2. EVIDENCE OF LOCAL CONNECTIVITY WITHIN THE GP

The results of early Golgi-impregnation studies in the mouse, rat and primate demonstrated the presence of local axon collaterals arising from neurons of the GP (Iwahori and Mizuno, 1981; Francois et al., 1984; Millhouse, 1986). Similarly, intracellular labelling, juxtacellular labelling, single-axon tracing studies (Park et al., 1982; Kita and Kitai, 1994; Bevan et al., 1998; Sato et al., 2000) and immunohistochemical labelling techniques (Kita, 1994) have revealed the presence of local axon collaterals of GP neurons that give rise to terminal arborisations and boutons that appose unlabelled structures, some of which could be identified as perikarya. Whilst these studies have been instrumental in qualitatively characterising the local axon collaterals of GP neurons, a quantitative characterisation is critical in defining their role within the GP.

We have performed a detailed single-cell juxtacellular labelling study of GP neurons and their local collaterals in the rat (Sadek et al., 2003, 2004; Sadek, Magill and Bolam, 2005 *in preparation*). Following the juxtacellular labelling and recovery of the neurons we digitally reconstructed them in three dimensions using the Neurolucida system, obtained quantitative anatomical data and performed an electron microscopic analysis of their local collaterals. These analyses revealed that all GP neurons possess organised and extensive local axonal arborisations, but may be divided into two groups on the basis of their location within the GP and the number of boutons contained within their local axonal arborisations. One group is located in a band apposing the striatum (within ~100 μm of the striatal border) i.e., the lateral and rostral poles of the GP (hereafter referred to as 'lateral neurons'). The second group is located in the more medial and caudal aspects of the GP (hereafter referred to as 'medial neurons'). Neurons in both groups possess extensive local axonal arborisations and there is a correlation between the location of the neurons and the numbers of boutons in the arborisations, such that lateral neurons possess an average of 264 boutons whereas medial neurons possess an average of 581 boutons (Table 1). The local axonal arborisations are not homogeneously distributed within the GP but are divided into sub-regions that are located at different positions with respect to the parent cell body. The axons of both groups possess an arborisation located within the parent dendritic arborisation (hereafter referred to as the *proximal arborisation*) and an arborisation that is located caudo-ventro-medial to the soma (hereafter referred to as the *distal arborisation*), along the course taken by the striatofugal fibre bundles passing through the GP. Medial neurons also possess an intermediate axonal arborisation located within or very close to the dendritic arborisation, but it will be considered together with the proximal arborisation for the purposes of this discussion. The numbers of boutons contained within the different arborisations are summarised in Table 1.

Electron microscopic analysis of the local axonal arborisations revealed that the boutons identified at the light microscopic level, are indeed synaptic boutons, forming symmetrical synapses with the dendrites and perikarya of other GP neurons. This analysis revealed that the majority of terminals form synaptic contact with perikarya and proximal (first order) dendrites (61% for lateral neurons and 72% for medial neurons). This is consistent with the light microscopic observations because boutons of the local arbours are often seen closely associated with unstained perikarya and large diameter proximal dendrites. Thus, like the synapses formed by GP neurons in other regions of the basal ganglia, local axon collaterals predominantly make contact with such proximal

domains of neighbouring GP neurons. The remainder of the synapses are formed at a greater distance from the perikaryon i.e., on higher-order dendrites.

3. QUANTITATIVE ESTIMATES OF LOCAL CONNECTIVITY

With knowledge of the numbers of axon terminals in a local axonal arborisation, and knowledge of their synaptic targets and pattern of innervation, we can make estimates of the degree of connectivity between GP neurons. However, certain assumptions have to be made.

