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CHARACTERISATION OF COLOUR-SENSITIVE NEURONES IN THE CAT THALAMUS

Ph.D. thesis

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1. Introduction

The domestic cat (*Felis catus*) is a classic animal model in visual neuroscience. Hence, we know a lot about the anatomical and functional features of its visual system. However, even if the investigation of the cat colour vision has an about 50-year-long history, we can find controversial reports in the literature, and the central mechanism lying behind colour distinction has not been cleared yet.

Cats are known as animals having excellent nocturnal vision, but their retina is adapted to daylight conditions as well: in addition to rods, they also have cones, and their retina contains an area (so called *area centralis*) similar to the primate fovea, where the cones are abundant (25-30.000 cones/mm^2, Linberg & al., 2001). These characteristics make this species suitable for further examinations to obtain deeper insight to the dichromatic colour vision of subprimate animals. Thus, we can expand our knowledge on the evolution of the primate trichromatic colour vision. Primary goal of the experiments present in this study were to find and characterise neurones as fully as possible in the cat thalamus, that play a role in colour vision.

Some of the early cat experiments failed to show the capability of colour vision (Ducker, 1964) supporting a vulgar error that cats can not discriminate colours. In fact, it has been known for a long time that there are two cone types in the cat retina (Ahnelt & Kolb, 2000; Szél & al., 2000): the S-cones (sensitive in the shorter wavelength range, absorption peak 450 nm, Guenther & Zrenner, 1993) and ML-cones (preferring the medium/long wavelengths, absorption peak 553, Yokoyama & Radlwimmer, 1999) allow dichromatic colour vision (Loop & Bruce, 1978; Loop & al., 1987; Jacobs & al., 2001; van Arsdel & Loop, 2004; Van Hooser & Nelson, 2006).

However, simply having cones with spectrally different photopigments is not sufficient for colour vision, but a – so called opponent – neural network is required as well to compare the outputs of the cone types. This system is fairly known in primates, but there is much to be revealed in species being lower on the phylogenetic tree. We distinguish three major pathways in cats as well: pathways corresponding to the primate parvo- and magnocelullar systems are called X- and Y-system, respectively, based upon the retinal ganglion cells and cell types of the *lateral geniculate nucleus* (LGN; Enroth-Cugell & Robson, 1966; Cleland & al., 1971). The third – so called W-system – in cats is homologous to the primate koniocellular pathway (Stone & Fukuda,

1974). This XYW system was described first and most detailed in cats. X-cells are characterised by brisk, sustained response and linear summation of the inputs on their small (0.2-1.0°) receptive fields, while the Y-cells produce brisk but transient response and have larger receptive fields (0.5-2.5°) and non-linear contrast function (Enroth-Cugell & Robson, 1966; Cleland & al., 1971). Receptive field size of the W-cells is similar to that of the Y-cells (0.4-2.5°) and their activity is described as sluggish (Cleland & Levick, 1974a,b). There is a correlation between this function-based classification of retinal ganglion cells (X, Y, W) and Boycott's and Wässle's anatomical division (1974; α, β, γ).

In cats, the structure of the LGN and the distribution of its cells are different as well, compared to what we can find in primates: X-cells are located mainly in the dorsal layers (A and A1) and in layer C in smaller proportion, while the Y-cells are more abundant in layer C and less common in layer A and A1. W-cells were described in layer C, C1 and C2 (Guillery, 1966; LeVay & Ferster, 1977; Friedlander & al., 1981; Stanford & al., 1981). Similarly to the primates, layers receive their inputs from the contra- and ipsilateral eyes alternatively.

Experiments showed in present study aimed – using electrophysiological methods – to learn as much as possible about neurones responsible for colour-opponency and their inputs in cats, thus uncover some aspects of the evolutionary prelude of the primate colour vision.

2. Objectives

We know few things only of the subprimate S-cone-driven pathway, but some studies suggest that early mammals (and their current nonprimate descendants) have S-cone opponent system similar to what was described in primates (Yeh &al., 1995a; Lee & al., 2000; Solomon, 2002; Blessing & al., 2004; Martin & al., 2011). Antagonistic responses induced by activation of the S- and ML-cones were reported from retinal ganglion cells (Michael, 1966; Cleland & Levick, 1974b; Vaney & al., 1981; Hemmi & al., 2002; Ekesten & Gouras, 2005; Yin & al., 2009) and relay cells of the LGN as well (Daw & Pearlman, 1970; Michail, 1973; Cleland & al., 1976; Wilson & al., 1976) in several species. However, the spatial structure of their receptive fields have not been examined systematically yet, and it is also unclear whether this system consists of one or more coherent channels. In the experiments providing the basis of this thesis, we set the following objectives:

1. Using electrophysiological techniques and cat-adapted versions of cone-specific stimuli previously used in studies of the primate visual system, we sought features that can be used to reliably distinguish colour-sensitive (chromatic opponent) and achromatic cells.

2. Based upon earlier studies, it is known, that colour-sensitive neurones prefer colour contrast over luminance contrast (Wiesel & Hubel, 1966; Livingstone & Hubel, 1984), and that the primate koniocellular LGN neurones have linear contrast function (Tailby &al., 2008a,b). Therefore, we were interested in the cone-specific and achromatic contrast function of the cat colour-sensitive LGN neurones.

3. We aimed to examine the structure, size and S- and ML-cone weights of the receptive fields of cells with S-cone input. In order to determine the receptive field sizes, we analysed the cells' spatial frequency tuning functions.

4. We were also interested in the cone weights that can be expected to shape the receptive field surround if we assume a randomly built retinal network. To answer this question, we simulated receptive fields on digitised retinal areas.

5. There are several papers suggesting that visual perception based upon colours is slower than the luminance-based sensation (De Lange, 1958; Kelly & van Norren, 1977; McKeefry & al., 2003; Smithson & Mollon, 2004; Bompas & Sumner, 2008). Based upon this idea, we intended to compare the temporal frequency tuning of colour and achromatic cells.

6. A detailed *in vitro* study (Crook & al., 2009) on macaque retina showed, that small bistratified ganglion cells (SBG) have type II receptive fields. However, in the literature, there is no solid view regarding the timing of the antagonistic inputs of the S-cone-driven opponent pathway. Some authors found differences in primate retina or LGN between the response latencies (Chichilnisky & Baylor, 1999; Reid & Shapley, 2002; Field & al., 2007) and temporal frequency tuning (Tailby & al., 2008a) of the S-ON and (L+M)-OFF inputs, others reported that these differences were negligible (Yeh & al., 1995b;

Solomon & al., 2005; Crook & al., 2009). We aimed to examine the temporal frequency tuning of the S- and ML-cone inputs and test if their timing is in balance.

7. In cats, thalamic cells of the W-system – probably homologous to the primate koniocellular channel – were described from layer C, C1 and C2 of the LGN (Stanford & al., 1981). We wondered about which layers of the LGN contain the colour-sensitive neurones we characterised.

3. Materials and methods

3.1. Animals and ethical approval

In the experiments included in present study, we used 7 adult (1-5 years old) domestic cats (*Felis catus*, 2 females, 5 males). Some animals were derived from our own rearing (animal facility of Institute of Physiology, Medical School, University of Pécs), others from animal facilities of University of Debrecen and University of Szeged. Animals were kept and the experiments performed according to Hungarian and European legislation (Regulation 40/2013. (II.14.) of the Hungarian Government on animal experiments and Directive 2010/63/EU and 2012/707/EU of the European Parliament). All procedures were approved by the Directorate for Food Chain Safety and Animal Health of the Baranya County Government Office (license no. BA02/2000-1/2010 and BAI/35/51-15/2016).

3.2. Surgical preparation

All experiments were performed in anaesthesia induced with intramuscular injection of mixture of ketamine (7 mg/kg, *Calypsol, Richter, Budapest, Hungary*) and xylazine (1 mg/kg, *CP-Pharma, Burgdorf, Germany*). One femoral artery was catheterised. This catheter was used for direct blood pressure measurement and fluid supply (6 ml/h Ringer solution infusion, *B. Braun, Melsungen, Germany*). Infusion contained glucose as nutriment supply (24 mg/kg/h) and vecuronium bromide to paralyse the animal (6 mg/kg/h, *Norcuron, Organon, Roseland, NJ, USA*).

From the point on when paralysis had developed, cats were ventilated artifically through a tracheal cannula with a 1:2 mixture of O_2 and N_2O supplemented with 0.5-2.5% isoflurane *(CP-Pharma)*.

