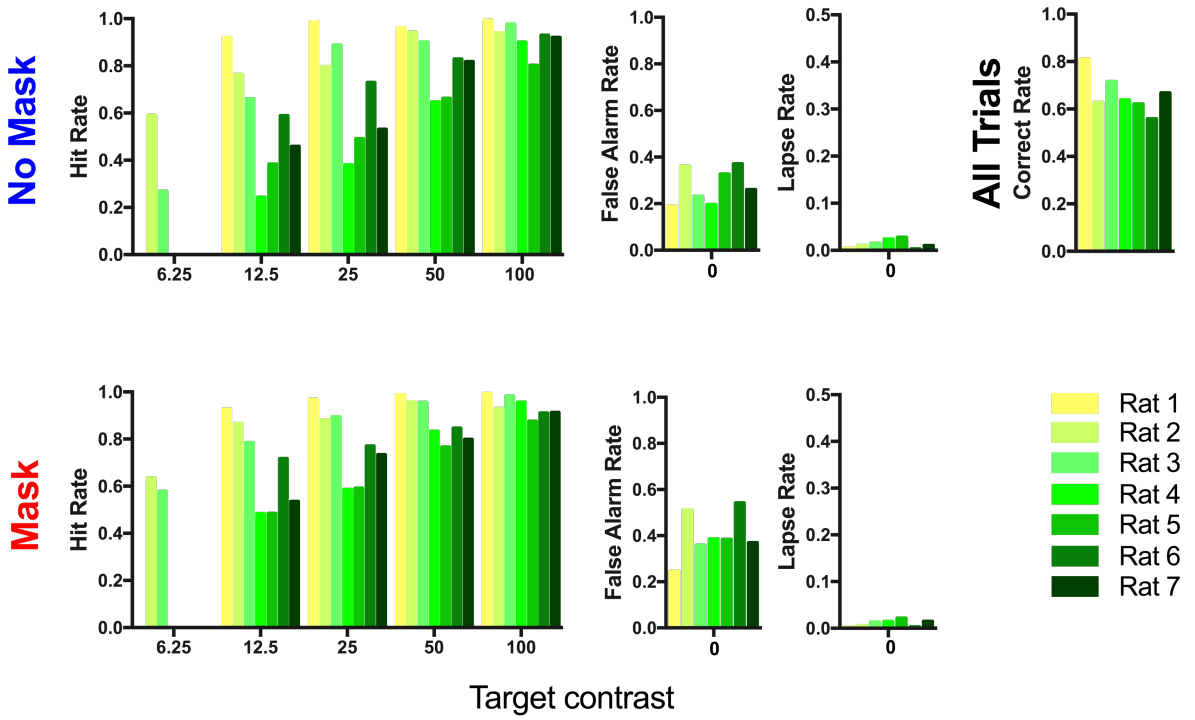

Figure 5.3. Behaviour was consistent across animals. Hit, false alarm and lapse rates for trials without a mask (top panels) and with a mask (bottom panels). Correct rates calculated from all trials indicate the rate at which the rats were rewarded. Each colour represents the performance of a single rat. A target stimulus was presented on every trial at either a 400 ms or 1200/1300 ms delay from trial onset. Only the targets that were presented in the early interval varied in contrast, and were used to measure the rate of hits and misses. Target stimuli presented in the late interval were always 100% contrast and were used to calculate the false-alarm rate. Only Rats 2 and 3 were tested with target contrasts of 6.25%.

5.4.3 Target detectability is affected by the contrast of the target

We examined the effects of target contrast and the presence of a mask on target detectability using d', because it accounts for an animal's tendency to respond in the absence of a stimulus (false alarm rate). Note that, although rats performed trials with either a 50% or 100% contrast mask, our training results demonstrated that each animal was performing the task near their psychophysical threshold. As in the human data, we found that d' significantly increased with target contrast regardless of if a mask were present (Figure 5.4A; pContrast<0.001, $F_{3,48} = 6.972$). The effect of target contrast on detectability was significant for all animals ($p_{Rat1}<0.0001$, $F_{3,75} = 11.7$; $p_{Rat2}<0.0001$ $F_{4,120} = 59.89$; $p_{Rat3}<0.0001$, $F_{4,144} = 144.6$; $p_{Rat4}<0.0001$, $F_{3,93}= 104.6$; $p_{Rat5}<0.0001$, $F_{3,102} = 88.51$; $p_{Rat6}<0.001$, $F_{3,93} = 28.4$; $p_{Rat7}<0.0001$, $F_{3, 72}= 51.75$; two-way ANOVA).

If visual masking impaired target perception, we would expect that d' would be lower in masked trials compared with target only trials. While on average this was true, there was no significant effect of the mask on target detectability across the population (Figure 5.4A; $p_{Mask}=0.3128$, $F_{1,48} = 1.04$). In general, individual animals performed worse in masked trials across all target contrasts, as indicated by the positive values in Figure 5.4D. However, this trend was significant for only three of seven animals ($p_{Rat1}=0.013$, $F_{1,25} = 7.19$; $p_{Rat2}=0.14$, $F_{1,30} = 2.349$; $p_{Rat3}=0.11$, $F_{1,36} = 2745$; $p_{Rat4}=0.33$, $F_{1,31}= 0.97$; $p_{Rat5}=0.17$, $F_{1,34} = 1.95$; $p_{Rat6}=0.001$, $F_{1,31} = 13.28$; $p_{Rat7}=0.048$, $F_{1, 24}= 4.30$; two-way ANOVA). Unlike in humans, we did not see any systematic change in the effect of the mask across target contrasts. Given that the strength of a mask tends to increase with its contrast (Breitmeyer and Ogmen, 2006), we were interested to see if the effect of the mask was greater for the rats performing the task with a 100% contrast mask. However, counter to expectations, we found that the three rats whose performance was significantly impaired by the presence of a mask were all performing the task with a 50% contrast mask (Figure 5.4B & E; rats 1, 4, 6 & 7; stats reported above). Rats performing the task with a 100% contrast mask were not significantly impaired by its presence, although two of these animals still tended to perform worse in the presence of the mask (Figure 5.4C & F; rats 2,3 & 5; stats reported above).

To determine if the animals that were significantly affected by the presence of a mask were related in any other aspect of the task or their behaviour, we examined the response window duration (700 vs 800 ms); their overall performance; their false alarm rates; the duration of their training; and the number of sessions and trials they completed in the final task. We found no relationship between any of these factors and the effect of the mask on their behaviour.

Altogether our results suggest that target detectability is affected by target contrast in a similar manner to that which occurs in humans. However, the effects of the mask on target detectability were inconsistent, with performance being significantly impaired in only three of seven animals. The difference in the effects of the mask between animals appears to correlate with the contrast of the mask. However, the animals performing the task with a 50% contrast mask, were doing so because they were unable to reach criterion to progress into the final task with a 100% contrast mask. It is therefore possible that the effects of the mask differed between animals' due to differences in the animals perceptual/cognitive capabilities rather than the contrast of the mask.

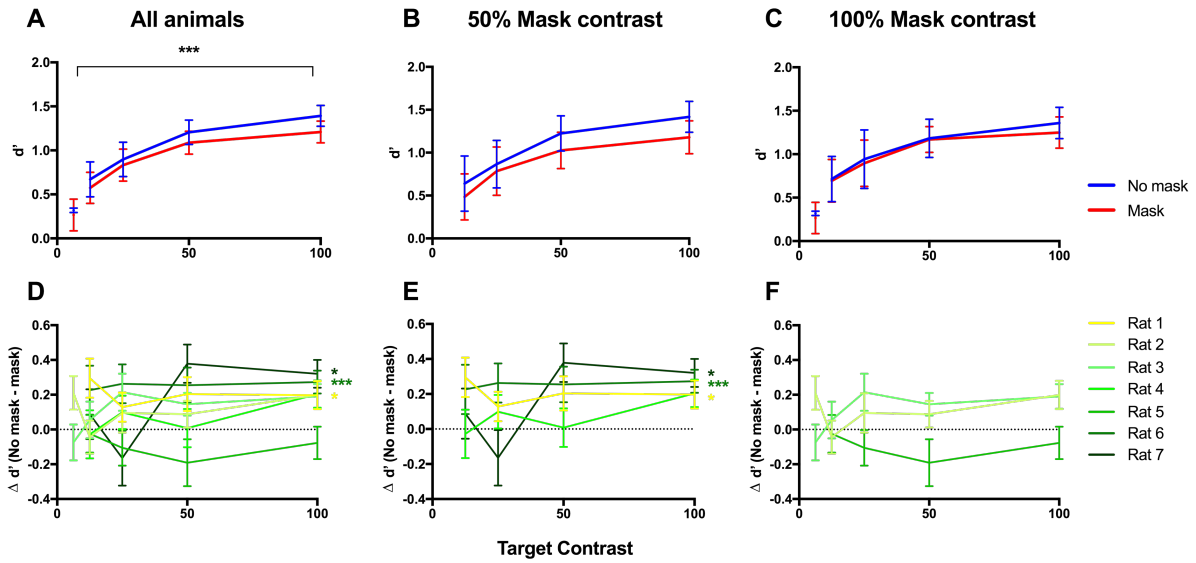

Figure 5.4. Target detectability decreases with target contrast but is not consistently impaired by a mask. A) Detection performance (d') was averaged across test sessions and across animals. This is shown separately for animals performing the task with a B) 50% and C) 100% contrast mask. D) The difference in target detection accuracy (d') between target only and masked trials is represented for each animal and shown separately for animals performing the task with a E) 50% and F) 100% contrast mask. The positive values indicate that most animals performed better in the target only trials, with the exception of rat 5, who performed better in the presence of a mask. 6.25% contrast condition represents the average performance of 2 animals (rats 2 & 3). Error bars represent ± SE across testing sessions; (*) p<0.05; (***) p<0.001;

5.4.4 Uninformative mask stimuli increase the bias to respond

In a Go/No-Go detection task, animals are commonly rewarded for responding (e.g. licking) when a target stimulus is present, and withholding the response when a target is absent. Therefore, impulsivity, or a bias to respond (lick) will increase the hit rate, at the expense of increased false alarms. Given our observation in Chapter 4 that rats respond impulsively, often failing to wait for visual cues, we specifically included trials in which the target was absent in the early interval. In these trials, animals were required to respond to a high contrast target that was presented in the late interval. This allowed us to monitor the rate of false alarm responses in the absence of a target stimulus, and critically, how the presence of a mask impacted this response bias. In signal detection theory, response bias is traditionally calculated as the criterion c = z(hit rate)+z(false alarm rate)/2. However, given that our noise distribution (false alarm rate) was the same for each target contrast, whereas the hit rate necessarily changes with contrast, the criterion metric does not provide any additional information compared to the false alarm rate on its own. We therefore quantified the effect of the mask on response bias by comparing the rate of false alarms in late-target trials in which a mask was present or absent during the early window. Rats responded impulsively (i.e. before or during the early response window, in the absence of a stimulus), in 28% of the late target trials (Figure 5.5A). The presentation of a mask significantly increased the average rate of false alarms by 12% (Figure 5.5A; $p<0.001$, $t_6= 6.175$; paired t-test). Altogether this indicates that while the animals were biased to respond early, the mask exacerbated this bias.

In Figure 5.5B we show the cumulative probability of response throughout the duration of a trial, for the late target only and late target + mask conditions. In the absence of any visual stimulus (blue trace), rats tended to respond impulsively within the early response window, and notably, at times that were consistent with when an early target would have appeared. In the presence of a mask (but with no target), rats responded more frequently in the early response window, in a manner that was time-locked to the appearance of the mask (divergence of red and blue traces). This suggests that the rats used trial timing-cues to respond.

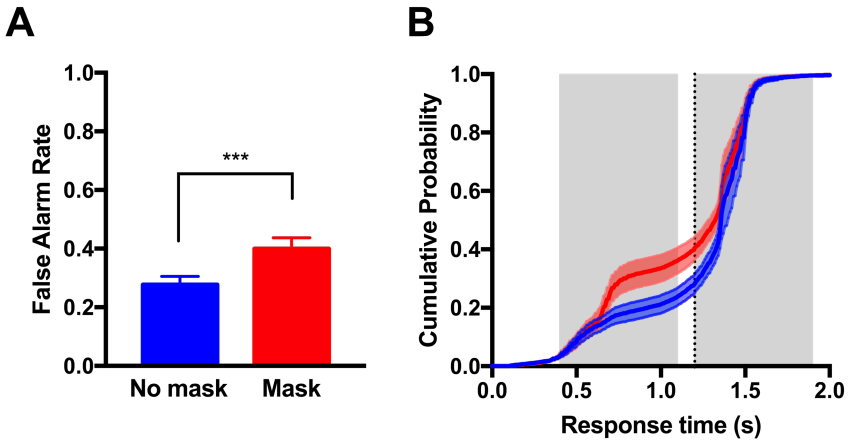

Figure 5.5. Rodents respond impulsively and are biased to respond in the presence of a mask. A) Response bias, measured as the false alarm rate was averaged across animals (n=7). Animals were biased to respond and this bias was exacerbated by the presentation of a mask. B) The cumulative probability of response over time was averaged across animals for late target trials. The gray shaded windows indicate the early and late target response windows. The vertical dotted line indicates the onset of the late target. Error bars represent ± SE. (***) p<0.001.

