Chapter 6

Animal Models of Amblyopia

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Introduction

Unquestionably, the last six decades of research on various animal models have advanced our understanding of the mechanisms that underlie the many complex characteristics of amblyopia as well as provided promising new avenues for treatment. While animal models in general have served an important purpose, there nonetheless remain questions regarding the efficacy of particular models considering differences across animal species, especially when the goal is to provide the foundations for human interventions. Our discussion of these issues culminated in three recommendations for future research to provide cohesion across animals models as well as a fourth recommendation for acceptance of a protocol for the minimum number of steps necessary for translation of results obtained on particular animal models to clinical trials. The three recommendations for future research arose from discussions of various issues including the specific results obtained from use of different animal models, the degree of similarity to the human visual system, the ability to generate animal models of the different types of human amblyopia as well as the difficulty of scaling developmental timelines between different species.

In considering animal models of all human diseases, including developmental disorders such as amblyopia, there is a concern as to whether experiential or other manipulations imposed in early postnatal life on animals having a normal genetic background can adequately mimic the human situation where there is a possible genetic contribution to the experiential abnormality. For example, in human amblyopia there is a potential genetic contribution to anisometropia, strabismus and media opacities that are the experiential abnormalities associated with the common subtypes of amblyopia and their presumed cause. Although research performed with animal models cannot at present mimic a possible genetic susceptibility for these amblyogenic factors in certain patients with amblyopia, researchers are aware that experiential manipulations of the early visual input alone in animal models may not precipitate the entire set of molecular, anatomical and physiological events that occur in all human patients with amblyopia.

The four specific recommendations we make emerged from a wide-ranging discussion of the value of the various commonly employed animal models for amblyopia from rodents to non-human primates (NHP). The obvious advantages of NHP (such as monkeys) that possess similarly organized visual pathways and vision to humans (such as a fovea, smooth pursuit eye movements, excellent spatial vision including stereoscopic vision, semi-decussated visual pathways and multiple visual cortical areas) are offset by many considerations that have motivated the choice of alternative models. Among the barriers to widespread use of NHP have been their long gestation time, small litter size, and the protracted length of key critical periods in visual system development. Added to these barriers are the attendant regulatory requirements and costs associated with establishment and maintenance of a primate breeding colony required to produce infant animals of known

ages. Discussion of the value of various animal models and models of strabismic amblyopia in particular, prompted debate among participants on the importance of a fovea or a region of central retinal specialization with respect to the ability of detection of strabismus or eccentric fixation. In passing it was noted that although carnivores such as cats do not possess a rod-free fovea they do have a central region of retinal specialization with a high cone density, the area centralis. The decline in resolution with eccentricity in cats has been documented by both behavioral techniques (Berkeley, Kitterle and Watkins, 1975; Pasternak and Horn, 1991) and from electrophysiological recordings of the spatial resolution of retinal ganglion cells (Clelan, Harding and Tulunay-Keesey, 1979). Both measurements reveal a regular decline from a central peak but with a more gradual slope than observed in humans. The four recommendations are discussed below in turn.

Recommendation 1. Documentation of the Perceptual Performance Space of Present and Putative Animal Models

Consensus on the behavioral and perceptual repertoire of each species will identify the most appropriate features of amblyopia to address with particular animal models. Furthermore, discovery of similarities in experimental results regarding the performance spaces of two or more species would promote efforts to establish the extent of consensus across results from use of these species as models of various forms of amblyopia.

Because of the complexity and diversity of amblyopia in humans, as well as species differences in the organization of the retina and central visual pathways, it is not surprising that certain animal models do not allow a perfect recapitulation of the clinical symptomology. Primate models exhibit remarkably similar behavioral deficits to those observed in the

