Supplemental Materials 1

Details and Validation of Method

Apparatus

The remote haploscopic videorefractor (RHV) presented targets at five fixation distances while collecting continuous recordings of eye position and accommodative response. The method and calibration procedures have been described in detail previously (Horwood & Riddell, 2008).

There are two optical pathways designed so that data collection and target presentation can be separated. The eye position and refraction data are collected continuously from each eye at 25Hz via an infra-red "hot" mirror (E in Figure 1) using a PlusoptiXSO4 PowerRefII photorefractor (Plusoptix GmbH,Nurnberg, Germany). The mirror transmits visible light so that the participant has an unimpeded view of the target, but reflects infra-red so that the camera sensors can be placed in the same optical plane as the target but without obscuring it. The participants view a monitor screen via two concave mirrors arranged so that the virtual image of the monitor is seen to move backwards and forwards directly in front of the participant (Figure1). The advantage of using these mirrors for the target presentation pathway is that one eye's view of the target can be occluded remotely by covering half the upper concave mirror in the stimulus pathway (F). The participants can then only see the target with one eye, but photorefraction of both eyes can still take place via the other optical pathway. Having the occluder remote from the participants' face makes it particularly suitable for use with infants. Typically, approximately one third of older participants in our lab are aware they have been occluded, one third are aware "something has happened to the image" but cannot define what it was, while a further third are completely unaware of the occlusion. During testing, older infants frequently tried to touch the nearest target images, and adults, when asked to try to touch the nearest images at the end of testing, pointed to appropriate points in space, confirming it is a realistic target.

The target monitor moves such that the image is placed optically at 0.25m, 0.33m, 0.5m, 1m and 2m from the participant's eyes, representing response demand of 4, 3, 2, 1 and 0.5 diopters (D) and meter angles (MA). By using meter angles as a the unit of measurement of vergence we were able to compare vergence and accommodation responses in relation to target demand more accurately between participants with widely different inter-pupillary distances and also plot both on the same scales e.g. a 0.5m target demands 2D of accommodation and 2MA of vergence. A pseudo-random testing order of 3, 0.5, 4, 1, 2 D and MA demand was used so that near and farther targets alternated. Data from the 4D & MA demand target was discarded due to excessive pupillary constriction preventing collection of many readings at 25cm, but this target was retained in the testing sequence to maintain the near/distance alternation. We also excluded this target because of the possibility that off-axis differences in peripheral refraction might induce inaccuracy, although even at 25cm and even if only one eye was doing most of the ocular rotation due to a head turn, the vergence angle would only be just over approximately

10°, well within the limits within which peripheral refractive errors are insignificant(Calver, Radhakrishnan, Osuobeni, & O'Leary, 2007).

The monitor was moved by a belt powered by a motor outside the apparatus and beyond the farthest target distance at 2.75m from the participants. While the motor could be heard during target motion, so alerting the participants that movement was occurring, it gave no clues to the target position or direction of movement. The target screen moved at 0.4 meters per second.

Target Validation

The clown target to stimulate accommodation as much as possible, containing high contrast, coloured edges of a wide range of spatial frequencies, down to one pixel in size, and in particular to include facial features with eyes, mouth and a "hairline" so as to be most interesting to infants, with two versions with different details alternating at 1Hz.

Gabor targets have been used to open the accommodative loop in adult studies (Tsuetaki & Schor, 1987) but to our knowledge there are no guidelines as to which type of Gabor in terms of contrast gradient maximally opens the accommodation loop best (particularly in infants compared to adults).

We made a pragmatic choice of Gabor target to use from those available in high enough resolution from the online literature. We needed a clear contrast gradient and high enough spatial frequency to allow accurate motor fusion of the image. The target chosen was downloaded from Figure 4 of Allard et al *Journal of Vision* February 22, 2006 vol. 6 no. 4 article 3 http://www.journalofvision.org/content/6/4/3.full, with a formula of

$$S(x, y) = c\sin(fx + p)\exp(-\frac{x^2 + y^2}{2\sigma^2}),$$

and this image seemed clear and of good resolution. In our paradigm, although disparity cues were completely eliminated, our scaled Gabor target contained higher spatial frequencies at 2m (1.58) than the <0.5cpd recommended by Tsuetaki& Schor (Tsuetaki & Schor, 1987). We chose this slightly higher spatial frequency since we were keen to keep an adequate stimulus for accurate fusion. Also, mindful of failure by others when testing infants with a completely diffuse target (Currie & Manny, 1997), we aimed to retain some cognitive interest within the target itself to maintain attention and retain disparity cues while minimizing detail.