5.4.5 Rats' responses are primarily visually evoked

Given the possibility that the rats were responding in a timing dependent manner, we specifically analysed when the rats were exiting the central sensor to report their detection (Figure 5.6). In general, the rats tended to respond within one of the two possible response windows. When they missed an early, low-contrast, target they were most likely to respond within the first few hundred milliseconds of the late response window (just after the late target should have appeared). In the late target trials, the rats rarely missed the target (see lapse rates; Figure 5.2), and any false alarms tended to occur within the early response window. Collectively this indicates that the rats understood that rewards could be obtained by responding in one of two response windows. However, regardless of the target contrast or the presence of a mask, the rats were most likely to respond shortly after target presentation. There was a narrow distribution of response times for both hits and correct rejects, in fact, for the 100% contrast early target, more than 80% of responses occurred within a 200 ms period beginning 100 ms after the target onset. This suggests that the rats sometimes responded according to a timing cue, but in most cases correctly responded to the target stimulus.

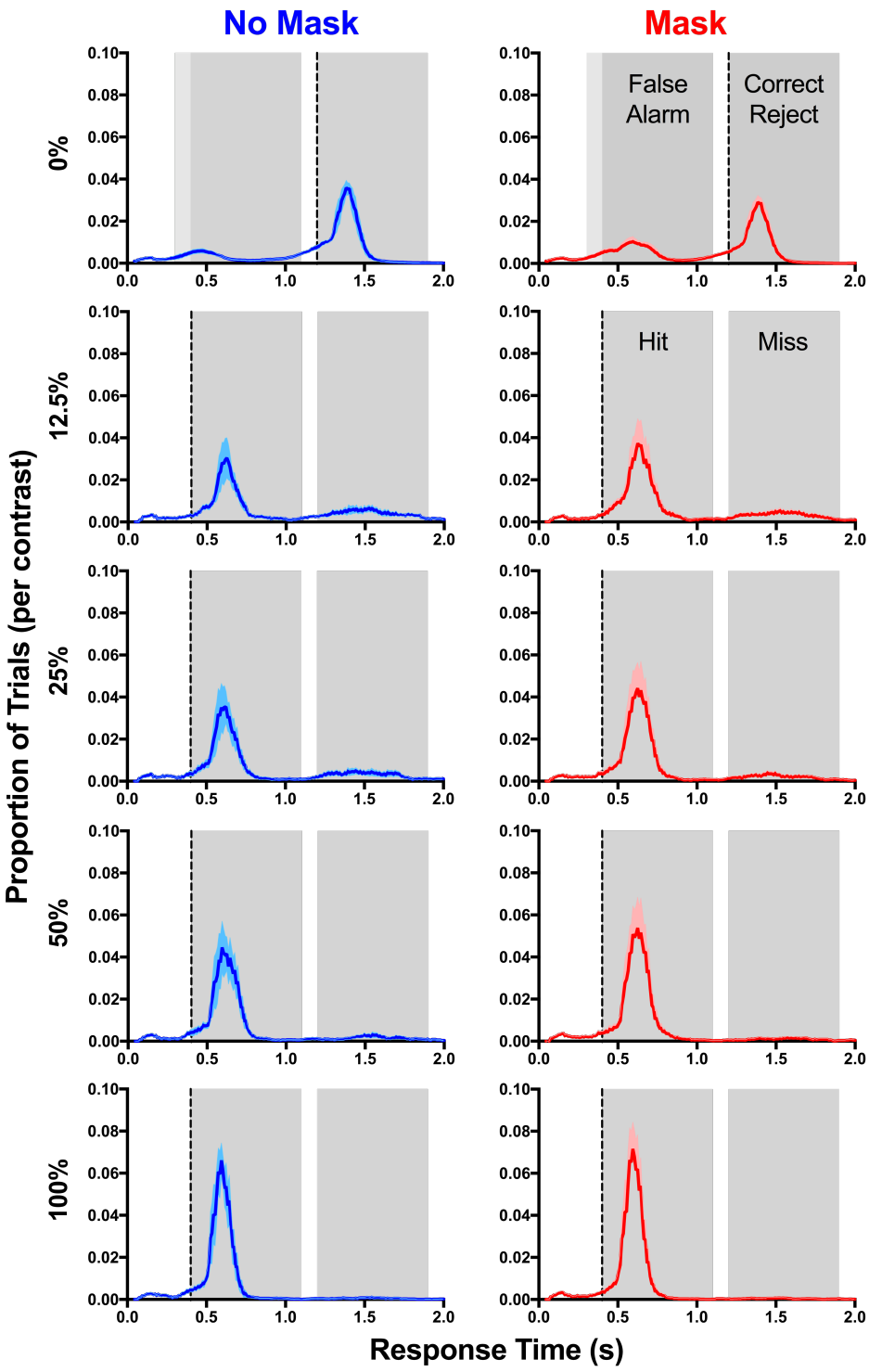

Figure 5.6. Rodent responses are predominantly visually driven. The distributions of response times were aligned according to the onset of the target stimulus (vertical dotted line) and averaged across animals (n=7). The gray shaded windows illustrate the early interval and late interval response windows. The 0% target condition represents the trials with a late target, where there was no target presented in the early interval but a 100% target presented in the late interval. Note that for this condition the response times were aligned to the late target onset, meaning that animals expected the early response window to begin either 800 or 900 ms prior to the late target onset depending on the late target delay. Only trials where the rat activated the flanking report sensor within 2 seconds of exiting the central sensor were included in these analyses. The red and blue shaded regions represent ± SE.

5.4.6 Response times are faster for high target contrasts

To further understand how the rat response times were affected by the target and mask stimuli, for each response type (hit, miss, false alarm and correct reject) we calculated response times from the onset of the early target, to the time that the rats exited the central sensor. In general, response times were less variable when the animals responded correctly (Figure 5.7A & B; hits and correct rejects) when compared with incorrect responses (Figure 5.7C & D; misses and false alarms). For hit trials, the response times were influenced by target contrast ($p_{Hit}<0.01$, $F_{3,48} = 4.889$; two-way ANOVA), with response times becoming significantly shorter at the higher target contrasts. However, response times were not significantly affected by target contrast in miss trials, with the response times remaining approximately the same across all target contrasts ($p_{Miss}=0.9587$, $F_{3,42} = 0.1016$; two-way ANOVA). These results suggest that in correct trials, the animals were responding to the target stimulus and were thus affected by the contrast of the target, while in the incorrect trials, the animals were responding independently of the target stimulus, presumably in a time-dependent manner. To further explore this possibility, we compared the distribution of response times between hits with the lowest contrast tested for all animals (12.5%) and false alarm responses. We expected that if rats were responding according to a timing-cue in the incorrect trials, the reaction times would be similar between these groups. We found this to be true; there were no significant differences between hit and false alarm response times ($p=0.2742$, $F_{1,6} = 1.448$; two-way ANOVA). In all response types, there were no significant effects of the mask on response times ($p_{Hit}=0.6638$, $F_{1,48}= 0.1914$; $p_{Miss}=0.9630$, $F_{1,42}= 0.0021$; two-way ANOVA; $p_{CorrectReject\_1300}=1.00$, $t_4= 0.003$; $p_{CorrectReject\_1200}=0.99$, $t_6= 0.007$; $p_{FalseAlarm}=0.86$, $t_{12}= 0.186$; paired t-test).

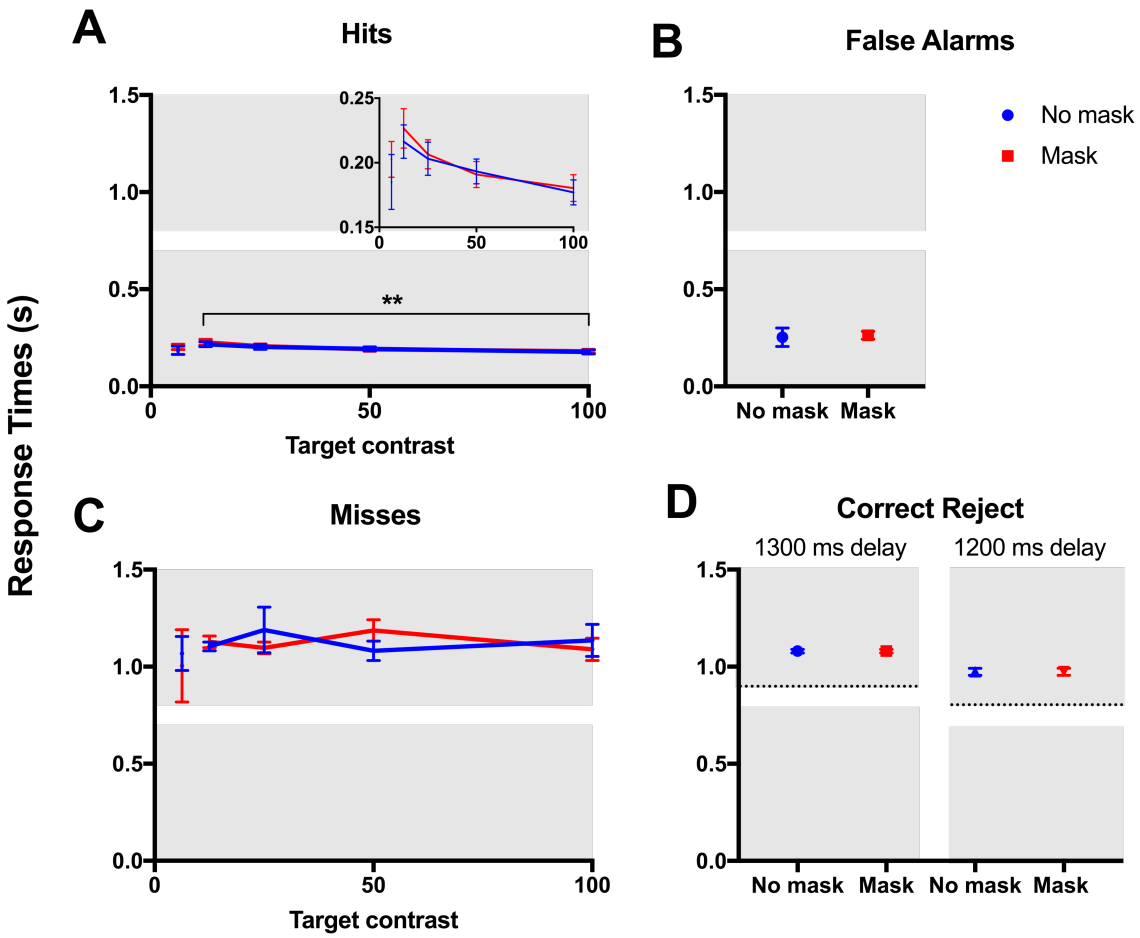

Figure 5.7. Response times are affected by target contrast. Reaction times were measured from the time of target onset in the early interval, regardless of whether the target was presented in the early or late interval. The median response times were averaged across animals (n=7) for A) hit, B) false alarm, C) miss and D) correct reject trials. The inset in panel A shows a zoomed axis of the effect of target contrast on reaction times in the hit trials. Incorrect responses where the rodents C) missed the target or B) responded in the absence of a target tended to fall within the incorrect response window. Trials were considered a correct reject when the animal correctly withheld a response during the early interval and then correctly responded to the 100% contrast target presented in the late interval. Late targets were presented at either a 1200 ms delay from trial onset (rats 1,2,4 & 5) or a 1300 ms delay (rats 3,6 &7). Reaction times for the 6.25% contrast condition represent an average of only 2 animals (rat 2 & 3). The dotted lines in plot D) represent the late target onset, the gray boxes illustrate the response windows for the early and late intervals. Error bars represent ± SE. (**) $p<0.01$.

5.5 Discussion

We sought to determine if perceptual masking was present in rats performing a detection task. In humans, we found that detection performance systematically improved with increasing target contrast, and was reduced by the presence of a mask. In order to determine if similar perceptual deficits occurred in a rodent species, we trained rats to perform a two-interval detection task. Similar to our human data, rodent detection performance was significantly affected by the contrast of the target and was generally reduced by the presence of a mask. However, the effect of the mask on target detection was only significant for three of seven animals, all of which were performing the task with a 50% contrast mask. Counter-intuitively, rats performing the task with a 100% contrast mask were unaffected by its presence. Below we discuss: 1) the possible influence of mask contrast on rodent behaviour; 2) the limitations of a rodent model for research in visual masking and perception; and 3) the differences between rodent and human perception that may have affected our results.

Given that previous perceptual studies have shown that the strength of a mask increases with its contrast (Weisstein, 1972; Breitmeyer and Ogmen, 2006), we expected that the effect of the mask on rodent perception would be greater for animals performing the task with a 100% contrast mask. Counter to this, we only found a significant effect of the mask on target detection in 3 of 4 rats performing the task with a 50% contrast mask. The difference in the effect of the mask across the population could not be attributed to any other aspect of the animal's behaviour or training. Although unexpected, this result is not unprecedented given that the shape of the trend across SOA can depend on the relative energy (contrast x duration x size) of the target and mask stimuli (Breitmeyer and Ogmen, 2006). For example, in humans the peak masking effect has been shown to shift from an SOA of 56 to 36 ms when the duration of the mask was increased from 2 to 32 ms (Breitmeyer, 1978). Similarly, the peak masking effect is expected to shift to shorter SOAs as the contrast of the mask is increased (Francis, 2003). In this way, it is possible that the peak masking effect shifted with the contrast of the mask so that perceptual deficits were only evident at an SOA of 50 ms when the contrast of the mask was 50%. Unfortunately, it is impossible to determine whether this was actually the case without additional data from another SOA or from multiple mask contrasts within the same animal.

