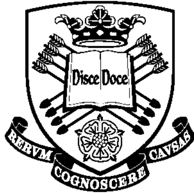

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Cortical and subcortical somatosensory regulation of dopaminergic neurons: role of the superior colliculus

The University of Sheffield

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A thesis submitted for the Degree of Doctor of Philosophy

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'You', your joys and your sorrows, your memories and your ambitions, your sense of personal identity and free will, are in fact no more than the behaviour of a vast assembly of nerve cells and their associated molecules"

Francis Crick, The astonishing hypothesis: The scientific search for the soul

Cortical and subcortical somatosensory regulation of dopaminergic neurons: role of the superior colliculus

Abstract

Dopaminergic (DA) neurons exhibit a short-latency, phasic response to unexpected biologically salient stimuli, including rewards. Despite extensive research on this DA signal, very little is known about the sources of sensory information reaching DA neurons. Previous research has identified the superior colliculus (SC) as the primary, if not exclusive route of short latency visual input to DA neurons. However, more recent research has suggested that the phasic DA response comprises two components; a short latency (50-110 ms), stimulus insensitive component, and a longer latency component (110-260 ms) that can reflect complex stimulus characteristics including reward value – more complex than might arise from intrinsic collicular processing. A solution to this apparent paradox may be suggested by recent studies that have demonstrated longer latency colour related responses in SC neurons. As the SC does not receive direct retinal input from colour sensitive cells, but it does receive input from a wide range of cortical structures, it is possible that cortical activation might underlie longer latency responses in the SC, which may in turn underlie longer latency responses in DA neurons. The aim of the research presented in this thesis was to investigate whether the cortex was capable of modulating the activity of DA neurons, and whether the SC was the relay for this cortical influence. In the anaesthetised rat, single pulse electrical stimulation of the barrel field of the primary somatosensory cortex (S1Bf) produced a short latency, short duration response in the SC, but DA neurons were largely insensitive to the stimulus. After disinhibition of the SC with the $GABA_A$ antagonist bicuculline, responses in the SC to S1Bf stimulation were enhanced, and DA neurons became responsive to S1Bf stimulation, suggesting that the SC is the route of cortical input to DA neurons. This was confirmed in the subsequent experiment. Responses were produced in DA neurons without the need for SC disinhibition by stimulating S1Bf with a high frequency train of pulses. This response in DA neurons was suppressed or eliminated by suppressing SC activity. Finally, the contribution of cortical and subcortical input to DA neuron responses was examined by stimulating the trigeminal nucleus. Trigeminal stimulation produced responses in the SC comparable to multiwhisker deflection, and produced responses in almost all DA neurons. Disinhibition of the SC differentially modulated phases of the SC response previously demonstrated to be produced by trigeminal and cortical input, and differential changes were seen in initial and later components of DA neuron responses, which were often associated with changes in the SC response. The results of these studies suggest that cortical inputs to the SC could provide a mechanism through which responses are produced in DA neurons that can reflect complex stimulus attributes. However, research in this thesis and elsewhere suggests that the activity of DA neurons is insufficiently discriminatory to reflect the full range of potentially rewarding stimuli, and hence it is suggested that DA neurons provide a salience signal, which can be biased by a pre-saccadic estimate of previously established reward value, but which does not communicate reward value per se.

Craig Alexander Bertram
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Thesis Contents