We had to adapt this Gabor image by merging it with a 2D Gaussian blob on a black background using the Adobe Photoshop "Merge Layer" ("normal" setting) feature to further retain fusion, direct attention, and ensure the two targets were of similar subjective size (although this was of course difficult with the Gabor where there are no edges). We needed to have a black surround so that the screen edge within the black shuttering was a minimally visible as possible.

As with the clown target we needed two Gabor versions, and the overall size of the stimulus needed to be equivalent, so Photoshop was used to further manipulate the image. The image was coloured either green or yellow (so that duochrome effects of using colours at more extremes of the visible spectrum would be minimized). Different sizes of this blob were trialled subjectively on adults at the pilot stages so that the Gabor section of the target merged invisibly with the black surround with no apparent edges and appeared subjectively the same size as the clown, and both the green and yellow versions appeared the same size. We also checked subjectively that inducing refractive blur with lenses up to +/- 4D (broadly the levels of optical blur likely to be induced by the stimuli and levels of refractive error we were interested in) made little difference to the subjective clarity of the image at 33cm. The yellow version of the Gabor initially subjectively appeared slightly bigger despite identical processing, so when alternating with the green version there was a jumping backwards and forwards illusion, so we reduced the size of the blob surround slightly so that this illusion disappeared, although the spatial frequency of the central grating portion remained the same.

The paper shows that the *o* condition still drove some residual responses. These could not have been due to disparity cues because these are entirely excluded by the remote occlusion. It has been suggested that our DoG target may not sufficiently minimize blur cues, so we have carried out a subsequent study of 29 young naive adult participants, comparing accommodation and vergence responses to three alternative low detail targets. The first was the target used for this study (A). The second (B) was an image with a grating with resolution of 0.99 cycles/deg at 2m and 0.16 cycles/deg at 33cm when unscaled, and 0.99 cycles /deg at all distances when scaled. The third (C) was the diffuse spot target (see Figure) we had used for the Gabor target processing. They were matched for luminance across the target and because the target edge was diffuse, also for subjective impression of size and brightness.
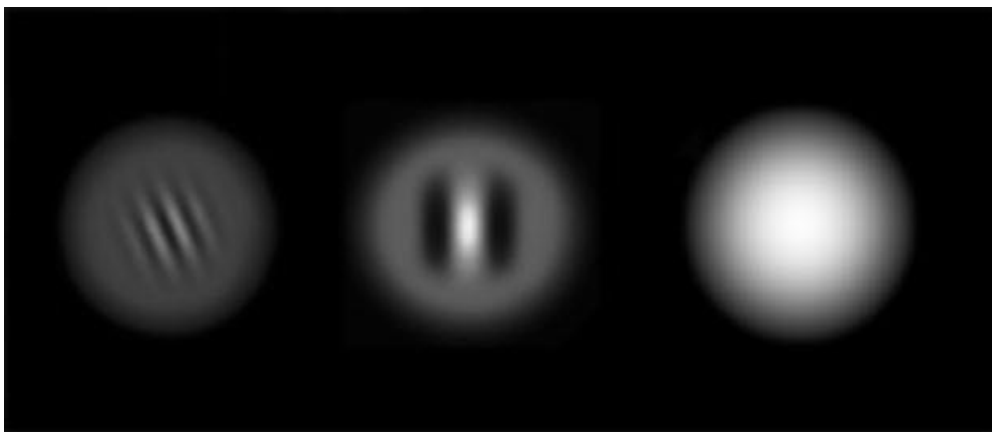
There were no significant differences between the two Gabor targets in terms of vergence and accommodation responses and response gains in any of the eight target conditions. In comparison to the diffuse