various forms of human amblyopia so that their cortical correlates are of particular interest for an understanding of their underlying cause. By contrast, the ability to replicate certain forms of amblyopia such as strabismic or anisometropic amblyopia is limited or impossible in other species. Cats and particularly rodents show a declining similarity to humans in their manifestation of deprivation amblyopia. Deprivation amblyopia is the only form of amblyopia that can be modeled in all species, but the reduction in acuity upon monocular deprivation is dramatically different across species. While rodent models exhibit a single octave reduction in grating acuity, macaques remain effectively blind in the deprived eye after this manipulation (Harwerth, Smith, Boltz *et al.*, 1983). Kittens also appear blind immediately after monocular deprivation (MD) but show some recovery afterward (Giffin and Mitchell, 1978; Mitchell, 1988). Participants discussed but without reaching consensus, whether the discordant magnitude of the effects of MD reflected fundamental anatomical or physiological dissimilarities in the organization of visual pathways across species, or else arose from species differences in the plasticity of neural circuitry.

As an offset to considerations based solely on the inability for adequate modeling of all forms of amblyopia and their poor vision, rodents by far possess the highest throughput and flexibility of experimentation, which has allowed visual circuits to be probed down to the cellular and molecular level in ways that are unfeasible for other species and particularly NHP models. The power of these two contrasting arguments with respect to the use of rodents versus NHP suggests that it would be imprudent to discard any given species on the basis of an individual shortcoming but to use each animal model based on its strengths.

Discussion of the value of various animal models revealed substantial gaps in our knowledge of the perceptual performance of many species and especially rodents. The lack of knowledge of specific visual thresholds became evident in discussion of the role of particular anatomical or physiological features or the magnitude of the perceptual deficits on different measures of spatial resolution such as grating acuity, Snellen acuity and the various hyperacuities. Another example arose on contemplation of the role of binocular cells in the visual cortex of rats and mice. Although the recent observation (Schol, Burge and Priebe 2013) that cells in the binocular zone of the visual cortex of mice were tuned to retinal disparity, albeit in a crude fashion with respect to that observed in the cat, discussion of their possible functional role was hampered by the absence of a clear demonstration of stereoscopic vision in mice. A number of studies of depth perception have been made on rats that test either their ability to jump across a gap or their performance on a visual cliff (Howard, 2002). Because these studies did not compare monocular with binocular performance or control for the use of motion cues such as motion parallax, the possible contribution of stereoscopic vision was unclear. Nonetheless, a recent study (Baroncelli, Braschi and Maffei, 2013) that tested rats on a graded series of depth differences between the two sides of a visual cliff apparatus provided evidence of superior performance with use of both eyes suggestive of the presence of stereopsis. However, the possible use of motion parallax could not be ruled out. Innovative new tests of the depth perception of rats and mice that target the specific use of retinal disparity cues are needed to establish whether they possess true stereoscopic vision.

Table 6.1 lists the results of existing measurements of various visual thresholds, the methods of assessment as well as the presence or absence of particular key anatomical and

physiological features in the central visual pathways for the various common animal models for amblyopia. As with clinical screening for amblyopia in humans and to assess progress during and following treatment, assessment of visual performance in animal models is most commonly made in terms of the effects on visual acuity. The depth of amblyopia in humans is graded according to the specific acuity measure employed with the severity of the deficit increasing from grating acuity to letter acuity to the various hyperacuities such as vernier acuity. Grating acuity has been measured in all the commonly employed species including mice and spans a range of six octaves from 0.5 cycles per degree in mice (Prusky and Douglas, 2003) to 30 cycles per degree in macaques, or 100-fold with respect to humans. The vast discrepancies of grating acuity across species reflect fundamental differences in retinal anatomy that include the lack of a fovea in species other than primates, or variation in the extent of central retinal specialization with respect to cone density. Vernier acuity, which is likely a more accurate reflection of behavioral amblyopic deficits, has not yet been measured in mice, but has been measured in rats (Seymoure and Juraska, 1997).

Table 6. 1.

The perceptual performance space for the different species that are commonly employed as animal models of amblyopia and the assessment methods employed. Also shown are the presence or absence of various anatomical and physiological features in the central visual pathways.