Title page i

Acknowledgements ii

Abstract iii

Thesis Contents iv

1 Introduction 1

1.1 Chapter Summary 1

1.2 The midbrain dopaminergic systems 1

1.3 The function of dopamine 2

1.3.1 Activity of DA neurons 3

1.4 Proposed functions of the phasic dopamine response 4

1.4.1 Reward prediction error hypothesis 4

1.4.2 Identifying the function of dopamine from its sensory inputs 6

1.4.3 Determining action-outcome associations of unexpected events 8

1.5 Two components of the phasic DA signal 10

1.5.1 Determining the source of longer latency input to the DA signal 12

1.6 The superior colliculus and its cortical afferent connections 12

1.6.1 Anatomy of the superior colliculus 12

1.6.2 Primacy of sensory corticotectal projections 14

1.6.3 Visual cortex 14

1.6.4 Auditory cortex 15

1.6.5 Somatosensory cortex 16

1.7 Whisker pathway as a useful investigatory tool 18

1.7.1 Trigeminal connectivity and anatomy 18

1.7.2 Differentiating direct and indirect somatosensory input 19

1.8 Rationale of identifying cortical input to DA neurons 20

1.9 Overview of the thesis 21

2 Materials and methods 22

2.1 Chapter summary 22

2.2 Electrode and cannula construction 22

2.2.1 Construction of glass microelectrode 22

2.2.2 Construction of multiunit electrode-cannula assembly 22

2.3 Experimental design 23

2.4 Subject preparation and surgical procedures 24
2.5 Implantation of electrodes 25
2.5.1 Stimulus generation 26
2.6 Experimental procedures 26
2.6.1 Identification of putative dopamine cells 26
2.6.2 Experimental procedure 27
2.7 Histological techniques 27
2.8 Data analysis 28
2.8.1 SC processing 28
2.8.2 SC analysis 30
2.8.3 DA processing 33
2.8.4 DA neuron waveform measurement 34
2.8.5 DA analysis 34
3 The effects of disinhibition of the superior colliculus on the responsiveness of dopaminergic neurons to stimulation of the barrel cortex 36
3.1 Chapter summary 36
3.2 Introduction 36
3.2.1 Tonic changes in DA activity in response to stimuli 36
3.2.2 Phasic changes in DA activity in response non-noxious stimuli 37
3.2.3 Changes in DA activity in response to aversive stimuli 38
3.2.4 The SC as a blocked route of sensory input in the anaesthetised rat 39
3.3 Experiment rationale 39
3.4 Method 40
3.4.1 Experimental procedure 40
3.4.2 Data analysis 41
3.4.3 Optical imaging spectroscopy procedure 42
3.4.4 OIS Data analysis 44
3.5 Results 47
3.5.1 Inclusion criteria 47
3.5.2 Activity in the superior colliculus 52
3.5.3 Activity of DA cells unresponsive until BMI injection 55
3.5.4 Differentiating between inhibitory and excitatory responses 56
3.5.5 Coincident spontaneous bursting in SC and DA 58
3.5.6 Dopaminergic response to non-reinforced, familiar stimuli 59

3.5.7 Activity of DA neurons responding before BMI injection ... 60
3.5.8 Effect of interleaved stimulation on response ... 61
3.5.9 Optical Imaging ... 62
3.6 Discussion ... 62
3.6.1 Summary of findings ... 62
3.6.2 Discussion of findings ... 63
3.6.3 Remaining questions ... 68
4 The effects of collicular suppression by injection of muscimol on the responsiveness of dopaminergic neurons to stimulation of barrel cortex with pulse trains ... 69
4.1 Chapter summary ... 69
4.2 Introduction ... 69
4.2.1 Stimulation of the SC can induce cortical desynchronisation ... 70
4.2.2 Producing a response in DA cells without BMI ... 70
4.2.3 Suppressing SC activity ... 71
4.2.4 Experiment rationale ... 73
4.3 Method ... 73
4.3.1 Experimental procedure ... 73
4.3.2 Data analysis ... 75
4.3.3 Optical imaging spectroscopy ... 77
4.4 Results ... 77
4.4.1 Inclusion criteria ... 77
4.4.2 Eliminating habituation as an alternative explanation ... 80
4.4.3 Eliminating EEG change as an alternative explanation ... 81
4.4.4 Activity in the superior colliculus ... 82
4.4.5 Activity of DA cells ... 84
4.4.6 Topographic distribution of response directions ... 86
4.4.7 Optical Imaging ... 87
4.5 Discussion ... 88
4.5.1 Summary of findings ... 88
4.5.2 Discussion of findings ... 89
4.5.3 Conclusion ... 93
5 The effects of collicular disinhibition on the responsiveness of dopaminergic neurons to trigeminal nucleus stimulation ... 94
5.1 Chapter summary ... 94

5.2 Introduction 94
5.2.1 Response characteristics of vibrissae sensitive SC neurons 94
5.2.2 DA responses to trigeminal stimulation 96
5.2.3 Experiment rationale 97
5.3 Method 97
5.3.1 Experimental procedure 97
5.4 Results 98
5.4.1 Inclusion criteria 98
5.4.2 Activity in the SC 101
5.4.3 BMI differentially modulates DA neuron multiphasic responses 108
5.4.4 Dopaminergic response to familiar, non-rewarded stimuli 111
5.4.5 Effect of interleaved stimulation on response 111
5.5 Discussion 111
5.5.1 Summary of findings 111
5.5.2 Discussion of findings 112
5.5.3 Final conclusions 116
6 Discussion 117
6.1 Chapter summary 117
6.2 Discussion of results 117
6.3 Broader functional implications 122
6.4 Alternative/further experiments 126
6.5 Final conclusions 128
References 130

1 Introduction

1.1 Chapter Summary

Dopaminergic (DA) neurons are undoubtedly involved in reinforcement learning, although their exact role is still unclear. The role of DA neurons in learning is often couched in terms of reward; however, the fact that DA neurons exhibit robust responses to a wider class of stimuli than those unambiguously related to reward suggests that the phasic DA signal may have a broader remit. The aim of this chapter is to provide the theoretical and experimental background to the work presented in this thesis. Firstly, the nature and function of the phasic DA response is detailed, including recent research suggesting the possibility of multiple components to the response. This is followed by a description of the SC and research indicating its role as a relay of visual input. Finally, the case will be made that the somatosensory vibrissal system provides an ideal tool for investigating whether cortical and subcortical sensory input can modulate DA activity via the SC, a possibility that this thesis will demonstrate is likely to be the case.

1.2 The midbrain dopaminergic systems

The monoamine neurotransmitter DA is produced in several regions of the brain. Dahlström and Fuxe, (1964) divided DA neurons of the midbrain into three groups. These were designated A10 (approximately corresponding to ventral tegmental area, or VTA), A9 (DA neurons predominately within the substantia nigra pars compacta, or SNc), and A8 (a dorsal and caudal extension of A9). These populations of neurons and their projections form the DA neurotransmission system.

