STUDIES IN THE NEURAL CONTROL OF AVIAN LOCOMOTION

By

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We accept this thesis as conforming to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

March 1989

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Date 23/03/89
This study examines aspects of the neural substrate for locomotion in birds. Electrical stimulation of mid- and hindbrain revealed three previously undefined brainstem regions from which locomotion was elicited in the decerebrate animal. The sites lie within the medial longitudinal fasciculus (MLF), intercollicular nucleus (ICo) and medial mesencephalic reticular formation (mMRF). These and previously defined avian locomotor regions were further examined utilizing the microinjection of agonists and antagonists to acetylcholine, GABA, the excitatory amino acids and Substance P to determine the locomotor effects of potential neurotransmitters at these sites. Cholinergic agonists were effective at eliciting locomotion when injected into the MLF, pontobulbar locomotor strip (PLS) and medullary reticular formation. GABAergic antagonists evoked locomotion when infused into the PLS, ICo and pontine and medullary reticular formation. NMDA injection into the PLS, MLF, mMRF and medullary reticular formation elicited locomotion or reduced the electrical stimulation threshold for locomotion, while Substance P injection evoked locomotion when injected into the pontine reticular formation. Phasic peripheral afferent input was found not to be essential for the production of an array of avian locomotor patterns when examined in the spontaneous, electrically stimulated and neurochemically stimulated paralyzed preparations. However, afferent feedback may have a role in setting the activation level required to initiate and set the frequency of locomotor patterns. The preservation of caudal diencephalic neural structures allowed spontaneous locomotion in
the high decerebrate bird, implicating the nucleus of the ansa lenticularis, subthalamic nucleus and lateral hypothalamic area as possibly modulating more caudal locomotor regions. Utilizing an integrated approach with the literature data collected from a variety of vertebrates, my results in birds suggest that locomotor-related neural pathways are highly conserved across a broad phylogenetic range.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>ii</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>iv</td>
</tr>
<tr>
<td>List of Tables</td>
<td>viii</td>
</tr>
<tr>
<td>List of Figures</td>
<td>ix</td>
</tr>
<tr>
<td>Table of Abbreviations</td>
<td>xi</td>
</tr>
<tr>
<td>Acknowledgement</td>
<td>xvi</td>
</tr>
<tr>
<td><strong>Chapter 1 - Review of the Literature and General</strong></td>
<td>1</td>
</tr>
<tr>
<td>Introduction</td>
<td>2</td>
</tr>
<tr>
<td>Review of the Literature</td>
<td>5</td>
</tr>
<tr>
<td>Spinal Cord</td>
<td>9</td>
</tr>
<tr>
<td>Cortex</td>
<td>11</td>
</tr>
<tr>
<td>Lateral Vestibular Nucleus and Tectum</td>
<td>14</td>
</tr>
<tr>
<td>Red Nucleus</td>
<td>15</td>
</tr>
<tr>
<td>Reticular Formation</td>
<td>18</td>
</tr>
<tr>
<td>Locomotor Regions and Locomotion-Related Structures</td>
<td>18</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>20</td>
</tr>
<tr>
<td>Pontobulbar Locomotor Strip</td>
<td>23</td>
</tr>
<tr>
<td>Mesencephalic Locomotor Region</td>
<td>29</td>
</tr>
<tr>
<td>Subthalamic Nucleus and Subthalmatic Locomotor Region</td>
<td>31</td>
</tr>
<tr>
<td>Basal Ganglia</td>
<td>32</td>
</tr>
<tr>
<td>Limbic Structures</td>
<td>34</td>
</tr>
<tr>
<td>Conclusions and Purpose of Studies in the Thesis</td>
<td>41</td>
</tr>
<tr>
<td>The Decerebrate Preparation</td>
<td>46</td>
</tr>
<tr>
<td>Nomenclature</td>
<td></td>
</tr>
<tr>
<td><strong>Chapter 2 - Electrical Stimulation of Mesencephalic and</strong></td>
<td>48</td>
</tr>
<tr>
<td>Pontine Regions Elicits Locomotion in Decerebrate Birds</td>
<td></td>
</tr>
<tr>
<td>Introduction</td>
<td>49</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>52</td>
</tr>
<tr>
<td>Figure 3</td>
<td>54</td>
</tr>
<tr>
<td>Results</td>
<td>58</td>
</tr>
<tr>
<td>Figure 4</td>
<td>59</td>
</tr>
<tr>
<td>Figure 5</td>
<td>61</td>
</tr>
<tr>
<td>Figure 6</td>
<td>64</td>
</tr>
<tr>
<td>Figure 7</td>
<td>67</td>
</tr>
<tr>
<td>Figure 8</td>
<td>71</td>
</tr>
<tr>
<td>Discussion</td>
<td>73</td>
</tr>
<tr>
<td>Conclusions</td>
<td>79</td>
</tr>
<tr>
<td>Chapter 3 - Characterization of Avian Mid- and Hindbrain Sites that Produce Locomotion with Local Intracerebral Infusion of Neurotransmitter Agonists and Antagonists (I): Acetylcholine.</td>
<td>81</td>
</tr>
<tr>
<td>Introduction</td>
<td>82</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>85</td>
</tr>
<tr>
<td>Results</td>
<td>88</td>
</tr>
<tr>
<td>Table 1</td>
<td>89</td>
</tr>
<tr>
<td>Figure 9</td>
<td>90</td>
</tr>
<tr>
<td>Figure 10</td>
<td>92</td>
</tr>
<tr>
<td>Figure 11</td>
<td>96</td>
</tr>
<tr>
<td>Figure 12</td>
<td>99</td>
</tr>
<tr>
<td>Figure 13</td>
<td>101</td>
</tr>
<tr>
<td>Figure 14</td>
<td>105</td>
</tr>
<tr>
<td>Discussion</td>
<td>108</td>
</tr>
</tbody>
</table>

| Chapter 4 - Characterization of Avian Mid- and Hindbrain Sites that Produce Locomotion with Local Intracerebral Infusion of Neurotransmitter Agonists and Antagonists (II): γ-Aminobutyric Acid (GABA). | 133 |
| Introduction | 134 |
| Materials and Methods | 137 |
| Results | 138 |
| Table 2 | 139 |
| Figure 15 | 140 |
| Figure 16 | 143 |
| Figure 17 | 145 |
| Figure 18 | 148 |
| Figure 19 | 151 |
| Figure 20 | 154 |
| Discussion | 157 |

| Chapter 5 - Characterization of Avian Mid- and Hindbrain Sites that Produce Locomotion with Local Intracerebral Infusion of Neurotransmitter Agonists and Antagonists (III): Excitatory Amino Acids and Substance P. | 166 |
| Introduction | 167 |
| Materials and Methods | 168 |
| Results | 169 |
| Table 3 | 170 |
| Figure 21 | 171 |
| Figure 22 | 174 |
| Figure 23 | 176 |
| Figure 24 | 181 |
| Figure 25 | 187 |
| Figure 26 | 191 |
| Discussion | 195 |
# TABLE OF CONTENTS (CONTINUED)

<table>
<thead>
<tr>
<th>Chapter 6 - Avian Locomotion in the Absence of Phasic Afferent Input - The 'Fictive' Preparation</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>209</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>211</td>
</tr>
<tr>
<td>Results</td>
<td>213</td>
</tr>
<tr>
<td>Figure 27</td>
<td>214</td>
</tr>
<tr>
<td>Figure 28</td>
<td>216</td>
</tr>
<tr>
<td>Figure 29</td>
<td>219</td>
</tr>
<tr>
<td>Figure 30</td>
<td>222</td>
</tr>
<tr>
<td>Figure 31</td>
<td>224</td>
</tr>
<tr>
<td>Figure 32</td>
<td>226</td>
</tr>
<tr>
<td>Figure 33</td>
<td>229</td>
</tr>
<tr>
<td>Discussion</td>
<td>231</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chapter 7 - Transection Level Determines Spontaneous Motor Activity in the Decerebrate Avian Preparation</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>237</td>
</tr>
<tr>
<td>Table 4</td>
<td>238</td>
</tr>
<tr>
<td>Figure 34</td>
<td>239</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>241</td>
</tr>
<tr>
<td>Results</td>
<td>243</td>
</tr>
<tr>
<td>Figure 35</td>
<td>244</td>
</tr>
<tr>
<td>Figure 36</td>
<td>246</td>
</tr>
<tr>
<td>Figure 37</td>
<td>248</td>
</tr>
<tr>
<td>Discussion</td>
<td>253</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chapter 8 - Summary Discussion</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>258</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chapter 9 - List of References</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>273</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Appendix I</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>291</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Appendix II</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>294</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table 1 - Time course, latency and concentrations of acetylcholine agonists and antagonists  
Page 89

Table 2 - Time course, latency and concentrations of GABA, its agonists and antagonists  
Page 139

Table 3 - Time course, latency and concentrations of glutamate, its agonists and antagonists and Substance P  
Page 170

Table 4 - Cat decerebration levels.  
Page 238
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Chapter 1</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1. - Diagram of cat decerebration levels.</td>
<td>6</td>
</tr>
<tr>
<td>Figure 2. - Electromyographic activity, blood pressure and heart rate resulting from electrical stimulation in TTD</td>
<td>44</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chapter 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 3. - Diagram of avian experimental apparatus.</td>
<td>54</td>
</tr>
<tr>
<td>Figure 4. - Composite diagram of electrical stimulation sites in pons and mesencephalon.</td>
<td>59</td>
</tr>
<tr>
<td>Figure 5 - Stimulation sites on coronal sections in the avian brain.</td>
<td>61</td>
</tr>
<tr>
<td>Figure 6. - EMG records of locomotion elicited by MLF stimulation.</td>
<td>64</td>
</tr>
<tr>
<td>Figure 7. - EMG records of locomotion elicited by mMRF stimulation and affects of stimulation frequency.</td>
<td>67</td>
</tr>
<tr>
<td>Figure 8. - EMG records of locomotion elicited by ICo stimulation.</td>
<td>71</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chapter 3</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 9. - Composite diagram of cholinergic agonist &amp; antagonist neurochemical injection sites.</td>
<td>90</td>
</tr>
<tr>
<td>Figure 10.- Stimulation and injection sites on coronal sections in the avian brain</td>
<td>92</td>
</tr>
<tr>
<td>Figure 11.- EMG records of locomotor activity elicited by electrical stimulation and carbachol injection into the PLS.</td>
<td>96</td>
</tr>
<tr>
<td>Figure 12.- EMG records of locomotor activity elicited by electrical stimulation and carbachol injection into the Cnd.</td>
<td>99</td>
</tr>
<tr>
<td>Figure 13.- EMG records of locomotor activity elicited by electrical stimulation and carbachol injection into the Cnv.</td>
<td>101</td>
</tr>
<tr>
<td>Figure 14.- EMG records of locomotor activity elicited by electrical stimulation and carbachol injection into the MLF.</td>
<td>105</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chapter 4</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 15.- Composite diagram of GABAergic agonist and antagonist neurochemical injection sites.</td>
<td>140</td>
</tr>
<tr>
<td>Figure 16.- EMG records of locomotor activity elicited by electrical stimulation and picrotoxin injection into the PLS.</td>
<td>143</td>
</tr>
<tr>
<td>Figure 17.- EMG records of locomotor activity elicited by picrotoxin and bicuculline injection into the PLS.</td>
<td>145</td>
</tr>
</tbody>
</table>
LIST OF FIGURES (CONTINUED)

Figure 18.- EMG records of locomotor activity elicited by electrical stimulation of the PLS blocked by GABA infusion.  

Figure 19.- EMG records showing GABA-reversible locomotor activity elicited by picrotoxin injection into Cnv.  

Figure 20.- EMG records showing GABA-reversible locomotor activity elicited by electrical stimulation and picrotoxin infusion into RP.  

Chapter 5  

Figure 21.- Composite diagram of EAA & SubP agonist and antagonist neurochemical injection sites.  

Figure 22.- EMG records showing locomotor activity elicited by electrical stimulation and NMDA injection into the PLS.  

Figure 23.- EMG records showing GDEE-reversible motor elicited by electrical stimulation and NMDA injection into the Cnd.  

Figure 24.- EMG records showing GDEE-reversible locomotor activity elicited by electrical stimulation and NMDA injection into the Cnv.  

Figure 25.- EMG records showing locomotor activity elicited by electrical stimulation, Substance P and NMDA injection into the RP.  

Figure 26.- EMG and ENG records showing locomotor activity elicited by electrical stimulation and NMDA infusion into the MLF.  

Chapter 6  

Figure 27.- Bilateral alternating walking activity in a spontaneously locomoting bird before and after paralyzation.  

Figure 28.- Histogram of electrical stimulation-induced and spontaneous step frequency during pre-paralyzed and paralyzed 'fictive' stepping.  

Figure 29.- Bilateral alternating walking activity evoked by focal electrical stimulation of the hindbrain before and after paralyzation.  

Figure 30.- Co-activation of leg and wing activity evoked by focal electrical stimulation of the midbrain before and after paralyzation.  

Figure 31.- Bilateral synchronous flying activity evoked by focal electrical stimulation of the hindbrain before and after paralyzation.  

Figure 32.- Histogram of electrical stimulation-induced wingbeat frequency during pre-paralyzed and paralyzed 'fictive' flapping.
LIST OF FIGURES (CONTINUED)

Figure 33.- Bilateral 'fictive' hindlimb activity elicited by microinjection of carbachol and NMDA into the pons and medulla.

Chapter 7

Figure 34.- Diagram of a sagittal section through the cat brainstem showing neuraxis transection levels and locomotor sites important to motor control.

Figure 35.- Diagram of transection levels of the avian brain which permit or eliminate spontaneous locomotion in the decerebrate bird.

Figure 36.- Bilateral alternating walking activity in a spontaneously locomoting bird before and after paralyzation.

Figure 37.- EMG records showing spontaneous stepping and flying activity in a high decerebrate bird.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACh</td>
<td>acetylcholine</td>
</tr>
<tr>
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</tr>
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</tr>
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</tr>
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</tr>
<tr>
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</tr>
<tr>
<td>b.p.</td>
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</tr>
<tr>
<td>CA</td>
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</tr>
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</tr>
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</tr>
<tr>
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</tr>
<tr>
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</tr>
<tr>
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</tr>
<tr>
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</tr>
<tr>
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</tr>
<tr>
<td>ChAT</td>
<td>choline acetyltransferase</td>
</tr>
<tr>
<td>CHCS</td>
<td>corticohabenular and corticoseptal tract</td>
</tr>
<tr>
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</tr>
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</tr>
<tr>
<td>Cnd</td>
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</tr>
<tr>
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</tr>
<tr>
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</tr>
<tr>
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</tr>
<tr>
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</tr>
<tr>
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</tr>
<tr>
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</tr>
<tr>
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</tr>
<tr>
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</tr>
<tr>
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</tr>
<tr>
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<td>decussation of the brachium conjunctivum</td>
</tr>
<tr>
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</tr>
<tr>
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</tr>
<tr>
<td>DMA</td>
<td>dorsomedial anterior thalamic nucleus</td>
</tr>
<tr>
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<td>dorsomedial posterior thalamic nucleus</td>
</tr>
<tr>
<td>DSCT</td>
<td>dorsal spinocerebellar tract</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>DSD</td>
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</tr>
<tr>
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</tr>
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</tr>
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<td>GCT</td>
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</tr>
<tr>
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<td>HA</td>
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<td>hertz (cycles/second)</td>
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</tr>
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</tr>
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</tr>
<tr>
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</tr>
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<td>nucleus isthmi, pars parvocellularis</td>
</tr>
<tr>
<td>ITC</td>
<td>iliotibialis cranialis muscle (sartorius muscle)</td>
</tr>
<tr>
<td>i.v.</td>
<td>intravenous</td>
</tr>
<tr>
<td>KCl</td>
<td>potassium chloride</td>
</tr>
<tr>
<td>LC</td>
<td>locus ceruleus</td>
</tr>
<tr>
<td>LFM</td>
<td>supreme frontal lamina</td>
</tr>
<tr>
<td>LH</td>
<td>hyperstriatal lamina</td>
</tr>
<tr>
<td>LHA</td>
<td>lateral hypothalamic area</td>
</tr>
<tr>
<td>LITC</td>
<td>left iliotibialis muscle</td>
</tr>
<tr>
<td>LMD</td>
<td>dorsal medullary lamina</td>
</tr>
<tr>
<td>lMLR</td>
<td>lateral mesencephalic locomotor region (see CF)</td>
</tr>
<tr>
<td>LOCO</td>
<td>locomotion</td>
</tr>
<tr>
<td>LPA</td>
<td>lateral preoptic area</td>
</tr>
<tr>
<td>LPECT</td>
<td>left pectoralis muscle</td>
</tr>
<tr>
<td>LPG</td>
<td>locomotor pattern generator</td>
</tr>
<tr>
<td>LPO</td>
<td>parolfactory lobe</td>
</tr>
</tbody>
</table>
LVN lateral vestibular nucleus (Dieters' Nucleus)
LRF parvocellular (lateral) reticular formation
M molar
MesV mesencephalic trigeminal nucleus (see MNV)
MLd lateral mesencephalic nucleus, dorsal part
MLF medial longitudinal fasciculus
MLR mesencephalic locomotor region
mM millimolar
mMLR medial mesencephalic locomotor region (see PPN)
mMRF medial mesencephalic reticular formation
MNV mesencephalic trigeminal nerve nucleus (see MesV)
MPv mesencephalic nucleus, pars profundus
MRF mesencephalic reticular formation
MUSC muscimol
MV motor trigeminal nucleus
MW molecular weight
N neostriatum
NA nucleus accumbens
nAL subthalamic nucleus (named nucleus of the ansa lenticularis in birds)
NC caudal neostriatum
neurochemical a neurotransmitter, its agonist or antagonist
NICO nicotine
NR no response
nipride sodium nitroferricyanide
NMDA N-methyl-D-aspartate
NII oculomotor nerve
NV trigeminal nerve
NVI abducens nerve
NX vagus nerve
NXII hypoglossal nerve
OI inferior olivary nucleus (see IO)
OMd dorsal part, oculomotor nucleus
OMv ventral part, oculomotor nucleus
OT optic tectum
Ov ovoid nucleus
P pineal (Figure 32 only)
P pons
PaM paramedian nucleus
PDA cis-2,3-piperidine carboxylate
PECT pectoralis muscle
PH plexus of Horsley
PICRO picrotoxin
PILO pilocarpine
PLS pontobulbar locomotor strip
PMH posterior part, posterior hypothalamic nucleus
PMI paramedian internal thalamic nucleus
POA anterior preoptic nucleus
POM medial preoptic nucleus
PPN pedunculopontine nucleus (see mMLR)
PRESTIM
previous to stimulation
PRF
pontine reticular formation
PrV
principle trigeminal sensory nucleus
PT
pretectal nucleus
PVM
periventricular magnocellular nucleus

R
raphe nucleus
R
red nucleus (Figures 1 & 31 only)
Rgc
gigantocellular reticular formation
RGC
medullary gigantocellular reticular nucleus
RITC
right iliotibialis cranialis muscle
RL
lateral reticular nucleus
RP
caudal pontine reticular formation nucleus
RPECT
right pectoralis muscle
RPgc
gigantocellular part, pontine reticular nucleus
Rpc
pontine nucleus, parvocellular part
RPC
caudal pontine reticular nucleus
RPO
pontine reticular nucleus, oral part
Ru
red nucleus

S
solitary nucleus
SC
superior colliculus
SCE
external cellular stratum
SCI
internal cellular stratum
SCOP
scopolamine
SG
substantia gelatinosa
SGC
central gray stratum
SI
substantia innominata
SL
lateral septal nucleus
SLR
subthalamamic locomotor region
SM
medial septal nucleus
SN
substantia nigra
SNC
substantia nigra, pars compacta
SP
subpreptectal nucleus
SpL
lateral spiriform nucleus
SRCT
spinoreticulocerebellar tract
SSP
supraspinal nucleus
ST
subtrigeminal nucleus
STIM
stimulation
SV
trigeminal sensory nucleus
T
trapezoid body
TFS
trigeminal field stimulation
Th
thalamus
TO
olfactory tubercle
TPC
substantia nigra, pars compacta (named nucleus tegmentipedunculopontinus in birds)
TSM
septomesencephalic tract
TTD
descending trigeminal tract and nucleus
TU
tuberal nucleus
UDV
unidirectional ventilation
V  ventricle
VeD  descending vestibular nucleus
VeL  lateral vestibular nucleus (see LVN)
VeM  medial vestibular nucleus
VIP  vasoactive intestinal polypeptide
vm  ventromedial internal cerebellar nucleus
VS  trigeminal sensory nucleus (see PrV)
VSCT  ventral spinocerebellar tract
VTA  ventral tegmental area of Tsai

WGA-HRP  wheat germ agglutinin horseradish peroxidase

ZI  zona incerta

III  oculomotor nerve and nucleus
IV  trochlear nucleus
VI  abducens nucleus
VII  facial nucleus
X  dorsal motor nucleus vagus

↑TH  increased electrical threshold for locomotion
↓TH  decreased electrical threshold for locomotion

µA  microamp
µl  microliter
µM  micromolar
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ENDORSEMENT

It is my wish that no agency should ever derive military benefit from the publication of this thesis. Authors who cite this work in support of their own are requested to qualify similarly the availabiltiy of their results.
CHAPTER 1

REVIEW OF THE LITERATURE

AND

GENERAL INTRODUCTION
REVIEW OF THE LITERATURE

Over the last approximately 100 years a variety of techniques have been brought to bear on how the central and peripheral nervous systems exert control over the skeletal muscles which are the effectors of movement. Originally, techniques as simple as the isolation of various parts of the nervous system provided investigators with a good deal of information about what was being controlled by those regions. Lesion or ablation experiments are still used to eliminate certain inputs or outputs in an effort to delineate regional function following selective removal. Nevertheless, a variety of new techniques have generated a vast amount of information which may both elucidate and obfuscate, but will undoubtedly complicate, the understanding of the mechanisms of motor control subserved by the nervous system.

Powerful techniques such as localization of neurotransmitters with immunocytochemistry and their receptors with receptor autoradiography give us information regarding the neurotransmitters used by the neurons and their postsynaptic targets. Focal electrical stimulation has elucidated several brain regions which, when stimulated, evoke locomotor patterns in a variety of vertebrate species. Extra- and intracellular recording from selected neuronal populations provides information about the firing patterns and phase relationships of those cells in relation to other neuronal populations in addition to establishing hodological relationships, while retrograde and anterograde neuroanatomical tracing techniques
have increased the number of described pathways and attendant neural circuits to surprising numbers. As will be made clear from the summary of the literature below, a great deal remains to be elucidated regarding the pathways and mechanisms by which the central nervous system controls locomotion.

Vertebrates require the organization and integration of neural components at all levels of the neuraxis to perform their normal range of locomotor behaviours. The organization is essentially hierarchical, being least complex at the level of the spinal cord and most complex in the forebrain. The most rudimentary motor response occurs at the level of the spinal reflex where only two neurons, comprising the neural circuitry of the monosynaptic reflex arc, can subserve a relatively simple motor behaviour such as occurs in response to a simple mechanical stimulus (e.g. stretching of the intrafusal muscle fibres). More complex organization can also occur at spinal levels via networks of intrinsic spinal neurons (spinal locomotor rhythmic oscillator or pattern generator) which interact to produce rhythmic movement of the limbs. The most complex level of motor organization is found within and between nuclei of the hind-, mid- and forebrain which are responsible for the control of volitional (i.e. goal-directed) locomotion in response to external or internal stimuli.

Before discussing the hierarchical organization of central motor structures, it is first necessary to examine several of the types of preparation used in the study of motor control (see Figure 1, page 6). Historically, studies attempting to elucidate the motor function of different levels of the neuraxis initially
utilized selective removal or partition of portions of the brain to examine any deficits of motor capability which resulted from the loss of this neural circuitry (for review see Grillner, 1975; Wetzel and Stuart, 1976). A number of investigators found that ablation of the telencephalon (cerebral cortex - decorticate preparation) in species including fish (Bickel, 1900 cf. Grillner, 1975), frogs (Bickel, 1900, cf. Grillner, 1975) and, sub-primate mammals (Goltz, 1892 cf. Grillner, 1975) (see Figure 1, line A) did little to interrupt spontaneous locomotor behaviours (Grillner, 1975). Indeed, Hinsey et al. (1930), using chronic preparations, reported that even thalamic (Figure 1 - line B) and hypothalamic (Figure 1 - line C) cats and rabbits walked following surgery. In their preparation, if a transection was made from the rostral superior colliculus to the optic chiasm (see Figure 1 - line B), leaving a rostral portion of thalamus intact (pre-collicular preoptic preparation), the animals would walk spontaneously and display behaviours which resembled motivated activities. Brainstem transection leaving only the caudal third of the thalamus intact (thalamic preparation) (Figure 1, line C), however, produced animals which were more rigid and locomoted only with strong exteroceptive stimulation (Laughton, 1924). Later studies in chronic cats (Bard and Macht, 1958) demonstrated that animals with even more caudal transections could locomote spontaneously. However, transection of the neuraxis from the rostral border of the superior colliculus to the caudal border of the mammillary bodies (pre-collicular post-mammillary or mesencephalic preparation) (Figure 1 - line D) (Hinsey et al., 1930; Orlovsky
and Shik, 1976) eliminated any spontaneous locomotion.

The above preparations, which will be discussed more fully below, have enabled investigators to examine locomotion under more easily controllable conditions. The various manipulations performed include, for example, eliciting locomotion by electrical or neurochemical stimulation of specified regions of the brain, examining spontaneous locomotor patterns in paralyzed animals and examining motor related structures at levels of the neuraxis below the transection during controlled locomotor behaviours. Such studies have provided a great deal of information regarding the control of locomotion exerted by the central nervous system.

Spinal Cord

Historically, Freusberg (1874), Freusberg and Goltz (1874) and Sherrington (1910) were the first to study motor performance by isolating components of the motor system. After high spinal cord transection or decapitation, they found that cats and dogs

1 Selective electrical stimulation presumably affects voltage sensitive channels located on any portion of a neuron (e.g. terminal, axon, cell body) (for review see Hille, 1984), and therefore may effect a host of pre- and postsynaptic changes.

2 Injection of neurotransmitters, their agonists and antagonists (neurochemicals), presumably effect changes in the specific neurotransmitter receptors for which they are efficacious (for review, see Carpenter and Reese, 1981). Unlike electrical stimulation (see previous footnote), it is possible, using injection of selected neurochemicals, to localize the receptor sites at which the agonist or antagonist is effective. The selectivity of neurochemical binding is the basis for the autoradiographic localization of neurotransmitter receptors. Whether the neurochemical acts presynaptically, postsynaptically, or both, and the receptor changes which it elicits are dependent on a variety of factors not the least of which is receptor subtype. In vivo, the downstream effects of neurochemical-induced receptor activation/inactivation are dependent upon the neural circuitry.
Figure 1. Diagram of a saggital section through the cat brainstem showing neuraxis transection levels and locomotor sites important for the study of locomotor control. Transection levels are designated by letters A-E. Transection level: A - thalamic, B - precollicular premammillary (hypothalamic of Hinsey et al., 1930), C - precollicular postmammillary, D - precollicular post-occulomotor, E - midcollicular pre-occulomotor. Locomotor sites include the subthalamic locomotor region (SLR) and mesencephalic locomotor region (MLR). The hatched lines surrounding RPC and RGC represent the pontine and medullary reticular formation that are thought to be the major motor information projection systems to the spinal cord. Abbreviations: CM - mammillary body, CO - optic chiasm, IC - inferior colliculus, MLR - mesencephalic locomotor region, P - pons, R - red nucleus, RGC - medullary gigantocellular reticular nucleus, RPC - caudal pontine reticular nucleus, SC - superior colliculus, SLR - subthalamic locomotor region, T - trapezoid body, Th - thalamus, III - oculomotor nerve. See text for additional explanation. This figure is redrawn from: 1) Shik et al., 1968, 2) Orlovsky, 1970a, 3) Grillner and Shik, 1973 and 4) Wetzel and Stuart, 1976.
continued to step in a rhythmic alternating manner. This "spinal stepping" provided the first direct evidence that intact supraspinal influences were not essential for the production of the basic pattern of locomotion. Sherrington (1910) suggested that post-transection locomotion was dependent on reflex actions activated by peripheral (sensory) input. However, Graham-Brown (1911) disproved this suggestion by demonstrating that previously deafferented animals also performed stepping movements following spinal cord transection. Graham-Brown later (1914) hypothesized that a neural circuit within the spinal cord acts as an intrinsic rhythm oscillator capable of producing the spinal stepping in each limb (half-centre hypothesis). The oscillating circuit of each limb was postulated to interact with the rhythmic oscillators of other limbs to produce the rhythmic coordinated movements of locomotor progression (Grillner, 1975). The rhythm was postulated to be an intrinsic property of the interconnections of antagonistic nerve cells (Graham Brown, 1914).

More recently, Grillner and Zangger (1979) demonstrated that a spinal-transected paralyzed cat which is devoid of any rhythmic peripheral feedback would also produce patterns of alternating motor activity as recorded from peripheral nerve efferents. The electroneurograms (ENGs) (for complete abbreviations list, see pages xi-xv) recorded during such fictitious or "fictive" (Perret et al., 1972) locomotion showed the same pattern as the ENGs recorded during normal (unparalyzed) locomotion. Although the above findings have been generalized to a variety of vertebrates (for review see
McClellan, 1986), it has yet to be demonstrated that 'spinal stepping' can occur in the adult of any primate species (Eidelberg, 1981). Several investigators have hypothesized that the absence of 'spinal stepping' in adult primates results from an increased dependency of spinal stepping circuits on supraspinal influences (Eidelberg et al., 1981a,b; Grillner, 1975). Eidelberg et al. (1981a) postulated that, in primates, increased descending tonic facilitatory influences are necessary for the pattern generators to trigger output from spinal motorneurons.

Despite the inability to demonstrate 'spinal stepping' in primates, spinal cord pattern generators (rhythmic oscillators) that are capable of producing the rhythmic alternating output necessary to evoke locomotor activity have been found in all lower vertebrates examined (Grillner, 1975; McClellan, 1986). Spinal lesion studies in the lamprey and cat demonstrate that the neural circuits which comprise the generators can be localized to as few as 2-3 spinal segments, but the circuits themselves have yet to be fully characterized (Cohen and Wallen, 1980; Grillner and Zangger, 1979).

The generators are undoubtedly modulated by peripheral feedback during locomotion (Grillner, 1975). Indeed, Jordan and co-workers (Shefchyk and Jordan, 1985; Jordan, 1986) have postulated that flexor reflex afferents (FRA) and pathways descending from supraspinal levels may impinge on a common interneuron first described by Jankowska et al. (1967). However, afferents descending from supraspinal regions to the spinal cord pattern generators appear to be the major controlling factor for
the initiation and ongoing control of locomotion (Kuypers, 1982). The supraspinal nuclei thought to be involved in motor control which provide direct descending connections to the spinal cord include the cortex, red nucleus, lateral vestibular nucleus, tectum and pontine & medullary reticular formation (for review see Kuypers, 1982; Holstege & Kuypers, 1988).

Cortex

Telencephalic structures are known to be directly involved in motivational or volitional commands which elicit locomotor responses (Wetzel and Stuart, 1976). In mammals, the premotor and motor cortex of the precentral gyrus gives rise to descending corticospinal neurons of the pyramidal system (Kuypers, 1982). Stimulation of parts of the motor cortex elicit discharge in motoneurons innervating both proximal and distal limb muscles (Marsden et al., 1981; Kuypers, 1964). However, evidence suggests that these direct corticospinal connections are considerably more important to the highly fractionated muscle movements of the distal extremities than to contractions of the proximal limb muscles essential to basic locomotor patterns (e.g. walking) (Lawrence and Kuypers, 1968a,b; Kuypers, 1982).

High intensity electrical stimulation of pyramidal tract neurons has been shown only to disrupt locomotion (Orlovsky, 1972a), while stimulation at lower current strengths served to increase flexor or extensor activity during the appropriate portion of the step cycle (Shik et al., 1966; Orlovsky, 1972a;
for review see Armstrong, 1986). Bilateral pyramidotomy at the level of the caudal brainstem did not inhibit volitional locomotion in the monkey, but severely reduced the animal's ability to perform precise movements using the distal extremities (Lawrence and Kuypers, 1968a,b). In the chronic cat, lesion of the lateral corticospinal tract at midthoracic levels did not prevent recovery of hindlimb walking (Yu and Eidelberg, 1981). Also, Steeves and Jordan (1980) utilized a precollicular-postmammillary cat preparation to demonstrate that locomotion induced by stimulation of a region in the midbrain (mesencephalic locomotor region (MLR)) was not blocked by lesion of the lateral corticospinal tracts. In the precollicular postmammillary cat, the initiation of locomotion by stimulation of the MLR was unaffected by bilateral medullary pyramidotomy. However, electrical stimulation of pontine corticofugal fibres rostral to the lesion, which send collaterals to the reticular formation that are antidromically activated by the electrical stimulation (Shik et al., 1968; Orlovsky, 1972a), elicited locomotion which could be blocked by complete destruction of the MLR or mid-collicular transection (Shik et al., 1968). The above findings indicate that the corticofugal contribution to the initiation and control of voluntary locomotion, even in primates, may be mediated via the phylogenetically older brainstem structures of the extrapyramidal motor system.

Neuroanatomical investigations have demonstrated that non-mammalian vertebrates including fish, amphibians, reptiles, and birds lack direct telencephalo-spinal projections (Cabot et al., 1982 (bird); Woodson and Kunzle, 1982 (reptiles, turtle);
Leonard et al., 1979 (fish). Indeed, these animals exhibit few locomotor deficits following decortication when compared to intact animals (Leonard et al., 1979; Gabbott and Jones, personal communication). Further, sub-primate mammals, in which the corticospinal connections are less extensive than in primates, exhibit minimal locomotor impairment following cortical ablation (for review see Wetzel and Stuart, 1976; Grillner, 1975).

Lateral Vestibular Nucleus (LVN) (Dieters' Nucleus) and Tectum

The lateral vestibular nucleus (LVN) gives rise to descending axons which travel in the spinal cord ventrolateral funiculus and impinge on extensor motoneurons in the spinal cord (Pompeiano, 1984; Wilson and Yoshida, 1968). Orlovsky (1972b) found that vestibulospinal neurons arising predominantly from the LVN are rhythmically modulated during locomotion, exert facilitatory effects on extensor motoneurons, and that electrical stimulation of these cells or their descending axons yields the same facilitatory results during both rest and locomotion (Orlovsky, 1972a). Arshavsky et al. (cf. Arshavsky and Orlovsky, 1986) found that the rhythmical activity of vestibulospinal cells is removed by cerebellar ablation while removal of afferent peripheral input with paralyzing agents ('fictive' preparation) did not affect this rhythmical activity (Arshavsky and Orlovsky, 1986).

Wilson and Yoshida (1968) used electrophysiological techniques to show that the LVN's strongest linkage, which was
monosynaptic, occurred between vestibulospinal neurons and neck extensor motoneurons. They found that vestibulospinal fibres form synapses on some hindlimb extensor motoneurons (gastrocnemius-soleus) but could find no monosynaptic links to forelimb extensor neurons in cats. They were, however, able to demonstrate a strong polysynaptic connection between vestibular efferents and extensor motoneurons at all levels of the cord. Their findings support the view that LVN neurons receive tonic afferent labyrinthine input and in turn exert labyrinthine righting reflexes on neck musculature. They postulated that "the monosynaptic link to neck motoneurons is less subject to descending and segmental control than is the polysynaptic pathway", thus placing the lateral vestibular nucleus in an excellent position to modulate righting reflexes both at rest and during locomotion. Pompeiano (1984) suggested that postural adjustments of hindlimb muscles during labyrinth reflexes are mediated by the vestibulospinal pathway and that the "LVN exerts an excitatory influence on ipsilateral extensor motoneurons". Considering the lumbosacral influence of vestibulospinal neurons, he postulated that the vestibulospinal and reticulospinal neurons have synergistic influences that result from neck and macular vestibular input to groups of motoneurons innervating hindlimb muscles (Pompeiano, 1984).