As the rats tested with each mask contrast differed, it is also possible that our results reflect inter-animal differences rather than a true effect of mask contrast. The animals performing the task with a 50% contrast mask were doing so because they were unable to reach our criterion (70% correct) to progress into the final task with a 100% contrast mask. Thus, in a sense, the animals were grouped according to their cognitive or perceptual capabilities. It is therefore possible that the changes in target detection that we observed in three animals, reflect the same behavioural features that prevented the animals from being able to perform the task with a 100% contrast mask in the first place. At the very least, the difference in the learning capabilities that we observed between rats implies there may be significant limitations for studying complex visually-driven behaviours in rodents.

Behavioural performance is always affected by an amalgamation of sensory, and non-sensory factors. In rodents, the tendency to respond impulsively can be a particularly dominant, non-sensory factor (Schwarz et al., 2010; Busse et al., 2011). In our study, we found that responses were clearly visually driven, but that the rats had a strong prior about when the stimulus should occur. Thus, impulsive responses, in the absence of a stimulus, most often occurred in the early target response window. This suggests that the animals were highly sensitive to trial timing cues and had a preference for shorter duration trials. That is despite the fact that the target stimulus was presented in the late interval in the majority (66%) of trials. Thus, while impulsive responses were made at strategic times within the trial, their timing-based strategy favoured speed over optimal reward acquisition across a testing session. This is similar to the findings of a motion discrimination task, where rat responses were predominantly governed by the time that had elapsed from the onset of the trial, rather than a criterion for evidence accumulation (Reinagel, 2013). Together these results demonstrate that rodent behaviour can be strongly influenced by trial timing cues. The influence of such non-sensory factors is difficult to avoid in an animal task, but should be considered in task design and ideally monitored throughout data acquisition.

Although rodents provide a valuable model for visual research, it is important to consider the limitations of their behavioural capabilities. Our investigations of visual masking (here and in Chapter 4) have demonstrated that rats are capable of learning and performing complex visual tasks, but that the presence of distractor stimuli significantly impairs their performance. While all our rats were able to detect target stimuli with high performance levels in the presence of a 50% contrast mask, more than half were unable to reach criterion to perform the final task with a 100% contrast mask. In general, the presentation of a mask significantly increased the animals' bias to respond. A similar issue was reported for a detection

task in which rats were required to report the presence or absence of a target Gabor that was sometimes presented between two flanking Gabors. In this study, the rats were unable to reach adequate target detection performance when the contrast of the flankers exceeded 40% (Meier et al., 2011). The researchers found that the flankers biased the rats to report the presence of a target, and that this bias increased with the contrast of the flankers (Meier and Reinagel, 2011). These findings suggest that rat behaviour may be limited in ways that impact the feasibility of some visual investigations. For example, should we have found that masking was only evident with a 100% contrast mask (and absent with the 50% contrast mask), more than half of our animals would not have been capable of performing the task with the stimulus parameters necessary to produce perceptual masking.

The greatest challenge of research in visual masking, is that the changes in stimulus parameters that increase the likelihood of perceptual deficits arising (i.e. higher mask contrast, shorter target duration, smaller target size, less separation between the target and mask stimuli) also reduce an observer's overall performance, and therefore the sensitivity of the data to any perceptual deficits that could be occurring (Alpern, 1953; Schiller and Smith, 1968; Weisstein, 1972; Breitmeyer, 1978; Oğmen et al., 2003; Breitmeyer, 2008). This is a particularly acute problem in animal studies, where having sufficiently high performance is necessary to maintain animal motivation, because correct responses are tied to rewards. Therefore, it is possible that perceptual masking does occur in rats, but that it only occurs with stimulus parameters that would severely compromise their baseline performance in an experimental scenario, thus preventing the perceptual deficits from being observed consistently across animals. During training, we focused on reducing the separation between stimuli and increasing the contrast of the mask to increase the likelihood of masking. The fact that four of our rats were unable to reasonably perform the task with a 100% mask contrast indicates that the task parameters were already pushing the limits of the animal's capabilities. This suggests there was little room to manipulate other stimulus parameters in a way that might increase the masking effect, such as shortening the target duration or decreasing the target size. Unfortunately, if the perceptual effects of visual masking cannot be consistently measured in a rodent species, then the benefits of any neuronal investigations are limited.

Rodents have become a popular choice for visual research, however, it is clear that they are not a perfect model of human visual processing and perception. The effect of contrast on stimulus detection provides a good example of when the psychophysical results in rodents closely replicates that of humans, albeit with greatly increased contrast detection thresholds (Busse et al., 2011; Histed et al., 2012). However,

the perceptual effects of contextual modulation are often different between species. In Chapter 4 we did not find convincing evidence of perceptual masking in rodents performing an orientation discrimination task, even under similar experimental conditions to that which caused perceptual deficits in humans. In the present study, the effects of the mask on target detection were only significant in three rats, and did not systematically change with target contrast, as it did in humans. Similarly, the influence of spatial context on target perception has been shown to be different between rats and humans, where, despite controlling for stimuli, task and learning procedures, the presentation of collinear flankers enhanced target detection in humans but impaired detection in rats (Meier and Reinagel, 2013). Whether these perceptual differences arise from variations in the retinal structure, representational capacity of neurons in visual cortex or other attention/cognitive factors is hard to say. Either way, it is clear that perception can vary between species, thus it is important to consider perceptual differences in any visual investigation that uses a rodent model.

We have shown that rats were capable of learning and performing a complex visual masking detection task and that their ability to detect target stimuli was affected by the contrast of the target in a similar manner to that of humans. However, the effect of the mask on target detection was only significant in three of seven animals. It is possible this inconsistency was the result of the mask contrast, which varied between animals, or alternatively it may reflect differences in the animals' cognitive/perceptual capabilities. In order to observe the effects of masking consistently across animals, it may be necessary to alter stimulus parameters so that the strength of the masking effect is increased (i.e. shorter target duration), however, these parameter manipulations would increase the difficulty of the task. Given that the animals were already performing at the boundaries of their capabilities, our data suggest it may not be possible to consistently observe perceptual masking in a rodent species.

6 Conclusion

In visual masking, the perception of a brief target stimulus may be impaired by a mask that is presented nearby in space and time. Manipulating the temporal separation of two stimuli (i.e. their stimulus onset asynchrony) systematically alters the effect of the mask on the neuronal representation and perception of the target stimulus (Breitmeyer, 2008). In this way, target perception can be altered without changing any of the physical properties of the target stimulus itself. Thus, visual masking provides a powerful tool to investigate the neuronal mechanisms of perception. Despite this, only a few studies have recorded neuronal responses to visual masking stimuli (Schiller and Chorover, 1966; Schiller, 1968; Vaughan and Silverstein, 1968; Fehmi et al., 1969; Levick and Zacks, 1970; Coenen and Eijkman, 1972; Bridgeman, 1975, 1980; Schwartz and Pritchard, 1981; Rolls and Tovee, 1994; Kovács et al., 1995; Macknik and Livingstone, 1998; Rolls et al., 1999; Macknik and Martinez-Conde, 2004), and even fewer have collected perceptual data within the same species (Fehmi et al., 1969; Bridgeman, 1980; Kovács et al., 1995). Given that perceptual data is necessary to determine if the changes observed in neuronal processing actually coincide with perceptual deficits, the lack of simultaneously obtained neuronal and perceptual data means that existing models of masking are based on extremely impoverished data sets.

Visual masking is likely to arise through a complex interaction of mechanisms occurring throughout the retina, thalamus and cortex (Fehmi et al., 1969; Levick and Zacks, 1970; Rolls et al., 1999; Macknik and Martinez-Conde, 2004; Alwis et al., 2016). However, the cortical mechanisms are of particular interest to the study of perception. In cortical neurons, the response to the target consists of two components, an early transient and a late sustained response (Bridgeman, 1975; Rolls and Tovee, 1994; Macknik and Livingstone, 1998). To date, the neuronal correlates of visual masking have been defined as a suppression of target evoked activity. In backward masking, this response suppression occurs specifically in the late sustained component of the response to the target (Bridgeman, 1975; Rolls and Tovee, 1994; Macknik and Livingstone, 1998; Lamme et al., 2002). This suggests that the sustained activation, not just the transient component, is important for the development of perception. Yet, the origin of this late component and the method of its suppression remain highly contentious. In V1, although backward masking causes a suppression of the late sustained component in all cells, the pattern of this response suppression across

stimulus onset asynchrony only matches the psychophysical trend in a small subset (~25%) of cells (Bridgeman, 1975; Schwartz and Pritchard, 1981; Macknik and Livingstone, 1998). Uncovering what defines this unique sub-population could provide important insights into the development of conscious visual perception.

Recently, rodents have become a popular choice in vision research due to a number of cost and experimental advantages (Lee et al., 2012; Reinagel, 2014; Juavinett and Callaway, 2015). For example, rodents provide the opportunity to monitor and manipulate specific neuronal cell types and circuitries in large cohorts of awake and behaving animals (Huberman and Niell, 2011; Lee et al., 2012; Juavinett and Callaway, 2015). Despite possessing lower spatial acuity and contrast sensitivity than non-human primates (Prusky et al., 2002; Busse et al., 2011; Histed et al., 2012), it is clear that rodents are capable of performing detection and discrimination tasks with similar performance levels to that of primates (Busse et al., 2011; Meier et al., 2011; Histed et al., 2012; Tafazoli et al., 2012; Zoccolan, 2015; Bossens and Op de Beeck, 2016). The Long Evans rat, especially, has an excellent track record for performing complex visual tasks such as object discrimination and Gabor detection in the presence of distractors (Meier et al., 2011; Meier and Reinagel, 2011; Tafazoli et al., 2012). For these reasons, we sought to investigate visual masking, and the neuronal correlates of perception in a Long Evans rat model.

6.1 Key findings of this thesis

In Chapter 2, we showed that both spatially overlapping and spatially distinct mask stimuli reduced the target-evoked firing rate and orientation selectivity of V1 responses in the anaesthetised rat. For both forward and backward masking conditions, the response suppression was greatest at short stimulus onset asynchronies, which is a trend that has been observed in other mammalian species. However, unlike previous studies, we did not identify any unique populations of cells where the neuronal response to the target was affected at different stimulus onset asynchronies. Our investigation provides novel findings, in that visual masking can reduce the selectivity of the response, even in response components where the firing rate is unchanged. For example, under backward masking conditions, the selectivity of the response was reduced in the early transient component despite the firing rate remaining the same. This indicates that the neuronal effects of backward masking are not limited to the sustained component of the response, as has previously been suggested.

In order to determine if the changes observed in neuronal processing were accompanied by perceptual deficits, and therefore potentially related to the development of perception, it is necessary to collect perceptual reports from the same species. For this reason, we developed a sophisticated behavioural paradigm that can be easily coupled with neuronal recordings to study visual behaviour in rats. In Chapter 3 we described the development of both discrimination and detection visual masking tasks that were used to assay rodent perception. Rodents were trained to perform these tasks within ~50-100 days and we described the factors affecting the trajectory of learning.

In Chapter 4, we show that the neuronal data from Chapter 2 predicts that Long Evans rats would experience perceptual deficits at short stimulus onset asynchronies in an orientation discrimination task. Despite human perception following these predicted trends for both spatially overlapping and spatially distinct stimulus configurations, we found that rodent performance, although reduced by the presence of a mask, did not always change systematically across stimulus onset asynchrony (as is typical of perceptual masking). This was true for all spatial and temporal configurations of stimuli. However, given that these results did not exclude the possibility that visual masking might be observed in a different type of task, we decided to further assess the perceptual effects of visual masking in a detection task.

In Chapter 5, we examined the effects of target contrast and the presence of a spatially distinct mask on target detectability in rodents and humans. As the contrast of the target was increased so too did target detection performance in both humans and rats. In humans, the presentation of a mask significantly impaired performance and while the mask generally reduced target detectability in rats as well, the effect was only individually significant in 3 of 7 animals.

6.2 Interpretation of key findings

Our neuronal investigation of visual masking revealed that the firing rate and selectivity of the neuronal response to the target was reduced at short stimulus onset asynchronies. Apart from the special case of blindsight, it seems uncontroversial that V1 is critical for visual perception and performing visual tasks in all species (Lamme et al., 2000). We therefore expected that rodent perception would be impaired under similar conditions to those used in our electrophysiological study, however, perceptual masking was not observed consistently across animals in our discrimination and detection tasks. Why was perceptual masking not observed when we know that similar stimuli can alter neuronal processing in V1? First, it could be that the changes in neuronal processing associated with our stimuli were not sufficiently large so as to alter rodent perception. Second, for methodological reasons, we used slightly different visual stimuli in our electrophysiological and perceptual studies. Thus, it is also possible that the few small differences between our stimuli in their size, duration and spatial structure (sine-wave vs. square-wave) were sufficient to remove, or significantly reduce, the effect of the mask in our perceptual investigations. Finally, it could be that perceptual masking occurs in rodents, but that the stimulus parameters necessary to elicit perceptual deficits also significantly reduce the behavioural performance of the animals, thereby compromising our ability to observe small changes in performance. We discuss each of these possibilities in further detail below.

