Research report

Variation in visual acuity within pigmented, and between pigmented and albino rat strains

Glen T. Prusky\textsuperscript{a,}\textsuperscript{*}, K. Troy Harker\textsuperscript{a}, Robert M. Douglas\textsuperscript{b}, Ian Q. Whishaw\textsuperscript{a}

\textsuperscript{a} Department of Psychology and Neuroscience, Canadian Centre for Behavioural Neuroscience, University of Lethbridge, 4401 University Drive, Lethbridge, AB, Canada T1K 3M4

\textsuperscript{b} Department of Ophthalmology, University of British Columbia, 2530 Willow Street, Vancouver, BC, Canada V5Z 3N9

Received 16 February 2002; received in revised form 3 May 2002; accepted 3 May 2002

Abstract

Many researchers assume that laboratory rats have poor vision, and accordingly, that they need not consider differences in the visual function of rats as a consequence of strain or experience. Currently, it is not specifically known whether rat domestication has negatively affected the visual function of laboratory rat strains, what the effects of strain albinism are on rat visual function, or whether there are strain differences in the visual function of laboratory rats that are independent of pigmentation. In order to address these questions, we measured psychophysically the vertical grating acuity of three pigmented (Dark Agouti, Fisher–Norway, Long-Evans) and three albino (Fisher-344, Sprague–Dawley, Wistar) strains of laboratory rats, and compared their acuity with that of wild rats. The grating thresholds of Dark Agouti, Long-Evans and wild strains clustered around 1.0 cycle/degree (c/d) and did not significantly differ from one another. Fisher–Norway rats, however, had a significantly higher threshold of 1.5 c/d. The grating thresholds of Fisher-344, Sprague–Dawley, and Wistar strains, which were clustered around 0.5 c/d, were significantly lower than those of the pigmented strains. These data demonstrate that there is significant strain variability in the visual function of laboratory rats. Domestication of Long-Evans and Dark Agouti strains does not appear to have compromised visual acuity, but in the case of Fisher–Norway rats, selective breeding may have enhanced their acuity. Strain selection associated with albinism, however, appears to have consistently impaired visual acuity. Therefore, a consideration of strain differences in visual function should accompany the selection of a rat model for behavioral tasks that involve vision, or when comparing visuo-behavioral measurements across rat strains.

\textsuperscript{*} Corresponding author. Tel.: +1-403-329-5161; fax: +1-403-394-2775

E-mail address: prusky@uleth.ca (G.T. Prusky).

Keywords: Visual acuity; Albino rat; Pigmented rat; Rat visual perception; Visual–spatial behavior; Visual cortex; Retina; Visual discrimination; Morris water maze

1. Introduction

The Norway rat (Rattus norvegicus) was the first animal species in which the primary motive for domestication was to develop an animal model for scientific research. Since its domestication, the laboratory rat has become one of the major animal models of bio-medical and behavioral research. Numerous inbred strains and outbred stocks have been created and maintained in an effort to control genetic variation within experiments; however, the selection pressures associated with rat domestication have resulted in strains with obvious visual abnormalities, such as albinism. Nonetheless, different strains of laboratory rat, including albinos, are regularly used and compared in vision-mediated behavioral tasks, such as the Morris water task [16], the Olton radial arm maze [30] and the Barnes maze [3], without specific knowledge of their visual capacity.

Our lab [18] and others [7,13,22,23] have reported that the adult grating acuity of normal Long-Evans strain rats is around 1.0 cycle/degree (c/d). We have also reported that visual deprivation in rats during a physiologically-defined critical period early in life [26] results in significantly reduced visual acuity [19]. This suggests that mature visual function in the rat, like that
of other mammals [6], is dependent on the nature of visual experience during development. Blind rats [27], rats with significant photoreceptor loss [17], and rats with reduced visual acuity [20] are all impaired at place learning in the Morris water task, indicating that visual deficits in rats can compromise the interpretation of data derived from behavioral tasks that are dependent on visual function.

It is likely that strain differences in visual function, independent of age or developmental visual experience, can also affect the performance of rats in visual-behavioral tasks, and thereby compromise the ability of researchers to make cross strain comparisons in behavioral function. At least one study has reported that pigmented rats are superior to albino rats at place learning in the Morris water maze [28]. This difference may be due, in part, to the reduced visual acuity associated with albinism [5]; however, no systematic study has quantified and compared the visual-behavioral function of pigmented and albino rats. Also, it is not known whether there are significant disparities in visual function, independent of pigmentation, between rat strains of, or whether rat domestication has had a detrimental effect on rat vision.

