**Mesopic visual acuity is less crowded**

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Revision Submitted: February 2018

**Acknowledgments**

We thank Jitka Ošťádalová for help with data collection and Dr Harold Bedell for his thoughtful comments and suggestions on the manuscript.

ACCEPTED FOR PUBLICATION

**Abstract**

**Purpose**: The decrease in visual acuity under low luminance conditions is well known. Recent laboratory evidence showed that crowding under low luminance (mesopic) light levels is less robust than under photopic conditions. The present study examines whether such differences in crowding influence clinical measurements of mesopic visual acuity, including test-retest repeatability. **Methods**: Twenty adult subjects with normal or corrected to normal visual acuity were recruited for the study. Monocular visual acuity was measured under photopic (228 cd/m2) and mesopic (0.164 cd/m2) luminance conditions using a letter chart, similar in principle to the ETDRS logMAR chart, presented on a computer monitor. Three rows of 5 letters, each row differing in size by 0.05 logMAR from largest to smallest were displayed at the center of the monitor. The level of crowding was varied by varying the separation between horizontally adjacent letters from 100% optotype size to 50%, 20% and 10% optotype size. Inter-row spacing was proportional to optotype size. Observers read the letters on the middle row only. Measurements continued by reducing the size of the letters, until 3 or more errors on the middle row were made. Each correctly identified letter contributed 0.01 to the recorded logMAR score. All measurements were repeated for each subject on two separate days. **Results**: Visual acuity (logMAR) was significantly better under photopic than mesopic luminance conditions with a mean difference of 0.48 logMAR. Visual acuity also decreased with decreasing letter separation (i.e. increase in crowding). However, the decrease in visual acuity for the smallest letter separation was less under the mesopic luminance condition, even after accounting for the increased size of threshold acuity letters. Test-retest repeatability for mesopic and photopic conditions was not significantly different. **Conclusions**: Crowding under mesopic luminance conditions has less impact on visual acuity than under photopic luminance.

**Keywords**

Visual acuity; crowding; photopic; mesopic; luminance; logMAR

**Introduction**

The measurement of visual acuity (VA) is an important part of any ophthalmic examination [[1](#_ENREF_1)]. It is used as a measure of performance for example, to inform clinical decisions such as the correction of refractive error [[2](#_ENREF_2)] and the management of patients with cataract [[3](#_ENREF_3)], and is also used extensively as an outcome measure in vision and eye related research [[4](#_ENREF_4), [5](#_ENREF_5)]. Standards for the construction and development of reliable VA charts exist [[6](#_ENREF_6)] and reflect known variables in measuring VA [[7](#_ENREF_7)]. A widely recognised VA chart format, particularly for research purposes, is the Early Treatment Diabetic Retinopathy Study (ETDRS) logarithm of the minimum angle (logMAR) chart [[8](#_ENREF_8)] which is based on the principles originally espoused by Bailey and Lovie [[9](#_ENREF_9)]. The ETDRS chart uses the Sloan [[10](#_ENREF_10)] letter set and comprises rows of letters that progress in size in 0.1 log unit steps. Each row consists of 5 different letters equally spaced at 1 letter width apart (i.e. edge-to-edge separation). The spacing between rows also is fixed as a function of the size of the letters in the line directly below.

Measurements of high contrast visual acuity using the ETDRS chart have been obtained in numerous studies, the vast majority conducted under photopic luminance conditions. However, measurements of visual acuity under less optimal, low luminance conditions may also be clinically relevant. The reduction of foveal visual acuity under low luminance conditions has been well established experimentally, worsening as the level of illumination decreases and reaching total insensitivity under scotopic conditions [[11-15](#_ENREF_11)]. Clinically, measurements of visual acuity under mesopic luminance conditions may be important in the assessment of some ocular diseases [[16-19](#_ENREF_16)], and more generally for assessing driver performance [[20](#_ENREF_20), [21](#_ENREF_21)]. Surprisingly, however, there are no accepted standards for measuring mesopic visual acuity. Recently some authors investigated clinical measurements of mesopic visual acuity, determining test-retest repeatability of standard high contrast ETDRS letter charts [[22](#_ENREF_22)] or electronic E-ETDRS charts [[23](#_ENREF_23)]. For a mesopic luminance of 0.75cd/m2, the mean visual acuity was 0.24 logMAR worse than under photopic conditions [[23](#_ENREF_23)], consistent with previous laboratory findings [[12](#_ENREF_12), [24](#_ENREF_24), [25](#_ENREF_25)]. Repeatability of mesopic acuity was of the order of about ± 0.1 logMAR [[22](#_ENREF_22), [23](#_ENREF_23)], consistent with the known repeatability of logMAR using photopic ETDRS charts [[26](#_ENREF_26)].