3.1. Assumptions

3.1.1. Number of contacts formed by a single GP local axon on a single postsynaptic neuron

The facts that lateral neurons possess a mean of 264 boutons in their axonal arbour and medial neurons possess a mean of 581 boutons indicate that the maximum number of neurons that each cell type could contact is 264 and 581, respectively, if their local axon collaterals give rise to only a single synapse per target neuron. However, this is clearly not the case because both light and electron microscopic analyses indicate that single GP axons make multiple appositions or synapses with their targets (Park et al., 1982; Kita, 1994; Sadek et al., 2003, 2004; Sadek, Magill and Bolam, 2005 *in preparation*). We frequently observe single axons making multiple appositions to the proximal domains of target neurons at the light microscopic level and on higher order dendrites in the electron microscope. The maximum number of boutons from a single axon apposed to the proximal domains (defined as the primary dendrites and soma) of a single target neuron that we have observed is 14, of which 10 have been shown to make synapses (Sadek et al., 2003, 2004; Sadek, Magill and Bolam, 2005 *in preparation*). An earlier electron microscopic study identified that a single axon can form up to 9 boutons onto a single dendrite (Park et al., 1982). We thus make the assumption that the maximum numbers of synapses formed by a single axon on the proximal domains and higher-order dendrites of a single postsynaptic neuron are 14 and 9, respectively. It is difficult to define a hard figure for the minimum number of contacts formed by a single axon with a single postsynaptic neuron without complete reconstruction and identification of the postsynaptic structures of individual labelled axons. From our light and electron microscopic analyses we estimate that an individual axon will make a minimum of 3 synapses with an individual postsynaptic neuron. This assumption is not without at least a degree of risk. It could perhaps be argued that the most conservative minimum estimate is that an individual axon only makes a single synaptic contact with any other neuron. However, we consider this conservative estimate to be too low for the reasons stated above, and because an axon may contact more that one dendrite of a postsynaptic neuron. We also assume that the connectivity is the same for the two classes of neurons.

3.1.2. Volume of GP occupied by proximal axonal arborisation

Previous studies have touched upon the issue of finding an accurate method of elucidating the volume of an axonal arborisation (Kincaid et al., 1998; Zheng and

Wilson, 2002). This is not the physical volume of the axonal arborisation, but rather the volume of tissue in which it can potentially make contacts. The best analogy to describe this is the volume enclosed by a net thrown over the branches of a tree. The total volume of the branches themselves is not of interest here, but rather the empty volume enclosed by the net is most instructive; the latter could be equivalent to the volume of GP innervated by a single axonal arbour. The volume of the axonal arborisation, or the bouton cloud, is difficult to obtain without mathematical cluster analysis, as it depends on the spatial distribution of boutons. However, we know that the proximal axonal arborisations of GP neurons are located within the volume of GP occupied by the dendritic arborisations. Because of the characteristic shape of the dendritic arborisations of GP neurons we can estimate the volume of GP that the dendrites occupy by approximating their shape to either a rectangle or circle for lateral and medial neurons, respectively, and assigning a depth. This is an approximation of the volume occupied by the principal plane of each neuronal type. Estimates made from 5 neurons of each type (Sadek, Magill and Bolam, 2005 *in preparation*) give the mean volume occupied by a lateral neuron as 0.015 mm^3 (s.d. = 0.002 mm^3) and that of a medial neuron as 0.028 mm^3 (s.d. = 0.003 mm^3). Of course, the axonal arborisation does not occupy the *whole* volume occupied by the dendritic arborisation; we estimate that the axonal arborisation occupies 20% of that volume occupied by the dendritic tree. Thus, any GP neuron within this volume is a potential target of the axonal arborisation.

3.1.3. Number of neurons in the volume occupied by the proximal axonal arborisations

The distribution of the 45,960 neurons within the rat GP (Oorschot, 1996) is not homogeneous (Kita and Kita, 2001). The neuronal density in the lateral regions of the GP is lower than that in the more medial regions. The lateral regions contain 15,264 neurons/mm^3 whereas the medial regions contain 18,708 neurons/mm^3 (Kita and Kita, 2001). On the basis of these figures, the number of neurons with somata located within the dendritic arborisation of a lateral neuron is 229, and that number within the dendritic arborisation of a medial neuron is 523. Because we estimate that the proximal axonal arborisation probably only occupies about 20% of this volume (see section 3.1.2), the maximum number of neurons that an average lateral and an average medial neuron may contact through their proximal axonal arborisation is 46 and 105, respectively. This figure of course does not take into account neurons with cell bodies located outside of this region but whose dendrites course through the arborisation.

3.1.4. Volume of GP occupied by the distal axonal arborisations

The spatial organisation of the distal axonal arborisation of both lateral and medial neurons is not as well defined as that of the proximal axonal arborisation and is not contained within the dendritic arborisation. Furthermore, the shape of the arborisation is qualitatively different between neurons of both groups. We have thus not attempted at this stage to make estimates of its volume.

3.2. Connectivity of the *Proximal Arborisation* of Lateral Neurons

On average, the proximal axonal arborisation of a lateral neuron possesses 166 boutons, of which 133 (80%) form contacts onto the proximal domains of other GP

neurons and 33 form contacts onto higher-order dendrites (Table 1; Sadek, 2004; Sadek, Magill and Bolam, 2005 *in preparation*). Based on the assumptions described above we estimate that the proximal arborisation of a lateral neuron may contact between 10 and 44 neurons in their proximal domains, and between 4 and 11 neurons on their higher-order dendrites. Thus, taking into account both proximal and distal dendritic contacts, lateral neurons are in a position to form synapses with 14-55 neurons through their proximal axonal arborisation. This represents between 30% and 100% of the neurons within the volume occupied by the proximal axonal arborisation and between 6 and 24% of the neurons located within the dendritic arborisation (see Table 1 for calculations).