Anatomical and physiological features

	Macaque	**Marmoset**	**Cat**	**Rat**	**Mouse**
Ocular dominance columns	+	±	+	-	-
Visual processing streams	+	+	+	?	+ [1]
Disparity selectivity	+	+	+	?	+ [2]

Physiological and anatomical MD effects	Severe shrinkage of OD columns, drastic OD shift	Appearance of deprived-eye columns, drastic OD shift [3]	Severe shrinkage of OD columns, drastic OD shift	Moderate OD shift	Moderate OD shift (reduction in OD index by ~0.3)
Cortical Suppression	+ [4, 5]	?	+ [6, 7]	+ [8]	?

Perceptual performance space

	Macaque	**Marmoset**	**Cat**	**Rat**	**Mouse**
Stereopsis	++	+	+	+? [9]	?
First-order (grating) acuity	30 c/deg	>10 c/deg	6 c/deg	1 c/deg [10]	0.5 c/deg [11]
Second-order (Vernier) acuity	10-12 sec arc [12]	?	1.2 min arc [13]	34 min [14]	?
Contrast sensitivity	~100 at 5 c/deg [15]	?	~100 at 0.3 c/deg [16]	~30 at 0.05 c/deg [17]	C57BL/6 mice 6%, [18]
MD effects on visual function	Fct blindness	?	Fct blindness	1 octave reduction in acuity	1 octave reduction in acuity

Assessment methods

	Macaque	**Marmoset**	**Cat**	**Rat**	**Mouse**
Single- or multi-unit recording	+	+	+	+	+
VEP / EEG recording *	+	+	+	+	+
Intrinsic signal imaging	+	+	+	+	+
Two-photon imaging	(+)	+	+	+	++
Neuro-anatomical markers (IEG	+	(+)	+	+	+

expression)					
Genetic modification	-	+ [19]	-	+	++
Behavioural tests of acuity	+	+	+	+	+

* Note that this is the only technique that is also widely used in humans

References for specific data entered in Table 6.1 are as follows: 1. Wang *et al.*, 2012; 2. Scholl *et al.*, 2013; 3. Sengpiel *et al.*, 1996 ; 4. Smith *et al.*, 1997; 5. Sengpiel and Blakemore, 1996; 6. Chino *et al.*, 1994; 7. Sengpiel *et al.*, 1994; 8. Pietrasanta *et al.*, 2014; 9. Baroncelli *et al.*, 2013; 10. Prusky *et al.*, 2000; 11. Prusky *et al.*, 2003; 12. Kiorpes, 1992; 13. Murphy and Mitchell, 1999; 14.Seymoure and Juraska, 1997; 15. De Valois *et al.*, 1974; 16. Bisti and Maffei, 1974; 17. Birch and Jacobs, 1979; 18. Yeritsyan *et al.*, 2012; 19. Sasaki *et al.*, 2009.

Not only is acuity substantially lower in typically reared rodents compared to primates, but the reduction in acuity upon monocular deprivation is dramatically different across species. While rodent models exhibit a single octave reduction in grating acuity, macaques remain effectively blind in the deprived eye after this manipulation (Harwerth, Smith, Boltz *et al.*, 1983). Kittens also appear blind immediately after MD but show some recovery afterward (Giffin and Mitchell, 1978; Mitchell, 1988). Participants discussed but without reaching consensus, whether the discordant magnitude of the effects of MD reflected fundamental anatomical or physiological dissimilarities in the organization of visual pathways across species, or else arose from species differences in the plasticity of neural circuitry.

In connection with the discussion of the effects of MD some participants questioned whether this manipulation was the most appropriate way to model deprivation amblyopia as a way to mimic the development of cataracts. And, as debated in other Targeted Sessions, amblyopia is at its root a binocular condition so that the ability to probe the status of binocular vision across different species including tests of stereopsis and suppression is important. While mice exhibit neural correlates of disparity selectivity in visual cortex (Scholl,

Burge and Priebe, 2013), it is not known whether they possess stereopsis and if so whether it is affected by experiential manipulations such as MD.

Recommendation 2. Comparative measurements of key perceptual abilities across species by use of the same or very similar techniques.

