General design principle for scalable neural circuits in a vertebrate retina

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Unlike mammals, fish continue to grow throughout their lives, to increase the size of their eyes and brain, and to add new neurons to both. As a result of visual system growth, the ability to detect small objects increases with the age and size of the fish. In addition to the birth of new retinal ganglion cells (RGCs), existing cells increase the size of their dendritic arbors with retinal growth. We have used this system to learn design principles a vertebrate retina uses to construct its neural circuits, and find that the size of RGC arbors changes with retina and eye size according to a power law with an exponent close to 1/2. This power law is expected if the retina uses a strategy that, independent of eye size, simultaneously optimizes both the accuracy with which each RGC represents light intensity and the image spatial resolution provided to the fish's brain.

ganglion cells | optimality

As fish grow and their eyes become larger, the area of the retina increases, new retinal ganglion cells (RGCs) are added, and the RGCs that are already present increase the size of their dendritic arbors (1–5). Because the same visual world is imaged on a larger retina containing more RGCs (6), the resolution of the visual system also increases with growth (7). The retina therefore presents a neural circuit with a scalable architecture (one in which performance is increased by simply making the circuit larger) and offers the possibility of studying the design principles underlying this scalability because fish retinas are available over a large range of sizes. Our goal is to discover design principles governing the size RGC dendritic arbors.

The size of RGC dendritic arbors determines two important functional properties of the eye. First, because of way the eye's imaging system and retinal circuits are organized, the retinal area covered by an RGC dendritic arbor determines what region of the image is sampled by the RGC and thus its "pixel" size; the smaller this pixel size, the greater the resolution of information provided to the brain about spatial properties of the image. Second, a dendritic arbor collects information about the light intensity in the RGC's patch of the visual world, and, because neural signals are noisy, the accuracy with which this intensity information is represented by the RGC depends on the extent of averaging and thus also on the pixel size: larger arbors average over a larger amount of the retinal area and thus provide the brain with more accurate intensity information. These two properties of the eye (spatial resolution and accuracy in the representation of intensity) therefore depend in opposite ways on the RGC dendritic arbor size and present the retina with a problem in how to optimize the information about the image passed on to the brain.

One can imagine three distinct design principles for scalable retinal circuits that RGCs could use to deal with these conflicting demands in retinas of different sizes. The first principle is for evolution to find a RGC dendritic size that represents light intensity with an acceptable accuracy (accuracy is measured here as the ganglion cell's signal-to-noise ratio for the light intensity information gathered by the arbor) and then to keep the area of RGC arbors and the accuracy of the cell's intensity estimate fixed

as the eye grows and the number of RGCs increases. Like increasing the number of megapixels in a camera, this strategy gives progressively better resolution as the eye becomes larger and spatial information about the image is provided by a larger number of RGCs.

The second design principle would have evolution discover an arbor size that provides sufficient resolution and then to have the RGC arbor grow in proportion to the area of the retina so that, by gathering more light signal information to average, the accuracy with which each RGC determines the light intensity for its patch of the visual world increases. For this situation, the resolution would not change with retinal size because the number of pixels covering the visual world would be constant.

Perhaps, however, evolution has selected a strategy that is a compromise between these first two cases. In this third case, both spatial resolution and accuracy with which intensity is represented by each RGC would increase with eye size in a scale-free way that keeps the ratio of resolution to accuracy at some constant, optimal value. Of course, it is possible that none of these three scale-free strategies is followed.

For the maximum spatial resolution situation (1st principle), the RGC dendritic arbor area a is independent of the retinal area A , and for maximum accuracy (2nd principle), the RGC dendritic arbor area a is proportional to A . When spatial resolution and accuracy (with accuracy measured as the signal-to-noise ratio of the RGC signal to the brain) are balanced (3rd principle), we show below that a is proportional to the square root of retina area A . Which of these design principles is followed, then, can be determined by measuring pixel size (the area of retina covered by individual RGC dendritic arbors) for different sized retinas. When the logarithm of pixel size is plotted against the logarithm of retina area, the three design principles make three different predictions for the slope of the plot: the slope is 0 for the 1st principle, 1 for the 2nd principle, and $\frac{1}{2}$ for the 3rd principle. For the teleost retina (we used goldfish and zebrafish), we find (see Fig. 3) that all or most RGCs follow the third design principle.