3.3. Connectivity of the *Proximal Arborisation* of Medial Neurons

For medial neurons, the proximal axonal arborisation possesses an average of 447 boutons, of which 215 (48%) form synapses with the proximal domains of other medial GP neurons and 232 with higher-order dendrites (Table 1). Hence between 15 and 72 neurons may be contacted in their proximal domains by the proximal axonal arborisation of a medial neuron and 26 to 77 neurons on their higher-order dendrites. Thus the total number of neurons the proximal axonal arborisation of a medial neuron may contact is in the range of 41 to 149. This represents between 39% and 100% of the neurons within the volume occupied by the proximal axonal arborisation of a medial neuron and 8 to 28% of the neurons within the dendritic arborisation (see Table 1 for calculations).

3.4. Connectivity of the *Distal Arborisation* of Lateral Neurons

Of the mean of 98 boutons contained within the distal axonal arborisation of a lateral neuron, 63 (64%) make synaptic contact with the proximal domains of other GP neurons and the remaining 35 make contact with higher-order dendrites. Boutons forming contacts with the proximal domains of neighbouring neurons could innervate 5 to 21 different neurons, whereas boutons in contact with distal dendrites may innervate another 4 to 12 neurons. Thus the distal axonal arborisation of a lateral neuron may contact between 9 to 33 (medial) neurons located caudo-ventro-medially in the GP (see Table 1 for calculations).

3.5. Connectivity of the *Distal Arborisation* of Medial Neurons

The distal axonal arborisation of medial neurons give rise on average to 134 boutons, 99 (74%) of which make contact proximal domains of their targets and 35 (26%) with higher-order dendrites. The boutons in the distal arborisation may thus contact 7 to 33 neurons in their proximal domains and 4 to 12 neurons at the level of higher-order dendrites. Thus, the distal axonal arborisation of a medial neuron is in a position to contact 11 to 45 other medial neurons (see Table 1 for calculations).

4. SUMMARY AND CONCLUDING REMARKS

- All GP neurons give rise to extensive and complex local axonal arborisations.
- There are quantitative differences between the local axonal arborisations of lateral

Table 1 Quantitative model of connectivity between GP neurons

Lateral Neurons	
1. Mean number of boutons in the local axonal arborisation	264
2. Mean number of boutons in the proximal axonal arborisation	166 (63%)
3. Mean number of boutons in the distal axonal arborisation	98 (37%)
4. Mean number of contacts with proximal domains formed by the proximal arborisation	133 (80%)
5. Mean number of contacts with distal dendrites formed by the proximal arborisation	33 (20%)
6. Maximum number of boutons from one GP neuron contacting the dendrite of another GP neuron (Park et al., 1982)	9
7. Maximum number of boutons from one GP neuron contacting the proximal domain of another GP neuron	14
8. Numbers of neurons contacted in their proximal domains by the proximal arborisation of a lateral neuron (i.e. minimum: #4 divided by #7; maximum: #4 divided 3)	10-44
9. Number of neurons receiving contacts onto their dendrites from the proximal arborisation of a lateral neuron (minimum: #5 divided by #6; maximum: #5 divided by 3)	4-11
10. Theoretical total number of GP neurons innervated by the proximal arborisation of a lateral neuron (sum of maxima and minima in #8 and #9)	14-55
11. Mean number of contacts with proximal domains formed by the distal arborisation	63 (64%)
12. Mean number of contacts with dendrites formed by the distal arborisation	35 (36%)
13. Numbers of neurons receiving contacts onto proximal domains formed by the distal arborisation of a lateral neuron (minimum: #11 divided by #7; maximum: #11 divided by 3)	5-21
14. Numbers of neurons receiving contacts onto their dendrites from the distal arborisation of one lateral neuron (minimum: #12 divided by #6; maximum: #12 divided by 3)	4-12
15. Theoretical total number of GP neurons innervated by the distal arborisation of one lateral neuron (sum of maxima and minima in #13 and #14)	9-33
16. Total number of neurons a single lateral neuron may contact through its local axonal collaterals (sum of maxima and minima in # 10 and #15)	**23-88**
Medial Neurons	
18. Mean number of boutons in the local axonal arborisation	581
19. Mean number of boutons in the proximal axonal arborisation	447 (77%)
20. Mean number of boutons in the distal axonal arborisation	134 (33%)
21. Mean number of contacts with proximal domains formed by the proximal arborisation	215 (48%)
22. Mean number of contacts with distal dendrites formed by the proximal arborisation	232 (52%)
23. Numbers of neurons contacted in their proximal domains by the proximal arborisation of a medial neuron (i.e. minimum: #21 divided by #7; maximum: #21 divided by 3)	15-72
24. Number of neurons receiving contacts onto their dendrites from the proximal arborisation of a medial neuron (minimum: #22 divided by #6; maximum: #22 divided by 3)	26-77
25. Theoretical total number of GP neurons innervated by the proximal arborisation of one medial neuron (sum of maxima and minima in #23 and #24)	41-149
26. Mean number of contacts with proximal domains formed by the distal arborisation	99 (74%)
27. Mean number of contacts with dendrites formed by the distal arborisation	35 (26%)
28 Numbers of neurons receiving contacts onto proximal domains formed by the distal arborisation of a medial neuron (minimum: #26 divided by #7 and a maximum: #26 divided by 3)	7-33
29. Number of neurons receiving contacts onto their dendrites from the distal collateral of one medial neuron (minimum: #27 divided by #6; maximum: #27 /3)	4-12
30. Theoretical total number of GP neurons innervated by the distal collateral of one medial neuron (sum of maxima and minima in #28 and #29)	11-45
32. Total number of neurons a single medial neuron may contact through its local axonal collaterals (sum of maxima and minima in #25 and #30)	**52-194**