While the expansion of technologies in physiology, imaging, and molecular biology has allowed neural circuits to be probed at unprecedented resolution, many of these techniques are not equally applicable across species and especially not to humans. Mice in particular offer high throughput molecular characterization of visual circuits and changes to them during development and in response to experiential manipulation. On the other hand, NHP such as macaques easily outperform rodents on visual tasks that accurately reflect human amblyopic deficits. Comparison of the perceptual abilities on various visual tasks has been made difficult by differences in the techniques employed across species. Rather than an exclusive focus on identification of the techniques best suited for each individual species, participants thought it would be valuable to propose a common technique that could be deployed with ease across species including humans. Discussion focused quickly upon the use of electrophysiological measures and particularly the use of various types of visual evoked potentials (VEP) and, in particular, upon the steady-state VEP, or the SSVEP (Norcia, Applebaum, Ales *et al.*, 2015). A VEP corresponds to electrical changes in large populations of neurons in the cortex, and can be recorded from the surface of the brain non-invasively using electroencephalography (EEG). High-density EEG recordings can be used to improve source localization of this common technique and are applicable across species, including humans. By use of a fan of electrode contacts placed strategically over the

scalp it is possible to obtain a high yield analysis of visual responses across different visual areas. A particular form of SSVEP, the sweep VEP that measures the changes in response to a stimulus that is swept (varied) over a range of values is used widely to measure various visual thresholds. For assessment of acuity or contrast sensitivity the SSVEP is measured in response to parametric sweeps of gratings of different spatial frequency or contrast. The method is fast and has been used on human infants and on various species including NHP (Nakayama & Mackeben, 1982) and rodents under light anesthesia (Guire, Lickey and Gordon, 1999; Xu, Tian, Zhang *et al.*, 2015).

On the basis of this discussion, participants proposed that use of the SSVEP would be the most efficient way to assess visual thresholds across species and in the various animal models of amblyopia. In addition to measurement of key visual thresholds across species by use of the SSVEP, the suggestion was made that certain methods of non-invasive imaging could also be used to assess the functional integrity of the visual cortex and possibly other visual areas in the various animal models of amblyopia.

Recommendation 3. Advocacy for the use of marmosets as an animal model of amblyopia.

Despite the value of rodents and carnivores such as cats as animal models of amblyopia, there are issues for which there is arguably no alternative to the use of NHPs. In addition to the need for their use to refine the optimum timing and the dosage for projected interventions in human patients with amblyopia, as a species with a highly developed fovea, NHP may provide the only valid choice to model strabismic amblyopia. The presence of a similar organization of higher visual cortical areas to that observed in humans and which are

likewise specialized for processing complex visual stimuli, attracts use of non-human primates for study of the underlying neural basis for the deficits of various higher visual functions observed in amblyopia. Macaque monkeys are easily trained to make very repeatable behavioral observations and the documented perceptual deficits associated with early amblyogenic manipulations are remarkably similar to those demonstrated by human patients with amblyopia.

In addition to the case that can be made for the use of NHP to model human amblyopia, it is very important to recognize their participation in translation of the results from animal studies to clinical trials. These issues are raised in the discussion of Recommendation 4 that follows.

Most of the earliest studies of the functional anatomy and physiology of the visual cortex and its development, including investigations of the consequences of early periods of selected visual deprivation for anatomy, physiology and behavior, were conducted on macaque monkeys. As such, there exists a large body of data documenting the close similarity of spatial vision, oculomotor characteristics as well as organization of higher visual cortical areas between macaques and humans, which makes the choice of the former the ideal primate animal model. Unfortunately, there are considerable practical barriers to their use. Macaques are expensive to purchase and to house. There are regulatory barriers as well as vociferous resistance to their use from the public and media expressing growing ethical concerns. Many of these issues are exacerbated upon consideration of their use as animal models of amblyopia where experimental manipulations must be made in infancy. The long gestation (5.5 months), the unitary litter size, the length of critical period of vulnerability to

monocular deprivation (> 1 yr) together with the need for experimental interventions near birth, in most cases requires the existence of an on-site breeding colony. The recent closure of the New England Primate Center and the continual pressures from diverse sources directed against remaining macaque colonies (in North America as well as in Europe) indicate that the ability to initiate a new macaque breeding colony would be close to impossible. As many of the same barriers to use of macaque monkeys apply to familiar New World monkey species, participants at the Targeted Session Group discussed the potential use of marmosets as a primate model of amblyopia. This discussion benefited from the insight provided by Dr. Sam Solomon (University College, London) who has conducted a number of investigations of the central visual pathways of marmosets in recent years in collaboration with Dr. Marcello Rosa and others at Monash University (Melbourne, Australia).