6.2.1 Changes in V1 processing may not be sufficient to elicit perceptual deficits

When we decoded the neuronal responses from V1 to predict how rodent performance would be affected by visual masking in an orientation discrimination task, we found that performance was expected to decrease towards shorter SOAs. A limitation of our decoder was that we decorrelated the neuronal data set, which means that our decoder may have had access to more or different information than the real brain, as the information from each neuron was treated as though it were independent. If anything, this suggests that the decoder should be have been more sensitive and better able to extract information about target orientation, even when the mask was presented. Therefore, the fact that we saw a reduction in the decoder performance at short SOAs, suggests that there was a real and significant loss of target information in V1. However, there were no obvious perceptual deficits at the predicted SOAs in rodents performing the orientation discrimination task. Of course, it is true that there were important differences in the animals' state of consciousness between experiments, with the neuronal data collected under anaesthesia and the perceptual data collected from awake animals. However, given that anaesthesia tends to reduce or abolish the neuronal effects of contextual modulation (Lamme et al., 1998; Nothdurft et al., 1999), if anything we would expect that the effects of visual masking would be stronger in our awake animals. Thus, it is especially surprising that perceptual masking was not observed in our discrimination task. These results provide cause to consider whether a loss of information in V1 is sufficient to alter rodent perception.

Although it is clear that the activity of V1 influences, and is necessary, for visual perception (Lamme et al., 2000), small changes that occur in the neuronal activity of V1 may not be a good reflection of changes in any perceptual outcome. In U-shaped masking, it has been shown that while all cells in V1 display response suppression, the pattern of response suppression only matches the psychophysical results in a small subset of cells (Bridgeman, 1975; Schwartz and Pritchard, 1981; Macknik and Livingstone, 1998). This means that the majority of V1 cells follow a different, monotonic, pattern of response suppression that is apparently unrelated to perceptual experience. Altogether, this suggests that the existing neuronal correlates of visual masking are limited, and may only be applicable to certain cell types, yet it is not clear what defines these cell types. Furthermore, given that we were unable to identify any unique subpopulation of neurons in our own neuronal investigation, it is entirely plausible that the changes that we observed in rat V1 processing occurred in cells that were uninformative of rodent perception. Put another way, these neurons were primarily responding to, and representing the properties of the stimulus. This is consistent with previous studies in macaque monkeys, which have suggested that it is unusual for V1

neurons to have choice probabilities (which quantify correlations between neuronal activity and perceptual choices on single trials) that differ from chance (Nienborg and Cumming, 2006). If this was indeed the case, then it is not surprising that rodent perception was not affected under the same stimulus conditions that caused reductions in the firing rate and selectivity of V1 responses.

6.2.2 Stimulus differences may have prevented perceptual masking

Although we attempted to minimise the stimulus differences between our neuronal and perceptual investigations, there was some variation in the size, duration and spatial frequency content of our stimuli. Therefore, it is important to consider whether the stimuli used in our perceptual study simply didn't cause strong masking at the neuronal level (and therefore, we observed little perceptual masking).

Given that the likelihood of perceptual masking increases as the strength of the mask is increased, the absence of perceptual masking could have been the result of a decrease in the strength of the mask in our perceptual investigations. This would require a decrease in the duration, contrast or size of the mask or in the physical similarities between the target and mask stimuli (Turvey, 1973; Ishikawa et al., 2006; Saarela and Herzog, 2009). Counter to this, the duration of the mask stimulus actually increased from 33 ms in our neuronal investigation to 42 ms in our discrimination task and 70 ms in our detection task. Furthermore, our use of plaid masks rather than hyperplaids (created as a binarised sum of 12 gratings of differing orientations) in our perceptual investigations only increased the similarities in orientation and spatial frequency information between the target and mask stimuli. Thus, if anything, the strength of the mask was greater in our perceptual investigations than in our neuronal investigation.

Given that the likelihood of perceptual masking also tends to increase as the energy (size, duration, contrast) of the target stimulus is reduced (Breitmeyer, 2008), we must also consider the possibility that perceptual masking was absent due to an increase in target energy. This could occur as an increase in the target duration, size or contrast. This was certainly true when we consider the duration of the target stimulus, which increased from 33 ms in our neuronal study, to 42 ms in our discrimination task and 48 ms in our detection task. The energy of the target was also increased by the stimulus size in our detection task, where the diameter of the target was 51° compared to 10-40° in our neuronal study, where targets were optimised to the receptor field size of the majority of units on the electrode array. However, stimulus

size could not have contributed to the absence of perceptual masking in our discrimination task, as the size of the target stimuli were similar, if not smaller in size than those in our neuronal investigation. Finally, it is also unlikely that our use of sine-wave, rather than square-wave gratings in both psychophysical investigations decreased the likelihood of masking, as sine-wave stimuli generally elicit weaker responses and require higher contrasts to be detected than square-wave gratings (Campbell and Robson, 1968). This suggests that sine-wave stimuli are lower in energy than square-wave stimuli.

In summary, there was a slight increase in the energy of the target in our perceptual investigations, which occurred namely through an increase in the target duration. Although this difference in target duration was relatively large (9-15 ms), in humans, similar increases in target duration have not been sufficient to remove the masking effect. In fact, a spatially overlapping pattern masking study showed that an increase in the target duration from 24 to 40 ms, did not alter the range of SOAs where target perception was impaired (Turvey, 1973). However, that is not to say that the magnitude of the effect was not reduced. Furthermore, the changes in target duration may be of larger consequence in the rodent visual system, as it has a lower temporal acuity than that of humans (Legg, 1986; Davis et al., 2015). Thus, it is possible that the difference in target duration reduced or removed the effect of the mask on neuronal processing and therefore resulted in an absence of perceptual masking. Unfortunately, when we attempted to use shorter target durations (16 ms) in our discrimination task we found that the animal's baseline performance was severely compromised, which would have prevented our ability to observe perceptual deficits, even if they were present.

6.2.3 The requisite task may prevent the observation of perceptual masking

Perceptual masking was not observed consistently across animals in either our discrimination or detection tasks, however, that does not necessarily mean that perceptual masking never occurs in rats. It is possible that perceptual masking was occurring, but that the effects were too slight to be observed in animals performing the tasks with reasonably high error rates (even in the absence of a mask). Unfortunately, the biggest challenge of research in visual masking is that the parameter changes that increase the likelihood and strength of perceptual masking (i.e. an increased mask contrast, shorter target duration or smaller target) also reduce the observers baseline performance and therefore compromise the sensitivity of the data to any perceptual deficits that could be occurring (Alpern, 1953; Schiller and Smith, 1968; Weisstein, 1972; Breitmeyer, 1978; Oğmen et al., 2003; Breitmeyer, 2008). This is particularly problematic for research

in animals, where a relatively high performance level is necessary to maintain motivation levels, as the correct responses are tied to rewards. For example, although we found our rats were capable of discriminating target orientation with reasonably high performance levels (80+%) when the target duration was long (~100 ms), performance dropped precipitously for shorter target durations. Furthermore, in both our discrimination and detection tasks, the introduction of a mask stimulus significantly reduced performance, even on easy trials, saturating below 75% in many animals. In our detection task, this reduction in performance prevented us from increasing the contrast of the mask past 50% in more than half of the cohort. Altogether, this suggests that the animals were already performing at the limits of their capabilities, meaning that there was little room to adjust stimulus parameters in a way that might increase the masking effect (i.e. shorter target duration). Unfortunately, this problem is particularly acute in rodents as the limitations of their visual system (poor spatial acuity and contrast sensitivity) demand baseline target stimuli that are relatively large and high in contrast to maintain reasonable baseline target detection or discrimination performance. This makes it difficult to generate mask stimuli that are higher in energy, particularly if they are spatially distinct. Ultimately, it may not even be possible to perceptually mask the high-energy targets that are necessary to maintain adequate performance levels. Overall our behavioural results suggest that it may difficult to consistently observe perceptual masking in a rodent species as the requisite task pushes the boundaries of their perceptual and cognitive capabilities. Unfortunately, if the perceptual effects of visual masking cannot be consistently measured, then a rodent model will be of limited benefit to the study of visual masking.

6.3 Limitations of this thesis

A caveat of our neuronal investigation of visual masking was that our recordings were collected from anaesthetised animals. Although anaesthesia does not influence the classical receptive field tuning properties of cells in V1 (Snodderly and Gur, 1995), it can affect contextual modulation (Lamme and Spekreijse, 2000). For example, anaesthesia can weaken modulations associated with perceptual pop-out and completely abolish the effects of figure-ground segregation (Lamme et al., 1998; Nothdurft et al., 1999). However, not all contextual modulations are affected by anaesthesia; surround suppression may be observed in both anaesthetised and awake animals (Knierim and Van Essen, 1992; Polat and Sagi, 1993; Kapadia et al., 1995). In visual masking, although one study has addressed the effects of anaesthesia, the duration of the stimuli (target: 60 vs.100 ms; mask: 110 vs. 100 ms) and the location of the masks relative

to the classical receptor field (inside vs. outside) were altered between awake and anesthetised preparations (Macknik and Livingstone, 1998). Therefore, the researchers could not have distinguished between the differences caused by the stimulus presentation methods versus the effects of anaesthesia. Thus, it remains to be seen whether the effects of visual masking in anaesthetised animals, are an accurate reflection of the changes that would occur in an awake animal. Furthermore, it is known that anaesthesia can affect particular cell types more than others (Ikeda and Wright, 1974). In this way, anaesthesia could have prevented us from observing any unique populations of cells in V1 that were affected by visual masking at different timescales, as have been observed in V1 of awake cats and monkeys (Bridgeman, 1975; Macknik and Livingstone, 1998). Further investigation, using the same stimulus presentation methods between anaesthetised and awake animals, would be necessary to determine if the effects of visual masking were significantly altered by anaesthesia.

6.4 Concluding remarks

We explored the neuronal and perceptual effects of visual masking in the Long Evans rat. In V1, the neuronal responses to target stimuli were suppressed by the mask in a similar fashion to that which has been observed in other mammalian species. Yet, despite using similar stimuli we did not find consistent effects of the mask on rodent perception in a discrimination or detection task. We believe that the neuronal data did not accurately predict the perceptual outcome because of stimulus differences and the effects of anaesthesia. It is also clear that the stimulus parameters that are necessary to produce perceptual deficits in rats push the boundaries of the animals' perceptual capabilities. If the perceptual effects of visual masking cannot be observed consistently in a rodent species, then the benefits of further neuronal investigations are limited. While rodents provide a useful model for vision research, they may not be an ideal candidate for investigations requiring highly complex visual tasks, as is requisite of visual masking.

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8 Appendix

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Masking reduces orientation selectivity in rat visual cortex

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Alwis DS, Richards KL, Price NS. Masking reduces orientation selectivity in rat visual cortex. *J Neurophysiol* 116: 2331–2341, 2016. First published August 17, 2016; doi:10.1152/jn.00366.2016.—In visual masking the perception of a target stimulus is impaired by a preceding (forward) or succeeding (backward) mask stimulus. The illusion is of interest because it allows uncoupling of the physical stimulus, its neuronal representation, and its perception. To understand the neuronal correlates of masking, we examined how masks affected the neuronal responses to oriented target stimuli in the primary visual cortex (V1) of anesthetized rats ($n = 37$). Target stimuli were circular gratings with 12 orientations; mask stimuli were plaids created as a binarized sum of all possible target orientations. Spatially, masks were presented either overlapping or surrounding the target. Temporally, targets and masks were presented for 33 ms, but the stimulus onset asynchrony (SOA) of their relative appearance was varied. For the first time, we examine how spatially overlapping and center-surround masking affect orientation discriminability (rather than visibility) in V1. Regardless of the spatial or temporal arrangement of stimuli, the greatest reductions in firing rate and orientation selectivity occurred for the shortest SOAs. Interestingly, analyses conducted separately for transient and sustained target response components showed that changes in orientation selectivity do not always coincide with changes in firing rate. Given the near-instantaneous reductions observed in orientation selectivity even when target and mask do not spatially overlap, we suggest that monotonic visual masking is explained by a combination of neural integration and lateral inhibition.

forward masking; backward masking; V1; primary visual cortex

NEW & NOTEWORTHY

We examined how masks that preceded or succeeded oriented target stimuli affected neuronal responses in rat primary visual cortex. Regardless of the spatial or temporal arrangement of stimuli, the greatest reductions in firing rate and orientation selectivity occurred when target and mask appeared closely in time. On the basis of our neuronal data, we suggest that monotonic patterns of perceptual visual masking are explained by a combination of long neural integration windows and lateral inhibition.

VISUAL MASKING describes a phenomenon in which perception of a target stimulus is reduced or entirely abolished by another stimulus, the mask (Breitmeyer 2008). By varying the relative presentation times of the target and mask, the perception and neuronal response to the target stimulus may be systematically altered. In this way, masking reveals a disconnect between the physical stimulus, its neuronal representation, and its perception. Uncovering the precise mechanisms involved in visual masking will provide important insights into how neuronal activity leads to conscious visual perception.

The effects of masking on target perception depend on a range of spatial and temporal stimulus factors and are likely to involve a diverse family of mechanisms (Breitmeyer 2008; Macknik and Martinez-Conde 2007). As such, masking phenomena are commonly categorized according to *1*) the temporal relationship of the target and mask (forward vs. backward masking); *2*) the temporal dynamics of the influence of the mask on the target (A- and B-type masking); and *3*) the spatial configuration of mask and target (spatially overlapping vs. center-surround masking). The stimulus timing categories of visual masking include forward and backward masking, in which the mask either precedes or succeeds the target stimulus, respectively. Backward masking illusions are of particular interest as the perception of the target stimulus is retroactively reduced by mask-evoked neuronal activity; the timing means that, unlike forward masking, this cannot be explained through photochemical depletion in the retina or adaptation in the thalamus (Crawford 1947). Furthermore, psychophysical studies of backward masking have shown that mask presentation in one eye can reduce the visibility of a target presented to the other eye (Turvey 1973; Weisstein 1971). This binocular interaction of target and mask responses suggests that cortical mechanisms are involved (Kinsbourne and Warrington 1962).