Results

Fig. 1 *A–C* show sections through three eyes from goldfish of different sizes. Measurements of the pupil (photographed while the fish was alive but anesthetized), the lens diameter, and the nodal distance (from the center of the lens to the retina) in eyes of different sizes confirm that large eyes are just scaled up versions of small eyes (1, 8). We measured the retina area by the surface of rotation determined from eyes of various sizes and found that, for 11 eyes whose retina areas ranged from 14.3 to

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Abbreviation: Dil, 1,1'-diocetadecyl-3,3',3'-tetramethylindocarbocyanine perchlorate; RGC, retinal ganglion cell.

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Fig. 1. Sections through eyes of large, medium, and small goldfish. (A) Section through the eye of a large goldfish (191-mm standard length) with lens diameter (shorter horizontal line), eye diameter, and nodal distance (vertical line) indicated. Pupil diameter is 4.55 mm and retina area is 120.6 mm². Scale bar applies to all three eyes. (B) Medium sized fish (80-mm length) with pupil diameter of 3.13 mm and retina area of 77.8 mm². (C) Small fish (46-mm length) with pupil diameter of 1.82 mm and retina area of 14.3 mm².

200.8 mm², the pupil diameter (measured in millimeters) squared is proportional to the retina area (Pearson's correlation coefficient = 0.99) with a proportionality constant 6.4; this relationship permitted us to estimate the retina areas for fish used to measure arbors areas. Fig. 2A is an example 1,1'-diiodo-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI)-stained RGC arbor, and Fig. 2B shows the Neurolucida reconstruction of this dendritic tree enclosed by the convex hull used to estimate arbor area. Approximately 12 different RGC types are recognized in the teleost retina, although the criteria used to differentiate between the various types cannot be applied automatically (5, 9). Ideally, the relationship between each cell type and the retina area would be determined separately, but this approach is not practical because the classification system for RGCs is not completely objective, some cell types are quite rare, and a prohibitively large number of RGCs would have to be reconstructed to identify all or many cell types in fish that range over an order of magnitude in eye sizes. Even within a single RGC type, the dendritic arbor size varies considerably within a single retina (5, 10, 11). We therefore started by finding the average RGC arbor areas as a function of retina sizes for fish of different sizes.

Fig. 3 presents, in a double logarithmic plot, the average arbor area, measured from 70 DiI-stained arbors from 26 fish (11 zebrafish and 15 goldfish), as a function of retina area (areas ranged from 4.21 to 44 mm²) associated with each average arbor size. The least squares fit to a power law relation between RGC arbor area and retina area in Fig. 3 (lighter solid line) gives an exponent of 0.62 ± 0.11 and this is not statistically different from the exponents of 0.5 or 0.625 predicted by the theory described in *Materials and Methods*. The best linear fit (on the double logarithmic plot) with a slope of $\frac{1}{2}$ in Fig. 3 (darker line) clearly provides a satisfactory description for the data, and is significantly different from a slope of 0 (best resolution principle) and a slope of 1 (best accuracy principle). We conclude that the 3rd design principle in which resolution and accuracy are balanced accounts for our data in Fig. 3 and that these data are inconsistent with the other two candidate design principles.

As noted above, the fish retina is known to possess a variety of different RGC types with different sized arbors (5, 9), and perhaps the relative abundance of RGC types differs systematically with eye size. This would mean that the average arbor size could depend on how many of which RGC types are present in a retina of a particular size. Or perhaps each cell type obeys a different scaling law relating arbor size to retina area so that the power law in Fig. 3 is an artifact of our averaging across cell types. To detect differences in relative abundance of RGC arbor sizes, we have compared the shape of arbor area distributions for large and small goldfish. Our sample contained 10 arbors from small

goldfish whose retina areas were $5.4 (\pm 0.8)$ mm² and 9 arbors from large goldfish whose retina areas were $43.2 (\pm 0.7)$ mm²; the mean RGC arbor area ranged from 4,509 (small fish) to 14,760 (large fish) μ m². The cumulative distribution of RGC areas for the small goldfish is plotted in Fig. 4 (solid fine line) and the distribution for the large fish is, after compressing the abscissa by 0.3, superimposed (dotted line); these distributions are not different at the 0.1 level of significance (Kolmogorov-Smirnov test). Thus the shape of the distribution of RGC arbor areas does not change detectably over the range of goldfish sizes we have studied.