and medial neurons.

- The local axonal arbours of lateral neurons are in a position to contact between 23 and 88 other GP neurons when considering the arborisation as a whole. Their proximal arborisations are in a position to contact between 14 and 55 neurons, which accounts for between 30% and 100% of the neurons with somata located inside the volume occupied by that part of the arborisation.

- The local axonal arbours of medial neurons are in a position to contact between 52 and 194 other GP neurons when considering the arborisation as a whole. Their proximal arborisations are in a position to contact between 41 and 149 neurons i.e. between 39% and 100% of the neurons with somata located within the volume occupied by that part of the arborisation.

- These findings predict that for a neuron in the lateral GP there is a 6-24% chance of it being connected to another GP neuron within its dendritic arborisation and this figure rises to 30-100% chance for neurons located within the volume occupied by its proximal axonal arborisation. Similarly, for a neuron in the medial GP there is a 8-28% chance of it being connected to another GP neuron within its dendritic arborisation and this figure rises to 39-100% chance for neurons located within the volume occupied by its proximal axonal arborisation

- Since the average number of boutons in the proximal arborisation of a lateral GP neuron is 166, then, if we assume that connectivity is homogeneous across the GP (which of course may not be the case), a lateral GP neuron will *receive* an average of 166 synaptic boutons from neighbouring GP neurons.

- Similarly, because the average number of boutons in the proximal arborisation of a medial GP neuron is 447, a medial GP neuron will thus *receive* an average of 447 synaptic boutons from neighbouring GP neurons *plus* any additional boutons derived from the distal arborisation of lateral neurons.

- The degree of convergence of local axons of GP neurons onto other GP neurons is given by the number of neurons that are estimated to be contacted within an arborisation. Thus lateral neurons will receive convergent input from 14 to 55 other GP neurons. Medial neurons on the other hand, will receive convergent input from 41 to 149 other GP neurons *plus* any additional convergence carried by the distal arborisations of later neurons.

- The complex local circuitry, the quantitative and qualitative differences in the connectivity of lateral and medial neurons, and the rostro-lateral to caudo-medial organisation of the distal axonal arborisations implies complex information processing within the GP. These data, together with the known connections of individual GP neurons with all other regions of the basal ganglia, suggest that the GP should not be considered as an homogeneous relay nucleus transmitting striatal information to the subthalamic nucleus and basal ganglia output nuclei, but rather a nucleus that is involved in complex information processing within its borders and the spatio-temporal selection of neurons at every level of the basal ganglia.

- These data call for the re-examination and redefinition of current models of GP, and the basal ganglia in general, to incorporate intra-pallidal connectivity.

- Finally, it should be emphasised that the quantitative features of connectivity that we calculate are necessarily based upon many assumptions. These assumptions may ultimately prove to be inadequate or inaccurate. With the availability of new data in the future, the calculations presented here can be revised and incorporated into new and more precise models of the local connectivity of GP neurons.

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