In perceptual masking studies, two psychophysical trends have been described: A- and B-type masking (Kolers 1962). In A-type masking, target perception is maximally impaired when target and mask stimuli are presented simultaneously and monotonically improves with increasing stimulus onset asynchrony (SOA) between the target and mask. In most cases forward masking produces an A-type trend (Bachmann 1994). On the other hand, in B-type masking the greatest impairment in target perception occurs at SOAs of 30–100 ms (Lefton 1973). B-type masking is often obtained if the target and mask stimuli do not spatially overlap; however, the same stimuli can cause A-type masking if the energy (i.e., contrast and duration) of the target is considerably lower than that of the mask (Hernandez and Lefton 1977; Schiller and Smith 1966).

Two prevailing theories are commonly used to explain psychophysical masking: neural integration and neural interruption (Scheerer 1973). Neural integration proposes that the reduction in target visibility is due to limits in the temporal resolution of the visual system, therefore causing the neuronal representation of the target to fuse with that of the mask. This has been the most widely accepted mechanism explaining A-type masking for both forward and backward masking conditions (Eriksen and Lappin 1964; Pilz et al. 2013; Stoper and Banffy 1977). Neural interrup-

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tion instead proposes that target processing is disrupted by the arrival of the response to the mask, leading to reductions in target perception. While a prominent explanation for B-type masking, the origins of the different processing delays for target and mask and the specific means through which interruption occurs remain highly contentious (Breitmeyer and Ganz 1976; Breitmeyer and Ogmen 2006; Francis 1997; Keysers and Perrett 2002; Macknik and Livingstone 1998; Macknik and Martinez-Conde 2007).

In macaque primary visual cortex (V1), target-evoked activity is reduced under stimulus conditions that cause perceptual masking, directly reflecting the psychophysical trends (Bridgeman 1975, 1980; Macknik and Livingstone 1998; Schiller 1969; Schiller and Chorover 1966; Vaughan and Silverstein 1968). In the absence of a mask, brief target stimuli evoke biphasic activity, consisting of an early transient component and a late sustained component that can persist for hundreds of milliseconds. Under backward masking conditions, only the late component of the response to the target is reduced at SOAs that cause perceptual deficits (Bridgeman 1975, 1980; Macknik and Livingstone 1998). Interestingly, only ~25% of cells in V1 show a temporal pattern of response reduction consistent with B-type masking (Bridgeman 1975; Macknik and Livingstone 1998); what defines this particular neuronal subpopulation remains unclear.

The majority of physiological studies have focused on how visual masking affects stimulus detectability. However, it is equally important to understand how the ability of neurons to support stimulus discrimination is affected. In the inferior temporal cortex (IT), neuronal discriminability of shapes is impaired by masking (Kovács et al. 1995; Rolls et al. 1999); however, these changes may be inherited from earlier processing regions such as V1. Indeed, in the context of figure-ground textures, orientation selectivity in V1 was shown to be weakly impaired at short SOAs (Lamme et al. 2002), but this study was limited to backward masking conditions and only used stimuli with two orientations.

To evaluate the effects of visual masking on neuronal discriminability in V1, we recorded responses to brief, oriented gratings presented before or after plaid mask stimuli. Firing rates and orientation selectivity were reduced at short SOAs, reflecting an A-type trend, for both spatially overlapping and non-spatially overlapping stimuli under forward and backward masking conditions. This demonstrates that visual masking affects stimulus visibility and also discriminability. We also observed biphasic responses to our target stimuli, and comparisons between transient and sustained response components revealed separate effects of masking on firing rate and selectivity. Notably, we demonstrate that the effects of backward masking are not limited to the sustained component; orientation selectivity is affected throughout the entire response to the target, often in the absence of significant changes in responsivity. When responses of neurons in supragranular, granular, and infragranular layers were analyzed separately, no lamina-specific differences were revealed. We propose that A-type visual masking cannot be explained by neural integration alone; other mechanisms such as lateral inhibition are necessary to account for near-instantaneous reductions that occur in orientation selectivity.

MATERIALS AND METHODS

Experiments were conducted in accordance with the Code of Practice for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council, Australia) and received approval from the Monash Animal Research Platform Animal Ethics Committee (MARP/2013/081). Adult male Long-Evans rats (n = 37; 320–350 g) were obtained from the Monash University Animal Research Precinct (MARP) and housed under 12:12-h light-dark cycles with food and water provided ad libitum.

Surgery and extracellular recordings. Animals were placed in an induction chamber and anesthetized with 5% halothane (in 1 l/min O_2). Once surgical anesthesia was established (confirmed by the absence of a hindpaw withdrawal reflex), animals were intubated with a 16-gauge polymer tube to allow mechanical ventilation (75–80 breaths/min) with a constant maintenance of anesthetic (1–2.5% halothane in 0.3 l/min O_2). A thermostatically controlled heating pad and rectal probe were used to maintain body temperature at 37–38°C throughout the duration of the experiment. Depth of anesthesia was regularly monitored via the withdrawal reflex and palpebral reflex and via ECG and EMG recordings taken from the upper forelimbs.

Animals were placed in a stereotaxic frame, and a scalp incision was made to expose the skull overlying the known binocular zone in V1 (~1.8 mm rostral from lambda and 4.5 mm lateral to the midline suture). A craniotomy of ~4-mm diameter was drilled over V1 and a durotomy performed to allow electrode penetration. Neuronal activity was recorded with a single-shank linear electrode array with 32 contact points (<1.2 MΩ, 50-μm contact spacing; A1x32-6mm-50-177-A32, NeuroNexus Technologies). Electrodes were inserted up to a depth of 2,000 μm to span all cortical layers. Neuronal signals were amplified, filtered between 0 and 250 Hz (for local field potentials) and between 0.75 and 5 kHz (for spikes), and recorded at a sampling rate of 30 kHz with a Cereplex Direct data acquisition system (Blackrock Microsystems). Raw signals were spike sorted offline (Plexon Offline Sorter) to separate multiunit and single-unit activity.

Visual stimuli. Stimuli were generated with Psychtoolbox in MATLAB (Brainard 1997; Pelli 1997) and presented on a 120-Hz refresh rate VIEWPixx/3D LCD monitor (VPixx Technologies; Ghodrati et al. 2015) at a viewing distance of 30 cm.

Receptive fields (RFs) were mapped for each of the array's 32 channels with a stimulus consisting of 5° white dots presented at random positions on a 9 × 17 grid across the monitor. Dots were presented on a black background (50 ms flash on, 50 ms flash off). Once RF locations and sizes were characterized, flashed static square-wave gratings were used to probe orientation selectivity. Orientation tuning stimuli were optimized to the location and size of the RFs of the majority of the units on the array and consisted of gratings randomly presented at six orientations (0–150°, 30° increments; 50 ms flash on, 500-ms interstimulus interval) and two phases (0 and 180°) on a gray background.

Responses to spatially overlapping and non-spatially overlapping (center-surround) forward and backward masking stimuli were recorded with square-wave gratings as the target stimuli. These were visible within a circular aperture matching the size and shape of the RFs of the majority of units on the array. The target grating had 100% contrast and was randomly presented at 1 of 12 different orientations (0–165°, 15° spacing) and 4 different phases (90° spacing) for 33 ms. The mask stimulus was also presented for 33 ms and consisted of a black-and-white hyperplaid generated randomly for each trial by binarizing the sum of 12 gratings with each possible target orientation, and randomized phase (see Fig. 2*A*, *inset*). Mask stimuli were either presented at the same spatial location and dimensions as the target stimulus or presented with a center-surround arrangement, where the masks were full-screen with an aperture matching the target size and location. As the surround masks did not overlap the classical RF, we expected them to evoke little or no response. The relative time of the target and mask stimuli, referred to here as stimulus onset asynchrony

(SOA), was measured from the onset of the first stimulus to the onset of the second stimulus. To examine the effect of spatially overlapping forward and backward masking, target and mask stimuli were presented at SOAs between ±33.3 and ±333.3 ms; forward and backward masking are associated with negative and positive SOAs, respectively. For center-surround forward and backward masking, SOAs ranged from ±8.3 to ±333.3 ms, including SOAs with temporal overlap of target and mask presentation. In both masking paradigms, an SOA of ±333.3 ms was used as a control. Note that forward and backward masking form a continuum—an SOA of 0 ms simply indicates that target and mask are presented simultaneously. However, to ensure stability of recordings during a single type of masking, we presented forward and backward masking in separate blocks. When no target or mask was visible, the screen displayed a blank gray background (luminance = 53.2 cd/m^2) during nonoverlapping SOAs. The intertrial interval was set to 500 ms, and each unique stimulus condition was presented 8–10 times.

Determination of cortical depth. Current source density (CSD) was calculated as the second spatial derivative of the local field potential collected in response to full-screen flashes alternating between black on white and white on black (flash duration = 8.3 ms; blank duration = 408.3 ms). The CSD traces were examined in order to identify the boundary between layers 4 and 5 as indicated by a reversal from current source to current sink (Mitzdorf 1985). The current sinks identified at the boundary, in combination with depth-from-cortical-surface measurements, were used to define the granular layer. Units were then assigned to one of three depth categories, supragranular, granular, or infragranular, according to their location relative to the granular layer.

Masking analysis. Initially, to check the orientation tuning of multiunits and single units, spikes were counted in a 50–150 ms window from target onset and a von Mises function fitted to the mean spike rates at each SOA in response to every orientation. Comparing the distribution of responses to preferred and antipreferred orientations, units with *d'* values above 0.3 at the control SOA (±333.3 ms) were classified as tuned and were included in our analysis, with single-unit and multiunit responses pooled together. These selection criteria yielded 73 and 95 tuned units in the spatially overlapping forward and backward masking conditions, respectively, and a total of 42 and 63 tuned units in the center-surround forward and backward masking conditions, respectively. Note that relatively few units are strongly selective, as we used brief flashes of static gratings. Forward and backward masking recordings for both paradigms were taken from the same penetrations as far as possible; however, the difference in numbers of responsive units reflects that forward or backward conditions, and spatially overlapping or center-surround conditions, were tested in separate blocks.

Transient and sustained target responses were found in the time windows 50–100 ms and 100–300 ms after target onset, respectively. To examine the effect of response integration on orientation selectivity, three spike counting windows (80–100, 80–120, and 50–150 ms relative to target onset) were used to determine orientation tuning for each masking condition. These windows were chosen as they centered around the average peak latency for orientation selectivity, and so could be used to probe the effect of integration window size on our ability to discriminate target orientation using the response of a single neuron. Responses to the preferred (θ_{pref}) and orthogonal (θ_{null}) orientations were used to calculate an orientation selectivity index (OSI) across time for each SOA:

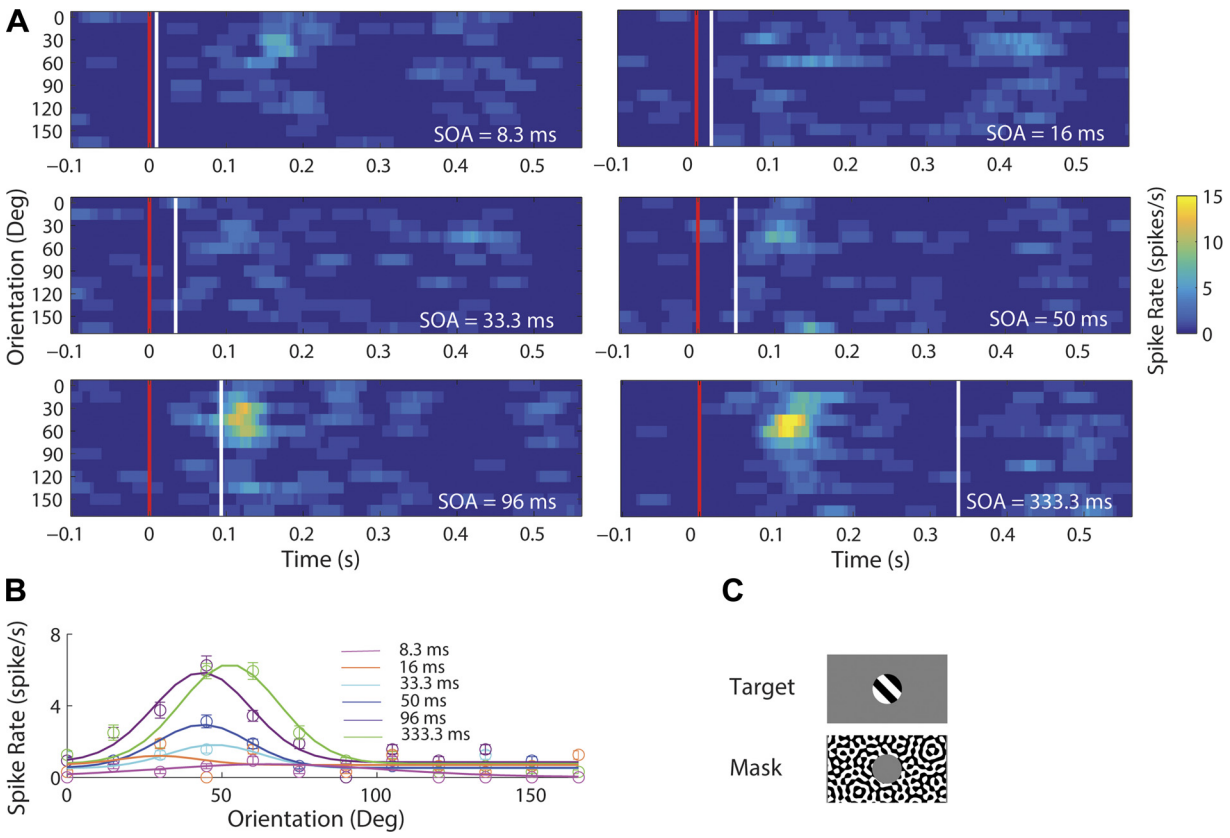

Fig. 1. Orientation tuning is reduced at short stimulus onset asynchronies (SOAs). *A*: responses of a single unit to 12 target orientations with a center-surround backward masking stimulus (examples of stimulus configuration shown in *C*). Responses are averaged in sliding 40-ms time windows. Vertical red and white lines indicate the time of target and mask onset, respectively. Note that the mask in isolation (control; SOA = 333.3 ms) evokes little response. *B*: tuning curves for the unit shown in *A* based on responses in a time window 50–150 ms relative to target onset. *C*: example target and mask configuration in the center-surround paradigm.