If some RGC types followed a different scaling law (say, RGC area were independent of retina size or varied linearly with it), the shape of the arbor size distribution should change when the square root scaling law (see Fig. 3) is used to adjust all RGC areas to the mean of distribution for small fish (Eq. 5 in *Materials and Methods*). This adjusted RGC area distribution for all RGCs is shown by the thick line in Fig. 4 and is not different from the other distributions at the 0.1 level of significance (Kolmogorov-Smirnov test). We conclude that all or most of the RGC arbors represented in our sample follow approximately the same scaling law. Of course, we can make no statement about scaling of some possibly low abundance RGC types that are not well represented in our sample.

Discussion

We have found, then, that most or all RGC types in zebrafish and goldfish retinas appear scale according to the relationship

$$a = 0.002A^p$$

where a is the convex hull RGC dendritic area (mm²), A is the retina area (mm²), and p is either $\frac{1}{2}$ (heavy line in Fig. 3) or 0.625 (light line in Fig. 3), depending on which version of the theory is selected. This scaling law is expected if evolution has selected an RGC area for each RGC type for some standard size retina and then increased or decreased the arbor area so that the ratio of spatial resolution to the signal-to-noise ratio for that cell type is always constant as the eye size varies. That is, the fish retina can be described as following design principle 3 rather than 1 or 2.

In one calculation of the accuracy with which a particular RGC reported the light intensity in the patch of the world it covers, we supposed that the noise that determined the signal-to-noise ratio arises in the inner nuclear layer neural circuits that combine data from different cones. If the dominant source of noise arises from the cone signal itself, then (as we show in *Materials and Methods*) the relationship between a and A is $a \propto A^{0.625}$ rather than $a \propto A^{0.5}$. Both of these versions of the theory are consistent with the data because, as noted above, the least

Fig. 2. Example of an RGC arbor. (A) Typical Dil-stained RGC arbor. (B) Reconstruction of arbor shown in A. The convex polygon surrounding the arbor defines the area we assigned to the arbor.

squares fit to the data in Fig. 3 has an exponent of 0.62 ± 0.11 , which is not statistically different from either 0.5 or 0.625. We cannot, then, decide which noise source dominates the information supplied to the RGCs, but in either case, we can conclude that the design principle with balanced resolution and accuracy is in agreement with our observations.

We have defined spatial resolution above as proportional to $\sqrt{A/a}$, which would coincide with the usual definition of retinal resolution (12) if the coverage factor (the number of receptor arbors that cover a particular point on the retina) is constant. For the human retina, the coverage factor is indeed found to be constant for midget and parasol RGCs (13, 14), a situation believed to hold in general for the mammalian retina (15), and the coverage for one class of goldfish RGCs has also been reported not to vary with retina size (16). But if coverage were

Fig. 3. Double logarithmic plot of average RGC arbor area as a function of retina area. Each point shows the average area and standard error of ≈ 5 RGC arbors. The thin line is a least squares fit of a power law to the data; this fit gives an exponent of 0.62 ± 0.11 . The thick line is the fit to equation (4) to the data with a prefactor of 0.002.

not constant as retina area changed, our definition of spatial resolution would diverge from the standard one. We have identified resolution associated with a single RGC, but some central visual center might be able to extract better resolution than we calculate from a population of RGCs whose arbors overlap by varying amounts in retinas of different sizes. Because we compare spatial resolution with the single RGC signal-to-noise ratio, we must use our single-arbor definition of resolution to ensure that our measures of spatial resolution and accuracy are comparable.

One might expect that mammalian eyes would also follow at least some of the same design principles that fish use. Testing this notion is difficult, however, because many mammalian retinas use the strategy of systematically varying resolution with eccentricity, and it is not immediately clear what parameter to identify with the fish retina area.