Detailed information relevant to the breeding and housing of marmosets is provided in a multi-center review (Tardiff, Smucny, Abbott *et al.*, 2003). The arguments for their use in visual neuroscience (Solomon and Rosa, 2014; Mitchell and Leopold, 2015) and in particular as a model for amblyopia are worth serious consideration. Marmosets possess a fovea with a peak cone density of 200,000 cones/mm^2 similar to that observed in macaque monkeys and humans. While the volume of the marmoset brain is approximately 12 times smaller than that of macaques, the cerebral cortex is relatively smooth so that the vast majority of the visual cortex lies exposed and not buried in sulci. Importantly V2, as well as the third tier visual also lie exposed on the cerebral surface. Important as these features of the marmoset cortex are for investigations of the neural basis for vision, they are more than matched by the short gestation time, large litter size and the comparative ease of housing. The fact that

they can be housed socially in groups of 5 or 6 reduces the costs of colonization. In a multi-colony database of 3,714 marmosets, the litter size ranged from 1-4, with twins as the most common but more than a third were triplets or quadruplets (see Table 2 from Tardiff, Smucny, Abbott *et al.*, 2003). The gestation time of 143 days is shorter than that for macaques (164 days) but longer than that for cats (63 days). Although data as yet is somewhat conflicting, ocular dominance columns in V1 of marmosets, like other New World monkeys, may be transitory or even variable across animals (Roe, Fritsches and Pettigrew, 2005) but have been shown to exhibit experience-dependent change in response to MD (Sengpiel, Troilo, Kind *et al.*, 1996; Fonta, Chappert and Imbert, 2000) or enucleation (Ribic, Flugg, Schlumbohm *et al.*, 2011).

A major additional argument for the use of marmosets beyond the development of a new non-human primate model for amblyopia is the potential for genetic modification (Sasaki, Suemizu, Shimada *et al.*, 2009). As summarized in a recent *Nature News* item, our Recommendation is reinforced by the launch of a Brain/MINDS project in Japan to study cognition and cognitive disorders in marmoset models (Cyranoski, 2014). Higher visual functions including contrast sensitivity or vernier acuity have not yet been experimentally investigated in the marmoset, but a physiological substrate for stereopsis has been documented (Table 6.1). Our recommendation for the increased use of marmosets as a NHP model for amblyopia should not be taken to mean that they replace completely the well-established macaque model as the latter holds a number of advantages over marmosets for application of particular techniques. For example, the larger body size of macaques allows for both much longer daily behavioral measurements as well as longer awake

behavioral recording experiments. Moreover, their longevity makes them invaluable for extended behavioral studies that also serve in part to mitigate against their high initial cost.

Recommendation 4. A balanced suggestion for replication of a laboratory finding made on one animal model to a "higher" species as a necessary step to a clinical trial: a "two-species rule."

For the most part, the use of animal models in the study of amblyopia should have a clear goal of applying the findings of such studies eventually to clinical practice. In some cases, this would mean the development of a therapy, either behavioral of pharmacological, that could be tested in clinical trials. It is therefore prudent to understand how to most efficiently and safely transition between the world of animal models and human patients.