The lateral vestibulospinal tract travels in the ventrolateral funiculus of the spinal cord. Several investigators, including Steeves and Jordan (1980) in the cat, Eidelberg et al., (1981b) in the monkey and Sholomenko and Steeves (1987) in the bird, found that lesions of the
ventrolateral funiculi abolished hindlimb locomotion in these animals, it appeared likely that the vestibulospinal pathway was essential for descending locomotor control. However, Yu and Eidelberg (1981) found that locomotor recovery could occur following bilateral vestibular nuclei lesions in chronically maintained cats, although their ability to walk at higher velocities on a treadmill was reduced compared to unlesioned or sham operated animals. Further, they found only a transitory reduction in joint extensor drive, particularly in the hindlimbs. They postulated that the vestibulospinal pathways arising from LVN play an adjunctive role in the control of the spinal cord pattern generators which control extensors during the step cycle. Jell et al. (1985) also lesioned the LVN bilaterally and were still able to elicit locomotion by MLR stimulation in the mesencephalic cat. They found no major deficits in either the amplitude or timing of flexor and extensor EMGs of the hindlimbs.

Taken together, the above findings implicate the descending LVN-spinal pathway in the control of both the righting reflexes via monosynaptic connections to neck motoneurons and the limb extensor postural muscles via a polysynaptic pathway. It therefore appears likely that the vestibulospinal projection plays its most significant role in the control of balance and posture during locomotion, while having little effect on the basic locomotor rhythm.

Similar to the role played by the vestibulospinal pathways in fine tuning locomotion, the tectum appears to influence motor behaviours in the cat (Huerta and Harting, 1982). The tectum has
been demonstrated to impinge monosynaptically on reticular formation neurons and, in addition, gives rise to the tectospinal tract. However, the tectospinal tract has been demonstrated to descend to only cervical spinal cord levels, thus presumably eliminating a role for this pathway in the control of hindlimb CPGs. The main role of the tectospinal tract is postulated to be in the "coordination of head and eye movements" (Huerta and Harting, 1982), and therefore may be involved in visual guidance during locomotion, but the pathway has not, to my knowledge, been postulated to affect the basic locomotor pattern.

Red Nucleus (Ru)

The red nucleus (Ru) gives rise to crossed rubrospinal fibres which facilitate flexor motoneurons via polysynaptic pathways (Hongo et al., 1969a,b) during the swing phase of locomotion (Orlovsky, 1972c). Cells in the nucleus are rhythmically active during swing (Orlovsky, 1972c) and electrical stimulation of the Ru enhances flexor activity during this phase of locomotion (Orlovsky, 1972a). The red nucleus receives major afferent inputs from the cerebellum, cerebral cortex (Orlovsky, 1972c), basal ganglia (Fanardzhyan and Sarkisyan, 1985) and dorsal column nuclei (Fanardzhyan and Sarkisyan, 1985). As in the case of the LVN, cerebellar ablation eliminates the Ru rhythmic activity during locomotion (Orlovsky, 1972c), while cortical ablation has little effect on its coincidence with the locomotor swing phase (Orlovsky, 1972c).
However, bilateral ablation of the red nuclei or rubrospinal tracts (Steeves and Jordan, 1980; Yu and Eidelberg, 1981; Shik et al., 1968; Ingram and Ranson, 1932; Jell et al., 1985; Sholomenko and Steeves, 1987b; Lawrence and Kuypers, 1968b) has little effect on locomotion in any chronic or acute animal studied. While the red nucleus appears to have little importance for the basic patterns of locomotion, Kuypers (1982) postulated that the rubrospinal tract adds a second level of resolution to the structures which subserve basic locomotor needs, particularly with respect to the independent movement of distal parts of individual limbs. Similarly, Fanardzhyan and Sarkisyan (1985) envisage the red nucleus as an important motor/sensory integration centre for the monitoring and correction of an initiated movement. It appears, then, that the red nucleus acts to "fine tune" motor control, but is not essential in the control of the basic motor rhythm.

Reticular Formation

Mid- and hindbrain reticular formation neurons are the origin of the reticulospinal pathway which plays an important role in the control of locomotion (Lawrence and Kuypers, 1968b; Eidelberg et al., 1981b; Eidelberg, 1981; Steeves and Jordan, 1980; Afelt, 1974; Steeves et al., 1987; Sholomenko and Steeves, 1987; Orlovsky, 1970a,b). Lesion studies in the monkey (Lawrence and Kuypers, 1968b; Eidelberg et al., 1981b), cat (Steeves and Jordan, 1980; Afelt, 1974; Eidelberg, 1981b), and bird (Sholomenko and Steeves, 1987b) demonstrate that interruption of
the reticulospinal pathway or ablation of its cells of origin severely impairs locomotor ability. Orlovsky (1970b), using intracellular recording in the acute decerebrate cat, found that a majority of reticular formation neurons were rhythmically modulated during: 1) spontaneous locomotion in the thalamic preparation, 2) MLR-electrically stimulated locomotion in the mesencephalic preparation and 3) subthalamic locomotor region (SLR)-stimulation in the thalamic preparation. He also established the existence of direct excitatory monosynaptic links between the regions stimulated (MLR or SLR) and reticular formation neurons. In addition, Orlovsky (1970a) found that electrical stimulation of the pyramids rostral to a pyramidal transection elicited locomotion in the precollicular postmammillary preparation that was mediated monosynaptically via antidromic activation of reticular formation neurons. Corticofugal neurons, therefore, presumably send collaterals to reticular formation neurons and may exert some degree of motor control via this pathway. Recent evidence shows that electrical stimulation of the reticular formation elicits locomotion in the cat (Mori et al., 1978; Garcia-Rill and Skinner, 1987b; Jordan, 1986; Noga et al., 1988), rat (Kinjo et al., 1988) and bird (Steeves et al., 1986).

Studies using the comparatively new approach of eliciting or blocking locomotor behaviour by injecting neurotransmitter agonists and antagonists into electrophysiologically identified locomotor regions has corroborated and extended previous findings. This approach yields two types of information. First, neurotransmitter agonists and antagonists are thought to act at
receptors (Goodchild et al., 1982). Receptors are found on cell bodies, dendrites and terminals but have not been localized to axons in appreciable numbers (Goodchild et al., 1982). Thus, activity evoked by direct intracerebral neurochemical infusion would likely be due to the activation of these receptors and not due to the stimulation of axons of passage which may traverse an electrical stimulation site. Second, the selective injection of these "locomotor" affecting neurochemicals leads to the characterization of receptor types, thereby aiding in the identification of neurons involved in the locomotor process. Chemical stimulation with various neuroactive agents injected into the reticular formation has demonstrated that locomotion can be activated by cholinergic agonists (Garcia-Rill et al., 1985; Sholomenko and Steeves, 1987a), GABAergic antagonists (Garcia-Rill et al., 1985; Sholomenko and Steeves, 1987a; Noga et al., 1988), excitatory amino acid agonists (glutamate, aspartate, NMDA) (Garcia-Rill et al., 1985; Sholomenko and Steeves, 1987a; Noga et al., 1988) and Substance P (Noga et al., 1988; Garcia-Rill et al., 1986).

The combination of results from lesion, stimulation and neurochemical injection studies strongly implicate reticulospinal neurons as being the origin of the major descending pathway controlling spinal cord rhythmic oscillators. However, the neurotransmitter(s) utilized by the reticulospinal pathway itself has yet to be determined (Jordan, 1986).
Locomotor Regions and Locomotion-Related Structures

Higher order brainstem structures that apparently do not have direct descending connections to the spinal cord locomotor generators or motoneurons have been implicated as driving and/or modulating the brainstem nuclei that give rise to the direct spinal pathways described above. Some of these structures do not have easily identifiable neuroanatomical substrates and have been physiologically identified as being involved in locomotion by virtue of the finding that electrical stimulation of these regions can produce locomotor alterations. Thus, controversy still exists over the exact location and hodological substrates for the actions observed with stimulation of the pontobulbar locomotor strip (PLS), the mesencephalic locomotor region (MLR) and the subthalamic (SLR) locomotor region. Other higher order structures such as the cerebellum, the limbic system and basal ganglia are also strongly implicated in motor control.

Cerebellum

The cerebellum has the remarkable attribute of receiving direct and indirect information from most peripheral receptors and from all of the CNS motor centres (Arshavsky and Orlovsky, 1986). After processing the data, it returns the integrated information back to all of the motor centres. Support for this supposition comes from Arshavsky's group who have found that the dorsal spinocerebellar tract (DSCT), ventral spinocerebellar tract (VSCT) and spinoreticulocerebellar tract (SRCT) relay
different types of information to the cerebellum (for review see Arshavsky and Orlovsky, 1986). The DSCT relays information concerning actual movements from the peripheral motor system and is silent in the absence of muscle contractions, while both the VSCT and SRCT transmit centrally generated neural information to the cerebellum even in the absence of rhythmic peripheral feedback such as is found in a fictive (paralyzed) preparation. Further, Arshavsky’s experiments support the hypothesis that these pathways carry information from central rhythmic oscillator elements rather than from output elements such as spinal motoneurons (Arshavsky and Orlovsky, 1986). The cerebellum also receives information concerning the visual system via the superior colliculus, the state of equilibrium via the vestibular system, the head and neck sensory system via the trigeminal system and from the cortex via the pontine nuclei. Its major hindbrain outputs include the reticular formation nuclei, red nucleus and LVN which give rise to the output pathways discussed above. The reticular formation, red nucleus and vestibular neurons all appear to be rhythmically modulated by output from the cerebellum, in whose absence, the rhythmicity and spontaneous activity of neurons was reduced (Orlovsky, 1970a,b; 1972a,c). In addition, the cerebellum also influences the cortex via the well characterized thalamic loop. Arshavsky and Orlovsky (1986) describe the cerebellum as an organ which can organize the interaction of a variety of locomotor synergisms. Thus, it can monitor information both from the

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3 Bernstein proposed the hypothesis of the multi-level system of control of movements. According to his hypothesis higher sections of the nervous system determine the chains of motor activity, the lower level ties movements to spatial coordinates. Still lower levels solve the motor problem as such by
environment and the current state of each locomotor synergism, "select essential data" from this information, and "regulate the transmission of signals from one part of the nervous system to another" (Arshavsky and Orlovsky, 1986).

Although the cerebellum appears to play a strong role in locomotor control, cerebellar ablation in the cat does not prevent: 1) spontaneous locomotion in the thalamic preparation, 2) locomotion initiated by electrical stimulation of the SLR in the thalamic preparation or, 3) locomotion in the mesencephalic preparation evoked by stimulation of the MLR (Orlovsky, 1970a,b; 1972a,b,c). The above results indicate that while cerebellar efferents can affect the quality of locomotor output via a variety of output pathways, the cerebellum is not essential for the genesis of the basic locomotor rhythm. This supposition does not, of course, negate the obvious importance of the cerebellum in the control of ongoing motor activity in the intact animal.

**Pontobulbar Locomotor Strip (PLS)**

The pontobulbar locomotor strip (PLS) was physiologically defined by the finding that electrical stimulation of this region elicited locomotion in the precollcular postmammillary cat (Shik and Yagodnitsyn, 1977; Mori et al., 1977). More recent studies have generalized the existence of the PLS to birds (Steeves et al., 1987) and to a wide range of vertebrate species (for review, see McClellan, 1986). The strip ranges along the organizing the necessary interaction of elements (muscles, joints, limbs) and operatively controlling this work." (cf. Shik et al., 1966). At each level, "the neuronal mechanisms controlling a given motor act" (Arshavsky and Orlovsky, 1986) may be described as a locomotor synergism.
extent of the descending tract and nucleus of the trigeminal nerve (Mori et al., 1977; Shik and Yagodnitsyn, 1977, 1978) rostrocaudally from the level of the trigeminal mesencephalic nucleus (MesV) to the caudal medulla (Mori et al., 1977; Shik and Yagodnitsyn, 1977, 1978). Although not in agreement with the region proposed by Garcia-Rill et al. (1986, 1987), Jordan (1986) postulated that the rostral head of the PLS (MesV) is synonymous with the medial mesencephalic locomotor region (mMLR) defined by Garcia-Rill and Skinner (1986) and is thought to project intranuclear interneurons to more caudal PLS neurons (Jordan, 1986). A recent review, (Garcia-Rill et al., 1986), however, designates the pedunculopontine nucleus (PPN) as the medial MLR. Evidence to supports this suggestion comes from neuroanatomical tracing studies which demonstrate that injection of retrograde tracers into the PLS label predominantly MesV neurons and only small numbers of PPN neurons, while anterograde neuroanatomical tracers injected into the mMLR label mainly ventromedial reticular formation structures (Garcia-Rill et al., 1983b).

The name PLS may be a misnomer because the strip also appears to extend continuously caudalward into the dorsolateral funiculus (DLF) of the spinal cord (Kazennikov et al., 1980, 1983a,b; Dubner and Bennett 1983; for review see McClellan, 1986). It has been postulated that the PLS may be a polysynaptic propriospinal pathway (Mori et al., 1977; Shik and Yagodnitsyn, 1978; Kazennikov et al., 1983a,b) whose cells of origin lie ventromedial to its descending fiber tract (Selionov and Shik, 1984). Garcia-Rill (1983) originally described the PLS as being
coextensive with Probst’s tract (i.e. trigeminal nuclear fibers coursing just ventral to the nucleus from rostral medulla to Cl (Garcia-Rill and Skinner, 1986)) which receives afferents from the mesencephalic trigeminal nucleus (Garcia-Rill et al., 1983b). Jordan and co-workers (Jordan, 1986; Noga et al., 1988) argue strongly that cell bodies in the lateral reticular formation and descending trigeminal nucleus constitute the PLS (Noga et al., 1988), and have a dual output both to downstream trigeminal (propriospinal) cells (Matsushita et al., 1981,1982) and to nuclei in the medial reticular formation (Steeves and Jordan, 1984). This hypothesis is supported by several observations including: 1) cooling of the ipsilateral medial reticular formation during locomotion evoked by electrical stimulation of the MesV/mMLR reversibly blocks locomotion (Shefchyk et al., 1984). This finding suggests a blockade of the pathway from mMLR to PLS to reticular formation to spinal cord between the PLS and reticular formation linkage, in addition to a block of the mMLR to reticular formation to spinal cord pathway between the mMLR and reticular formation. 2) cooling of the PLS abolishes locomotion produced by mMLR stimulation (Shefchyk et al., 1984). 3) PLS stimulated locomotion is not abolished by transection of the propriospinal PLS-DLF pathway at C2-C3 (Noga et al., 1988) and 4) exteroceptive stimulation of the trigeminal receptive field and a variety of other sensory afferents [e.g. pinna (Aoki and Mori, 1981) and flexor reflex afferent (FRA) stimulation, (Jankowska et al., 1967)] evokes locomotion (for review see Jordan, 1986; Noga et al., 1988). Both Jordan and co-workers (Jordan, 1986; Noga et al., 1988) and
Garcia-Rill and Skinner (1986) now suggest that the locomotion elicited by stimulation of the PLS-DLF system mimics that which results from afferent trigeminal input to this locomotor region. This places the descending trigeminal nuclear complex in a position very similar to that of the lateral vestibular nucleus in which afferent input from the periphery, which may be modulated by comparator systems such as the cerebellum, is able to alter motor output but is not responsible for the primary locomotor rhythm.

Recent studies utilizing neurochemical stimulation to elicit locomotion suggest that PLS/trigeminal stimulation-induced locomotion is under GABAergic inhibitory control (Noga et al., 1988). Noga et al. (1988) found that locomotion could be elicited by injection of the GABAergic antagonist picrotoxin into the PLS. Further, they found that trigeminal field stimulation, which alone was ineffective at eliciting locomotion, would evoke locomotion following picrotoxin injection into the PLS. Other neurochemicals, including glutamate and Substance P, also produced locomotor behaviour when injected into the PLS (Noga et al., 1988). However, the anatomical substrates through which these neurotransmitters affect locomotor control have yet to be determined.

Mesencephalic Locomotor Region (MLR)

The classical MLR was characterized by Shik et al. (1966) when they found that focal high frequency electrical stimulation
of a region later identified as lying in close proximity to the cuneiform nucleus (Shik et al., 1967) evoked locomotion in a mesencephalic cat preparation. They also found that variation of the stimulus parameters could modulate the frequency of stepping, force of stepping and gait, thereby making this preparation ideal for the study of motor control mechanisms. Later investigators have attempted to clarify the location and input/output relations of the MLR in a variety of animals including the monkey (Eidelberg et al., 1981), cat (Garcia-Rill et al., 1983a,b; Garcia-Rill, 1983; Steeves and Jordan, 1984), rat (Mogenson et al., 1985; Mogenson and Wu, 1986) and bird (Sholomenko and Steeves, in preparation; Webster and Steeves, in preparation). As previously stated, Orlovsky (1970a) found that the MLR had monosynaptic connections with descending reticulospinal neurons. Steeves and Jordan (1984), using anterograde autoradiographic tracing techniques ([3H]proline & [3H]leucine), demonstrated descending projections from the MLR to the ipsilateral (heavy projection) and contralateral (light projection) pontine and medullary reticular formation. They also found ascending projections to the more rostral cuneiform nucleus, inferior colliculus, superior colliculus, central tegmental field, ipsilateral periaqueductal gray, substantia nigra (both pars compacta and reticulata), fields of Forel and ventral tegmental area of Tsai (VTA). At diencephalic levels, terminals were found in nuclei which included the thalamic intralaminar nuclei, zona incerta, medial subthalamic nucleus and lateral hypothalamic nucleus. Electrophysiological and neuroanatomical tracing techniques demonstrated afferent
projections to the feline MLR from the pars reticulata of the substantia nigra, entopeduncular nucleus, central gray, nucleus of the ansa lenticularis, medial and lateral hypothalamus and central nucleus of the amygdala (Garcia-Rill et al., 1983a,b; Garcia-Rill, 1983). Anterograde transport studies using the tritiated amino acid [³H]leucine injected into the MLR gave slightly different results than those of Steeves and Jordan (1984). While Steeves and Jordan (1984) found a predominance of ipsilateral labelling in the pontine and medullary reticular formation, Garcia-Rill’s group (Garcia-Rill et al., 1983b) found a higher proportion of contralateral reticular formation labelling after injection. However, differences in projection laterality could be attributed to a more medial placement of the injection by Garcia-Rill’s group. In addition, Garcia-Rill et al. (1983b) found a projection from the mMLR to Probst’s tract which was not reported by Steeves and Jordan (1984). More recently however, Garcia-Rill and Skinner (1986, 1987b) reinterpreted this result such that the apparent MLR-Probst’s tract projection probably arises from the trigeminal mesencephalic nucleus which is known to send projections to the descending trigeminal nuclear complex (Garcia-Rill and Skinner, 1986). Combined with Orlovsky’s finding (1970a) that MLR neurons have monosynaptic connections with spinal projecting reticular formation neurons, it seems likely that the MLR, whatever its anatomical substrate, exerts control over motor function via its monosynaptic connection with the reticular formation.

Recently, two divisions of the MLR have been postulated. Thus, the classical MLR described by Shik et al., (1966) [now
called the lateral MLR (lMLR) [Jordan, 1986; Noga et al., 1988] and a MLR (PPN/MLR) located within the confines of the pedunculopontine tegmental nucleus (PPN) [Garcia-Rill, 1986] have been described. Support for the existence of the PPN/MLR also comes from studies in the rat, where electrical stimulation of the PPN evoked locomotion in the decerebrate animal [Garcia-Rill and Skinner, 1986] and increased motor activity in the intact freely moving animal [Mogenson et al., 1985; Brudzynski et al., 1988]. As will be discussed more fully below, Brudzynski et al. (1988) also described more lateral sites, possibly equivalent to the PPN/MLR, which evoke locomotion upon electrical stimulation but which show different characteristics with respect to chemical stimulation.

Mogenson et al. (1985), in reviewing neuroanatomical connections to the rat PPN, listed the nucleus accumbens, the subpallidal area (including the substantia innominata and lateral preoptic area), the zona incerta, the medial preoptic area, the subthalamic nucleus and the anterior hypothalamus/preoptic area as all sending direct connections to the PPN. They proposed that the accumbens, subpallidal and zona incerta projections to PPN may play a role in limbic ("motivational") control over locomotor behaviour.

In an attempt to evoke or block locomotion, Garcia-Rill et al. (1985) injected a variety of neurochemicals into the PPN/MLR of the mesencephalic cat. They found that injection of the GABAergic antagonists picrotoxin and bicuculline into the mMLR elicited locomotion which could be blocked by the infusion of GABA or muscimol at the same location. Further, they found that
glutamate (at high concentration) reduced the electrical threshold and acetylcholine was ineffective in eliciting locomotion. Also, studies from this group (Garcia-Rill et al., 1985; Garcia-Rill and Skinner, 1986) showed that Substance P injection into the mMLR elicited long lasting locomotion in the cat. In the intact freely moving rat, injection of picrotoxin and glutamate (Brudzynski et al., 1988) into the PPN/MLR significantly increased motor activity, while injection of the cholinergic agonist carbachol reduced motor activity (Brudzynski et al., 1988). Why glutamate was more effective in the rat than the cat remains to be elucidated, but several possible explanations may be given. First, glutamate is a natural neurotransmitter which is rapidly taken up or metabolized and unlike electrical stimulation, where current spread and therefore recruitment (activation) is both intensity dependent and instantaneous with the onset of stimulation, the diffusion of glutamate through the tissue to a sufficient number of cells to evoke locomotion or change threshold would likely occur more rapidly and with less degradation in the smaller more compact rat PPN than in the cat. Second, Garcia-Rill et al. (1985) used the mesencephalic preparation in which activation level plays a major role in the elicitation of locomotion (see Mori et al., 1982), while Brudzynski et al. (1986, 1988) utilized an intact freely moving animal in which the activation level was already high and in some cases was made even higher by the introduction of amphetamine into the nucleus accumbens. It is likely that in the intact animal with high baseline activation, the recruitment of a small number of locomotor modulating neurons will cause a
significant increase in locomotor activity, while in the mesencephalic preparation, the activation level must first be increased from a lower baseline before locomotion can be elicited. Brudzynski et al. (1988) also found that injections of the cholinergic agonist carbachol into the PPN reduced locomotion, while more dorsal and lateral injections into the region bordered by the cuneiform nucleus, PPN and periaqueductal gray, possibly coextensive with the 1MLR, increased locomotion in the freely moving animal. Both effects could be reversed by atropine injection into the same site. These different effects may reflect the previously discussed differential distribution of receptors and/or projecting fibres from the two MLRs. Unlike Brudzynski et al. (1988), Garcia-Rill et al. (1985) found no effect when acetylcholine was injected into the PPN/MLR of the mesencephalic cat preparation. Reasons for this discrepancy remain to be determined, however, the use of the shorter acting, quickly metabolized acetylcholine instead of the longer acting carbachol may be responsible for some of these experimental differences (Taylor, 1985a,b).

Putative nuclear origins of pathways impinging on the MLRs include the: 1) laterodorsal tegmental nucleus, which may send cholinergic (Brudzynski et al., 1988; Fibiger and Semba, 1988) and Substance P (Vincent et al., 1983) projections to the PPN, 2) substantia innominata and lateral preoptic area, which may send cholinergic projections to the PPN (Brudzynski et al., 1988), 3) intrinsic cholinergic neurons present in the PPN (Goldsmith and Van der Kooy, 1988), 4) Substantia nigra, pars reticulata, which appears to send a GABAergic input to PPN.
(Garcia-Rill and Skinner, 1986), 5) entopeduncular nucleus, which sends input to PPN (Garcia-Rill and Skinner, 1986) and 6) Nucleus accumbens, which sends input to the PPN and cuneiform nucleus (Garcia-Rill and Skinner, 1986). Other projections to the MLRs have been described above, but the type of neurotransmitters employed in these pathways remains to be elucidated.

Subthalamic Nucleus and Subthalamic Locomotor Region

The subthalamic nucleus and subthalamic region (including the posterior and lateral hypothalamus) also appear to be involved in motor control. While bilateral destruction of the subthalamus in an otherwise intact cat does not prevent locomotion (Haertig and Masserman, 1940), unilateral destruction of the subthalamic nucleus underlies hemiballismus in humans (Hammond et al., 1979). In addition, electrical stimulation of this region elicits alternating stepping movements in the intact (Siegel and Flynn, 1968), lightly anaesthetized (Waller, 1940; Haertig and Masserman, 1940), thalamic (Orlovsky, 1969b), and hypothalamic cat (Eldridge et al., 1985), as well as in the decerebrate monkey (Eidelberg et al., 1981b). Further evidence implicating the subthalamic region as being important to locomotion comes from the observation that animals with a transection of the neuraxis rostral to the subthalamic nucleus (a precollicular-premammillary decerebration) will spontaneously locomote, while a transection removing this nucleus (a precollicular-postmammillary decerebration) eliminates
spontaneous activity (Shik et al., 1967; Garcia-Rill and Skinner, 1986). Orlovsky (1969) found that stimulation of the subthalamic locomotor region (SLR), a site dorsomedial to, but not within, the subthalamic nucleus evokes treadmill locomotion in an acute thalamic cat. Further, he found that bilateral lesion of the MLR did not interrupt SLR-stimulated locomotion but did reduce the bouts of spontaneous locomotor activity associated with the thalamic preparation (Orlovsky, 1969). Also, decerebrate cats with bilateral destruction of the SLR can be made to run and walk with electrical stimulation of MLR (Shik et al., 1966; Sirota and Shik, 1973). Thus, the SLR appears to send efferents to both the MLR and reticular formation (Orlovsky, 1970b). Anatomically, the SLR appears to be coextensive with the zona incerta (ZI) and lateral hypothalamic area (LHA) which were labelled with the anterograde tracer PHA following injection into the rat substantia innominata (Mogenson et al., 1985). The ZI/LHA region, like the subthalamic nucleus, would be spared in a spontaneously locomoting precollicular-premammillary transection and may be damaged with the type of cerebrovascular accident associated with human hemiballismus. Also, the ZI receives afferent projections from the cortex, mesencephalic reticular formation and superior colliculus, while sending efferents to the PPN and basal ganglia (Mogenson and Wu, 1986). Mogenson and Wu describe the ZI region as being strategically located in a position to integrate information between the basal ganglia, limbic system and MLR (Mogenson and Wu, 1986). Recently, Eldridge et al. (1985) and Waldrop et al. (1988) have demonstrated that injection of the GABAergic antagonist
picrotoxin into the feline SLR evoked both actual and 'fictive' locomotion which could be blocked by muscimol (GABA$_A$ receptor agonist) infusion. This data implicates cell bodies or dendrites contained within the SLR in the activation of locomotion and contradicts the suggestion by Garcia-Rill and Skinner (1986) that electrical stimulation of the SLR activates basal ganglia-thalamic or entopedunculo-pedunculopontine nuclear en passant fibres. As will be discussed below, the region of the SLR may act as a step in the transduction of motor information from limbic systems (Mogenson et al., 1985).

**Basal Ganglia**

Damage to or degeneration of the basal ganglia nuclei has been shown to lead to movement disorders such as Parkinson's disease (Substantia nigra, pars compacta), and Huntington's chorea (caudate nucleus) (cf. Carpenter, 1978). Although these disorders constitute severe impairments to motor performance, the major deficits associated with these diseases appear to be of a postural or intentional nature (Wetzel and Stuart, 1976; for review see Garcia-Rill and Skinner, 1986).

Electrical stimulation of the globus pallidus produces inconsistent locomotor responses in the lightly anaesthetized cat (Waller, 1940). Monkeys, dogs and cats with bilateral ablation of various nuclei of the basal ganglia do not show incapacitating impairment or loss of coordination once locomotor progression has begun (Waller, 1940; Denny-Brown, 1966; Hinsey et al., 1930). Therefore, the basal ganglia, while important to
the initiation of voluntary locomotion, do not appear to affect the stepping mechanism itself (Martin, 1967) but probably modulate locomotor activity through connections with extrapyramidal motor circuitry including the PPN/MLR (Garcia-Rill and Skinner, 1986). In accordance with this information, the substantia nigra, pars reticulata has been shown to send a GABAergic projection to the PPN/MLR (Childs and Gale, 1983; McGeer et al., 1984; Garcia-Rill et al., 1985), while entopeduncular nucleus and globus pallidus GABAergic efferents also impinge on the PPN/MLR (Garcia-Rill and Skinner, 1986; McGeer et al., 1984). In addition, the basal ganglia-cortical loop, via the centromedian, ventral anterior and ventral lateral thalamic nuclei, may exert some influence over the MLR, as cortical efferents with an as yet unspecified neurotransmitter (possibly excitatory amino acids (EAA) such as glutamate) appear to connect with the PPN/mMLR (Garcia-Rill and Skinner, 1986).

Limbic Structures

Limbic structures have been implicated in motivational aspects of locomotor behaviours such as procuring food or escaping from predators (Brudzynski and Mogenson, 1985). The neural circuit underlying these behaviours is believed to be, in part, mediated via the PPN/MLR (Brudzynski and Mogenson, 1985). Two limbic regions, the hippocampal formation and amygdala, have been demonstrated to project to the subpallidal area [substantia innominata (SI) & lateral preoptic area (LPA)] and nucleus
accumbens (NA) in the rat (Mogenson et al., 1985; Mogenson and Wu, 1986, 1988). The amygdala appears to send a direct projection to the MLR (Garcia-Rill et al., 1983b). The NA sends GABAergic efferents to the pedunculopontine and cuneiform nuclei (Garcia-Rill and Skinner, 1986; Jones and Mogenson, 1980) and also to the subpallidal region (Mogenson et al., 1985). The subpallidal region, in turn, sends a projection to PPN (Mogenson et al., 1985). The subpallidal region also projects to the ZI/LHA (SLR) which, in turn, sends efferents to PPN (Mogenson et al., 1985). Evidence to support the importance of this limbic motor circuit comes from a variety of studies using the freely moving rat. These studies have found that: 1) the substantia nigra, pars compacta sends a dopaminergic projection to NA. Intra-accumbens injection of dopamine or agonists that increase NA dopamine release (e.g. amphetamine) increased locomotor activity (Brudzynski and Mogenson, 1985), 2) injecting procaine, which blocks synaptic and axonal transmission (for review, see Brudzynski and Mogenson, 1985) into the PPN significantly reduced hyperactivity elicited by amphetamine injection into NA (Brudzynski and Mogenson, 1985), 3) Injection of GABA antagonists (e.g. picrotoxin) into the subpallidal region (SI/LPA) increased locomotor activity (Jones and Mogenson, 1980) and 4) injection of procaine into the ZI/LHA after SI picrotoxin-induced hyperactivity reduces the activity (Mogenson et al., 1985). Combining the above findings with the neuroanatomical data showing that these regions are connected to the major locomotion-associated centres, it appears possible that limbic motor integration may occur through this neural
Conclusions and Purpose of Studies in the Thesis

The survey of the literature presented reiterates the point that, while a great deal is now known concerning pathways and mechanisms in the CNS control of locomotion, much remains to be elucidated. For example, it is generally accepted that rhythmic oscillators which are capable of producing the basic locomotor patterns exist in the spinal cord. However, the neural circuits which comprise the oscillators remain unknown.

The origins of descending supraspinal pathways controlling the rhythmic oscillators are perhaps the most clearly delineated components of the control system in non-primate vertebrates. In these animals, the reticulospinal pathway appears to play an obligatory role, as lesion of the reticular formation or its descending tracts abolishes voluntary locomotion. The vestibulospinal, rubrospinal and corticospinal pathways are thought to add postural and "fine" control features to the system. However, little is known about the exact connections and neurotransmitters the descending pathways utilize to exert control. Furthermore, while only limited knowledge is available concerning the neural elements impinging on these descending systems, more is known about the hodology than about the neurotransmitters which act to control these pathways. Very little is known regarding the differences between the locomotor control systems of primates versus lower vertebrates which could account for the occurrence of spinal stepping found in all
vertebrates except primates.

Higher order structures including the locomotor regions, basal ganglia, limbic system, sensory systems (e.g. trigeminal, vestibular) and comparator systems (e.g. cerebellum) appear to exert control over the descending supraspinal pathways, but comparatively little is known with certainty regarding the specifics of this control.

As described above, the majority of locomotor studies have been carried out in complex mammalian systems (e.g. cat, rat) that employ quadrupedal locomotion (Dietz, 1987). The use of quadrupedal animals imposes complications to the study of motor systems (e.g. intragirdle coordination, gait conversion) that may, in part, be overcome by the study of a bipedal system. The bird seemed to me to be ideal for the study of locomotor behaviour. Like humans, the bird is a true biped during overground locomotion. Also, like humans, presumed interactions between forelimbs and hindlimbs which occur in quadrupedal animals are reduced or absent except during the transition from walking to flying. The two independent modes of avian locomotion also make the bird an ideal model for the study of locomotor behaviour, as it may be possible to more easily isolate the neural circuitry involved in these two different modes of locomotion. Perhaps most importantly, the bird does not possess the corticospinal tract which complicates the study of motor control in mammalian species and primates (Cabot et al., 1982; Webster and Steeves, 1988; Reiner and Karten, 1982). This absence strongly implicates more caudal brainstem structures in the control of locomotion and allows one to study a complex
motor system that is devoid of corticospinal influences, even in the intact state. Lastly, birds possess CNS motor circuitry equivalent to all mammalian vertebrates (including primates) to the level of the basal ganglia (Reiner et al., 1984), thus making possible the comparison of hind- and midbrain locomotor mechanisms across a broad phylogenetic range.

The above attributes make the bird an excellent model not only for the elucidation of neuronal elements controlling vertebrate motor systems, but also for the study of other aspects of motoricity, including locomotor development and repair.

Prior to the beginning of this work, a review of the literature indicated that very little was known about the physiology and anatomy of supraspinal structures controlling locomotion in birds (Eidelberg, 1981), with the exception that birds were capable of making stepping movements in the absence of supraspinal input ("spinal stepping") (Tarchanoff, 1895; ten Cate, 1960). My studies have examined aspects of the supraspinal mechanisms responsible for the initiation and descending control of avian locomotion (Weinstein et al., 1984; Sholomenko and Steeves, 1987a,b; Steeves et al., 1987). The Canada goose, Branta canadensis was chosen as the main experimental animal as it is an excellent walking bird as well as a remarkable long distance flier. Further, it is a large animal, is readily available for study, is easy to handle in captivity and is not an endangered species. The domesticated non-flying, Pekin duck, Anas platyrhynchos and the domesticated Brandies goose were also utilized in some of the acute brainstem stimulation experiments
to determine the applicability of the experimental findings in the goose to other avian species.

My previous studies have included: 1) defining an index of normal locomotor muscle patterns (Weinstein et al., 1984) using electromyographic techniques to determine which forelimb and hindlimb muscles best define the flight and walking patterns respectively. The muscles which best define the flight phase include the pectoralis muscle (PECT) (major wing depressor) and deltoideus major muscle (DM) (wing levator). Muscles which define the stance phase and swing phase of walking include the flexor cruris lateralis (FCL) and iliotibialis cranialis (ITC) (synonymous with the mammalian sartorius muscle), respectively, 2) using selective lesions of the low thoracic spinal cord to determine the descending spinal cord pathways essential to hindlimb locomotion in both chronically maintained and in acute decerebrate brainstem stimulated preparation. Results from these studies demonstrated that motor information descending in the ventral funiculi is essential for locomotion in both preparations. Further, the pathway most strongly implicated as essential to locomotion was the reticulospinal pathway travelling as a diffuse projection in the ventral spinal cord (Sholomenko and Steeves, 1987b) and 3) defining regions in the mid- and hindbrain which, when electrically stimulated, produce locomotor movements at low current intensity. Mapping of these stimulation sites has determined the location of locomotor sites in the avian brainstem which are equivalent to those found in mammals, thereby providing a better understanding of the supraspinal influences which initiate, maintain and modulate
locomotion in birds (Steeves et al., 1987). Locomotion evoking stimulation sites found to date include: 1) a strip extending from the caudal pons to caudal medulla subjacent to and within the nucleus and tract of the descending trigeminal system. This region appears to be equivalent to the mammalian pontomedullary locomotor strip (PLS or Probst's Tract of Garcia-Rill et al., 1983b), 2) sites in the ventral gigantocellular and magnocellular reticular formation of the hindbrain which lie within the dorsal and ventral central reticular nuclei and 3) a region in the midbrain which lies slightly medial to the lateral spiriform nucleus.