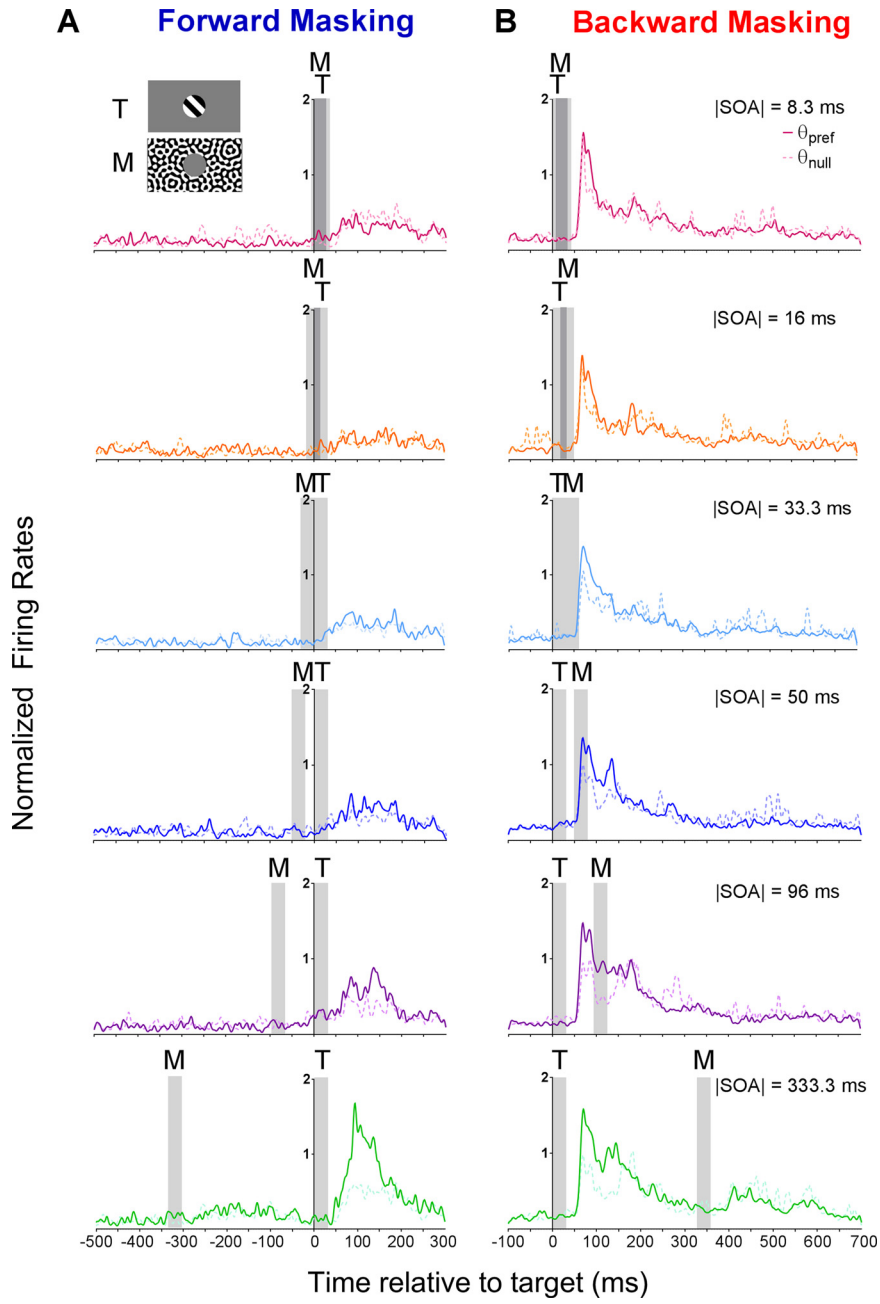

Fig. 2. Population responses in center-surround forward (*A*) and backward (*B*) masking conditions. Peristimulus time histograms for preferred and null orientations were aligned to target onset and averaged across units ($n_{forward}$ = 42; $n_{backward}$ = 63). Firing rates were normalized to the maximum response to the target at the longest stimulus onset asynchrony, measured over a window of 50–150 ms. Gray bars indicate the periods in which target (T) and mask (M) are visible. *Inset*: example target and mask stimuli for the center-surround configuration.

$$OSI = \frac{R_{pref} - R_{null}}{R_{max}}$$

where R_{pref} refers to the mean response to the preferred orientation, R_{null} refers to the mean response to the orientation orthogonal to the preferred, and R_{max} refers to the maximum preferred response across the entire stimulus presentation window (−500 to 400 ms relative to target onset in the forward masking condition and −100 to 700 ms relative to target onset in the backward masking condition).

Latencies. Response latencies (t_{resp}) were calculated as the time taken to reach a response that was greater than the mean + 3SD of the spontaneous firing rate, over 10 ms. Latency to selectivity (t_{sel}) values were calculated as the time taken to reach an OSI that was greater than the mean OSI before stimulus presentation + 3SD of the mean prestimulus OSI, over 20 ms. Units were only included in latency analyses if t_{resp} or t_{sel} was under 250 ms, which was the maximum length for a target response in the control (±333.3 ms) SOA condition.

RESULTS

Neuronal firing rates are reduced at low stimulus onset asynchronies. To determine the effect of our experimental masking paradigms on neuronal responses, we obtained extracellular recordings from populations of neurons in V1 in response to spatially overlapping and center-surround visual masking stimuli under both forward and backward masking conditions. As the target stimulus was an oriented grating, we used changes in firing rates and orientation selectivity as measures of the strength of masking. Across the population of recorded neurons, we found that orientation tuning was sharper

at long SOAs and that tuning became broader as SOAs decreased (see Fig. 1 for single-unit example).

Figure 2 illustrates the population-average peristimulus time histograms for center-surround masking stimuli. Before averaging, firing rates for each unit were normalized relative to the peak response to the target with the longest SOA, measured across 50–150 ms relative to the target onset. With this stimulus configuration, the mask is outside the classical RF and evokes little response; however, the target stimulus elicited a significant response regardless of orientation. Previous studies have characterized transient and sustained components of the response to the target and explored how they are affected by the presence of a preceding or succeeding mask. In the case of forward masking, we found that both the transient and sustained components of the target response are greatly reduced when SOAs are <100 ms (Fig. 2*A*). In backward masking conditions, the transient target response is unaffected even at the shortest SOAs (Fig. 2*B*); however, the sustained target response is suppressed at short SOAs and recovers with increasing SOAs. For each analysis in the following sections, we report our results first for spatially overlapping configurations and then for center-surround configurations.

Given that the transient and sustained target responses appear differently affected under forward and backward masking conditions, we separately quantified how masking affected transient (50–100 ms) and sustained (100–300 ms) responses. Recordings were initially segregated according to cortical layers based on CSD analyses; however, we found no significant differences between supragranular, granular, and infragranular layers, and the data were henceforth combined.

In the spatially overlapping paradigm, we found higher firing rates in response to the preferred vs. the null target orientation in both transient (Fig. 3*A*; $P_{forward} < 0.001$, $F_{1,720} = 45.7$; $P_{backward} < 0.001$, $F_{1,884} = 91.0$; 2-way ANOVA) and sustained ($P_{forward} < 0.001$, $F_{1,720} = 40.8$; $P_{backward} < 0.001$, $F_{1,884} = 53.0$; 2-way ANOVA) time windows. Critically, target-evoked firing rates were lower with shorter SOAs, and SOA significantly affected firing rates for both transient ($P_{forward} < 0.001$, $F_{4,360} = 8.1$; $P_{backward} = 0.04$, $F_{4,443} = 2.5$; 1-way ANOVA) and sustained ($P_{forward} = 0.02$, $F_{4,360} = 3.1$; $P_{backward} = 0.002$, $F_{4,443} = 4.3$; 1-way ANOVA) time windows. This demonstrates that the firing rates of both transient and sustained components of the target response are affected by changing SOAs in spatially overlapping forward and backward masking.

In the center-surround paradigm, transient firing rates were significantly higher in response to preferred target orientations for both forward and backward masking (Fig. 3*B*; $P_{forward} < 0.001$, $F_{1,738} = 21.3$; $P_{backward} < 0.01$, $F_{1,1053} = 59.1$; 2-way ANOVA) but sustained firing rates were only significantly higher in the forward masking condition ($P_{forward} < 0.001$, $F_{1,738} = 24.8$; $P_{backward} = 0.23$, $F_{1,1053} = 1.5$). Transient firing rates significantly decreased with SOA for forward ($P < 0.001$, $F_{8,369} = 5.6$; 1-way ANOVA) but not backward ($P = 0.18$, $F_{8,531} = 1.4$) masking. However, sustained firing rates were significantly affected by SOA for both forward and backward masking ($P_{forward} < 0.001$, $F_{8,369} = 9.4$; $P_{backward} < 0.001$, $F_{8,531} = 5.5$; 1-way ANOVA). These results demonstrate that the presence of a surround mask before a target affects both transient and sustained components of the target response. In center-surround backward masking, where presentation of the mask follows target presentation, only the firing rates of sustained target response components are affected. Therefore, in the center-surround masking paradigm, firing rates for the transient and sustained components are affected differently according to the temporal position of the mask.

Masking differently alters transient and sustained orientation selectivity. As the target stimuli used in the present study were oriented gratings, we calculated OSIs to determine whether the masking-induced changes in spiking rate also affected orientation selectivity. In spatially overlapping masking, OSIs calculated with a transient window were significantly higher than those with a sustained window under forward, but not backward, masking (Fig. 4*A*; $P_{forward} = 0.03$, $F_{1,720} = 8.8$; $P_{backward} = 0.07$, $F_{1,896} = 3.4$; 2-way ANOVA). Furthermore, in forward masking OSIs were significantly reduced in the

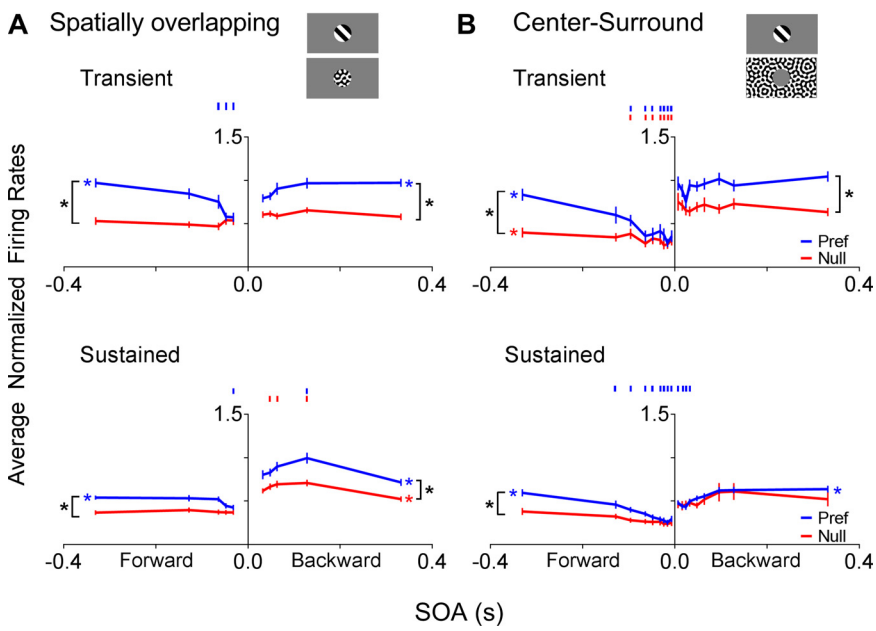

Fig. 3. Comparison of transient and sustained responses. Firing rates were calculated for preferred and null target orientations using transient (50–100 ms relative to target onset) and sustained (100–300 ms relative to target onset) time windows. Responses were calculated for spatially overlapping (*A*) and center-surround (*B*) paradigms. Vertical colored bars indicate specific SOAs where OSIs are significantly different from controls (SOA = −0.333 or 0.333 s, forward and backward masking, respectively; $P < 0.05$), in response to either the preferred or null target orientation. Black asterisks indicate a significant main effect for differences in the responses to preferred vs. null orientations ($P < 0.05$). Colored asterisks indicate significant main effects of SOA ($P < 0.05$). Error bars show SE.