In order to address these issues, we measured the visual function of several pigmented and albino strains of laboratory rats, most of which are commonly used in behavioral neuroscience, and compared their vision with that of wild rats. We chose to use visual acuity as a measure of visual function because it can best assess the quality of visual information that is available to rats when they are engaged in behaviors that are dependent upon visual stimuli. Sinusoidal grating acuity was measured specifically because the receptive fields of neurons in primary visual cortex are spatial frequency and orientation tuned, and a sinusoid presents a single spatial frequency. In addition, any complex visual stimulus can be decomposed into component sine waves and the mammalian visual system displays many of the functional features of Fourier analysis [15].

2. Materials and methods

2.1. Animals

Seven strains (Dark Agouti, Fisher 344, Fisher–Norway, Long-Evans, Sprague–Dawley, wild, Wistar) of male laboratory rats were used in these experiments. Table 1 outlines defining characteristics of each strain. Long-Evans rats were bred from stock originally obtained from Charles River, raised at the University of Lethbridge vivarium, and behaviorally tested there as adults. All other animals were acquired from commercial suppliers or university laboratories as adults, and maintained and tested at the University of Lethbridge vivarium. Animals were housed in hanging wire cages (26 cm L × 67 cm W × 14 cm H) in groups of 3–4 in a
room with an ambient temperature of 21 °C, 35% relative humidity, 12/12 light/dark cycle, and where food and water were available ad libitum.

2.2. Testing apparatus and procedure

Visual acuity was assessed psychophysically using the Visual Water Task [18]: a two-alternative, forced-choice visual discrimination task in which the threshold of animals to discriminate a sine wave grating from gray of the same mean luminance was assessed.

2.3. Visual water box

A trapezoidal-shaped (140 cm L × 80 cm W × 25 cm W) tank (55 cm H) made of Plexiglas was positioned on a solid table and tap water (22 °C) was added to the tank to a depth of 15 cm. The 80 cm end wall of the tank was transparent, but the inside of the remaining walls were painted flat black to reduce reflected light within the pool. A midline divider (40 cm H) was placed in the tank that extended 46 cm into the pool from the transparent wall, creating a maze with a stem and two arms. A transparent, moveable Plexiglas platform (37 cm L × 13 cm W × 14 cm H) was submersed in the pool at the end of one of the arms. (Fig. 1A and B). Screen light reflecting off the surface of the water rendered the platform invisible from water level.

2.4. Computer hardware and software

Two computer monitors (17” VGA: Viewsonic E70F) were positioned side-by-side outside the clear end of the tank, each facing into one of the two arms of the maze (Fig. 1A and B). The bottoms of the screens were situated at water level (Fig. 1B). The black level and contrast settings of the monitors were equated and the mean luminance of the visual stimuli measured from the end of the divider was 43 cd/m². The monitors were controlled by PCI video cards (ATI Rage Orion 128) operated over video extension cables by an Apple Macintosh computer (PowerPC G4; 400 MHz). The gamma response was measured for each monitor and used to linearize the output to the screens. A computer program (Vista ©; http://www.cerebralmechanics.com) was used to generate visual stimuli on the monitors. High contrast sine wave gratings were drawn on the video screen on the positive side above the platform. For the other screen, a homogeneous gray stimulus was generated in the same way except that contrast was set to zero. The spatial frequencies displayed on the screens were restricted to those with full sine wave cycles to ensure there were no differences in the mean luminance of the monitors. Vista © also controlled the left/right randomization pattern of the stimuli, recorded behavioral responses with the aid of a remote control box, provided control of parameters for individual animals while still testing animals within a group, calculated the visual angle of the sine wave gratings, and plotted the data.

2.5. Procedure

Rats are instinctive swimmers and the Visual Water Task capitalizes on their natural inclination to escape from water to a solid substrate, the location of which is directly paired with a salient visual stimulus. Animals are first pre-trained to distinguish a low spatial frequency grating from gray with high reliability before the limit of that ability is tested at higher spatial frequencies. The end of the divider within the pool sets a choice point for the animals that is as close as they can get to the visual stimuli without entering one of the two arms (Fig. 1A)- the length of the divider, therefore, sets the effective spatial frequency of the sine wave gratings for the animals.