Processes of the midbrain DA neurons form ascending projections, which target several forebrain structures. These projections can be broadly divided into three pathways based on their points of origin and targets. The nigrostriatal pathway comprises neurons from the SNc, projecting to the dorsal striatum. The mesolimbic pathway comprises neurons from the VTA, projecting to areas of the limbic system (nucleus accumbens, ventral striatum and the amygdala). The mesocortical pathway comprises neurons from the ventral tegmental area, projecting to cortical regions (medial, prefrontal, cingulate and entorhinal cortices) (Marsden, 2006). Figure 1-1 shows an illustration of these projections in the rat brain.

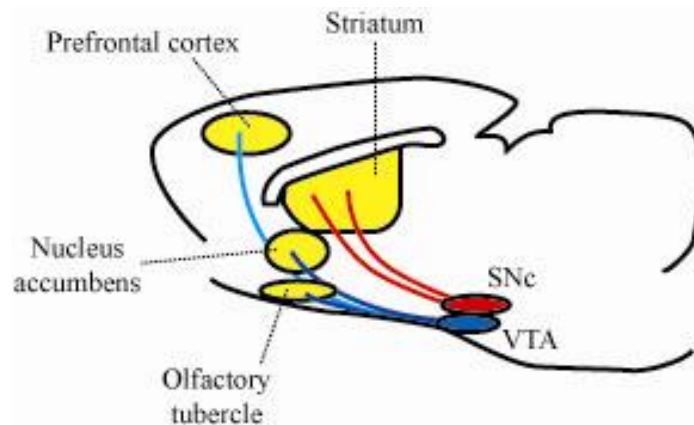


Figure 1-1 The pathways of the ascending DA system and some of the target structures. Nigrostriatal (red) mesocortical (light blue), and mesolimbic (dark blue)

While the distinction between pathways is not absolute, and there is overlap in the projection targets of each group of neurons, it is widely used and can be regarded as a "convenient heuristic when considering the DA system" (Björklund and Dunnett, 2007).

1.3 The function of dopamine

The DA neurotransmission system has been implicated in a wide range of both normal cognitive and behavioural functions, including associative learning, action selection and movement coordination. The malfunction of DA systems have been suggested to be involved in conditions as diverse as schizophrenia (Snyder, 1972; Meltzer and Stahl, 1976), Parkinson's disease (Bernheimer et al., 1973; Lloyd et al., 1975; Birkmayer and Hornykiewicz, 1998), Huntington's disease (Bernheimer et al., 1973; Sourkes, 1981), Tourette's syndrome (Sweet et al., 1976; Ross and Moldofsky, 1978; Cohen et al., 1979), and ADHD (Swanson et al., 2007). However, the precise role of DA in many conditions remains unclear (e.g. the mechanism underlying the effect of DA levels in Parkinson's disease; Grace, (1991)). Due to the apparently disparate conditions in which DA function is involved, it is difficult to infer a broad function of DA from examining its effects. Instead, a better approach may be to explore the sources of input to DA neurons. DA neurons can only communicate the input they receive, albeit in a processed form, therefore it follows that the function of DA will relate to the function of the structures that provide DA neurons with input. By identifying the structures that provide DA neurons with input, and considering what function they serve, the role of DA might be better understood. Before

considering their inputs, the activity of DA neurons will be described, and a distinction made between phasic and tonic DA release.

1.3.1 Activity of DA neurons

DA neurons typically exhibit resting activity of 1-9 spikes/s. As well as the baseline firing rate, DA neurons also exhibit bursts of typically 2-6 spikes – a burst being defined as starting when two spikes occur within 80 ms, and ending when two spikes occur more than 160 ms apart, with subsequent spikes in the burst decreasing in amplitude, increasing in duration and increasing in interspike interval (Grace and Bunney, 1983, 1984a, 1984b). Figure 1-2 shows an illustration of such a burst. Although the cause of spontaneous burst firing in DA neurons in anaesthetised animals is not readily apparent, some researchers (Overton and Clark, 1997) do not consider it to necessarily be acausal, and so refer to the bursts as 'natural' rather than spontaneous.

As well as natural bursting, DA neurons also show the same bursting activity in response to external sensory stimuli (Strecker and Jacobs, 1985; Schultz, 1986; Ljungberg et al., 1992; Horvitz et al., 1997; Dommett et al., 2005). The activity of DA neurons releases DA at terminals throughout the forebrain. While the level of extracellular DA is usually maintained at a fairly stable level, high frequency activity of DA neurons in a burst results in a release of DA that is greater than the release that would be expected from activity with the same mean frequency, but with the spikes evenly distributed (Gonon, 1988; Garris and Wightman, 1994). This evoked burst of action potentials and the resulting release of DA are known as the phasic DA response.