The above findings have demonstrated that structures responsible for avian motor control are similar to those found in other vertebrates. The purpose of the studies found in this thesis was to further characterize these and other regions in the avian brain which may contribute to the control of locomotor behaviours. The characterization of neural circuitry which controls locomotion is a necessary prerequisite to the functional repair of this circuitry following central nervous system (CNS) injury or degeneration.

In Chapter 2, a systematic survey using focal electrical stimulation was performed in an attempt to localize other avian locomotor regions which may exert control over the regions described previously. Three previously uncharacterized locomotion-evoking electrical stimulation regions have been found. One region lies in close proximity to the nucleus intercollicularis (ICo) of the tectal midbrain. The second can be localized to the medial midbrain reticular formation (MRF). A
third site lies within the confines of the pontine and rostral medullary medial longitudinal fasciculus (MLF).

Following the localization of the electrophysiologically-defined locomotor regions, a series of experiments were performed using microinjection of neurochemicals into these regions. While necessarily incomplete, this survey was undertaken for two reasons. First, neurochemical activation of locomotion from a locomotor region should differentiate between locomotion elicited by activation of neurotransmitter receptors and locomotion evoked by stimulation of axons of passage. Second, selective injection of different locomotion-evoking neurochemicals may provide information concerning both the neurotransmitters involved in the locomotor circuitry and the potential neural pathways which contain these neurotransmitters.

Chapter 3 describes and discusses the results of direct intracerebral injection of cholinergic neurotransmitter agonists and antagonists into locomotion-evoking electrical stimulation sites. Cholinergic muscarinic agonists elicited long lasting and reversible (antagonist) locomotion when introduced into the pontobulbar locomotor strip (PLS), dorsal part of the medullary central nucleus (Cnd), ventral part of the medullary central nucleus (Cnv), and MLF. Injection of agonist into the medial mesencephalic reticular formation (MRF) reduced the threshold for electrically stimulated locomotion.

Chapter 4 describes and discusses the results from the introduction of γ-aminobutyric acid (GABA) antagonists into a variety of electrical stimulation-defined sites, including the PLS, Cnd, Cnv, pontine reticular formation (RP) and ICo.
Antagonist injection evoked long lasting locomotion which was transiently reversed by GABA. Chapter 5 describes and discusses the results from microinjection of excitatory amino acid and Substance P injection into a variety of locomotor regions. Injection of the glutamatergic agonist N-methyl-D-aspartate (NMDA) into sites including the PLS, Cnd, Cnv, MLF and MRF elicited, or reduced the electrical threshold necessary to induce, locomotion. These effects were reversible with the glutamate antagonist glutamic acid diethyl ester (GDEE). Injection of Substance P into the pontine and medullary reticular formation elicited walking or reduced the threshold for electrically stimulated locomotion.

Phasic peripheral afferent input has been shown to have a role in locomotor control in a variety of vertebrates (see Chapter 6). However, the extent to which this input may be important in avian locomotion has not been determined. Therefore, studies designed to examine whether phasic peripheral afferent input was essential for avian locomotor patterns were undertaken (Chapter 6). My results demonstrate that locomotor patterns were evoked by both electrical and chemical stimulation of several mid- and hindbrain sites in the decerebrate paralyzed bird ('fictive' preparation). Furthermore, 'fictive' locomotion was found in high decerebrate spontaneously locomoting birds. These results are discussed in Chapter 6.

The multilevel organization of neural circuitry subserving locomotor control has been demonstrated by selective transection of the neuraxis in a variety of decerebrate preparations. As described in the general introduction (see Locomotor Regions and
Locomotion-related Structures), decerebration to a level which preserves the mammalian subthalamic locomotor region allows spontaneous locomotion in the preparation. To determine whether a similar organization exists in birds, and to identify a region which may exert control over locomotor behaviour, varying levels of decerebration were performed to examine their effects on spontaneous versus non-spontaneous locomotion in both paralyzed and unparalyzed decerebrate animals (Chapter 7). Results from these studies show that in birds, as in mammals, structures lying near the region of the subthalamic nucleus appear to subserve spontaneous locomotion and removal of these structures by more caudal transection eliminates this spontaneous activity.

The Decerebrate Preparation

The unanaesthetized decerebrate preparation has been utilized in a broad range of neurobiological studies including the study of high threshold tactile stimuli (e.g. Besson and Le Bars, 1979; Kajander and Giesler, 1987), respiration research (e.g. Eldridge et al., 1985; Funk et al., (submitted)) and locomotor control research (e.g. Aoki and Mori, 1981; Bard and Macht, 1958; Budakova and Shik, 1970; Eidelberg et al., 1981b; Garcia-Rill, 1983; Garcia-Rill et al., 1983a,b,c; Garcia-Rill and Skinner, 1987; Grillner and Shik, 1973; Hinsey et al., 1930; Jell et al., 1985; Kazennikov et al., 1983a,b; Orlovsky, 1969, 1970a,b, 1972a,b; Selionov and Shik, 1984; Shefchyk et al., 1984; Sherrington, 1910, 1915; Shik et al., 1966; Sholomenko et al., 1987; Steeves and Jordan, 1980; Steeves et al., 1987;
Villablanca, 1962; Waller, 1940). Decerebrate animals are devoid of any conscious perception of pain, allowing experimental manipulations which are not acceptable in intact, unanaesthetized animals which are unable to "indicate or arrest the onset of suffering" (e.g. paralyzed) (Wall, 1975; Wall and Sternbach, 1976).

The following quotation, which supports the view that decerebrate animals are devoid of pain, (Adams, 1980) relates to human pain perception.

"Perception of pain. Only upon the arrival of pain impulses at the thalamocortical level of the nervous system is there conscious awareness of the pain stimulus. Clinical study has not informed us of the exact localization of the nervous apparatus for this mental process. It is not entirely abolished by a total hemispherectomy, including the thalamus on one side. It is often said that impulses reaching the thalamus create awareness of the attributes of sensation and that the parietal cortex is necessary for the appreciation of the intensity and localization of the sensation. This seems an oversimplification. Probably a close and harmonious relationship between thalamus and cortex must exist in order for a sensory experience to be complete. The traditional separation of sensation (in this instance awareness of pain) and perception (awareness of the nature of the painful stimulus) has been abandoned in favor of the view that sensation, perception, and the various conscious and unconscious responses to a pain stimulus comprise an indivisible process."
This view is further supported by the finding in the freely moving rat with varying levels of decerebration that the medulla and brainstem support reflex and startle and flight reactions to painful stimuli, while the rhinencephalon and cortex support affective and intellectual alertness, respectively (Charpentier, 1968; for review of pain levels, see Loeser and Black, 1975).

In regard to pain resulting from the decerebration procedure, strong evidence is available from human studies that surgical manipulation of brain tissue does not elicit pain responses. Many neurosurgical procedures are often performed in conscious patients with only the use of local anaesthetics at incision sites; such patients never report the perception of pain (Emmers, 1981). In this thesis, all surgical procedures, including decerebration procedures, were performed under halothane/nitrous oxide inhalation anaesthesia with direct infiltration of local anaesthetic at all incision sites and pressure points. Following completion of the decerebration procedure and subsequent removal of halothane/nitrous oxide anaesthesia, local anaesthetic at pressure points and incision sites was continued until the termination of the experiment (for details of all surgical procedures, see Materials and Methods, Chapters 2,3,6,7). No signs of discomfort (e.g. writhing, vocalization, elevated blood pressure, elevated heart rate) were observed in any decerebrate animal (see APPENDIX II) and, as shown in Figure 2, electrical stimulation caused no significant increase in heart rate or blood pressure, while the locomotion evoked by the stimulation elicited distinctive cardiovascular changes.
Figure 2. Electromyographic activity, blood pressure and heart rate changes resulting from ramped electrical stimulation in the region of the descending trigeminal tract and nucleus. The stimulation trace (STIM) demonstrates the ramped stimulation intensity from $0\mu A$ on the left to $80\mu A$ at the right. Blood pressure (BP) remained relatively constant from the beginning of stimulation until threshold for locomotion was reached at approximately $60\mu A$ intensity. Upon the onset of locomotion, as demonstrated by electromyographic records from the left (LPECT) and right (RPECT) pectoralis muscles (major wing depressor muscles) and left (LITC) and right (RITC) iliotibialis cranialis muscles (major hip flexor synonymous with the mammalian sartorius muscle), the blood pressure increased (see calibration bar at right) with the locomotion and returned to resting levels following the trial (return to resting BP not shown). The heart rate (HR) also remained relatively constant until the threshold for locomotion was reached and appeared to increase with exercise, returning to resting levels following the trial (not shown) (nb. during strong wing flapping, movement artifact makes it difficult to distinguish the HR signal. However, the signal can be observed during the interval between bout of strong flapping).
Further indirect evidence demonstrating that decerebrate animals were not capable of perceiving a painful stimulus come from studies examining respiratory changes as a consequence of evoking locomotion with brainstem stimulation. In both cat [electrical and neurochemical stimulation - subthalamic locomotor region (Eldridge et al., 1985)] and bird [electrical stimulation - trigeminal nucleus and tract (Funk et al., submitted) also see Appendix II and Fig. 2], the ventilatory responses to electrical or chemical stimulation-induced locomotion were similar to those observed in unoperated intact animals. Taken together, the above evidence supports the contention that noxious stimulation was not responsible for the results observed in this study.

NOMENCLATURE

To prevent confusion concerning structures which have homologous nomenclature but are not equivalent in the avian versus the mammalian literature, I have used the mammalian names where advisable. Thus, the avian Nucleus tegmentipedunculopontinus, pars compacta (TPc) will be called by the name of its mammalian equivalent, the substantia nigra, pars compacta (SNc) (Brauth et al., 1978). Also, the avian nucleus of the ansa lenticularis (nAL) will be called the subthalamic nucleus, its mammalian equivalent (Brauth et al., 1978). Where homologies exist between avian and mammalian structures with different nomenclature, I have utilized the avian nomenclature and provided the appropriate mammalian counterpart (e.g.
iliotibialis cranialis (ITC) muscle is equivalent to the mammalian sartorius muscle) (Weinstein et al., 1984).
CHAPTER 2

ELECTRICAL STIMULATION OF MESENCEPHALIC AND PONTINE REGIONS
ELICITS LOCOMOTION IN DECREBRATE BIRDS
INTRODUCTION

The initiation and control of spinal locomotor mechanisms by supraspinal structures has been studied in many vertebrate species (McClellan, 1986). Until recently, however, very little information was available concerning the role played by these structures in the neural control of locomotion in birds (Eidelberg, 1981; Steeves et al., 1987). We are investigating avian locomotion for several reasons. First, birds display a strong separation between the functional activity of wing muscles involved during flight from those used by the legs during bipedal walking. This suggests a functional uncoupling of forelimb and hindlimb spinal locomotor pattern generators in birds (ten Cate, 1962), and may also suggest that the two pattern generators are controlled by different descending supraspinal pathways (Jacobson and Hollyday, 1982). Second, birds do not possess a telencephalo-spinal projection analogous to the mammalian corticospinal tract (Cabot et al., 1982). Finally, unlike most vertebrates, birds are true bipeds whose overground locomotor patterns resemble human walking (Weinstein et al., 1984).

Earlier reports from our laboratory (Steeves et al., 1986, 1987; Sholomenko and Steeves, 1987a,b) have demonstrated that focal electrical stimulation of discrete hindbrain regions in the low decerebrate bird preparation can elicit the entire repertoire of avian locomotor behaviours. The behaviours include bipedal alternating stepping, bipedal synchronous hopping, stepping with wing flapping and flying alone.
One locomotion-evoking region was located within the ventromedial pontomedullary reticular formation (specifically, the gigantocellular reticular formation (Rgc)), while the second lay dorsolaterally within the parvocellular reticular formation (LRF), subjacent to or within the descending trigeminal tract and nucleus (TTD) (see Chapter 3, Figs. 9 & 10).

Retrograde tracing combined with electrical stimulation studies demonstrated that neuronal cell bodies in Rgc, co-localized with locomotion-evoking stimulation sites, project directly to both cervical and lumbar spinal cord (Steeves et al., 1987a; Webster and Steeves, 1987). These results in birds, as in a variety of species including lamprey (McClellan and Grillner, 1984), stingray (Leonard et al., 1979), teleost fish (Kashin et al, 1975), turtle (Kazennikov et al., 1980), cat (Mori et al., 1978; Shik and Yagodnitsyn, 1977) and monkey (Eidelberg et al., 1981b) demonstrated that reticulospinal neurons play a major role in the descending control of spinal cord rhythmic oscillators which are essential for the basic motor pattern (Sholomenko and Steeves, 1987b; ten Cate, 1960, 1962; Grillner, 1985; Dubuc, 1988).

The dorsolateral region of the reticular formation appears to be the avian homologue of the mammalian pontobulbar locomotor strip (PLS) seen in cats (Steeves et al., 1987; Selionov and Shik, 1984; for review see McClellan, 1986) and stimulation of this region also elicited locomotion in birds.

The above evidence strongly suggests a high degree of conservation of brainstem and spinal cord motor neuronal circuitry across a broad phylogenetic range of species. To
further test this hypothesis and examine to what extent the conservation of circuitry observed in the medulla and pons is carried to more rostral brain structures, we utilized electrical stimulation in the decerebrate bird to systematically examine regions of the pons and mesencephalon to determine if avian counterparts of more rostral mammalian locomotor regions, including possible equivalents of the mesencephalic locomotor region (MLR), could be localized.

Our findings demonstrate that locomotion could be evoked by focal electrical stimulation of regions of the mesencephalon and pons. Two centres were localized to the avian midbrain, one within the medial mesencephalic reticular formation (mMRF) and a second in close proximity to the intercollicular (Ico) and isthmal (Ipc) nuclei of the tectum. The pontine site was localized to the dorsal midline of the pons and rostral medulla within the confines of the medial longitudinal fasciculus (MLF).
MATERIALS AND METHODS

Surgery

Each bird (either a Canada goose, *Branta canadensis* or a Pekin duck, *Anas platyrhynchos*) was anaesthetized throughout all surgical procedures with halothane (1-3%) and nitrous oxide (20-30%) administered through an endotracheal tube (Figure 3). The anterior air sac was cannulated to facilitate unidirectional (flow through) ventilation (UDV) of the lungs with O$_2$/CO$_2$ (95%/5%). The carotid artery and jugular vein were cannulated unilaterally for monitoring blood pressure and fluid/chemical infusion respectively. Body temperature was monitored with a temperature probe inserted into the oesophagus and maintained (39-41°C) via a rectal cooling/warming probe.

The bird was then fixed in a stereotaxic head holder and the body supported in a sling mounted over a motorized treadmill. Following a craniotomy, a suction decerebration was performed along a plane extending dorsally from the caudal margin of the habenular nucleus to the ventrocaudal portion of the optic chiasm. To prevent blood loss during the decerebration procedure in the geese, blood pressure was transiently depressed by intravenous (i.v.) infusion of sodium nitroferricyanide (nipride) (15mg/100ml) in 10% dextrose solution. In ducks, no depression of blood pressure was necessary. Anaesthesia was discontinued following decerebration and the birds were allowed a minimum of 30 minutes for all effects of the anaesthetic to wear off before any form of brain stimulation was initiated. All
pressure points (e.g. ear bars) and surgical sites were routinely infused with xylocaine hydrochloride (2%) after general anaesthetic removal. The level of transection described above usually eliminated spontaneous locomotion in the decerebrate animal, however, reflex responses to deep pressure and foot web pinch stimulation appeared normal.

Bipolar electromyographic (EMG) electrodes were implanted percutaneously in the pectoralis (PECT) and iliotibialis cranialis (ITC) muscles to monitor muscle activity (Weinstein et al., 1984). During flight, the PECT muscles act in-phase as the major depressors of the wings. The ITC muscles of the legs (synonymous to the mammalian sartorious muscles) function as the major hip flexors and also as weak knee extensors. All EMGs were recorded with the treadmill on. EMG signals were amplified (Grass P15/Pramp) and filtered (band pass 200-10K Hz) prior to monitoring on an oscilloscope and recording on chart recorder (Gould ES100B) and tape (Akai)(Figure 3).

Brain Stimulation

Focal electrical stimulation of localized regions within the avian brainstem was completed on 16 Canada geese and 11 Pekin ducks. Two types of monopolar stimulating electrode were utilized. The first type was a commercially available stainless steel electrode (Kopf model SNE 300, tip diameter = 0.1mm, impedance 60-70kΩ) while the second was constructed by inserting 0.0762mm stainless steel wire (exposed tip length = 0.1mm, impedance = 60-70kΩ) through one barrel of a pulled three barrel
Brain Stimulation Experimental Apparatus

venous cannula

arterial cannula

b.p

EMG

movement potentiometer

head holder

inhalation anesthetic

internal carotids ligated

ventilatory outflow

sling

treadmill

ccu

SB8
micropipette (total tip diameter = 0.1mm). Constant current stimulation trials, (Grass Model S88/ Grass Model CCU1A) were undertaken with the following standard stimulation parameters: square wave pulse duration = 1.0-2.0ms; pulse frequency = 60Hz; current strength = 10-170μA.

Stimulation trials were undertaken by incrementally lowering the electrode stereotaxically (Karten and Hodos, 1967; Zweers, 1971) into the brainstem while stimulating with a current intensity of 50-100μA. When locomotion was observed, the current intensity was reduced to zero and then slowly increased until threshold was reached. The optimal electrode tip position for evoking locomotion was then established by slowly lowering the electrode to the point where coordinated reproducible (stimulus linked) locomotor movements were initiated with the lowest stimulation current (Steeves et al., 1987).

After recording the stimulation-evoked electromyographic (EMG) activity, the stimulation site was marked for neuroanatomical identification with an electrolytic lesion made by passing a direct current of 3 milliamps for 5 seconds. At the end of each experiment, the animal was deeply anaesthetized (5% halothane/70% nitrous oxide) and sacrificed with a bolus intravenous injection of 2M KCl.

The brainstem was removed and placed for at least 2 days in 4% paraformaldehyde, 0.1M phosphate buffer (pH = 7.4). It was then placed in two changes of sucrose cyroprotectant (25% sucrose/10% glycerine/0.1M phosphate buffer (pH =7.4)) for at least 4 days. Serial sections, at 50μm thickness, were cut coronally on a freezing microtome, mounted on gelatinized slides
and stained with Eosin/Cresyl Violet dyes. The location of electrolytic lesions, indicating effective locomotor stimulation sites, were identified according to the atlases of Karten and Hodos (1967) and Zweers (1971).
RESULTS

Medial Longitudinal Fasciculus (MLF)

Electrical stimulation of the medial longitudinal fasciculus along a considerable rostrocaudal extent (Figs. 4 & 5) elicited varied locomotor patterns in seven birds (6 Canada geese, 1 Pekin duck) at threshold stimulation intensities ranging from 30-160μA (mean = 92μA). The patterns included bilateral stepping in 3 birds (Fig. 6A), combined stepping and flapping in 3 animals (Fig. 6B) and wing flapping alone in one preparation. These varied patterns did not appear to correlate with any rostrocaudal or lateral orientation of the stimulation sites. Electromyographic records taken from 2 birds showed alternating stepping and stepping with flapping (Figure 6). Electrical stimulation of the latter animal (Figure 6B) elicited alternating stepping at low stimulation intensity (not shown) which gave way to stepping & wing flapping as the current intensity was increased. This pattern, while found in only one of the seven birds stimulated, was reminiscent of that seen during electrical stimulation of the medullary reticular formation (Steeves et al., 1986). As also seen during MRF stimulation, ramp increases in the stimulus intensity above threshold values during MLF stimulation appeared to increase the force and frequency of the locomotor pattern (Steeves et al., 1986).
Figure 4. Composite diagram of coronal sections through the pons and mesencephalon illustrating sites from which locomotion was evoked by electrical stimulation. The rostrocaudal extent of the sections is indicated by the numbers in upper left corner of each section [A=anterior, P=posterior (in millimeters (mm))]. Unfilled circles represent effective stimulation sites which are discussed in the text. Rostrocaudal extent is indicated by stereotaxic levels in the upper left corner of each section.


(Redrawn from Karten and Hodos, 1967).
Figure 5. Coronal sections through varying levels of the neuraxis illustrating sites (L) from which locomotion could be elicited by electrical stimulation. A: Coronal section through the caudal pons showing a locomotion-evoking stimulation site (L) in the MLF. B: Coronal section through the mesencephalon demonstrating a locomotion-evoking stimulation site (L) in the medial mesencephalic reticular formation (MRF). C: Coronal section through the rostral mesencephalon illustrating an electrically stimulated locomotor site (L) in the isthmal parvocellular nucleus (Ipc). D: Coronal section through the mesencephalon illustrating a locomotion-evoking stimulation site (L) in the intercollicular nucleus of the tectum. See Results for details. Abbreviations: FRL - lateral mesencephalic reticular formation, FRM - medial mesencephalic reticular formation, ICo - intercollicular nucleus, Ipc - isthmal nucleus, parvocellular part, L - stimulation site marked by an electrolytic lesion, MLd - dorsal part, lateral mesencephalic nucleus, MLF - medial longitudinal fasciculus, MRF - medial mesencephalic reticular formation, NV - trigeminal nerve, Omd - dorsal part, oculomotor nucleus, Omv - ventral part, oculomotor nucleus, PrV - principal sensory trigeminal nucleus, R - raphe nucleus, RPgc - gigantocellular part, pontine reticular formation, Ru - red nucleus, III - oculomotor nerve
**Figure 6.** Electromyographic (EMG) records showing locomotor activity elicited by electrical stimulation of the medial longitudinal fasciculus (MLF). A: Alternating stepping represented by EMG patterns from the right (RITC) and left (LITC) iliotibialis cranialis muscles elicited by electrical stimulation of the MLF. ITC is the major avian hip flexor and is synonymous with the mammalian sartorius muscle. B: Stepping together with wing flapping EMGs evoked by electrical stimulation of the MLF. The top two traces are records from the right (RPECT) and left (LPECT) pectoralis muscles which are the major wing depressor muscles. The bottom 2 traces are records from the left and right ITC flexor muscles.
Stimulation of the medial mesencephalic reticular formation (Figure 4) at threshold current intensities ranging from 25-150\(\mu\)A (mean = 78\(\mu\)A) elicited locomotor patterns in 14 animals (4 Canada geese, 10 Pekin ducks) that were similar to the patterns seen in response to stimulation of the MLF. Five birds displayed walking alone with no wing participation even at stimulation intensities up to 170\(\mu\)A (maximum), while in five others, stepping and flapping began simultaneously (e.g. Figure 7A) at the threshold intensity. Flying behaviour alone was elicited in 4 other animals.

The effects of changing the frequency of stimulation while maintaining other stimulation parameters constant were examined in several animals. Figure 7B illustrates the hindlimb flexor (ITC) and wing depressor (PECT) EMGs from one bird which demonstrated walking and flying together at threshold stimulation intensity (100\(\mu\)A). Increasing the stimulus frequency from 50Hz to 80Hz in 10Hz increments decreased the frequency of both flapping (2.5-0.83Hz) and stepping (2.3-1.3Hz) (only one leg and wing are shown for each frequency).

**Nucleus Intercollicularis (ICo) and Nucleus Isthmi, pars parvocellularis (Ipc)**

Locomotion was elicited in nine Canada geese by focal electrical stimulation (threshold intensity range 25-100\(\mu\)A, mean = 71\(\mu\)A) of a region in close proximity to the tectal
Figure 7. EMG records showing locomotor activity produced by electrical stimulation of the medial mesencephalic reticular formation (mMRF). A: Stepping together with wing flapping EMGs elicited at a stimulation threshold of 100μA. Records are from the left (LITC) and right (RITC) ITC muscles together with the left (LPECT) and right (RPECT) muscles. B: EMG records from the same animal showing the effects of changing stimulation frequency on locomotion. The traces are paired to illustrate the simultaneous stepping (LITC) and flapping (LPECT) behaviour elicited at a constant stimulation intensity of 100μA and square wave pulse duration (2.0ms). The frequency of stimulation was varied from 50Hz in the top pair to 80Hz in the bottom pair in 10Hz increments. The frequency of stepping decreased with increasing frequency of stimulation (50Hz - 2.3 steps/sec, 60Hz - 1.7 steps/sec, 70Hz - 1.5 steps/sec, 80Hz - 1.3 steps/sec). The frequency of wing flapping also decreased with increasing stimulation frequency (50Hz - 2.5 wingbeats/sec, 60Hz - 2.0 wingbeats/sec, 70Hz - 1.0 wingbeat/sec, 80Hz - 0.83 wingbeats/sec).
intercollicular and isthmal nuclei (Figures 4 & 5). Threshold stimulation produced stepping together with wing flapping in all animals. An example illustrating the coactivation of leg and wing locomotor patterns is displayed in Figure 8.
Figure 8. EMG records illustrating the locomotor activity elicited by electrical stimulation of the intercollicular nucleus of the mid-brain. The records were taken from the right (RPECT) and left (LPECT) pectoralis muscles simultaneously with the right (RITC) and left (LITC) ITC muscles. The coactivation of both limb girdles in response to electrical stimulation was typical for sites in this region.
Previous studies in our laboratory have demonstrated that locomotion can be elicited in birds by electrical stimulation of reticular formation nuclei that give rise to the descending reticulospinal pathways essential for the initiation and ongoing control of locomotion (Steeves et al., 1986, 1987; Sholomenko and Steeves, 1987b; Webster and Steeves, 1988). Furthermore, a region corresponding to the mammalian pontobulbar locomotor strip was described (Steeves et al., 1986, 1987). The PLS appears to play a role in the sensorimotor activation of locomotion in a variety of species (McClellan, 1986; Jordan, 1986; Noga et al., 1988; see Chapter 1).

To examine structures which may control or modulate the locomotor-related reticular formation nuclei, we have utilized electrical brainstem stimulation to examine systematically more rostral portions of the avian brainstem. Equivalents of the mammalian mesencephalic locomotor region (MLR) and subthalamic locomotor region (SLR) (Shik et al., 1966; Orlovsky, 1969) have not been previously identified in birds (Eidelberg, 1981).

Our present findings demonstrate one region in the pons and two in the midbrain from which locomotion may be electrically induced. These include the medial longitudinal fasciculus (MLF), the medial mesencephalic reticular formation (mMRF) and the intercollicular (ICo) and parvo cellular isthmal (Ipc) nuclei of the tectal midbrain.

In birds, as in mammals (Karten and Hodos, 1967; Carpenter and Sutin, 1983), the MLF describes a fibre tract which carries
a variety of projections. The rostrocaudal extent of the MLF is
from the level of the Edinger-Westphal nucleus to the caudal
medulla (Karten and Hodos, 1967). Projections which traverse the
MLF include: 1) descending axons travelling from the pontine
reticular formation (PRF) to the medulla and spinal cord,
2) descending axons from the interstitial nucleus of Cajal (INC)
to spinal cord, Probst's tract, medial and descending vestibular
nuclei and inferior olive (Carpenter and Sutin, 1983; Skinner et
al., 1984), 3) vestibulospinal fibers which send collaterals to
the medullary reticular formation (Carpenter and Sutin, 1983)
and 4) visual internuclear fibres from cranial nerve nuclei VI,
IV and III. In addition, some fibres which impinge on the
pedunculopontine tegmental nucleus (PPN) also project via the
MLF (unspecified origin) (Rye et al., 1987).

Several of the above descending projections travelling in
the MLF have been implicated in locomotor control mechanisms
which may subserve our results. Thus, electrical stimulation of
these fibres may activate locomotion in a non-specific manner.
Fibres arising from the vestibular nuclei are carried in the MLF
and project to the medullary reticular formation and spinal cord
and thus could be responsible for the locomotor responses
associated with MLF stimulation. However, direct stimulation of
the lateral vestibular nucleus does not activate locomotion
(Orlovsky, 1972a) but only facilitates extensor tone in the
decerebrate cat. Similarly, although INC sends direct
projections via the MLF to the motor associated Probst's Tract
(Garcia-Rill et al, 1983a), electrical stimulation of the INC
elicits only head or conjugate vertical and rotatory eye
movements (Skinner et al., 1984). Activation of pontine reticular formation fibres have been implicated in the control of postural tonus in the cat (Mori et al., 1978, 1982). These fibres have been demonstrated to impinge on the medullary reticular formation and spinal cord structures in birds (Steeves et al., 1987, Webster and Steeves, 1988, in preparation) and could also account for the effects of MLF stimulation on locomotion. Electrical stimulation of axons terminating on neurons of the PPN, a mesencephalic nucleus which appears to form a portion of the mesencephalic locomotor region (MLR) (Garcia-Rill et al., 1986) could also be responsible for eliciting locomotion through MLF stimulation.

However, while electrical stimulation of the axonal projections arising from these nuclei may have some role in locomotion evoked from the MLF region, results from neurotransmitter (or agonists/antagonists) injection studies which will be subsequently discussed (Chapters 3 & 5) indicate that it is unlikely that the results of MLF stimulation-induced locomotion resulted from the stimulation of en passant fibres alone. While the neuroanatomical connections of cells in this region which may be responsible for locomotion have yet to be determined in birds, serotonin-containing neurons which stain positively for acetylcholinesterase have been identified in close proximity to the locomotion-evoking stimulation sites (Dube and Parent, 1981; Taccogna et al., in preparation). These serotoninergic cells appear to project to the nucleus pretectalis of the midbrain, a region lying in close proximity to electrically identified locomotor sites which will be
discussed below (Reiner et al., 1982). In the rat, injection of the GABAergic agonist muscimol into either the dorsal or median raphe nuclei increases locomotor activity which is blocked by pre-treatment with bicuculline (Paris and Lorens, 1987). Also, ibotenic acid lesion of cell bodies within the median raphe produced hyperactivity in the rat (Asin and Fibiger, 1983). These results, therefore, implicate this region in locomotor processes in the intact mammal. While the observed locomotor behaviour elicited by electrical stimulation of the MLF in birds may result from activation of neurons in this region, the mechanism and neuroanatomical pathways through which the locomotion is evoked remains to be determined.

Electrical stimulation of a second region, the medial mesencephalic reticular formation (mMRF), also elicited locomotion in decerebrate birds. The rostrocaudal extent of the effective stimulation sites (from caudal red nucleus to rostral pons) indicates the diffuse nature of this locomotor region.

In birds, anterograde and retrograde tracing studies have shown that the mMRF has reciprocal connections with deep layers (layers 8-13) of the tectum (Hunt et al., 1977; Hunt and Brecha, 1984). In turn, deep tectal layers receive the bulk of their innervation from the lateral spiriform nucleus (SpL), a relay nucleus which itself receives the major outflow of the avian equivalent of the mammalian basal ganglia (Reiner et al., 1984). Bilateral destruction of SpL results in deficits in birds that are similar, as in monkeys with pallidal lesions and also in Parkinsonian patients, to those seen following disruption of the outflow pathway from the mammalian basal ganglia (Bugbee, 1979,
cf Reiner et al., 1982). Thus, this pathway may have a role in controlling the initiation of ongoing or impending movements which coordinate the bird’s position in space (Bugbee, 1979, cf Reiner et al, 1982; Reiner et al., 1984).

The mMRF projects ipsilaterally both to the high cervical spinal cord and to the gigantocellular region of the medial medullary reticular formation (Webster and Steeves, in preparation). This circuit may, therefore, provide a portion of the outflow loop from the basal ganglia to hindbrain structures which underlies some aspects of motor performance in birds (Reiner et al., 1984). Within the constraints of the available data concerning avian mMRF connections, those which have been identified above resemble the afferent and efferent pathways of the mammalian mesencephalic locomotor regions (Garcia-Rill et al., 1983a; Steeves and Jordan, 1984; Rye et al., 1988). However, further comparison of this avian region to the mesencephalic locomotor regions of mammals awaits increased avian and mammalian neuroanatomical, immunohistochemical and receptor subtype information.

Locomotion was elicited in the low decerebrate bird preparation through electrical stimulation of a third site, the deep tectal regions including the intercollicular nucleus (ICo) and parvo cellular isthmal nucleus (Ipc). The neuroanatomical connections through which these regions elicit locomotor behaviour are presently unknown. However, some pathways which may underlie our results have been identified.

ICo receives input from the spinal cord (Hunt and Kunzle, 1976; Webster and Steeves, in preparation), deep tectal layers
(Hunt et al., 1977; Wild, 1987) and body-related afferent input from the cuneate and gracile nuclei (Wild, 1987). It sends direct projections to the ipsilateral rostral descending trigeminal tract and nucleus (TTD), ipsi- and contralateral gigantocellular reticular formation (Rgc), ipsilateral central medullary nucleus, ventral part (Cnv) (Reiner and Karten, 1982; Webster and Steeves, in preparation) and ipsilateral high cervical spinal cord (Reiner and Karten, 1982). Thus, ICo maintains connections to locomotion-related nuclei and may be a relay of the output circuit through which the avian basal ganglia affect locomotor control (Reiner et al., 1984). These connections are reminiscent of those found for the mammalian mesencephalic locomotor regions (MLR) which project directly to reticular formation structures (Garcia-Rill et al., 1983a; Jordan, 1986; Noga et al., 1988). Indeed, Cabot et al. (1982) compare these connections to those of the mammalian cuneiform nucleus, a portion of which, in mammals, is believed to be the lateral MLR (Shik et al., 1966; Steeves and Jordan, 1984; Jordan, 1986; Noga et al., 1988).

On the other hand, electrical stimulation of the more ventrally located Ipc also evoked locomotion in our preparation. Ipc, a tectal nucleus which stains positively for both choline acetyltransferase (CHAT) (Taccogna et al., in preparation) and acetylcholinesterase (AChE) (Hunt et al., 1977) receives cholinergic input from the superficial (visual) tectal layers (layers 2&3) (Hunt et al., 1977) and also receives input from the deep tectal layers (layers 8-13) (Reiner et al., 1982). In the turtle and frog, ChAT-positive isthmal nucleus neurons have
been demonstrated to send a cholinergic projection to the tectum (Desan et al., 1984), while in the cat, ChAT-positive neurons have been localized to the parabigeminal nucleus [thought to be the mammalian equivalent of Ipc (Vincent and Reiner, 1987)]. In birds, based on uptake of $[^3]$H]choline and HRP transport studies, a cholinergic projection has been postulated from Ipc to layer 2d of the lateral and caudal tectum (Hunt et al., 1982). Similarly, radioactive uptake studies using $[^3]$H]glycine and $[^3]$H]GABA suggest that Ipc neurons project glycinergic and GABAergic efferents to the superficial tectal layers (Hunt et al., 1977), indicating that Ipc, while histologically homogeneous, may be heterogeneous with respect to neurotransmitter type (Hunt et al., 1982). In retrograde labelling studies, however, Ipc does not appear to directly innervate motor-related brainstem reticular formation (Rgc, Cnv) or sensorimotor (TTD) nuclei (Webster and Steeves, in preparation). Therefore, while electrical stimulation of Ipc evokes locomotion in decerebrate birds, it appears that the behaviour is evoked indirectly via an Ipc to tectal neuronal circuit, the output of which remains to be determined.

**CONCLUSIONS**

The results of this study demonstrate the presence of pontine and mesencephalic locomotor regions in birds. Further investigation is required to determine whether these regions are analogous to the more rostral locomotor regions (MLR or SLR) of higher vertebrate species (for review, see Noga et al., 1988).
One locomotor site, located close to or within the intercollicular nucleus of the tectal midbrain, appears to possess several of the hodological characteristics of the mammalian MLR. However, considerably more study is required to determine whether this site is an avian equivalent of the mammalian MLR.