Head of the animals was fixed in a stereotaxic frame and a craniotomy of ~5 × 5 mm with its centre at Horsley-Clark coordinates of anterior 4.7, lateral 9.2, was made for vertical access to the left LGN.

Each experiment has lasted for 3-5 days to maximise the amount of data collected. General physiological parameters were monitored continuously and kept in normal ranges (end-tidal CO_2: 3-4%, systolic blood pressure: 100-140 mmHg, body temperature: 38°C). The eyes were protected against dehydration using contact lenses with zero optical power.

3.3. Visual stimuli

Visual stimuli were generated using Open GL commands controlled via freely available software *(Expo, courtesy of Peter Lennie, University of Rochester, Rochester, NY, USA)* running on an Apple Power Mac G5 computer and presented on a computer monitor (IBM P275, frame refresh rate: 96 Hz) at 28.5 or 57 cm from the cat's eyes.

Stimuli were circular patches whose colour and contrast were calibrated to modulate either both cone types together (achromatic stimuli) or S-cones or ML-cones (cone-isolating stimuli). The technique of silent substitution allows us to increase the activity of one of the cone types compared to the background, while the other type is kept at a constant level. Thus, the response recorded can be considered as a result of the modulation of the affected cone type (Donner & Rushton, 1959). Cone nomograms (Lamb, 1995) for cat photopigments were calculated using peak absorption wavelengths of 450 nm (Guenther & Zrenner, 1993), and 553 nm (Yokoyama & Radlwimmer, 1999) for S- and ML-cones, respectively.

The screen had a mean photopic luminance of 42 cd/m^2. Light levels of >10 cd/m^2 can be considered to be photopic for the cat (Loop & al., 1987), so rod-derived responses are negligible.

The pupils were dilated with 1% atropine sulfate and glass lenses were placed in front of the cat's eyes to focus the reflected image of the tapetum lucidum onto the stimulus monitor. On encountering the first well isolated single unit, the correction lens was adjusted so as to maximise

the spatial frequency cutoff of its receptive field for achromatic gratings. This procedure was performed for each eye separately.

3.4. Search strategy

The major goal of the study was to identify and characterise neurones in the cat LGN responding to modulation of S- („blue") cones. Accordingly, we probed each recording location with achromatic and S-cone-isolating gratings of low spatial (~ 0.05 cycles per degree) and temporal (~ 3 Hz) frequency as we moved along vertical penetrations (n = 29) through the LGN. Achromatic stimuli evoked cell responses much more often, so – by covering one eye or the other – we could use them to follow the transitions from one layer to the next, because layer A, C and C2 receive inputs from the contralateral eye, layer A1 and C1 have them from the ipsilateral eye (Casagrande & Norton, 1991). If a cell could be heard on the audio monitor to respond to the S-cone-isolating stimulus, we always attempted to isolate and characterise all single units based on their spike shape at that location. Additionally, units without any apparent S-cone response were also picked up and characterised using the same battery of tests for comparison. Together, we isolated 201 units at 85 locations. We could confirm specific S-cone responses of 23 units at 21 of these locations. Fifty-six neurones (16 blue-ON and 40 achromatic) had sufficiently complete datasets to be included in this study. However, the numbers of elements at the different analyses may vary because in some cases, we could not go with the full palette of tests through or it turned out later that the recorded data is not clear enough for an appropriate analysis.

3.5. Single-unit electrophysiology

Single channel stainless steel microelectrodes *(FHC, Bowdoin, ME, USA)* or custom-made seven-channel electrodes *(„heptodes", Neuronelektród, Budapest, Hungary)* were used for extracellular recording. Signals were bandpass filtered between 700 and 5000 Hz, amplified and digitised at 22 or 48 kHz sampling rate. Action potentials of single units were separated using principal component analysis of the waveforms in the *Expo* program.

3.6. Data analysis

3.6.1. Testing the receptive field

At each recording location, we first determined the dominant eye, that is, the eye through which visual stimulation evoked single or multiunit activity of higher amplitude. The depth where receptive fields were clearly contralateral and their location well defined within a few degrees of visual field was taken as the start of layer A. From here, systematic change of the dominant eye indicated the successive layers A1, C, C1 and C2. In some of the tracks, we could not isolate units from the deepest one or two layers. The nondominant eye was covered for further characterisation of the receptive field.

The stimuli were centred on the receptive field of the cell (or that of the unit with the most vigorous discharge if multiple spikes were isolated at the same location) by moving the stimulus patch on the screen and gradually decreasing its size. The diameter of the patch was then set at a value between 10 and 30° (average: 19.5°) for the remaining of tests at the given recording site. We chose these large patch sizes because behavioural data (Loop & al., 1979) indicated that the cat's colour vision system is extremely low-pass and to include any receptive field surrounds for complete characterisation of spatial frequency tuning. Analysis of the spatial frequency response of the cells showed that stimuli were 4.8 ± 3.4 times (geometric mean ± SD, $n = 44$) larger than the receptive field size of the same cell, including surround.

Adjusting the elevation of the stimulus allowed on-line estimation if we were recording from a blue-ON or an achromatic cell, but regardless of the cell type, we tried to run the full set of tests, including the achromatic, the ML- and S-cone-isolating, and the S+ML– stimuli. In the DKL (Derrington–Krauskopf–Lennie; Derrington & al., 1984) colour space, elevation means the angle of rising from the isoluminant plane – determined by the S- and ML-cone-isolating axes – along the luminance axis. The rotation inside the isoluminant plane is called azimuth. Both parameters are given in degree. Elevation and azimuth of the S-cone-isolating stimulus is 0 and 90°, respectively, while the achromatic stimulus has 90° of elevation. Achromatic cells had their minimum response at around 0° of elevation and the maximum at 90°, while blue-ON cells responded most at around 0°. Based upon this, we could use this measurement to check the calibration of the S-cone-isolating stimulus.

Then, we tested cone inputs qualitatively using spatially uniform, square-wave modulated achromatic and cone-isolating stimuli. In the first, exploratory one-half of the study (27 units),

temporal frequency was set to 5 Hz for the remaining tests. In later experiments (29 units), temporal frequency tuning was determined using large (diameter: 20°), spatially homogenous patches, whose contrast was modulated sinusoidally in time between -50 and +50% of cone contrast values along the ML- and S-cone-isolating axes. In the remaining tests, we used the optimal temporal frequency of the given cell. Temporal frequency function was recorded at 1, 2, 4, 6, 8, 12, 16, 32 and 48 Hz stimulus frequencies, and the result of the measurement was used for calculation of visual latencies of the LGN cells, as well. Then, we measured contrast sensitivity, and receptive field size of these cells, and cone weights of their inputs, too.

3.6.2. Histology

At the end of each experiment, animals were overanaesthetised using 5% isoflurane, killed using intrapulmonary injection of T61 (embutramide 250 mg/kg, tetracaine-HCl 6.25 mg/kg, mebezonium-iodide 63 mg/kg; *Intervet International B.V., Boxmeer, Netherlands*). Then, animals were perfused through the left ventricle with 0.1 M phosphate buffer, pH 7.4, followed by 4% paraformaldehyde prepared in the same buffer. After perfusion, brain and eyes were removed and postfixated in 4% paraformaldehyde.

3.6.2.1. Cresyl violet staining of brain sections

Tissue blocks containing the LGN were removed, then cryoprotected in 30% sucrose. Frozen sections of 40 µm thickness were cut in the frontal plane and stained using cresyl violet stain. The location of electrode tracks was determined in the light microscope. Only units that were recorded along a track passing through the laminated part of the dorsal LGN were included in the dataset.

