DIFFERENCES IN BEHAVIOUR AND IN FORELIMB CORTICAL NEURONS OF TWO RAT STRAINS FOLLOWING REACH-TRAINING

BY

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Abstract

The brain undergoes structural changes in response to new experiences like learning a new skill. Skilled motor movements depend greatly on the primary motor cortex for their execution. Recent studies describe rat strain differences in motor performance related to differential synaptic efficacy in the motor cortex of rats. Previous studies identified differences in motor performance related to differential dendritic morphology and strain related differences in synaptic function in the motor cortex. Strain differences are one way of investigating anatomical organization and behaviour of the motor system. The object of this research was to examine strain-related differences in dendritic morphology in layer II / III pyramidal cells of the forelimb area of the sensory motor cortex in both Long-Evans and Fischer 344 rats after reach-training. This research also examined whether changes in reaching behaviour could be attributed to changes in dendritic morphology. Rats were trained once a day for 30 days to reach for a food pellet through a slot in a reaching box. Pyramidal cells in the motor sensory forelimb (MSF) cortex were stained with the Golgi-Cox method. Subsequent analysis of Sholl and branch order data of cell drawings determined that there were no significant differences in any measure of dendritic length or dendritic length at branch order 3, 4, 5 of pyramidal cells in layer II/III of the MSF cortex between the Long-Evans and Fischer 344 rat strain. The only significant strain-related difference was that the Fischer 344 strain exhibited fewer reaches for each food pellet obtained, demonstrating greater reaching proficiency than similarly trained Long-Evans rats. These findings suggest that further research examining strain comparisons is required to understand the neural mechanisms underlying the differences in motor behaviour observed in these rat strains.

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Introduction

Enriched Environments and Plasticity

The brain has the natural ability to change and reorganize in response to environmental experiences. Coleman & Buell (1985) suggested that factors responsible for the regulation of dendritic growth in the developing brain may also mediate changes in aging dendrites as they respond to their local environment. It is reasonable to assume that factors that appear to promote the proliferation of cell processes or cell turnover in early development would be suitable for the regulation of dendritic growth in adult cells.

Hebb (1947) performed one of the earliest studies addressing how learning and environmental experiences can change the brain. He studied the behaviour of rats exposed to an enriched environment in an experiment contrasting a group of rats blinded at birth with another group of rats blinded as adults. Hebb examined the role of early experience in perceptual development and suggested that rats blinded at birth would be deprived of the sensory events from the environment that are necessary for normal development. Rats blinded at birth would not have encountered sensory events that produce synaptic modifications that lead to specific motor facilitation in sighted rats. The experiment had two conditions. The first condition employed a rote memory task: both groups of rats performed a task that did not require any training to produce results, the second condition consisted of a complex learning task that required a great deal of training to produce results. Rats blinded as adults performed significantly better in the learning condition than the rats blinded at birth. These results demonstrate the brain's ability to respond to learning and

environmental experiences. Hebb's later research focused on examining how environmental experience could change the brain and subsequently change behaviour.

Experiments on animals housed in a complex environment have been used to demonstrate that the sensory system is able to adapt and consequently change the structure of the brain. The manipulation of sensory input in the environment can produce changes in higher-order dendritic branching and promote the development of more synapses per neuron as is evident in research done on the effects of environmental enrichment on the neural morphology of the visual cortex of the outbred Long-Evans (LE) rat. The LE rat (*Rattus norvegicus*) is a cross between Wistar females and a wild Norway rat and is commonly used in studies examining motor behaviour. Volkmar & Greenough (1972) were among the first researchers to investigate how dendritic fields were altered as a result of an enriched environment. They suggested that manipulating afferent signals would increase neuronal size and strengthen existing excitatory and inhibitory synaptic connections in an experience-dependent manner. Volkmar & Greenough (1972) compared animals housed in an enriched environment to those housed in standard laboratory cages. Animals assigned to the enriched environment (also referred to as a complex environment) condition were housed in a large cage filled with a number of different toys to play with, allowing both object play and extensive social interaction. The toys were changed daily and the animals in the enriched condition were allowed access to another room with different toys for one hour each day. Rats housed in standard laboratory cages were deprived of this environmental stimulation. At the end of the experiment the animals were sacrificed and the researchers discovered a thicker occipital cortex in the animals in the enriched condition. The results indicated that higher order dendritic branching in the visual cortex increased in direct

proportion to environmental complexity. Bennett, Rosenzweig, & Diamond, (1969) also found that rats raised in an enriched environment had greater cortical weight than rats raised in standard laboratory cages. The results indicated that the brain was able to adapt to the enriched environment by changing its structure. These changes in structure provide evidence of plasticity in the brain and suggest that increased neuronal firing in response to environmental stimulation in learning a task promotes proliferation of dendritic processes and therefore an organizational change in the brain.

Motor Learning and Plasticity

The enriched environment paradigm exposes rats to different opportunities to experience new things. The subsequent changes in the brain encompass many different areas as evidenced by increased cortical weights. A more focused environmental manipulation would provide a method that would make it possible for researchers to detect changes in brain structure related to changes in behaviour. Learning a skilled reaching task provides a focused behaviour that would allow researchers the opportunity to examine the area of the brain underlying the performance of the behaviour.

Dendritic communication and cortical structure play an important role in determining the connectivity patterns within the brain. The sensory and motor cortex of the rat both consist of six layers or lamina. In both cortices, layers I to IV are mainly input or afferent layers. The motor cortex has a smaller layer IV than does the sensory cortex. Layer V and VI in the motor cortex are mainly output or efferent layers that convey, in some instances, the final output to the spinal cord and motoneurones (Kolb & Whishaw, 1985). Layer V cells, also known as cortical motoneurones, are large output neurons that receive

input along descending fibers from Layer II/III cells. Most of the inter-cellular communication between cortical layers is from above or below. This vertical structure can be examined in animals by placing a recording electrode in the somatosensory cortex between layer I and layer VI and observing the activity in cells when a distal stimulus is presented (Kolb & Whishaw, 1985). Kolb & Whishaw (1985) described how peripheral stimulation is first signalled by the activation of interneurons in layer IV of the sensory cortex, which receive direct afferent input. These interneurons in layer IV then project vertically to synapse on other interneurons or efferent pyramidal cells in layer V of the motor cortex. Layer V cells are activated later because the signal must travel through interneurons in layer IV before reaching the cortical motoneurones in layer V. Mapping experiments using peripheral stimulation while recording in the somatosensory cortex examined the importance of afferent input in modulating cortical reorganization. Peripheral nerve stimulation of the digits on the rat forepaw evoked action potentials that were recorded from an electrode placed in the somatosensory cortex (Li, Waters, McCandlish, & Johnson, 1996). This procedure produced a map of digit representations that are organized into bands running medial to lateral with a specific site responsive to each glabrous digit tip. By stimulating the tip of the third digit in the anesthetised rat, Li et al. (1996) demonstrated that there was an expanded representation of the stimulated digit in the forepaw area in the somatosensory cortex. Increased stimulation produced larger digit representations. The results of these experiments suggest that this series of projections, starting at the periphery and ending in the somatosensory cortex, play a role in the organization of representations in the somatosensory cortex. A study examining sensory deprivation of the cockroach cercal system in deafferented and control subjects indicates

that the axonal terminals of sensory afferent neurons mostly affect development of high-order (i.e. more distal) dendritic segments, with little effect on the lower-order (i.e. more proximal) dendritic segments (Mizrahi & Libersat, 2002).

The reorganization of the sensorimotor cortex can be characterized by area expansion and / or by increases in dendritic branching as described above. Greenough, Larson, & Withers, (1985) demonstrated that learning a skilled-reaching task was responsible for altering neuronal morphology in the contralateral hemisphere of the LE rat. Specifically, changes occurred in layer V pyramidal cells in trained hemispheres as a result of learning a skilled reaching task. Greenough et al. (1985) used a skilled reaching paradigm in which LE rats were trained to reach through a tube for pieces of cookie. The clear Plexiglas reaching cage had a moveable wall that was positioned along the midline of the cage close to the tube. The wall could be positioned in such a way as to allow reaching with only one forepaw. The rats were allowed to practice reaching for the cookie pieces, allowing researchers to determine paw preference. Rats were divided into four groups: one group was trained using the forepaw opposite their preferred forepaw (REV), control (CONT) rats did no reaching but were placed in a cage with no tube and allowed to eat the cookie pieces off the floor of the cage, a third group of rats was trained with the preferred forepaw (PRAC), and the final group was trained with both forepaws on alternating (ALT) days. In this experiment, reach training took place over 16 days, after which the rats were sacrificed and the brains were removed and stained using the Golgi-Cox method. The stained brains were sectioned, removing a 7 mm thick coronal slice containing the motor sensory forelimb (MSF) cortex defined as including both the somatosensory and the somatomotor cortex (Donoghue & Wise, 1982). These sections, each 150 microns thick,

were fixed and mounted on slides. Layer V pyramidal cells were analysed because they receive information from layer II/III cells and their axons terminate in the forelimb area of the rat spinal cord. Only cells that were within the middle 100 microns of the 150 micron sections were selected; cells that were within 25 microns of the top or bottom of the section were rejected because their dendrites were likely to have been truncated during sectioning. Cells were drawn using the camera lucida system and the cells were then traced using a 3-dimensional computer-assisted dendritic tracking system.

