The Classification of gait parameters of Rat models of stroke during over-ground locomotion

Key Words: MCAO, gait, temporal gait, posture, movement analysis, instability, grafting

Word Count: 4850

Illustrations

ABSTRACT

Animal models of stroke have been investigated for many years to understand the behaviour and function following the human condition. The aim of this study was to utilize an established motion analysis technique and the Cardiff Classification tool based around Dempster-Shafer theory (DST) to quantify temporal gait and postural gait parameter of a rat model of stroke. Marker based motion analysis (Qualisys, Sweden) was used to investigate the effects of Middle Cerebral Artery Occlusion (MCAO) on rat locomotion along a wide beam compared to controls. Significant differences (p<0.05) were found between the MCAO and control rats for swing time and roll ROM during one gait cycle. Furthermore the Cardiff DST enabled the objective classification of the rat cohorts into a MCAO group and a control group. Roll ROM and swing time data were transformed into a set of belief values that the animals had MCAO gait or normal gait. The belief values were then represented on a simplex plot, for visualisation. The DST tool was able to classify rats with an accuracy of greater than 81% accuracy. Swing time and roll were identified as the most influential variables in distinguishing differences in gait after MCAO lesion. DST therefore improves comparisons between groups when compared to ANOVA alone by taking into account a level of uncertainty and producing a clear visual comparison between the cohorts.
Introduction

MCAO in the rat is used to mimic large vessel occlusion in humans. As is the case with humans, the territory of the middle cerebral artery is the largest of all the cerebral arteries, and the proximal branches supply the posterior striatum and internal capsule (Paxinos, 1995). The MCAO technique should, theoretically, produce restriction in blood supply both in the cortex and in striatum. However, blood flow from parallel (collateral) blood vessels can contribute to maintenance of cerebral blood flow that sustains tissue viability in the cortex. This means that the behaviour exhibited by the rat is dependent on the location and size of the lesion. The duration of the MCAO can be as short as 30 minutes (Shen and Wang, 2010) although some studies perform it for as long as 3 hours (Lim et al., 2008). There are advantages and disadvantages to the difference in duration of the occlusion. The longer the duration the larger the infarction caused. This difference can be seen in both pathology and behaviour, i.e., motor deficits increase with an increase in the infraction size (Wegner 2005).

Embryonic grafting is a technique that is used to replace neuronal populations within the lesioned brain. Grafting embryonic tissue into the damaged areas of rat’s brain has been shown to restructure synaptic, neuro-chemical and behavioural deficits in rat models of neurological dysfunction such as Parkinson’s disease (PD) and Huntington’s disease HD (Dunnett, 1992). Striatal tissue grafted within the damaged striatum partially restores an appropriate anatomical distribution of neuro-chemicals, decreases lesion induced hyperactivity and reintegrates into the Striatal circuitry (Dunnett et al., 1988, Björklund, 1992). Therefore the use of embryonic tissue for grafting can provide a functional recovery to the damaged brain.

The most common symptom of human MCAO is total or partial inability to move one side of the body and the loss of sensation on one side of the body. The most common behavioural tests for rodents with stroke were designed to examine the differences in function between the intact (ipsi-lateral) and impaired (contra-lateral) side of the body using asymmetry tests (Schallert et al., 2003, Ungerstedt et al,1968). This paper applies a 3D marker based motion analysis (3DMA) technique which has been shown to work for a cohort of PD models Madete et al., 2010 and 2011 where temporal and postural gait parameters were successfully processed and discussed.

Behavioral and functional studies that involve the use of animal models to study diseases that affect motor abilities, such as PD and stroke, have been mainly non-automated and subjective (Whishaw et al., 2002, ;Schallert and Hall, 1988). This study approaches animal data acquisition from a biomechanics point of view using 3DMA techniques. By calculating
3D co-ordinate data of reflective markers placed at intrinsic locations on the subject, it’s possible to analyse time and distance variables from the position and location of segments and bodies during gait.

We investigated temporal and postural patterns in three groups of rats: MCAO rats, Grafted rats and healthy control rats. Temporal gait (stance time, swing time, stride length, speed and cadence) and posture variables were analysed by measuring rotations in the three Cartesian axes: X-axis known as ‘Roll’; Y-axis known as ‘Pitch’ and the Z-axis known as ‘Yaw’.