Studies which combine electrical with neurochemical stimulation of these locomotor regions will help to determine whether neuronal populations or axons of passage are being stimulated. Some studies using this experimental paradigm have already been undertaken (see Chapters 2-4). Furthermore, neuroanatomical tract tracing using anterograde and retrograde tracers combined with receptor autoradiographic binding studies of locomotor-effecting radioactive neurochemicals would help to determine the neuronal subpopulations from these regions involved in locomotor control.
CHAPTER 3

CHARACTERIZATION OF AVIAN MID- AND HINDBRAIN SITES THAT PRODUCE LOCOMOTION WITH LOCAL INTRACEREBRAL INFUSION OF NEUROTRANSMITTER AGONISTS AND ANTAGONISTS (I): ACETYLCHOLINE
INTRODUCTION

The initiation and ongoing control of normal vertebrate locomotion depend upon the production, integration and transfer of both centrally and peripherally generated information interacting at many levels of the central nervous system (CNS). In order to understand the possible contribution made by each level of the complex network which controls locomotion, one approach has been to examine the system by utilizing only portions of the CNS in reduced preparations (Grillner and Zangger, 1979; Grillner and Kashin, 1976). One example which has yielded valuable information is the decerebrate electrically stimulated cat preparation pioneered by Shik and his co-workers (Shik et al., 1966). This type of preparation has been used for the study of locomotion in a variety of vertebrate species (Garcia-Rill, 1983, 1986; Jordan, 1986; for review see Grillner, 1975), including birds (Sholomenko and Steeves, 1987a; Steeves et al., 1987).

My studies with decerebrate geese and ducks have shown that electrical stimulation of regions in the mesencephalon, pons and medulla, including the pontobulbar locomotor strip (PLS) mesencephalic reticular formation (MRF) and pontine and medullary reticular formation, elicit all patterns of avian locomotion from walking to flying. These findings, complementary to those found in a variety of both higher and lower vertebrates (for review see McClellan, 1986), strongly implicate nuclei of the mid- and hindbrain as playing a major role in locomotor control.
Although locomotion can be elicited by electrical stimulation in well circumscribed regions of the brainstem (for review see Grillner, 1975), this type of stimulation gives only limited information concerning the neural structures underlying the behaviour. Electrical stimulation activates all neuronal elements including distantly originating axons of passage (en passant) which may traverse the point of stimulation (Goodchild et al., 1982; Garcia-Rill et al., 1985, 1987; Noga et al., 1988). To circumvent this problem and to more closely mimic the physiological activation of locomotion related regions, we utilized pharmacological stimulation by injecting neurotransmitters, or neurotransmitter agonists and antagonists (neurochemicals) into sites which produce locomotion when electrically stimulated. These neurochemicals are thought to activate neurotransmitter receptors situated on dendrites, cell bodies and terminals (Goodchild et al., 1982). Receptors have not, however, been localized on axons (Goodchild et al., 1982). Activation or blockade, then, of locomotor behaviour by neurochemical injection into an electrically defined locomotor region yields two types of information. First, it differentiates between the activation of receptors and en passant fibers. Second, it aids in the definition of receptor types present on cells believed to give rise to pathways involved in locomotor control. Correlation of this type of finding with neuroanatomical information makes neurochemical stimulation a powerful paradigm for the study of locomotor control.

The choice of neurochemicals used in this study was based on: previous investigations using chemical stimulation
(Garcia-Rill et al., 1985, 1987; Noga et al., 1988; Eldridge et al., 1985; Brudzynski et al., 1986, 1988 (see Appendix I, pp. 291); neuroanatomical information; and autoradiographic and immunocytochemical localization of receptors types and transmitter profiles for nuclei in the regions previously shown to elicit locomotion when electrically stimulated. The neurochemicals utilized in this chapter examine the locomotor effects of cholinergic agonist and antagonist injection into previously defined avian locomotor regions. Because six regions have been found, and the number of neurotransmitters extensive, this study was designed as a survey of these regions, with more detailed neurochemical characterization of individual regions left to future investigations.

Our results demonstrate that neuroactive chemicals were effective at stimulating or blocking avian locomotor patterns in sites previously defined using only electrical stimulation (Steeves et al., 1987). Locomotion was elicited or the threshold for electrically-induced locomotion decreased in a variety of sites following injection of cholinergic agonists. The locomotion could be blocked or electrical threshold increased with the corresponding antagonist. The effective agonists/antagonists appeared to possess individual characteristics for activation or blockade, with varying times to onset and longevity of action (see Table 1).

These results in the bird will be compared to recent findings in the cat and rat, with a view to defining mid- and hindbrain pathways involved in motor control.
MATERIALS AND METHODS

Surgery

The surgical and decerebration procedures and electromyographic recordings from the leg flexor muscles (ITC) and wing depressor muscles (PECT) have been previously described in Chapter 2.

Electroneurographic records (ENGs) from ventilated animals paralyzed with d-tubocurarine (0.025ml/kg) were recorded using bipolar platinum hook electrodes placed on peripheral nerves to ITC and PECT with the same equipment used for EMGs but at higher gain (10,000x) and with the treadmill off.

Brain Stimulation

Both electrical and chemical stimulation of localized regions within the brainstem were used to elicit locomotion from the preparation. A monopolar stimulating electrode, constructed by inserting 0.0762mm stainless steel wire (exposed tip length = 0.1mm, impedance = 60-70kΩ) down one barrel of a pulled three barrel micropipette (total tip diameter = 0.1mm), was positioned stereotaxically into sites previously shown to elicit locomotion (Steeves et al., 1987; Sholomenko and Steeves, 1987a). The other two barrels of the micropipette were filled with neurotransmitter agonists, antagonists or saline. These neurochemicals were delivered to the micropipette from a microsyringe (Hamilton) via flexible vinyl tubing. Constant
current stimulation trials (Grass Model S88/ Grass Model CCU1A) were undertaken with the following stimulation parameters: square wave pulse duration - 1.0-2.0ms, pulse frequency - 60Hz, current strength - 25-170μA.

Stimulation trials were undertaken by incrementally lowering the electrode into the brainstem while stimulating with a current intensity ranging from 50-100μA. When locomotion was observed, the stimulation was turned off. The current intensity was then reduced to zero. The stimulator was turned on and the current intensity was slowly increased until threshold was reached. The optimal electrode tip position for evoking locomotion was then established by slowly lowering the electrode to the point where coordinated locomotor movements were initiated with the lowest stimulation current (Steeves et al., 1987). Once optimal electrode position was established, chemical stimulation was attempted by injection of neurotransmitter agonists or antagonists (agonist/antagonist) into the site (see Table 1). The injection rate was 0.2μl/minute to a maximum volume of 1.0μl for any one neurochemical solution (pH7.2-7.4) (unless otherwise stated, 1μl volumes were injected). The concentration of each solution varied, with initial concentrations based on those used by other investigators (Noga et al., 1988; Garcia-Rill et al., 1985; Eldridge et al., 1985; Brudzynski et al., 1986, 1988). Over the course of many experiments, an attempt was made to systematically titrate the concentration of each agonist/antagonist to find the lowest suprathreshold concentration that would still elicit or block locomotion (see Table 1). To examine the efficacy of each
neurochemical and its time course, the following manipulations were performed to determine the effects of agonist/antagonist injection upon locomotion; 1) counteracting a locomotion producing neurochemical by injection of its corresponding antagonist and vise versa 2) electrical stimulation following injection of agonist/antagonist to examine the effect of injection upon electrical stimulation threshold and 3) replication of each injection either in the same site and/or in the homologous site contralaterally.

After recording the locomotor activity (EMGs and/or ENGs) following electrical stimulation and neurochemical injection, the stimulation/injection site was marked for neuroanatomical identification with an electrolytic lesion made by passing a direct cathodal current of 3 milliamps for 5 seconds.

Histological procedures and stimulation site identification were the same as those described in Chapter 2.
RESULTS

Acetylcholine Agonists and Antagonists

A variety of cholinergic muscarinic (AChm) and nicotinic (AChn) receptor agonists and antagonists were infused into sites in the brainstem in an attempt to delineate the receptor subtypes activated. The neurochemicals used were: carbachol, an AChm+N agonist, pilocarpine, an AChm agonist; nicotine, an AChn agonist; atropine sulfate, an AChm antagonist and; scopolamine, an AChm antagonist. Neurochemical injection sites, lowest effective concentrations and time course of activity are listed in Table 1 (also see Appendix I) and described in the text.

The agonists or antagonists were injected into six regions of the mid- and hindbrain from which locomotion was elicited by electrical stimulation (Figs. 9 & 10). These regions included the pontobulbar locomotor strip (PLS) (a region which lies within or in close ventromedial apposition to the descending trigeminal tract and nucleus (TTD)), the dorsal part of the central nucleus (Cnd) in the medullary reticular formation, the ventral part of the central nucleus (Cnv) in the medullary reticular formation, the gigantocellular part of the pontine reticular nucleus (RPgc), the mesencephalic reticular formation (MRF) and the medial longitudinal fasciculus (MLF) of the pons. A composite diagram of the electrical stimulation/neurochemical injection sites is shown in Figure 9 and examples of lesions indicating effective sites are shown in Figure 10.
TABLE 1
Acetylcholine Agonists and Antagonists

<table>
<thead>
<tr>
<th>Animal</th>
<th>Site</th>
<th>Chemical</th>
<th>pH</th>
<th>Concentrations Injected</th>
<th>Lowest Effective Concentration</th>
<th>Volume</th>
<th>Rate</th>
<th>Time Course (min)</th>
<th>Latency Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>decerebrate TTD</td>
<td>Carbachol</td>
<td>7.2-7.4</td>
<td>25mM-100mM</td>
<td>25mM</td>
<td>1.0ul</td>
<td>0.2uMin</td>
<td>2.2-12</td>
<td>7-45</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Scopolamine</td>
<td>&quot;</td>
<td>25mM</td>
<td>none</td>
<td>&quot;</td>
<td>&quot;</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nicotine</td>
<td>&quot;</td>
<td>25mM</td>
<td>none</td>
<td>&quot;</td>
<td>&quot;</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Atropine</td>
<td>&quot;</td>
<td>25mM</td>
<td>25mM</td>
<td>&quot;</td>
<td>&quot;</td>
<td>6</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pilocarpine</td>
<td>&quot;</td>
<td>50mM</td>
<td>none</td>
<td>&quot;</td>
<td>&quot;</td>
<td>7</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Cnd</td>
<td>Carbachol</td>
<td>&quot;</td>
<td>11mM-100mM</td>
<td>11mM</td>
<td>&quot;</td>
<td>&quot;</td>
<td>3-3.6</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Scopolamine</td>
<td>&quot;</td>
<td>25mM</td>
<td>none</td>
<td>&quot;</td>
<td>&quot;</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Atropine</td>
<td>&quot;</td>
<td>30mM</td>
<td>30mM</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&lt;5</td>
<td>&gt;26</td>
<td></td>
</tr>
<tr>
<td>Cnv</td>
<td>Carbachol</td>
<td>7.2-7.4</td>
<td>27-100mM</td>
<td>27mM</td>
<td>&quot;</td>
<td>&quot;</td>
<td>2.2-6</td>
<td>7-45</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Scopolamine</td>
<td>&quot;</td>
<td>25mM</td>
<td>none</td>
<td>&quot;</td>
<td>&quot;</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nicotine</td>
<td>&quot;</td>
<td>100mM</td>
<td>none</td>
<td>&quot;</td>
<td>&quot;</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Atropine</td>
<td>&quot;</td>
<td>5-50mM</td>
<td>20mM</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&gt;7.5</td>
<td>21-40</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pilocarpine</td>
<td>&quot;</td>
<td>50mM</td>
<td>none</td>
<td>&quot;</td>
<td>&quot;</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>RP</td>
<td>Carbachol</td>
<td>&quot;</td>
<td>64mM</td>
<td>none</td>
<td>&quot;</td>
<td>&quot;</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>MRF</td>
<td>Carbachol</td>
<td>&quot;</td>
<td>100mM</td>
<td>100mM (RT)</td>
<td>&quot;</td>
<td>&quot;</td>
<td>—</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>MLF</td>
<td>Carbachol</td>
<td>&quot;</td>
<td>27-100mM</td>
<td>27mM</td>
<td>&quot;</td>
<td>&quot;</td>
<td>9-10</td>
<td>23-40</td>
<td></td>
</tr>
</tbody>
</table>

ABBREVIATIONS:
Cnd — dorsal part, medullary central nucleus
Cnv — ventral part, medullary central nucleus
MLF — medial longitudinal fasciculus
MRF — mesencephalic reticular formation
RP — pontine reticular nucleus
RT — reduced threshold for electrically stimulated locomotion
TTD — descending trigeminal tract and nucleus
Figure 9. Composite diagram of cholinergic agonist and antagonist neurochemical injection sites. The diagram of coronal sections through various levels of the avian neuraxis [numbers in upper left corner of each level, A=anterior, P=posterior (in mm)] illustrates the locomotor effects of each neurochemical in brainstem regions from which locomotion was first elicited by electrical stimulation. Where atropine is shown as filled triangles, its effect was to block locomotion.

Key Abbreviations: ATRO - atropine (triangle), CARB - carbachol (square), NICO - nicotine (circle), PILO - pilocarpine (hexagon), SCOP - scopolamine (diamond); LOCO - locomotion (except for atropine), \( \downarrow \) TH - decreased electrical threshold intensity for locomotion, \( \uparrow \) TH - increased electrical threshold intensity for locomotion, NR - no response.

Figure 10 Coronal sections through the avian brainstem illustrating electrical and neurochemical stimulation sites which elicited locomotion in the decerebrate bird. A: Coronal section through the caudal medulla showing a stimulation/injection site (L) in TTD. B: Coronal section through the caudal medulla showing a stimulation/injection site (L) in Cnd. C: Coronal section through the caudal medulla demonstrating a stimulation/injection site (L) in Cnv. D: Coronal section through the pons illustrating a stimulation/injection site in the pontine reticular formation (RP) near the raphe nucleus (R). For the effects found in each region, see Results. Abbreviations: cc - central canal, Cnd - dorsal part, central medullary nucleus, Cnv - ventral part, central medullary nucleus, IM - intermediate nucleus, IO - inferior olivary nucleus, L - lesion made at stimulation/injection site, MLF - medial longitudinal fasciculus, NVIII - glossopharyngeal nerve, R - raphe nucleus, RP - pontine reticular formation, SSP - supraspinal nucleus, TTD - descending trigeminal tract and nucleus, X - dorsal motor nucleus of the vagus.
Pontobulbar Locomotor Strip (PLS)

Electrical stimulation of the pontobulbar locomotor strip (PLS) elicited locomotor movements at low threshold intensities ranging from 30-80μA in nine animals. In four out of four birds, following the establishment of a locomotion producing electrical stimulation site (walking) (Figure 11A), injection of carbachol (25-100mM) into PLS elicited a pattern of alternating walking behaviour (no wing flapping) with a mean latency to the first detectable movement following initial injection of 8.2 minutes (range 2.2-12 minutes). The stepping (Figure 11B) was long lasting (mean 23 minutes: range 7-45 minutes), during which time electrical stimulation enhanced the vigor of walking in an intensity dependent manner.

In the only animal tested, infusion of atropine (25mM) into the same site during carbachol-induced locomotion completely blocked not only the chemically induced behaviour but also all electrically evoked stepping up to the maximal stimulation intensity (170μA).

Introduction of pilocarpine (50mM) into PLS produced intermittent short bursts of walking and flying behaviour in one animal with a time to onset of 7 minutes. The bursts were maintained for approximately 8 minutes. This period was followed by extension of both wings and legs, at which time electrical stimulation was ineffective at eliciting locomotor patterns.

Neither scopolamine (N=1) (25mM) nor nicotine (N=2) (25mM) was effective at blocking, eliciting or changing the threshold of PLS electrically stimulated locomotion.
Figure 11. Electromyographic records (EMGs) showing locomotor activity elicited by electrical stimulation and carbachol injection into the pontobulbar locomotor strip (PLS). A: Alternating stepping represented by EMG patterns from the right (RITC) and left (LITC) iliotibialis cranialis muscles (major hip flexor muscle) elicited by electrical stimulation of the PLS. B: Alternating stepping EMGs elicited by injection of carbachol (100mM) into the same site.
Central Nucleus, dorsal part (Cnd)

Infusion of carbachol (100mM) into the dorsal part of the medullary central nucleus (Cnd) produced long lasting stepping in only one of five birds tested (Fig. 12A,B). In two animals, however, the thresholds for electrically evoked locomotion were reduced (60μA to 30μA and 80μA to 60μA) after carbachol (11mM & 50mM) infusion. Introduction of atropine (30mM) during carbachol stimulated locomotion blocked the ongoing locomotion, as well as further attempts to induce locomotion electrically and chemically (carbachol). Similarly, introduction of atropine into one of the animals that showed decreased threshold (carbachol, 30mM) completely blocked any further electrically evoked activity. Scopolamine (25mM) did not change the carbachol-induced decrease in locomotor threshold of the other animal.

Central Nucleus, ventral part (Cnv)

In the ventral part of the medullary central nucleus (Cnv), electrical stimulation, used to establish optimum electrode position, elicited bilateral alternating hindlimb walking in 9 out of 10 birds, and running and flying in 1 out of 10. Subsequent injection of carbachol produced long lasting walking behaviour in 6 of the 10 animals injected (Fig. 13, carbachol = 27mM). One bird showed both walking and flying behaviour in response to carbachol injection, while electrical threshold for walking was reduced in another. Two animals demonstrated no
Figure 12. Electromyographic records (EMGs) showing locomotor activity elicited by electrical stimulation and carbachol injection into the central medullary nucleus, dorsal part (Cnd). A: Alternating stepping represented by EMG patterns from right (RITC) and left (LITC) iliotibialis cranialis muscles elicited by electrical stimulation of the Cnd. B: Alternating stepping EMGs elicited by injection of carbachol (100mM/1.0μl) into the same site.
Figure 13. Electromyographic records (EMGs) showing locomotor activity elicited by electrical stimulation and carbachol infusion into the central medullary nucleus, ventral part (Cnv). PRESTIM: EMGs from right (RITC) and left (LITC) iliotibialis cranialis muscles illustrating the lack of spontaneous locomotor activity in the pre-stimulation decerebrate preparation. STIM: Alternating stepping represented by EMG patterns from the same muscles during electrical stimulation (40μA) of the site (Cnv) shown by the squares in the coronal section through the caudal medulla (bottom right). CARB: Carbachol injection into the same site (filled square) elicited alternating stepping, as demonstrated by the EMGs from RITC and LITC. ATRO: The carbachol-induced locomotor activity was blocked by injection of atropine (unfilled square) into the same site. In addition, after atropine injection, as shown during electrical stimulation of the site, stimulus intensities up to 170μA did not evoke locomotor patterns (some stimulus bleed through can be seen in the EMG traces).
effects of carbachol injection. Five animals injected with atropine (25mM) following carbachol-induced locomotion, including the walking/flying bird, ceased all locomotor activity within a mean time of 7.5 minutes post-injection (Fig. 13). In all cases where carbachol-induced locomotion was blocked by atropine, locomotion did not return in the absence of electrical stimulation during the experimental period. However, the return of electrically stimulated locomotion following atropine injection appeared to relate to the concentration injected, with higher concentrations of atropine (50mM (range 25-50mM) blocking for periods of up to 40 minutes (stimulation maximum 170μA).

Injections of nicotine (N=2) (100mM) and pilocarpine (N=2) (50mM) were ineffective at producing chemically stimulated locomotion when infused into Cnv and did not appear to have any effect on either the threshold or type of locomotion elicited by electrical stimulation. Scopolamine (N=1) (25mM) also had no effect when injected into this site.

**Pontine (RP) and Mesencephalic (MRF) Reticular Formation**

Injection of carbachol (54mM) into the pontine reticular formation (RPgc) (N=1) had no effect on electrically stimulated locomotor threshold or on locomotor pattern. In the MRF, however, carbachol (100mM) injection decreased the electrical stimulation threshold for locomotion (100→60μA) in one animal, but was ineffective at eliciting locomotion or changing the electrical stimulation threshold intensity for locomotion in a second bird.
Medial Longitudinal Fasciculus (MLF)

Electrical stimulation (70-80μA) of the MLF evoked hindlimb and forelimb locomotion in five birds. Low stimulus intensities evoked walking alone (Fig. 14A) which gave way to flying and running at higher intensities. Injection of carbachol (27-100mM) into these sites in three out of five animals evoked either walking (N=1) (Fig. 14B) or running and flying (N=2). The animals were initially electrically stimulated, then curarized, and the stimulation intensity necessary to evoke locomotor patterns was increased over the pre-paralyzed condition to a mean intensity of 170μA (see Chapter 6). However, the concentrations of carbachol necessary to evoke locomotor patterns in these paralyzed birds ('fictive' locomotion) were not significantly different from those found in unparalyzed preparations.

COMPARISON BETWEEN ELECTRICAL AND CHEMICAL STIMULATED LOCOMOTION

The locomotion elicited by electrical stimulation typically varied with different intensities of stimulation and from region to region in the bird (Sholomenko and Steeves, 1987). For example, electrical stimulation in some sites produced walking at low current intensity (e.g. 30μA), running and flying at higher intensity (e.g. 90μA) and flying alone at still higher intensity (e.g. 120μA) (see Fig.3, Steeves et al., 1987). In other sites, threshold intensity stimulation would evoke running and flying together, while in still other locations, only
Figure 14. Electromyographic records (EMGs) showing locomotor activity elicited by electrical stimulation and carbachol injection into the medial longitudinal fasciculus (MLF). A: Alternating stepping as represented by EMGs from right (RITC) and left (LITC) iliobibialis cranialis muscles during electrical stimulation of the MLF. B: Alternating stepping EMGs elicited by carbachol (27mM) infusion into the same site.
walking could be elicited at all stimulation intensities. However, electrical stimulation always evoked the same pattern of behaviour in a stimulation dependent and site specific manner. Thus, repeated trials in the same site always elicited the same locomotor pattern. These patterns, with few exceptions, were replicated by the neurochemical injections which elicited locomotion.
DISCUSSION

Neurochemical stimulation of selected regions in the avian mid- and hindbrain elicited a spectrum of locomotor patterns in decerebrate birds. My results demonstrated that cholinergic agonists evoke locomotion and muscarinic antagonists block locomotion when injected into a variety of sites. These results will be discussed region by region and conclusions will be drawn as to the possible avian neuroanatomical pathways involved. These data from birds will be compared with those found in mammalian species for which similar data exists.

Pontobulbar Locomotor Strip

Electrical stimulation of the pontobulbar locomotor strip (PLS) elicits locomotion in birds (Steeves et al., 1987; Funk et al., submitted) and in a wide range of vertebrate species from lamprey to cat (for review, see Chapter 1 & McClellan, 1986). As will be discussed in greater detail below, the neuroanatomical input/output relations of the avian TTD are similar to those found in a variety of vertebrates with the exception that the major trigeminothalamocortical relay found in mammals is replaced in birds by a direct telencephalic projection (Arends et al., 1984; Arends and Dubbeldam, 1984; Wild et al., 1984).

My previous studies in birds demonstrate that electrical stimulation of the descending trigeminal nucleus and the Plexus of Horsley (PH), in addition to adjacent regions of the
pontomedullary lateral reticular formation (parvocellular reticular nucleus), which are virtually indistinguishable from the nucleus interpolaris of TTD (Arends and Dubbeldam, 1984), elicits locomotion in the decerebrate animal, thus defining this region as the avian equivalent of the mammalian PLS (Steeves et al., 1987).

In my experiments, locomotion was elicited by introduction of carbachol or pilocarpine into the PLS. Carbachol, a cholinergic agonist with a structure similar to that of acetylcholine (Taylor, 1985) is an effective cholinomimetic at both nicotinic and muscarinic acetylcholinergic receptor subtypes (Burgen, 1983), including four currently recognized muscarinic receptor subtypes which have been identified in the CNS (Buckley et al., 1988). Receptor binding studies demonstrate that carbachol binds to high and low affinity receptors in the CNS, although the binding varies by a factor of 100 depending upon the location (Burgen, 1983). The binding studies also demonstrate that at carbachol concentrations of 100mM, all muscarinic (M1 & M2) receptor sites should be bound (Potter et al., 1983). Thus, at the site of carbachol injection into locomotor regions in the avian brain at high concentrations (100mM), all muscarinic receptors should be activated. It was not possible to determine, however, the concentration gradient from the injection point to the periphery of the affected volume. In this study, it was also not possible to distinguish between the different receptor subtypes activated, as no agonists or antagonists are yet available which are specific for a single muscarinic receptor subtype (Buckley et al., 1988).
Therefore, it was difficult to distinguish between the carbachol induced activation of muscarinic receptors which 1) increase potassium conductance in some neurons, 2) decrease the potassium conductance in some neurons, 3) increase the cation (most importantly sodium) conductance in some neurons or 4) reduce calcium conductance in some neurons (North, 1985). Similar difficulties come in determining the specific receptor subtype activated by injection of the muscarinic agonist pilocarpine into the PLS, as pilocarpine recognizes all muscarinic receptor subtypes, although with differing affinities (Brown, 1983). However, the activation of locomotion following pilocarpine injection lends credence to the hypothesis that cholinergic receptors are involved in the activation, although this agonist was employed in a single trial only.

Further evidence implicating muscarinic receptors as being responsible for the induction of locomotion was that carbachol induced locomotion could be blocked by injection of the muscarinic antagonist atropine sulfate into the same site. Atropine has been demonstrated as a non-selective antagonist which blocks all muscarinic receptors (Mitchelson, 1983), and which has no nicotinic action (Mitchelson, 1983). However, as only a single PLS site was injected with atropine, this result can only be considered suggestive of a role for muscarinic receptors underlying the activation of locomotion from this region. Correlative support for this suggestion comes from the finding that nicotine, the classic cholinergic nicotinic receptor agonist, had no effect when injected into this region. However, scopolamine, a potent muscarinic antagonist (Kilbinger,
1983), rather unexpectedly also had no effect on electrical stimulation-induced locomotion when injected into the PLS in one animal. These above results in the bird, while somewhat equivocal, do suggest a role for cholinergic muscarinic receptors underlying the observed results. The results are also supported, in part, by neuroanatomical and receptor localization studies discussed below.

N-[^3]H]methylscopolamine (AChM antagonist) binding studies in pigeon brain show heavy labelling of cholinergic receptors in TTD and substantia gelatinosa (SG) of the medulla and spinal cord, (Dietl et al., 1988 see Figure 1H,J,K,G,I). Thus, this region contains the type of receptor which correlates with our observed results. However, to our knowledge, no cholinergic inputs to TTD or SG have been reported in birds. In an attempt to isolate possible cholinergic inputs to these regions, our laboratory has utilized immunohistochemical techniques to localize choline acetyltransferase (ChAT) containing cell bodies in the avian brain (Steeves and Taccogna, unpublished observations). This study has been combined with retrograde transport studies using fluorescein- and rhodamine conjugated dextran amines (Glover et al., 1986) into TTD (Webster and Steeves, unpublished observations) to locate putative cholinergic projections into this region. These findings indicate that possible sources of cholinergic input to TTD may arise from ChAT positive neurons originating in: 1) the pontine and medullary reticular formation, including the lateral reticular nucleus (RL), gigantocellular reticular nucleus (Rgc) and oral part of the pontine reticular formation (RPO), 2) the
nuclei of VII, which send efferents to TTD in regions caudal to the obex, 3) the descending vestibular nucleus (VeD), which projects to rostral regions of TTD, 4) the nucleus raphe pallidus, which impinges on rostral TTD, 5) the paramedian nucleus, also projecting to rostral TTD and 6) the parabrachial region which lies ventral to the ventral subceruleus nucleus and projects to rostral TTD. ChAT containing neurons have also been localized to TTD and the subtrigeminal nuclear regions, allowing for the possibility that intrinsic cholinergic interneurons may modulate TTD neural circuits and subserve the carbachol-induced locomotion from this region.

In mammals, afferents from the trigeminal and glossopharyngeal nerves impinge on the descending trigeminal nucleus and are thought to contain Substance P, cholecystokinin (CCK), vasoactive intestinal polypeptide (VIP) and somatostatin (Dubner and Bennett, 1983) but are apparently not cholinergic. In the cat the ChAT-containing cells (Vincent and Reiner, 1987) of PPN/mMLR may send a small cholinergic innervation to PLS neurons (Garcia-Rill, 1985) which, in turn, project upon the brainstem reticular formation and propriospinal pathways outlined above. Also in mammals, MesV is known to send internuclear fibres to TTD (Garcia-Rill et al., 1983; Ikeda et al., 1984) but no ChAT activity has been found in the MesV (Vincent and Reiner, 1987). This precludes the possibility that MesV provides cholinergic input to TTD (Reiner and Vincent, 1987). Other possible cholinergic inputs to TTD may arise from the caudal cuneiform nucleus, where both ACh and AChE have been localized in the rat (Palkovits and Jacobowitz, 1974;
Ramon-Moliner and Dansereau, 1974) and cat (Kimura et al., 1981). However, some controversy exists as to whether the cuneiform nucleus contains ACh neurons, as Reiner and Vincent (1987) found no evidence of ChAT containing neurons in this nucleus (for review see p. 521 of Rye et al., 1987).

Pharmacological evidence of a role for ACh in TTD stimulation-induced locomotion has also been found in the rat where iontophoretic application of ACh increased firing rates of cells in the nucleus caudalis of TTD (Salt and Hill, 1981). While, to our knowledge, no cholinergic afferents to the region we injected have been unequivocally established in birds, our studies point to several potential cholinergic pathways which may play some role in locomotor control via the PLS pathway. However, the precise role of these projections in motor control remains to be determined.

In birds, TTD efferents project to a variety of regions including cerebellum, spinal cord dorsal horn (C4), Cnv, Cnd, ST, PH, RL, Rpc, dorsal column nuclei, PrV, and parabrachial nucleus (Arends and Dubbeldam, 1982, 1984; Arends et al., 1984; Webster and Steeves, personal communication). The spinal cord projection may be equivalent to the trigeminal propriospinal network found in mammals, while the efferents to PrV and the reticular nuclei have been implicated in the control of grasping, feeding and pecking in birds (Wild et al., 1984). Connections of TTD with the reticular formation nuclei will be discussed below in relation to locomotion elicited from these regions. On the basis of its hodological connections, combined with its locomotor eliciting properties following electrical and
chemical stimulation, however, TTD appears to play a major role in the sensory control of a variety of motor behaviours in birds. This data, therefore, agrees with the proposal by Jordan and co-workers (Jordan, 1986; Noga et al., 1988) for mammalian species that the descending trigeminal nucleus is concerned with the sensorimotor control of locomotion. Our studies suggest that this control is mediated, in part, by cholinergic muscarinic mechanisms.

Central Nucleus, dorsal part (Cnd)

Previous studies have shown that electrical stimulation of the dorsal part of the medullary central nucleus (Cnd) elicits locomotion in the decerebrate bird (Steeves et al., 1986). Retrograde transport studies using True Blue (Steeves et al., 1987) and HRP (Cabot et al., 1982) demonstrated that Cnd gives rise, in part, to the reticulospinal fibers travelling in the spinal cord ventral funiculus, which have been shown through selective low thoracic spinal cord lesions, to be essential for voluntary or electrically induced walking both in chronic and decerebrate birds (Sholomenko and Steeves, 1987b), cat (Steeves and Jordan, 1980; Eidelberg, 1981) and monkey (Eidelberg et al., 1981). Thus, it appears that cells originating from this nucleus give rise to a descending pathway important for the descending control of locomotion.

Further evidence for a role of Cnd in locomotor control in birds comes from neuroanatomical studies which show that Cnd receives afferent input from the subnuclei caudalis and
interpolaris of TTD (Arends et al., 1984; Arends and Dubbeldam, 1984), the nucleus intercollicularis (ICo) (Webster, personal communication), the tectum (Hunt and Kunzle, 1976) and from the archistriatum intermedium via the occipitomesencephalic tract (Wild, 1985, Wild et al., 1984). The afferent input from the archistriatum has been implicated as being part of a circuit terminating in the pontomedullary reticular formation which is essential for the control of feeding, grasping and pecking in the pigeon (Wild et al., 1984). Input to Cnd from the deep tectal layers (Hunt and Kunzle, 1976; Reiner and Karten, 1982) appears to be part of the outflow circuit involving the avian basal ganglia (TPc)/lateral spiriform nucleus/tectum, which is postulated to be involved in visuo-spatial motor control (Reiner et al., 1984). In turn, Cnd sends ascending projections to the motor nuclei of cranial nerves V & VII, gigantocellular reticular nucleus (Rgc) and the parabrachial nucleus (Arends and Dubbeldam, 1984), in addition to its descending reticulospinal component. The projections from PLS/TTD to brainstem reticular nuclei, including Cnd, appear to have direct sensorimotor implications as discussed from our results above. In birds, ICo, and FRL are thought to contain neuronal populations homologous to those found in the the mammalian cuneiform nucleus (Cabot et al., 1982; Edwards, 1975) and will be discussed subsequently. The efferent connections of Cnd to the parabrachial and cranial nerve V and VII motor nuclei implicate this region in the control of feeding and drinking behaviours (Arends and Dubbeldam, 1982). These hodological relationships, therefore, place Cnd in an excellent position to act as an integratory and
descending output region from which both sensory and higher brain centers may influence motor control.

To further our understanding of Cnd's role in locomotor control, we have begun to examine its neuropharmacology. Carbachol infusion into Cnd elicited (or reduced threshold for) walking behaviour which was blocked by the muscarinic antagonist atropine, while nicotinic agonists were ineffective at producing locomotion or changing the threshold for electrically stimulated locomotion.

 Autoradiographic studies of receptor binding with N-[^3]H)methylscopolamine indicate the presence of muscarinic cholinergic receptors in the Cnd of the pigeon (Dietl et al., 1988). Although no cholinergic afferents to Cnd have been unequivocally identified in birds, neuroanatomical (Webster and Steeves, unpublished observations) and immunohistochemical studies from our laboratory (Steeves and Taccogna, unpublished observations) demonstrate the presence of ChAT containing cell bodies in the subtrigeminal nucleus, TTD, pontine reticular formation, laterodorsal tegmental nucleus and nucleus isthmi, pars parvocellularis, all of which have been shown to project to Cnd. Cnd itself also contains ChAT positive neurons.

Similarly, in mammals, the equivalent structure, FTL, (lateral tegmental field) receives afferent input from the region of the PLS/trigeminal area (Noga et al., 1988; Garcia-Rill et al., 1983b) and also appears to receive descending cholinergic input from the MesV region and PPN/mMLR (Garcia-Rill et al., 1983b). Further, in cat, cell bodies containing ChAT have been localized in the reticular tegmental field (Vincent
and Reiner, 1987), possibly implicating intrinsic cholinergic neurons and muscarinic receptors as producing some of the observed results.

Taken together with those from mammalian studies, our results provide strong evidence that Cnd neurons give rise to part of the descending reticulospinal pathway which is essential for locomotor control in vertebrates. Further, it appears to act as an integration centre for sensory information from the descending trigeminal nucleus and also from higher brain structures controlling locomotion in birds. Based on the findings that carbachol injection elicited atropine-reversible locomotor behaviour, the nucleus appears to be in part under cholinergic muscarinic control and possible control pathways have been described. However, as only a single animal directly demonstrated locomotion following carbachol infusion, it may be possible that neurochemical spread to Cnv or TTD was responsible for the locomotion. Studies utilizing smaller injection volumes (presumably with less spread) may alleviate this problem. Also, why scopolamine did not block locomotion in the one animal injected awaits further replication. Future studies are also required to determine both the mechanisms and source(s) of cholinergic input (or intrinsic neurons) which may exert control over this descending pathway.

Central Nucleus, ventral part (Cnv)

The Cnv also appears to play an important role in locomotor control. Electrical stimulation of the ventral part of
the medullary central nucleus (Cnv) produced locomotion in birds (Steeves et al., 1986; Sholomenko and Steeves, 1987b). Evidence from the bird (Sholomenko and Steeves, 1987), as in the cat (Steeves and Jordan, 1980) and monkey (Eidelberg et al., 1984), demonstrated that a lesion of the descending pathways travelling in the spinal cord ventral funiculus blocks voluntary locomotion in the intact animal and electrically stimulated locomotion in the decerebrate preparation.