Greenough et al. (1985) reported that the rats in the REV group did not reverse their original forepaw preference. All rats in the PRAC, and most of the rats in the ALT group, maintained their forepaw preference. These results suggest that forepaw reversal was not permanent but specific to the reach training. For dendritic branching analysis and to determine the effects of training, all trained hemispheres were grouped together, including both ALT hemispheres and contralateral hemispheres from REV and PRAC rats. These were compared to both CONT hemispheres and ipsilateral hemispheres from both REV and PRAC rats to look for a main effect of reach training on dendritic morphology. Results indicated that neurons from trained hemispheres had significantly larger apical dendritic fields than untrained hemispheres, with two apparent trends. Trained hemispheres had a greater number of branches at all orders other than 3rd order branches. In addition, terminal branches were longer in trained hemispheres. With-in and between-subjects comparisons were made by comparing trained and untrained hemispheres in the REV and PRAC rats and comparing the combined hemispheres of the ALT rats with combined hemispheres from the CONT rats. Again, these comparisons indicated that there was a significant main effect of training on branching in the contralateral hemispheres. Lastly, the untrained hemispheres of

the REV and PRAC rats were compared with both hemispheres of the CONT rats. This last comparison was done to determine whether there were any effects of training that were not specific to the trained hemisphere. Results revealed significant effects of training in that untrained hemispheres in the REV and PRAC rats had greater dendritic length than untrained hemispheres in the CONT rats. Taken together, the results from Greenough et al. (1985) provide evidence that reach training increases the size of apical dendritic fields of Layer V pyramidal cells in the MSF. The enlarged cortical fields suggest increased representations in the MSF cortex consistent with cortical reorganization.

Greenough et al. (1985) examined layer V pyramidal cells in the MSF because these cells represent the final output of the spinal tract to the forelimbs. It follows that examining cells above layer V cells might reveal cortical reorganization in cells projecting to the layer V cells. Withers & Greenough, (1989) reported that reach training produced changes in the subpopulation of forked-shafted apical dendrites of layer II/III cells in the MSF cortex which project to the layer V pyramidal cells examined by Greenough et al. (1985). When between-hemisphere differences were examined, forked-shafted apical cells in both hemispheres of CONT rats and ALT rats were compared, and both the ALT rat hemispheres had significantly more ring intersections in the Sholl analysis (Sholl, 1956) and therefore greater dendritic length. The changed forked-shafted apical cells of layer II/III may be the location where sensory information was incorporated in the cells that then projected to layer V cells which terminated on or near spinal motoneurones. Withers and Greenough, (1989) also reported that although the number of neurons did not increase, the size of the neurons and surrounding tissue did, and this suggests that enhanced dendritic structure would reflect greater opportunities for synaptic transmission.

As Greenough et al. (1985) and Withers & Greenough (1989) had done, Kleim, Barbay, & Nudo, (1998) used a skilled reaching paradigm to examine morphological changes within the cortex of the LE rat. They measured cortical reorganization by examining dendritic branching which may support rats learning a skilled motor task. In electrophysiological mapping experiments, Kleim et al. (1998) concluded that the trained or contralateral hemisphere of the motor cortex exhibited an increase in wrist and digit representations in the caudal forelimb area compared with the same area in the untrained hemisphere. This is consistent with the hypothesis proposed by Greenough et al. (1985) that changes in layer V cells in trained hemispheres would reflect improvement in skilled reaching. Karni et al. (1998) provided evidence that in humans morphological changes in the cortex underlie changes in behaviour. Karni et al. (1998) reported that increased performance of a skilled task over time corresponded to functional MRI images indicating increased activity and more extensive representations of the training experience in the motor cortex. A study mapping forelimb movement representations found that although rats typically reach the asymptote of behavioural performance after seven days of training, the motor maps show reorganization only after ten days of training (Kleim et al. 2001). Kleim et al. (2004) also investigated the timing of acquisition of motor skills in rats.

They further examined the different patterns of activity in the motor system supporting different phases in the acquisition of motor skills. They used standard intracortical microelectrode stimulation to produce high-resolution maps of the motor cortex. In their experiment, the skilled reaching paradigm was used to train the rats. Kleim et al. (2004) proposed that gathering information about the experience was characterized by a phasic pattern of changes in the caudal forelimb area of the motor cortex; an early phase

showing rapid improvement in reach success, and a late, slow phase that showed moderate gains in performance based on changes in cortical maps. They noted a significant expansion of distal movement representations only after day 10 of training. Therefore, the changes occurred during the slow late phase. These results demonstrate a non-linear pattern of brain plasticity in which initial gains in performance were not reflected in an increase in synapse formation or reorganization of motor maps until the late phase of training. Perhaps the early phase of acquisition is supported by generating a great deal of activity in existing synapses as suggested by Karni et al. (1998).

Communication within the nervous system is accomplished mainly at the synapse. Modifications of their structure or an increase in their number in response to stimulation have been postulated as the major mechanisms underlying plasticity. The final phase of skill acquisition may represent a fine tuning of inter-cellular communication involving synaptogenesis which might allow increased efficacy of information transmission creating specific pathways that correspond to the trained movements.

Rat Strain Comparisons

As discussed above, motor skill learning has been shown to produce both synaptogenesis and dendritic changes in the adult rat brain. However, understanding of the functionality of these changes remains in its infancy. Therefore, research describing strain differences may add to our knowledge of the motor system and thereby aid researchers in selecting an appropriate rat strain for behavioural studies. Webb, Gowribai, & Muir (2003) examined motor skills and sensory differences in five rat strains: the outbred LE, and the Fischer (F344), Wistar, Lewis, and Sprague-Dawley inbred albino strains. Motor skills

were evaluated using a number of different measures and sensory ability was assessed by von Frey testing. Von Frey filaments were used to evaluate the force required to elicit hindlimb withdrawal. Webb, Gowribai, & Muir, (2003) found that the F344 strain was deficient in motor and sensory performance compared with the other four rat strains. The F344 rat is an inbred rat strain that is commonly used for cancer research, toxicology and aging studies and is not usually used for behavioural studies. VandenBerg, Hogg, Kleim, & Whishaw (2002) examined the behavioural characteristics, dendritic morphology, and synaptic communication of the LE and the F344 strains of rats. VandenBerg et al. (2002) used the skilled reaching paradigm to train rats from both strains to reach for a food pellet through an opening in the front of a reaching cage. They then compared behavioural measures of reaching performance and quality of reaching movement of the two strains of rats. VandenBerg et al. (2002) reported that the LE rats were better at reaching than the F344 rats. The LE rats had a significantly higher overall hit percentage or reaching performance than the F344 rats: LE rats started reaching at a higher success percentage than the F344 rats and the LE rats never fell below the F344 rats' success percentage for the entire experiment. LE rats scored significantly better on all the measures of the quality of the reaching. The F344 rats scored significantly lower than the LE rats in aiming the forepaw, pronating the forepaw, and grasping the food pellet. This result is also consistent with how Webb et al. (2003) described the performance of the F344 strain on motor skill movements. VandenBerg et al. (2002) also reported that cortical maps of the contralateral forepaw in the LE rats were significantly larger than those of F344 rats. These cortical maps changed as a function of the reach training in both rat strains. Dendritic fields in the contralateral hemisphere were significantly larger than the dendritic fields in the ipsilateral

hemisphere in both strains. In addition, the authors used intracortical microstimulation to map the hemisphere contralateral to the forepaw used for the reaching task in order to detect both reaching condition and strain-related differences in synaptic communication in the MSF cortex. They used 350 Hz pulses of stimulation through an electrode, placed in a specific area in layer V of the MSF cortex to elicit movements that were recorded. The movements were elicited by the lowest intensity of stimulation, ≥ 60 μA. This was the first study to compare differences in the cortical maps of these two strains of rats performing a behavioural task. The area of the forebrain that VandenBerg et al. (2002) chose to stimulate included representations of the rostral forelimb area, the caudal forelimb area, and the hind limb area. They found that the LE rat had significantly lower activation thresholds than the F344 rat for eliciting movement in the contralateral forelimb area. This meant that the F344 rats had a higher activation threshold required to elicit movement than the LE rats. A high activation threshold could possibly account for the smaller cortical map area found in the F344 rats, because cells with high activation thresholds do not promote cellular communication as well as do cells with lower activation thresholds. This suggests that differences in synaptic communication could possibly account for strain differences in cortical alteration and subsequently reaching performance.

Wawryko, Ward, Whishaw, & Ivanco (2004) examined synaptic transmission in the motor cortex of LE and F344 rat strains using both long term potentiation (LTP) and short term potentiation (STP). LTP and STP at present enjoy widespread use in the study of learning and memory (Ivanco, Racine, and Kolb, 2000). Wawryko et al. (2004) did not find any difference between LE and F344 rat strains in synaptic efficacy (facilitation of firing via lowered activation thresholds), contrary to the findings of VandenBerg et al. (2002).

The findings of Wawryko et al. (2004) suggest that strain differences may not be explained by differences in the synaptic communication of cells comprising the MSF cortex in LE and F344 rat strains as described by VandenBerg et al. (2002). Indeed, strain differences may be attributable to differential dendritic growth responses to reach training, suggesting that enhanced dendritic structure would reflect greater opportunities for synaptic transmission rather than altered activation thresholds.

Experiment

The mammalian brain has the ability to generate both structural and behavioural changes in response to motor learning. Evidence of these changes include changes in dendritic structure (Greenough et al. 1985; Volkmar & Greenough, 1972), synaptic connections (Li et al. 1996; VandenBerg et al. 2002; Wawryko et al. 2004) and behaviour (VandenBerg et al. 2002). Research describing strain differences has added to our current knowledge of how the motor system can adapt to new experiences and this information can aid researchers in selecting an appropriate rat strain for behavioural studies. VandenBerg et al. (2002) describe how differences in synaptic modification were responsible for strain differences in performance on the skilled reaching task. This finding was called into question by the findings of Wawryko et al. (2004) who found no evidence of inter-strain differences in synaptic facilitation or efficacy. If strain differences cannot be attributed to synaptic modification affecting synaptic efficiency when performing the skilled reaching task, then they may result from differential dendritic growth in response to experiential input. This research addresses the possibility that changes in dendritic morphology within rats of two different strains may be associated with changes in motor behaviour after learning a skilled reaching task.