The effect of MCAO lesion and embryonic grafting on gait was investigated, and a group of naive controls were also tested for age matched comparisons. In the present study, we extend and validate our initial description of the 3D movement analysis method by providing a detailed analysis of the temporal and postural adjustments made by animal model in traversing the beam and characterising quantitatively the movement deficits exhibited by the animals with MCAO lesions.

**Methods**

Initially, all 50 rats were habituated to walk along the beam following which marker based 3D digital video recordings, using a seven Qualisys PRO-reflex optoelectronic camera system (Qualisys, Sweden), were captured as described. This was the first motion analysis session (MA1). After MA1, 40 of the rats underwent MCAO surgery and the remaining 10 rats were used as naive control rats. The surgeries were carried out by a licensed member of staff at the Cardiff Brain Repair Group. 24 hours later they had MRI scans that excluded those rats that did not exhibit a lesion, or showed signs of haemorrhage. 23 rats had suitable lesions and were included in the further study. Seven to 12 days after the MCAO surgery 10 of the 23 lesioned rats received embryonic grafts. Six weeks after the MCAO surgery the rats were re-trained on the beam and a second MA beam walking trial was performed (MA2).

**Middle cerebral artery occlusion surgery**

All procedures were carried out in accordance with the United Kingdom Animals (Scientific Procedures) Act, 1986. 50 adult male Wister rats were used in this experiment (Harlan, UK). Rats were anaesthetised and the rat's core body temperature was kept at 36.7 ± 1°C. The filament (390 or 410μm, Doccol Company, USA) was inserted and the loose suture was tightened around the filament to allow release of the micro clip. The filament was removed after 30 minutes, the micro clip was replaced. The incision in the CA was sealed with
electrocoagulation using bipolar diathermy probes (Aesculap, Germany) attached to a cautery unit (Diathermo MB122, Veterinary Instrumentation, UK), prior to release of all sutures so that complete reperfusion of all vessels was achieved.

**Grafting**

7-12 days following MCAO surgery, 10 of the 23 rats received grafts of E14 whole ganglionic eminence tissue. The embryos were removed and the whole ganglionic eminence was carefully dissected out, as done previously (Björklund, 1992 S.B., 1992. The tissue pieces were then dissociated into cell suspensions as described in (Björklund, 1992 S.B., 1992). 500,000 cells, in a 2µl solution were injected into the lesioned hemisphere, using a 10µl Hamilton microsyringe connected to a thin-walled widebore needle (dia = 0.25mm). The rats were anesthetized with isoflurane (Abbott, Queensborough, UK) and were stereotactically injected unilaterally with the cells. The coordinates were set according to bregma and dura: tooth bar -2.3, anterior / posterior +1.4, lateral -3, dorso-ventral -4 and -4.5. Injection volume was 2 µl and the injection rate was 1 µl over 90 seconds, with 1µl deposited at each depth. The needle was left in place for 3 min before withdrawal, cleaning and suturing of the wound. Paracetemol (Boots, UK) was provided in the drinking for 3 days after surgery to assist with pain relief.

**3D motion Data collection and processing**

Temporal and postural gait parameters were obtained and subsequent classification analysis was performed for 30 Wister rats. The rats were divided into three different cohorts: 10 naive controls, 10 that had undergone MCAO lesion and 10 that received an embryonic graft following lesion. This paper presents outputs from data following two beam walking trials; MA1, carried out on all the rats before any surgery and MA2, carried out six weeks later following the MCAO lesion surgery and grafting.

Temporal gait parameters were quantified by calculating the position vectors of the markers attached to the four limbs of the rat as described in Madete et al., 2010. The data was input into in house software that calculated the stance time, swing time, stride length, speed and cadence for comparisons between the control, lesioned and grafted cohorts while walking along a WD elevated beam.

Postural gait was quantified by calculating the rat’s body displacement and orientation using Euler angles from markers placed on appendicular parts of the rat’s skeletal structure as described in Madete et al., 2011. These markers effectively define a “rigid body” attached to
the trunk and enable six degrees of freedom (6DOF) calculation as displacement angles defined as the roll, pitch and yaw.