In the pre-training phase of the experiment, a low spatial frequency, vertical sine wave grating (0.12 c/d; 4 sine wave cycles on the computer screen) was displayed on one monitor (+ stimulus; Fig. 1A and B) and uniform gray of the same mean luminance was displayed on the other (— stimulus; Fig. 1A and B). The platform was located directly below the monitor displaying the sine wave grating, regardless of its left/right location. Rats were shaped to associate swimming to the platform with escape from the water. On their first trial, animals were removed from their holding cage and released into the pool facing the screen a few centimeters from the platform. Upon being released, animals usually swam directly forward, touched the platform, and then climbed upon it. They were allowed to remain on the platform for a few seconds and were then returned to their holding cages. On the next trial, the location of the grating and platform was switched to the opposite arm of the pool and another trial was completed. After this routine was repeated a few times, the release distance from the platform was increased gradually until the animals could swim reliably to the platform from a release point (Fig. 1A) at the end of the pool opposite the monitors.

In the training phase of the experiment, animals were shaped to distinguish the same sine wave grating used in pre-training, from gray, with high reliability. The alternating pattern of the grating/platform location was substituted with a Left (L), Right (R) LLRLRR sequence [9]. We have determined previously (unpublished observation) that a LRLLRLRR sequence commencing each day from a different position in the sequence, cannot be memorized by the rats. Using this sequence, however, mitigates side biases in animal’s responses when a stimulus is displayed three or more times on the same side in succession. On all trials,
animals were required to swim until they located the platform. If animals swam to the platform without entering the arm that displayed gray, the trial was considered correct (Fig. 2A). If they swam into the side of the tank with the monitor displaying gray, the trial was scored as incorrect, and the animal is forced to swim until it finds the platform. The task rewards animals that take a direct swim path to the monitor displaying the sine wave grating, and negatively reinforces animals for choosing the gray stimulus by prolonging the trial.

A method-of-limits procedure was used in the testing phase of the experiment in which incremental changes in the spatial frequency of the stimulus were made within blocks of trials until the accuracy of animals to distinguish the grating from gray fell below 70%. A LRLLRLRR trial-by-trial schedule was used to determine which monitor displayed the sine wave grating. Throughout testing, animals were released from the end of the pool opposite the monitors (Fig. 1A) and allowed to swim until they found the platform. If animals made a correct choice, one sine wave cycle was added to the grating (0.03 c/d) on the next trial. This procedure was used through the low spatial frequencies, thereby minimizing the number of trials far away from threshold. If an error occurred, a criterion test was initiated in which additional trials were run at the same spatial frequency until four correct responses were made in a block of 10 trials. After trials covering approximately 1/2 of the animal’s projected range to threshold were completed, the minimum number of trials at a spatial frequency was increased to three, and then again increased to four around 3/4 of the projected range. The same criterion testing as described for the low spatial frequencies was applied at the higher frequencies. A preliminary grating threshold was established when animals failed to achieve 70% accuracy at a spatial frequency. In order to determine the validity of this estimate, the spatial frequency of the grating was reduced by 3–4 cycles (0.9–1.2 c/d), and the experimental procedures described above were repeated a number of times until a stable pattern of performance was established. This method of sequential testing made use of 30 different spatial frequencies between 0.12 and 1.0 c/d. Performance at each spatial frequency was averaged and a frequency-of-seeing curve was constructed for each animal. The point at which the curve intersected 70% accuracy was recorded as the grating acuity. Each strain was trained and tested as a group in sessions of 10–15 interleaved trials (1st rat, 2nd rat, . . . last rat, 1st rat, etc.) with each session lasting 45–60 min. No more than three sessions, separated by at least an hour, were performed in a single day.

2.6. Data analysis

The effects of pigmentation and strain on grating acuity were statistically examined with Univariate Analysis of Variance and Bonferroni post-hoc comparisons. Post-hoc mean differences were considered significant at the P = 0.05 level.