Methods

Subjects

Subjects were 14 male Long-Evans (LE), and 14 male Fischer 344 (F344) rats. Both strains were ordered from Charles River Laboratories, Inc., Wilmington, MA USA. Upon arriving at the Psychology Holding Facility in the Duff Roblin Building, animals of each strain were assigned to standard laboratory cages (45 cm long x 23.5 cm wide x 20 cm high) containing either two or three littermates each. Animals were separated into two groups, each of which was comprised of seven LE rats and seven F344 rats. This was done to avoid complications of running 28 animals at one time and also to ensure that all 28 animals were approximately 60 days old at the start of the experiment. Food (ProLab 5P00 RMH 3000) and water were available ad lib until behavioural testing began. Animals were maintained on a diurnal cycle of 12:12 (lights on at 0700 CST) in a temperature controlled (21 ± 2^0 C) colony room. Age at the start of training was 54 days old (d.o.) for the first group of animals, and 63 d.o. for the second group. The body weight of the animals at the start of testing ranged from 279 - 304 g for the LE animals in group 1, 140 – 167 g for the F344 animals in group 1, 302 – 363 g for the LE animals in group 2, and 153 – 202 g for the F344 animals in group 2. Thus, animals in group 1 were, on average, 9 days younger than the animals in group 2 and their weights were correspondingly lower as well.

Reach training Procedure

Reaching Box

Training took place in a clear Plexiglas reaching box, 32 cm long x 23 cm wide x 25.5 cm high (Figure 1), to allow for videotaping from a ventral perspective. There was one, 1 cm wide x 15 cm high slot at the front of the box 10.5 cm from each of the sidewalls

and 0.2 cm from the bottom. In front of the slot was a shelf on the outside wall. The shelf was 2 cm deep x 3.75 cm wide x 2 cm high, and was 3.5 cm from the bottom of the apparatus. The shelf had two small indentations 0.75 cm from the end of the shelf and 0.75 cm from each side. Placing the food pellet in either indentation would limit the retrieval of the pellet to either the left or the right forepaw only. The reaching box was placed on a glass-top stand that had an inclined mirror located below to allow videotaping from the ventral perspective.

Paw preference

All animals were placed on a reduced diet until they reached 90% of adult body weight and their weight was maintained at approximately that level throughout training. This slight food deprivation was used to encourage the animals to take part in the reach training. To assess paw preference, the animals were allowed to reach freely through the slot with either paw for the food pellet. This was done both so that the researcher could observe the use of the preferred paw in each animal and so that the animals could practice pellet retrieval. Food pellets used in reach training were 45g Precision Dustless pellets from Bio-Serv®. The food pellet was placed between the indentations allowing the animal to grasp the food pellet with their preferred paw. Paw preference was determined when the researcher observed each animal in the skilled reaching condition perform 20 consecutive retrievals with the same paw.

Reach training

Members from littermate pairs of each strain were randomly assigned in a manner that equally distributed littermates across experimental conditions. Seven LE and seven F344 rats were assigned to either the reaching condition in which the rat reached through

the slot in the front of the reaching box for each food pellet or to a control condition in which the control littermate was not required to reach for the food pellets. The control animal was placed in an identical reaching box beside the box containing the reach-trained animal but was permitted simply to eat food pellets off the floor of the box. The order in which the pairs of animals were used was the same each day. Reach-trained animals were allowed to retrieve 50 food pellets placed in the indentation on the shelf opposite the preferred paw, forcing the animals to reach for the food pellet using their preferred paw. Untrained animals were simply allowed to eat an equal amount of food pellets off the floor of their cage. Each pair of animals was trained or fed pellets once per day for a total of 30 days. The training was conducted to allow for a short pause after a food pellet was retrieved at which time, another food pellet was dropped into the back of the reaching box. This would ensure that the rat would reposition itself at the slot in the front of the reaching box for the next food pellet.

Videotaping

The behaviour of reach-trained animals was videotaped and recorded from the ventral perspective in order to clearly see each reach attempt for the food pellet. A CANON™ ZR50 mc video camera set to the 'Sports' program was used for all videotaping. This setting enabled the shutter speed to vary between 1/250 and 1/1000 second. Illumination for videotaping was provided by one Lowel™ Caselite 2 fluorescent light source which was colour-balanced for daylight at 5300^{o} K and set to 160 foot candles measured at the bottom of the reaching box. Frame-by-frame analysis was done using a Sony™ VHS playback unit to determine the number of reach attempts made during pellet retrieval.

Reach scoring procedure

In this study, a reach attempt made by the rat was defined as a movement of the forelimb through the slot, an individual paw grasp, or any combination of the two, regardless of whether the pellet was missed or grasped. If a successful grasp occurred and the pellet was retrieved, then the reach was considered a retrieval. Each of the 30 training days consisted of the reach-trained animal retrieving 50 food pellets. However, only the number of reach attempts made by the rat for the first ten retrievals of the food pellets on each training day were used for analyses because the number of reach attempts made by the rat for the remaining 40 food pellets was not made available for analysis in this study. Reaching proficiency for each rat was calculated by dividing the 10 pellets retrieved by the number of reach attempts required to retrieve those pellets (i.e. 10/x). This number was then multiplied by 100 to determine a percentage measure of reaching proficiency for each rat at the end of each training session

$$\left(\frac{\text{10 food pellets retrieved}}{\text{number of reach attempts}} \text{ x } 100 = \text{reaching proficiency percentage}\right)$$

. Thus, ten reach attempts made by each rat for the retrieval of ten food pellets would be 100% reaching proficiency for that training day. A mean was then derived for each rat and strain for each training day.

Histology

Following the last day of training, all the animals were euthanized with an overdose of sodium pentobarbital (100 mg/kg) and perfused transcardially with 0.9 % saline. The whole brain was removed and placed in a Golgi-Cox solution (as per Gibb & Kolb, 1998). The brains were stored in the dark for 21 days after which they were removed from the Golgi-Cox solution and placed in a 30 % sucrose solution for 10 days before sectioning. Areas of the brains that were rostral of, and caudal to, the MSF cortex in the coronal plane

were removed and the remaining block of brain tissue that contained the MSF cortex was mounted on a sectioning stage with cyanocacrylic glue. The block of cortical tissue was aligned such that the apical arborisation was contained within the plane of section. For this study, the region of the anterior cortex involved in performing the reaching task was identified from evidence provided by Donoghue & Wise, (1982) where they used cytoarchitecture and microstimulation mapping to identify a band of overlapping regions including both somatosensory and somatomotor areas. Each section was also compared to photomicrographs in an atlas (Paxinos & Watson, 2005) of the rat brain with stereotaxic coordinates from bregma 2.28 to -1.2 (plate 14 to 43) in the acceptable range of sections (Figure 2). Bregma is the craniometric point at the junction of the sagittal and coronal sutures at the top of the cranium. The sectioning stage was mounted in a vibratome (model VT1000S), the reservoir of which was filled with 10 % sucrose solution. The block of cortical tissue was sectioned at a thickness of 200 microns. Wet sections were pressed onto slides and blotted to remove excess water which prevented the sections from coming off the slide. Slides were processed by the Golgi-Cox method (as per Gibb & Kolb, 1998) and cover slipped with Permount®.

Quantitative morphology

For this project, basilar dendrites of layer II / III pyramidal cells were analyzed. Only one type of pyramidal cell, the forked-apical cell was drawn from the mounted sections. The forked-apical cells were identified as having an apical branch with a fork within 75% of the total height of the apical field above the soma. Forked-apical cells are found in layer II/III, as described in Withers & Greenough, (1989). The cells were drawn by the camera lucida system, which allows the operator to draw the soma and dendritic

field of the neuron using a 60X objective lens. Stage coordinates and the section and slide number of each drawn cell were recorded in a logbook in order to prevent the accidental re-drawing of a cell. An independent observer coded slides so the experimenter was not aware of the training condition or strain of the animal from which the brain tissue came. Once drawn, each neuron was analysed using the Sholl method (Figure 3). The Sholl analysis utilizes a series of concentric rings drawn on acetate that were centered over the soma of the drawn cell. The number of times the dendritic tree intersected each of these concentric rings was counted, and estimates of the length of the dendrites were calculated by multiplying the number of intersections by the area of each ring, which was 10 microns. The number of intersections represents an estimate of dendritic length because this analytical procedure uses a 2-dimensional representation of a 3-dimensional object, thereby compressing the Z axis. This method does not account for dendritic processes that are not parallel with the section surface. For example, a cell may have one very long dendrite with a high number of ring intersections and very few shorter dendrites or lower dendritic complexity. The method of branch ordering that was used is described in Kolb, Forgie, Gibb, Gorny, and Rowntree (1998). This method of ordering starts at the origin of the dendritic tree and works out towards the terminations with the root segment receiving the branch order number one and all other branches receiving a branch order one larger than the parent segment. The length of each branch order was determined by counting the number of ring intersections the branch order made starting from when the branch order first intersects a ring and ending at the last ring intersection before the start of the next branch and multiplying this number by the area of each ring as described above. This method would account for cells with low number of ring intersections but greater dendritic

complexity. Comparisons of dendritic length at each branch order could then be made between the cerebral hemisphere contralateral to the paw used in the reach-training (the 'trained' hemisphere) and the hemisphere ipsilateral to the paw used in reach-training (the 'untrained' hemisphere) of both rat strains. Total dendritic length provides an overall measure of the effects of reach training while branch order analysis provides a measure of dendritic complexity at each segment. The number of branches ≥ 3 is also used as measure of branching complexity. For all drawn cells, each dendritic process was drawn in a different colour in order to differentiate one process from the other. The number of branches ≥ 3 was counted for each dendrite and the length of branches that did not bifurcate was measured. Any branches that were cut off by the surfaces of the sections were excluded from analysis.