An analysis of variance (ANOVA) was used to compare the mean kinematic data between the three groups of rats. Subgroups were categorized based on side of lesion (left versus right), time point (before or after stroke), surgery (lesion, graft and control). All variable were compared statistically between using an ANOVA (p<0.05) where \( F_{1,\text{degrees of freedom}} \) followed by a SIDAK post hoc test. An ICC was carried out

The inter-trial repeatability of gait parameters was calculated by the one-way random intra-class correlation coefficient (ICC), using one way ANOVA (Oken et. al., 2008; Shwatrts et al., 2004; Rankin et. al., 1998). The evaluation criteria and standards for ICC values are accepted as follows: values ≥0.75 represent excellent repeatability, 0.4–0.74 represents adequate repeatability, and values ≤0.40 represent poor repeatability.

**Results**

Temporal Postural data were acquired as the rats were walking along the beam. Stance time, swing time, stride length, speed, cadence roll, pitch and yaw of the body axes were analysed by means of 3D video analysis calculating the variable and the postural range of motion.

**Temporal Gait Parameters**

The data was recorded as the average values of two walking trials to test the hypotheses that: There is no difference in gait variables between the two test points (MA1 and MA2) and that there are no differences in gait between the three cohorts (controls, lesioned and grafted) and between the right and left limbs. To observe the interactions related to age, lesion and graft, all the data was analysed together using an ANOVA. This also allowed to comparisons of each rat back to its own baseline performance in MA1. Following an ANOVA, grafted data from MA1 and MA2 was eliminated from the overall statistical analysis because initial results revealed that there was insufficient data for accurate evaluation of interactions between the three groups. The ANOVA therefore was carried to compare the control and lesioned rats for the two test points (MA1 and MA2). The performance was stable across test points for all the variables, with the exception of the fore and hind limb swing times, the difference was significantly lower for MA1 data for both the control and lesioned groups (p<0.05).
**Comparing two test points**

This section compares the data from MA1 and MA2. The mean and standard mean errors (SME) for the temporal gait parameters of the rat models and their controls were recorded in Table 1. The data illustrates the impact of age, MCAO lesion surgery and embryonic grafting on gait.

The main observations comparing the data from MA1 and MA2 were as follows:

1. POST_CN cohort walked with fewer steps per minute, a longer stance time, a longer swing time and shorter stride length on each limb compared to the PRE_CN cohort.
2. POST_LE cohort walked with slower gait, fewer steps per minute, a longer stance time and a longer swing time for each limb with the exception of RBL compared to the PRE_LE cohort.
3. POST_GRa cohort walked slower, fewer steps per minute, a significantly longer stance time, a significantly longer swing time on the RBL and the LFL and a significantly shorter stride length was recorded for the RBL as compared to the PRE_GRa cohort.