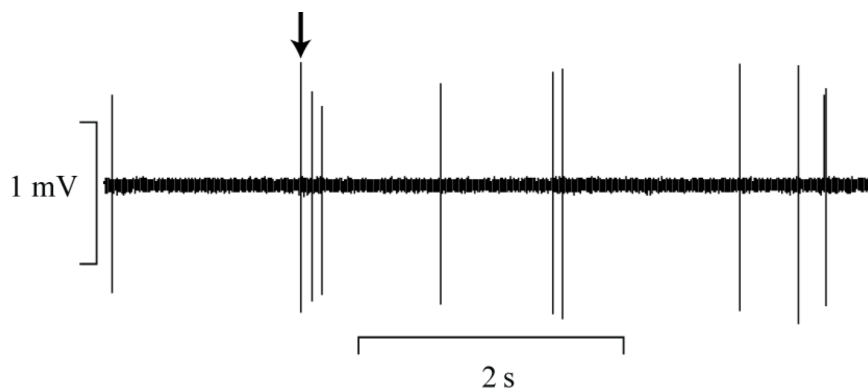


Figure 1-2 Extracellular recording of a spontaneous burst of three spikes in a DA neuron (indicated by the arrow) during resting activity.

The phasic DA response typically occurs following unexpected presentation of a primary reward, such as food, as well as presentation of stimuli that are salient by virtue of their novelty or intensity, but not necessarily inherently rewarding (Chiodo et al., 1980; Romo and Schultz, 1990; Schultz and Romo, 1990; Ljungberg et al., 1992; Horvitz et al., 1997). If a stimulus not associated with reward is presented repeatedly, the response habituates (Ljungberg et al., 1992). The DA response is largely stereotyped with regard to sensory modality or situation (Schultz et al., 1997), with DA neurons responding to visual, auditory and somatosensory stimuli (Strecker and Jacobs, 1985; Schultz, 1986; Ljungberg et al., 1992; Horvitz et al., 1997; Dommett et al., 2005), in both SNc and VTA (Dommett et al., 2005).

It has been demonstrated that the phasic DA response can shift from a primary reinforcer to an arbitrary stimulus if the reward is reliably predicted by the stimulus (Schultz, 1986; Romo and Schultz, 1990; Ljungberg et al., 1992). If, under these circumstances, a predicted reward fails to materialise, there is a brief pause in the on-going activity of DA neurons (Hollerman and Schultz, 1998). If a previously reinforced stimulus ceases to be reinforced, the response habituates rapidly (Ljungberg et al., 1992).

1.4 Proposed functions of the phasic dopamine response

The close association of the phasic DA response with biologically salient stimuli and its ability to respond to predictors of reward have led to suggestions of a role in associative learning. Several potential functions have been proposed, although a currently popular hypothesis is that the phasic DA signal constitutes a reward prediction error signal.

1.4.1 Reward prediction error hypothesis

Based on the ability of DA neurons to show a positive response to unpredicted reward, unpredicted neutral stimuli that reliably predict a reward, and a negative response in the absence of an expected reward, it has been suggested that the phasic DA signal forms a reward prediction error signal: a signal of the value of a stimulus compared to the value expected by the organism (Schultz, 1997; Schultz et al., 1997). If an event is more rewarding than expected, or if an unexpected reward occurs, there is a brief increase in the firing rate of the DA neuron. If the event is as rewarding as expected, the firing rate of the neuron does not change. If the event is less rewarding,

or there is an absence of an expected reward, then there is a brief decrease in firing rate, or a pause in the activity of the neuron.

However, while this explanation is currently popular, it has been criticised on several points (Redgrave et al., 1999). Despite the characterisation of the phasic DA response as a reward related signal, DA neurons exhibit strong responses to a wider class of stimuli than those that could be considered rewarding, or reward related. DA neurons respond to unexpected sensory stimuli with no appetitive value (Horvitz et al., 1997; Horvitz, 2000). There is also evidence that DA neurons respond with an excitation to aversive events (Kiyatkin and Zhukov, 1988; Brischoux et al., 2009). Responses to both novel stimuli without any knowledge of the reward value, as well as aversive stimuli, which presumably are not rewarding, suggest that the function of the phasic DA signal extends beyond communicating reward related information. Further, as previously mentioned, the DA response is largely stereotyped with regard to sensory modality or situation. A system that possesses little variation in the responses of the majority of its neurons in the majority of situations would not lend itself well to communicating information about a complex and unpredictable world. However, not only do DA neurons respond to non-rewarding stimuli, they also do not always respond to rewarding stimuli – a peculiar property for neurons in a 'reward' system. The proportion of DA neurons reported as not responding to primary rewards is typically around 10-25% (Romo and Schultz, 1990; Mirenowicz and Schultz, 1994; Hollerman and Schultz, 1998; Kobayashi and Schultz, 2008), although it has been reported to be as high as 75% (Schultz et al., 1993).