In the cat, Garcia-Rill’s group (Garcia-Rill and Skinner, 1987b) utilized retrograde transport of bisbenzimide and nuclear yellow from electrically/chemically defined locomotor sites in the VRN to demonstrate its afferent input. They found ipsilateral and contralateral projections to VRN from the central gray, cuneiform nucleus, subcuneiform nucleus, PPN, midbrain reticular formation, anterior lateral reticular nucleus, lateral reticular nucleus and scattered reticular formation cells. Steeves and Jordan (1984) utilized anterograde transport of radioactive amino acids to demonstrate direct projections to the pontine and medullary reticular formation from the classical MLR, while Orlovsky, (1970a) used electrophysiological techniques to show a monosynaptic linkage between the 1MLR and medullary reticular formation.

In birds, neuroanatomical retrograde transport studies demonstrate that cells in Cnv give rise to reticulospinal neurons travelling the length of the spinal cord in the ventral funiculus (Steeves et al., 1987; Webster and Steeves, 1988) and that electrical stimulation in and around these cells elicits locomotion (Steeves et al., 1987). In birds, retrograde (True
blue, HRP, dextran amines) and anterograde (WGA-HRP) neuroanatomical tracing techniques demonstrate that afferents to the medial medullary reticular formation (Cnv) include the nucleus intercollicularis (ICo), cerebellum, Area ventralis of Tsai (AVT), Red nucleus, nucleus raphe magnus, lateral and medial mesencephalic reticular formation (FRL, FRM), more rostral pontine reticular formation, (RPC, RPgc) and TTD (Webster, personal communication). All of the above afferent structures have been implicated in motor control (as discussed above; for review see Kuypers, 1981) and similar to Cnd, place Cnv in an ideal position to integrate and output motor information to the spinal cord. Hodological considerations implicate Cnv as being homologous to the mammalian ventral reticular nucleus (Garcia-Rill and Skinner, 1987a,b) (magnocellular reticular formation of Noga et al., 1988).

To examine the motor-associated role which acetylcholine may have in the activation of locomotion from this region, cholinergic agonists and antagonists were injected into Cnv sites from which locomotion could be electrically induced. Carbachol injection into the ventral part of the medullary central nucleus (Cnv) produced atropine-reversible locomotor behaviour in birds in a manner similar to that found following injection of acetylcholine, carbachol or acetylcholinesterase inhibitors into the cat medullary ventral reticular nucleus (Garcia-Rill and Skinner, 1987a). However, pilocarpine injection did not elicit any locomotor behaviour. Although it is presently unknown whether any avian afferent projections to Cnv are definitively cholinergic, studies from our laboratory point to
several potential nuclei which possess both ChAT containing neurons and have been found to project to Cnv. These include, from caudal to rostral, TTD, Nucleus reticularis gigantocellularis (Rgc), Nucleus reticularis parvocellularis (Rpc) and Nucleus mesencephalicus, pars profundus (MPv). Also, as in the cat (Jones et al., 1986), Cnv itself encompasses ChAT containing neurons. We can speculate that TTD, Rgc and Rpc are the most likely candidates to have this cholinergic effect on Cnv neurons, as electrical stimulation of these regions elicits locomotion. However, these pathways are still only potentially cholinergic. Further study is needed to resolve this problem.

In the rat, PPN projections to VRN have been shown to contain acetylcholine (Rye et al., 1987, 1988). Some controversy exists regarding these connections, as Goldsmith and Van Der Kooy (1988), using NADPH-diaphorase histochemistry, found no evidence of descending cholinergic projections from PPN in the rat. However, Rye et al. (1988) have recently reported a significant cholinergic projection from PPN to the reticular formation in the rat. Jones et al. (1986), also in the rat, used retrograde transport of [³H]Choline to find evidence for the presence of cholinergic neurons descending from the PPN to high cervical levels. In addition, they demonstrated retrogradely labelled cells in the medial gigantocellular reticular formation of the medulla which descend to cervical spinal levels, possibly implicating these descending cholinergic neurons in the control of locomotion. Cells arising from the VRN are known to give rise to reticulospinal neurons important to locomotor control (Steeves et al., 1987). As in the bird, ChAT immunoreactivity
has been found in the tegmental field of the cat (medial to lateral tegmental field and dorsal to inferior olive) (Vincent and Reiner, 1987), supporting the possibility that neurons intrinsic to this region may provide interneuronal input (or be output neurons) to descending reticulospinal neurons. The above studies indicate that in mammals, as in birds, the definitive locomotor related cholinergic inputs to the medullary reticular formation responsible for the observed results remain to be elucidated. It appears likely, however, that reticulospinal neurons originating from the avian Cnv, like the mammalian VRN, are at least partially under cholinergic muscarinic control.

Pontine Reticular Formation (RP)

Electrical stimulation of the ventral pontine reticular formation has been shown to elicit locomotion in birds (Sholomenko and Steeves, 1987). Previous investigators have utilized both chemical and electrical stimulation of the pontine reticular formation to evoke a variety of motor responses from this region. Interestingly, Katayama et al. (1984) found that injection of carbachol into the dorsal aspects of the pontine reticular formation in the intact cat produced postural atonia similar to that seen with dorsal midline pontine electrical stimulation in the mesencephalic cat (Mori et al., 1978). Mori et al. (1978) described two regions in the pontine tegmentum which gave very different results when electrically stimulated. The first region, which lay dorsally within the central superior pontine nucleus, produces a decrease in hindlimb extensor muscle
tone when stimulated. The second more ventral region, lying within the boundaries of the nucleus raphe magnus, produces an increase in extensor tone when stimulated. In birds, raphe-spinal connections are known to impinge on preganglionic sympathetic neurons in the spinal cord and have not been implicated in motor control (Cabot et al., 1982). Like Mori's results in cats, in birds, ventral pontine electrical stimulation resulted in locomotion.

In birds, the pontine reticular formation is known to receive afferent input from the nuclei caudalis and interpolaris of TTD (Arends and Dubbeldam, 1982; Webster, personal communication) and from the tectum (Hunt and Kunzle, 1976). It projects efferents to caudal TTD, motor nuclei V and VII, nucleus parabrachialis (Arends and Dubbeldam, 1982) and to lower brainstem reticular formation nuclei (Webster, personal communication). RP also gives rise to sparse descending projections to both cervical and lumbar spinal cord which travel in the ventral funiculus of the spinal cord (Cabot et al., 1982; Webster and Steeves, 1988), a region which lesion studies show supports locomotion (Sholomenko and Steeves, 1987).

Mammalian neuroanatomical studies demonstrate that RP receives projections from the region of the ventral tegmental area, caudal cuneiform (Steeves and Jordan, 1984) and PPN (Garcia-Rill et al., 1983) in the cat and rat (Garcia-Rill et al., 1986). It also receives afferent input from the laterodorsal tegmental nucleus (TLD) in the rat (Brudzynski et al., 1988).

Injection of carbachol into RP was ineffective at eliciting locomotion or affecting the stimulus threshold necessary to
evoke locomotion in the one preparation tested. Further study is required to determine whether cholinergic agonists and antagonists affect locomotor patterns in this region. However, injection of other neurotransmitter agonists and antagonists were effective at producing locomotor changes in this region and will be discussed in subsequent chapters (Chapters 4&5).

Mesencephalic Reticular Formation (MRF)

Electrical stimulation of the mesencephalic reticular formation, including sites in the nucleus intercollicularis (ICo), lateral mesencephalic reticular formation (FRL) and medial mesencephalic reticular formation (FRM), elicited locomotion in the decerebrate bird (Sholomenko and Steeves, 1988). ICo receives afferent input from the spinal cord, dorsal column nuclei, and tectum (Hunt and Kunzle, 1976; Webster, personal communication). It sends efferent projections to the pontine and medullary reticular formation, as well as to the high cervical spinal cord (Reiner and Karten, 1982; Webster, personal communication). FRL and FRM appear to have similar afferents and efferents to those of ICo. Cabot et al. (1982) compared these connections to those of the mammalian cuneiform nucleus (see Chapter 2), a portion of which, in mammals, is believed to be the lateral MLR (Steeves and Jordan, 1984; Shik et al., 1966).

In one of two birds, injection of carbachol into the MRF reduced the electrical stimulation threshold for locomotion. This equivocal result suggests that detailed study is required
to determine any possible role for acetylcholine in this locomotor region. As is discussed below, such a study in the bird may provide important information concerning the control of locomotion by the currently identified mammalian mesencephalic locomotor regions.

\[ N^-{^3}H \text{methylscopolamine binding studies in the bird show the presence of muscarinic cholinergic receptor binding in ICo but little binding in either FRL or FRM (Dietl et al., 1988).} \]

ChAT immunohistochemical studies demonstrate sparse or no labelling of potential cholinergic cells in these regions (Taccogna and Steeves, unpublished observations), thus negating any homology between these midbrain structures and the ACh-containing cell bodies of the mammalian PPN/mMLR defined by Garcia-Rill and co-workers (Garcia-Rill et al., 1983a).

Interestingly, Garcia-Rill et al. (1985) found that injection of acetylcholine into the PPN/MLR was ineffective at eliciting locomotion in the decerebrate cat and Brudzynski et al. (1988) found that carbachol injection into PPN decreased locomotion in the freely moving intact rat. However, carbachol injections placed between the PPN, cuneiform nucleus and periaqueductal gray, possibly within the region of the rat equivalent of the cat 1MLR, increased locomotion in the rat (Brudzynski et al., 1988).

The above results in cat and rat appear to lend credence to the existence of two MLRs, each possessing different characteristics that may be more fully defined by neurochemical injection studies. The pharmacological properties of neurons in corresponding anatomical structures in the midbrain of birds and
mammals indicate that different populations of neurons are under somewhat different control mechanisms, but are both apparently active in the control of locomotor behaviours. We suggest that regions in the avian midbrain, specifically in the regions of the nucleus intercollicularis and mMRF, are equivalent to those proposed as the lateral and medial MLRs of mammals. Further studies are required, however, before any homology between the mammalian and avian MLRs can be completed.

Medial Longitudinal Fasciculus (MLF)

Locomotion was elicited by electrical stimulation of the pontine and rostral medullary medial longitudinal fasciculus (MLF). Carbachol injection into the MLF elicited similar locomotor patterns.

The MLF is a fibre tract which carries ascending projections from all vestibular nuclei to visual motor nuclei and projections to the interstitial nucleus of Cajal (InC) (Carpenter and Sutin, 1983). It carries fibres from the 1) pontine reticular formation to the spinal cord (Carpenter and Sutin, 1983), 2) InC, which forms the interstitio-spinal tract (Carpenter and Sutin, 1983), to the Probst’s tract (Skinner et al., 1984), 3) rostral brainstem (e.g. InC) nuclei to the inferior olivary nucleus (Carpenter and Sutin, 1983; Skinner et al., 1984)), 4) vestibulospinal tract which sends collaterals to the medullary reticular formation (Carpenter and Sutin, 1983) and 5) internuclear visual motor nuclei (III, IV and VI) (Carpenter and Sutin, 1983). In addition, the MLF carries fibres
which impinge on the pedunculopontine nucleus in the rat (source unspecified) (Rye et al., 1987).

While it was not surprising that electrical stimulation of this tract elicited locomotor responses, as many of its fibre tracts are related to locomotor function (e.g. pontine reticular formation nuclei, pedunculopontine nucleus), it was surprising that neurochemical stimulation with the cholinergic agonist carbachol elicited long lasting locomotor behaviours. To our knowledge, no cholinergic receptors have been reported in the MLF, although serotonin-containing neurons which stain positively for acetylcholinesterase have been localized in this region in the chicken and duck (Dube and Parent, 1981; Taccogna et al., in preparation) (see Chapter 2). The hodological features of these neurons and their possible relation to locomotor function, however, remain to be determined.

PHARMACOLOGICAL CONSIDERATIONS

Neurochemical injection into some of the above locomotor regions demonstrated that these regions contain neurotransmitter receptors which can be affected by cholinergic agonists and antagonists. The mode of action by which the neurochemical affects the receptor is dependent upon the agent utilized. Agonists are believed to induce conformation changes in receptor proteins. This can be achieved through direct coupling to the ionophore or indirectly via activation of a second messenger system (muscarinic receptors - see Buckley et al., 1988). The agonist presumably produces an alteration in the ionic
permeability of the membrane, leading to a neuronal response which elicits downstream responses (e.g. locomotion). Antagonists, on the other hand, are believed to form an inert complex with the receptor protein, which, in the case of a competitive antagonist, can be displaced by an appropriate agonist at specific concentrations (for review of competitive binding studies, see Watson et al., 1984).

In my study, the receptors, when activated or inactivated by the injected agent, presumably affect a sufficient number of neurons to elicit or block decerebrate avian locomotion. The activation (or blockade) of neurons necessary to evoke (or block) locomotion is dependent on the ability of the neurochemical to spread through the tissue (activating or inactivating receptors) until enough neuronal receptors (and therefore neurons) have been recruited so that the outward behavioural signs of locomotion can be recorded with EMGs or ENGs. Thus, neurochemicals which are not readily metabolized [e.g. acetylcholinesterase which does not hydrolyze carbachol (Taylor, 1985)] will be more effective stimulators/inhibitors than readily metabolized neurochemicals such as acetylcholine (Taylor, 1985). Also, the ability of one neurochemical (e.g. atropine) to counteract the effects of another neurochemical (e.g. carbachol) is dependent upon their relative binding affinities [atropine>>carbachol (Furchgott and Cherry, 1984)] and the concentration of each agent, if the pair are acting at the same site.

In the case of the carbachol versus atropine mainly used in this study, atropine has been shown to displace receptor bound
carbachol (and pilocarpine) at muscarinic receptors (Taylor, 1985). Recent findings (Christie and North, 1988) suggest that the competitive antagonism of carbachol by atropine at muscarinic receptors is not directly on the ionophore, but on muscarinic receptors located distant to the ionophore (Christie and North, 1988). While it is not yet possible to determine the exact nature of the locomotion-associated cholinergic receptors being stimulated by the neurochemical injection technique. It appears likely that muscarinic receptors can be implicated in this control.

The pharmacological activation of locomotion is not, however, without limitations. First, it is difficult to titrate the injection dose to produce threshold activation. Second, the locomotion elicited by neurochemical stimulation is not as readily modified as is electrical stimulation-induced locomotion. Third, like electrical stimulation, the lack of a response to any given neurochemical may reflect the efficacy of the preparation at the time of injection. Fourth, the spread of neurochemical, and therefore, effective distribution, is difficult to control (see below). Fifth, the concentrations of intracerebrally injected neurochemicals are probably not physiological, although it may be argued that in order to recruit a sufficient number of neurons to elicit locomotion, it is necessary to use higher than physiological concentration at the injection point. Sixth, the intracerebral infusion technique provides no information concerning the pre- or post-synaptic location of the receptors. Lastly, the technique cannot account for the possibility that the neurochemical is activating
receptors present on neurons which normally have no afferent inputs associated with that transmitter and which may not normally be involved in the locomotor process (e.g. a cholinergic receptor may be present on a cell which has no cholinergic input. also see Stone and Burton, 1988).

The limitations of this technique do not, however, preclude its usefulness in demonstrating that the electrophysiologically-defined locomotor regions also contain neuronal receptors (presumably dendritic, somal or on terminals) which may underlie the physiologically evoked locomotor responses. Further, the technique provides information concerning possible neurotransmitter controlling inputs to the locomotor regions and has predictive value in determining the nature of neural pathways involved in locomotor control. The intracerebral neurochemical infusion technique also has predictive value for the use of other techniques. For example, prospective neurotransmitters identified with neurochemical infusion could be examined using in-vivo microdialysis (Sabol and Freed, 1988; Becker et al., 1988; Ajima and Kato, 1988; Phillips et al., 1988). The dialysis technique may be used to quantify the release of neurotransmitters during evoked locomotion and thereby elucidate which neurotransmitters have a role in brainstem locomotor control.

NEUROCHEMICAL SPREAD AND TIME COURSE OF ACTIVATION/INACTIVATION

Although this study and those in the following chapters (Chapters 4 & 5) did not include the injection of dye marker
chemicals into the injection sites to mark the degree of neurochemical spread, it is likely that the size of our stimulation sites were smaller than those shown in the cat (Garcia-Rill et al., 1985, Garcia-Rill and Skinner, 1987). The slow injection rate used in the present study (0.2μl/min), as compared to that utilized by other groups (e.g. Garcia-Rill et al., 1985 - 1μl/min; Garcia-Rill and Skinner, 1987a - 1μl/min; Noga et al., 1988 - 1μl/min; Lai and Siegel, 1988 - 0.5μl/min) and the small volumes (maximum 1.0μl) as compared to others (e.g. Garcia-Rill et al., 1985 - 1.5-3.0μl; Noga et al., 1988 - 5μl (see Appendix I)) injected would have served to reduce the neurochemical spread through the tissue. Also, affected areas were probably within <0.5mm radius, for it was noted in several trials of the present study that infusion of a neurochemical 0.5mm from an effective site did not elicit locomotion. Studies utilizing autoradiographic tracing of dispersed silver grains following injection of radioactive agonists and antagonists into injection sites might serve to confirm the degree of spread after intracerebral infusion of neurochemicals.

With regard to the latency for activity of the various neurochemicals utilized, there appears to be a general trend that small molecular weight neurochemicals (e.g. carbachol (molecular weight (MW) = 182.6) (this chapter), GABA (MW = 103.1) (Chapter 4) and NMDA (MW = 147.1) (Chapter 5)) acted more quickly than those with large molecular weight (e.g. atropine (MW = 676.8) (this chapter), picrotoxin (602.6) (Chapter 4)). Whether this trend results from a differential diffusion rate through the tissues remains to be determined. Also, the time
course (see Table 1) over which the agonists and antagonists maintained their locomotor effects appeared to be similar to those reported by other investigators (see Appendix I). For example, atropine injection into the cat ventral reticular nucleus (NRV - see Appendix I) blocked electrical stimulation-induced locomotion for 1-2 hours (Garcia-Rill and Skinner, 1987a), while in this study, atropine injection into an equivalent avian brainstem region, the ventral reticular formation (Cnv), blocked electrically stimulated locomotion for at least the length of the experimental period (21-40 min). Thus, atropine appears to produce long lasting effects in both bird and cat, although it is difficult to compare these effects due to differences in both concentration and injection volume.

COMPARATIVE CONSIDERATIONS

The neuroanatomical and neurophysiological comparison of birds with mammals is somewhat hindered by the different neuroanatomical terminology used for the two groups. However, it appears that neural circuitry in the bird midbrain and hindbrain is comparable to that of mammals (Reiner et al., 1984), only diverging at the level of the output of the basal ganglia (Reiner et al., 1984). Also, the hindbrain descending pathways in the bird appear to be fundamentally comparable to those of mammals (Cabot et al., 1982; Webster and Steeves, 1988). My results, that demonstrate both electrical and neurochemical cholinergic stimulation (carbachol, pilocarpine) of restricted brainstem regions produce avian locomotion, are similar to those
brainstem regions in mammals. These similarities therefore underscore the suggestion that the neuroanatomical substrate underlying the control of locomotion is highly conserved for these groups of vertebrates. Furthermore, our results demonstrate that several locomotion-promoting regions in the avian hindbrain are under cholinergic muscarinic control, although the precise neuroanatomical substrate for this control remains to be elucidated.
CHAPTER 4

CHARACTERIZATION OF AVIAN MID- AND HINDBRAIN SITES THAT PRODUCE LOCOMOTION WITH INTRACEREBRAL INFUSION OF NEUROTRANSMITTER AGONISTS AND ANTAGONISTS (II): γ-AMINOBUTYRIC ACID (GABA)
INTRODUCTION

In vertebrates, the control of locomotor behaviour is dependent upon central nervous system (CNS) circuitry at all levels of the neuraxis. Attempts to characterize this circuitry physiologically have utilized a variety of techniques including ablation, selective lesions, extra- and intracellular recording, electrical stimulation (for review see Grillner, 1975; Orlovsky and Shik, 1976) and more recently, neurochemical stimulation (Garcia-Rill et al., 1985; Noga et al., 1988; Sholomenko and Steeves, 1987a). Neuroanatomical studies have provided valuable information regarding the origin, course and terminations of CNS nuclei thought to be involved in locomotion (Kuypers, 1981; Holstege and Kuypers, 1987; Steeves and Jordan, 1984; Garcia-Rill et al., 1983a). Recent studies have aided in the identification and localization of neurotransmitters and their receptors which may be involved in the locomotor process (e.g. Mugnaini and Oertel, 1985). The present state of knowledge, therefore, allows for the initiation of an integrated approach to the study of locomotor control.

The previous chapter (Chapter 3) takes this approach in the description of the locomotor effects of cholinergic agonist and antagonist injections into sites in the avian mid- and hindbrain. Results from that study demonstrated that cholinergic muscarinic agonist stimulation of a variety of locomotor sites in the hindbrain of the decerebrate bird (Canada goose or Pekin duck) evoked locomotor behaviours. Furthermore, attempts were made to elucidate the cholinergic substrate involved in
locomotor control and generalize these findings to other vertebrates. This chapter describes the locomotor effects of injection of ɣ-aminobutyric acid (GABA), its agonists and antagonists into these same sites. The study was designed to survey whether GABAergic neurochemicals were effective in these regions as a prelude to more detailed pharmacological characterization.

Since the discovery of GABA by two independent research groups in 1950 (Roberts and Frankel, 1950; Awapara et al., 1950), GABA has been well characterized as a rather ubiquitous inhibitory neurotransmitter which acts both pre- and postsynaptically (Olsen, 1981; Bloom, 1985). Recent studies in the cat (Garcia-Rill et al., 1985, 1987; Eldridge et al., 1985; Noga et al., 1988) and rat (Brudzynski et al., 1988) have demonstrated that locomotion can be blocked by GABA infusion into selected locomotor regions of the neuraxis, while injection of GABA antagonists has been shown to induce or increase locomotor behaviour in these preparations.

In the bird, GABA injection into sites from which locomotion could be elicited by electrical stimulation was effective at blocking or increasing the threshold for electrically stimulated locomotion in the decerebrate preparation. In addition, GABA antagonists elicited locomotion when injected into some of these same sites. Because the neurochemicals infused are believed to act on neurotransmitter receptors (Goodchild et al., 1982), it is likely that these locomotor sites contain neuronal populations or terminals which are involved in the locomotor process. Our results in the bird
are similar to those described for mammals (Garcia-Rill et al., 1985, 1987; Noga et al., 1988) and therefore underlie our contention that avian CNS motor circuitry, at least at mid- and hindbrain levels, is homologous to that of mammalian species.
The materials and methods, including the decerebration procedure, electrical stimulation methodology, neurochemical injection parameters and histological procedures for the localization of stimulation sites have been previously described in Chapters 2 & 3.
RESULTS

GABA Agonists and Antagonists

Introduction of GABA, or GABAergic agonists and antagonists into electrically stimulated locomotion promoting sites in the hind- and midbrain were effective at blocking and eliciting a variety of locomotor patterns in decerebrate birds. The neurochemicals injected included: GABA; picrotoxin, a non-competitive GABA antagonist; muscimol, a GABA A receptor agonist and; bicuculline, a GABA A receptor competitive antagonist. Neurochemical injection sites, the lowest effective concentration and time course of activity are listed in Table 2 and described in the text. A composite diagram of the injection sites is shown in Figure 15.

Pontobulbar Locomotor Strip (PLS)

Five of eight animals which received picrotoxin injections (3-20mM/1.0μl) into PLS produced long lasting locomotor patterns. The mean latency to onset for locomotion following the initial injection was 13.5 minutes (range 4-22 minutes) with the periods of activity lasting between 35 and 60 minutes (at which point the experiment was terminated). The site-dependent modes of locomotion included walking alone, running and flying, and flying alone. In three birds, the initial locomotor reaction to picrotoxin injection involved small unilateral leg excursions (similar to the bilateral stepping elicited at threshold
TABLE 2
GABAergic agonists and antagonists

<table>
<thead>
<tr>
<th>Animal</th>
<th>Site</th>
<th>Chemical</th>
<th>pH</th>
<th>Concentrations Injected</th>
<th>Lowest Effective Concentration</th>
<th>Volume</th>
<th>Rate</th>
<th>Time Course</th>
<th>Latency</th>
<th>Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>decerebrate TTD</td>
<td>GABA</td>
<td>7.2-7.4</td>
<td>0.3-0.5M</td>
<td>0.3M</td>
<td>1.0ul</td>
<td>0.2ul/min</td>
<td>1-5</td>
<td>2-21</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Picrotoxin</td>
<td>&quot;</td>
<td>9-20mM</td>
<td>3mM</td>
<td>&quot;</td>
<td>&quot;</td>
<td>4-22</td>
<td>35-60</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bicuculline</td>
<td>&quot;</td>
<td>10mM</td>
<td>10mM</td>
<td>&quot;</td>
<td>&quot;</td>
<td>15</td>
<td>&gt;30</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Muscimol</td>
<td>&quot;</td>
<td>6.5-25mM</td>
<td>6.5mM</td>
<td>&quot;</td>
<td>&quot;</td>
<td>6-10</td>
<td>30-70</td>
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</tr>
<tr>
<td>Cnd</td>
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<td>&quot;</td>
<td>&quot;</td>
<td>none</td>
<td>none</td>
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</tr>
<tr>
<td></td>
<td>Muscimol</td>
<td>&quot;</td>
<td>6.25mM</td>
<td>none</td>
<td>&quot;</td>
<td>&quot;</td>
<td>none</td>
<td>none</td>
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<tr>
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<td>&quot;</td>
<td>0.5M</td>
<td>0.5M</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&lt;1</td>
<td>9-14</td>
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<td>5mM</td>
<td>&quot;</td>
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<td>4-22</td>
<td>30</td>
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<tr>
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<td>&quot;</td>
<td>6.25mM</td>
<td>6.25mM</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&gt;9</td>
<td>&gt;30</td>
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<tr>
<td>RP</td>
<td>GABA</td>
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<td>0.5M</td>
<td>0.5M</td>
<td>&quot;</td>
<td>&quot;</td>
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<td>12-21</td>
<td></td>
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<tr>
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<td>3mM</td>
<td>&quot;</td>
<td>&quot;</td>
<td>10</td>
<td>35</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Muscimol</td>
<td>&quot;</td>
<td>6.25mM</td>
<td>none</td>
<td>&quot;</td>
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<td>—</td>
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<tr>
<td>MRF</td>
<td>GABA</td>
<td>&quot;</td>
<td>0.5M</td>
<td>0.5M</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&lt;1</td>
<td>2-12</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Picrotoxin</td>
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<td>5mM</td>
<td>&quot;</td>
<td>&quot;</td>
<td>11-13</td>
<td>&gt;30</td>
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</table>

ABBREVIATIONS:

Cnd — dorsal part, medullary central nucleus
Cnv — ventral part, medullary central nucleus
MRF — mesencephalic reticular formation
RP — pontine reticular nucleus
TTD — descending trigeminal tract and nucleus
Figure 15. Composite diagram of GABAergic agonist and antagonist neurochemical injection sites. The diagram of coronal sections through various levels of the avian neuraxis [numbers in upper left corner of each level, A=anterior, P=posterior (in mm)] illustrates the locomotor effects of each neurochemical in brainstem regions from which locomotion was first elicited by electrical stimulation. Where GABA (filled square) and muscimol (filled circle) are shown as filled, their effects were to block locomotion.

Key Abbreviations: BICUC - bicuculline, GABA - γ-aminobutyric acid, MUSC - muscimol, PICRO - picrotoxin; LOCO - locomotion (except for GABA and muscimol), TH - decreased electrical threshold intensity for locomotion, TTH - increased electrical threshold intensity for locomotion, NR - no response.

electrical stimulation as seen in Fig. 16A) followed by bilateral treadmill stepping which increased in force as time progressed. In these animals, weak and then stronger bilateral wing flapping (Fig. 16B) also became incorporated into the behaviour in a manner similar to the transition between walking and flying in the normal animal. This change in locomotor pattern was similar to that seen in these animals as electrical stimulation intensity was gradually increased from threshold. GABA antagonist injection elicited walking alone in one bird (Fig. 17A, picrotoxin), while in a second, wing flapping alone was observed with bilateral leg extension (Fig. 17B, bicuculline), as has been seen during electrical stimulation (13A). One animal, not included in the locomotion group, displayed only tonic extension (convulsant-like) of both wings and legs without any discrete locomotor movements. This behaviour resembled that seen after a prolonged period of picrotoxin-induced locomotion in several of the animals.

In four of the above picrotoxin-stimulated birds, trigeminal field stimulation (TFS) (either air puffs or stroking the surface of the head near the eyes) appeared to increase the intensity of the locomotor behaviour being performed. Thus, after picrotoxin injection but before the onset of locomotion, this stimulation, which prior to injection was ineffective at eliciting locomotion, would elicit short bouts of walking or flying behaviour. Also, after picrotoxin-induced locomotion was established, TFS seemed to increase the force of walking or would cause a conversion from walking to flying. Similar to the mechanical TFS stimulation, loud noise would occasionally elicit
Figure 16. Electromyographic records (EMGs) showing locomotor activity elicited by electrical stimulation and picrotoxin injection into the pontobulbar locomotor strip (PLS). A: Alternating stepping represented by EMG patterns from the right (RITC) and left (LITC) iliotibialis cranialis muscles (major hip flexor muscle) elicited by electrical stimulation of the PLS. B: Stepping and wing flapping EMGs elicited by injection of picrotoxin into the same site. The top two traces illustrate the in-phase activity of the right (RPECT) and left (LPECT) pectoralis muscles, the major wing depressors used for flight. The bottom two traces are from the leg ITC flexor muscles as in A.
A

RITC

LITC

3 sec

B

RPECT

LPECT

RITC

LITC

1 sec
Figure 17. Electromyographic records (EMGs) showing locomotor activity elicited by picrotoxin and bicuculline injection into the pontobulbar locomotor strip (PLS). A: Alternating stepping represented by EMG patterns from right (RITC) and left (LITC) iliotibialis cranialis muscles elicited by picrotoxin injection into the PLS. B: Simultaneous (in-phase) wing flapping EMGs from the right (RPECT) and left (LPECT) pectoralis muscles elicited by injection of bicuculline into the PLS of a different animal.
bouts of locomotor activity in these animals.

GABA (0.3-0.5M) infused into all animals tested (N=7) rapidly (mean <1.5 minutes; range 1-5 minutes), transiently (2-21 minutes; mean 10.7 minutes) and reversibly blocked locomotion (or increased electrical threshold for locomotor activity) elicited by either electrical stimulation or picrotoxin infusion (Fig. 18A,B). The GABA-induced locomotor blockade decayed with time such that electrical stimulation immediately post-injection was ineffective at eliciting locomotion. However, over time (mean 10.7 min.), the electrical threshold for locomotion decreased until the blockade wore off and activity similar to that seen previous to GABA injection returned. In two birds in which locomotion was produced by electrical stimulation alone, GABA injection (0.5M) replicably and reversibly increased threshold above the stimulation intensity maximum (170μA) from a pre-injection threshold mean of 50μA. In these animals, the reversible nature of the GABA effects were seen after 3 trials of GABA infusion (GABA injection followed by recovery), in which the electrical threshold required to initiate locomotion returned to near pre-GABA values (70μA). In three birds, the tonic extensor activity which was evident after the prolonged picrotoxin-induced locomotion or the extensor hypertonicity described above disappeared for short periods following GABA injection into these sites. As the GABA block appeared to wear off, locomotor movements reappeared for a brief period before extensor hypertonicity reasserted itself.

Muscimol, the GABA agonist (6.5-25mM/1.0μl), produced
Figure 18. Electromyographic records (EMGs) showing locomotor activity elicited by electrical stimulation (A) of the pontobulbar locomotor strip (PLS) which was blocked by GABA infusion into the same site (B). A: Alternating hindlimb locomotion elicited by electrical stimulation as demonstrated by the EMGs from right (RITC) and left (LITC) iliotibialis cranialis muscles. B: The stepping locomotion elicited in A was blocked by injection of GABA into the same site. The traces from right and left ITC muscles were taken during ramp electrical stimulation (0-170μA) of this PLS site.
irreversible block of electrical and picrotoxin (and bicuculline) induced locomotor behaviour in 2 animals (N=2). The latency to muscimol-induced block was 5-10 minutes after the initial injection (0.2µl) and lasted throughout the course of the experiment (30 & 70 minutes). Additional injections of picrotoxin and electrical stimulation were ineffective at eliciting further locomotion from the muscimol injection site in either animal.

In one animal (N=1), bicuculline (GABA<sub>A</sub> antagonist) injection (10mM/1µl) into PLS produced locomotor activity (onset of 15 minutes lasting for 30 minute experimental period) which appeared similar to that seen following picrotoxin injection.

**Central Nucleus Medulla, dorsal part (Cnd)**

Injection of picrotoxin (5mM) into Cnd (N=3) was ineffective at promoting chemical-induced locomotion or reducing electrical stimulation threshold. Muscimol (6.25mM) (N=1) injection had no blocking effect on electrical stimulation-induced locomotor behaviour in one animal.

**Central Nucleus Medulla, ventral part (Cnv)**

Injections of picrotoxin into Cnv (5-20mM) in 4 out of 8 birds elicited long lasting walking (hopping in 1 bird) (see Figure 19A,B) with an onset of first activity appearing at a mean time of 13.5 minutes (range 4-22 minutes). In one of the remaining animals, picrotoxin (25mM) decreased electrical
Figure 19. Electromyographic records (EMGs) showing GABA-reversible locomotor activity elicited by picrotoxin injection into the central nucleus of the medulla, ventral part (Cnv). A: Alternating stepping as represented by EMGs from right (RITC) and left (LITC) iliotibialis cranialis muscles following picrotoxin injection into Cnv. B: The stepping locomotion elicited by picrotoxin injection was blocked by infusion of GABA into the same site. The traces from right and left ITC muscles were taken during ramp electrical stimulation of this Cnv site (0-170μA, arrow under bottom trace indicates 170μA).
threshold for locomotion from 100 to 70μA, while in yet another, picrotoxin injection (10mM) resulted in long lasting extension of both wings and legs without any rhythmic patterns of locomotion. All of the above locomoting animals displayed some degree of both wing and leg extensor activity following the period of locomotor behaviour. Only one of eight birds displayed no reaction to picrotoxin injection.

GABA (0.5M) infusion into Cnv (N=3) rapidly (<1 minute), transiently (mean = 11.5 minutes; range = 9-14 minutes) and reversibly blocked or increased threshold for picrotoxin and electrically stimulated locomotion (Fig. 19B), while muscimol (6.25mM) (N=2) irreversibly blocked (time to onset of the block <9 minutes) both picrotoxin and electrical stimulation-induced locomotion in one of two birds.

Pontine Reticular Formation (RP)

Picrotoxin (5mM) stimulated locomotion (latency to onset 10 minutes: lasting >35 minutes) or reduced the threshold (3mM) for electrically stimulated locomotion (100->50μA) in two of four birds when injected into RP (Figure 20).

GABA (0.5M) blocked the picrotoxin response in both animals with a fast onset (<1 minute). The block lasting for 12 minutes in one bird and 21 minutes in the other (Figure 20). Similar to the GABA injections into Cnv, the block was transitory and time dependent, with the most effective block (for electrical stimulation) occurring immediately after completion of the injection. GABA (0.5M) was effective at blocking electrically
Figure 20. Electromyographic records (EMGs) showing GABA-reversible locomotor activity elicited by electrical stimulation and picrotoxin infusion into the ventral pontine reticular formation (RP). **STIM:** EMGs from right (RPECT) and left (LPECT) pectoralis muscles and right (RITC) and left (LITC) iliotibialis cranialis muscles during electrical stimulation of the site shown (filled triangle) in the coronal section at the bottom right. Threshold electrical stimulation evoked alternating hindlimb stepping as shown by the activity of the ITC muscles. **PICRO (T10):** Ten minutes (T10) after picrotoxin infusion into the same site, locomotor activity appeared both in the wings (PECT) and legs (ITC). **PICRO (T30):** The locomotion continued, as evidenced by the EMGS showing alternating stepping activity, at T30. **GABA:** GABA injection at T35 blocked the picrotoxin-induced locomotion transiently, as seen from the ITC EMG traces. **PICRO:** The picrotoxin-induced locomotion returned, however, as seen by the hindlimb EMG traces at 55 minutes post-injection (T55). Abbreviations: BC - brachium conjunctivium, MLF - medial longitudinal fasciculus, N VI - abducens nerve, N VII - vestibular nerve, R - raphe nucleus, RP - nucleus reticularis pontis, Rpc - parvocellular part, pontine reticular formation, VI - abducens nucleus, VS - trigeminal sensory nucleus.
induced locomotion (stimulation maximum 170μA) in two other birds in which picrotoxin injection was ineffective at eliciting locomotion.