The fixed relative separation of letters and rows of letters in the ETDRS chart provides a consistent crowding effect, defined as the detrimental impact on visual acuity arising from the presence of adjacent letters [[27](#_ENREF_27)]. Crowding using letter charts includes contour interaction (as a result of the lateral spatial interaction between the target letter and the flanking letters), together with unstable and imprecise fixational eye movements and inaccurate or inappropriate deployment of attention [[27](#_ENREF_27)]. In laboratory settings, the magnitude (but not the extent) of contour interaction has been shown to be significantly reduced under mesopic luminance conditions [[28](#_ENREF_28), [29](#_ENREF_29)] confirming previously suggested differences in contour interaction with differences in luminance [[30](#_ENREF_30), [31](#_ENREF_31)]. The reduction in contour interaction under mesopic luminances may help to explain previously reported differences in visual acuity as a function of optical blur and luminance [[12](#_ENREF_12), [24](#_ENREF_24), [25](#_ENREF_25)].

Given the potential importance of visual acuity measurements under mesopic luminance conditions, and the likelihood that crowding is reduced under such conditions, we investigated whether such reduced crowding could affect clinical measurements of mesopic visual acuity, including test-retest repeatability.

**Methods**

Subjects

Twenty participants, recruited from the Palacky University community in Olomouc, Czech Republic, took part in the study (2 males and 18 females, age range 18-28 years). The number of observers recruited was sufficient to find an acuity difference, if it existed, of 0.1 logMAR between measurements, based on a significance level of 0.05 with a power of 80% (paired t-test). Observers were free from ophthalmic pathology or any systematic condition known to affect vision and had normal or corrected-to-normal vision of at least 0.0 logMAR (6/6).

The research was conducted in accordance with the tenets of the Declaration of Helsinki, and written informed consent was obtained from each subject before participating and after all of the procedures and risks were explained. The study met the requirements of the institutional research ethics processes.

Stimuli

The stimuli consisted of 3 horizontal rows of black Sloan letters (C D H K N O R S V Z) with each row containing 5 letters of the same size (selected at random but with a constraint that they were different), presented at the center of a display monitor on a white background (Fig 1). The monitor (ASUS VW 220TE, LCD) measured 56 cm diagonally, with 1680 x 1050 pixel resolution and an unattenuated background luminance of 228 cd/m2. The luminance of the letter stimuli was 4.5 cd/m2 (i.e. Weber contrast of –98 %), measured using luminance meter LMT L1003 (<http://www.lmt.de/>) under the same dim ambient illumination used during the experiments. Letter size decreased from the upper to the lower row in accordance with exponential scaling using a step size of 0.05 logMAR between rows. The edge-to-edge letter and row spacing was adjustable (see below). The letter stimuli were generated using custom software designed by one of the authors (FP). The exposure duration was unlimited.

*Insert Fig 1 about here*

Procedure

Measurements were performed monocularly using a pinhole in front of the tested eye, with appropriate refractive correction determined under photopic testing, if needed. The sighting dominant eye of each subject, established using the “hole-in-the-card” method, was used for testing. The non-viewing eye was occluded. Ambient illumination in the experimental room remained dim and constant during all measurements. Each observer was asked to read aloud the letters on the middle row, from left to right. She or he was encouraged to guess if the letters were indistinct. Testing began at a letter size where all letters on the row could be read. The row was considered as resolved if at least 3 letters on the row were identified correctly. If appropriate, the heights of all rows were then reduced in size by a step corresponding to 0.05 logMAR and testing continued. The final visual acuity (logMAR) was determined using an interpolation method and letter-by-letter scoring [[32](#_ENREF_32)]. Each letter read correctly on each row was scored as 0.01 log units.

The stimuli were viewed under two different background luminances, corresponding to photopic (228 cd/m2) and mesopic (0.164 cd/m2) light levels. The photopic luminance corresponded to the unattenuated background luminance of the monitor. The mesopic level represented an attenuation of the luminance by 3.14 log units. The edge-to-edge, inter-optotype separation of the letters in each row was varied to produce 4 different spacing conditions of 100%, 50%, 20% and 10% of the letter width. For each condition, row spacing was fixed at the optotype size of the row below. The different inter-optotype separations were presented in random order for each luminance condition tested. The viewing distance was 12 m for the photopic and 6 m for the mesopic conditions.