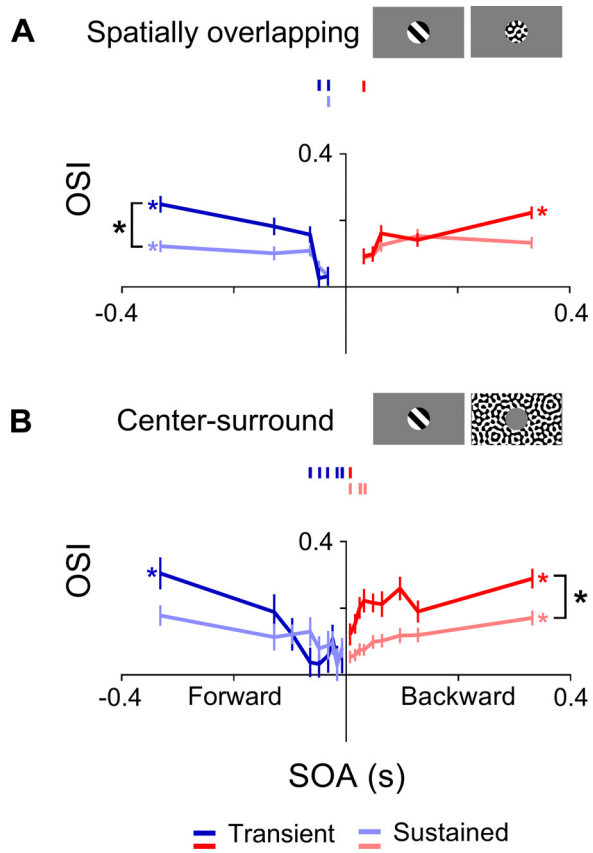

Fig. 4. Orientation selectivity is higher in the transient response. OSI calculated with transient (50–100 ms) and sustained (100–300 ms) integration windows is shown for spatially overlapping (*A*) and center-surround (*B*) conditions. Vertical colored bars indicate specific SOAs where OSIs are significantly different from controls (SOA = −0.333 or 0.333 s, forward and backward masking, respectively; $P < 0.05$) in either the transient or sustained windows. Black asterisks indicate a significant main effect for differences in the OSIs between transient and sustained windows ($P < 0.05$). Colored asterisks indicate significant main effects of SOA ($P < 0.05$). Error bars show SE.

shortest SOAs for both transient and sustained windows (Fig. 4*A*; $P_{transient} < 0.001$, $F_{4,360} = 13.1$; $P_{sustained} = 0.006$, $F_{4,360} = 3.6$; 1-way ANOVA). In backward masking, there was only a significant decrease in OSI at the shortest SOA, and this was found only with the transient window ($P_{transient} < 0.001$, $F_{4,448} = 5.9$; $P_{sustained} = 0.08$, $F_{4,448} = 2.1$; 1-way ANOVA).

In center-surround masking, OSIs calculated with the transient response were significantly higher than those with the sustained component, but only in the backward masking condition (Fig. 4*B*; $P_{backward} < 0.001$, $F_{1,1116} = 72.4$; $P_{forward} = 0.86$, $F_{1,738} = 0.03$; 2-way ANOVA). OSIs in the forward masking condition were only significantly lower than control values at the five shortest SOAs using a transient window ($P < 0.001$, $F_{8,369} = 3.7$; 1-way ANOVA), with no differences when using a sustained window ($P = 0.34$, $F_{8,369} = 1.1$; 1-way ANOVA). In backward masking, OSIs were significantly lower than the control at only the shortest SOA in the transient window and at the three shortest SOAs in the sustained window ($P_{transient} = 0.02$, $F_{8,558} = 2.3$; $P_{sustained} = 0.001$, $F_{8,558} = 3.2$; 1-way ANOVA). In general, while we routinely found changes in firing rate as a result of masking, these did not always guarantee a change in OSI in either the spatially overlapping or center-surround masking paradigms.

Orientation selectivity is reduced at short SOAs, regardless of spike counting window. To assess the effect of the mask on the evolution of orientation selectivity, we averaged OSIs, calculated in sliding 40-ms windows, across units (see Fig. 5). In center-surround masking, selectivity is clearly highest with the longer SOAs, and the effects of forward masking are more profound than those of backward masking (Fig. 5, *A* and *C*). We also observed that the duration over which neurons were selective appeared shorter with short SOAs, especially for backward masking. Similarly, under spatially overlapping masking conditions, OSIs for forward masking were markedly decreased as SOAs approached zero, with only a small decrease evident for backward masking (Fig. 5, *B* and *D*). As the interesting temporal dynamics of selectivity mostly overlap with the transient window used in previous analyses, for each masking condition we quantified orientation selectivity in three new integration windows: 80–100, 80–120, and 50–150 ms relative to target onset. This allows us to manipulate the proportion of mask-evoked activity that was included in calculations of target orientation selectivity. These windows were chosen as they centered around the average peak latency for orientation selectivity, but the exact choice of window has little effect on the results reported below.

The following analyses were performed to determine whether the masking effects seen in the firing rates and OSIs are consistent with the theory of neural integration. We first investigated whether changing the size of the integration window affected OSIs but found no effect for spatially overlapping conditions in forward or backward masking (Fig. 6*A*; $P_{forward} = 0.85$, $F_{2,1080} = 0.16$; $P_{backward} = 0.29$, $F_{2,1344} = 1.2$; 2-way ANOVA). To determine whether selectivity was affected by SOA in specific integration windows, we calculated OSIs separately at all SOAs, for each integration window. OSIs were compared with those at their respective control SOAs (±333.3 ms), as in these conditions neuronal responses to target and mask stimuli are temporally well separated. In the forward masking condition, OSIs measured at the three shortest SOAs (−33.3, −50, and −66.7 ms) were significantly lower than at the control using the 100-ms integration window and the two shortest SOAs were significantly lower than the control using the 40- and 20-ms integration windows (Fig. 6*A*; $P_{100} < 0.001$, $P_{40} < 0.001$, $P_{20} < 0.001$; 1-way ANOVA), with no differences between OSIs at other SOAs ($P > 0.05$, ANOVA). In the backward masking condition, for all integration windows OSIs were significantly lower at only the shortest SOA (33.3 ms) compared with the control (Fig. 6*A*; $P_{100} < 0.001$, $P_{40} < 0.001$, $P_{20} < 0.001$; 1-way ANOVA).

In center-surround masking conditions (Fig. 6*B*), we found significant overall differences in OSI between integration windows and OSIs significantly decreased as the SOA was shortened for both forward (integration window: $P = 0.03$, $F_{2,575} = 5.60$; SOA: $P < 0.001$, $F_{8,575} = 7.12$) and backward (integration window: $P = 0.03$, $F_{2,774} = 3.53$; SOA: $P < 0.001$, $F_{8,774} = 6.12$) masking. We also investigated the effect of integration window size on the effect of masking (reduced OSI with shorter SOAs). There was no effect of SOA on OSI in the 20-ms integration window. However, using both the 100-ms and 40-ms integration windows, we found that forward masking OSIs were significantly lower in the shortest three SOAs (−8.3, −16.7, and −25 ms) compared with

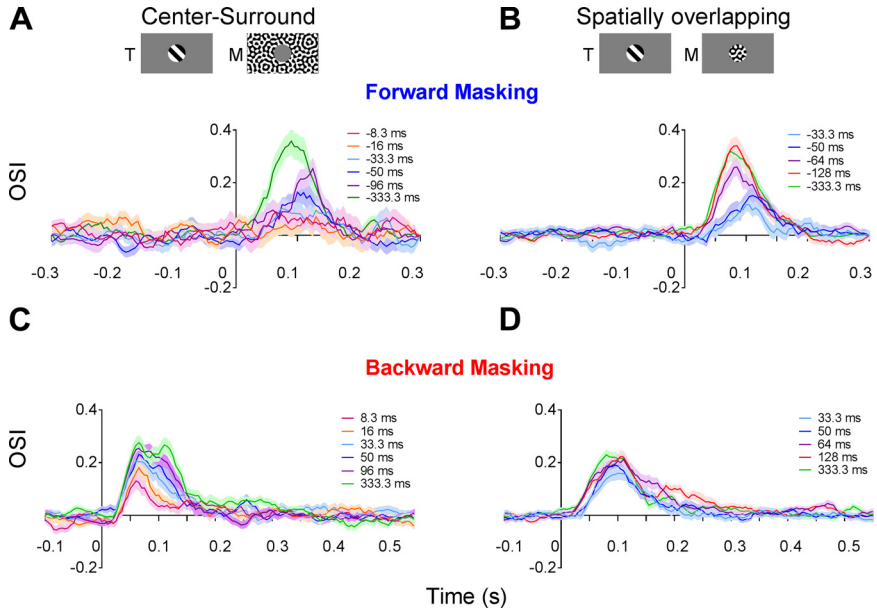

Fig. 5. Population OSIs decrease at shorter SOAs. Effect of SOA on orientation selectivity calculated with a sliding 40-ms window for center-surround (*A*, $n_{forward} = 42$; *C*, $n_{backward} = 63$) and spatially overlapping (*B*, $n_{forward} = 73$; *D*, $n_{backward} = 95$) masking paradigms. Solid lines and shaded regions indicate mean and SE, respectively.

the control SOA (−333.3 ms; $P_{40} < 0.001$, $P_{100} < 0.001$; 1-way ANOVA). Similarly, in the backward masking condition, using a 100-ms integration window we found a significant reduction in OSIs at the five shortest SOAs (8.3–50 ms) compared with the control ($P < 0.001$; 1-way ANOVA), with no differences in OSI at any of the other SOAs. OSIs were only significantly reduced at the shortest SOA with the 40-ms integration window, with no effects of SOA on OSI in the shortest 20-ms window.

Finally, we examined whether spatially overlapping and center-surround masking affect OSI differently. This is important because isolated masks (control; SOA = ±333.3 ms) induce a response in the spatially overlapping but not the center-surround conditions. Focusing on the 100-ms integration window, we found no differences in OSIs between the two masking types in either the forward or backward masking condition ($P_{forward} = 0.36$, $F_{1,545} = 0.83$; $P_{backward} = 0.12$, $F_{1,713} = 2.45$; 2-way ANOVA).

The neural integration theory of masking predicts that the effect of SOA on OSI will be smaller with shorter integration windows, reflected by a flattening of the OSI curves. This was not observed for the spatially overlapping masking condition. Regardless of the integration window size, the orientation selectivity was significantly affected by the mask at short SOAs. In the center-surround condition, the effect of SOA was removed in the 20-ms integration window; however, this finding is likely due to the increase in variance rather than neural integration. This suggests that neural integration occurring solely in V1 is not a plausible mechanism for explaining how target discriminability decreases with decreasing SOA.

Effect of masking on latencies to response and selectivity. As response amplitude, selectivity, and latency often interact, we examined whether masking affected the latency of responses to the target (t_{resp}) or the latency until orientation selectivity emerged (t_{sel}). Again, we found little to no layer-specific effects of SOA on latency in either spatially overlapping (t_{resp}: $P_{forward} = 0.17$, $P_{backward} = 0.60$; t_{sel}: $P_{forward} = 0.80$, $P_{backward} = 0.68$) or center-surround (t_{resp}: $P_{forward} = 0.39$, $P_{backward} = 0.07$; t_{sel}: $P_{forward} = 0.04$, $P_{backward} = 0.49$; 2-way ANOVA) masking paradigms.

In the spatially overlapping paradigm, response latencies were shorter in forward than backward masking, but the magnitude of these latency differences was small ($P < 0.001$; 2-way ANOVA). Between SOAs, t_{resp} was not significantly different in the backward masking condition and approached significance for the forward masking condition (Fig. 7*A*; $P_{forward} = 0.05$, $P_{backward} = 0.72$; 1-way ANOVA). Because of the response evoked by a spatially overlapping mask, it is difficult to interpret the changes in target response latency when considering forward masking. The response to the mask is likely to interfere with measures of target response latencies, particularly at short SOAs, creating the appearance of a shorter latency. In fact, the delay observed in the latency to selectivity suggests that the response to the target may in fact be delayed under these conditions, similar to what is observed in center-surround masking.

In the center-surround paradigm, response latencies were shorter in backward than forward masking ($P < 0.001$; 2-way ANOVA), and in the forward masking condition there was a weak, but significant main effect of SOA on t_{resp} (Fig. 7*B*; $P_{forward} = 0.04$; $P_{backward} = 0.74$; 1-way ANOVA). Again, these latency differences were small. Finally, we found no influence of SOA on t_{sel}, in either forward or backward masking conditions ($P_{forward} = 0.51$, $P_{backward} = 0.49$; 2-way ANOVA). However, similar to the response latencies, there was a significant main effect of latencies to selectivity between the forward and backward masking conditions, where latencies were significantly shorter in backward masking than in forward masking ($P < 0.001$; 2-way ANOVA). Collectively, our latency data suggest that the presentation of a mask immediately before the target causes inhibition that delays the onset of target processing. This agrees with response patterns observed in monkey V1, in which the response to a briefly presented preferred orientation sinusoidal grating is delayed if the grating is preceded by the antipreferred orientation rather than a blank screen (Bair et al. 2002).