The reward prediction error hypothesis has also been criticised on the basis that the phasic response occurs at latencies too short to allow the reward value of an unexpected stimulus to be judged. The phasic response typically begins at around 70-100 ms after stimulus presentation and is approximately 100 ms in duration (Schultz, 1998). This precedes the gaze shift required to bring the stimulus onto the fovea (typically 150-200 ms) (Hikosaka and Wurtz, 1983; Jay and Sparks, 1987). Hence, the onset of the response precedes post-saccadic cortical analysis that would be able to identify and value the stimulus (Thorpe and Fabre-Thorpe, 2001; Rousselet et al., 2004). However, although the response precedes post-saccadic cortical analysis, it does not necessarily precede all cortical activity. Eyes are not blind outside the fovea, and given that areas of the cortex are dedicated to extra-foveal input, albeit with less

detail than fovea related cortical processing, they may be able to contribute pre-saccadically.

1.4.2 Identifying the function of dopamine from its sensory inputs

The answer to the question of the function of DA can be answered at least partly by examining the properties of structures providing input to the DA systems. For example, for DA neurons to respond to visual stimuli at short latencies, they would have to receive information from a structure that deals with visual information at similarly short timescales. Studies have discovered a projection from the SC to DA containing regions of the midbrain, the tectonigral pathway, and suggested that the SC is the primary, if not sole, source of short latency visual input to DA neurons (Coizet et al., 2003; Comoli et al., 2003; Dommett et al., 2005).

The SC and the tectonigral pathway

The superior colliculus ('optic tectum' in non-mammalian vertebrates) is a subcortical structure located on the dorsal surface of the midbrain. Its function is to direct the sensory organs and the head toward objects of interest. In animals such as primates, who rely on a well-developed visual system to explore the world, the SC is dominated by visual input and its function is direct the eyes and head (May, 2006). The SC is also located early in the visual processing pathway, receiving direct input from the retina. A connection between the SC and midbrain DA neurons has been demonstrated both anatomically and functionally. A direct projection from the SC to VTA was demonstrated by Comoli et al. (2003), and also by Geisler et al. (2007), although the projection from the SC to SNc – the tectonigral pathway – is stronger (Comoli et al., 2003; May et al., 2009). The presence of the tectonigral pathway has been demonstrated in the rat, cat, and monkey (Comoli et al., 2003; McHaffie et al., 2006; May et al., 2009). Further anatomical study showed that the majority of the synapses formed by the tectonigral pathway were on TH negative neurons, with approximately 13% of anterogradely labelled butons found on TH negative neurons (Comoli et al., 2003). Tectonigral neurons were found to form both asymmetric and symmetric synapses on both TH negative and TH positive neurons (Comoli et al., 2003). This might suggest both an excitatory and inhibitory effect of the tectonigral projection, as asymmetric and symmetric synapses are often considered to correspond to excitatory and inhibitory synapses; however, this is not necessarily the case (Klemann and Roubos, 2011). Neither can the effect of the tectonigral pathway

presently be inferred from the neurotransmitters involved, which, according to Redgrave et al. (2010) “remain unknown”. Nevertheless, some reasonable suggestions can be made. If the TH negative neurons are inhibitory interneurons, the presence of projections directly onto TH positive neurons, and onto TH negative neurons suggest that the tectonigral projection might be able to produce opposing effects on nigral DA neurons.

The SC as a relay for short latency visual input to DA neurons

Combined with the demonstration of a direct tectonigral pathway, the functional properties of the SC made it an ideal candidate for providing the necessary short latency input to DA neurons to drive the phasic signal. The primate SC shows two bursts of activity in response to a visual stimulus. The first is a sensory response, typically ~50 ms after the onset of the stimulus, and a longer latency (<150 ms) presaccadic motor burst (Wurtz and Goldberg, 1972; Jay and Sparks, 1987). The sensory response is short enough to precede that of the DA neurons, and as such, it could be the source of input to trigger the phasic DA response. SC neurons respond to rapid changes in luminance; the appearance, disappearance or movement of an object in the visual field (Wurtz and Albano, 1980; Sparks, 1986), and so the SC is ideally suited to perform the role of signalling the unexpected occurrence of stimuli.

The functional connectivity of the SC and midbrain DA neurons, and that the SC provides visual input to DA neurons were demonstrated by Coizet et al. (2003) and Dommett et al. (2005). Simultaneous recording of the deeper layers of the SC, and DA neurons in anaesthetised rats showed that both structures were initially unresponsive to visual stimuli. Following disinhibition of the deeper layers of the SC with an intracollicular injection of bicuculline methiodide (BMI), the deeper layers of the SC became responsive to visual stimuli. The responses of the SC were closely associated with responses in DA neurons of the VTA and SNc, and the onset latencies of responses in the SC were reliably shorter than those of DA neurons. Disinhibition of the primary visual cortex alone affected LFP responses, but produced no change in spiking activity. Although DA neurons responded to visual stimulation with both increases and decreases in activity, electrochemical recording confirmed that the visual stimulation produced a phasic increase in DA levels in the striatum.

These results demonstrated that the SC was a relay for short latency visual information to midbrain DA neurons, which was not only capable of driving DA

neuron activity, but also produced phasic DA release in a target structure of the ascending DA systems.