To vary the luminance of both the stimuli and the background, observers viewed the computer monitor through a calibrated Thorlabs glass neutral density filter (http://www.thorlabs.com). The filter was mounted in a pair of light-tight goggles, which included an opaque shield to occlude the non-viewing eye. A 2.5 mm pinhole was located in front of the test eye at the spectacle plane to stabilise the retinal illuminance. This aperture also limited the field of view of the test eye. When a spectacle correction was required, the pinhole was positioned at the center of the ophthalmic lens. Observers were sufficiently adapted to the low luminance condition before testing was begun. The goggles and pinhole were used for both photopic and mesopic conditions the only difference being the use of the filter for the mesopic luminance.

The visual acuity measurements for each observer under all conditions were performed twice (on different days) to assess repeatability. Before any measurements, each observer was familiarised with the optotypes and the procedures. Trial measurements were included for practice and performed under the photopic luminance condition using an inter-optotype separation of 100% letter width.

Data analysis

Visual acuity (logMAR) under different luminances and inter-optotype separations was analysed using repeated-measures ANOVAs with a significance level of 5%. When necessary, the levels of statistical significance included a Huynh-Feldt correction for departures from sphericity [[33](#_ENREF_33)]. Test-retest repeatability was expressed as the limits of agreement (LoA) defined as the interval that includes 95% of the measurements (95% LoA = mean difference ±1.96 x standard deviation of the differences) [[34](#_ENREF_34), [35](#_ENREF_35)]. The 95% confidence intervals for the LoAs were calculated as suggested by Carkeet [[36](#_ENREF_36)]. Coefficients of repeatability (CoR = 1.96 x standard deviation of the differences) are also reported [[23](#_ENREF_23)]. Paired t-tests were used to assess differences in the means.

**Results**

Mesopic visual acuity was markedly reduced relative to photopic luminance conditions. On average, across all inter-optotype separations and test-retest conditions, mesopic logMAR was 0.48 higher than the average photopic logMAR. A summary of the measurements for each inter-optotype separation and luminance condition is shown in Table 1 (mean logMAR and standard deviations).

Fig. 2 plots the average visual acuity (logMAR) across all observers as a function of the inter-optotype separation in terms of percent letter width (top panel) and min arc (bottom panel). The open symbols represent the photopic luminance condition and the closed symbols the mesopic condition. The first measurements are represented by the circles and the second, repeat measurement by the triangles. The error bars represent ± 1 standard error (SE). The figure shows that logMAR increases as both inter-optotype separation and level of luminance decreases. The increase in logMAR (i.e. poorer visual acuity) as the inter-optotype separation decreases (i.e. as crowding increases) is more marked for the photopic luminance condition. Under the closest inter-optotype separation (10% letter width) photopic logMAR increased by almost twice as much as the comparable inter-optotype separation condition under mesopic luminance. This is supported by the outcome of the repeated measures ANOVA, which revealed highly significant effects of separation (*P* < .0001), luminance (*P* < .0001) and a significant separation x luminance interaction (*P* < .0001). If the inter-optotype separation is expressed in min arc, photopic logMAR increases more rapidly with decreasing separation than in the mesopic condition even at similar min arc separations. The critical angular separation, at which crowding begins to reduce the visual acuity, appears to be roughly unchanged for both luminances.

*Insert Fig 2 about here*

*Insert Table 1 about here*

The measurements of repeatability are presented in Table 2. Shown are the mean differences (*VA)* in visual acuities of test (*VA*1) and retest (*VA*2) measurements, the associated standard deviations, and the relevant CoRs for each condition of luminance and optotype separation. The corresponding Bland-Altman plots [[34](#_ENREF_34)] with the LoAs are presented in Fig. 3. In each panel of Fig. 3, the difference in logMAR between the test and retest measurements is plotted against their average. The panels on the left side show the results for each optotype separation under photopic luminance while the panels on the right side show the equivalent results for the mesopic luminance condition. In each panel, the dashed lines represent the 95% LoAs and the dotted lines the mean differences. The error bars shown in each panel represent the 95% confidence intervals for the Bland-Altman LoAs [[36](#_ENREF_36)]. Mean differences between test and retest measurements were statistically insignificant except for 2 photopic separation conditions of 10% and 50% letter width (*P* = .027 and *P* = .008, respectively).