3.6.2.2. Immunohistochemistry on the retinae

Fixated retinae were detached from the choroid and pigment epithelium, and with horizontal and vertical cuts through the optic disc, were divided into four quadrants. For modelling the retinal networks, fluorescent immunohistochemistry using photopigment-specific antibodies was performed on these retina pieces. S-cones were labelled with OS-2 mouse

monoclonal antibody *(Prof. Ágoston Szél, Semmelweis University, Budapest, Hungary,* Lukáts & al., 2002), ML-cones were labelled with Anti-Opsin Red/Green 5405 rabbit polyclonal antibodies *(EMD Millipore, Temecula, CA, USA,* Arango-Gonzalez & al., 2010). OS-2 primary antibody was reacted with biotinylated anti-mouse IgG *(Vector Laboratories, Burlingame, CA, USA),* and that was revealed by streptavidin-Alexa Fluor 488 conjugate *(Invitrogen, Carlsbad, CA, USA).* Anti-Opsin Red/Green 5405 primary antibody was revealed by Texas Red-conjugated goat anti-rabbit IgG *(Jackson ImmunoResearch, West Grove, PA, USA).*

3.6.3. Receptive field modelling - simulation

Localisation of the cones was digitised at retinal eccentricities corresponding to the locations of receptive fields of neurones characterised electrophysiologically. In the simulations of inhibitory surrounds, circles with different diameters were placed around each S- and ML-cones using a custom-made *Matlab*® program. We intended to model the receptive fields with various cone composition, that results from local patterns of the cone mosaic. Size of these circles was chosen so that they match the size of retinal areas covered by the smallest (*CBa2*, dendrite tree diameter: 30 μm) and the largest (*CBa6*, dendrite tree diameter: 102 μm) OFF-type cone bipolar cells (Pourcho & Goebel, 1987), and the A-type horizontal cells (*HC-A*, dendrite tree diameter: 80-220 μm, Wässle & al., 1978). These cells could be the ones that build the surround part of the receptive fields up.

Assuming random wiring, cone weights present in the inhibitory surround were determined from the number of different cones inside the circles fit on the digitised retinal images.

3.6.4. Statistics

Statistical analysis of the data was performed using *Matlab*® and *Microsoft Excel*® programs. Our tests included Wilcoxon's signed-rank and rank sum test, Student's paired t-test, F-test and ANOVA. Level of significance (p) was usually 0.01, but the exact values are given at each analysis.

4. Results

4.1. Division of achromatic and blue-ON cells

4.1.1. On the basis of achromatic and S-cone response

Together, 56 neurones are included in the present analysis: 40 in the magnocellular layers of the LGN (A, A1 and C), 15 in the parvocellular layers (C1 and C2), and one undetermined. At most recording sites, achromatic stimuli evoked strong multiunit activity, whereas the S-cone-isolating stimulus minimised the multiunit response as if contrast was decreased to zero. On rare occasions (21 of 200 recording sites tested), the S-cone-isolating search stimulus caused clear modulation of the spike rates of one or more single units, while the activity of the other units was minimised. Here, the achromatic stimulus evoked a slight elevation only in the ON phase of the stimulus. However, these cells show a tonic OFF response to the ML-cone isolating stimulus and a tonic ON response to the S-cone-isolating stimulus. Moreover, the amplitudes of the cone-isolating responses appeared nearly equal at equal cone contrast. This suggests that the achromatic response is minimal because equal and opposite functional inputs (excitatory vs. inhibitory) from the two cone types cancelled each other. We called these neurones blue-ON cells, because all of them responded in the S-ON phase of the stimulus. We show 16 cells of this type in the present study.

4.1.2. On the basis of DKL-elevation function of the cell response

In the other test used for quick discrimination of achromatic and blue-ON cells, we adjusted the modulation of the colour of the stimulus between the achromatic and S-cone-isolating cone contrast values in several steps. Practically, this corresponds to going around the circle perpendicular to the S-cone axis (elevation) in the DKL colour space. Polarity of the curve fitted on the elevation function of the cell response shows the cell type consistently: peak of the V-shaped curve points downward in achromatic cells ($n = 32$), and upward in blue-ON cells ($n = 16$). Achromatic cells had their minimum point of the curve at $1.42 \pm 6.37°$ of elevation (mean ± SD), while blue-ON cells had their response peak at $1{,}20 \pm 7{,}65°$.

4.2. Contrast response

After measuring the contrast response of the cells using our achromatic and two cone-isolating stimuli, we estimated contrast gain using a stepwise fitting procedure described in detail in the 'Materials and methods' chapter of the full thesis. The slope at the steepest point of the fitted curve was calculated and used as contrast gain.

A minority of cells in our sample (14 of 45; 31%) showed vigorous responses to S-cone contrast, whereas achromatic contrast evoked no significant change in their response (ANOVA, $p = 0.33$); therefore ML-cone contrast gain was set to zero. The cells whose S-cone sensitivity exceeded their achromatic sensitivity were defined as blue-ON cells ($n = 14$), while cells with higher achromatic sensitivity were labelled as achromatic cells ($n = 31$). The definition might seem arbitrary at this point, but we present further evidence hereinafter that blue-ON cells are a distinct population in cat LGN. By definition, each cell group has higher contrast gain for its preferred stimulus. The median achromatic contrast gain for achromatic cells was 0.86 spikes / s / % contrast, and the median S-cone contrast gain for blue-ON cells was 0.22 spikes / s / % contrast.

Many of the blue-ON cells showed a linear contrast response. In 6 of the 12 cases in which the full S-cone contrast response was measured, the best contrast model found by stepwise fitting was linear. Achromatic cells, however, typically showed saturation at higher contrasts; the best model for 85% (22 of 26) achromatic contrast response was saturating or supersaturating (also see 'Materials and methods').

4.3. Spatial frequency response

For most cells ($n = 44$), we recorded spatial frequency transfer functions for ML-cone-isolating as well as for achromatic and S-cone-isolating gratings. These measurements allowed spatiochromatic decomposition of the receptive field into two spatial (centre and surround) and two chromatic (ML- and S-cone) components. The data were used to evaluate the relative weights of functional inputs from the two cone types to the receptive field centres, the sign (ON or OFF) and phase of these inputs relative to the stimulus, and the relative sizes of the chromatic receptive field components. In addition to that, we determined the cone weights in the surround

of the recpetive fields as well, to compare them to the data obtained from our random wiring model of the retina.

In the achromatic group, responses can be explained by assuming only ML-cone input to the receptive field: achromatic response amplitudes were statistically indistinguishable from ML-cone-isolating responses in all achromatic cells at all spatial frequencies (Student's paired t-tests between the two conditions showed $p > 0.01$). The S-cone-isolating stimuli rarely evoked response in these cells.

A common property of blue-ON cells was their very weak achromatic response, which in approximately one-half of the cases (7 of 13 cells), did not show any statistically significant variation with spatial frequency (ANOVA, $p > 0.1$). The S- or ML-cone-isolating stimuli were always effective in blue-ON cells, although the shape of their spatial frequency characteristics showed substantial diversity. In 10 of the 13 cells tested, S-cone-isolating gratings produced bandpass tuning curves; in 4 of them, ML-gratings showed similarly bandpass characteristics. In 6 cells, ML-gratings produced low-pass spatial frequency tuning. Low-pass tuning curves for S-cones were found in three of the blue-ON cells.

Spatial antagonism in receptive fields can be quantified by the "low-cut ratio", which is the ratio of the responses to the lowest and the optimal spatial frequencies (Tailby & al., 2008b). Low-cut ratio is zero if the attenuation of the response is complete at low spatial frequencies (meaning that surround inhibition cancels the central excitation), and it is 1 if the spatial frequency response is low pass (so, there is no surround inhibition). The highest low-cut ratios (that is, most low-pass responses) were seen in the ML-cone fields of blue-ON cells (0.81 ± 0.20; mean ± SD) followed by their S-cone fields (0.75 ± 0.15). By contrast, achromatic cell responses to achromatic gratings showed significantly more lowfrequency attenuation (0.60 ± 0.22, $n = 31$; Student's t-tests against ML- and S-cone fields of blue-ON cells: $p = 0.0049$, $n = 13$, and $p = 0.0309$, $n = 13$, respectively).

The difference between the response phases to S- and ML-cone-isolating gratings was narrowly distributed and, on average, very close to 180° (171.3 ± 26.6; $n = 13$) in the blue-ON cell population. A similar calculation for achromatic cells showed a mean phase difference of $80.2 \pm 74.8°$ ($n = 31$) and the S- versus ML-cone phase difference was evenly distributed.

4.4. Cone weights

Weight of the S-cone inputs can be calculated as $w_S = R_S / (R_S + R_{ML})$, where R_S represents the maximal response amplitude evoked by the S-cone-isolating gratings (using the optimal spatial frequency), and R_{ML} represents the maximal response for the ML-cone-isolating gratings. In achromatic cells, the low responsivity to S-cones was mirrored in low S-cone weights (median: 0.11; $n = 31$). The same calculation for blue-ON cells resulted in significantly higher values ($p < 0.01$, Wilcoxon's rank sum test). In blue-ON cells, S-cone weights were narrowly distributed around 0.5 (median: 0.51; $n = 13$), implying that the inputs from the two cone classes to the receptive field centres were well balanced.