Statistical Analyses

Changes in basilar dendritic morphology of pyramidal cells from layer II/III of the MSF cortex in the hemispheres of reach-trained versus non-trained rats were determined for each rat strain. Within-animal comparisons were not conducted because only one cell was drawn in a single hemisphere in 17 of 28 animals (64%). All dendritic data were evaluated to determine the normality of distribution and the homogeneity of variance. D'Agostino's D-test (Statworks ^{TM}) for normality was used to determine whether the data for total dendritic length and branch order analysis could have been derived from populations with underlying normal distributions. D'Agostino's D-test determined that only dendritic length for branch order 3, 4, 5 and branch order ≥ 3 were normally distributed and could therefore be analyzed using a parametric test. The F_{max} test was used to determine if the dependent variables of total dendritic length and dendritic length for branch order analyses met the

assumption of homogeneity of variance. This test determined that all but one dependent variable examined met this assumption (Table 2). Differences were considered significant where $p \leq 0.05$.

The reaching proficiency of each rat strain was analyzed utilizing a repeated-measures ANOVA comparing reaching proficiency for each rat strain across training days. Differences between rat strain and reaching condition on the total dendritic length data were analyzed using the non-parametric Mann-Whitney U-test for independent groups. To determine if the reach-training experience had any effect on the Sholl-derived measure of total dendritic length within each rat strain the non-parametric Kruskal-Wallis test was utilized. Differences between rat strain and reaching condition on the distribution of dendritic length as a function of branch order 3, 4, and 5, would determine if there was an interaction of reach training and rat strain. Repeated-measures ANOVA tested for strain differences in reaching proficiency, treating proficiency data from individual rats across training days as a repeated measure. All the number of branches ≥ 3 data were pooled within the contralateral and ipsilateral hemispheres for each rat strain. Comparisons of the number of branches ≥ 3 across reaching conditions and strains utilized the independent-samples t-tests. Within-strain comparisons were made utilizing a paired-samples t-test to determine if the number of branches ≥ 3 differed significantly between the contralateral and ipsilateral hemispheres in trained animals.

The Sholl method also derives a measure of total dendritic length for each cell that sums the number of ring intersections for each basilar dendrite of a cell. Independent samples t-tests were utilized to determine if the number of ring intersections from the contralateral and ipsilateral hemispheres differed significantly between rat strains.

Results

Reaching Proficiency

A repeated-measures ANOVA used to test for strain differences in reaching proficiency treated proficiency data from individual rats across training days as the repeated measure. There was a significant difference in the main effect of reach-training between rat strain $F_{1, 12} = 28.50$, $p = 0.0002$ (Figure 4). Overall, the F344 rats retrieved the ten food pellets with fewer reach attempts than did the LE rats. There was also a significant interaction of reaching proficiency scores with training days, $F_{29, 348} = 3.20$, $p < 0.0001$. Visual inspection of the graph depicting reaching proficiency in both rat strains indicated that as training progressed the F344 rats got better or more proficient at retrieving the food pellets while the LE rats did not. Visual inspection of the graph also suggests that the improvement in reaching of the F344 rats reached a plateau around day 11, whereas the LE rats appeared to improve over the first week of training but failed to achieve the proficiency of the F344 rats.

Total Dendritic Length

The Sholl Method analysis produced the dependent measure of the total dendritic length for each strain of rat. The Mann-Whitney U-test was utilized to determine that the total dendritic length measured from cells in the trained hemisphere of the reach-trained rats was not significantly different between the LE and F344 strains; $U_{6,6} = 10$, $p = 0.20$. Further, the total dendritic length measured from cells in the untrained hemisphere in reach-trained rats was not significantly different between the LE and F344 strains; $U_{6,7} = 16$, $p = 0.47$. Also total dendritic length from the left and right hemispheres of control animals was not significantly different; $U_{7,7} = 24$, $p = 0.95$, and $U_{6,7} = 19$, $p = 0.48$ (Figure

5). The non-parametric Kruskal-Wallis test was utilized to determine within-strain differences in total dendritic length. Comparisons were made between the total dendritic length of all cells in the contralateral and ipsilateral hemispheres of reach-trained rats and from all cells in the left control and the right control hemispheres of untrained rats. There were no significant differences in total dendritic length across training condition in either strain, LE; $H_{3, 4} = 2.41$, $p = 0.49$, and F344; $H_{3, 4} = 0.76$, $p = 0.86$: (Figure 6).

Independent samples t-tests were used to determine if there were differences between reaching conditions or strains in the data derived from the Sholl analysis. There were no effects of reach training on the Sholl-derived measure of the number of ring intersections between contralateral and ipsilateral hemispheres in either rat strain; LE contralateral versus F344 contralateral hemisphere; $t_{62} = 0.43$, $p = 0.67$, ± SE, LE = 1.71, F344 = 2.7, and LE ipsilateral versus F344 ipsilateral hemisphere; $t_{62} = -0.33$, $p = 0.75$, ± SE, LE = 2. F344 = 2.8: (Figures 7 & 8). Also, the within-strain comparison of concentric ring intersections in the contralateral and ipsilateral hemispheres in each strain was examined utilizing a paired samples t-test. The comparison of the number of concentric ring intersections found that the concentric ring intersections in the contralateral hemisphere differed significantly from that in the ipsilateral hemisphere within the LE strain, $t_{21} = 3.288$, $p = 0.004$, ± SE 0.572. The same comparison in the F344 strain of the number concentric ring intersections found that the contralateral hemisphere did not differ significantly from the ipsilateral hemisphere, $t_{18} = 0.975$, $p = 0.342$, ± SE 0.507: (Figures 9 & 10).

Segment analysis for Branch Order 3, 4, 5

Segment analysis utilized repeated-measures ANOVA, at branch order 3, 4, and 5, across reaching condition and rat strain. There were no significant differences between strain and reaching condition on the measure of dendritic length for branch order 3, 4 or 5: Branch order 3 for rat strain; $F_1 = 0.002$, $p = 0.96$, reaching condition; $F_3 = 0.071$, $p = 0.98$, and the interaction of strain and reaching condition; $F_3 = 0.56$, $p = 0.65$. Branch order 4 for rat strain; $F_1 = 0.12$, $p = 0.73$, reaching condition; $F_3 = 0.14$, $p = 0.94$, and the interaction of strain and reaching condition; $F_3 = 0.313$, $p = 0.82$. Branch order 5 for rat strain; $F_1 = 0.21$, $p = 0.65$, reaching condition; $F_3 = 0.23$, $p = 0.83$ and the interaction of strain and reaching condition; $F_3 = 0.74$, $p = 0.54$: (Figures 11, 12, & 13).

Number of Dendritic Branches for branch order ≥ 3

Independent samples t-tests were used to determine if the measure of the number of branches ≥ 3 were different across reaching condition and rat strain. There were no significant differences in the number of branch order ≥ 3 between reaching condition and strain, LE; $t_{18} = -1.57$, $p = 0.13$, and F344; $t_{18} = 0.76$, $p = 0.46$: (Figure 14). There were no significant within-strain differences in the number branch order ≥ 3 contrasting the contralateral versus ipsilateral hemispheres within each rat strain; LE contralateral versus ipsilateral hemisphere, LE; $t_5 = 0.35$, $p = 0.84$, and contralateral versus ipsilateral hemispheres, F344; $t_5 = 0.21$, $p = 0.74$.

Discussion

This research examined the possibility that changes in dendritic morphology would reflect changes in motor behaviour after LE and F344 learned a skilled reaching task. In

particular, if strain-differential performance in the skilled reaching paradigm documented in a previous study (VandenBerg et al. 2002) cannot be attributed to differential synaptic efficiency when performing the skilled reaching task as Wawryko et al. (2004) have suggested, then strain differences may be accounted for by differential dendritic growth in response to experiential input. In this research, reach training did not produce any significant differences in measures of dendritic morphology.

The F344 rats were, however, superior reachers, but changes in dendritic morphology quantified in my research did not seem to underlie this differential proficiency. In fact, the only statistically significant difference in dendritic morphology apparent in this research was the greater number of ring intersections in the ipsilateral relative to contralateral hemispheres of reach-trained Long-Evans rats, a finding which is the reverse of that predicted based upon any influence of reach-training on dendritic morphology. These findings are not consistent with previous research examining strain differences between LE and F344 rats in motor behaviour and dendritic morphology. When describing the motor behaviour of the F344 rats, VandenBerg et al. (2002) suggested that a structural defect in synaptic connections might be responsible for the abnormal motor behaviour in the F344 rats. No such behavioural deficiency in the F344 rats was noted in my research. It must be said that the more comprehensive analysis of motor behaviour conducted by VandenBerg et al. (2002) was not an objective of this study. What was noted in my research was the F344 rats were more efficient at retrieving food pellets than the LE rats. Therefore, it may be necessary to include the more complex behavioural analysis in future research examining strain differences.

The lack of evidence for differential dendritic growth in the contralateral versus ipsilateral hemispheres within reach-trained animals is not consistent with previous research by Greenough et al. (1985). The Sholl and branch order analysis used in this study have proven to be good methods for examining dendritic growth in past research. The inability of these methods to detect any differential dendritic growth attributable to reach-training in my study may be due to the fact that there were too few cells to analyse. It is also unlikely that the length of time the animals were trained in this study may have affected differential dendritic growth. Others have trained LE rats for much shorter periods of time; VandenBerg et al. (2002) trained rats for ten days whereas this study trained rats for 30 days. A previous study using LE rats has shown that although significant improvements in reaching performance were observed after the first 3 days of skilled reach training, there were no significant changes in the in cortical maps of movement representations in the SMF region (Kleim et al. 2002). Kleim suggested that only after the rats had reached behavioral asymptote, between 7 and 10 days, could the reorganization be detected. To this date, no studies have reached-trained rats for 30 days. Further research that would look at changes in dendritic morphology at different time points during reach-training over 30 days might reveal differential dendritic growth as reach-training progresses.