Muscimol (6.25mM) injected into one site did not change the quality or the threshold for electrically stimulated locomotor activity. Picrotoxin injection (5mM) following muscimol infusion was ineffective at changing the stimulation parameters necessary to evoke locomotion from this site.

Mesencephalic Reticular Formation (MRF)

Bilateral hindlimb stepping produced by electrical stimulation of the mesencephalic reticular formation was blocked transiently by GABA infusion (0.5M) (latency <1 minute; lasting 8-12 minutes) in two birds. In one of three animals, infusion of picrotoxin (20mM) decreased the threshold for electrically induced walking from 160 to 90μA over a 70 minute period. GABA (0.5M) infused into the same site increased the threshold (220μA). In two other birds, picrotoxin (5mM & 10mM) was effective at eliciting long lasting flying behaviour with tonic leg extension which was also reversible by GABA infusion. Injection of picrotoxin (3mM) into a more medial injection site in the MRF of one animal had no effect on electrically induced locomotion.
DISCUSSION

Pontobulbar Locomotor Strip (PLS)

The injection of GABA antagonists picrotoxin and bicuculline into the PLS evoked locomotion which could be blocked by GABA or muscimol. These results are similar to those found in the cat, where picrotoxin injection elicited muscimol/GABA-reversible locomotion (Noga et al., 1988) and further verify our contention of the homology between the avian and mammalian PLS. Noga et al. (1988) suggest that the PLS is under GABAergic inhibitory control and state as evidence that locomotion could be elicited by trigeminal field stimulation following PLS injection of picrotoxin, neither of which alone activated the behaviour. They argue from these results that the PLS is synonymous with the descending tract of the trigeminal nerve (TTD) that sends efferents via propriospinal pathways to more caudal TTD and also to reticular formation neurons. They also argue that stimulation of PLS (TTD) results in locomotion through secondary activation of the reticular neurons that play a direct role in the initiation of locomotion (Noga et al., 1988) (for discussion of efferent TTD pathways see Chapter 3).

As seen in the cat, trigeminal field stimulation (air puffs or stroking of the head) of the bird shortly following picrotoxin infusion but before the onset of locomotion elicited by picrotoxin alone appeared to initiate locomotion. Loud noise also elicited bouts of locomotion prior to the onset of neurochemical induced locomotion. In addition, stroking of the
head region following the onset of picrotoxin-induced locomotion appeared to augment the force of stepping and/or flapping behaviour. It appears from these results, therefore, that peripheral stimulation, acting through TTD, may increase the overall gain of the system and facilitate the induction of locomotor behaviour from PLS/TTD stimulation.

While no projections of GABAergic neurons to the PLS/TTD have been elucidated in bird, in the rat, Mugnaini and Oertel (1985) report the presence of both GABA-containing cell bodies and terminals in TTD along its rostrocaudal extent. McGeer and McGeer (1981) report a variety of proposed GABAergic pathways, many of which are interneuronal in origin. However, internuclear GABA-containing afferents to TTD have not been reported (E. McGeer, personal communication). Although the present data indicate that TTD GABAergic interneurons may subserve the motor effects observed in our experiments, the veracity of this supposition remains to be determined.

The role that GABA plays in the control of PLS-associated locomotion is presently unknown, but combined with the hypothesis of Noga et al. (1988) that the PLS/trigeminal/LRF system "provides a substrate for sensorimotor reflex initiation of locomotion", I hypothesize that GABAergic interneurons may modulate afferent trigeminal and possibly centrally generated information by down-regulating or dampening the effects of this afferent input on TTD before TTD sends locomotion-producing signals to reticular formation or other locomotor-related structures.
Central Nucleus Medulla, dorsal part (Cnd)

Injection of GABA agonists and antagonists were ineffective at modulating locomotor behaviour following injection into Cnd. These results correlate with those found by Noga et al. (1988) for the cat, where electrical stimulation elicited, but picrotoxin injection failed to elicit, locomotion. They suggest that the electrical stimulation was activating fibers travelling from the PLS/TTD to more medial brainstem reticular formation structures. Our results, however, demonstrate that injection of cholinergic and excitatory amino acid agonists into Cnd elicits locomotion, indicating that Cnd intrinsic neurons are capable of activating locomotion. A different explanation for the lack of GABAergic effects in this region may lie in the differential distribution of GABAergic receptors and terminals in the brainstem. Although little information is available for GABA distributions in birds, in the rat, Mugnaini and Oertel (1985) show only low levels of GABA terminals in the regions medial to TTD (Cnd in bird; FTL in cat), while higher levels were found in TTD. It is likely that with electrical stimulation of Cnd, both fibers of passage from TTD and cell bodies (dendrites) in Cnd are being activated, while chemical stimulation activates only Cnd receptors. Thus, while acetylcholine and the excitatory amino acids appear to be involved in locomotor control via Cnd, GABA does not appear to be active as a neurotransmitter in this region of the brainstem in either the bird or cat (Noga et al., 1988).
Central Nucleus Medulla, ventral part (Cnv)

GABAergic antagonists injected into Cnv produced locomotion or caused a decrease in electrical stimulation threshold required to initiate locomotion. Both effects could be blocked by GABA or muscimol injection. These results are similar to those found in the cat (Noga et al., 1988; Garcia-Rill and Skinner, 1987) following injection of GABA agonists/antagonists. Garcia-Rill and Skinner (1987) reported block of electrically stimulated locomotion with GABA or muscimol infusion into the medial reticular formation, but found that picrotoxin or bicuculline injection produced convulsions at concentrations greater than 5mM. In the bird, only one animal of seven demonstrated convulsant type activity immediately following picrotoxin infusion. However, all animals in which picrotoxin was effective at producing locomotor behaviour eventually displayed tonic extensor activity of both wings and legs to varying degrees. As discussed by Noga et al. (1988), and similar to our studies in birds using electrical stimulation (unpublished observations), it is possible that a locomotor somatotopic organization exists in the reticular formation. Our observations have shown that small lateromedial translocations of the stimulating electrode will often change the pattern of hindlimb walking from contralateral unilateral stepping to bilateral stepping. These results are, however, seldom seen for wing locomotion, where flapping is bilateral in the vast majority of cases. Taken together with the information that in neurochemical-induced locomotion trials, some feline muscle
groups showed a loss of phasic activity (Noga et al., 1988), it appears that the convulsant activity demonstrated after injection of the long acting picrotoxin (Franz, 1985) may result from diffusion and subsequent disinhibitory action of picrotoxin on centres controlling different extensor and/or flexor muscles which are normally modulated out of phase.

GABAergic cell bodies and terminals have been found in the rat ventral reticular formation (Mugnaini and Oertel, 1985). Equivalent structures may serve as the neuroanatomical substrate for the observed changes in locomotor behaviour induced by GABA or its agonists and antagonists in the bird. To our knowledge, GABA neuroanatomy and neurochemistry have been explored in the bird using retrograde transport of $[^3]$H-GABA only with respect to GABAergic striatotegmental projections (Hall et al., 1984) and no reports of GABAergic innervation or intrinsic GABA-containing cell bodies in this region are available. However, our results suggest the probability that Cnv cells giving rise to reticulospinal fibers (Webster and Steeves, 1988) are under GABAergic inhibitory control. Whether this control arises from intrinsic or extrinsic neurons remains to be determined both for birds and mammals.

Pontine Reticular Formation (RP)

Chemical stimulation of the ventral RP region with the GABA antagonist picrotoxin produced locomotion which was blocked by GABA infusion. While no data is available for GABAergic cell bodies or terminals in the bird, in the rat, medium to low
levels of GABA terminals and low to very low levels of GABAergic cell bodies have been found in the pontine reticular formation (Mugnaini and Oertel, 1985). It appears unlikely, therefore, that GABAergic neurons intrinsic to the pontine reticular formation are responsible for this response. However, cell bodies in the pontine reticular formation appear to be under GABAergic inhibitory control. The greater concentration of GABAergic terminals relative to cell bodies suggests an extrinsic source of input. Neural pathways which underlie this control are, however, presently unknown, leaving any significant locomotor role for GABA in the pontine reticular formation undetermined.

Mesencephalic Reticular Formation (MRF)

Infusion of GABAergic antagonists and agonists into the MRF was effective in eliciting or blocking locomotion in birds. These results are similar to those found following the infusion of GABAergic neurochemicals into the MLR both in the decerebrate cat (Garcia-Rill et al., 1985) and the freely moving rat (Brudzynski and Mogenson, 1986). GABA is present in the cuneiform nucleus (CN) and pedunculopontine nucleus (PPN) of the rat, with higher levels of both GABA-containing cell bodies and terminals being found in the cuneiform nucleus (Mugnaini and Oertel, 1985). Possible locomotion-related GABAergic projections from the substantia nigra, pars reticulata, nucleus accumbens and entopeduncular nucleus to the PPN/MLR have been reported in the cat (Garcia-Rill et al., 1983b, Garcia-Rill and Skinner,
1986) and rat (Garcia-Rill et al., 1986). These results were supported, in part, by the description of nucleus accumbens GABAergic projections to both PPN and CN in the rat (Mogenson et al., 1985; Mogenson and Wu, 1986; Brudzynski et al., 1988). As discussed in the previous chapter, the mammalian CN and PPN, which may represent the neuroanatomical substrates for the lateral and medial mesencephalic locomotor regions respectively, (Garcia-Rill et al., 1985; Noga et al., 1988; Brudzynski et al., 1988), appear to be equivalent to the avian mesencephalic regions stimulated in our study. While to our knowledge, no studies localizing GABA-containing cell bodies or terminals have been carried out for these regions in the bird, comparison of the mammalian results with those found above in birds provides evidence which supports the presence of an avian equivalent of the mammalian MLR. However, due to the small number of mesencephalic injection sites and the inability to elicit locomotion following a single injection of picrotoxin into the more medial MRF, further testing of this hypothesis is required before any firm equivalency can be established.

Pharmacological Considerations

Picrotoxin injection into a variety of electrically identified locomotor regions also elicited locomotion which was transiently reversed with GABA infusion, and in some cases, appeared to be irreversibly blocked by muscimol injection. Furthermore, subthreshold (for electrically evoked locomotion) injection of picrotoxin decreased the electrical stimulation
intensity necessary to evoke locomotion.

Pharmacologically, these results appear somewhat perplexing, as picrotoxin has been demonstrated to act at different receptor sites than GABA, muscimol and bicuculline (Olsen, 1981). How then does GABA or muscimol reverse the locomotor action of picrotoxin? Picrotoxin is a potent antagonist at both the $\text{GABA}_A$ and $\text{GABA}_B$ receptor subtypes (Krogsgaard-Larsen et al., 1983). The $\text{GABA}_A$ receptor (defined by its sensitivity to the competitive antagonist bicuculline), is thought to control a chloride ionophore (Krogsgaard-Larsen et al., 1983) and has been demonstrated to possess five different binding sites including a GABA agonist/antagonist (including muscimol and bicuculline) site, a benzodiazepine site, a picrotoxin site, a depressant site (e.g. barbiturates) and a site(s) which binds the channel-permeating ions (Barnard et al., 1987). The $\text{GABA}_B$ receptor (defined by its sensitivity to the agonist baclofen), on the other hand, is believed to exert its effects by restricting presynaptic (voltage dependent) calcium influx (Desarmenien et al., 1983).

As discussed in Chapter 3, the elicitation of locomotion by neurochemical injection is viewed as a recruitment phenomenon whereby a sufficient number of neurons must be activated or blocked by the neurochemical to initiate/block the visible signs of locomotion (see Chapter 3). In this study, it is not possible to determine whether the inhibitory action of picrotoxin on GABA receptors is acting pre-synaptically, postsynaptically or both, although the effectiveness of bicuculline at eliciting locomotion would suggest that $\text{GABA}_A$ and not $\text{GABA}_B$ receptors...
mediate the effect. Introduction of a high concentration of GABA should not displace the bound picrotoxin, but may exert its locomotor inhibitory effects by binding to GABA receptors which are not occupied by picrotoxin, thereby short circuiting the picrotoxin inhibition and blocking locomotion. This may also explain the high concentration of GABA necessary to reverse the picrotoxin-induced locomotion. The rapid breakdown of GABA in vivo (Franz, 1985) would account for the return of locomotion induced by the more persistent binding of picrotoxin. The inhibitory action of muscimol, a potent, long lasting GABA agonist (Bloom, 1985) on picrotoxin-induced locomotion presumably utilizes the same mechanism and underlies the suggestion that GABA receptors may be the target of the GABA agonist and antagonist locomotor effects. Furthermore, the electrical stimulation intensity necessary to evoke locomotion following subthreshold (for locomotion) picrotoxin infusion may augment the number of neurons activated by picrotoxin, thereby eliciting locomotion at reduced electrical threshold.

In future studies, differentiation of the avian GABA receptor subtype underlying the locomotion observed in this study may be possible utilizing more specific GABA agonists and antagonists such as the newly reported GABA antagonist phaclofen (Karlsson et al., 1988). Such studies would provide further valuable information concerning the role of GABA in the control of locomotion.
CHAPTER 5

CHARACTERIZATION OF AVIAN MID- AND HINDBRAIN SITES THAT PRODUCE LOCOMOTION WITH INTRACEREBRAL INFUSION OF NEUROTRANSMITTER AGONISTS AND ANTAGONISTS

(III): EXCITATORY AMINO ACIDS AND SUBSTANCE P
Previous chapters (3 & 4) in this thesis have demonstrated that site specific intracerebral microinjection of cholinergic and GABAergic neurotransmitter agonists and antagonists are effective at eliciting or blocking locomotor behaviours in decerebrate birds. Furthermore, attempts were made to describe a possible neuroanatomical substrate for those effects. This chapter surveys the effects of injection of glutamate, its agonists or antagonists into several locomotor regions previously identified by electrical stimulation (Steeves et al., 1986, 1987; Sholomenko and Steeves, 1987a,b, 1988). Results of Substance P infusion into several sites will also be described.

The results demonstrate that microinjection of the glutamate agonist NMDA into various locomotor regions was effective at evoking locomotor behaviour in birds. Substance P elicited locomotion in only one region injected. These results will be correlated with interspecific neuroanatomical, immunohistochemical and receptor autoradiographic data in an attempt to elucidate and compare the neural substrate controlling locomotion across a broad range of vertebrate species.
MATERIALS AND METHODS

The materials and methods, including the decerebration procedure, electrical stimulation methodology, neurochemical injection parameters and histological identification of stimulation/injection sites have been previously described in Chapters 2 & 3.
RESULTS

Excitatory Amino Acids Agonists, Antagonists and Substance P

Excitatory amino acid (EAA) neurotransmitters, their agonists, antagonists and Substance P were infused into regions in the hind- and midbrain to determine their effectiveness at evoking or inhibiting locomotion at sites from which locomotion can be elicited by focal electrical stimulation (Steeves et al., 1986). NMDA, the glutamate agonist most effective at the NMDA receptor subtype, and glutamate were infused to excite putative glutamatergic receptors. Glutamic acid diethyl ester (GDEE), a relatively non-specific EAA antagonist was used in an attempt to block EAA receptors (Noga et al., 1988; Lai and Siegel, 1988; Stone and Burton, 1988). Substance P was also injected into several electrically identified locomotor sites to determine its effects on locomotor behaviour. The sites injected were distributed within regions of the mid- and hindbrain which included the pontobulbar locomotor strip, dorsal and ventral parts of the medullary reticular formation, pontine reticular formation, mesencephalic reticular formation and medial longitudinal fasciculus. Neurochemical injection sites, lowest effective concentration and time course of activity are listed in Table 3 and described in the text. A composite diagram of injection sites is shown in Figure 21.
### TABLE 3
Excitatory Amino Acids and Substance P

<table>
<thead>
<tr>
<th>Animal Site</th>
<th>Chemical</th>
<th>pH</th>
<th>Concentrations Injected</th>
<th>Lowest Effective Concentration</th>
<th>Volume</th>
<th>Rate</th>
<th>Time Course (min)</th>
<th>Latency</th>
<th>Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>decerebrate TTD</td>
<td>Glutamate ‡</td>
<td>0.5-1.0M</td>
<td>none</td>
<td>1.0μl</td>
<td>‡</td>
<td>‡</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>bird</td>
<td>NMDA ‡</td>
<td>80mM</td>
<td>80mM</td>
<td>0.2μl</td>
<td>&lt;1</td>
<td>10</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>GDEE ‡</td>
<td>2-80mM</td>
<td>2mM</td>
<td>0.4-1μl</td>
<td>4-8</td>
<td>35-55</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Substance P ‡</td>
<td>5.44mM</td>
<td>none</td>
<td>1.0μl</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Cnd</td>
<td>NMDA ‡</td>
<td>20-83mM</td>
<td>20mM</td>
<td>0.2μl</td>
<td>&lt;1.5</td>
<td>3-24</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>GDEE ‡</td>
<td>80mM</td>
<td>80mM</td>
<td>0.8μl</td>
<td>&lt;5</td>
<td>&gt;35</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Substance P ‡</td>
<td>6.44mM</td>
<td>none</td>
<td>1.0μl</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Cnv</td>
<td>Glutamate ‡</td>
<td>1M</td>
<td>none</td>
<td>1.0μl</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>NMDA ‡</td>
<td>4-83mM</td>
<td>4mM</td>
<td>0.4μl</td>
<td>&lt;1</td>
<td>3-4</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>GDEE ‡</td>
<td>2-80mM</td>
<td>2mM</td>
<td>0.2μl</td>
<td>4-6</td>
<td>35-50</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Substance P ‡</td>
<td>5.44mM</td>
<td>none</td>
<td>1.0μl</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>RP</td>
<td>NMDA</td>
<td>7.2-7.4</td>
<td>81-83mM</td>
<td>83mM</td>
<td>0.2μl</td>
<td>&lt;1-1.25</td>
<td>4-9</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Substance P ‡</td>
<td>5.44mM</td>
<td>5.44mM</td>
<td>1.0μl</td>
<td>4</td>
<td>&gt;15</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>MRF</td>
<td>NMDA ‡</td>
<td>5-34mM</td>
<td>5mM (RT)</td>
<td>0.2μl</td>
<td>5</td>
<td>15</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>MLF</td>
<td>NMDA ‡</td>
<td>20-34mM</td>
<td>34mM</td>
<td>0.4μl</td>
<td>&lt;1</td>
<td>8</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

**ABBREVIATIONS:**
- Cnd — dorsal part, medullary central nucleus
- Cnv — ventral part, medullary central nucleus
- MLF — medial longitudinal fasciculus
- MRF — mesencephalic reticular formation
- RP — pontine reticular nucleus
- RT — reduced threshold for electrically stimulated locomotion
- TTD — descending trigeminal tract and nucleus
Figure 21. Composite diagram of glutamatergic agonist and antagonist neurochemical injection sites. The diagram of coronal sections through various levels of the avian neuraxis [numbers in upper left corner of each level, A=anterior, P=posterior (in mm)] illustrates the locomotor effects of each neurochemical in brainstem regions from which locomotion was first elicited by electrical stimulation. Where glutamic acid diethyl ester (GDEE) (filled square) is shown as filled, its effect was to block locomotion.

Key Abbreviations: GDEE - glutamic acid diethyl ester, GLUT - glutamate, NMDA - N-methyl-D-aspartate, SUBP - substance P; LOCO - locomotion (except for GDEE), TH - decreased electrical threshold intensity for locomotion, ↑TH - increased electrical threshold intensity for locomotion, NR - no response.

Pontobulbar Locomotor Strip (PLS)

Following establishment of a low intensity stimulation point for evoking locomotion, injection of NMDA (80mM/0.2μl) (N=3) elicited repeatable bouts of locomotion in 2 of 3 birds injected (Fig. 22). The locomotion followed NMDA injection with a rapid onset (<1min) and occurred in vigorous bouts (1-2min) for approximately 10 minutes. Both NMDA and electrically stimulated locomotion were irreversibly blocked by GDEE (80mM/0.4μl and 2mM/0.2μl) for the duration of the experiment (>40min) (Figure 22C). Glutamate infusion (0.5-1.0M/1.0μl) (N=2) into PLS had no effect on the threshold of electrically stimulated locomotor behaviour. Substance P (N=1) (5.44mM/1.0μl) also had no effect when infused into this site.

Central Nucleus, dorsal part (Cnd)

Injection of NMDA into Cnd (81-83mM/0.2μl) in 2/5 birds elicited repeatable bursts of running and flying behaviour (20-40sec) (Fig. 23B). The locomotion occurred with fast onset (1.0 & 1.5 minutes) and lasted for 3 and 14 minutes. Prior to chemical injection, electrical stimulation in one of these animals produced only bilateral stepping movements, while in the other, both running and flying were displayed at electrical threshold (Fig. 26A). One of five of the birds displayed rapid onset (<1 min) and long lasting (24 minutes) walking behaviour following NMDA stimulation (20mM/0.2μl) which was similar to that produced by pre-injection electrical stimulation. In the
Figure 22. Electromyographic records (EMGs) showing locomotor activity elicited by electrical stimulation and NMDA injection into a site in the pontobulbar locomotor strip (PLS) shown in the coronal section of the medulla (bottom right). **STIM:** Alternating stepping represented by EMG patterns from the right (RITC) and left (LITC) iliotibialis cranialis muscles (major hip flexor muscle) elicited by electrical stimulation of the PLS. No activity is present in the right (RPECT) and left (LPECT) pectoralis muscles which are the major wing depressor muscles. **NMDA:** NMDA injection into the same site evoked bursts of stepping and wing flapping as shown by the EMGs for the paired wings (PECT) and legs (ITC) at 90 seconds post-injection (only 1st burst is shown). **STIM:** Electrical stimulation at 20 minutes post-injection elicited hindlimb stepping as shown by the EMGs of right and left ITC muscles. **GDEE:** Hindlimb EMG records following GDEE infusion into the same site which blocked further NMDA and electrical stimulation-induced locomotion.

Figure 23. Electromyographic records (EMGs) showing locomotor activity elicited by electrical stimulation and NMDA injection into the dorsal part of the central medullary nucleus (Cnd) A: Alternating stepping and wing flapping represented by EMG patterns from right (RITC) and left (LITC) iliotibialis cranialis muscles and right (RPECT) and left (LPECT) pectoralis muscles elicited by electrical stimulation of Cnd. B: Simultaneous (in-phase) wing flapping EMGs (PECT) and alternating stepping (ITC) EMGs evoked by injection of NMDA (83mM/0.2μl) into the same site. C: EMG traces from wings and legs after injection of GDEE (80mM/0.6μl) into the site. Traces were taken during electrical stimulation (170μA) of the site 4 minutes after the infusion of NMDA (83mM/0.2μl).
bird which displayed running/flying behaviour both with electrical and NMDA stimulation, GDEE (80mM/0.6µl) infusion into this site irreversibly blocked further electrical and NMDA stimulated locomotion (Fig. 23C). In one bird which was paralyzed following establishment of an alternating walking electrical stimulation site (see Chapter 6), injection of 20mM/0.2µl aliquots of NMDA repeatably initiated bouts (~1.5 minutes long) of bilateral in-phase 'fictive' leg movements (hopping) within 1 minute post-injection. The electroneurographic activity appeared strongest immediately post-injection and became weaker over time. No bouts were observed after approximately 6 minutes post-injection (see Chapter 6, Fig. 33).

Substance P (5.44mM) injection produced no changes in electrical stimulation threshold when injected into Cnd in one animal.

Central Nucleus, ventral part (Cnv)

NMDA injection into Cnv produced locomotion in 3 of 3 birds. Electrical stimulation, carried out prior to chemical stimulation, produced running and flying behaviour at threshold intensity (Fig. 24A). Chemical stimulation (81mM/0.2µl; 4mM/0.4µl) in both of these animals resulted in short bouts (5-15 seconds) of running and flying locomotion (latency to onset <1 min) which continued for 3 minutes in one animal and 4 minutes in the other and was similar to that seen in response to electrical stimulation (Fig. 24B). GDEE at both
Figure 24. Electromyographic records (EMGs) showing locomotor activity elicited by electrical stimulation and NMDA injection into the ventral part of the central medullary nucleus (Cnv) A: Alternating stepping and wing flapping represented by EMG patterns from right (RITC) and left (LITC) iliotibialis cranialis muscles and right (RPECT) and left (LPECT) pectoralis muscles elicited by electrical stimulation of Cnv. B: Simultaneous (in-phase) wing flapping EMGs (PECT) and alternating stepping (ITC) EMGs evoked by injection of NMDA (4mM/0.4μl) into the same site. C: EMG traces from wings and legs after injection of GDEE (2mM/0.2μl) into the site. Traces were taken 4 minutes after the infusion of NMDA (4mM/0.4μl).
B

RPECT

LPECT

RICTC

LITC

1 sec
high (80mM/0.4μl) and low (2mM/0.2μl) concentrations irreversibly blocked both NMDA and electrically induced locomotion when infused into these sites (Fig 24C). In the third animal, electrical stimulation produced continuous bilateral walking behaviour which was repeatably (5 trials) mimicked by NMDA (83mM/0.2μl) injection with short latency (<1 minute) and time course (~4 min). Substance P (5.44mM/1.0μl) injection at this site had no effect either on the quality of chemically induced locomotion or electrical stimulation threshold.

In two other animals which received Substance P (5.44mM/1.0μl) injections into Cnv, no changes were observed in the locomotor pattern or electrical threshold needed to initiate locomotion. One of the above birds received an injection of glutamate (1M/1μl) into the same site 20 minutes after Substance P injection. Although the glutamate injection did not induce locomotion or affect any changes to stimulation intensity threshold, increases in breathing frequency and force of expiration were observed which were similar to those seen resulting from electrical stimulation in this site.

**Pontine Reticular Formation (RP)**

Introduction of NMDA into sites in the pontine reticular formation elicited locomotion in 3 of 5 birds. One of these animals displayed running/flying both at electrical stimulation threshold and following NMDA (81mM/0.4μl) infusion. The latency to onset of running/flying was 1.25 minutes with short bouts (15-25 seconds) of this behaviour occurring for up to 4.25
minutes post-injection. The two other birds with RP NMDA injections (83mM/0.2μl) elicited walking only (similar to pre-injection electrically stimulated locomotion). One of the above animals repeatably produced walking movements within 1 minute post-injection which lasted approximately 4 minutes. The second animal received NMDA 15 minutes after injection of Substance P (5.44mM/1μl) into the site (Fig. 25A,B). The NMDA (83mM/0.2μl) injection significantly increased the force of the weak bilateral alternating stepping (and initiated flapping behaviour) (Fig. 25C) produced by a Substance P injection (onset 4 minutes post-injection lasting up to 15 minutes at which time NMDA was injected) within 1 minute. The effect of NMDA lasted approximately 9 minutes and decreased in a time dependent manner.

Mesencephalic Reticular Formation (MRF)

NMDA injection (5mM/0.2μl) into the MRF in one of two birds reduced the threshold for electrically stimulated walking from 100μA to 60μA but did not elicit locomotion independently, while NMDA injection in the second bird (paralyzed) (34mM/0.6μl) had no effect either on locomotor pattern or threshold for electrically stimulated locomotion.

Medial Longitudinal Fasciculus (MLF)

Infusion of NMDA (34mM/0.4μl) into a locomotion promoting stimulation site (walking and flapping, Fig. 26A) into the MLF
Figure 25. Electromyographic records (EMGs) showing locomotor activity elicited by electrical stimulation, Substance P and NMDA injection into the pontine reticular formation (RP). A: Alternating stepping evoked by threshold electrical stimulation of RP. The bottom two traces demonstrate the EMG activity in the right (RITC) and left (LITC) iliotibialis cranialis muscles, while the top traces (RPECT & LPECT), taken from the pectoralis muscles show no activity. B: EMG activity demonstrating only alternating hindlimb stepping in the bird following injection of Substance P into the same site. The rhythmic pattern of EMG activity seen in the PECT traces is breathing. C: Stepping and flapping activity evoked following the injection of NMDA into the site previously injected with Substance P as shown by the EMGs from the leg flexor muscles (ITC - bottom 2 traces) and wing depressor muscles (PECT - top 2 traces).
B

RPECT

LPECT

RITC

LITC

2 sec
Figure 26. Electromyographic records (EMGs) showing locomotor activity elicited by electrical stimulation and NMDA infusion into the medial longitudinal fasciculus (MLF). A: EMGs from the right (RPECT) and left (LPECT) pectoralis and right (RITC) and left (LITC) iliotibialis cranialis muscles illustrating the coactivated stepping (ITC) and flapping (PECT) elicited by electrical stimulation of the MLF. B: Electroneurographic records (ENGs) taken from the left pectoralis muscle (LPECT) and right (RITC) and left (LITC) iliotibialis muscles during paralysis demonstrating the alternating pattern of 'fictive' hindlimb activity elicited by injection of NMDA (34mM/0.4μl) into the same site as in A.
replicably (4 times) evoked 'fictive' bilateral alternating stepping within one minute post-injection which lasted for approximately 8 minutes in one animal (Fig. 26B). NMDA injection (20mM/0.2μl) into a paralyzed bird from which 'fictive' walking could be elicited by electrical stimulation of the MLF showed no effects.
DISCUSSION

Neurochemical injection into selected regions of the avian mid- and hindbrain with EAA agonists, antagonists and Substance P produced a variety of locomotor responses in decerebrate birds. Introduction of the glutamate agonist NMDA, but not glutamate itself, elicited locomotion or reduced the current intensity threshold for locomotion when injected into electrical-stimulation defined locomotor regions. The induced locomotion could be blocked by the introduction of the glutamate antagonist GDEE into the same site. Substance P also elicited locomotion following its injection into the pons. These results will be discussed for each locomotor region and an attempt will be made to elucidate the neuroanatomical pathways which subserve them. The data from our study will be compared to that found in other species in which similar investigations have been performed.

Pontobulbar Locomotor Strip (PLS)

Injection of NMDA into the PLS, the physiologically defined locomotor region which appears to be synonymous with the descending trigeminal tract and nucleus (TTD) (Noga et al., 1988), evoked locomotion in birds. The effective injection sites were histologically identified as lying on the dorsomedial border between TTD and Cnd. These results are similar to those found by Noga et al., (1988) in the cat, in which injection of glutamate elicited locomotion or decreased the electrical
threshold intensity necessary to evoke walking. The avian neuroanatomical connections which subserve the observed locomotion are presently unknown, but in the rat, EAA corticofugal pathways descending to the brainstem may provide glutamatergic input to this region (Fagg and Foster, 1983). Salt and Hill (1981, 1983) demonstrated that PDA (cis-2,3-piperidine carboxylate), a broad spectrum EAA antagonist (acts at all receptor types), but not the specific NMDA antagonist, D-α-amino adipate, could block vibrissal and noxious mechanical but not noxious thermal afferent input to cells in the nucleus caudalis of TTD in the urethane anaesthetized rat. They did not, however, examine the types of stimulation which have been shown to elicit locomotion in the decerebrate preparation discussed above (e.g. pinna stimulation – Aoki and Mori, 1981). Although NMDA receptor binding studies in mammals show low densities of NMDA receptors in the region of TTD and surrounding lateral reticular formation, Monaghan and Cotman (1985) surmise that "there is a trend for motor-associated, ventrally located, glutamate-using pathways to have a lower density of NMDA sites than the cortical, limbic and sensory-associated glutamate using pathways". However, from these preliminary findings, it appears that glutamatergic pathways, possibly impinging on NMDA receptors in the PLS/TTD region, may play some role in the control of locomotor behaviour. Why glutamate itself was not effective in the PLS is unknown, although it is possible that after injection, the neurotransmitter is taken up before it can spread to a sufficient number of neurons to elicit locomotion. NMDA, on the other hand, is less susceptible to removal (Stone
and Burton, 1988) and through diffusion through the tissue, may therefore exert its action over a larger group of neuronal receptors (see Chapters 3 & 4).

The neuroanatomical connections through which NMDA/glutamate serve a locomotor role have yet to be determined. Also, other EAA receptor types cannot be ruled out, as the concentrations of NMDA injected, even at the lowest concentrations necessary to evoke locomotion, may cross react with other glutamate receptor subtypes (H. McLennan, personal communication).

While Noga et al. (1988) found that Substance P injection into the caudal PLS evoked locomotion in the cat, injections into comparable sites in the bird were ineffective at eliciting locomotor behaviour. Substance P has been localized in trigeminal ganglion cells and appears to be a transmitter involved in pain perception (Kishida et al., 1985; for review see Dubner and Bennett, 1983). In the cat, locomotion elicited by Substance P injection mimics that seen following a noxious stimulus (Salt and Hill, 1983) and thus would appear to support the hypothesis that PLS/TTD stimulation-induced locomotion is involved in the sensorimotor initiation of locomotion. In the bird, further studies are required to examine a possible role for Substance P in locomotor control.

Central Nucleus, dorsal part (Cnd)

The dorsal part of the medullary central nucleus (Cnd) gives rise, in part, to the reticulospinal pathway which
descends to all spinal cord levels in birds (Cabot et al., 1982; Webster and Steeves, 1987). Selective lesion studies have demonstrated that the reticulospinal pathway plays a major role in the descending control of locomotion in all vertebrates studied (Sholomenko and Steeves, 1987; for review see Grillner, 1976). In birds, the infusion of the glutamatergic agonist NMDA into Cnd evoked locomotor behaviour which was blocked by the antagonist GDEE. The efferent contribution of Cnd to the reticulospinal pathway and its importance to the descending control of locomotion in the bird and other species has been discussed previously (See Chapters 1 & 2). However, no previous injections of glutamate agonists into this region have been reported. More medial injections of glutamate and homocysteic acid into the gigantocellular tegmental field have been reported to induce locomotion in the cat (Noga et al., 1988). In the lamprey, interneurons containing excitatory amino acids have been identified as specifically impinging upon reticulospinal neurons which are involved in the descending control of locomotion (Dubuc et al., 1988). Furthermore, Dubuc and co-workers (Dubuc et al., 1988) have established the presence of NMDA receptors on lamprey reticular formation neurons. In addition, these EAA containing interneurons receive trigeminal, vestibular and ascending spinobulbar pathways input (Dubuc et al., 1988). Whether comparable interneurons exist in birds remains to be determined.

Receptor binding studies in the rat (Monaghan and Cotman, 1985) report low levels of NMDA/glutamate receptors in the hindbrain reticular formation. Low levels of receptor do not,
however, preclude a modulatory role for this neurotransmitter (Monaghan and Cotman, 1985) in the control of locomotion from forebrain structures which have been shown to impinge on this region in mammals (Fagg and Foster, 1983). It is unknown at this time whether telencephalic to reticular formation EAA pathways comparable to those in mammals also exist in birds. However, a number of neuroanatomical retrograde transport studies have established a variety of locomotor-related afferents to this region which could also be the source of EAA input to Cnd (see Chapters 3 & 4).

The above results, gathered from a broad phylogenetic range of species, strongly implicate an excitatory role for glutamate in the control of locomotion from reticular formation structures. Furthermore, the glutamate NMDA receptor subtype appears to be involved in this control. However, the glutamatergic pathways and exact location of glutamate involvement, be it directly on reticulospinal neurons, interneurons or both, remains to be elucidated.

Central Nucleus, ventral part (Cnv)

Similar to Cnd, neurons localized within Cnv give rise to a portion of the descending reticulospinal projection which is essential for the initiation and maintenance of locomotion in birds (Webster and Steeves, 1987; Sholomenko and Steeves, 1987) and other species (Grillner, 1985). Focal electrical stimulation of this region elicits locomotion in a wide range of species (Steeves et al., 1986). Previous papers in this series have
established that Cnv, like Cnd, appears to function as an integratory and descending output centre for a variety of sensory and centrally generated locomotor related inputs.