Another of our methods is not only eligible to measure cone weights, but for division of achromatic and blue-ON cells as well. This time, we measured the weights of ML- and S-cone inputs to each cell using the 'colour circle' stimulus (in detail, see the 'Materials and methods' chapter of the full thesis) that has been applied before to characterise retinal ganglion cells of macaques (Sun & al., 2006a,b) and LGN neurones of marmosets (Tailby & al., 2008b).

The phase of a cell's response to a sinusoidally modulated stimulus is chiefly determined by phototransduction delay, neural conduction and transmission delays, and phase offsets generated by the neural filters. In order to discount these delays we presented the stimulus sequence in clockwise and counter-clockwise directions around the colour circle, then averaged the resulting response phases.

The preferred colour angle of each cell was determined as the y-axis intercept of a regression line fitted to response phase. Three populations are readily distinguished. Two populations are aligned with the ML (horizontal) axis, which implies exclusive input from ML-cones. These cells correspond to achromatic ON (ML+) and OFF (ML–) cell populations as revealed by their mean phase angles (ON: 5.42 ± 6.92°, $n = 17$; OFF: 177.74 ± 6.20°, $n = 9$). The weights of ML- and S-cone inputs can be calculated as the x- and y-components of the preferred colour angle (Sun & al., 2006a,b). The slight residual deviation of the mean angles from the ML-axis may be attributable to weak S-cone input (S-cone weight 0.10 ± 0.06; mean ± SD) or to deviation of the actual ML-cone axis from our calculation. As it was showed in the chapter of 'Contrast response', achromatic cells have high contrast gain, making them sensitive to such residual contrast. Even though we cannot differentiate between these two possibilities, the main conclusions of our study are not affected.

The blue-ON cells were characterised by a preferred colour angle of 135.24 ± 9.96° (n = 8), placing them close to halfway between the S+ and ML– points of the colour circle. The chromatic optima of blue-ON cells thus indicate equal but opposite inputs from the opponent cones (S-cone weight: 0.50 ± 0.10), in other words, these cells form a well-balanced colour-opponent system. We found no colour opponent neurones with blue-OFF input, which would appear around 315° in the opposite, S–ML+ quadrant (Tailby & al., 2008b). Blue-OFF cells are encountered more rarely than blue-ON cells in primate retina or LGN (Krüger, 1977; Malpeli & Schiller, 1978; de Monasterio, 1979; Valberg & al., 1986; Lee & al., 2005; Szmajda & al., 2006; Tailby & al., 2008a,b; Roy & al., 2009). On the basis of our sample this may be the case in the cat LGN as well, or there are no such cells at all.

4.4.1 Cone weights of the receptive field surround

We showed that both chromatic components of blue-ON cell receptive fields can exhibit surround inhibition albeit weaker than usually seen in achromatic cells. Blue-OFF surround component could be determined from the DOG (difference of gaussians) model fitted on the response data evoked by the S-cone-isolating stimulus, ML-ON surround component was derived from that of the ML-cone-isolating stimulus. Interestingly, low-cut ratios for S- and ML-cone components of blue-ON cell receptive fields were statistically similar to each other (Student's paired t-test: p = 0.2203; Pearson's correlation: r = 0.55, p = 0.0534, n = 13). This result shows these cells to balance their chromatic opponent spatial surrounds, which would, again, favor responses to colour over achromatic stimuli.

Functional S-cone weights of the recpetive field surround (mean: 28.7%) were calculated as *S-cone weight = S-OFF response / (S-OFF + ML-OFF response)*.

4.5. Receptive field size and structure

Behavioral studies indicate that the spatial resolution of S-cone vision in cats is much worse than it is for luminance stimuli (Berkley & Sprague, 1979; Loop & al., 1979). One reason for this may be that the density of S-cones is ~1/5 to 1/20 of the ML-cone density in the central part of cat retina (Linberg & al., 2001). Thus, even if the colour pathway is set up to achieve maximum spatial resolution, its receptive fields are expected to be larger than those of a high-

acuity system sampling only from ML-cones. Further increase in receptive field size can result from neuronal convergence and optical blur (Hammond, 1974; and Wässle, 1971; Robson & Enroth-Cugell, 1978, respectively).

By the application of cone-isolating gratings, we determined the sizes of both S- and ML-cone components of the recorded cells. Achromatic cells (median radius: 0.50°) are substantially smaller than the S-cone radius (median: 1.99°) or ML-cone radius (median: 2.30°) of blue-ON cell receptive fields (Wilcoxon's rank sum test: $p < 0.001$).

Our present data indicate that the S- and ML-cone fields of cat blue-ON cells are similar in size, as evidenced by their significant correlation (Pearson's correlation of log-transformed data: $r = 0.75$, $p = 0.0031$, $n = 13$). Closer inspection reveals that S-cone fields are slightly smaller (median S/ML radius ratio: 0.91), although the difference is on the margin of statistical significance (Wilcoxon's signed-rank test: $p = 0.08$, $n = 13$). Thus, the spatial extents of S- and ML-cone mediated excitatory inputs to cat blue-ON cells are essentially equal.

To estimate the relative size of achromatic and blue-ON receptive fields, we need to take into account the eccentricity-dependent increase in receptive field size in cats. The dependence of receptive field size on eccentricity is well approximated by an exponential function (Troy, 1983; Enroth-Cugell & Freeman, 1987; Rowe & Cox, 1993). To obtain an estimate of this trend in our dataset, we fitted an exponential by least squares minimisation to the receptive field centre radii of achromatic cells (as determined using ML-cone-isolating gratings). The resulting regression line (equation: $y = 0{,}33\ e^{0{,}032x}$) is approximately halfway between similar data series published for X- and Y-cells of cats (Troy, 1983; Enroth-Cugell & Freeman, 1987). The eccentricity-dependent increase in RF radius was slightly less for blue-ON cells (equation: $y = 0{,}92\ e^{0{,}021x}$).

We normalised the RF radii of each cell by dividing the actual radius in degrees by the radius expected for achromatic cells at the same eccentricity. For achromatic cells, the distribution of normalised radii was centred around 1 as expected (median: 1.02°; $n = 31$). The normalised RF centre radii of blue-ON cells were significantly larger, showing a median value of 2.60 and 2.77 for the S- and ML-cone fields, respectively ($n = 11$; $p < 0.01$ in Wilcoxon's rank sum test against data of achromatic cells). Thus, blue-ON cells had receptive fields ~2.7 times greater than achromatic cells at any eccentricity.

4.6. Receptive field modelling

To learn more about the receptive field surround of the blue-ON cells and the cone weights of the OFF-type inputs emerging there, we designed a simulation (see 'Materials and methods'). In this simulation we measured morphometric parameters on 8 digitised retinal areas obtained from retina whole mounts labelled with fluorescent cone-specific antibodies.

Density of both cone types was decreased exponentially with eccentricity as expected (Linberg & al., 2001). Average proportion of the S-cones was 4.5% of the total number of cones. In the size range of most OFF-type bipolar cells, up to a few S-cones are expected only to occur in the dendritic field. Nevertheless, horizontal cells are estimated to cover several (6-20) S-cones. The likelihood of S-cones in the simulated receptive fields increased with field size, reaching 100% for sizes above the average S-cone distance (52.51 ± 16.60 µm; mean ± SD).

If we suppose that S- and ML-cones contribute to the cells' response proportionally, then average S-cone weight would be 4.5% corresponding to their ratio. Since S-cone density in the retinal cone mosaic – based upon the former condition – limits the maximal S-cone weight, 29% of S-cone weight measured by electrophysiology can not be achieved assuming equal strength of S- and ML-cone inputs. However, if we suppose that S-cone inputs contribute 10 times stronger than ML-cones, then the S-cone weights obtained here would be very similar to what we got from the electrophysiological data. This 10-fold conversion factor is just an estimation; its exact value is less important than the conclusion, that the S-cone weights provided by the pure S-cone density have to be multiplied somehow to reach the values measured by electrophysiology. Such an increased contribution of the S-cones to the cells' response can be achieved by enhanced synaptic strength (increased amount of neutransmitter released, more postsynaptic receptors or dendritic spines).

4.7. Temporal frequency tuning

We recorded temporal frequency tuning functions in response to temporal (1-48 Hz) sine-wave modulated, spatially uniform fields. Stimuli were modulated either along the achromatic, ML-cone-isolating or S-cone-isolating colour axes.

One group of achromatic cells (n = 16) showed band-pass frequency tuning over the range of frequencies tested (1–48 Hz) with optimum frequencies close to 10 Hz (10.72 ± 6.94 Hz;

geometric mean ± SD). Other achromatic cells showed high-pass (n = 9) characteristics for temporal modulation. Cells with band-pass and high-pass responses did not show distinct properties on other spatial or temporal response dimensions tested.