Admittedly, the number of cells drawn in this research is substantially less than others have used. A total of 864 drawn cells were used for Sholl analysis by Withers & Greenough, (1989); 400 of these cells were forked-shafted apical type and the other 464 cells were single shaft cells. They did not find a significant difference between the contralateral and ipsilateral hemispheres in single shaft cells. In the present study, a total of

140 forked-shafted apical cells were drawn by camera lucida and increasing the number of cells drawn would have increased statistical power. Also, a computerized cell drawing system may help reveal dendrites that could not be drawn using the camera lucida technique. Neurolucida® uses a 3D system that accounts for dendritic processes that are perpendicular to the surface of the section. Any cell dendrites drawn on the Neurolucida® system as opposed to the camera lucida system would have greater length and surface area.

Rats provide a useful model for the examination of brain function and neurodegenerative diseases that may affect motor control. Studies investigating neural plasticity and learning and memory can use strain differences as a way of examining the behavioural and anatomical organization of the motor system and would help in understanding how neural plasticity relates to changes in behaviour. The rat model also provides the means to explore how experience influences neuronal morphology in humans particularly in planning rehabilitative strategies after injury.

This study has demonstrated that one rat strain may be more proficient than another at the reaching task. In this study there were no significant differences in dendritic morphology that would have supported the differences in reaching proficiency in either rat strain. This suggests that additional experiments are required to elucidate the neural mechanisms underlying the differences in motor behaviour observed in these rat strains.

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Table 1

D'Agostino's D-test determined if the data for the dependent variables of total dendritic length and branch order analysis came from a normal distribution.

Dependent Variable	n	D	*p* - Value
Total Dendritic Length	53	0.29	0.017
Branch Order 3	53	0.23	0.051
Branch Order 4	50	0.22	0.059
Branch Order 5	43	0.24	0.061
Branch Order ≥ 6	26	0.22	0.135
Branch Order ≥ 3	53	0.18	0.101

The data were considered normally distributed when $p > 0.05$.

Table 2

The F_{max} test determined that the dependent variables of total dendritic length and branch order analysis met the assumption of homogeneity of variances with one exception.

Dependent Variable	Df	F_{max} - Value
Total Dendritic Length	13	4.53
Branch Order 3	13	1.37
Branch Order 4	12	2.72
Branch Order 5	11	1.73
Branch Order ≥ 6	7	23.86*

The F_{max} test determined that the variances of total dendritic length and branch order analysis were not significantly different except for branch order ≥ 6; the F_{max}-value of 23.86 exceeded the F_{max} critical value of 8.44 at alpha 0.05.

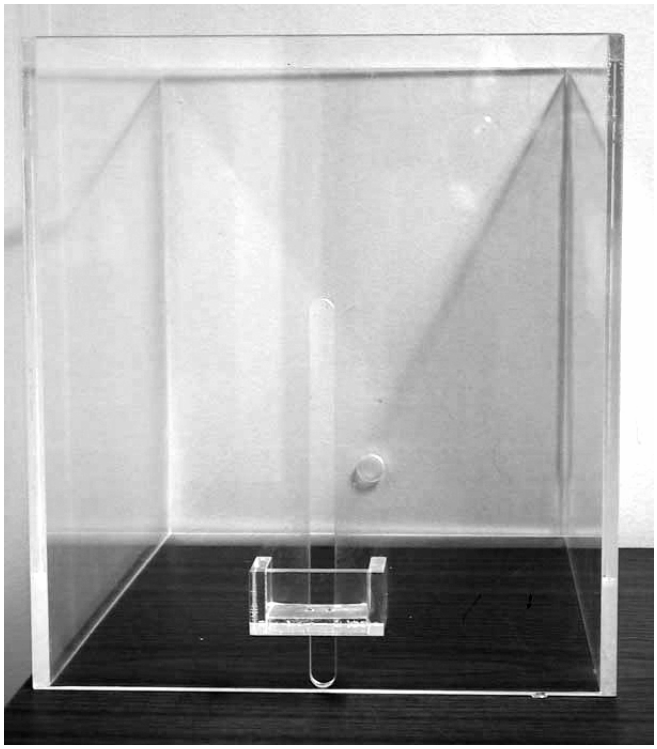

Figure 1. Reaching Box. Rats in the skilled reaching condition were placed inside this reaching box and allowed to reach for 50 food pellets placed in the indent on the shelf opposite the reaching paw. At the same time, control animals were placed inside an identical box and were allowed to eat 50 food pellets off the floor of the box.

Figure 2. Photomicrograph from Paxinos & Watson, 2005 atlas of the rat brain identifying the MSF area of the cortex where the layer II/III pyramidal cells were drawn from.

Figure 3. Sholl analysis is used to estimate dendritic length of the cell. This drawing illustrates concentric ring analysis where each ring is 10 nm apart. Counting the number of ring intersections provides a measure of dendritic length. Another measure of dendritic length is branch order analysis where the total number of dendritic branches can be analyzed by segment to give an estimate of where dendritic growth differs between segments.

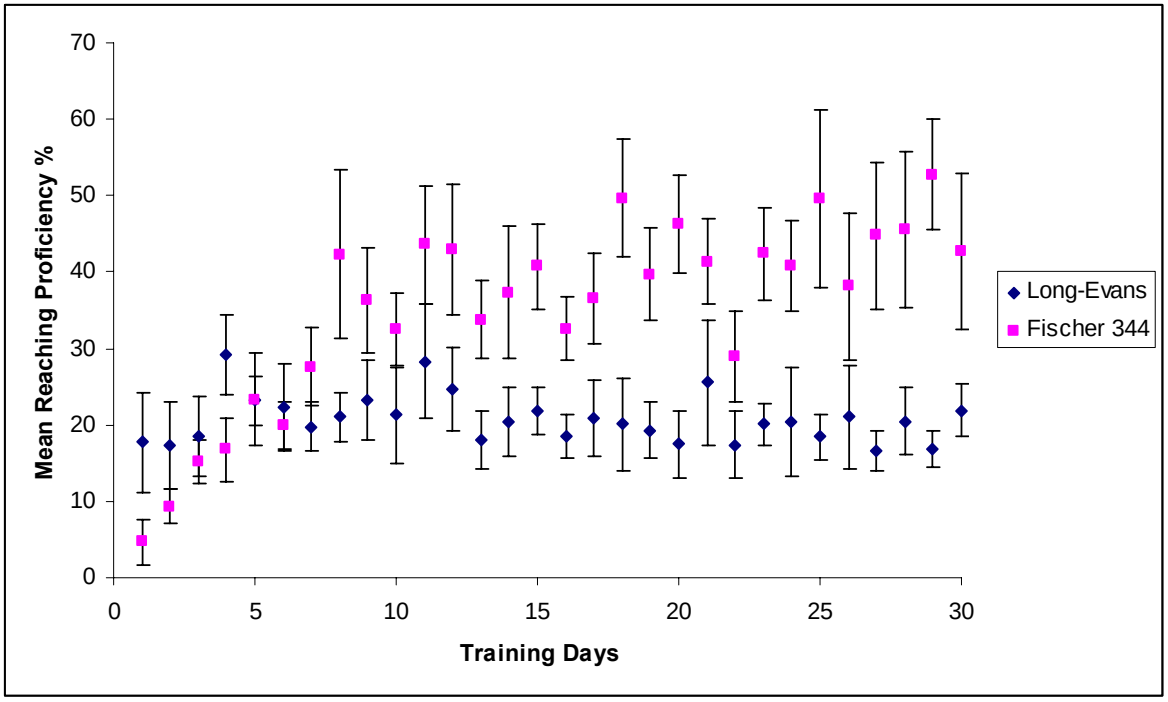

Figure 4. Skilled reaching proficiency (± SE) for the LE and F344 strains over 30 days of training. Fischer 344 rats (squares) improved to a higher level of proficiency during training than LE rats (diamonds).

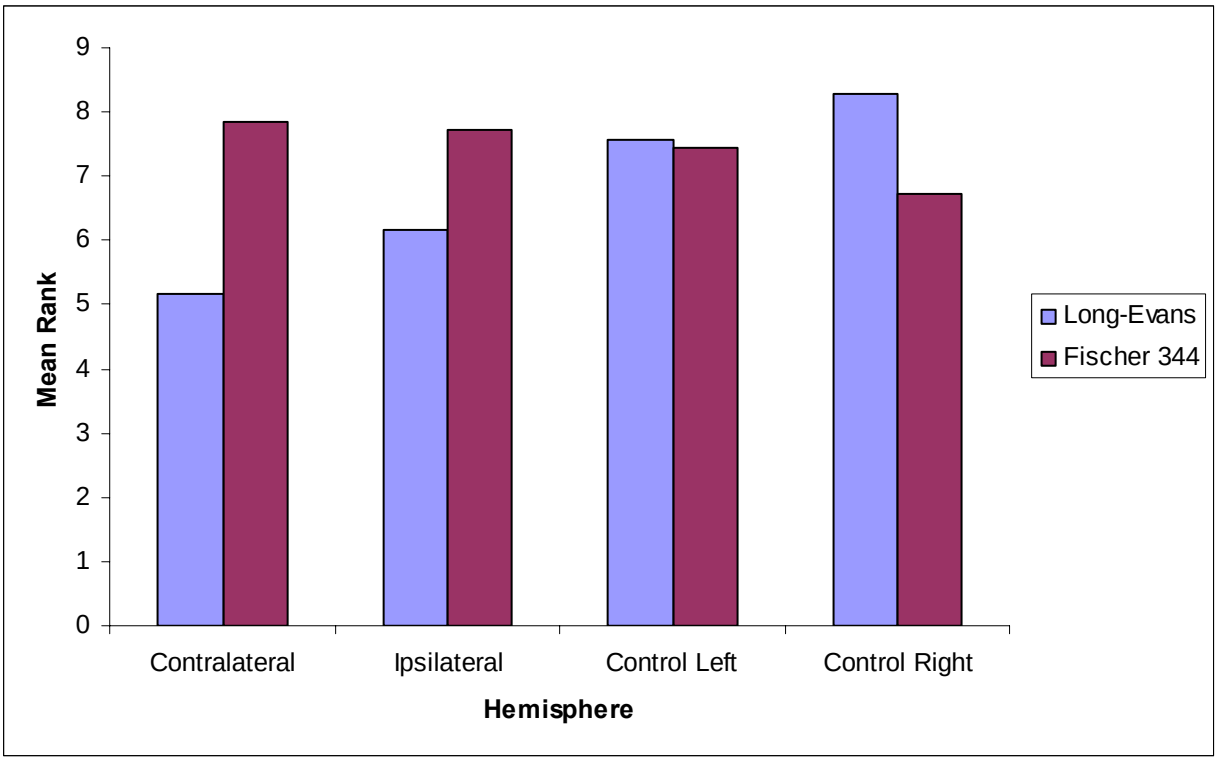

Figure 5. There was no significant difference in the total dendritic length between reaching condition and rat strain. The non-parametric Mann-Whitney U-test determined that the total dendritic length for both rat strains was not significantly different after reach training.