A nonvisual role of the SC

Although the studies of Coizet et al. (2003) and Dommett et al. (2005) focussed on visual input, the role of the SC in relaying sensory input to DA neurons need not be similarly restricted. The SC can indeed be regarded as a visual midbrain structure, and it is referred to as the 'optic' tectum in non-mammalian vertebrates, but it also receives input in other sensory modalities. Animals with different sensory priorities show different strengths of projections from other sensory structures, e.g. rodents have a stronger trigeminotectal projection than primates (May, 2006), whilst Huber and Crosby observe that it is "equally true that the tectum is a sensory correlation centre" (Huber and Crosby, 1933). As well as directing gaze shifts, the SC may also direct the mouth (Redgrave et al., 1996), pinnae (Stein and Clamann, 1981), or limbs (Werner et al., 1997).

The SC responds strongly to the occurrence of sensory stimuli in multiple modalities. It is arranged in topographic maps of retinal space in the case of visual input, and local space in the case of somatosensory and auditory input, with the location of cells in SC responding to a stimulus corresponding to a spatial location. Neurons responding to auditory, somatosensory and/or visual stimuli are located within the intermediate and deep layers of the SC (Gordon, 1973; Dräger and Hubel, 1976; Stein et al., 1976; Chalupa and Rhoades, 1977; Harris et al., 1980; King and Palmer, 1985; Meredith and Stein, 1986). The sensory maps of each modality are in register with each other and with motor maps that direct the orienting behaviour (Stein et al., 1975). Like the visual responses in the intermediate and deep SC and DA neuons demonstrated by Coizet et al. (2003) and Dommett et al. (2005), the neurons of the SC that respond to other sensory modalities could likewise relay sensory input to DA neurons at short latency.

1.4.3 Determining action-outcome associations of unexpected events

Although neurons of the SC are responsive to the appearance and movement of stimuli, they are thought to be largely insensitive to static contrast, velocity, wavelength and geometric configuration of visual stimuli due to them receiving little or no input from the division of the visual system that processes these details – the parvocellular system (Wurtz and Albano, 1980; Sparks, 1986; Sumner et al., 2002;

Vuilleumier et al., 2003). Consequently, DA neurons relying on this input would be unable to discriminate between static stimuli and their reward value, an ability fairly fundamental to a reward prediction error system. Thus, as an alternative to the reward prediction error hypothesis has been proposed. Namely, that the DA signal acts to bias the reselection of action immediately preceding an unexpected event to help determine action-outcome associations (Redgrave and Gurney, 2006; Redgrave et al., 2008).

One of the major projection targets of the DA system, particularly neurons of substantia nigra, is the dorsolateral striatum (Marsden, 2006). The dorsolateral striatum receives input for a wide variety of neural structures; however, the potential interaction of three of these inputs with the presence of DA means the striatum is ideally placed to control action selection. Many of the neurons of the tectonigral pathway have branching collateral projections to areas of the thalamus that project to the striatum (Coizet et al., 2007). This projection provides glutamatergic sensory input in response to an unexpected stimulus that would also produce a phasic release of DA to the striatum (McHaffie et al., 2005). Contextual information – the general sensory, metabolic and cognitive state of the animal – affects the activity of striatal neurons (Apicella et al., 1997; Nakahara et al., 2004; Samejima et al., 2005). This provides the animal with a record of the internal and external 'state' it is in, and is likely to come from cortical, limbic and subcortical (thalamic) sources (Redgrave et al., 2008). Finally, both cortical and subcortical sensorimotor structures that provide input to the brainstem also provide input to the striatum via branching collaterals (Crutcher and DeLong, 1984; Bickford and Hall, 1989; Lévesque et al., 1996; Mink, 1996; McHaffie et al., 2005).

If the DA signal biases the action selection of the striatum towards recent behaviour, the repetition of the behaviour leading up to the occurrence of an event would allow an organism to determine the precise sequence of actions that results in the occurrence of an event, and in what context. On occasions when an unexpected event is a consequence of actions by an agent, there would be a conjunction of the context and motor copy, the glutamatergic, and DA representations of the unexpected event. If the behaviour was not the cause of the unexpected event, its absence after the repetition of behaviour expected to trigger an event would cause a decrease in DA activity, biasing selection away from the behaviour.

One of the benefits of considering the role of the phasic signal from nigral DA neurons as a 'timestamp' to be applied to the behaviour and contextual efference copy present in the striatum rather than signalling reward prediction error is that it only requires the detection of a stimulus, rather than identification. This function could be performed solely with the input of simpler sensory structures, such as the SC.

1.5 Two components of the phasic DA signal

Redgrave and Gurney (2006) suggested that DA neurons could not support the reward prediction error signal based on the capabilities of the structures providing sensory input at latencies short enough to trigger the phasic DA response. However, the fact remains that some many studies have apparently demonstrated value related responses in DA neurons at such latencies.