The steep response elevation at higher frequencies that characterises the high-pass group may be attributable to a well-established property of retinal ganglion cells (Frishman & al., 1987). The explanation is a temporal delay between excitatory and inhibitory inputs summated on the cell. The effect of the delay is that as stimulus frequency is increased, inhibition tends to miss the peak of excitation. At higher frequencies, the two inputs become synergistic, i.e. the peak of excitation approaches in time the removal of inhibition, causing high amplitudes of response modulation. In addition, high-frequency responses can be generated by subharmonics of the stimulus monitor frame refresh rate (96 Hz). For our purposes the exact cause of high-pass responses is not important, except insofar as that the high-pass property distinguishes achromatic cells from blue-ON cells.

Blue-ON cells receive ON-type excitation arising in S-cones and OFF-type excitation arising in ML-cones. We showed above that the sample of blue-ON cells reported here exhibits predominantly low-pass ('Type II') spatial tuning for S- and ML-cone selective gratings. The opponent S- and ML-cone inputs thus can be isolated to a large extent using cone-isolating stimuli. The cells' sensitivity covered low frequencies so that at 1 Hz (the lowest frequency tested) the cells still responded at 64 ± 20% (geometric mean ± SD; n = 11) of the maximum. The optimal stimulus frequencies were around 3 Hz for both cone types (3.44 ± 4.30 Hz for S- and 3.31 ± 8.85 Hz for ML-cones, geometric mean ± SD; Wilcoxon's signed-rank test: p = 0.88).

High frequency responses of blue-ON cells fell to noise level at 11 ± 12 Hz (geometric mean ± SD; n = 8) and above, with the exception of three cells that still responded at 48 Hz (the highest frequency tested). The high-pass behaviour seen in many achromatic neurones was not exhibited by the blue-ON cells in our sample. A single exception may be the cell with response optima at 16 Hz for S-cone flicker and 32 Hz for ML-cone flicker.

The similarity of responses to the opponent cone types extended across all frequencies tested. Pairs of response amplitudes from the S- and ML-cone frequency tuning curves were in robust correlation (r = 0.8, $p < 0.001$).

4.8. Temporal frequency-dependent phase shift and visual latency

As shown in the previous section, blue-ON cells can be considered 'sluggish' in the sense that they prefer considerably lower temporal frequencies than achromatic cells do. Another aspect of the temporal response is latency to visual stimulation, which as noted above, may be due to conduction time and/or to the time constants of filters in the upstream neural pathway. Measuring the temporal frequency dependence of steady state neural responses to sinusoidally modulated stimuli offers a way of directly assessing visual latencies (Enroth-Cugell & al., 1983; Frishman & al., 1987). As temporal frequency is increased, the response delay represents an increasing proportion of the stimulus cycle and is evident as a linear shift in response phase.

The curves of frequency-phase relationship for ML-cone isolating responses of achromatic cells ($n = 26$) cluster into two groups representing ON- and OFF-type cells. The average phases (measured at 1 Hz) of the two clusters are $157.35 \pm 32.68°$ and $353.62 \pm 28.67°$, respectively (i.e. a difference of 196.27°). A similar pattern appeared when plotting phase shifts of S- and ML-cone responses of blue-ON cells ($n = 11$) with the two cone types eliciting responses in opposite phases.

The phase values at 1 Hz were subtracted from each data point of the particular cell in order to pool ON- and OFF-type responses. Here, latency can be calculated by simple linear regression whereby steeper slopes correspond to longer latencies. Linear regression performed for each cell separately confirmed the assumption that a constant delay was the main contributor to phase shifts in our dataset; the goodness of fit (R^2) values were 0.98 ± 0.018, 0.97 ± 0.025 and 0.98 ± 0.020 for achromatic cells and the ML- and S-cone responses of blue-ON cells, respectively.

Achromatic cell response latency to ML-cone isolating stimuli is 56 ± 8 ms (mean ± SD). Latencies obtained with achromatic stimuli were not significantly different to this value (57 ± 19 ms, $p = 0.73$, Wilcoxon's signed-rank test, frequency range tested 1–16 Hz). We measured the latencies of blue-ON cell responses using either ML- (63 ± 8 ms) or S-cone isolating stimuli (69 ± 3 ms). Although there was little latency difference between ML- and S-cone responses for individual blue-ON cells ($p = 0.05$, Wilcoxon's signed-rank test), the distributions were different. In particular, S-cone latencies were tightly clustered and they were always > 60 ms. ML-cone latencies on the other hand, showed larger variance.

Altogether, ML- and S-cone response latencies of blue-ON cells were longer than latencies of achromatic cells ($p = 0.02$ and $p < 0.01$, respectively, Wilcoxon's rank sum test).

Taken together, the weak achromatic responses, matched temporal frequency transfer functions and similar S vs. ML latencies indicate that the temporal properties of ML- and S-cone inputs to blue-ON cells in cats are functionally balanced.

4.8.1 Relationship to recpetive field size

As we showed earlier, cat blue-ON cells have about three times larger receptive field radius than the average achromatic cell. Comparison of the receptive field centre sizes to the temporal frequency optima and the visual latencies of the cells show three major things: (1) they illustrate the upshot of our analyses so far: blue-ON cells are characterised by a combination of large RF size, low temporal frequency optimum and long visual latency; (2) achromatic cells and blue-ON cells form a continuum along the three parameter dimensions illustrated, and (3) the data also show robust correlations between each pair of the parameters in question.

The strong negative correlation ($r = -0.58$; $p < 0.001$) between log temporal frequency optimum and visual latency demonstrates that latency is dictated by the frequency transfer limits of the afferent neural network. Temporal frequency optimum is inversely correlated with RF radius ($r = -0.56$; $p < 0.001$; for log transformed data) suggesting that across all cell types, larger fields prefer lower temporal frequencies. Similarly, cells with larger receptive fields tend to respond with longer latency ($r = 0.44$; $p < 0.001$; for log transformed data).

4.9. Laminar distribution of the cells in the LGN

We tested altogether 201 recording sites using S-cone-isolating stimuli in the LGN. Units with preferential S-cone responses were generally rare (found in 10.4% of the sites tested) (also see 'Materials and methods' and 'Search strategy'). Note, that the real proportion of the blue-ON cells is certainly lower in the population, because we were searching for these cells selectively using S-cone-isolating stimuli.

Blue-ON cells of our present sample are all from layer C or from the two parvocellular layers C1 and C2 and were never encountered in the upper two (A and A1) layers. The segregation of S-cone responses to the deep layers suggests that these cells may constitute an

anatomically separate group. The laminar distribution of our blue-ON cells corresponds to the distribution of W-type relay cells (Cleland & al., 1976; Wilson & al., 1976), a heterogeneous cell group mainly defined by their afferentation from slow conducting retinal axons.

We did not find significant differences ($p > 0.37$ in all following t-tests) in S-cone or achromatic contrast gains, spatial frequency low-cut ratios, or normalised receptive field sizes of blue-ON cells between the magnocellular (C) and parvocellular (C1, C2) laminae. Thus, our data do not show evidence for further anatomical segregation of response properties among blue-ON cells.

5. Discussion

Our results extend earlier findings in two respects. First, we show that S-cone-responsive cells in the main visual relay nucleus of a nonprimate mammal constitute a functionally coherent population specialized for colour vision. Second, the physiological properties of blue-ON cells in the cat LGN show strong functional similarity to blue-ON cells in monkeys.

5.1 Relay of blue cone signals in the cat LGN

We aimed at creating conditions where only S- and ML-cones contributed to the stimulus-evoked responses. The influence of rods was minimised by applying photopic mean light levels of >10 cd/m^2 (Loop & al., 1987). The isolation of each cone type can under these conditions be judged by considering how well the other cone type was silenced. We tested this property of our stimuli by recording elevation function of the cell responses. Based upon our results, we can conclude that the S-cone-isolating stimuli used fulfilled these requirements, we could distinguish the colour-opponent neurones reliably from the achromatic ones. The achromatic cell population showed very low sensitivity to the nominal S-cone-isolating modulation. The residual responses to the S-cone-isolating stimulus sometimes seen in these cells can be attributed to either weak S-cone input or to incomplete silencing of the ML-cone mechanism, possibilities that cannot be distinguished currently. The fact that blue-ON cells responded preferentially to the same stimulus that minimised the responses of achromatic cells, demonstrates that S-cone stimuli were effective in driving a second receptor mechanism.