No species matches the efficiency (both cost and time) or the genetic toolbox available to probe the visual system for new approaches to treatments as the mouse. Unfortunately, the relative simplicity of their visual system is the least comparable to humans, although recent studies have found underappreciated complexities in the mouse visual pathway (e.g. Scholl, Burge and Priebe, 2013) as well as sophisticated perceptual abilities such as the perception of motion coherence (Douglas, Neve, Quittenbaum *et al.*, 2006). They also exhibit qualitative differences in visual plasticity compared to other models such as cats and primates. For example, mice living in environmentally enriched conditions display ocular dominance plasticity well beyond the classical critical period (Greifzu, Pielecka-Fortuna, Kalogeraki *et al.*, 2014), a finding not replicated in other mammals. Mice also exhibit a form of plasticity in adulthood that is not observed in cats or NHP (Satwell, Frenkel, Philpot *et al.*, 2003). In addition, strategies shown to be successful in one species may be less so in another. For

example, reverse occlusion but not binocular visual exposure is quite effective in promoting recovery when combined with various approaches to enhance plasticity in rats (He, Ray, Dennis *et al.*, 2007), while cats benefit from binocular visual experience (Mitchell, Cynader and Movshon, 1977; Mitchell, 1988; Mitchell, Gingras and Kind 2001). Dark exposure is effective in promoting recovery from long-term monocular deprivation in adult rats (He, Ray, Dennis *et al.*, 2007), but is only effective in juvenile cats (Duffy and Mitchell, 2013; Holman, Duffy and Mitchell, 2014; Duffy, Lingley, Holman *et al.*, 2016). Varied responses across species are also observed in response to pharmacological treatments. For example, enzymatic digestion of perineuronal nets with chondroitinase restores ocular dominance plasticity in adult rats (Pizzorusso, Medini, Landi *et al.*, 2006) but has much less of a beneficial effect in cats (Vorobyov, Kwok, Fawcett *et al.*, 2013); Fluoxetine promoted recovery from MD in rats (Maya-Vetencourt, Sale, Viegi *et al.*, 2008) but according to a Press Release (http://www.evaluategroup.com/Universal/View.aspx?type=Story&id=453937) failed to show an enhancement of results above that achieved by video training in a recent Phase II human clinical trial.

As researchers we are torn between two extreme viewpoints with respect to the steps to a clinical trial. At one extreme is the view that a result from a single species provides sufficient evidence to mount a Proof of Principle clinical trial. Of course, there are variations of this viewpoint depending on the specific species employed; results obtained from NHP would likely meet with wider acceptance than data from other species. The opposing and more conservative viewpoint is that it is necessary for confirmation of the initial result in at least one other species, with a NHP serving as the second species in the extreme interpretation of such a "two-species" rule.

The strongest argument for the acceptance of results from a single species as sufficient to mount a clinical trial is to accelerate the time between laboratory discovery and a clinical trial. However, such hasty action may jeopardize the clinical trial because of failure to optimize the treatment parameters, the patient population or the timing of the intervention. Because a negative finding on a clinical trial subtracts from the ability to conduct trials in the future it is important that the urge to "fast-track" a laboratory finding to the clinic be tempered by a careful evaluation of the various parameters of the intervention. Failure to optimize the treatment parameters such as dosage on a clinical trial has the potential to jeopardize conduct of future trials of a different and potentially very effective dosage of the same treatment. Moreover, a negative clinical trial based on results from a single species also diminishes the ability in the future to argue for the relevance of that particular species as a viable model of amblyopia. It is also important to recognize that failure of a clinical trial provides ammunition for activist groups opposed to the use of all animal models. As it was perceived that treatment parameters on a clinical trial based upon results obtained from species other than NHP's would need to be tweaked by data obtained from a second species, participants vacillated between various versions of the alternative viewpoint.

The viewpoint that discoveries from rodent or cat animal models be replicated first on NHP's prior to a clinical trial inevitably introduces delay and reduces the number of possible treatments that could be explored. The suggestion of a two species "rule" with the caveat that two rodent species would probably not qualify, was considered a useful practical compromise as it increased substantially the ability to test numerous treatments. Upon progression to a cat model of a potential treatment based on findings in a rodent, a negative

finding would make it unnecessary to proceed to tests on a NHP. An understanding of the efficacy and timing of manipulations in at least two species can elucidate how likely these treatments are to translate to humans. This consideration is made pointedly in consideration of pharmacological interventions in children. Because of the risk of off-target effects of pharmacological treatments, especially when prescribed to children with other brain regions likely undergoing sequential critical periods, special care should be taken to understand the efficacy of these drugs across species prior to clinical trials, not only for safety, but also to ensure translation of the optimum timing and dosage from animal models to humans. An example of a "two-species" examination of the use of binocular retinal silencing with intra-vitreal injection of tetrodotoxin (TTX) as a replacement for darkness to treat the consequences of a prior early period of monocular deprivation has recently been published (Fong, Mitchell, Duffy *et al.*, 2016). The study was conducted in three different laboratories on two species (mouse and cat) and reported very similar outcomes and thus replication of the results in both species.