To observe the interactions related to age, lesion and graft, all the data was analysed together using an ANOVA. This also allowed to comparisons of each rat back to its own baseline performance in MA1. Following an ANOVA, grafted data from MA1 and MA2 was eliminated from the overall statistical analysis because initial results revealed that there was insufficient data for accurate evaluation of interactions between the three groups. The ANOVA therefore was carried to compare the control and lesioned rats for the two test points (MA1 and MA2). The performance was stable across test points for all the variables, with the exception of the fore and hind limb swing times, the difference was significantly lower for MA1 data for both the control and lesioned groups (p<0.05).
Table 1: Kinematic parameters of the rats for MA1 (PRE_prefix) and MA2 (POST_prefix) showing temporal and postural gait parameters for the CN; LE and GRa cohorts while walking along the beam. Where LFL= left fore- limb, LHL= left hind limb, RFL= Right fore- limb, RHL= right hind limb. The results are expressed as mean ± SME of each cohort.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Limb</th>
<th>PRE_CN (n=10)</th>
<th>PRE_LE (n=10)</th>
<th>PRE_GRa (n=7)</th>
<th>POST_CN (n=10)</th>
<th>POST_LE (n=10)</th>
<th>POST_Gra (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadence (steps per minute)</td>
<td>Average</td>
<td>251.50±21.66</td>
<td>242.50±17.70</td>
<td>267.20±15.24</td>
<td>258.20±32.29</td>
<td>261±12.89</td>
<td>315.20±30.62</td>
</tr>
<tr>
<td>Speed/cm/s</td>
<td>Average</td>
<td>28.72±0.38</td>
<td>26.21±0.17</td>
<td>35.33±0.47</td>
<td>26.91±0.30</td>
<td>26.41±0.26</td>
<td>26.23±0.29</td>
</tr>
<tr>
<td>Stance time/ ms</td>
<td>LHL</td>
<td>218.50±30.65</td>
<td>255.83±37.23</td>
<td>181.00±12.98</td>
<td>259.67±48.20</td>
<td>242.50±27.33</td>
<td>255.67±30.18</td>
</tr>
<tr>
<td></td>
<td>RHL</td>
<td>217±22.25</td>
<td>266.17±41.22</td>
<td>185.17±16.77</td>
<td>258±41.72</td>
<td>263.67±23.63</td>
<td>188.50±25.45</td>
</tr>
<tr>
<td></td>
<td>LFL</td>
<td>231.83±30.32</td>
<td>258.33±28.88</td>
<td>147.67±8.70</td>
<td>273.67±43.27</td>
<td>250.83±15.20</td>
<td>253.83±21.78</td>
</tr>
<tr>
<td></td>
<td>RFL</td>
<td>257.50±34.77</td>
<td>248.33±27.55</td>
<td>162.17±14.78</td>
<td>296±41.01</td>
<td>265.00±22.20</td>
<td>271.50±15.67</td>
</tr>
<tr>
<td>Swing time/ms</td>
<td>LFL</td>
<td>185.17±11.60</td>
<td>182±14.64</td>
<td>162.00±13.01</td>
<td>210.67±17.83</td>
<td>215.83±12.47</td>
<td>208.50±11.77</td>
</tr>
<tr>
<td></td>
<td>RFL</td>
<td>168±17.14</td>
<td>185.83±13.64</td>
<td>146.33±19.37</td>
<td>207.33±21.23</td>
<td>217±9.67</td>
<td>207.17±9.77</td>
</tr>
<tr>
<td></td>
<td>LHL</td>
<td>199.83±17.98</td>
<td>156.3±11.42</td>
<td>127.33±11.28</td>
<td>209±30.55</td>
<td>220.50±20.62</td>
<td>301.50±30.68</td>
</tr>
<tr>
<td></td>
<td>RHL</td>
<td>196±25.67</td>
<td>177.50±18.25</td>
<td>165.33±20.55</td>
<td>234±21.48</td>
<td>209.50±17.58</td>
<td>287.67±21.88</td>
</tr>
<tr>
<td>Stride length/mm</td>
<td>LFL</td>
<td>164.80±10.74</td>
<td>156.30±4.57</td>
<td>148.20±2.43</td>
<td>157.80±6.86</td>
<td>155.30±12.25</td>
<td>151±6.17</td>
</tr>
<tr>
<td></td>
<td>RFL</td>
<td>159.60±12.03</td>
<td>148.40±4.21</td>
<td>146.50±3.40</td>
<td>151±8.70</td>
<td>157.20±7.01</td>
<td>149.10±7.65</td>
</tr>
<tr>
<td></td>
<td>LHL</td>
<td>162.70±11.09</td>
<td>144.20±3.92</td>
<td>123.20±14.95</td>
<td>148±9.60</td>
<td>148.40±7.96</td>
<td>157.90±8.55</td>
</tr>
<tr>
<td></td>
<td>RHL</td>
<td>161.20±13.43</td>
<td>145.10±6.07</td>
<td>160.90±7.79</td>
<td>159.80±7.08</td>
<td>143.60±10.12</td>
<td>147.90±9</td>
</tr>
</tbody>
</table>
**Comparing the three cohorts**

This section investigates the impact of MCAO lesion surgery and graft on gait compared with an age-matched control cohort (POST_CN); and the impact of grafting compared to lesion on gait. The mean and SME for the temporal gait parameters of the rat models and their controls were recorded in Table 1. The main observations were as follows:

1. The POST_LE walked slower, fewer steps per minute; longer stance, longer swing times; and a shorter stride length compared with POST_CN cohort.
2. The POST_GRa walked slower, fewer steps per minute, a shorter stance time, a longer swing time as well as a shorter stride length compared with POST_CN cohort.
3. The POST_LE walked slower, fewer steps per minute, a longer stance time, shorter swing time and a shorter stride length compared with POST_GRa cohort.