2.7. Image filtering

Images were processed to provide an impression of the relative differences in the visual perception of rat strains in this study. A two-dimensional FFT (Image Processing Toolkit, Reindeer Games, Inc.) of the 1024 x 1024 pixel original was performed and all spatial frequencies beyond the different acuity limits were set to zero. To reduce ringing in the images from such a sharp frequency cut, the drop-off in contrast sensitivity close to the acuity limit was also modeled. Starting at a frequency 20% of the acuity, the contrast was decreased linearly from 100 to 22% at the acuity limit. These values are derived from the figures of Keller et al [13], but are somewhat more conservative. The low frequency roll-offs, and the overall lower contrast sensitivity of the rat versus the human that they report were not incorporated.

3. Results

3.1. Training and testing

Each strain learned quickly to associate swimming to the platform with escape from the water in the pre-
training phase. The testing criterion in the training phase was reached for all animals within four sessions. During testing, all animals maintained near flawless performance for many spatial frequencies before the accuracy of their choices fell below 70% correct. The spatial frequencies around those at which performance fell below 70% correct were then retested a number of times. Animals of all strains learned serendipitously during training to swim to the end of the divider, grasp it with their paws, and then look at each screen several times before making their choice. The amount of time spent inspecting the screens generally increased near the spatial frequencies at which their accuracy fell below 70%, probably reflecting an increase in the difficulty of discriminating between the stimuli. Average performance above 70% accuracy was maintained over many spatial frequencies for all animals, and then it declined rapidly around threshold.

3.2. Acuity of pigmented rat strains

The visual acuity of the four-pigmented strains of rats is illustrated in Fig. 3. The acuity of Dark Agouti animals was 1.00 c/d (S.E.M. = 0.038, N = 4), Fisher–Norway was 1.558 c/d (S.E.M. = 0.053; N = 6), Long-Evans was 1.03 c/d (S.E.M. = 0.018, N = 23), and wild was 1.05 (S.E.M. = 0.025, N = 6). There was a significant effect of pigmented strain on visual acuity ($F = 57.545$; $P = 0.0001$). Post-hoc analysis revealed no significant differences in the acuity of Dark Agouti, Long-Evans and wild strains (Dark Agouti Vs Long-Evans Sig. = 0.506; Dark Agouti Vs wild Sig. = 0.402; Long-Evans Vs wild Sig. = 0.692). In contrast, the acuity of the Fisher–Norway strain was significantly higher than other pigmented strains (Fisher–Norway Vs Dark Agouti Sig. = 0.0001; Fisher–Norway Vs Long-Evans Sig. = 0.0001; Fisher–Norway Vs wild Sig. = 0.0001) or their average of 1.033 c/d ($F = 178.03$, $P = 0.0001$).

3.3. Acuity of albino rat strains

The visual acuity of the three albino strains of rats tested in this study is illustrated in Fig. 4. Fisher-344 rats had an average acuity of 0.54 c/d (S.E.M. = 0.011, N = 6), Sprague–Dawley rats averaged 0.528 c/d (S.E.M. =
344, \( N = 6 \), and Wistar rats averaged an acuity of 0.538 c/d (S.E.M. = 0.032, \( N = 4 \)). There was no significant effect of strain on albino visual acuity (\( F = 0.100, P = 0.905 \)).

3.4. Comparison of pigmented and albino rat acuity

Pigmentation was a significant factor in the acuity of rats in this study. The average acuity of pigmented strains (including Fisher–Norway, 1.113 c/d; excluding Fisher–Norway, 1.033 c/d) was significantly higher than albino strains (0.536 c/d) regardless of whether Fisher–Norway thresholds were used (included, \( F = 115.653, P = 0.0001 \); excluded, \( F = 501.607, P = 0.0001 \)) to calculate the pigmented average (Fig. 5).

4. Discussion

The rat strains in this study clustered into three categories on the basis of their acuity: (1) Three pigmented strains with acuity in the range of 1.0 c/d. 2) The Fisher–Norway strain with an acuity of 1.5 c/d. (3) Three albino strains with acuity near 0.5 c/d.