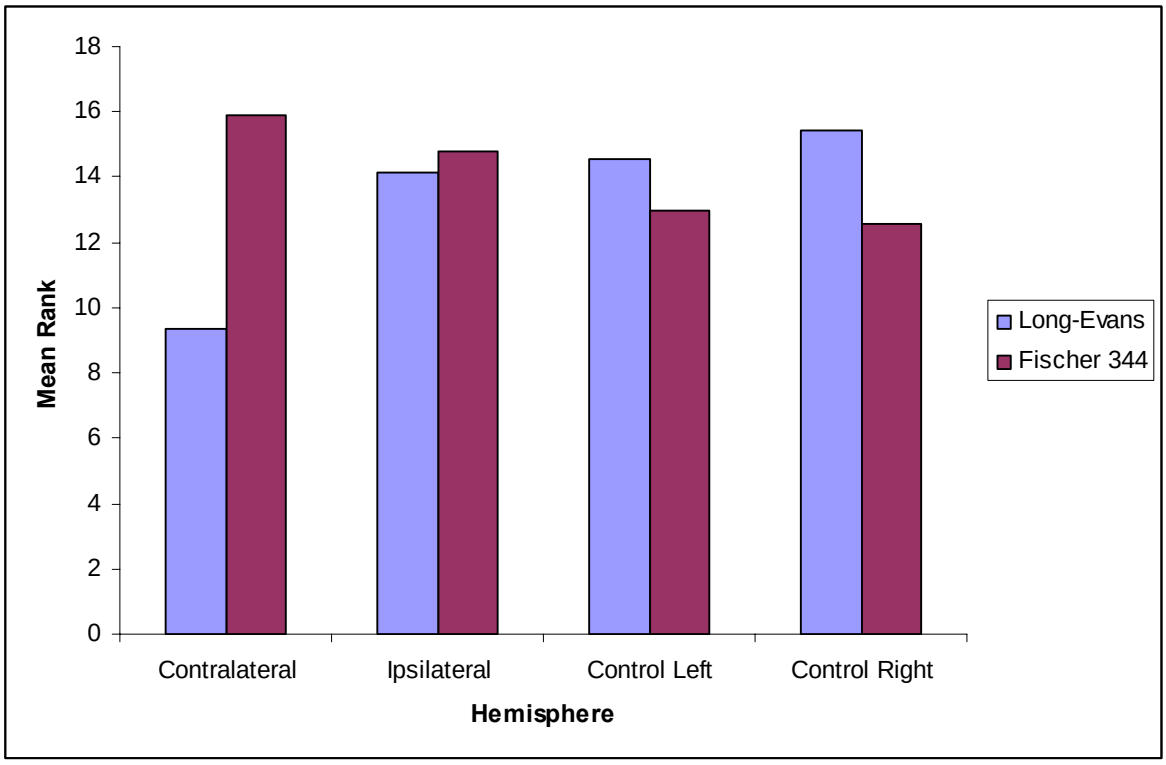

Figure 6. There was no significant difference in total dendritic length between reaching condition and rat strain. The non-parametric Kruskal-Wallis test was used for analysis.

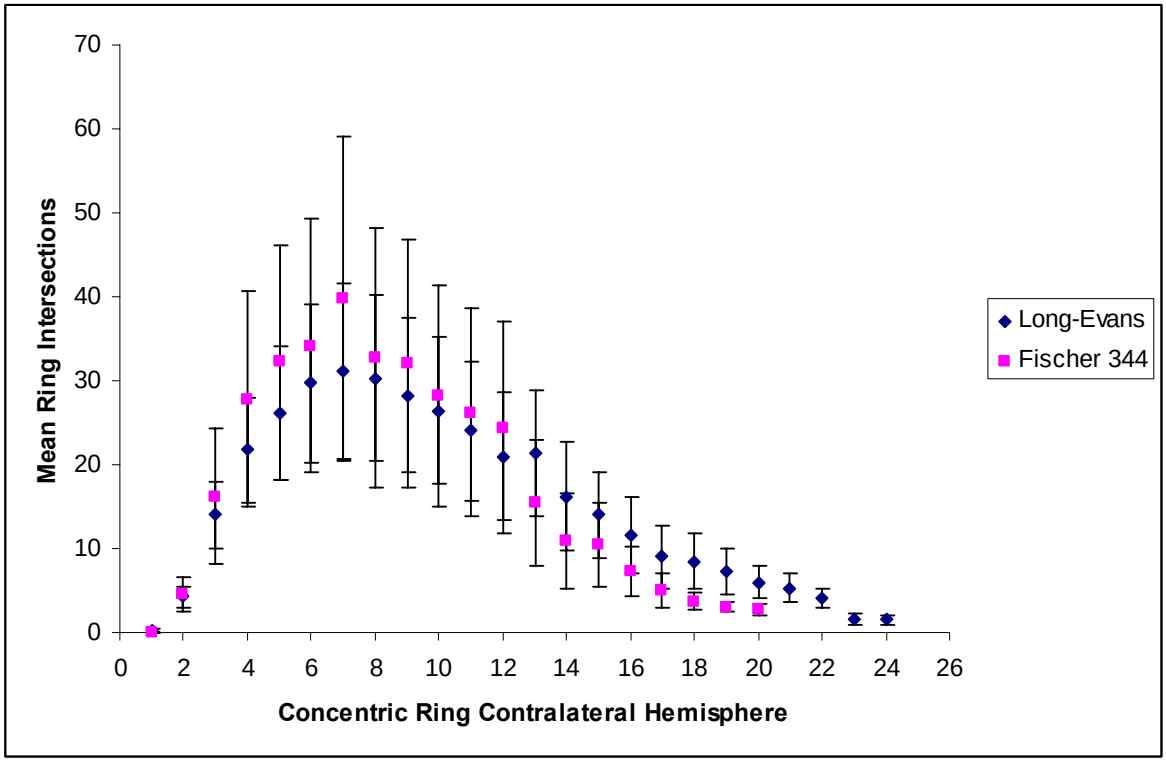

Figure 7. Sholl plot for the contralateral hemispheres from each rat strain showing the mean number of ring intersections (mean ± SE) along the dendrites from the center to the periphery. In Sholl analysis, the number of intersections is counted within each Sholl ring. In LE dendritic branches (square), the peak number of intersections is located at roughly 7 rings and the distribution of branch points ranges from 0 to 26 rings from the center. In F344 dendritic branches (diamond), the peak number of branch points is located at roughly 7 rings and the distribution of branch points ranges from 0 to 23 rings from the center.

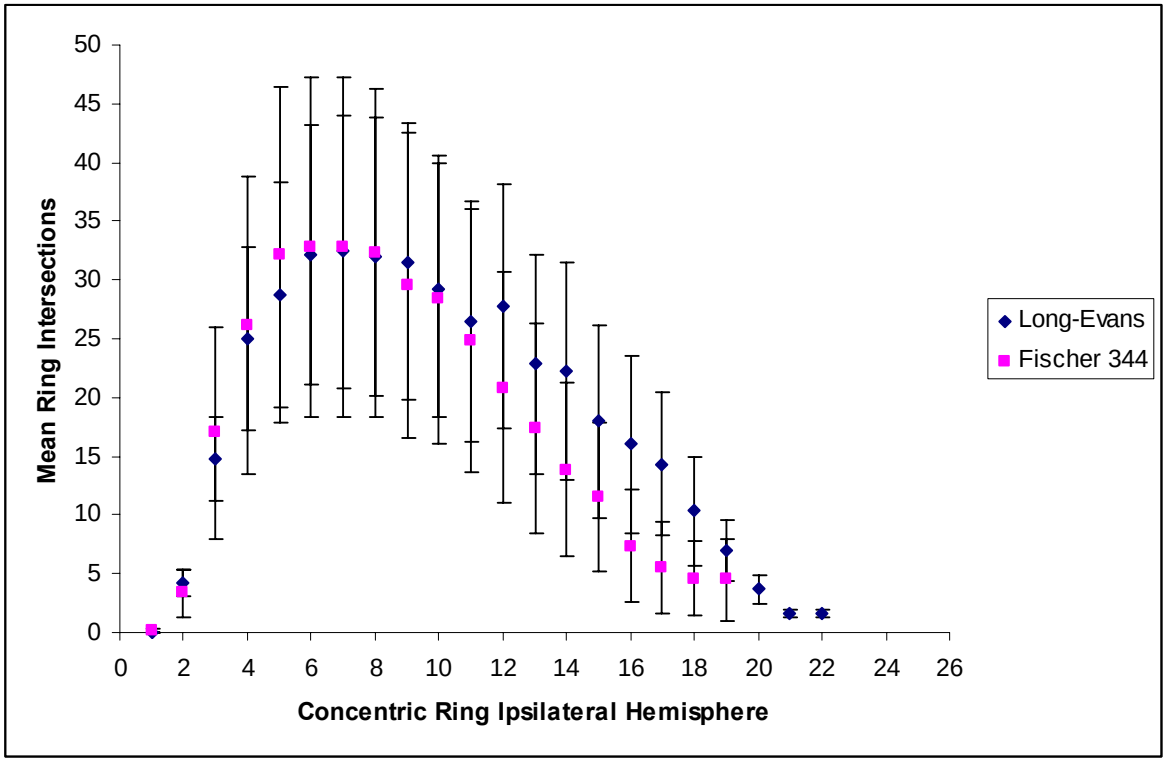

Figure 8. Sholl plot for the ipsilateral hemispheres from each rat strain showing the mean number of ring intersections (mean ± SE) along the dendrites from the center to the periphery. In Sholl analysis, the number of intersections is counted within each Sholl ring. In LE dendritic branches (square), the peak number of intersections is located at roughly 6 rings and the distribution of branch points ranges from 0 to 22 rings from the center. In F344 dendritic branches (diamond), the peak number of branch points is located at roughly 6 intersections and the distribution of branch points ranges from 0 to 27 rings from the center.