In this study, injection of NMDA, but not glutamate, into Cnv elicited locomotion which was blocked by GDEE infusion. Both Garcia-Rill and Skinner (1987a) and Noga et al. (1988) have found that glutamate infusion into the medial reticular formation elicited locomotion or decreased electrical stimulation threshold in the cat, although in some cases the behaviour was short-lived (Garcia-Rill and Skinner, 1987a). We utilized NMDA in an attempt to specify the receptor type underlying the production of locomotion by the excitatory amino acids. One possible difference between our results and those described in cat is the longevity of locomotor behaviour produced by NMDA injection. The apparent longer lasting action of NMDA versus glutamate may result from the slower uptake, and therefore, increased efficacy, of NMDA (Stone and Burton, 1988).

Like Cnd, the sources of glutamatergic input to Cnv remain to be characterized. However, EAA projections to Cnv may arise from more rostral structures (e.g. telencephalon), glutamatergic reticular formation interneurons and ascending spinobulbar projections (Dubuc et al., 1988; Fagg and Foster, 1983). The paucity of glutamate neuroanatomical, neurotransmitter and receptor profile data for birds and other species leaves the physiological role for glutamatergic control of reticular formation neurons unresolved. However, combined data from neurochemical stimulation studies in lamprey (Dubuc et al., 1988), bird and mammal (Garcia-Rill et al., 1985; Garcia-Rill
and Skinner, 1987a; Noga et al., 1988) suggest an important excitatory role for glutamate in the control of locomotion-related reticular formation structures.

**Pontine Reticular Formation (RP)**

Focal electrical stimulation of the ventral pontine reticular formation elicits locomotion in the decerebrate bird preparation (Steeves et al., 1986). Neuroanatomical tracing studies demonstrate that RP gives rise to a component of the descending reticulospinal pathway (Cabot et al., 1982; Webster and Steeves, 1987) In addition, RP projects to motor related structures such as TTD and more caudal reticular formation nuclei (Webster and Steeves, in preparation). In birds, as in mammals, RP receives afferent input from a wide variety of motor related sources including TTD, tectum, cerebellum, Cnv, Cnd and vestibular nuclei (Hunt et al., 1977; Wold, 1978; Webster and Steeves, in preparation). Thus, from a neuroanatomical viewpoint, the pontine reticular formation is in an ideal position to regulate motor control.

Following the establishment of electrically stimulated locomotion, infusion of NMDA into the ventral RP elicited locomotor behaviour in several animals. EAA pathways are known to impinge on the pontine tegmentum from forebrain structures in the rat (Fagg and Foster, 1983) and NMDA-sensitive binding sites have been localized in the pons of the rat (Monaghan and Cotman, 1985). Our data suggest that similar NMDA binding sites exist in the bird. However, it has yet to be determined whether the EAA
projections arise from the forebrain, pontine interneurons, ascending inputs from brainstem/spinal cord or other regions. Our results suggest that pontine reticular formation neurons are under glutamatergic excitatory control.

Interestingly, injection of Substance P into RP produced weak bilateral walking behaviour which appeared to be significantly enhanced by NMDA infusion into the same site. Reiner (personal communication) describes Substance P-containing fibres localized within the paramedian pontine reticular formation near the ventral midline. The origin of these fibres, however, is unspecified. Thus, speculation on the role which Substance P has in the control or modulation of locomotor behaviours in the pontine reticular formation would be premature.

Mesencephalic Reticular Formation (MRF)

Two locomotion-evoking electrical stimulation sites have been previously described in the avian mesencephalon (See Chapter 2 and Sholomenko and Steeves, 1987b). The more lateral site is situated in close proximity to the intercollicular nucleus (ICo) of the avian tectum (see Chapter 2, Figure 1). A second, more medial site, is found in the medial mesencephalic reticular formation ventrolateral to the red nucleus and medial to the nucleus of the ansa lenticularis (mMRF) (see Chapter 2, Figure 1). The mMRF receives a major afferent projection from the deep tectal layers (Hunt et al., 1977) and sends efferents to tectum, high cervical spinal cord and medial medullary
reticular formation (Reiner and Karten, 1982; Webster and Steeves, in preparation). These electrophysiological and hodological considerations, therefore, possibly implicate this region as an avian equivalent of the mammalian MLR described by Garcia-Rill and Skinner (1986). However, this equivalency is limited by the apparent lack of an avian equivalent of the mammalian acetylcholine-containing PPN neurons (Steeves and Taccogna, in preparation). Controversy still exists as to whether the cholinergic PPN neurons actually underlie the effects of MLR stimulated locomotion. Rye and co-workers (Rye et al., 1987, 1988), following a thorough examination of the cytoarchitectonic, cytochemical and hodological relationships of the pedunculopontine nucleus in the rat, postulated that the PPN was not part of the MLR and substituted in its place a region termed the midbrain extrapyramidal area. Only a complete profile of this region, possibly based on a combination of intracellular recording, immunohistochemistry, intracellular dye injection and neurochemical stimulation will alleviate this controversy.

In this study, NMDA was found to decrease the threshold for electrically stimulated locomotion when infused into the mMRF in one animal. Similarly, in the cat, Garcia-Rill et al. (1985) found that glutamate infusion into the PPN/mLMLR decreased the threshold for electrically stimulated locomotion but did not, in isolation, elicit locomotion. Mogenson and Brudzynski (1986) found that glutamate injection into the PPN/MLR increased locomotor activity in the freely moving rat. Reasons for the apparent discrepancy between the findings in bird and cat versus those found in rat are presently not known, although variation
in the spatial distribution of cells within the locomotor regions in these species may account for some of the differences (Mogenson and Brudzynski, 1986). In combination with our data from cholinergic injection studies discussed in Chapter 3, and the congruence of those data in the bird with those found in mammals, it appears possible that avian equivalents of the MLRs exist in birds. Further study will be required to lend greater strength to this hypothesis.

**Medial Longitudinal Fasciculus (MLF)**

Previous studies demonstrated that locomotion could be elicited both by electrical and *neurochemical* (carbachol) stimulation of the MLF at pontomedullary levels. The injection of NMDA into this region also elicited repeatable locomotion in the decerebrate bird, although only in one animal. As discussed previously (Chapters 2 & 3), the MLF is generally recognized as a fibre tract which contains a variety of intranuclear projections. Thus, it was surprising that *neurochemical* injection into this region elicited locomotion. However, serotonin-containing neurons which stain positively for acetylcholinesterase have been localized in close proximity to the injected site and may be activated by *neurochemical* injection (Dube and Parent, 1981). Whether these avian neurons also possess EAA receptors is presently unknown, but a variety of reports from mammalian species do not report EAA receptors localized in this region (Monaghan and Cotman, 1985; Monaghan et al., 1985; Greenamyre et al., 1984). At present, therefore, EAA
excitation of locomotion from this site lacks even a putative neuroanatomical substrate. The result of NMDA injection-induced locomotion in this region, however, suggests possibilities for future study.

Pharmacological Considerations

In this study, direct intracerebral infusion of NMDA, but not glutamate, proved effective at eliciting locomotion in several electrophysiologically defined avian locomotor regions. In some cases, the locomotion could be blocked by GDEE infusion into the same site. The neurotransmitter glutamate has been demonstrated to be effective at three different proposed receptor subtypes in the CNS. The receptors are classified according to their differential affinity for the agonists NMDA, kainate and quisqualate (for review, see Stone and Burton, 1988; Watkins and Olverman, 1987), although the agonist for each receptor cross reacts to some extent with the other two receptors (D. Magnusson, personal communication). At the NMDA receptor, NMDA has been found to be 10-1000 times more potent than glutamate and is relatively specific for its receptor (Watkins and Olverman, 1987). The potency is dependent on the type of preparation and may reflect the rate of NMDA (slow) versus glutamate (fast) uptake (Stone and Burton, 1988). Recent results suggest that the site of NMDA action may be both pre- and postsynaptic (for review, see Stone and Burton, 1988). The action of NMDA is complex and involves a magnesium and voltage dependent increase in membrane permeability probably to calcium
and sodium ions. This is distinct from the action of the non-NMDA receptors (e.g. quisqualate and kainate), whose activation results in a voltage-independent increase in membrane conductance mediated by sodium (quisqualate and kainate - fast direct link to ionophore (quisqualate response potentiated by zinc, kainate not potentiated by zinc) or via an intracellular second messenger system (quisqualate) (for review, see Choi, 1988 & Stone and Burton, 1988).

GDEE, utilized in this study as a glutamate antagonist, was originally found to decrease neuronal sensitivity at glutamate receptors preferentially over aspartate receptors (Haldeman and McLennan, 1972). Subsequent to the findings of Haldeman and McLennan (1972), GDEE has been demonstrated to block all three glutamate receptor subtypes, but with greater efficacy at the quisqualate receptor than at the NMDA and kainate receptors (Watkins and Olverman, 1987; Stone and Burton, 1988).

The above glutamate pharmacology illustrates the inherent problems in assessing the exact type and location of receptors responsible for activating (or blocking) neurons involved in locomotion elicited by NMDA infusion. The NMDA concentrations at the injection point (see Table 3 and results) were higher than physiological concentrations, and probably activated all three glutamate receptor subtypes (D. Magnusson, personal communication), although the NMDA concentration, following diffusion into the tissues, would presumably have decreased as it spread through the CNS tissue. It is not possible to determine which type(s) of glutamate receptor was activated in the present experiments. Hopefully, future avian studies that
identify the neuroanatomical location of glutamate receptor subtypes will assist in determining which possible receptors underlie the observed results. Future studies utilizing more specific NMDA receptor antagonists, such as 2-amino-5-phosphonopentanoic acid (AP5) (Watkins and Olverman, 1987), may also be useful in this regard.
CHAPTER 6

AVIAN LOCOMOTOR PATTERNS IN THE ABSENCE OF PHASIC AFFERENT INPUT - THE 'FICTION' PREPARATION
INTRODUCTION

In vertebrates, both the initiation and ongoing control of locomotion are dependent upon a hierarchical organization of brain and spinal cord neural networks. These circuits, in turn, are modulated by peripheral afferent feedback during locomotion and the interaction between the two is responsible for normal production of locomotor pattern (for reviews see Grillner, 1985; McClellan, 1986). Previous avian studies have demonstrated that birds possess brainstem and spinal cord locomotor networks analogous to those found in higher and lower vertebrates (Jacobson & Hollyday, 1982; Steeves et al., 1986, 1987a,b; Ten Cate, 1960, 1962). These networks include: 1) spinal cord 'pattern generators', which in the absence of descending supraspinal input, can produce 'spinal stepping' (Jacobson & Hollyday, 1982; Sholomenko & Steeves, 1987; ten Cate, 1960, 1962), 2) descending pathways projecting through the ventrolateral funiculi that are essential for initiating locomotion and originate in the hindbrain reticular formation (Sholomenko & Steeves, 1987; Steeves et al., 1987), and 3) discrete brainstem regions, which when electrically or chemically stimulated in a decerebrate preparation, evoke walking and/or flying (Sholomenko & Steeves, 1987a,b, 1988; Steeves et al., 1987).

In this chapter, I examine whether forelimb and hindlimb phasic afferent input is a prerequisite for the production of avian locomotor patterns by the central nervous system. Phasic afferent feedback was eliminated through paralysis of the
animal and then brainstem locomotor regions were stimulated in the decerebrate bird while recording electroneurographic activity from locomotor-muscle nerves. The term 'fictive' has been used to describe this type of neural activity associated with locomotion or motor output during neuromuscular paralysis (Perret et al., 1972).

My results demonstrate that all unparalyzed locomotor patterns can be elicited in a paralyzed decerebrate animal including: 1) alternating (out of phase) leg stepping, 2) bilateral (in phase) jumping (or hopping), 3) coactivated walking and flying (i.e. combined lumbar and cervical cord activity) and 4) bilateral (in phase) wing flapping.
MATERIALS AND METHODS

Surgery

Surgical procedures have been previously described in detail in Chapter 2. Decerebration levels of spontaneous versus non-spontaneous animals are described in Chapter 7.

Brainstem Stimulation

Either intracerebral electrical or chemical stimulation of localized regions within the brainstem was used to evoke locomotion from a non-spontaneously locomoting preparation (for methods, stimulation parameters and procedures, see Chapters 2-5 this thesis).

After establishing an optimum brainstem stimulation site and recording EMG activity from the PECT and ITC muscles during evoked locomotion, each animal (17 Canada geese, 7 Pekin ducks) was paralyzed with tubocurarine chloride (initially 0.15mg/kg; supplemented as required) and unidirectional ventilation (UDV) was initiated. Electroneurograms (ENGs) were recorded with platinum hook electrodes placed on nerves directly innervating the main body of ITC and PECT muscles. Signals were amplified (10,000x), filtered and recorded in the same manner as the EMG signals. ENG recordings were made during spontaneous or evoked locomotion and EMG muscle activity was monitored to ensure that there was no movement in response to stimulation. At the conclusion of each experiment, the position of the micropipette
tip was marked with an electrolytic lesion made by passing a direct cathodal current of 3mA for 5 seconds at the stimulation site.

Histological identification of stimulation sites has been previously described (Chapter 2).
RESULTS

Spontaneous Locomotion

Several high decerebrate birds (N=6) exhibited spontaneous periods of walking in response to a moving treadmill belt (Fig. 27A). When the treadmill belt was stopped, several of the birds maintained an upright standing posture. After paralyzation, 2 of the 6 animals showed spontaneous alternating ITC neural activity patterns characteristic of walking (i.e. 'fictive' locomotion). As shown for one of these animals in Fig. 27, the long lasting (>30min) spontaneous alternating ITC activity patterns were similar both before (Fig. 27A, EMGs) and after (Fig. 27B, ENGs) paralyzation. However, the step frequency was reduced somewhat in both birds during 'fictive' walking relative to the preparalyzed state (Fig. 28, spontaneous). The other 4 animals did not demonstrate spontaneous 'fictive' locomotion in response to the moving treadmill belt. However, two of these animals did show short bursts (5-10 seconds) of 'fictive' walking in response to exteroceptive stimulation of the head. In the final 2 birds, 'fictive' locomotion was initiated and maintained only in response to focal electrical stimulation of brainstem locomotor regions.

Electrically Stimulated Locomotion

Electrical stimulation of brainstem regions including the pontobulbar locomotor strip, medullary reticular formation,
Figure 27. Bilateral alternating walking activity in a spontaneously locomoting bird before (A) and after (B) paralyzation. The transection level (dotted line) which allows post-decerebration spontaneous locomotion is shown in the sagittal section. A: EMG traces from left and right ITC (*) muscles during spontaneous treadmill walking. B: Subsequent ENG traces from left and right ITC nerves in the paralyzed animal showing spontaneous walking pattern activity.

* The iliotibialis cranialis (ITC) muscle is synonymous with the mammalian sartorius muscle.
A - PRE-PARALYZED EMG

LEFT ITC

RIGHT ITC

1 sec

LEVEL OF DECREASEBRATION

B - PARALYZED ENG

LEFT ITC

RIGHT ITC

2 sec
Figure 28. Histogram of electrical stimulation-induced and spontaneous step frequency during pre-paralyzed (small cross hatch) and paralyzed 'fictive' (large cross hatch) stepping. Pre-paralyzed data have been normalized to 100%. Paralyzed 'fictive' values are shown as % change relative to pre-paralyzed data. Actual pre-paralyzed and paralyzed 'fictive' step frequencies for each bird (numbers above record) were averaged from trials evoked at brainstem stimulation threshold current intensities (numbers in brackets).
ANIMAL # (Electrical brainstem stimulation) (Spontaneous)

= PRE-PARALYZED  = PARALYZED
rostral pontine reticular formation, mesencephalic reticular formation and medial longitudinal fasciculus produced locomotion in 16 animals. The variety of patterns in the evoked locomotion depended not only on the condition of the decerebrate animal, but also on the location of the stimulating electrode within the brainstem. However, the elicited locomotor pattern was constant for any given site. In one animal, for example, at moderately weak current strengths (25-100 μA), monopolar electrical stimulation of the descending trigeminal nucleus (TTD) (Karten & Hodos, 1967) evoked walking (Fig. 29A). After paralyzation, as determined by comparing alternating firing patterns of the ITC muscles and nerves, a similar walking pattern was elicited. However, a higher stimulation intensity was required to initiate walking following paralyzation (Fig. 29B).

Of the eight animals which walked in both the unparalyzed and paralyzed states, the averaged frequency of stepping observed at threshold stimulation levels decreased in seven birds from the unparalyzed (1.6Hz±0.27) to the paralyzed (1.0Hz±0.18) condition (Fig. 28, electrical brainstem stimulation). Although the mean stepping frequencies of the 2 groups were not significantly different (ANOVA, p=.075), the mean threshold stimulation intensity necessary to evoke walking in the paralyzed bird (176±25μA) was significantly greater (p<0.005) than in the unparalyzed animals (67±8μA).

Electrical stimulation of other locomotor regions characteristically elicited walking behaviour at lower stimulation intensities, with recruitment of wing activity as intensity increased. This pattern is shown for one animal
Figure 29. Bilateral alternating walking activity evoked by focal electrical stimulation of the hindbrain before (A) and after (B) paralyzation. The transection level is shown by the dotted line. The medullary stimulation site, marked by the triangle in the transverse and sagittal sections, was located in the descending tract of the trigeminal nerve (PLS). A: EMG traces from left and right leg ITC muscles during electrically stimulated walking. B: ENG traces from left and right ITC nerves showing evoked walking patterns in the paralyzed bird.
stimulated within the region of the mesencephalic reticular formation (MRF) prior to paralysis (Fig. 30A). At a stimulation intensity of 40μA, walking movements were initiated and increased in frequency as intensity was increased. When current intensity reached approximately 90μA, bilateral wing flapping was also observed. The same sequence of events was not observed in the paralyzed animal (Fig. 30B), but coactivation of the nerves from both legs and one wing was observed. As seen previously during walking alone, the electrical current strengths necessary to evoke rhythmic locomotor activity were at least two times greater in the paralyzed versus unparalyzed animal.

In-phase wing flapping alone, characteristic of flying behavior, was also often elicited in response to electrical stimulation of specified brainstem regions (Fig. 31A). After paralysis, a similar pattern of evoked motor activity could be recorded from the PECT nerves (Fig. 31B). The average current strength necessary, however, to evoke 'fictive' flying in the paralyzed birds (155±27μA) was significantly greater (ANOVA, p=0.036) than that required to initiate locomotion in the unparalyzed birds (71±26μA, n=6) (Fig. 32). In spite of this increased stimulus intensity, the six animals which elicited both unparalyzed flapping and 'fictive' flapping displayed a significant reduction (ANOVA, p=0.018) in evoked flapping frequency after paralysis was initiated (from 2.9Hz±0.4 to 1.6Hz±0.2) (Fig. 32).
Co-activation of leg and wing activity evoked by focal electrical stimulation of the midbrain before (A) and after (B) paralyzation. The transection level is shown by the dotted line. The midbrain stimulation site, marked by the triangle in both saggital and transverse sections, was located in the medial mesencephalic reticular formation. A: EMG traces from left and right Pectoralis (PECT) (top 2 traces) and left and right ITC (bottom 2 traces) muscles during electrically stimulation showing transition from walking alone to simultaneous walking/flying. B: ENG traces from left Pectoralis (top trace) and left and right ITC (bottom 2 traces) nerves showing coactivated evoked locomotor patterns of the legs and wing during electrical stimulation in the paralyzed animal. Due to recording difficulty, only a single wing trace is shown in these data. However, wings are normally phase locked and bilaterally synchronous during both paralyzed and actual locomotion (see Figure 31).
A - PRE-PARALYZED EMG

LEFT PECT

RIGHT PECT

LEFT ITC

RIGHT ITC

STIMULATION SITE

LEVEL OF DECREBRATION

B - PARALYZED ENG

LEFT PECT

LEFT ITC

RIGHT ITC

2 sec

2 sec
Figure 31. Bilateral synchronous flying activity evoked by focal electrical stimulation of the hindbrain before (A) and after (B) paralyzation. The transection level is shown by the dotted line. The medullary stimulation site, marked by the triangle in the transverse and sagittal sections, was located in the dorsal part of the medullary central nucleus (reticular formation). A: EMG traces from left and right Pectoralis muscles during electrically stimulated flying. B: ENG traces from left and right Pectoralis nerves showing evoked flying patterns in the paralyzed bird.
A - PRE-PARALYZED EMG

LEFT PECT

RIGHT PECT

1 sec

STIMULATION SITE

LEVEL OF DECEREBRATION

B - PARALYZED ENG

LEFT PECT

RIGHT PECT

2 sec
Figure 32. Histogram of brainstem stimulation-induced wingbeat frequency during pre-paralyzed (small cross hatch) and paralyzed 'fictive' (large cross hatch) flapping. Pre-paralyzed data have been normalized to 100%. Paralyzed 'fictive' values for each bird are shown as % change relative to pre-paralyzed data. Actual pre-paralyzed and paralyzed 'fictive' wingbeat frequencies (numbers above record) were averaged from flapping evoked at threshold stimulation intensities (numbers in brackets).
% WINGBEAT FREQUENCY

ANIMAL

1 = PRE-PARALYZED

2 = PARALYZED

- 2.1 Hz (25uA)
- 2.0 Hz (170uA)
- 1.6 Hz (180uA)
- 2.4 Hz (200uA)
- 2.6 Hz (40uA)
- 1.9 Hz (100uA)
- 3.5 Hz (30uA)
- 1.3 Hz (100uA)
- 3.4 Hz (80uA)
- 1.1 Hz (110uA)
- 3.9 Hz (70uA)
- 1.2 Hz (250uA)
Chemically Stimulated Locomotion

Direct intracerebral infusion of specific neurotransmitter agonists and antagonists also evoked locomotion in paralyzed decerebrate birds (see Chapters 3-5). Carbachol injection (N = 3) (1μl at 0.2ml/min, 27mM in PBS) into the ventromedial brainstem reticular formation [nucleus reticularis gigantocellularis (Rgc)] evoked alternating bursting activity in the right and left leg ITC nerves of a paralyzed goose (Fig. 33A). Carbachol activation of locomotor activity was also recorded in more distal leg muscle nerves (e.g. gastrocnemius) indicating that the paralyzed locomotor pattern was not restricted to just the major hip muscles. Carbachol-induced locomotor patterns mimicked those seen in response to focal electrical stimulation of the brainstem both before and after paralyzation.

Injection of N-methyl-d-aspartate (NMDA) in 2 animals (0.4μl at 0.2μl/min, 5 or 30mM in PBS) into the caudal reticular formation [nucleus centralis medulla oblongata, pars dorsalis (Cnd)] produced similar locomotor responses in both unparalyzed and paralyzed birds. Figure 33B shows NMDA activation of the left and right leg ITC nerves of a decerebrate paralyzed goose in an in-phase hopping or jumping locomotor pattern. This locomotor pattern was identical to that observed in a different unparalyzed bird after an intracerebral injection of NMDA. Both alternating (e.g. walking) and in-phase (e.g. hopping) locomotor patterns can be elicited by intracerebral injection of NMDA into the brainstem, however, it is not presently clear what
Figure 33. Bilateral 'fictive' hindlimb nerve activity elicited by neurochemical microinjection of carbachol and NMDA. A: ENG traces from the left and right ITC nerves showing alternating 'fictive' walking activity following injection of carbachol (1.0μl : 0.2μl/min : 100mM), a cholinergic agonist, into the pons. The site injected (triangle), as shown in the transverse section A, was located within the pontine gigantocellular reticular formation. B: ENG traces from the left and right ITC nerves showing synchronous in-phase activity ('fictive' hopping or galloping) after injecting NMDA (0.2μl : 20mM), a glutamatergic agonist, into the dorsal part of the medullary central nucleus (triangle in transverse section B).
A - CARBACHOL - PARALYZED ENG

LEFT ITC

RIGHT ITC

B - NMDA - PARALYZED ENG

LEFT ITC

RIGHT ITC

A CARBACHOL STIMULATION SITE

B NMDA STIMULATION SITE
determines which pattern will be expressed (Sholomenko & Steeves, in preparation). The time course of NMDA activation in both unparalyzed and paralyzed animals was similar, with the onset of locomotor activity beginning within 2 minutes of the intracerebral injection. Locomotion (both unparalyzed and paralyzed) ceased 10-15 minutes post-injection.
DISCUSSION

The main finding of the present study is that paralyzed decerebrate birds (i.e. 'fictive' preparations) are capable of producing all the same locomotor patterns as unparalyzed animals, regardless of whether the 'fictive' locomotion is generated: 1) spontaneously (Fig. 27B), 2) in response to focal electrical stimulation (Fig. 29B, 30B, 31B), or 3) in response to direct intracerebral chemical infusion into brainstem locomotor regions (33A,B). To my knowledge, this is the first example of 'fictive' bipedal locomotion in a vertebrate. Furthermore, the coactivation of both modes of locomotor behaviour (stepping and flapping) (Fig. 30A,B) illustrates that relatively complex activation of brainstem and spinal locomotor networks can also occur in the absence of phasic peripheral feedback.

It is clear from the present observations that rhythmic motor activity resembling walking and flying can be elicited in the absence of phasic peripheral input, thereby implicating the CNS in generating a considerable array of avian motor patterns. It has been argued that the 'fictive' locomotion observed in response to focal electrical stimulation or intracerebral infusion of neurotransmitter related chemicals into brainstem locomotor regions is due, in part, to the activation of central pathways that normally relay somatosensory information during locomotion [e.g. TTD or pontobulbar locomotor strip (PLS) electrical stimulation (Noga et al., 1988) (Fig. 29)]. Thus, this 'fictive' locomotor activity in paralyzed animals may not
reflect an inherent CNS locomotor pattern generating capacity. However, this possibility is eliminated, at least for avian walking, by the perseverance of 'fictive' leg locomotor activity after paralysis in the spontaneously moving animal (Fig. 27B), where the somatosensory pathways described by Noga et al. (1988) are not activated by phasic sensory input.

In spite of the capacity of the CNS to independently generate locomotor rhythm, it is important to note that proprioceptive feedback does alter motor pattern. One facet of this influence was evidenced by the greater stimulation intensity required to activate locomotion in the paralyzed birds, in addition to the lower locomotor frequencies observed in the paralyzed animals when locomotion was finally activated.

The higher threshold of the paralyzed birds might suggest that sensory input serves to lower the threshold for activating movement by increasing the overall gain of the animal's activation level. While the occurrence of spontaneous 'fictive' walking in paralyzed birds may argue against this suggestion, only 2 of the 6 animals that exhibited spontaneous locomotion prior to paralysis elicited spontaneous patterns during paralysis. It is possible that the activation state of the 2 spontaneous fictive animals was high enough to allow spontaneous 'fictive' walking, while the activation level in the remaining 4 was reduced to levels that precluded spontaneous activity following paralysis. This is supported by the finding that exteroceptive stimulation (non-noxious) could elicit short bouts of 'fictive' walking in 2 of the 4 birds that did not elicit spontaneous locomotion during paralysis. A further piece of
evidence, namely, the rare occurrence of fictive flying, indirectly supports the argument that afferent input serves to increase the animals activation level. From results presented here, where bilateral flapping is consistently observed to occur at stimulation intensities greater than that required to elicit walking, it appears, as seen in the cat (Shik et al., 1966, 1967), that the threshold for forelimb activation is greater than that for hindlimbs. Thus, where stimulation up to 170\(\mu\)A is often sufficient to elicit walking and/or wing flapping when afferent feedback is present, similar stimulation, although activating legs, is rarely sufficient to activate the forelimb pattern generators in the paralyzed bird.

The degree to which afferent input is important in establishing this "activation level", and, in turn, the role afferent input plays in determining the frequency of locomotor output remains unclear. However, studies in which the degree of afferent input is controlled may help to solve these issues. Afferent input could be applied, for example, by alternately lifting the legs of the animal (graded excursions) during the appropriate extensor phase of 'fictive' locomotion, thereby providing the bird with varying degrees of sensory feedback. This paradigm would presumably increase the activation level of the preparation and could be utilized to explore the effects of peripheral feedback on locomotion both in the spontaneous and electrically stimulated preparations.

In conclusion, this study has demonstrated that the central nervous system possesses neural networks which can produce a wide array of avian locomotor patterns in the absence of
afferent feedback. This study, however, has also alluded to the importance of afferent feedback in the production of a normal walking pattern. Removal of all feedback appears to lower the "activation level" of the animal such that greater stimulation is required to initiate locomotion and that, once initiated, the locomotor movements are reduced considerably.
CHAPTER 7

TRANSECTION LEVEL DETERMINES SPONTANEOUS MOTOR ACTIVITY
IN THE DECEREBRATE AVIAN PREPARATION
INTRODUCTION

In vertebrates, selective brain transection at different levels of the neuraxis yields varying degrees of spontaneous locomotor activity (for review see Shik and Orlovsky, 1976; Wetzel and Stuart, 1976; Armstrong, 1986). Table 1 briefly summarizes the motor capabilities of cats following transection of the neuraxis at the various levels shown in Figure 1. These results, with minor variation, are equally applicable to a variety of mammals including dogs (Magnus, 1924), rabbits (Hinsey et al., 1930) and rats (Woods, 1964; Skinner and Garcia-Rill, 1984). Differences in an animal's ability to produce spontaneous locomotion between the premammillary versus postmammillary acute preparation (transection B and C in Figure 1) have been attributed to the sparing of structures contained within the preserved wedge of neural tissue (Wetzel and Stuart, 1976; Shik and Orlovsky, 1976). Neural structures found in this region which could be responsible for the initiation of spontaneous locomotor behaviour include the subthalamic locomotor region (SLR) (Waller, 1940), subthalamic neurons, posterior hypothalamic nuclei and posterior thalamic midline neurons (Wetzel and Stuart, 1976).

Our studies with birds have demonstrated that the avian neural substrates for locomotion appear similar to those found in a variety of both higher and lower vertebrates (Steeves et al., 1986, 1987; Sholomenko and Steeves, 1987a,b; Sholomenko, this thesis; McClellan, 1986), thus implying a high degree of
<table>
<thead>
<tr>
<th>TRANSECTION LEVEL</th>
<th>ANIMAL PREPARATION</th>
<th>ACUTE PREPARATION</th>
<th>CHRONIC PREPARATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRECOLLICULAR PREMAMMILLARY CAT Fig 1-Line B</td>
<td>SPONTANEOUS LOCOMOTION (HINSEY ET AL., 1930) (SHIK ET AL., 1966)</td>
<td>SPONTANEOUS LOCOMOTION (HINSEY ET AL., 1930)</td>
<td></td>
</tr>
<tr>
<td>PRECOLLICULAR POST-OCULO-MOTOR NERVE CAT Fig 1-Line D</td>
<td>NO SPONTANEOUS LOCOMOTION (BARD AND MACHT, 1958)</td>
<td>LOCOMOTION IN RESPONSE TO STRONG EXTEROCEPTIVE STIMULATION (BARD AND MACHT, 1958)</td>
<td></td>
</tr>
<tr>
<td>MIDDICOLLICULAR PRE-OCULO-MOTOR NERVE CAT Fig 1-Line E</td>
<td>NO SPONTANEOUS LOCOMOTION (BARD AND MACHT, 1958)</td>
<td>ALTERNATING LIMB MOVEMENTS IN AN ANIMAL WHICH IS LYING PRONE (BARD AND MACHT, 1958)</td>
<td></td>
</tr>
</tbody>
</table>
Figure 34. Diagram of a sagittal section through the cat brainstem showing neuraxis transection levels and locomotor sites important for the study of locomotor control. Transection levels are designated by letters A-E. Transection level: A - thalamic, B - precollicular premammillary (hypothalamic of Hinsey et al., 1930), C - precollicular postmammillary, D - precollicular post-occulomotor, E - midcollicular pre-occulomotor. Locomotor sites include the subthalamic locomotor region (SLR) and mesencephalic locomotor region (MLR). The hatched lines surrounding RPC and RGC represent the pontine and medullary reticular formation that are thought to be the major motor information projection systems to the spinal cord. Abbreviations: CM - mammillary body, CO - optic chiasm, IC - inferior colliculus, MLR - mesencephalic locomotor region, P - pons, R - red nucleus, RGC - medullary gigantocellular reticular nucleus, RPC - caudal pontine reticular nucleus, SC - superior colliculus, SLR - subthalamic locomotor region, T - trapezoid body, Th - thalamus, III - oculomotor nerve. See text for additional explanation. This figure is redrawn from: 1) Shik et al., 1968, 2) Orlovsky, 1970a, 3) Grillner and Shik, 1973 and 4) Wetzel and Stuart, 1976.
conservation of motor circuitry across a broad phylogenetic range. To further this comparison and in an attempt to delineate the structures required for the initiation of spontaneous locomotion, we have examined the effects of different levels of brain transection on spontaneous locomotor capability in birds.

Our results indicate that in the acute avian preparation, as in mammals, animals with a rostral transection of the neuraxis (post-habenular/preoptic) display spontaneous locomotion, while more caudal transection (post-habenular/postoptic) eliminates any spontaneous behaviour. Diencephalic structures which are contained between these two transection levels presumably underlie this difference.
MATERIALS AND METHODS

Surgery

Surgical and EMG/ENG recording procedures have been previously described (Chapter 2) with the exception that following a craniotomy, a suction decerebration was performed along a plane extending dorsally from the caudal margin of the habenular nucleus to either: 1) the rostral or 2) the caudal edge of the optic chiasm ventrally.

Stimulation procedures for low decerebrate animals have been described in detail previously (Sholomenko and Steeves, 1987; this thesis, Chapters 2-5). In high decerebrate animals, after recording the spontaneous electromyographic (EMG) activity from the legs and/or wings, the birds were deeply anaesthetized and sacrificed with an intravenous injection of KCl (2M). Histological procedures have been described in detail in Chapter 2, with the exception that the level of decerebration was determined by the reconstruction of serial coronal sections according to the stereotaxic atlases of Karten and Hodos (1967) and Zweers (1971).

Assessment of Spontaneous versus Non-spontaneous

Two criteria were used to distinguish spontaneous from non-spontaneous birds. First was the bird’s response to foot web pinch stimulation following removal of anaesthetic. Strong
reflex withdrawal was common to all birds, however, those birds responding to web pinch with stepping motions and/or wing flapping met the first criteria for spontaneous preparations. Second, if the birds also displayed prolonged walking behaviour (longer than 1 minute continuous) in response to the tactile and leg movement stimulation produced when the treadmill was turned on, they were considered spontaneous.

Animals which did not meet the above criteria were classified as non-spontaneous. These animals required either electrical or neurochemical brainstem stimulation to elicit locomotor behaviours.
RESULTS

Ten animals (6 Canada geese, 4 Pekin ducks) with the rostral transection depicted in Figure 35 (line A) displayed spontaneous locomotion. Several of the animals displayed significant extensor tonus, resembling that of a standing posture, when the treadmill belt was motionless. When the belt was turned on, the legs were moved caudally and the animals would begin to make alternating stepping movements (Figure 36A,B). The stepping seldom appeared of sufficient force to be self-supporting, although total force was difficult to determine.

Of the total of ten spontaneous animals, only one bird displayed wing flapping behaviour combined with stepping in response to the treadmill belt stimulation alone (Figure 37). The flapping occurred in short bursts (approximately 10 seconds long) and subsided over several hours, even though belt-stimulated stepping continued for several hours.

In the spontaneous birds, tactile stimulation of parts of the body other than the feet (e.g. stroking around the eye or caudal back region) increased the force of hindlimb stepping when the animal was walking. Similarly, if an animal ceased stepping in response to the belt, tactile stimulation would often re-initiate the stepping behaviour.

Two birds which were paralyzed displayed alternating hindlimb 'fictive' stepping activity in the absence of any phasic afferent input (treadmill belt off) from the periphery.
Figure 35. Diagram of the transection levels of the avian brain which permit or eliminate spontaneous locomotion in the decerebrate bird. The diagram is of a near midline (lateral 0.5mm) sagittal section of the pigeon brain (Karten and Hodos, 1967). Line A designates the approximate level of section which allows spontaneous locomotion in the decerebrate bird. Animals with a transection at the approximate level of Line B do not show spontaneous locomotor activity.