For a given luminance level, the CoRs differ minimally between separation conditions. On the other hand, there appear differences in the CoRs between luminance conditions. The photopic measurements show smaller CoRs (0.12 logMAR on average) in comparison with the mesopic data (0.19 logMAR on average) (Table 2). However, 2 of the differences under the mesopic condition, (10% and 20% inter-optotype separations) show extreme (outlier) values. Moreover, the differences for these (and only for these) conditions are not normally distributed (Shapiro-Wilk test, *P* = 0.001 and *P* = 0.0006). Consequently, the rationale for the calculated CoRs (0.209 and 0.203 logMAR for the 10% and 20% separations, respectively) can fail resulting in overestimated values. If the extreme values are removed from each condition, the distribution is normal (Shapiro-Wilk test, *P* = 0.81 and *P* = 0.16) and calculated CoRs are now very close to photopic conditions (0.125 and 0.128 logMAR for the 10% and 20% separations, respectively). Inspection of the calculated 95% CI for the LoAs is consistent with this revised analysis and shows that the distributions of LoAs between the mesopic and photopic luminance conditions are not appreciably different. Thus, test-retest repeatability exhibits no dependence on the inter-optotype separation and little dependence on luminance.

*Insert Table 2 about here*

*Insert Fig 3 about here*

**Discussion**

Consistent with the established literature [[12](#_ENREF_12), [13](#_ENREF_13), [23](#_ENREF_23)], visual acuity (logMAR) tested under mesopic luminance conditions was considerably worse than when tested under photopic conditions. On average, across all test conditions (i.e. all inter-optotype separations and test and retest measurements), there was a decrease of 0.48 logMAR from photopic (228 cd/m2) to mesopic (0.164 cd/m2) luminances. The reduction in visual acuity from photopic to mesopic luminances in our study is similar to some previous reports [[12](#_ENREF_12)], but is more than the decrease reported in a more recent study [[23](#_ENREF_23)]. The discrepancy is explained by differences in the level of mesopic luminance used as lower luminances cause greater reductions in visual acuity [[14](#_ENREF_14)]. The mesopic luminance level we used (0.164 cd/m2) was similar to previous work (0.075 cd/m2), where decreases in visual acuity were consistent with our results [[12](#_ENREF_12)], but lower than the luminance used (0.75 cd/m2) in another recently reported study that found less of a decrease in acuity as a function of luminance [[23](#_ENREF_23)].

An additional finding of our study, one that has not previously been reported, is that the decrease in visual acuity as a function of luminance is dependent on the inter-optotype separation (i.e. the amount of crowding). Closer inter-optotype separations, where crowding is stronger, produced relatively greater decreases in logMAR (i.e. poorer acuity) under photopic luminance compared to the same inter-optotype separations under mesopic conditions. This effect can be direct consequence of luminance decrease, or can be mediated by the difference in visual acuity between both luminance conditions, i.e. by the difference in letters size. Previous results showed that foveal contour interaction (i.e. using bar flankers) [[28](#_ENREF_28), [37](#_ENREF_37), [38](#_ENREF_38)] and foveal crowding (i.e. using optotype flankers) [[39](#_ENREF_39)] occurs only within a fixed angular range of letter-to-flanker separations (edge-to-edge separation in min arc) irrespective of the letter size. The results of the present study are consistent with these findings. However, with regard to clinical relevance, the stimuli were constructed on the basis of an inter-optotype separation metric of % letter width. For this reason and because visual acuity is poorer for mesopic luminance conditions (i.e. letters sizes are bigger), the small angular separations (in min arc) reached for the photopic luminance condition, where crowding is strongest, were not assessed under mesopic luminance. However, comparing the data plotted as a function of angular inter-optotype separation (bottom panel in Fig. 2) (where the letter size does not influence the separation), the logMAR dependence on inter-optotype separation is steeper for the photopic than for the corresponding mesopic results. In other words, photopic crowding remains stronger and not as a result of letter size. Previous research in laboratory settings showed that the magnitude of contour interaction, the specific lateral spatial interaction between a target letter and adjacent flankers [[27](#_ENREF_27)], is reduced under mesopic luminance levels [[28-31](#_ENREF_28)]. In the current study, we extended those findings to include the influence of luminance on the more general crowding effect, which occurs when viewing letter charts [[27](#_ENREF_27)].

Our results were obtained for participants with normal vision. However, a similar conclusion was reached by a previous study where a reduction in crowding (or separation difficulty as it was termed) was found in observers with amblyopia under mesopic luminance conditions [[30](#_ENREF_30)]. Although the data are few, they suggest that the underlying mechanism responsible for the reduction of crowding at low luminance may be similar in normal and amblyopic vision. It is not known whether similar results would be found in other ocular conditions.