Similarly a MANOVA was carried out to compare the POST_CN and POST_LE data. There was no interactions that suggested effect of lesion on temporal gait parameters gait following a MANOVA: (cadence, \( F_{1,17} = 0, P=n.s; \) speed, \( F_{1,17} = 0.29, P=n.s; \) fore limb stance time, \( F_{1,17} = 0.14, P=n.s; \) hind limb stance time, \( F_{1,17} = 0.09, P=n.s; \) fore limb swing time, \( F_{1,17} = 0.31, P=n.s; \) hind limb swing time, \( F_{1,17} = 1.24, P=n.s; \) fore limb stride length, \( F_{1,17} = 0.22, P=n.s; \) hind limb stride length, \( F_{1,17} = 1.74, P=n.s. \) )

**Comparing the two sides: Left and right**

A MANOVA revealed that there was no effect of lesion on the right and left side therefore, no asymmetry was observed for the temporal gait parameters following lesion. (Fore-limb stance time, \( F_{1,17} = 1.70, P=n.s; \) hind limb stance, \( F_{1,17} = 0.38, P=n.s; \) fore limb stride length, \( F_{1,17} = 1.24, P=n.s; \) hind limb stride length, \( F_{1,17} = 0.59, P=n.s; \) fore limb swing time, \( F_{1,17} = 0.14 P=n.s; \) and hind limb swing time= \( F_{1,17} = 0.963, P=n.s)\)

**Postural parameters (ROM)**

The rotation angles of a rigid body are defined as: rotation around the X-axis is called roll; rotation around the Y-axis is called pitch; rotation around the Z-axis is called yaw. Positive rotation is defined as clockwise when looking in the direction of the axis. The angles are applied to the local reference system of the rigid body in the order: roll, pitch then yaw. These rotation angles are defined starting with the 6DOF body which is in alignment with the global reference system. The postural rotations of the defined rigid body are illustrated in
The roll, pitch and yaw are representative of body rotations of the rat during gait defined in Table 2.

**Table 2: Definitions of roll pitch and yaw**

| Roll | Motion of the body during walking in the axis parallel to the beam, x-axis.
|------|-------------------------------------------------------------------
|      | On the plane perpendicular to the beam, z-y plane
|      | showing the body rotation of the rat form side to side
| Pitch | Motion of the body during walking in the axis perpendicular to the beam, y-axis
|      | On the plane parallel to the beam, x-z plane.
|      | showing how much the animal is rotating the body up and down the beam
| Yaw  | Motion of the body during walking in the axis perpendicular to the beam, x-axis
|      | on the plane perpendicular to the beam, x-y plane
|      | showing body rotations from left to right as the rat walks to right

The displacements were recorded as average ROM of the roll, pitch and yaw angles. A MANOVA (without the GRa group) was carried out to compare the ROM values in Table 3 for the roll, pitch and yaw rotations. There was no effect of lesion on pitch, $F_{1,17} = 0.05$, $P=n.s.$ and yaw, $F_{1,17} = 2.36$, $P=n.s.$ and the ROM performance was stable across MA1 and MA2 ($Pitch= F_{1,14} =3.56$, $P=n.s.$ and $Yaw= F_{1,14} =3.77$, $P=n.s.$).

There was a significant difference between the CN and LE cohorts. The POST_LE cohort exhibited more roll, $F_{1,17} =15.18$, $P<0.01$ than the POST_CN cohort. There was also a significant difference in performance across MA1 and MA2 time points for the two cohorts independently ($F_{1,13} =5.14$) and after POST hoc SIDAK analysis, this effect showed interactions within the LE and CN cohorts $F_{1,13} =5.14$, $P<0.05$, with the control animals improving over the two test points, MA1 and MA2 ($T_{29.37} = 3.12$). The LE animals did not improve between MA1 and MA2 ($T_{29.37} = 0.20, p = n.s.$)
Table 3: Range of motion of the rats for MA1 (PRE_prefix) and MA2 (POST_prefix) showing postural gait parameters in terms of roll, pitch and yaw angles for the control (CN); Lesion (LE) and graft (GRa) cohorts while walking along the beam. The results are expressed as mean ± SEEM of each cohort.