It has been known since early studies of the cat visual system (Daw & Pearlman, 1970; Cleland & Levick, 1974; Wilson & al., 1976; Krüger, 1979; Rowe & Cox, 1993) that certain "colour-coded" retinal ganglion cells or thalamic relay cells respond vigorously to narrow-band blue light but are inhibited by longer wavelengths. Together, 13 examples of colour-coded neurones are known from earlier studies of the cat LGN (Daw & Pearlman, 1970; Pearlman & Daw, 1970; Cleland & al., 1976; Wilson & al., 1976). Two features of the 16 blue-ON cells presented here matched the properties of those neurones, suggesting that we analysed members of the same cell population. First, they receive spectral opponent input. Second, they are segregated to the C, C1, and C2 layers of the LGN. Together, our data suggest that blue-ON cells in LGN form the thalamic relay for cortical mechanisms serving colour discrimination in cats (Loop & Bruce, 1978; Loop & al., 1979).

5.2 Comparison to the primate S-cone system

Our data revealed several points of functional similarity between blue-ON cells in cats and monkeys:

(1) In our sample of blue-ON cells, the response to S-cone contrast increment was always ON-type. Blue-OFF cells may also exist in the cat, but they must be rare or difficult to record from, as reported consistently in recordings from monkeys or ground squirrel (Krüger, 1977; Malpeli & Schiller, 1978; de Monasterio, 1979; Valberg & al., 1986; Reid & Shapley, 2002; Chatterjee & Callaway, 2003; Lee & al., 2005; Szmajda & al., 2006; Tailby & al., 2008a,b; Chen & Li, 2012; Sher & DeVries, 2012). Thus, based on their frequency of occurrence, blue-ON cells are likely the major carriers of S-cone signals in the visual pathway of cats as well as primates.

(2) The contrast dependence of cat blue-ON cell responses resembled corresponding data from primates. Blue-ON cells in macaques and marmosets show nearly linear contrast response for S-cone-selective stimuli (Tailby & al., 2008a,b). We also observed only feeble responses to achromatic stimuli and balanced ML- and S-cone weights to the blue-ON cell centre mechanism. The closely linear relationship between cone contrast and the response of the blue-ON cells underlines their role in colour vision because it allows efficient coding of the relative activities of the two cone types.

There are two simple explanations for these findings. One would be a small S-cone input to the receptive fields of achromatic cells added to the ML-cone input. An alternative possibility is some residual stimulation of the nominally silenced cone type, that is, an ML-cone artifact in the S-cone-isolating stimulus. In both cases, the responses to the S-cone stimulus would increase with increasing achromatic sensitivity of the cell. We cannot rule out either possibility, but our further analysis does not depend on the cause of S-cone responses in achromatic cells.

It could be argued that separating X- and Y-cells would be a useful addition to our study. Finding a reliable diagnostic measure was, however, not easy. It is clear from the contrast response analysis that the achromatic cell group does not split into clusters with high and low contrast gain, which we may identify with X- and Y-types. Furthermore, contrast gain is known to depend on a number of factors including eccentricity and receptive field size. The null test (Hochstein & Shapley, 1976) is useful to identify linear and nonlinear cell types, but these groups are known to exist within the W-cell population (Sur & Sherman, 1982), which likely has also contributed to our achromatic cell sample. The only unequivocal diagnostic to our knowledge is the measurement of axonal conduction velocity, but we did not make this measurement.

(3) A third similarity between cat and monkey blue-ON receptive fields is their spatially coextensive S- and ML-cone inputs (Wiesel & Hubel, 1966; Chichilnisky & Baylor, 1999; Field & al., 2007; Crook & al., 2009). A classical example of this organisation is the type II receptive field described in monkeys (Wiesel & Hubel, 1966), which is characterised by just two, perfectly overlapping chromatic opponent mechanisms. Such neurones favor chromatic contrast over spatial contrast because an achromatic stimulus of any spatial structure evokes responses of equal magnitude but opposite sign in the S- and ML-cone fields (Wiesel & Hubel, 1966; Livingstone & Hubel, 1984). The detailed receptive field structure of cat blue-ON cells is, however, more complex than the typical type II organisation. In most cells, we observed additional, mainly S-cone-driven, surround inhibition. Similar inhibitory surrounds have been described in blue-ON cells of the primate LGN (Tailb & al., 2008a,b), although they are not normally manifest in their retinal ganglion cell counterparts examined *in vitro* (Dacey & Lee, 1994; Crook & al., 2009).

(4) A few cells showed a comparable degree of low-frequency response attenuation by both cone types, which suggests receptive field organisation known as “double-opponent” (Pearlman & Daw, 1970; Livingstone & Hubel, 1984; Johnson & al., 2008). However, canonical

examples of double-opponent cells show virtually complete low-frequency attenuation, whereas low-cut ratios of cat blue-ON cells always exceeded 0.5. Regardless of these details, it can be concluded that the inputs from the two cone types to blue-ON cells are functionally well balanced, which is evidenced by their generally low achromatic sensitivity.

(5) A further point of similarity of blue-ON cells in cats and monkeys is their relatively large receptive field size compared to cells carrying luminance signals. In the retina of macaque monkeys and in the LGN of marmosets, blue-ON cells have receptive field centre sizes ~3 times that of parvocellular cells and ~1.5 times that of magnocellular cells at the same eccentricity (Solomon & al., 2005; Tailby & al., 2008b); a comparable ratio between blue-ON and achromatic receptive field sizes (2.7) is seen in our dataset.

(6) Generally speaking, a neurone would favour chromatic contrast over luminance contrast if the opponent cone inputs are in perfect balance at any location in the receptive field. The antagonistic inputs may, however, become unbalanced due to spatial or temporal asymmetries. Spatial differences may arise simply because the density of S-cones is much lower in the retina than the density of the longer wavelength cones, which effectively results in centre-surround antagonism with small S-cone-ON subfields and larger ML-cone-OFF subfields. As shown recently for blue-ON and blue-OFF cells in marmosets and macaques (Tailby & al., 2008b, 2010; Roy & al., 2009), achromatic gratings of high spatial frequency can be powerful stimuli for such receptive fields because the bright and dark stripes can coincide with the regions of ON- and OFF-sign excitation, respectively. Although the distribution of S-cones is similarly sparse in the cat (Linberg & al., 2001), our results show that their blue-ON cells rarely respond to achromatic stimuli, even at high spatial frequencies. This result suggests balanced S- and ML-cone inputs across their receptive fields. A similar, type II receptive field organisation characterises M-ON/S-OFF opponent retinal ganglion cells of the tammar wallaby (Hemmi & al., 2002).

In common with spatial imbalance of opponent inputs, temporal imbalance can yield achromatic response as well. Surprisingly, there are no comprehensive studies of achromatic flicker sensitivity in monkey blue-ON cells, but the evidence consistently shows well-balanced temporal properties of S- and ML-opponent inputs (Lee & al., 1989; Chichilnisky & Baylor, 1999; Solomon & al., 2005; Field & al., 2007; Crook & al., 2009). We have shown here that blue-ON cells in cats show negligible response to achromatic flicker at frequencies up to 48 Hz.

(7) Another prominent feature of cat blue-ON cells was their preference for low temporal frequencies, with optima around 3 Hz. Typical achromatic cells were most responsive around 10 Hz, where blue-ON cell responses fell to noise level. Comparable data from the LGN of macaque monkeys (Tailby & al., 2008a) show remarkable similarities: blue-ON (as well as blue-OFF) cells are most responsive at 3 Hz when tested with stimuli similar to ours (low spatial frequency sine-wave modulation of S-cone contrast). In comparison, the optimal temporal frequencies for parvocellular and magnocellular neurones of the macaque LGN are known to be considerably higher at around 10 Hz and 20 Hz, respectively (Derrington & Lennie, 1984; Solomon & al., 1999).