If neural circuits outside the retina are to have a scalable architecture, principles underlying the design must be scale free; that is, design goals, such as best resolution, best accuracy, or a constant ρ/ν ratio, should apply to circuits at all scales. Here, we have provided evidence for the use of one such candidate principle in which the ratio of resolution and accuracy is constant

Fig. 4. Cumulative histograms of RGC dendritic arbor areas. The light solid line gives the distribution of arbor areas for 10 RGCs from small goldfish with a retina area of 5.4 ± 0.8 mm². The dotted line is the distribution of nine RGC arbor areas from large fish with retina areas of 43.2 ± 0.7 mm². To compare the shape of this histogram with that for the small fish, the abscissa has been compressed to 0.3 times the actual areas. The thick line is the arbor area distribution for 70 RGC arbors that have been adjusted according to Eq. 5 as described in *Materials and Methods* with $p = 1/2$ and $A_0 = 4$. None of the histograms are significantly different from the others at the 0.1 level (Kolmogorov–Smirnov test).

and therefore independent of the size of the circuit. Because the outputs of other neural circuits are based on local sampling of information distributed over some topographic map, and are scalable (the fish tectum is one example), we anticipate that the same sort of design principle could apply more widely.

Materials and Methods

We studied two teleost species, the zebrafish (*Danio rerio*), because of its common use in genetic studies, and the goldfish (*Carassius auratus*), because the structure, function, and development of its visual system have been extensively examined. Zebrafish were bred in the Salk Animal Facility, and goldfish were obtained from local pet stores. RGCs from retinas of 11 zebrafish, ranging in body length from 19 to 26 mm (standard length, measured from tip of nose to base of tail), and from 15 goldfish, ranging in body length from 25 to 146 mm, have been stained with DiI (Molecular Probes, Eugene, OR). After the fish was anesthetized by immersion in water containing 0.3% tricaine (Sigma-Aldrich, St. Louis, MO), the eye was removed and fixed in 4% paraformaldehyde in PBS (pH 7.4) (Roche Diagnostics, Indianapolis, IN) overnight. Small crystals of DiI were then inserted into the stump of the optic nerve and the eye was maintained in fixative for one week at 37°C. Retinas were whole-mounted in 1:1 glycerol/PBS and stacks of images of 70 well isolated RGC dendritic fields were obtained with a confocal microscope (LSM510; Carl Zeiss, Thornwood, NY; 25× NA0.8, 40× NA1.30, 63× NA1.25) and reconstructed with Neurolucida (Microbrightfield, Williston, VT). Because we find that the pupil diameter photographed in the anesthetized fish correlates well with the other dimensions of the eye, we used this measure as our size parameter; pupils ranged in size from 0.81 to 1.0 mm for zebrafish and from 1.14 to 3.93 mm for goldfish.

To measure the retina area, we used the frozen sections of freshly obtained eyes of various sizes and photographed the block face during sectioning (6). The retina area was estimated by finding the surface of revolution of the retina length exposed by a section through the optical axis of the eye. Parameters are specified as the expected value $\pm$ standard error.

Theory: Spatial Resolution. The first step in our derivation of the relationship between the RGC dendritic arbor area a and the area of the retina A is to find an equation that relates these two quantities. As the fish eye grows, the same visual world is imaged on a larger retina (6, 8). This means that the spatial resolution, determined by the Nyquist sampling theorem (17), is proportional to $\sqrt{N}$, where N is the number of pixels. We average RGC arbor areas for single retinas or average RGCs arbor areas across retinas of similar size to calculate the spatial resolution for the mean arbor area, our pixel size. The number of pixels (N) we use is found from the ratio A/a , where A is the retina area, and a is the average area of the RGC arbors in an eye with a retina of that area. Thus, the average spatial resolution ρ can be written

$$\rho = \sqrt{\frac{A}{a}}, \quad [1]$$

where we have, for convenience, chosen the proportionality constant units so that an equality results.