<table>
<thead>
<tr>
<th>Variable</th>
<th>PRE_CN (n=10)</th>
<th>PRE_LE (n=10)</th>
<th>PRE_GRa (n=7)</th>
<th>POST_CN (n=10)</th>
<th>POST_LE (n=10)</th>
<th>POST_Gra (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roll/o</td>
<td>41.09±3.21</td>
<td>46.52±2.39</td>
<td>26.09±4.63</td>
<td>27.45±3.57</td>
<td>48.90±3.27</td>
<td>21.38±2.16</td>
</tr>
<tr>
<td>Pitch/o</td>
<td>4.33±0.40</td>
<td>5.09±0.98</td>
<td>9.35±1.68</td>
<td>6.53±1.12</td>
<td>5.99±0.87</td>
<td>9.71±0.93</td>
</tr>
<tr>
<td>Yaw/o</td>
<td>6.58±0.58</td>
<td>5.93±0.66</td>
<td>9.41±1.30</td>
<td>9.38±1.52</td>
<td>7.43±1.07</td>
<td>6.02±1.16</td>
</tr>
</tbody>
</table>

Classification simplex plots

A summary of the classification method is presented in Jones et al., 2004 and Whatling 2009. Rat gait data is transformed into a set of three belief values Body of Evidence (BOE):

1. A belief that the rat has normal gait (*one side of simplex plot*)
2. A belief that the rat has gait that is characteristic of a model of stroke (*other side of simplex plot*)
3. An associated level of uncertainty (*The center as denoted by (Θ)*).
To classify, the collected data was trained using the DST classifier and presented as simplex plots to visually represent the differences in gait data between the following pairs:

**POST CN versus PRE CN**

The control cohort achieved 6 out of 10 dominant classifications six weeks apart, where $m_c (\{POST\_CN\}) > m_c (\{PRE\_CN\}) + m_c (\{\Theta\})$ see Figure 1 with an out of sample accuracy 85%. Most of the $\{PRE\_CN\}$ cohorts, except for 2, were accurately classified with positioning of their simplex coordinates within their dominant classification region of the simplex plot.

Figure 1 Simplex co-ordinate representations of the BOEc for the PRE\_CN classified with the POST\_CN cohorts
**POST LE versus PRE LE**

The lesioned cohort after surgery achieved 7 out of 10 dominant classifications where $m_c(\{POST\_LE\}) > m_c(\{PRE\_LE\}) + m_c(\{\theta\})$ see Figure 2. MCAO Lesion surgery reduced normal gait function allowing for a classification with an out of sample accuracy 86.31%. Most of the \{PRE\_LE\} cohort, had 6 out of 9 subjects accurately classified with positioning of their simplex coordinates within their dominant classification region of the simplex plot.

![Simplex coordinate representations of the BOEc for the PRE\_LE classified with the POST\_LE cohorts](image)

**Figure 2:** Simplex co-ordinate representations of the BOEc for the PRE\_LE classified with the POST\_LE cohorts.
POST_GRa Versus PRE_GRa

After classification the data revealed that the grafted cohort achieved 9 out of 10 dominant classifications after surgery where $m_c (\{POST\_GRa\}) > m_c (\{PRE\_GRa\}) + m_c (\{\emptyset\})$ see Figure 3. Grafting affected gait function allowing for a classification with an out of sample accuracy of 88%. Most of the \{PRE\_GRa\} cohort, except for 1, was accurately classified with positioning of their simplex coordinates within their dominant classification region of the simplex plot.

![Simplex Co-ordinate Representations](image)

**Figure 3** Simplex co-ordinate representations of the BOEc for the PRE_GRa classified with the POST_GRa cohorts
POST_LE versus POST_CN

8 of the 10 rats in the lesioned cohort were accurately classified after surgery with an out of sample accuracy of 83.15% where $m_c (\{POST\_LE\}) > m_c (\{POST\_CN\}) + m_c (\{\emptyset\})$ see Figure 4. MCAO affected gait function when compared to an age matched control. 6 of the $\{POST\_CN\}$ cohort were accurately classified with positioning of their simplex coordinates within their dominant classification region of the simplex plot.