4.1. Pigmented rat strains with acuity near 1.0 c/d

Dark Agouti, Long-Evans and wild rat strains were assessed with grating thresholds near 1.0 c/d; values that are comparable with those we have previously reported for Long-Evans rats [18–20]. One of the goals of the present study was to assess whether rat domestication, and the subsequent creation of distinct laboratory strains, has had a detrimental effect on the function of the visual system. One way to investigate this question would be to compare the acuity of various strains of laboratory rats with wild-caught rats. This, however, was not possible in the present study due to a variety of logistical and animal husbandry-related issues. Instead, we measured the acuity of laboratory-housed wild rats that were recently descended (25 generations) from a wild-caught population of rats [24]. If the acuity of the wild strain is representative of truly wild rats, our data indicate that the selection of Dark Agouti and Long-Evans strains has not resulted in animals with abnormal visual systems. Our study also found no evidence that inbreeding, independent of pigmentation, necessarily affects visual function. Inbred strains, distinguished by brother/sister matings for at least 25 successive generations, do not contain normal genetic variation because they are homozygous at all genetic loci, and therefore, could be homozygous for deleterious recessives that would rarely be homozygous in a wild population. Consequently, inbreeding could result in animals homozygous for recessive genes that negatively affect visual system function. Since the visual acuity of the Dark Agouti inbred strain did not differ from wild and Long-Evans outbred strains, our data do not support this possibility for Dark Agouti rats.

4.2. Acuity of the Fisher–Norway rat strain

Our study found that the Fisher–Norway strain had a significantly higher acuity than wild rats, as well as other pigmented and albino strains tested: their grating threshold was approximately 50% higher than other pigmented strains and 150% higher than the albino strains. There was no overlap in the threshold of any Fisher–Norway animal with any other animal in the study, and there was little variability in acuity within the Fisher–Norway strain, indicating that enhanced acuity is a strain trait. There were no obvious differences between the Fisher–Norway and other strains in the number of trials to learn the task, or in the number of errors during threshold testing. They did appear to be slightly calmer and more purposeful than other strains when performing in the Visual Acuity Task, but it is unlikely that this can account for their superior acuity.

A more likely explanation is that a genetic difference accounts for their enhanced acuity. The Fisher–Norway rats are a F1 cross between an inbred Fisher-344 female and an inbred Norway–Brown male. Although we did not measure the acuity of the Norway–Brown strain in this study, we did measure the acuity of Fisher-344 animals and found their acuity to be about 0.5 c/d. It is possible that the Norway–Brown strain possesses acuity higher than other pigmented strains in our study, and that the Fisher–Norway animals owe their high acuity to genes present in the Norway–Brown genome. The superior visual acuity of the Fisher–Norway strain also raises the possibility that the acuity of 1.0 c/d we measured for our wild animals is lower than that of native Rattus norvegicus, or that there is substantial heterogeneity in the visual acuity of wild rats.

It is also possible that the enhanced visual acuity of Fisher–Norway animals is not the result of a direct genetic inheritance from Norway–Brown rats. Fisher-344 and Norway–Brown strains may carry alleles that are deleterious for high-resolution vision within the strains, but the unique combination of genes in Fisher-344/Norway–Brown heterozygotes results in alleles that are beneficial to visual acuity. This may produce animals with enlarged eyes, smaller receptive fields in the visual cortex, or other structural changes that could lead to higher resolution vision. We are currently investigating these possibilities. The superior visual acuity and calm demeanor of the Fisher–Norway strain also makes them attractive as a model for studying the mechanisms of visual perception in nocturnal rodents.
4.3 Albino rat strains with acuity near 0.5 c/d

The three albino strains in the present study, Fisher-344, Sprague-Dawley and Wistar, constituted the group of strains with the lowest visual acuity; albino strains did not differ significantly in their acuity but possessed approximately half the visual acuity of the pigmented strains. The scale of the albino acuity deficit can be further illustrated by the facts that there was no overlap in individual acuity measurements of albino and pigmented animals, and the acuity of albino rats is effectively the same as that of a mouse [18], an animal with substantially smaller eyes.