Figure 36. Bilateral alternating walking activity in a spontaneously locomoting bird before (A) and after (B) paralyzation. The transection level (dotted line) which allows post-decerebration spontaneous locomotion is shown in the saggital section. A: EMG traces from left and right ITC (*) muscles during spontaneous treadmill walking. B: Subsequent ENG traces from left and right ITC nerves in the paralyzed animal showing spontaneous walking pattern activity.
* The iliotibialis cranialis (ITC) muscle is synonymous with the mammalian sartorius muscle.
A - PRE-PARALYZED EMG

LEFT ITC

RIGHT ITC

LEVEL OF DECEREBRATION

B - PARALYZED ENG

LEFT ITC

RIGHT ITC
Figure 37. Electromyographic records (EMGs) showing spontaneous stepping and flying activity. The EMGs were taken from the right (RPECT) and left (LPECT) pectoralis muscles and right (RITC) and left (LITC) iliotibialis cranialis muscles during a bout of spontaneous activity. The PECT muscle is the major wing depressor essential for flight and the ITC muscle is the major hip flexor in birds.
(Figure 36B) (see also Chapter 6). Both animals were strongly spontaneously active prior to paralyzation. Five other spontaneous animals which were subsequently paralyzed did not demonstrate any spontaneous 'fictive' locomotion after curarization and required either electrical or chemical stimulation to initiate 'fictive' locomotor patterns (see Chapters 2-6).

Non-spontaneous animals (N=122) required either: 1) focal electrical stimulation of brainstem locomotor regions (Steeves et al., 1986, 1987; Sholomenko and Steeves, 1987; Sholomenko, this thesis) or 2) intracerebral microinjection of neurotransmitter agonists or antagonists into the same locomotor regions (Sholomenko and Steeves, 1987b; Sholomenko, this thesis) to evoke locomotor behaviours (see Chapters 2-6). The approximate level of transection averaged from a random sample of 10 of these birds is displayed in Figure 35 (line B).

Decerebration Level

Spontaneous

The transection level in six of the spontaneous birds extended from the caudal border of the habenular nucleus dorsally to the rostral border of the anterior preoptic nucleus ventrally. This transection eliminated the entire telencephalon in addition to portions of the anterodorsal thalamus (DMA). Transections which eliminated more dorsocaudal structures (N=2),
including the posterior thalamic nuclei and ovoid nucleus, extended dorsally from caudal to the posterior commissure (CP) to anterior to the anterior preoptic nucleus ventrally. Two spontaneous birds had transections which ablated more caudal structures including the external (SCE) and portions of the internal (SCI) cellular stratum. One of these birds, with a transection that separated the entire thalamus, posterior commissure and part of the medial posterior hypothalamic nucleus from the brainstem, elicited prolonged hindlimb stepping in response to treadmill belt stimulation.

In all of the above spontaneous animals, the subthalamic nucleus (nucleus of the ansa lenticularis (nAL)), the substantia nigra (TPc), the lateral spiriform nucleus and caudal portions of the hypothalamic nuclei [e.g. posterior (AHP) and lateral (LHA) hypothalamic areas] remained intact.

Non-spontaneous

Neuroanatomic analysis of the ten birds chosen at random (9 Canada geese, 1 Pekin duck) from those which did not elicit spontaneous locomotion after the decerebration (Fig. 35B) demonstrated that the majority of animals had more caudal transection levels than those found in spontaneous birds. In two animals, the transection eliminated the most rostral portions of the Spl, but left the bulk of the nucleus intact. These transections completely removed the subthalamic nucleus (nAL) and the bulk of the posterior and lateral hypothalamic nuclei.
Also, transection levels in three animals damaged the most rostral region of the substantia nigra.

Slightly more rostral lesions also eliminated spontaneous locomotion in 4 birds. In these animals, the substantia nigra and Spl nuclei were spared, but again the nAL and lateral and posterior hypothalamic nuclei were excised.

One bird which did not perform spontaneously after decerebration had a transection which was of the same level as those seen in animals which exhibited spontaneous locomotion. All of the nuclei which remained in the spontaneous birds were also present in this animal. Reasons why this animal did not elicit spontaneous locomotion cannot be determined, but it is possible that the condition of the preparation deteriorated more rapidly than in the other birds.
DISCUSSION

The results from this study demonstrate that in birds, as in mammals, preservation of a region lying within the boundaries of the caudal diencephalon allows spontaneous locomotion in the acute decerebrate preparation. More caudal transections eliminate the spontaneity but do not alter the ability to generate locomotor rhythms in response to electrical and/or chemical stimulation of brainstem locomotor regions (Sholomenko and Steeves, 1987; Sholomenko, this thesis).

The nuclei which were present in the wedge of tissue lying between the two transection levels included the nucleus of the ansa lenticularis (nAL), nuclei of the lateral and posterior hypothalamic areas (LHA & PHA) and the most rostral portion of the compact part of the substantia nigra (TPc). The nAL, based primarily on hodological considerations, is comparable to the mammalian subthalamic nucleus (Brauth et al., 1978), while the TPc has been compared to the mammalian substantia nigra (Brauth et al., 1978; Reiner et al., 1984). The LHA and PHA also lie within the wedge of tissue which was removed in the non-spontaneous but preserved in the spontaneous birds. All but the most rostral portion of Spl, which receives the major outflow of the avian basal ganglia (Reiner et al., 1984), was spared in both rostral and caudal lesions.

Several explanations have been put forth in an attempt to account for the different levels of activity in precollicular-premammillary versus precollicular-postmammillary
transected mammals. Wetzel and Stuart (1976) suggest that the structures remaining in the precollicular-premammillary but removed from the precollicular-postmammillary preparation, consisting of the posterior hypothalamic, subthalamic and ventral posterior thalamic neurons, increase the general excitability of more caudal neurons. Similarly, Armstrong, in a recent review (1986), postulated that cells contained within the preserved slice of tissue, possibly arising from the posterior hypothalamus, zona incerta or H₁ and H₂ fields of Forel, provide a tonic (as opposed to rhythmic or patterned) excitatory input to downstream motor related structures. Garcia-Rill and Skinner (1986), on the other hand, suggested that tonic GABAergic subthalamic nucleus projections to the substantia nigra (SN), which are spared after the precollicular-premammillary transection, inhibit an inhibitory GABAergic projection from the SN to the pedunculopontine nucleus (mesencephalic locomotor region). Thus, locomotor patterns are effectively disinhibited (released) and can express themselves in the acute premammillary cat preparation. Their hypothesis accounts for the loss of spontaneous activity after a more caudal transection through the elimination of the inhibition from the SN to the PPN. This theory is supported by the finding that injection of GABA antagonists into the SN blocks spontaneous stepping in the premammillary preparation and that stepping can be reinstated by injection of GABA or muscimol into the SN (Garcia-Rill and Skinner, 1986). However, specific damage to the subthalamic nucleus, which should, according to Garcia-Rill and Skinner
(1986), through disinhibition of an inhibitory pathway, inhibit locomotion, only releases motor behaviours such as hemiballismus or chorea (Hammond et al., 1979).

Another diencephalic region, defined electrophysiologically as the subthalamic locomotor region (SLR), will elicit locomotion when electrically stimulated. It is intimately involved in locomotor control and lies dorsomedially to the subthalamic nucleus (Shik and Orlovsky, 1976; Orlovsky and Shik, 1976). Mogenson and co-workers (Mogenson, 1984; Mogenson et al., 1985; Brudzynski et al., 1988) used a variety of neuroanatomical tracing techniques to describe the SLR as lying within the zona incerta (ZI) and lateral hypothalamic area (LHA) in the rat. This region has been found to project to the pedunculopontine, cuneiform and reticular formation nuclei, which themselves are strongly implicated in motor control (Swanson et al., 1984; Mogenson et al., 1985; Orlovsky, 1970a,b; Steeves and Jordan, 1984; Garcia-Rill and Skinner, 1986). Their evidence corroborates that found by Orlovsky (1969), who used electrophysiological techniques to describe direct projections from the SLR both to the MLR and reticular formation. Evidence that the SLR-evoked locomotion results not from stimulation of axons of passage but from stimulation of neuronal receptors comes from the finding that infusion of the GABAergic antagonist picrotoxin into the SLR induced locomotion in the precollicular-premammillary cat (Eldridge et al., 1985). Of interest is the hypothesis that the SLR region underlies goal directed behaviour (Shik and Orlovsky, 1976; Mogenson, 1984;
Mogenson et al., 1985) and may be the locus through which the limbic system influences locomotor behaviours (Mogenson et al., 1985; Brudzynski et al., 1988). Thus, the SLR, in addition to the subthalamic nucleus is a possible source of the spontaneous locomotor activity in high decerebrate animals.

In birds, the structures conserved in the high decerebrate animal parallel those found in mammals. The nAL (subthalamic nucleus) projects to the avian equivalent of the substantia nigra (TPc) (Reiner et al., 1984), pallidum (paleostriatum primativum) and lateral spiriform nucleus (Brauth et al., 1978). It is presently unknown whether this pathway is equivalent to the GABAergic projection suggested in mammals (McGeer et al., 1984; Garcia-Rill and Skinner, 1986), but it is interesting to note that the structures to which nAL project are all motor-related in birds (Reiner et al., 1984). It remains unclear, however, whether the nAL is the neural substrate responsible for spontaneous locomotion in high decerebrate birds.

The afferent and efferent projections of the hypothalamic nuclei which are preserved in the high decerebrate spontaneous preparation (LHA and PHA) have not been elucidated in birds, although Berk and Hawkin (1985) make brief mention that in pigeon, as in rat (Mogenson et al., 1985), the parahippocampal region projects to the lateral hypothalamic area. Thus, although no avian equivalent of the SLR/zona incerta has yet been recognized in birds (Karten and Hodos, 1967; Zweers, 1971), some homology appears to exist between limbic to hypothalamic
connections in birds and mammals (Berk and Finkelstein, 1983) which may underlie the preservation of spontaneous locomotion after a rostral decerebration. In birds, as in mammals, more information is required to determine which nucleus or combination of nuclei in the conserved diencephalic tissue is essential for spontaneous locomotion. Furthermore, the exact role of this region, although possibly limbic-motor related, remains to be determined.

The lack of information regarding the avian pathways which lead to spontaneous locomotion after a rostral transection of the neuraxis make it impossible to delineate the neural substrate for the activity at this time (Armstrong, 1986). However, taken together with our previous results demonstrating that birds possess locomotor circuitry which is very similar to that of mammals, we hypothesize that the neural substrate and mechanisms involved in avian high decerebrate spontaneous locomotion will be the same as those found in the mammalian preparation.
CHAPTER 8

SUMMARY DISCUSSION
In this thesis, I have attempted to take an integrated or multimodal approach to the study of avian central nervous system motor control mechanisms. This approach compares my results from selective lesion, brainstem electrical stimulation and brainstem neurochemical injection studies in birds with those from other vertebrates. Results from avian and vertebrate neuroanatomy, electrophysiology, immunohistochemistry and receptor autoradiography have been incorporated in an attempt to further characterize the neuroanatomical pathways involved in locomotor control in birds.

My previous studies included the use of low thoracic spinal cord lesions to examine two aspects of motor function in birds. The first was to corroborate the finding that pigeons with an isolated lumbosacral spinal cord could elicit 'spinal stepping' (ten Cate, 1960, 1962). This result was supported by the finding that following complete transection of the low thoracic spinal cord, geese demonstrated the ability to 'spinal step'. This stepping has been attributed to the presence of spinal cord locomotor pattern generators (LPG) that are capable of producing a rhythmic pattern in the absence of descending influences (Sholomenko and Steeves, 1987). Similar spinal LPGs have been found in virtually all vertebrates examined, possibly excluding primates (Eidelberg et al., 1981; Graham-Brown, 1911, 1914; Wetzel and Stuart, 1976; Eidelberg, 1981). As will be discussed below, the oscillators are modulated both by pathways descending from the brainstem (Kuypers, 1982) and by afferent peripheral information (Grillner, 1985; McClellan, 1986).
The second aspect of my study was to determine the major brainstem descending pathways which impinge on the LPGs and are responsible for the initiation and ongoing control of the rhythmic locomotor oscillations. Selective lesions of the avian low thoracic spinal cord demonstrated that the essential pathways arise from the brainstem reticular formation nuclei (Steeves et al., 1986; Sholomenko and Steeves, 1987). This finding, that the reticulospinal pathways are necessary for voluntary locomotor patterns, is the same as that found for all vertebrates studied (Lawrence and Kuypers, 1968a,b; Orlovsky and Shik, 1976; Eidelberg et al., 1981; Steeves and Jordan, 1984; Armstrong, 1986). Electrical stimulation and neurochemical injection (Chapters 2-5) studies were then undertaken and have provided additional, although necessarily incomplete, information regarding the important role of the reticular formation in locomotor control.

I have identified four avian brainstem reticular formation regions from which locomotion can be elicited by electrical stimulation (Steeves et al., 1986; Sholomenko and Steeves, 1987; Steeves et al., 1987). Effective locomotion-inducing stimulation sites were localized to the dorsal (Cnd) and ventral (Cnv) parts of the central medullary reticular nuclei, the ventral pontine reticular nucleus (RP) and the medial mesencephalic reticular formation (mMRF). Two of these regions, Cnd and Cnv, contribute substantially to the descending reticulospinal pathways, while RP and mMRF, structures which will be discussed subsequently, project only sparsely to the spinal cord (Cabot et al., 1982;
Webster and Steeves, 1988).

The view that neurons in Cnd and Cnv give rise to the final common brainstem-spinal locomotor pathway is supported by lines of evidence other than lesion and electrical stimulation data. First, Cnd and Cnv neurons retrogradely labelled from the spinal cord have been co-localized with locomotion-inducing stimulation sites in the same bird (Steeves et al., 1987). Second, neuroanatomical results demonstrate that many locomotion-related brainstem nuclei send projections to Cnd and Cnv, thus placing Cnd and Cnv in an ideal position to integrate and project motor information to the cord. The motor related nuclei projecting to Cnd/Cnv, many of which have been identified electrophysiologically, include the RP, mMRF, gigantocellular (Rgc) and parvocellular (Rpc) reticular formation, trigeminal descending tract and nucleus region (TTD), nucleus intercollicularis (ICo), tectum, archistriatum intermedium, cerebellum, Area ventralis of Tsai, red nucleus and nucleus raphe magnus (Webster, personal communication; Arends et al., 1984; Arends and Dubbeldam, 1984; Hunt and Kunzle, 1976; Wild, 1984; Wild et al., 1985; Reiner and Karten, 1982). Finally, direct intracerebral neurochemical microinjection of specific neurotransmitter agonists/antagonists into these regions can elicit or block locomotion (Chapters 3-5). Neurochemicals which were effective at eliciting locomotion from these regions include cholinergic agonists (Cnd & Cnv), GABAergic antagonists (Cnv only) and glutamatergic agonists (Cnd and Cnv).

The studies utilizing neurochemical injection were combined
with available anatomical, immunohistochemical and receptor autoradiographic data from the literature in an attempt to differentiate afferents to Cnd and Cnv which excite or inhibit these nuclei. My analysis revealed several potential sources of motor related cholinergic input to Cnd and Cnv. Possible cholinergic afferents to Cnd arise from the subtrigeminal nucleus, TTD, RP, laterodorsal tegmental nucleus, and nucleus isthmi, pars parvocellularis, while those to Cnv may arise from TTD, Rpc, Rgc and the nucleus mesencephalicus, pars profundus. In addition, both Cnd and Cnv contain intrinsic cholinergic neurons. While it is possible that TTD and other reticular formation (e.g. RP, Rgc, Rpc) structures give rise to the cholinergic motor connection, as electrical stimulation of these regions gives rise to locomotion, insufficient information is available to unequivocally identify any single pathway, or combination of pathways, as being responsible for my results. It is clear, however, from the effects of carbachol on locomotion and the ability of atropine, a muscarinic antagonist, to block the locomotor effects elicited by carbachol, that neurons in both Cnd and Cnv appear to be under cholinergic control via muscarinic receptors.

While cholinergic agonist injection elicits locomotion in both Cnv and Cnd, GABAergic antagonists induce locomotion only when injected into Cnv. Equally, GABA injection blocks locomotion only when infused into Cnv. It is likely, therefore, that neurons in Cnd and Cnv play slightly different roles in locomotor control, receiving and integrating input from
different parts of the neuraxis. The different neuroanatomical inputs to these nuclei (described, in part, above) and their different cytoarchitecture (Karten and Hodos, 1967) would suggest that this is the case.

Locomotion is also elicited through the infusion of the glutamatergic agonist NMDA into both Cnd and Cnv in the avian preparation. While glutamatergic input to both nuclei remains to be characterized in birds, in other species, reticulospinal neurons have been demonstrated to receive glutamatergic input from several sources. In lamprey, intrinsic reticular formation excitatory amino acid-containing interneurons, which themselves contain NMDA receptors, have been reported to receive trigeminal, vestibular and ascending spinobulbar input. The neurotransmitters utilized by the latter pathways are undetermined (Dubuc et al., 1988). However, the interneurons have been demonstrated to impinge on reticulospinal neurons (Dubuc et al., 1988). In mammals, descending excitatory amino acidergic (EAA) input to this region from telencephalic structures has been reported (Fagg and Foster, 1983). Also in mammals, neurochemical stimulation using glutamate has been shown to be effective at eliciting locomotion when infused into the reticular formation (Garcia-Rill and Skinner, 1987a; Noga et al., 1988). In birds, to my knowledge, there is no information about glutamatergic pathways or excitatory amino acid-containing neuronal elements. However, combined with the above data from a variety of studies, my results suggest an important excitatory role for glutamate in the control of locomotion-related
reticular formation structures.

The above findings for Cnd and Cnv, which are similar to those found for synonymous structures in other vertebrates (Grillner, 1976; Armstrong, 1986), suggest that reticulospinal neurons in Cnd and Cnv serve as the brainstem liaison to spinal cord LPGs. The higher levels of control, the neural structures which impinge on and modulate the reticular formation nuclei, have not been characterized to the same extent. However, several regions have been identified which may exert control over Cnd and Cnv (Chapters 2-7).

Two rostral reticular formation nuclei, RP and mMRF are likely candidates. Both RP and mMRF project mainly to Cnd, Cnv and only sparsely to the spinal cord (Webster, personal communication; Webster and Steeves, 1988). RP receives afferent input from several motor related sources including TTD, tectum, cerebellum, vestibular nuclei and Cnd/Cnv. From a neuroanatomical standpoint, therefore, RP is ideally situated to integrate a broad range of sensory information and influence downstream motor structures. Evidence that this region has a role in locomotor control comes from electrical stimulation studies which demonstrate that RP electrical stimulation elicits locomotion in birds (Chapters 1-5). Furthermore, locomotion can be evoked by the injection of GABAergic antagonists and the glutamate agonist NMDA, but not cholinergic agonists, into RP (Chapters 4,5). While it is not yet possible to determine the pathways through which these neurotransmitters exert their effects on RP, the data (see Chapters 1-5) suggest that RP, like
several other locomotor-related structures, appears to elicit locomotor behaviour mainly through its projections to Cnd and Cnv. The neurotransmitters which subserve the control also remain to be determined, although, from the Cnd/Cnv data above, acetylcholine and glutamate are possible candidates.

Similar to RP, the mMRF projects mainly to the brainstem reticular formation nuclei and only sparsely to the spinal cord. The mMRF has strong reciprocal connections with the deep tectal layers (intercollicular nucleus, ICo) which appear to form one link of the avian basal ganglia loop (Chapter 2). Electrical stimulation of the mMRF elicits locomotion in birds (Chapter 2). In addition, neurochemical injection studies (Chapters 4, 5) demonstrate that the electrical threshold for locomotion can be reduced by the infusion of picrotoxin and NMDA into this region. Based on the above considerations and those from mammalian studies (Garcia-Rill et al., 1985; Mogenson and Brudzynski, 1986; see Chapter 5), it appears possible that the mMRF may form one component of an avian equivalent to the mammalian medial MLR. A second component, potentially equivalent to the mammalian lateral MLR, is found in the region of the midbrain intercollicular nucleus (ICo).

ICo receives input from the spinal cord (Hunt and Kunzle, 1976; Webster and Steeves, in preparation), deep tectal layers, and cuneate and gracile nuclei (Wild et al., 1987). It projects to TTD, Rgc, Cnv and high cervical cord (Reiner and Karten, 1982; Webster and Steeves, in preparation). Electrical stimulation of this region (Chapter 2) elicits locomotion in
birds. Its connectivity suggests this nucleus may be equivalent to the mammalian cuneiform nucleus (Cabot et al., 1982), a region which has been implicated through electrical stimulation studies as the lateral MLR (Shik et al., 1967; Noga et al., 1988). The mammalian lateral MLR is believed to exert motor control effects via projections to the medullary reticular formation (Steeves and Jordan, 1984; Noga et al., 1988). Further evidence correlating ICo with the mammalian MLR arises from neurochemical infusions into this region which, as seen in cats, demonstrates that the GABAergic antagonist picrotoxin elicits locomotor behaviour (Chapter 4; Garcia-Rill et al., 1983). While these results are indicative of a correlation between the mammalian and avian MLRs, further testing is required before any firm equivalency can be established. Part of the study required to establish equivalency of the avian MLRs with their mammalian counterparts is to describe the nuclei at the next level of the hierarchy which control the activity of the mesencephalic regions. Transection studies have shown that nuclei in the mammalian caudal diencephalon are implicated in such control, as preservation of these nuclei after selective transection allows spontaneous locomotor activity in decerebrate preparations (for review, see Armstrong, 1986). The spontaneous activity is attributed to the control which the preserved nuclei exert over more caudal locomotion-related nuclei.

To determine whether such regions exist in birds, decerebrations were performed at varying levels of the neuraxis (Chapter 7). Rostral decerebrations which preserved a wedge of
neural tissue containing the subthalamic nucleus (nucleus of the ansa lenticularis), the lateral and posterior hypothalamic nuclei and the most rostral portion of the substantia nigra yielded spontaneous locomotion in birds. Ablation of this region resulted in birds which would locomote only with electrical or neurochemical brainstem stimulation. Previous studies in mammals have demonstrated that similar diencephalic structures (e.g. subthalamic nucleus, subthalamic locomotor region (SLR), zona incerta and lateral hypothalamic area) are preserved in spontaneous decerebrate (acute premammillary precollucular preparation) animals (Waller et al., 1940). Also in mammals, these structures appear to send projections to the more caudal locomotor regions (for review see Chapter 1). My results, therefore, suggest that counterparts of these mammalian diencephalic regions are also found in birds. Whether they serve as the locus through which the avian limbic system governs goal directed locomotor behaviours, as has been suggested for mammals (Mogenson et al., 1985), awaits further information.

My studies have revealed a novel locomotor region in birds which lies within the confines of the pontine and rostral medullary medial longitudinal fasciculus (MLF) (Chapter 2). Electrical stimulation of this region repeatably elicits locomotor patterns. Furthermore, the locomotion can be repeatably evoked by neurochemical infusion (carbachol and NMDA), providing conclusive evidence that neurotransmitter receptors (not en passant fibres) were stimulated to produce this effect (Goodchild et al., 1982). Serotoninergic cell
bodies, which stain positively for acetylcholinesterase (Dube and Parent, 1981; Taccogna et al., in preparation) and lie in close proximity to the injection site have been implicated in the locomotor response. These cells have been shown to project to the region of the midbrain pretectal nucleus near sites from which locomotion can be elicited (Dube and Parent, 1981). The role of these cells in locomotor control and the neuroanatomical substrate through which the control may be exerted, however, has yet to be determined.

Sensory information also appears to have a role in motor control. At least one locus for the integration of this sensory information may lie within the trigeminal descending tract and nucleus (TTD), a region which receives a variety of sensory, as well as central afferents and which, in turn, projects to the hindbrain reticular formation nuclei (Cnd & Cnv) (see Chapters 1, 3-5). My studies in birds, similar to those in many vertebrates (for review see McClellan, 1986), have demonstrated that electrical stimulation of the pontobulbar locomotor strip (PLS) elicits locomotion in birds. The electrically induced locomotion (walking at threshold) can be maintained over considerable periods (>10 minutes) (Steeves et al., 1987; Funk et al., submitted), thereby indicating that the locomotion is not simply a transitory escape behaviour. In birds, as in mammals, the stimulation locus lies within the TTD and strongly implicates this region as being synonymous with the PLS (Jordan, 1986; Garcia-Rill and Skinner, 1986; Noga et al., 1988)(Figs. 1, Chapters 3-5). Furthermore, locomotion can be evoked by the
introduction of a variety of neurochemicals (carbachol, picrotoxin and NMDA) into TTD.

As discussed in Chapter 3, intra-TTD cholinergic agonist injection elicited long lasting locomotion in birds. The most likely inputs for this cholinergic innervation arise from the pontine and medullary reticular formation, the descending vestibular nucleus, the nucleus raphe pallidus and the parabrachial region, while TTD itself encompasses CHAT-containing neurons.

Although several potential pathways for cholinergic control of TTD have been postulated, the locomotor effects produced with injection of GABAergic and glutamatergic antagonists/agonists remain without a strong neuroanatomical substrate (Chapters 4,5). GABA-containing cell bodies and receptors have been localized to TTD in mammals (Mugnaini and Oertel, 1985), suggesting that GABA plays some role, possibly selectively down-regulating afferent information, in locomotor control via TTD. A role for glutamate, possibly antagonistic to that of GABA, may also be postulated (Chapter 5).

The above results in birds, taken together with those from other vertebrates (Grillner, 1976; Armstrong, 1986; Garcia-Rill and Skinner, 1986; Jordan, 1986; Noga et al., 1988), suggest that the PLS and TTD region are equivalent. Further, its neuroanatomical connections suggest that the TTD region is intimately associated with a variety of sensory inputs and may therefore serve as an integratory centre through which sensory information affects ongoing locomotor output. This hypothesis
supports the hypothesis of Noga et al. (1988), which states that PLS/trigeminal/lateral reticular formation system "provides a substrate for sensorimotor reflex initiation of locomotion". Further support for and extension of this hypothesis comes from a study designed to determine whether phasic peripheral afferent information is essential for the production of locomotor patterns in birds.

In the study (Chapter 6), I examined the role of phasic afferent feedback by comparing the locomotor patterns of animals prior to and following paralyzation. My results indicate that afferent input is not essential for the production of the wide array of avian locomotor patterns in both high decerebrate spontaneously locomoting or in stimulated (chemical and electrical stimulation) paralyzed animals. One of the results of the study was that locomotion could be elicited by trigeminal field stimulation in birds which previous to paralyzation were spontaneous, but after paralyzation did not show any spontaneous activity. This finding, together with the result that increased stimulation intensity was necessary to initiate locomotion after paralyzation in low decerebrate animals, suggests that afferent input may not only initiate reflex locomotion, but may also serve to set the animal's overall activation level for locomotion. If peripheral afferent input serves to set the activation level, TTD may well serve as the integratory centre for the information.

The results of studies presented in this thesis strongly suggest that birds, like mammals (Armstrong, 1986), possess a
hierarchical system of locomotor control. The lowest level of the hierarchy is found in the spinal cord LPG. The LPG appears to be controlled both by afferent input and, more importantly for voluntary locomotion, via reticulospinal input from the brainstem reticular formation nuclei (Cnd & Cnv). In turn, control is exerted on the reticulospinal neurons by sensory (e.g. via TTD) and centrally generated input (e.g. RP, ICo, mMRF,). Central structures themselves (e.g. subthalamic region, basal ganglia) appear to be under higher levels of control from limbic and telencephalic regions.

The general impression of my studies strongly suggests that avian locomotion-related structures and their interconnections are similar to those found in both higher and lower vertebrates. This similarity is discussed throughout the thesis (Chapters 1-7). While the neural circuitry which comprises the hierarchy appears to have been highly conserved during the evolutionary process, considerably more information is required for the understanding of neural locomotor control mechanisms.

However, in light of the currently available techniques, I believe that the study of motor control has reached a point from which it will now be possible to define and manipulate all of the major neural pathways involved in locomotor control. Techniques such as neuroanatomical tracing, both retrograde and anterograde, make it possible to define effectively the afferent and efferent connections of every brain region (Steeves and Jordan, 1984; Garcia-Rill et al., 1983a; Webster and Steeves, 1988). Immunohistochemistry allows description of some of the
neurotransmitters utilized by these pathways (Taccogna et al., in preparation). Receptor autoradiography defines some of the neurotransmitter receptors present both pre- and postsynaptically (Dietl et al., 1988). Electrophysiology allows us to record from individual neurons to examine their activity (Orlovsky, 1970a, 1972b,c; Garcia-Rill and Skinner, 1988) and in vivo microdialysis (Phillips et al., 1988) determines changes in neurotransmitter concentrations from specific regions during activity. Finally, selective intracerebral neurochemical infusion of neurotransmitter agonists and antagonists allows us to manipulate the process (Chapters 3-5; Garcia-Rill et al., 1985; Noga et al., 1988). While these techniques, though powerful, allow for the definition and manipulation of locomotor pathways, only the integration of information gathered from these techniques will increase the understanding of locomotor control and have predictive value for future studies. In this thesis, I have attempted to utilize this integrated approach in the study of avian locomotion.

The attributes which the bird possesses, including its bipedal locomotion, two separate modes of locomotion and absence of a corticospinal tract make the bird an excellent animal model for the study of many aspects of locomotion.
CHAPTER 9

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## APPENDIX I

### Neurochemical Injection Parameters for Altering Locomotion

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<th>Neurochemical</th>
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<th>Mouse</th>
<th>Rat</th>
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<td>1.5</td>
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<td>4.7</td>
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<td>4.7</td>
<td>4.7</td>
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<td>4.7</td>
<td>4.7</td>
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<td>4.7</td>
<td>4.7</td>
</tr>
<tr>
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<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
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<td>1.5</td>
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### Time Course (min)

- **Cat**: 1-5, 10-30, 1-5, 10-30, 1-5, 10-30
- **Mouse**: 1-5, 10-30, 1-5, 10-30, 1-5, 10-30
- **Rat**: 1-5, 10-30, 1-5, 10-30, 1-5, 10-30

### Concentration

- **Cat**: 0.01-0.1 M (RT), 0.1-1 M (NR)
- **Mouse**: 0.01-0.1 M (RT), 0.1-1 M (NR)
- **Rat**: 0.01-0.1 M (RT), 0.1-1 M (NR)

### Volume

- **Cat**: 0.1-1 ul
- **Mouse**: 0.1-1 ul
- **Rat**: 0.1-1 ul

### Rate

- **Cat**: 0.1-1 ul/min
- **Mouse**: 0.1-1 ul/min
- **Rat**: 0.1-1 ul/min

### Effective Concentration

- **Cat**: 0.1-1 M (RT), 0.1-1 M (NR)
- **Mouse**: 0.1-1 M (RT), 0.1-1 M (NR)
- **Rat**: 0.1-1 M (RT), 0.1-1 M (NR)

### Note

- See above results.
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<tr>
<th>Experimenters Animal</th>
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<th>Chemical</th>
<th>pH</th>
<th>Concentrations Injected</th>
<th>Lowest Effective Concentration</th>
<th>Volume</th>
<th>Rate</th>
<th>Time Course (min)</th>
<th>Latency Period</th>
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**GABAergic agonists and antagonists**

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**Excitatory Amino Acids and Substance P**

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<td>&quot;</td>
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292
ABBREVIATIONS: Appendix 1

AH — anterior hypothalamus
C — convulsant
Cnd — dorsal part, medullary central nucleus
Cnv — ventral part, medullary central nucleus
h — hours
I — irreversible
irr. block — irreversible block
LPA — lateral preoptic area
MLF — medial longitudinal fasciculus
mMLR — medial mesencephalic locomotor region (see PPN)
mrf — medial reticular formation (medulla)
MRF — mesencephalic reticular formation
na — not available
N.Acc — nucleus accumbens
NMC — magnocellular reticular formation
NPM — paramedian nucleus
NR — no repeatable response
NRG — gigantocellular reticular formation
NRV — ventral reticular formation
PLS — pontobulbar locomotor strip — equivalent to the descending trigeminal tract and nucleus together with the adjacent parvocellular reticular formation
PPN — pedunculopontine nucleus (see mMLR)
RP — pontine reticular nucleus
RT — reduced threshold for electrically stimulated locomotion
s — seconds
SLR — subthalamic locomotor region
TTD — descending trigeminal tract and nucleus
## APPENDIX II

### RESPIRATORY AND CARDIOVASCULAR VALUES IN DECREBERATE AND INTACT CANADA GEESE

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<tr>
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<th>DECREBERATE</th>
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<td>Rest n</td>
<td>Exercise</td>
<td>%Change n</td>
<td>Rest n</td>
<td>Exercise</td>
<td>%Change n</td>
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<td>$V_E$ (ml/min/Kg)</td>
<td>314±28 13</td>
<td>448±27 # 13</td>
<td>42.2 13</td>
<td>417±30 21</td>
<td>687±65 #* 119</td>
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<tr>
<td>$V_T$ (ml/Kg)</td>
<td>29.9±1.5 13</td>
<td>34.1±2.3 13</td>
<td>14.6 13</td>
<td>29.2±1.3 21</td>
<td>26.5±2.4 -4.6</td>
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<td>$f_v$ (min⁻¹)</td>
<td>10.7±1.0 13</td>
<td>13.4±0.8 13</td>
<td>25.2 13</td>
<td>14.8±0.80 21</td>
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<tr>
<td>$f_s$ (min⁻¹)</td>
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<td>57.5±5.0  --</td>
<td>--</td>
<td>13 --</td>
<td>55.2±2.3 --</td>
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<td>$V_{O2}$ (ml/min/Kg)</td>
<td>11.3±0.8 8</td>
<td>15.9±1.1 # 8</td>
<td>48.2 8</td>
<td>13.5±1.5 9</td>
<td>32.7±3.8 #* 142</td>
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<td>$V_{CO2}$ (ml/min/Kg)</td>
<td>7.6±0.7 8</td>
<td>12.6±0.6 # 8</td>
<td>66.3 8</td>
<td>13.1±1.3 * 8</td>
<td>29.9±3.8 #* 126</td>
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<td>$R$</td>
<td>0.67±0.03 8</td>
<td>0.8±0.03 # 9</td>
<td>-- 9</td>
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<td>0.81±0.03 --</td>
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<td>$T_b$ (°C)</td>
<td>40.4±0.7 5</td>
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<td>42.3 2</td>
<td>42.7 --</td>
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<tr>
<td>Ent %</td>
<td>--</td>
<td>28.9±3.3 --</td>
<td>-- 9</td>
<td>-- --</td>
<td>28.3±4.0 --</td>
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<td>Heart rate (min⁻¹)</td>
<td>198±24 12</td>
<td>207±21.5 12</td>
<td>4.5 12</td>
<td>284±28 4</td>
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<td>B.P.</td>
<td>89±4.0 4</td>
<td>108.3±6.5 4</td>
<td>21.7 4</td>
<td>164±7 3</td>
<td>162±8 # 11.0</td>
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</table>

Slope $\dot{V}_E/\dot{V}_{O2}$ 24.43 (R = 0.787) 18.48 (R = 0.985)

Slope $\dot{V}_E/\dot{V}_{CO2}$ 24.74 (R = 0.988) 18.67 (R = 0.982)

### Blood Gases

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<td>$P_{O2}$ (mmHg)</td>
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<td>94.9±7.3 8</td>
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<td>$P_{CO2}$ (mmHg)</td>
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<td>23.9±0.9 12</td>
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<td>27.5 1</td>
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<td>7.52 1</td>
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Appendix II: Respiratory values in decerebrate and intact Canada geese recorded prior to
and over the last 4 minutes of a 10 minute (decerebrate) and 6 minute (intact) walking period.

(mean ± S.E.)

n = number of experiments

% Change; percent change from rest to exercise.

#; significant difference between rest and exercise within a group.

*; significant difference between the two groups during rest and exercise.

$V_E$: minute ventilation

$V_T$: tidal volume

$f_v$: breathing frequency

$f_s$: stride frequency

$V_{CO2}$: CO2 production

$V_{O2}$: O2 Production

$T_b$: body temperature.

R: Pearson's Correlation Coefficient

#; not tested for significant difference