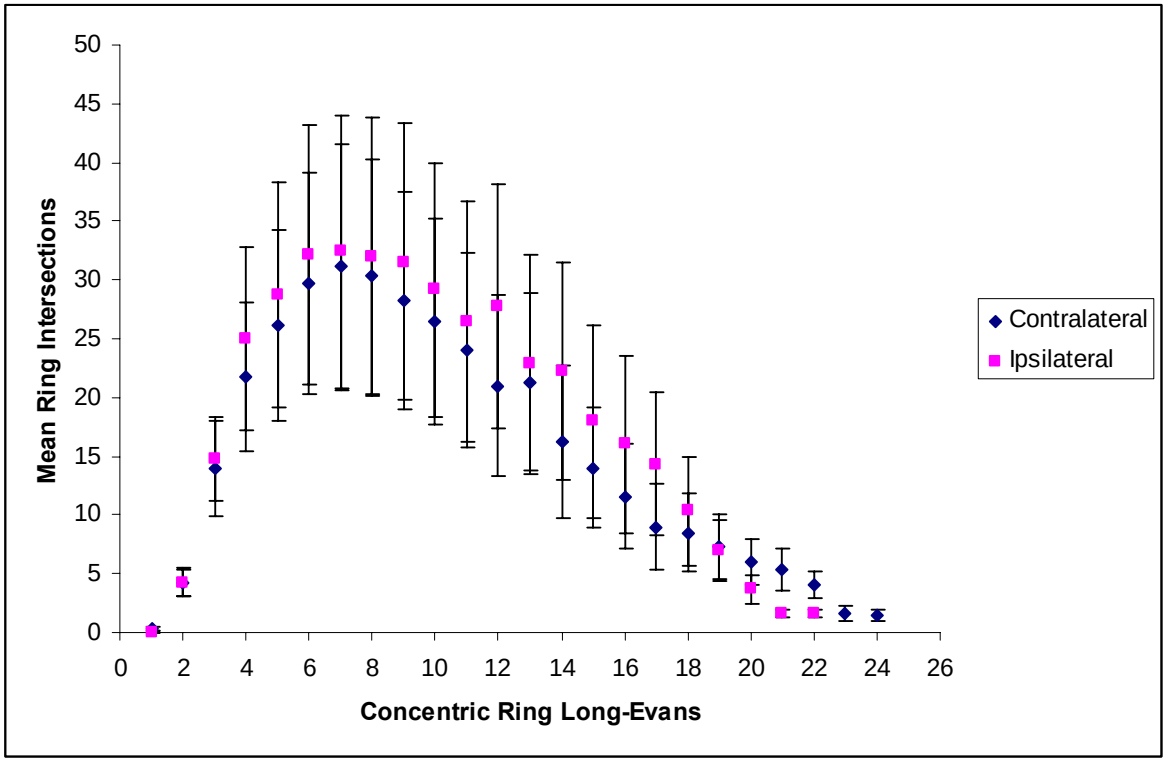

Figure 9. Sholl plot for the contralateral and ipsilateral hemispheres from the LE rat strain showing the mean number of ring intersections (mean ± SE) along the dendrites from the center to the periphery. In ipsilateral dendritic branches (square), the peak number of intersections is located at roughly 7 rings and the distribution of branch points ranges from 0 to 24 rings from the center. In contralateral dendritic branches (diamond), the peak number of branch points is also located at roughly 7 rings and the distribution of branch points ranges from 0 to 24 rings from the center. The ipsilateral hemispheres differed significantly from the contralateral hemisphere, there were more ring intersections in the ipsilateral hemisphere.

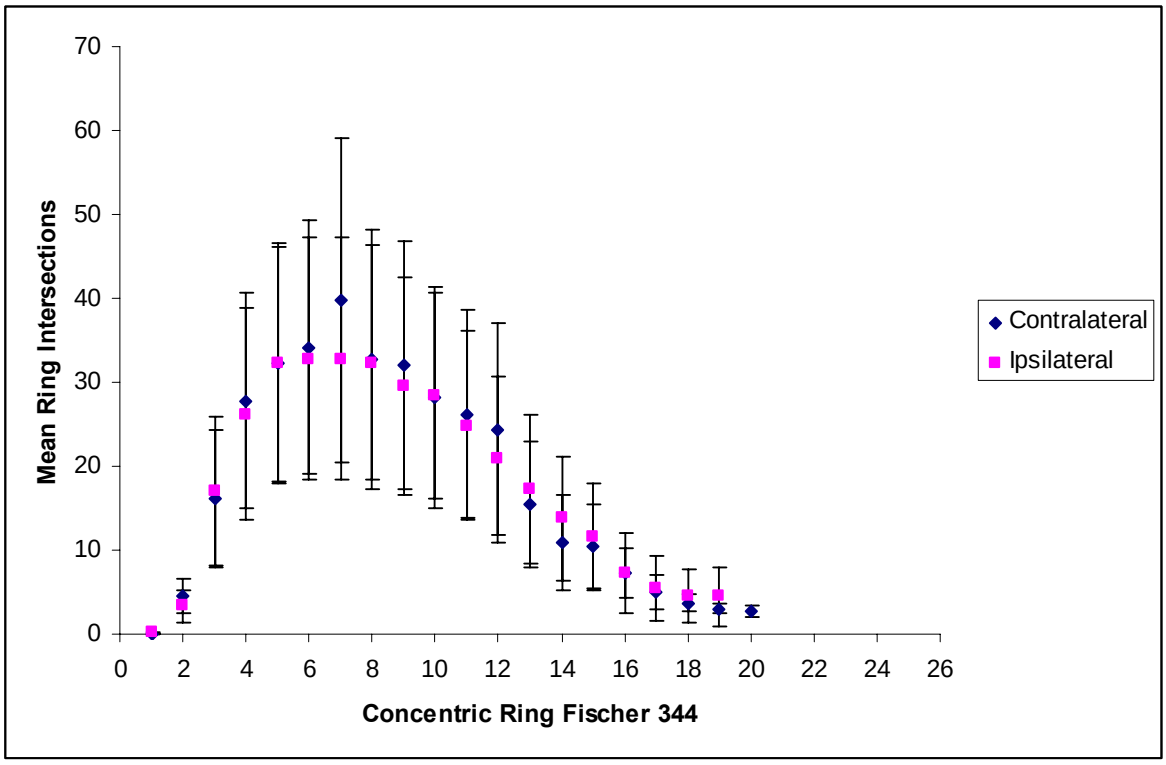

Figure 10. Sholl plot for the contralateral and ipsilateral hemispheres from the F344 rat strain showing the mean number of ring intersections (mean ± SE) along the dendrites from the center to the periphery. In ipsilateral dendritic branches (square), the peak number of intersections is located at roughly 7 rings and the distribution of branch points ranges from 0 to 32 rings from the center. In contralateral dendritic branches (diamond), the peak number of branch points is also located at roughly 7 intersections and the distribution of branch points ranges from 0 to 20 rings from the center. There was not a significant difference between hemispheres.

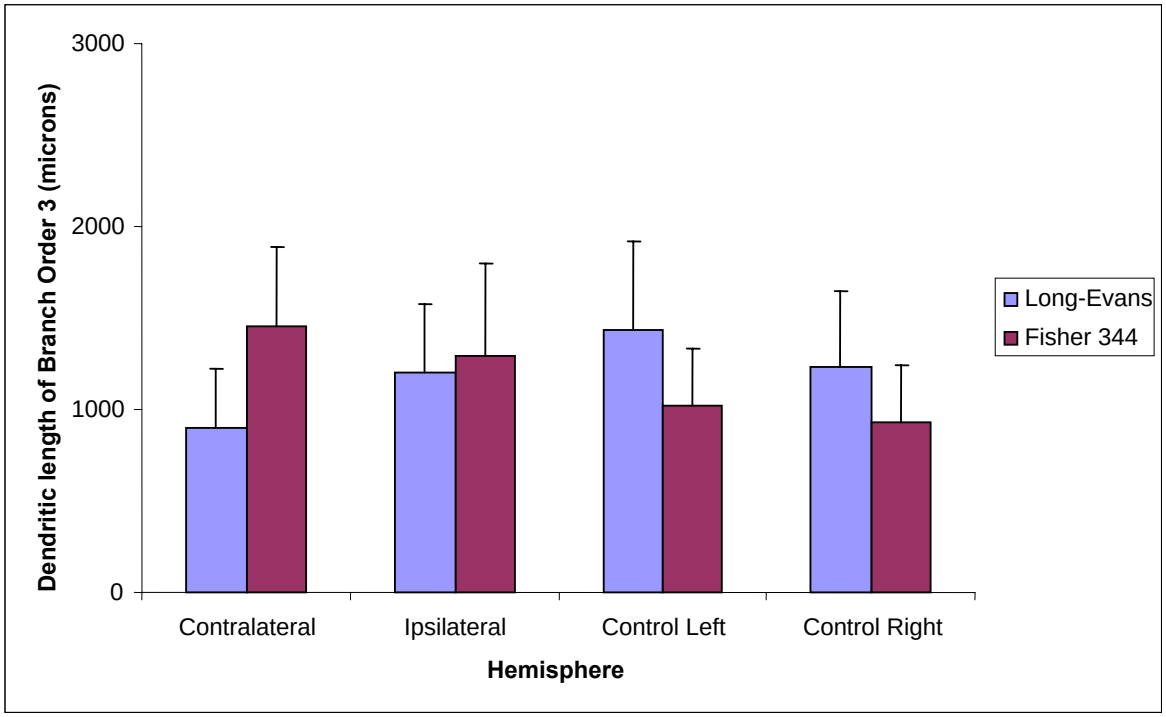

Figure 11. There was no significant difference in dendritic length at branch order 3 between reaching condition and rat strain. A repeated-measures ANOVA, dendritic length at branch order 3 across reaching condition and rat strain was used for analysis.