Albinism produces a number of structural abnormalities in the visual system, including neuro-retinal abnormalities resulting from un-pigmented retinal pigment epithelium [12], abnormal decussation of retinal ganglion cell axons at the optic chiasm [14], and abnormal interhemispheric connections of the visual cortex [2]. The most parsimonious explanations for reduced visual acuity in albino rats are that excessive light scattering within the retina [1] make the albino eye a rather poor image forming device, and light-induced retinal degeneration [4] results in poor spatial sampling. It is possible that deficient central visual processing also plays a role in the acuity deficits of albino rats. The absence of melanin or the melanin-related agent [21] responsible for anomalous axonal decussation at the optic chiasm in albinos [11] may also produce errors of interhemispheric connectivity of the visual cortex [2] and result in anomalous visual cortical processing. We have tested the visual acuity of pigmented acallosal mice [29], however, and found that their acuity of about 0.5 c/d was not significantly lower than that of control mice (unpublished observations). In addition, lesions of striate cortex [8], and monocular deprivation in Long-Evans rats throughout early life [19], reduce visual acuity by just 30%, not the 50% deficit that characterizes the albino strains in this study. It is possible that the anomalies caused by albinism produce a form of long-term deprivation for the visual system, which results in experience dependent abnormalities in both central and retinal visual processing, and this may produce a massive deprivation-induced deficit in acuity. A recent study lends support to this possibility: Long-term monocular deprivation in rats can lead to a deficit in acuity greater than that caused by removal of the visual cortex [25], suggesting that deprivation-induced subcor-
tical visual system abnormalities may also be contributing factors to an acuity deficit.

4.4. Strain variation in rat visual perception

The results of this study demonstrate that there is significant strain variability in the visual acuity of rats. Rats are commonly used as subjects in visual-behavioral tasks that measure cognitive functions, and in studies of visual perception and plasticity. Given the low visual acuity of all the rat strains in this study, relative to humans, one might conclude that the differences in acuity between rat strains we are reporting are of no real significance. However, we have recently found [20] that a reduction in acuity of as little as 30% can affect the place learning of Long-Evans rats in the Morris water task, one of the most widely used rodent tasks in behavioral neuroscience. Many of the strain differences in acuity we report here are greater than 30%, and therefore, might be expected to affect measures of behavioral function in tasks that rely on vision.

In order to illustrate how differences in visual acuity could affect visually-guided behavior, Fig. 6 presents a picture as it might appear to the different strains of rats in our study. The original image (top-left) has been filtered to mimic the effects of acuities of 1.5 c/d (top-right; Fisher–Norway), 1.0 c/d (bottom-left; Dark Agouti, Long-Evans, wild) and 0.5 c/d (bottom-right; Fisher-344, Sprague–Dawley, Wistar) when the image subtends 10 degrees. This approximates the size of the image if it were used as a visual cue in a typical visuo-behavioral task. See text for details.

Fig. 7. Visual perception of rat strains in visual-based behavioral tasks. The original image (top-left) has been blurred to model the perception of rats with acuities of 1.5 c/d (top-right; Fisher–Norway), 1.0 c/d (bottom-left; Dark Agouti, Long-Evans, wild) and 0.5 c/d (bottom-right; Fisher-344, Sprague–Dawley, Wistar) when the image subtends 10 degrees. This approximates the size of the image if it were used as a visual cue in a typical visuo-behavioral task. See text for details.
rodent visuo-behavioral tasks. In addition, researchers should consider differences in the visual information available to rats with different acuities before comparing their performance in behavioral tasks that rely on vision.

4.5. Rat strain differences in place- and matching-to-place learning

A recent study has shed some light on the implications of variation in rat visual acuity for performance in the Morris water task [10]. The results of this study indicate that visual acuity alone does not predict performance on place, or matching-to-place versions of the Morris water task. For example, in spite of having superior (Fisher–Norway) or equal visual acuity (Dark Agouti) relative to the Long-Evans strain, both of these strains are impaired relative to the Long-Evans strain. Even among albino strains, whose visual acuity does not differ in our study, Harker and Whishaw [10] report that there are significant strain differences in performance. These data do not rule out the possibility that visual acuity can influence rat behavioral performance in vision-dependent tasks, because all of the albino strains with poor visual acuity (Sprague–Dawley, Wistar, Fisher-344) in Harker and Whishaw’s [10] study were impaired relative to the Long-Evans (pigmented) strain. Therefore, it is likely that strain rates vary in a number of brain functions, including visual function, and this variation can contribute to differences in performance on complex behavioral tasks.

Acknowledgements

The authors thank Dr J.M. Koolhaas for generously donating wild animals for use in this study. In addition, the authors thank Yelena Arjannikova for her expertise in training and testing many of the animals, and Tatianna Arjannikova and Doug Wallace for helping with data analysis. Supported by research grants from the Natural Sciences and Engineering Research Council of Canada and the Canadian Stroke Network to G.T.P. and I.Q.W.

References