spot, both Gabor targets produced significantly greater accommodation responses gain than the spot in two of the eight conditions (the *DiPr* and *Di* targets). Examination of the data, however, also showed highly significant differences in vergence between these targets ($F = 51.32$, $p=<.0001$ and $F=20.74$, $p<.0001$) but only due to less vergence to the spot target at 3MA demand (*DiPr*: A = 2.81MA, B = 2.68 MA, C = 2.14MA; *Di*: A=2.71, B=2.73, C=2.63MA). If vergence response gain was used as a covariate, differences in accommodation gain between the targets were not significant ($F(2,67)=1.94$, $p=0.15$ and $F(2,67)=2.4$,$p=0.1$). We typically find disparity a stronger drive to accommodation than is blur in naive young adults, so it is likely that the poorer accommodation to the spot target is as much, or more, due to the target being an insufficient target to drive vergence (and so lead to poor accommodation indirectly via the CA/C linkage), rather than being a more impoverished a blur cue. We accept that the choice of such targets represents a compromise between minimizing blur while retaining adequate disparity and looming cues, but we feel this confirms the superiority of the Gabor targets as the optimal stimuli to minimize blur while retaining fusional potential. Reducing the spatial frequency of the Gabor target did not reduce responses significantly so we feel that a target with lower spatial frequency would have been not opened the accommodation loop further without also compromising fusion.

The other possible source of residual cues in the *Min* condition could have come from the size cues of the screen edges. The whole apparatus, including the black cloth screen is contained in black shuttering so that the target is seen against a dark background, but the minimal background screen luminance of the black target surround on the screen is still very dimly visible against the physical black screen edges. We have masked this with a diffuse gradient printed on an acetate overlay, but some minimal residual size cues remain, even though dynamic looming is eliminated by screening all target movement in the proximity-free conditions. It seems likely that these residual proximal cues are the source of the minimal-cue residual responses. This is supported by the data in the paper which finds that responses to the *Min* target reduce with age, in line with the decline in the influence of proximal cues. Other sources of residual responses could have been experience of repeated testing, or unquantifiable voluntary influences.

Data Collection

The tester watched the traces during testing and the target was only moved to the next position in the sequence when traces of both vergence and accommodation could be seen by the tester to have been stable for at least two seconds. Off-line, data were converted to vergence (in degrees) and accommodation (in D), and responses were charted against time for the whole run of all five target positions and visually inspected (Figure 5). The macro searched for spikes of data caused by blinks and removed data points immediately before and after them. Representative vignettes of the most stable 25 continuous data points were selected for each target position. Vignettes were only chosen from sections of the data where the response had settled and flattened out for at least 0.5 sec (Tondel & Candy, 2007), but before any tonic changes would be expected to have occurred, so although there may have been a dynamic cue to the target position, we did not assess a dynamic response in this study.

These responses were averaged, and the accommodative and vergence planes were calculated using a macro developed in our laboratory which uses raw data corrected for individual angle lambda and inter-pupillary distance (IPD), and a systematic error in increasing underestimation of accommodation in comparison to dynamic retinoscopy found during earlier calibration studies (using the formula $1.2385x+0.799$, where x equals the PlusoptiXSO4 accommodation measure (Horwood & Riddell, 2008)). We obtained the best estimate of angle lambda when fixing at infinity by plotting the y-intercept of the nasal displacement from the pupil center averaged across both eyes at all four fixation distances in the all cue (bdp) condition. True IPD was calculated from the y-intercept of PlusoptixSO4 IPD plotted against target distance at all four fixation distances in the bdp condition. This was used to calculate responses in MA for each participant. Individual accommodation calibration was not carried out in view of the long testing session for very young infants, but Blade & Candy (2006), using similar apparatus have shown that group means in infants and adults are similar. Inter- scorer reliability on masked scoring where each scorer was free to choose the vignette was excellent. For both vergence and accommodation, this analysis showed a high agreement: for vergence: $r=0.99$, mean inter-scorer difference = $0.037\pm95\%\text{CI}$ 0.37MA; for accommodation $r=0.99$, mean inter-scorer difference 0.0095 $\square\pm95\%\text{CI}$ 0.175D.

The testing order is described in the main article text.