(8) In terms of the other temporal property examined, the low frequency preference and long visual latency of blue-ON neurones of the cat is not unexpected. There is evidence that the pathways conveying signals for S- (‘blue’-) cone based vision in primates are in some way slower or more sluggish than the pathways carrying L- (‘red’-) and M- (‘green’-) cone signals alone (Stromeyer & al., 1991; Cottaris & De Valois, 1998; McKeefry & al., 2003; Smithson & Mollon, 2004; Lee & al., 2009; Pietersen & al., 2014). Our results confirmed that blue-ON cells of the retina and LGN are part of the W-cell system in cats (Daw & Pearlman, 1970; Cleland & Levick, 1974; Cleland & al., 1976; Wilson & al., 1976) and they are part of the koniocellular system in primates (Martin & al., 1997; White & al., 1998; Roy & al., 2009). The retino-geniculate axons of both the W- and the koniocellular pathways are characterised by slow conduction velocities on average (Stone & Hoffmann, 1972; Norton & Casagrande, 1982), although this does not necessarily apply to blue-ON cells in particular because the W- and koniocellular cell populations are functionally heterogeneous (Cleland & Levick, 1974; Silveira & al., 2005). Considering evoked responses to visual stimuli, sluggishness may present itself by preference for low stimulus frequency or by long latency to stimulation. Although these properties may be correlated, they are usually measured separately.

A question emerges, that where the delay between the opponent signals develop. The lag of S-opponent signals must arise in post-receptoral pathways (including the visual cortical networks) since the temporal responses of spectrally distinct cone types are very similar to each other in primates (Baylor & al., 1987; Schnapf & al., 1990; Stockman & al., 1993) as well as in the mouse (Nikonov & al., 2006). Furthermore, in vitro recordings from macaque retina (Dacey, 1993; Dacey & Lee, 1994) show that the peak of the S-cone signal reaches blue-ON (small

bistratified) ganglion cells about as rapidly as M- and L-cone signals reach the achromatic parasol cells (Chichilnisky & Baylor, 1999; Field & al., 2007; Crook & al., 2008, 2009). On the other hand, blue-cone inputs to colour-opponent neurones of the wallaby retina are delayed by 15 ms relative to M-cone inputs (Hemmi & al., 2002).

Pietersen & al. (2014) found that the visual latencies of blue-ON cells in marmoset LGN are 10–20 ms longer than those of parvocellular or magnocellular cells, however, another study from macaques (Tailby & al., 2008a) reported only trivial differences between blue-ON and parvocellular cells. Our results are broadly consistent with these results: we found visual latencies of blue-ON cells from the cat LGN lag behind those of achromatic cells by around 7–13 ms and the difference is on the margin of statistical significance. Based upon point (7) and (8), we can conclude that sluggishness of the S-cone derived signals may be a general, common characteristic of the mammalian vision.

Nevertheless, all of these similarities just make it probable that the W-system of cats and the koniocellular pathway of primates are homologues, since independent evolutionary pathways could also end up at similar functional structures due to advantages provided by colour vision (analogy). To confirm the former developmental path, we should examine the last common ancestor (*Boreoeutheria* taxon, diverged ~97 million years ago, Murphy & al., 2007), or – since they are extinct – some descendant diverged earlier in evolution.

5.3 The primordial colour system in cats

It is reasonably assumed that all mammals possess an ancient "primordial subsystem" of colour vision (Mollon, 1989; Jacobs, 1993), which in nonprimate mammals is based essentially on the same subcortical mechanism as the "blue–yellow" channel of trichromatic primates. This hypothesis is supported by studies of colour vision of New World monkeys, whose populations comprise trichromatic and dichromatic individuals (Mollon & al., 1984). Physiological and anatomical experiments have shown that the downstream circuitry is independent of the number of manifest cone types in these animals (Yeh & al., 1995b; Lee & al., 2000; Solomon, 2002; Blessing & al., 2004; Telkes & al., 2008; Martin & al., 2011), suggesting that the circuitry was left unchanged by the diversification of the ML-cone opsin gene into multiple spectral types. At

the same time, retinal part of this network – that is highly responsible for the receptive field properties of blue-ON cells – is largely unknown.

What is known from anatomical studies is that the cat retina features a distinct bipolar cell type with diffuse dendrites, called cb8 by Kolb & al. (1981) (also known as wb (Famiglietti, 1981), CBb5 (Pourcho & Goebel, 1987) or b5 (Cohen & Sterling, 1990)). These cells connect to only a small subset of cones within their dendritic field suggesting that cb8 bipolar cells could be specialized for the sparsely distributed S-cones (Linberg & al., 2001). A dedicated blue-cone bipolar cell type appears to be a general feature of mammalian retinas (*mouse:* Haverkamp & al., 2005; Li & DeVries, 2006; Puller & al., 2011; *rabbit:* Famiglietti, 2008; *cat:* Cohen & Sterling, 1990a,b; *primates:* Mariani, 1984; Kouyama & Marshak, 1992; Chan & al., 2001). In every known case, the putative blue cone bipolar cells arborise in the ON-sublamina of the internal plexiform layer, as do the cb8 bipolar cells of cats.

Inside the blue-cone pathway, level of the ganglion cells is best studied in monkeys as well (Dacey & Lee, 1994; Crook & al., 2009). The small bistratified ganglion cells (SBG) receive ON-sign excitation from blue-cone bipolar cells and OFF-sign excitation from long- and medium-wavelength selective diffuse bipolar cells (Ghosh & al., 1997; Calkins & al., 1998; Crook & al., 2009; Percival & al., 2009). In nonprimates, Yin & al. (2009) described a rarely encountered colour opponent ganglion cell type in guinea pig retina that has monostratified morphology quite different than that of small bistratified cells in monkey retina. It is known from electrophysiological studies that ‘colour-coded’ ganglion cells exist in the cat’s retina and it is straightforward to assume that they provide direct afferents to the blue-ON cells we have described in the LGN (Daw & Pearlman, 1970; Cleland & Levick, 1974; Cleland & al., 1976; Wilson & al., 1976; Saunders, 1977; Rowe & Cox, 1993), but morphological correlate of these retinal ganglion cells in cats is yet to be found, and is an important gap in our knowledge of this otherwise best-studied mammalian visual system. Although a similar circuit could be envisaged in the cat retina, some other models are equally plausible. The most parsimonious one derives from evidence that S-cones are spectrally opponent as a result of the longer-wavelength inhibitory input they receive from horizontal cells (Packer & al., 2010). The idea that cone-opponent responses are present in the S-cones is also compatible with our current physiological data from cats. In particular, the fine temporal balance of S- and ML-cone inputs could be best ensured if their subtraction occurred as early in the neuronal chain as possible. If on the other

hand different bipolar cells conveyed cone-specific signals to the opponent stage, their latencies and frequency transfer properties would have to be well-matched in order to avoid achromatic responses to the extent we find here.

Colour-coded neurones in cats are customarily attributed to the "third visual channel" represented by W-cells, comprising small-bodied neurones with slow conducting axons (Daw & Pearlman, 1970; Cleland & Levick, 1974; Cleland & al., 1976; Wilson & al., 1976). Although we did not classify our cells in the X, Y, and W categories, the laminar location of blue-ON cells in our sample corresponds to that of the W-cell population (Stone & Hoffmann, 1972; Fukuda & Stone, 1975; Cleland & al., 1976; Wilson & al., 1976). It is interesting that we recorded most blue-ON cells in the magnocellular C-lamina, which contains a lower proportion of W-cells (40%) than do the parvocellular laminae C1 and C2 (100%, Cleland & al., 1976; Wilson & al., 1976). This may be due either to the much greater thickness of lamina C or to some anatomical segregation among different subtypes of W-cells.

Little is known about the processing of chromatic opponent signals in the visual cortex of dichromatic nonprimates. It has been examined in rabbits (Polyanskii & al., 2006), squirrel (Van Hooser & Nelson, 2006) and tree shrew (Johnson & al., 2010), but we were short of data from cats. In general, blue-ON cells are best described as necessary but not sufficient to form a cortical representation of stimulus color because S-cone increments and ML-cone decrements result in equal changes in response of blue-ON cells. Based on the fact that other cells in the cat LGN had all or most of their input from ML-cones, the second dimension of the colour space of the cat brain must be an achromatic one fed by ML-cones, similar to the luminance channel of primates.

6. Summary

1. In addition to the 13 colour-opponent LGN neurones known from the literature, we have characterised 16 blue-ON cells using electrophysiological methods. We tested their response properties with several types of visual stimulus conditions and compared the results to the corresponding primate data and to the 40 achromatic cells that were also collected and characterised as controls. We found a number of parameters that allowed us to seperate the two cell types reliably. Such properties were the response phase, the elevation response and the cone weights of the inputs of neurones.

2. We showed that contrast response of the blue-ON cells is linear, similarly to the primate colour-opponent cells. This observation reinforces our idea that these LGN neurones play an important role in colour vision.

3. Measuring the spatial frequency tuning of the cells, we found the receptive field centre radii of the blue-ON cells – on average – 2.7 times larger than that of the achromatic cells. Cone inputs of the blue-ON cells were in perfect balance (S-cone weight: 0.50 ± 0.1), while in the achromatic cells only a weak S-cone input was detectable (0.10 ± 0.06).