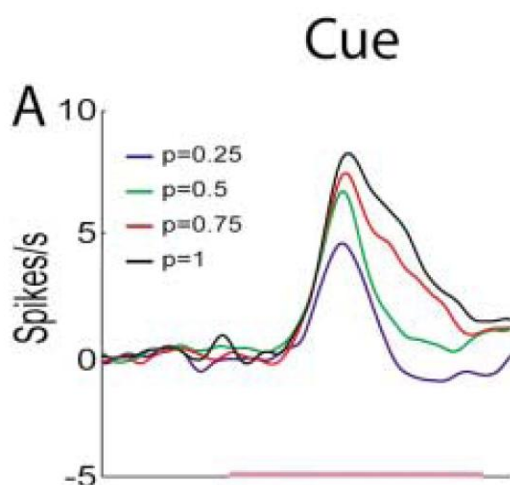


Figure 1-3 Responses of DA neurons to stimuli indicating different probabilities of reward delivery. The pink horizontal bar indicates the period used to quantify the responses – the first 400 ms after stimulus onset. From Morris et al. (2004).

Although Schultz (2007) makes a distinction between different functions of DA on different timescales as phasic and tonic DA, he seems to make the implicit assumption that the phasic DA response was a homogeneous signal serving a single function. However, there is an increasing amount of evidence to challenge this position. Morris et al. (2004) presented stimuli that were associated with reward with different probabilities and found that responses in DA neurons were longer than expected, and differences in response in the DA neurons to different stimuli could only be detected by extending the period over which activity was measured to 400ms. The differences in magnitude associated with reward value were largely in the latter

portion of the response. Thus, while DA neurons showed a differential response "reflecting the mismatch between expectation and outcome in the positive domain", a careful examination of the figure showing DA responses to stimuli associated with different reward probabilities (see Figure 1-3) appears to show that DA neurons responded similarly for the first ~150 ms. Only after this period does the magnitude of the DA response begin to differ.

Although there were incidental indication in earlier research, the possibility of multiple components of the phasic DA response has only recently begun to be formally addressed. Recent evidence has emerged to suggest that DA neurons can respond differentially on different timescales. Hudgins et al. (2009) demonstrated that DA neurons respond differently to stimuli if they are associated with different reward probabilities. However, they also showed that the response of DA neurons consists of a short component, 50-110 ms after stimulus presentation, which does not discriminate between stimuli, and a longer latency component around 110-250 ms which can discriminate between stimuli, and reflects reward probability (see Figure 1-4). This ability to discriminate beyond the capabilities of the SC may be the result of more information about the stimulus that triggered the DA response.

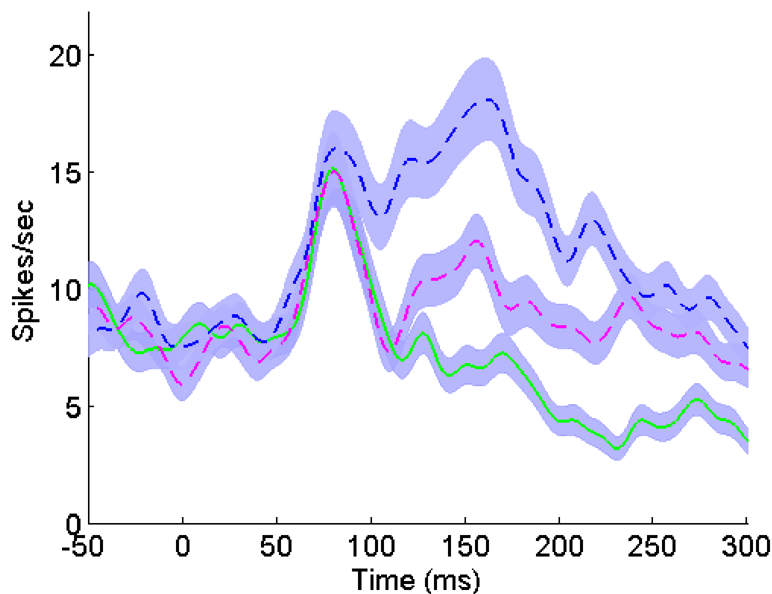


Figure 1-4 Population spike density plot showing the responses of 84 DA neurons to stimuli in fixed locations associated with different reward probabilities. Blue line p=1.0, magenta line p=0.5, green line p=0.0. Shaded regions represent standard errors around the mean. From Hudgins, (2010).

1.5.1 Determining the source of longer latency input to the DA signal

Assuming the DA response can be divided into a stimulus insensitive initial component and a later component that is sensitive to the reward probability associated with a particular stimulus, the possibility of different sources of input could be investigated. It is likely that the initial stimulus insensitive response is driven by SC input. As has been mentioned previously, it is likely to be the primary if not sole source of visual input at such latencies (Dommett et al., 2005). However, given the previously mentioned insensitivity of the SC to many stimulus features, the possibility that the SC relays the later, stimulus sensitive component has been, until recently, seen as less likely.

It was previously thought that that SC and other visual orienting structures were insensitive to visual properties beyond low spatial frequency luminance changes (Schiller et al., 1979). Recently, however, it was demonstrated that neurons in the intermediate layers of the monkey superior colliculus were in fact responsive to colour stimuli that were isoluminant with the background. Response latencies were on average 30 ms greater than their response to stimuli based on monochromatic luminance (White et al., 2009). White et al. suggest that the latencies involved imply a transcortical pathway, as the SC receives input from cortical areas that respond to colour (e.g. V4). This cortical input to the SC may be the source of input underlying the longer latency stimulus sensitive component. The following sections address the SC, and its cortical input.