Attention

Infant fixation was monitored in real time on the PlusoptiX video-monitor at the time of testing. Attention during each run was scored immediately after each run on an ordinal scale between 1 and 5, (1= totally calm and attentive throughout, 5= totally inattentive to the target). Only runs scoring 3 or less (3 = the infant was observed to be looking steadily at the target for at least two seconds despite mild fussiness) were analyzed. Infants were less engaged by the more impoverished targets and attention frequently waned towards the end of repeated testing. However, 82% of infants whose attention score reduced during testing in single cue conditions achieved a better attention score when the bdp target was re-presented immediately afterwards at the end of testing, demonstrating that fatigue was not the main reason for attention loss. Wilcoxon Signed Ranks tests showed no significant difference between the first and repeated bdp conditions ($z=-1.732$, $p=0.08$). Attention was significantly better with 3-cue vs 1-cue (1.1 vs 1.36; $z=-4.421$, $p<0.0001$), 2-cue vs 1-cue ($z=-4.13$, $p<0.0001$) and 1-cue vs "zero"-cue ($z=-4.06$, $p<0001$) conditions although there were no attention differences between targets within the 1 or 2 cue blocks. Infants became more distractible with the impoverished cues, so fewer runs were collectable but infants were just as likely to be distracted by being rendered monocular in the disparity-free conditions as they were by waiting for the screen to be removed in the proximity-free conditions or by being given the less salient DoG target in the blur-minimized conditions.

Refraction estimates

We needed to estimate refractive error as accurately as possible in the infants, since many would be expected to be significantly hyperopic, thus resulting in variability in accommodation demand for any given stimulus. Cycloplegic refraction on each visit would have provided a gold standard measure of maximum hyperopia, but was not attempted in order to maximize participant recruitment and retention during a longitudinal study dependent on repeated attendance, and in response to ethical constraints. We have reported that a reliable estimate of true hyperopic error when cycloplegia is not available is given by the maximally hyperopic refraction (MHR) found at any point during the whole testing session in our laboratory (Horwood & Riddell, 2009). In that study MHR correlated closely with cycloplegic retinoscopy (r=0.93) and was a better estimate than Mohindra retinoscopy (Mohindra, 1977). Results of cycloplegic retinoscopy were available for 17 of the infants in this study between three and six months of age and agreement was very good between these results and MHR ($r = 0.83$, 95%CI ±0.50D). In addition, while Mohindra retinoscopy was not possible with some fussier infants, a measure of MHR was obtainable at every visit. We therefore used MHR as the best estimate of refractive error for each infant each visit. Infants with anisometropia >1.00D or MHR of >+2.00 were excluded in this study. A continuous measure of astigmatism was not recorded by the PlusoptiX SO4 in PowerRefII mode, so was not considered here. Within the angular change in fixation (<10°) demanded by even the nearest target, off-axis errors or differences in peripheral refraction (Charman & Radhakrishnan, 2010) are not likely to have induced refraction artifacts.

References

Allard, R., & Faubert, J. (2006). Same calculation efficiency but different internal noise for luminance- and contrast-modulated stimuli detection. Journal of Vision, 6(4), 322-334.

Blade, P. J., & Candy, T. R. (2006). Validation of the PowerRefractor for measuring human infant refraction. *Optom Vis Sci, 83*(6), 346-353.

Calver, R., Radhakrishnan, H., Osuobeni, E., & O'Leary, D. (2007). Peripheral refraction for distance and near vision in emmetropes and myopes. *Ophthalmic Physiol Opt, 27*(6), 584-593.

Charman, W. N., & Radhakrishnan, H. (2010). Peripheral refraction and the development of refractive error: a review. *Ophthalmic Physiol Opt, 30*(4), 321-338.

Horwood, A., & Riddell, P. (2008). The use of cues to convergence and accommodation in naïve, uninstructed participants. *Vision Research, 48*(15), 1613-1624.

Horwood, A., & Riddell, P. (2009). Receding and disparity cues aid relaxation of accommodation. *Optometry & Vision Science, 86* (11), 1276-1286.

Mohindra, I. (1977). A non-cycoplegic refraction technique for infants & young children. *J Am Optom Assoc, 48*, 518-523.

Tondel, G. M., & Candy, T. R. (2007). Human infants' accommodation responses to dynamic stimuli. *Invest Ophthalmol Vis Sci, 48*(2), 949-956.