Fuelling the debate on this Recommendation were considerations of two main issues that related to the problem of translation of dosage and timing of interventions from animals to humans. The difficulty of testing dosage of treatments was emphasized by the recent report of the somewhat ambivalent preliminary findings of a clinical trial on the use of a re-purposed drug, the SSRI Citalopram, that was reported at the March 2016 meeting by Ben Thompson. The ability to employ a FDA-approved drug has many benefits, not the least being the ability to fast-track a clinical trial. On the other hand, because the prior approved use of the drug was for administration to adults, the dosage applied to children or young

adults in the amblyopia trial was deliberately conservative. This raised the issue of how to interpret the results of the trial, since it is possible that the drug was ineffective at the dosage applied. It also pointed to the many difficulties associated with the establishment of dosage levels. With respect to translation of dosage levels from adults to children guidance may be sought from the calculations made for the effective dosage levels of drugs, such as cancer drugs, that are commonly used on humans from children to adults where multiple considerations including weight, allometric and metabolic measures are applied.

A related concern is the issue of how to translate developmental and susceptibility timelines across species to humans so that the timing of treatment would be optimal. During this discussion it was pointed out that, for example, the common 4:1 multiple applied to translate developmental timelines from macaques to humans applies only to resolution acuity and does not apply to relative eye growth where the ratio is 3:1. Development of an equivalent ratio between species for stereoacuity is hampered by the existence of too many isolated studies of monkeys on small numbers and the use of very different stereo measures. For example, only a single study exists (O'Dell and Boothe, 1997) of the time of emergence of stereopsis in macaques. Extrapolation of the developmental timeline of the cat to humans is complicated by the delay in eye opening as well as the slow disappearance of the hyaloid artery around the crystalline lens in cats.

Conclusions

In contrast to the comparative sparsity of animal models of many other neurological clinical disorders, study of the basis of amblyopia and pursuit of new avenues for its treatment are guided by a rich variety of animal models that employ a common set of experiential

manipulations on divergent species. Our Recommendations for the application of Animal Models focused on the use of a monocular deprivation as the most common experiential manipulation, that has been employed widely with graded success across species from rodents to NHP's. The use of a common manipulation has aided comparison of the results across species but also permitted identification of gaps in our knowledge of the perceptual abilities of certain species including rodents and marmosets.

- Two of our Recommendations (1 & 2) suggest particular gaps in knowledge that need to be filled and also common methods of assessment that could be applied across species as an aid to comparison of perceptual performance. Sometimes assumptions are made about the perceptual abilities of a particular species without hard evidence. A case in point is the assumption that rodents do not possess stereopsis in the face of a lack of tangible evidence of their ability to employ stereoscopic vision. Tangible data on this issue would assist discussion of the role of binocular neurons in the rodent visual cortex. On the basis of the demonstration of large independent eye movements in freely moving rats, it has been suggested (Wallace, Greenberg, Sawinski *et al.*, 2013) that the main purpose of binocular neurons may be to ensure a large panoramic visual field above them to escape predation from raptors.

- Our third Recommendation for increased use of marmosets as a NHP species was suggested as a means to speed the path to clinical trials in situations where information from NHP's was deemed essential.

- Our final Recommendation could be deemed a principle or strategy to guide the path from results obtained from an animal model to a clinical trial. For translation to a clinical trial, participants advocated the principle of a "two-species replication" with the suggestion that the two species not both be rodents. Participants thought that a wider adoption of marmosets as models of amblyopia may lead eventually to the principle that one of the two species be a NHP.

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