Figure 4: Simplex co-ordinate representations of the BOEc for the POST_CN classified with the POST_LE cohorts
**POST_GRa versus POST_CN**

5 out of the 10 grafted rats were accurately classified with an out of sample accuracy of 81% where $m_c \left( \{POST_{GRa}\} \right) > m_c \left( \{POST_{CN}\} \right) + m_c \left( \{\Theta\} \right)$ see Figure 5. Similarly, only 5 out of 10 $\{POST_{CN}\}$ rats were accurately classified with positioning of their simplex coordinates within their dominant classification region of the simplex plot.

![Figure 5: Simplex co-ordinate representations of the BOEc for the POST_CN classified with the POST_GRa cohorts](image-url)
**POST_GRa versus POST_LE**

Grafted cohort achieves 9 out of 10 dominant classifications with an out of sample accuracy of 91.84% where 
\[ m_c ([POST_GRa]) > m_c ([POST_LE]) + m_c ([\Theta]) \]  
see Figure 6. Similarly, 9 out of 10 {POST_LE} rats were accurately classified with positioning of their simplex coordinates within their dominant classification region of the simplex plot. Gait function changed after grafting when compared to lesion rats.

\[ \text{Figure 6: Simplex co-ordinate representations of the BOEc for the POST_LE classified with the POST_GRa cohorts} \]
A seventh classification (Figure 7) was performed to investigate the effect of the MCAO and grafting. After classification the data revealed that the grafted cohort achieved 9 out of 10 accurately classified rats with positioning of their simplex coordinates within their dominant classification region of the simplex plot. The Lesioned rats achieved 10 out of 10 rats classification with most of the control rats having 5 out of the 10 classified in the dominant grafted region the five in the dominant lesioned region.

Figure 7: Simplex co-ordinate representations of the BOEc for the POST_LE classified with the POST_Gra and POST_CN cohorts.
Discussion

This study applies a 3D marker based motion analysis (3DMA) technique which has been shown to work for a cohort of PD models (Madete et al. 2010) where temporal and postural gait parameters were successfully processed and discussed. Gait parameters during over-ground locomotion of MCAO, grafted Wister rats and their controls before and after surgery were quantified to further validate the developed protocol. The rats were part of a larger study that was aimed at looked at various behavioural characteristics of MCAO.

Gait parameter data was obtained for 27 rats before surgery (MA1) and 30 rats after surgery (MA2) to provide a measure of different behaviour and functional characteristics of all four limbs. Eight different variables, (cadence, speed, swing time, stance time, stride length, roll ROM, pitch ROM and yaw ROM) were tested for significant differences between the three cohorts to quantify the effects of training and age on gait; the effect of MCAO lesion surgery and graft on gait between the two testing time-points and; body asymmetry between the impaired and the healthy side within the lesion cohort.

Data was further classified using the DST classification method developed by Jones et al., (2004) to assess the outcome of the data collected. The DST uses mathematical probability to quantify objective data and provides a means of interpreting several data sets simultaneously. More importantly the DST helps to deal with conflicting data produced from MA by assigning levels of support to each measurement variable; taking each piece of evidence to classify the data presented. The roll ROM and swing time variables were used to train classifier as they were variables that were found to be significantly different within the cohorts.

Temporal and postural gait parameters

The most common behavioural tests for rodents with stroke were designed to examine the differences in function between the intact (ipsi-lateral) and impaired (contra-lateral) side of the body using asymmetry tests (Schallert et al., 2003) (Ungerstedt et al., 1968). Many of these tests were originally developed for other unilateral models of basal ganglia disorders, such as PD and HD disease.

The characteristics of human Ischemic stroke are very diverse since the location of the damage plays an important part in determining the observed symptoms (Van der Staay et al., 1996, Corbett and Nurse, 1998). Similar to PD patients, spatio-temporal gait and posture are affected in patients with stroke. Patients walk slower than the average healthy subjects; their gait cycle is characterised by a low stride length, lower cadence, longer stance phase,
toe-drag during the swing phase and exhibit increased double support times (De Bujanda et al., 2004, Olney et al., 1994, Shumway-cook and Woollacott, 1995). MCAO in the rat is used to mimic large vessel occlusion in humans however the behaviour exhibited by the rat is dependent on the location and size of the lesion.