Theory: Signal-to-Noise Ratio. The second step in our derivation is to find a second equation that relates the area a covered by RGC dendritic arbors with the area of the entire retina A . The idea behind this second equation is to calculate the mean quantity of information about light intensity gathered by an RGC arbor and use this to estimate the signal-to-noise ratio. For a Poisson process, the variance σ^2 of a signal is proportional to the mean signal (18), and the average quantity of information about

luminance in the image gathered by a RGC sampling the output of the inner nuclear layer circuitry should be proportional to the mean RGC arbor area a , the area of an image (and the circuitry processing it) on the retina covered by the cell; here, we suppose that the RGC input noise is dominated by the neural circuits in the inner and outer plexiform layers rather than mostly arising from cones themselves. The Poisson assumption means that the noise standard deviation is $\sigma \propto \sqrt{a}$, because the average signal should be proportional to the area of the retina over which the RGC gathers information from inner nuclear layer circuitry about the image. The signal-to-noise ratio ν (which we term “accuracy”) is thus

$$\nu = \frac{a}{\sqrt{a}} = \sqrt{a}, \quad [2]$$

and again we have, for convenience, chosen the proportionality constant to give equality. Note that the product of spatial resolution and accuracy (signal-to-noise ratio) is

$$\rho\nu = \sqrt{A}. \quad [3]$$

Theory: Combining Resolution and Signal-to-Noise Ratios. Using these definitions and the last relation, we find that $a \propto 1$ for the first design principle (best resolution), $a \propto A$ for the second principle (best accuracy), and $\rho = \sqrt{A/a} \propto \nu = \sqrt{a}$, or

$$a \propto \sqrt{A} \quad [4]$$

for the third principle (balanced resolution and accuracy with the ratio ρ/ν constant).

For any particular retina, the areas of dendritic arbors usually vary considerably from one RGC to the next, but arbor areas also vary systematically with size of retina (5, 10, 11). Suppose that the arbor area a varies systematically with retina area A according to a power law $a(A) \propto A^p$ (p would equal 0, 1, and $1/2$ for design principles 1, 2, and 3), and further suppose all or most RGCs follow the same scaling rule. In this situation, it is possible to disentangle inherent arbor size variations within a single retina from the effect of retina size by using the relationship

$$a_0(A_0) = \left(\frac{A_0}{A}\right)^p a(A) \quad [5]$$

where $a_0(A_0)$ is the adjusted arbor area for a retina of standard area A_0 that would be predicted from an RGC arbor of area $a(A)$ in a retina of area A . This relationship was used to transform arbors from retinas of all sizes to the predicted size in a standard retina in Fig. 4.

Theory: Alternative Equation for the Signal-to-Noise Ratio. The calculation of accuracy ν above assumed that the dominant source of the noise arises from the neural circuits that process cone inputs, but one could also suppose that noise in the RGC signal is dominated by the cones themselves; this would be the case, for example, if the retinal networks added little noise and photon noise in cones is the major source of variability. If n is the number of cones covered by a RGC and σ_c is the cone-derived noise standard deviation, then $\sigma_c \propto \sqrt{n}$, and the cone-dominated accuracy ν_c is $\nu_c \propto n/\sqrt{n} = \sqrt{n}$. The cone density η is proportional to retinal area A to the $-1/4$ power (from figure 6a in ref. 1), so that the equation $\eta \propto A^{-1/4}$ holds. The number of cones covered by a RGC whose dendritic arbor area is a , then, is $n \propto \eta a \propto aA^{-1/4}$, and the cone-dominated noise gives an accuracy (signal-to-noise ratio) $\nu_c \propto \sqrt{aA^{-1/4}}$. The balanced resolution and accuracy principle ($\rho/\nu_c \propto 1$) holds that $\rho = \sqrt{A/a} \propto \nu_c \propto$

$\sqrt{aA^{-1/4}}$, which in turn means that the relationship between a and A is

$$a \propto A^{5/8} = A^{0.625} \quad [6]$$

for RGCs whose input noise is dominated by cones rather than

by the circuits processing cone input. Thus, we have two versions of the third strategy that predict an exponent of 4/8 or 5/8, depending whether the inner nuclear circuitry or the cone signal dominates the noise in the intensity signal from the cones.

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