Differences in crowding as a function of luminance have been suggested to partly explain some previous findings of a differential effect of optical blur on visual acuity under high and low luminances [[12](#_ENREF_12), [24](#_ENREF_24), [25](#_ENREF_25)]. Simpson et al [[25](#_ENREF_25)] used Landolt C optotypes, presented in a format which controlled for contour interaction [[40](#_ENREF_40)] (by ensuring the spacing between optotypes was held constant), to measure visual acuity under different levels of optical blur and luminance. Their results showed that while visual acuity is worse with blur under both photopic and mesopic luminances, the decrease in acuity (i.e. the effect of the optical blur) is less for the lowest mesopic luminance compared to the photopic conditions (see also Bedell [[24](#_ENREF_24)]). The authors speculated that differences in spatial processing at mesopic and photopic levels of luminance adaptation may account for the differential effect of blur at low luminance, but another suggestion is that the differential effect of blur on acuity may be due to differences in crowding under photopic and mesopic conditions [[12](#_ENREF_12)]. Although both Simpson et al [[25](#_ENREF_25)] and Bedell [[24](#_ENREF_24)] used Landolt C optotypes in a crowded format [[40](#_ENREF_40)], the inter-optotype separation was fixed at 1 optotype width (i.e. 100% letter width). Our results showed a differential effect of luminance only for closer inter-optotype separations; thus, the differences in crowding at mesopic luminance that we have shown cannot account for the differential effect of blur on visual acuity at low luminance.

Test – retest repeatability was of the order of about ±0.1 logMAR (or 1 line on a standard ETDRS chart) for the photopic luminance condition, consistent with previous reports [[26](#_ENREF_26)]. Repeatability of visual acuity under mesopic conditions may be slightly poorer but not significantly so. The CoRs for the mesopic test – retest conditions were larger than those for the photopic conditions (Table 2). However, careful statistical analysis did not support a difference. It may be that a larger sample size may show differences, although a previous study using a larger similar samples of subjects did not find a difference in repeatability between photopic compared to mesopic luminance, albeit for the ETDRS letter chart with 100% optotype spacing [[22](#_ENREF_22)].

There is evidence that a greater amount of crowding can steepen the slopes of the underlying psychometric functions for visual acuity measurements [[41](#_ENREF_41)] which, in turn, has led to the suggestion that measurements of visual acuity using more crowded charts could improve the reliability of measurements [[42](#_ENREF_42)]. If so, we would have expected to find better repeatability of photopic compared to mesopic acuity, especially for close inter-optotype separations where crowding was stronger. It may be that small differences in repeatability do exist but the relatively small sample size in our study precluded significance. In any case, even with a larger sample size, it is likely that any effect would be small.

 Our results describe new findings of a reduction in crowding for clinical measurements of visual acuity under low luminance (mesopic) conditions. However, the effect of such a reduction is evident only for relatively close inter-optotype separations. As such, for measurements under low luminance conditions (i.e. mesopic) using standard ETDRS charts [[22](#_ENREF_22)] or other standard formats [[23](#_ENREF_23)], where the inter-optotype spacing is fixed at 1 letter width (100% optotype), the reduction in crowding that we have demonstrated will have little or no impact. However, for other measurements, where the separation between target letters and flankers are closer, the reduction in crowding may be more important. Our results may also be important when using other, non-acuity targets that are subject to crowding effects (e.g. faces) or in pathological conditions that necessitate the use of a non-foveal locus where the effects may be quite different.

Funding: The Faculty of Science, Palacky University, Olomouc, Czech Republic provided financial support in the form of a grant IGA\_PrF\_2017\_003. The sponsor had no role in the design or conduct of this research.

Conflict of interest: All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee (Palacky University, Olomouc, Czech Republic) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

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**Figure Captions**

**Fig 1** An example of the experimental stimuli showing 100% spacing between letters. The size of each row of letters differs by 0.05 logMAR.

**Fig 2** The top panel shows theaverage logMAR across all subjects, plotted against edge-to-edge inter-optotype separation (% letter width) for photopic (open symbols) and mesopic (closed symbols) luminance conditions. Data for test (circles) and re-test (triangles) are shown. The bottom panel shows the same data but this time plotted against inter-optotype separation in min arc. It is evident that LogMAR increases both as a function of luminance and inter-optotype separation although the increase appears less under the mesopic conditions.

**Fig 3** Bland and Altman [[34](#_ENREF_34)] plots depicting the difference *VA* in logMAR between the first *VA*1 (test) and second *VA*2 (retest) measurements against their average. The panels on the left side depict results for each inter-optotype separation under the photopic luminance condition while the panels on the right side show the corresponding separations under the mesopic luminance condition. The dashed lines represent the 95% limits of agreement (LoA) and the dotted lines the mean differences. The error bars shown in each panel represent the 95% confidence intervals for the Bland-Altman LoAs [[36](#_ENREF_36)].