MCAO rat models are used to understand stroke in humans and results vary from study to study, depending on the strain of rat used and the nature of the behaviour analysis carried out (Whishaw and Kolb, DeVries et al., 2001). Locomotor activity has been reported in previous studies to either increase or decrease in experimental stroke rats depending on the model of stroke used. (Shen and Wang, 2010, Tomac et al., 2002, Ji et al., 2007). In the current study, POST_ LE rats walked slower than POST_CN. Subsequently, stance times were longer and swing times were shorter, suggesting that although POST_LE stayed in stance for longer, thus affecting their speed, the increase in swing times meant that the speed difference was not significant.

The rat data between time-points, MA1 and MA2, show that the rats are older and are accustomed to the beam thus an overall improved orientation on the beam is observed for the control cohort. The control group also showed improvements in the roll ROM and in the swing times between trials MA1 and MA2. The control cohort learnt how to navigate the beam after training whereas the lesion rats did not show differences in gait function, therefore the control group were better at learning the beam after training than the lesioned rats.

A 30 minute MCAO surgery has been shown to increase swing time, but does not affect the other temporal gait parameters. The lesion cohort data between the two time points was to illustrate the impact of MCAO lesion surgery on gait. Quantitative analysis of postural instability during over-ground locomotion revealed a greater difference between healthy and MCAO rats. The sensitivity of the protocol allowed for quantitative assessment of angular changes of body rotations during gait. Lesioned rats did not show any asymmetry toward the contra-lateral side as expected, but revealed a high (not significant p<0.05) roll ROM when compared to the control cohort. It is useful to note that impairments are mainly manifested in the first few days after MCA occlusion; studies have shown that there is complete recovery after 3-4 weeks of testing (Corbett and Nurse, 1998). Classification demonstrated deficits that are not evident using other behavioural studies such as those used by (Shen and Wang, 2010).

Embryonic grafting is a technique that is used to replace neuronal populations within the lesioned brain. Grafting embryonic tissue into the damaged areas of rat's brain has been
shown to restructure synaptic, neuro-chemical and behavioural deficits in rat models of neurological dysfunction such as PD and HD (Dunnett, 1992). Therefore the use of embryonic tissue for grafting can provide a functional recovery to the damaged brain. The grafted rats should approach normal values compared to the lesion cohort. Although the results show improvements in gait following grafting (the grafted rats walk faster than the lesioned rats which is reflected with shorter swing, shorter stance time, increased cadence and increased stride length), these differences were not found to be significant. The graft cohort compared to the controls had significantly longer stance and swing phase times thus they walked with a slower gait and less steps per minute compared with gait before surgery. Therefore grafting affected normal gait function.

Classification

Classification of data sets between the two test points (MA1 and MA2 data) for the three cohorts showed accurate classification of the rats using swing times and roll ROM. This suggested that there are differences in gait due to training (accurate classification between the PRE_CN and POST_CN); there are differences in gait after MCAO lesion (accurate classification between the PRE_LE and POST_LE) and there are differences in gait after grafting (accurate classification between the PRE_GRa and POST_GRa). With all classifications having an accuracy of above 80% with more than seven out of 10 rats accurately classified on the dominant region of the simplex plot.

Classification between the three cohorts also revealed greater than 80% accuracy. A better classification is observed when comparing the POST_LE cohort with the POST_CN cohort than when comparing it with the POST_GRa data, with 9 out of 10 rats achieving a dominant classification in the POST_LE group and only 5 in the POST_GRa cohort. More rats from the POST_GRa cohort approached normal gait than the POST_LE cohort.

The results strongly validate the novel marker-based optoelectronic MA protocol developed in Madete et al. 2010 by demonstrating that it can provide an effective and simple approach to quantifying temporal gait parameters for rat models of stroke and the effect of grafting. Swing time and roll ROM indicative of postural adaptation strategies, were found to be stronger variables for the classification of stroke rats.

The data can be used as a basis for correlation with healthy human data based on a similar 3D MA in human subjects. The results of this study demonstrate the sensitivity of the MA protocol to quantify functional characteristics of the stroke model. They also reveal the use of a powerful classification tool that has allowed data analysis in terms of comparison between
the three surgical interventions and their controls. It has also allowed the relationship
between outcomes of the rats’ pre and post surgery and the use of important variable that
distinguish between MCAO function.

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