4. We showed that receptive field surrounds of the blue-ON cells are mixed: their inhibitory (OFF) mechanism receives inputs from both cone types (S & ML). This raises the possibility that this mechanism is not selective, but functional cone weights depend on random wiring and the local distribution of the cone mosaic. Our simulation on real cone mosaics showed that the low S-cone density is not sufficient to build up an S-cone weight that we found with electrophysiology. We assume that S-cones compensate for their relatively low numbers with increased synaptic weight compared to that of the ML-cones.

5. In accordance with the primate data, temporal frequency optima of the colour-sensitive cells were much lower (3 Hz) than that of the achromatic cells (10 Hz).

6. We showed that temporal frequency tuning of the S-cone and ML-cone inputs of the blue-ON cells is very similar: optimum 3.44 ± 4.30 Hz and 3.31 ± 8.85 Hz, respectively (geometric mean ± SD, $p = 0.88$, Wilcoxon's signed-rank test). We confirmed that the two types of inputs arrive synchronously to the blue-ON cells of the LGN (S-cone latency: 70 ms, ML-cone latency: 69 ms), and that achromatic cells transmit the luminance signals faster (latency: 53 ms).

7. Colour-opponent neurones were found in layers C, C1 and C2 of the cat LGN, that matches the localisation of the W-cells.

7. Conclusion

Based upon our data, we can conclude that spatial and temporal properties of cat blue-ON LGN cell receptive fields are compatible with results from macaque blue-ON small bistratified ganglion cells in showing the S-ON-inputs are in spatial and temporal balance with the ML-OFF-inputs (Crook & al., 2009). In comparative context, these common features suggest that blue-ON receptive fields in cats and monkeys are adapted to detection of chromatic contrast. In ecological context, our results are consistent with proposed association of S-cone mediated signals with detection of illumination sources and shadows, to allow rapid navigation through terrestrial and arboreal environments (Chiao & al., 2000; Mollon, 2006; Conway, 2014).

8. List of abbreviations

CB – cone bipolar cell

DKL – Derrington–Krauskopf–Lennie colour space (Derrington & al., 1984)

DOG – Difference of Gaussians

HC – horizontal cell

IPL – inner plexiform layer

L – long wavelength sensitive

LCR – low-cut ratio

LGN – lateral geniculate nucleus

M – medium wavelength sensitive

ML – medium/long wavelength sensitive

S – short wavelength sensitive

SBG – small bistratified ganglion cell

SD – standard deviation

9. List of publications

9.1. Publications related to the thesis

Kóbor P., Petykó Z., Telkes I., Martin, P.R. és Buzás P. (2017) Temporal properties of colour opponent receptive fields in the cat lateral geniculate nucleus. *Eur J Neurosci,* 45:1368-1378. (2,941 impact factor)

Buzás P., **Kóbor P.**, Petykó Z., Telkes I., Martin, P.R. és Lénárd L. (2013) Receptive field properties of color opponent neurons in the cat lateral geniculate nucleus. *J Neurosci,* 33:1451-1461. (6,747 impact factor)

9.2. Other publications with impact factors

Takács I., Horváth S., Molnár A., Gáspár S., Hajós R., Meczker Á., **Kóbor P.**, Lantos J., Jávor S., Balatonyi B., Szekeres G., Róth E. és Wéber G. (2011) Comparative immunohistochemical study of tissue integration of macroporous and laminar meshes. *Histol Histopathol,* 26:821-830. (2,480 impact factor)

9.3. Citable abstracts, conference abstracts, presentations

Kóbor P.: Klonalitás vizsgálatok hypereosinophiliás szindrómában. *Tudományos Diákköri Konferencia,* Pécs, 2004. (előadás)

Kóbor P.: Kettős Philadelphia-kromoszóma jelenlétének és expressziójának összehasonlító vizsgálata interfázis cytogenetika és kvantitatív PCR segítségével CML-es betegekben. *Tudományos Diákköri Konferencia,* Pécs, 2006. (előadás)

Magyary I., **Kóbor P.** és Tóth A.: Effects of the psychotic drug dizocilpine maleate (MK-801) on locomotor activity and social behavior in zebrafish (Danio rerio). *5th European Zebrafish Genetics and Development Meeting,* Amsterdam, 2007. (poster)

Kóbor P.: Hiszto- és immunhisztotechnológia helye az őssejtkutatásban és a regeneratív medicinában. *Akkreditált immunhisztokémiai továbbképzés,* Pécs, 2007, 2008, 2009. (előadás)

Buzás P., Petykó Z., **Kóbor P.**, Telkes I. és Lénárd L. (2010) Blue-yellow chromatic opponent responses in the lateral geniculate nuclues of the cat. *Acta Physiol Hung,* 97:430-431. (poster ID: P039)

Kóbor P.: Színérzékeny sejtek receptív mezőinek tulajdonságai a macska talamuszában. *III. Nemzetközi, IX. Országos Interdiszciplináris Grastyán Konferencia,* Pécs, 2011. (előadás)

Kóbor P., Petykó Z., Telkes I., Lénárd L. és Buzás P.: Receptive field properties of colour cells in the cat lateral geniculate nucleus. *13th Conference of the Hungarian Neuroscience Society (MITT),* Budapest, 2011. (poster ID: P-5.2)

Kóbor P., Petykó Z. és Buzás P.: Receptive field properties of colour selective neurones in the cat lateral geniculate nucleus. *1st International Doctoral Workshop on Natural Sciences,* Pécs, 2012. (poster ID: O-13)

Kóbor P., Petykó Z., Telkes I., Lénárd L., Lukáts Á., Szél Á. és Buzás P.: Mixed cone inhibition in colour cells of the cat retina. *International Brain Research Organization Workshop,* Szeged, 2012. (poster ID: P5-15)

Kóbor P., Petykó Z., Papp L., Allston, M.A. és Buzás P.: Testing a novel 7-channel deep brain microelectrode for parallel single unit recording in the cat thalamus. *XIV. Conference of the Hungarian Neuroscience Society*, Budapest, 2013. (poster ID: P3.8)

Kóbor P., Petykó Z. és Buzás P.: Temporal frequency tuning of blue-ON cells in the lateral geniculate nucleus of the cat. *A Magyar Élettani, Farmakológiai és Mikrocirkulációs Társaságok 2013. évi közös tudományos kongresszusa,* Budapest, 2013. (előadás, absztrakt ID: A-0057)

Kóbor P., Petykó Z. és Buzás P.: Temporal frequency tuning of blue-ON cells is the lateral geniculate nucleus of the cat. *FENS Herie Winter School*, Obergurgl, Austria, 2013. (előadás)

Kóbor P., Petykó Z. és Buzás P.: Színopponens sejtek frekvencia hangoltsága macska corpus geniculatum laterale magjában. *XVIII. Magyar Látás Szimpózium,* Pécs, 2013. (előadás)

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Telkes I., Orbán J., **Kóbor P.**, Nyitrai M., Buzás P. és Völgyi B.: Connexin-36 distribution in the cat retina follows the general scheme of other mammalian species. *International Brain Research Organization Workshop,* Debrecen, 2014. (poster ID: P56)

Buzás P., **Kóbor P.** és Petykó Z.: A színlátás pályarendszerei. *Annual Congress of the Hungarian Ophthalmological Society,* Pécs, 2014. (előadás)

Telkes I., Orbán J., **Kóbor P.**, Nyitrai M., Völgyi B. és Buzás P.: Laminar and topographic distribution of connexin-36 and calretinin in the cat retina. *9th FENS Forum of Neuroscience,* Milan, Italy, 2014. (poster ID: 1636)

Kóbor P., Petykó Z. és Buzás P.: Temporal and chromatic properties of blue-on cells in the lateral geniculate nucleus of the cat. *9th FENS Forum of Neuroscience*, Milan, Italy, 2014. (poster ID: 2130)

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Radó J., **Kóbor P.**, Meczker Á., Török B., Jandó G. és Buzás P.: Measurement of visual contrast threshold and reaction time in awake behaving cats. *International Brain Research Organization Workshop,* Budapest, 2016. (poster ID: P1/96)

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Telkes I., Meczker Á., **Kóbor P.**, Szabó-Meleg E. és Buzás P.: Recoverin immunopositive bipolar cells of the cat retina. *Federation of European Neuroscience Societies Regional Meeting,* Pécs, 2017. (poster ID: P1-434)