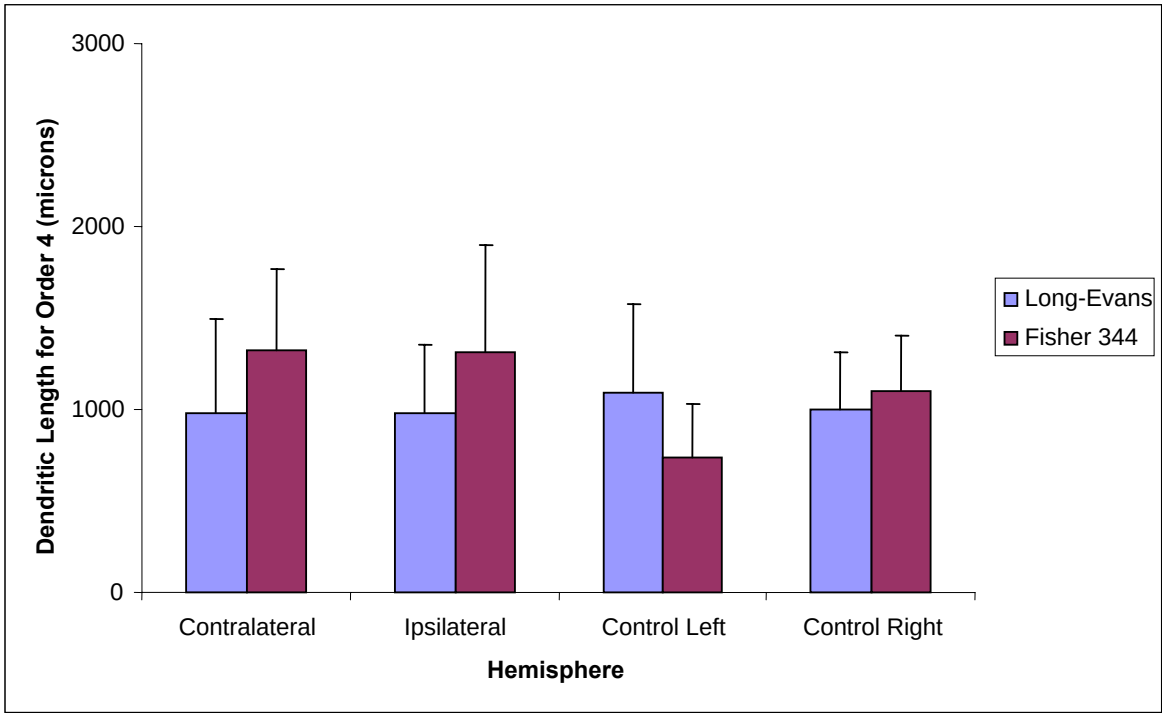

Figure 12. There was no significant difference in dendritic length at branch order 4 between reaching condition and rat strain. A repeated-measures ANOVA, dendritic length at branch order 4 across reaching condition and rat strain was used for analysis.

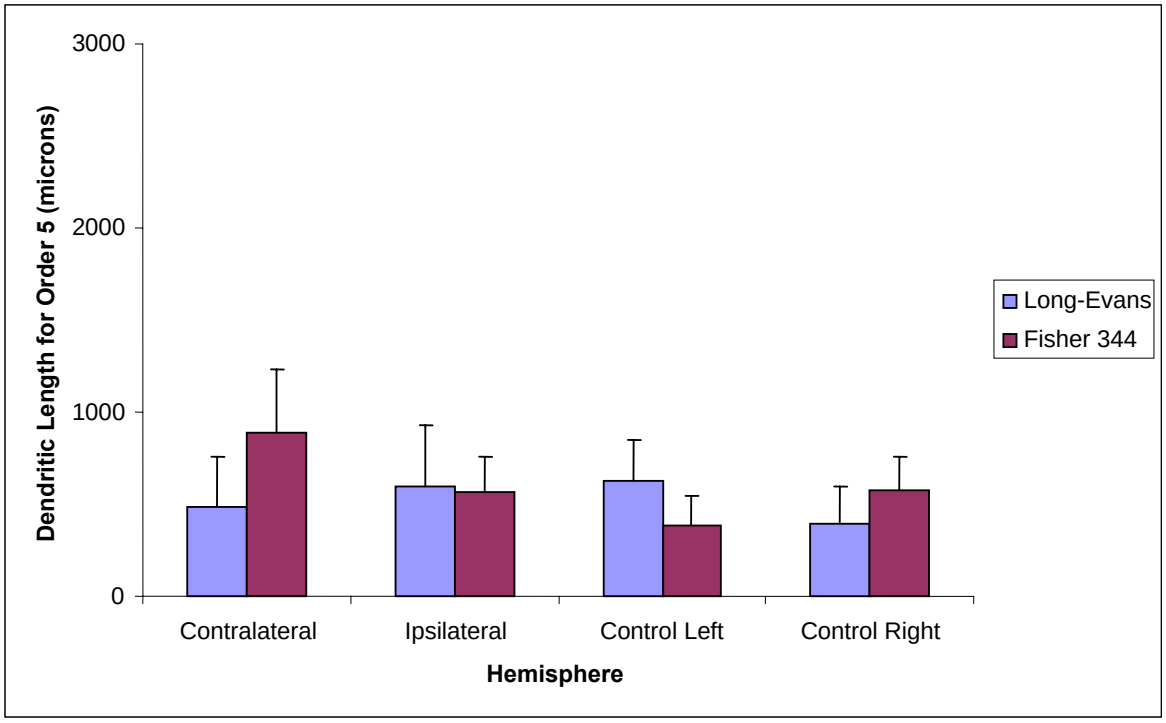

Figure 13. There was no significant difference in dendritic length at branch order 5 between reaching condition and rat strain. A repeated-measures ANOVA, dendritic length at branch order 5 across reaching condition and rat strain was used for analysis.

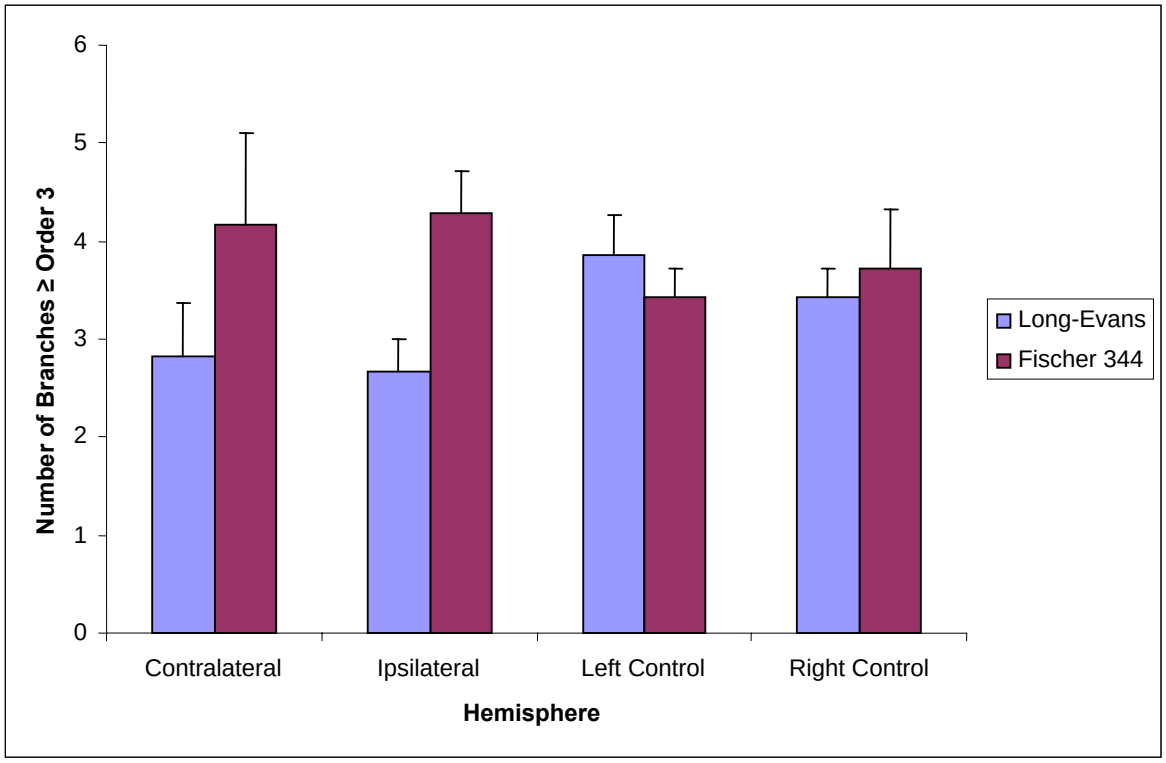

Figure 14. There was no significant difference in the number of branches ≥ order 3 between reaching condition and rat strain. An independent samples t-test was used to analyze the number of branches ≥ order 3 in the contralateral and control hemispheres of both rat strains.