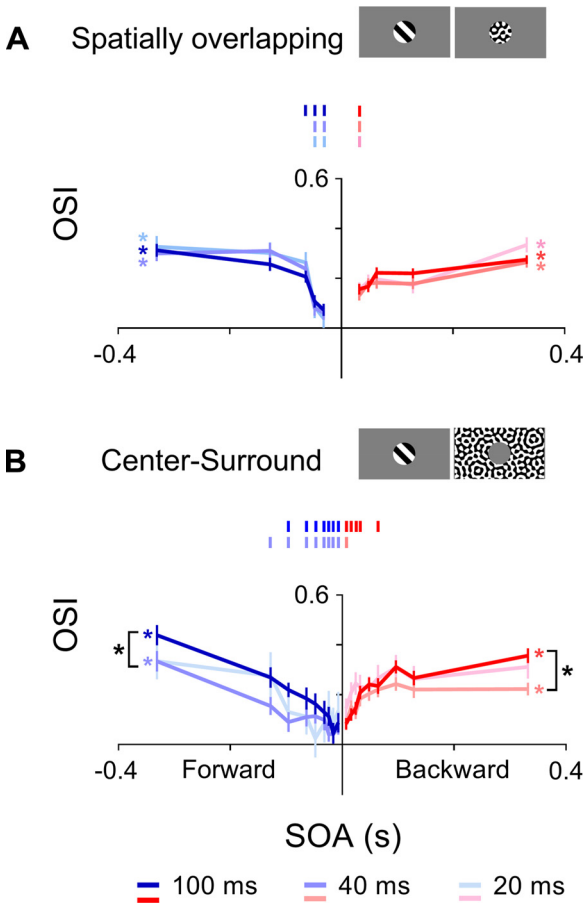

Fig. 6. Integration window has little influence on orientation selectivity. OSIs for spatially overlapping (*A*) and center-surround (*B*) conditions, calculated with 3 integration windows: 80–100 ms, 80–120 ms, and 50–150 ms relative to target onset. Vertical colored bars above plots indicate where OSIs are significantly different from control (SOA = −0.333 or 0.333 s, forward and backward masking, respectively; $P < 0.05$), at each of the integration windows. Black asterisks indicate a significant main effect for differences in the OSIs between integration windows ($P < 0.05$). Colored asterisks indicate significant main effects of SOA ($P < 0.05$). Error bars show SE.

DISCUSSION

We examined how the responsivity and selectivity of V1 neurons are affected by masking stimuli presented either before or after flashed target gratings. Neurons responded to 33-ms target stimuli with a two-component response: an initial transient component lasting 50–100 ms followed by a sustained component extending up to 300 ms. For all conditions our data followed an A-type visual masking trend; responsivity and selectivity were lowest with short SOAs. For spatially overlapping masks, the neuronal responses to target and mask stimuli began to merge and eventually became indistinguishable as the SOA approached zero. With center-surround masks that did not evoke a neuronal response in isolation, the entire response to the target was reduced when using forward masks while in backward masking only the sustained component was reduced at short SOAs. Below, we explore the different effects of masking on transient and sustained components, the difference between detection and discrimination tasks, and how our data are consistent with theories of neural integration and lateral inhibition.

Previous studies have suggested that the neural correlate of backward masking is a reduction in the sustained component of the response to the target (Bridgeman 1975; Lamme et al. 2002; Macknik and Livingstone 1998; Rolls and Tovee 1994). Therefore, we specifically examined how masking affected both transient and sustained responses and orientation selectivity during these periods. The long-lasting activation (100–300 ms) that we observed in response to a brief, 33-ms target grating in the absence of a mask is frequently reported in studies of V1 and IT (Bridgeman 1975; Lamme et al. 2002; Macknik and Livingstone 1998; Rolls et al. 1999; Rolls and Tovee 1994; Schiller 1969). With center-surround stimuli, primarily the sustained response was reduced under backward masking conditions. With spatially overlapping stimuli, it is impossible to determine whether the target response was similarly affected, as the responses to target and mask merge and become indistinguishable at short SOAs. However, analogous response reductions have been observed in IT with the use of spatially overlapping masks that, in isolation, did not elicit a response, such as faces or a pattern formed by "N" and "O" letters (Rolls and Tovee 1994). Interestingly, the trends in neuronal responsivity and selectivity were different. Orientation selectivity was affected by visual masking in the transient component for the spatially overlapping condition and in both the transient and sustained components for the center-surround condition. This is important because it demonstrates that the effects of backward masking are not limited to a reduction in the sustained firing rate; feature selectivity is also affected throughout the entire response to the target.

Similar to backward masking, the neural correlate of forward masking is a reduction in the response to the target; however, the precise response components affected vary between studies. Using a single line as the target, Bridgeman (1975) showed that target responses in area 17 of anesthetized cats were affected by the presence of parallel, flanking lines, with forward masking reducing only the sustained component. Despite using similar line stimuli, Macknik and Livingstone (1998) found that forward masking affected only the transient component in V1 of anesthetized monkeys and in awake monkeys inhibited the entire target response. Our forward masking data, although collected in anesthetized rodents, agree with the latter finding, showing a reduction in the entire response to the target. Yet, for the center-surround condition, orientation selectivity was significantly reduced only in the transient component. Thus response rate and selectivity follow an A-type masking trend regardless of the timing and spatial arrangement of stimuli. However, a reduced firing rate does not predict an impairment in selectivity; changes in selectivity may occur in isolation or accompany firing rate reductions.

Previous studies using non-spatially overlapping stimuli have often yielded a B-type (U shaped) backward masking trend (Bridgeman 1975; Macknik and Livingstone 1998), and this is commonly expected if the energy of the target is greater than or equal to that of the mask (Breitmeyer 1978; Fehrer and Smith 1962; Kolers 1962; Lefton 1974; Spencer and Shuntich 1970). This does not preclude observing B-type masking with high-contrast targets (Agaoglu et al. 2015; Bruchmann et al. 2010; Macknik and Livingstone 1998). In the past, stimulus energy has been defined as a function of the contrast and duration of a stimulus (Tapia et al. 2011). By this definition, our target and mask stimuli would be considered to have equal

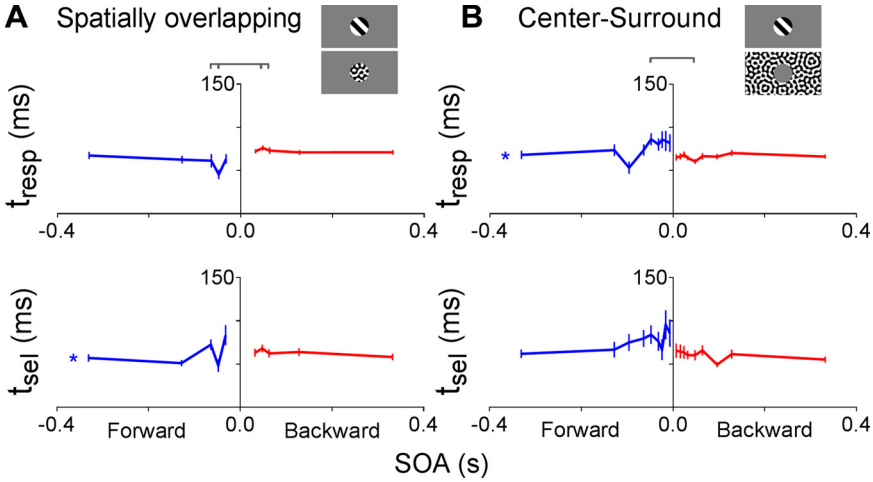

Fig. 7. Latencies to selectivity and responsivity in spatially overlapping (*A*) and center-surround (*B*) masking conditions: average latencies to responsivity (*top*) and orientation selectivity (*bottom*). Gray brackets above plots indicate specific SOAs where average latencies between forward and backward masking conditions are significantly different ($P < 0.05$). Colored asterisks indicate significant effects of SOA ($P < 0.05$). Error bars show SE.

energy, predicting B-type masking. However, since our surround stimuli were larger than traditionally used annuli, we believe that the size of our mask is an important factor determining our observed A-type trend. While it is possible that B-type masking trends occur under differing circumstances in rodents, this seems unlikely, as human psychophysical data with the same stimuli show similar A-type trends (manuscript in preparation).

Macknik and Livingstone (1998) and Bridgeman (1975) found that only units with distinct (rather than continuous) transient and sustained peaks showed a B-type trend in the late activity. As our neurons were not separated according to anatomical, functional, or response properties, we may not have been able to observe B-type masking. That said, when our neurons were separated according to their laminar location, we found no important differences. Furthermore, a study using electroencephalography, a technique that groups neuronal activity more broadly than our own approach, has also shown that the late target-evoked activity can reflect a B-type trend (Vaughan and Silverstein 1968). Thus it seems unlikely that our pooled analyses of the sustained activation were insufficient to show B-type masking if it were present.

Most psychophysical and electrophysiological studies of masking have focused on changes in visibility, or how masks affect detection performance (Bridgeman 1975, 1980; Macknik and Livingstone 1998; Mitov et al. 1981; Snowden 2001). Electrophysiologically, this requires observing a large mask-induced reduction in the target response. This is problematic, as a large reduction in firing rate need not correlate with changes in visibility, as evident if stimulus contrast is reduced. As V1 responds strongly to oriented stimuli, we examined how masking affects orientation discrimination performance. OSIs were lower than commonly reported, because our target stimuli were static and only briefly presented. The flashes mean that target stimuli elicited significant responses regardless of their orientation, but selectivity persisted throughout both transient and sustained periods, contrasting with previous results (Lamme et al. 2002). Generally, firing rates in response to preferred orientations decreased with shorter SOAs, while the firing rates induced by null orientations were less affected. As a consequence, the orientation selectivity decreased, and persisted for less time, with shorter SOAs. This is consistent with results in area IT, where the difference in the response to the "best" compared with the "worst" stimulus, and the amount of information about the target stimulus, decreased with SOA (Kovács et al. 1995; Rolls et al. 1999). Thus it is clear that cell selectivity also follows an A-type trend, suggesting that discriminability may be impaired, even with high-contrast stimuli where the visibility of the target should not have been significantly reduced.

While the SOAs affected by masking in our study might be explained purely through spatial and temporal summation in the retina, our results with large center-surround stimuli make it likely that thalamic and cortical processes further contribute. Recordings from retinal ganglion cells have shown that forward masking reduces the subsequent target response for SOAs of −80 up to −160 ms (Coenen and Eijkman 1972). In backward masking, retinal interactions can account for reductions in the target response for SOAs of up to 50 ms; after this point the target information will have already entered cortical regions before the mask impinges on the retina (Battersby et al. 1964). However, the spatial relationship and size of our center-surround stimuli make it unlikely that retinal interactions alone could account for our results. Furthermore, to discount the involvement of any cortical contributions would be to disregard a wealth of information: numerous psychophysical studies have shown that visual masking occurs even under dichoptic stimulus presentation, which, given that the first site of binocular combination is V1, implies some cortical involvement (Schiller and Smith 1968; Smith and Schiller 1966; Turvey 1973; Weisstein 1971).

One cortical-based theory that is frequently used to explain perceptual masking is neural interruption, where neuronal processing of the target is abandoned at the arrival of the response to the mask (Breitmeyer and Ogmen 2006). In this way, target processing is left unfinished, resulting in impaired perception. However, this requires presentation of the target to precede the mask and is therefore incapable of explaining forward masking. Furthermore, in backward masking, most neural interruption theories predict a B-type visual masking trend; therefore, we suggest that neural interruption is unlikely to contribute to the visual masking observed in this study.

Neural integration, where the neuronal representation of the target and mask stimuli are grouped together in a relatively long "perceptual window," is frequently used to explain A-type masking (Eriksen and Collins 1967, 1968). Integration predicts the perception of a fused image therefore reducing target perception. To evaluate whether integration was contributing

to the masking effect in our data, we restricted the duration of our spike counting windows to avoid the window including responses to both target and mask, in a sense artificially shortening the "perceptual window." If cortical integration were sufficient to explain perception, we would expect orientation information to be unaffected by masking when using a short "perceptual window." In our data this would be seen as the slope of the OSI curves flattening with shorter spike counting windows (Fig. 5*B*); however, this was not observed. Thus neural integration alone is not capable of explaining our A-type masking trends; something further is needed to explain the near-instantaneous reduction in selectivity that we observe even under center-surround conditions.

One likely explanation is lateral inhibition, a mechanism that is ubiquitous in neuronal circuitry and that has already been incorporated into a number of visual masking theories (Bridgeman 1971; Francis 1997; Herzog et al. 2003; Weisstein et al. 1975). To explain our data, it is not necessary for lateral inhibition to operate faster than the response to the target, completely abolish the target response (Fig. 2), or even affect the entire transient response (50 ms). It is only necessary for lateral inhibition to influence orientation selectivity in short time windows (20 ms; Fig. 5). Given that surround suppression via lateral connections travels at conduction speeds of 0.1–0.3 m/s (Angelucci and Bressloff 2006; Bringuier et al. 1999; Girard et al. 2001), and that our surround masks were immediately adjacent to the target, it is entirely plausible that orientation selectivity might be affected by masking in time windows as short as 20 ms.

Our results demonstrate that visual masking influences discriminability in V1. Regardless of the spatial and temporal arrangement of the target and mask, the firing rate and neuronal selectivity are reduced at short SOAs, reflecting an A-type trend. However, the impairment in stimulus discriminability occurs in the transient and/or sustained component of the response to the target in a manner that is not always predicted by changes in firing rate. We suggest that the effects of visual masking may be explained through a combination of neural integration and lateral inhibition occurring throughout the early visual processing hierarchy.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

D.S.A., K.L.R., and N.S.P. conception and design of research; D.S.A., K.L.R., and N.S.P. performed experiments; D.S.A., K.L.R., and N.S.P. analyzed data; D.S.A., K.L.R., and N.S.P. interpreted results of experiments; D.S.A. and K.L.R. prepared figures; D.S.A., K.L.R., and N.S.P. drafted manuscript; D.S.A., K.L.R., and N.S.P. edited and revised manuscript; D.S.A., K.L.R., and N.S.P. approved final version of manuscript.

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