1.6 The superior colliculus and its cortical afferent connections

1.6.1 Anatomy of the superior colliculus

Although the anatomy of the SC is broadly similar in most mammals, there are some differences between species. Detailed studies of collicular anatomy are available that focus on cat (Huerta and Harting, 1984), primate (Wurtz and Albano, 1980), and tree shrews (Hall and Lee, 1993, 1997; Lee and Hall, 1995). For comparisons between species, Lund (1972) focuses on the superficial layers, or see May (2006) for a comprehensive review of mammalian collicular anatomy. However, a full review of these differences in the anatomy of the SC is beyond the scope of this thesis. Instead, the following section will focus largely on rodent SC, and statements regarding SC will refer to the rat unless otherwise specified. Although effort will be made to

reference data from rodent species, more comprehensive work undertaken in other species will be referred to where little rodent work is available, or where comparisons to other species are particularly relevant.

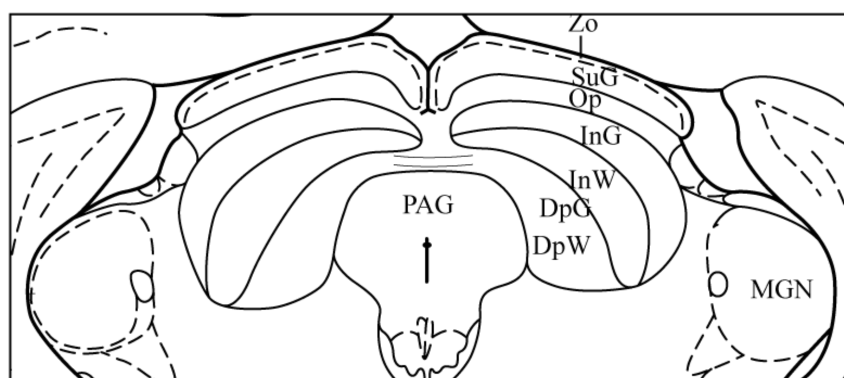


Figure 1-5 An illustration of the layers of the rodent SC, adapted from (Paxinos and Watson, 2004). Abbreviations as follows: Zo – zonal layer, SuG – superficial grey layer, Op – optic layer, InG – intermediate grey layer, InW – intermediate white layer, DpG – deep grey layer, DpW – deep white layer, PAG – periaqueductal grey, MGN – medial geniculate nucleus.

The mammalian superior colliculus is a layered structure on the dorsal surface of the midbrain, and is most clearly visible in coronal section. It is comprised of seven alternating cellular and fibrous layers that run broadly parallel to the dorsal surface of the brain. They are the *stratum zonale* ('zonal layer', Zo), *stratum griseum superficiale* ('superficial grey layer', SuG), *stratum opticum* ('optic layer', Op), *stratum griseum intermediale* ('intermediate grey layer', InG), *stratum album intermediale* ('intermediate white layer', InW), *stratum griseum profundum* ('deep grey layer', DpG), and *stratum album profundum*, ('deep white layer', DpW) (Paxinos and Watson, 2004). SuG is often divided into an upper (uSuG) and lower (lSuG) sub layer. In some species, e.g. cats, it is divided into three numbered layers, with layers 1 and 2 corresponding to uSuG and layer 3 to lSuG (May, 2006). May reports an alternative nomenclature "primarily used by primate physiologists and by some investigators that use the rat model", in which lSuG is considered to be InW, and InW and DpG of the first system constitute the DpG. Further, other researchers e.g. Helms et al. (2004) divide InG/InW into three sub layers designated SAIa, b and c, where SAIb contains rostrocaudally-running fibres. Given that the studies presented here focus on rats, the divisions of Paxinos and Watson (2004) will be used (see Figure 1-5 for an illustration).

1.6.2 Primacy of sensory corticotectal projections

The presence of a substantial corticotectal projection with a parallel projection from ascending pathways provides a useful tool for exploring the effect of cortical and subcortical sensory input on the SC and DA neurons. Although there is a significant projection form visual cortex, there are complications, such as the proximity of secondary cortices, which make visual cortical stimulation less than ideal for investigating the role of the SC in relaying cortical input. Reports of projections of auditory cortex to the SC are likewise complicated by the proximity of primary and secondary cortices, but also by the poorly understood auditory corticotectal projection.

In contrast, anatomical studies have also shown the rodent primary somatosensory cortex has a significant projection to the superior colliculus, and projections from regions of cortex corresponding to the whiskers and face cover an extensive anterolateral part of the colliculus (Wise and Jones, 1977; Kassel, 1982; Welker et al., 1988; Hoffer et al., 2005). This descending cortical projection is also in register with the ascending projection coming directly from sensory structures. Focal electrical stimulation of the primary somatosensory cortex produces responses in the SC, which are also responsive to peripheral tactile stimulation (Kassel, 1982). Thus, intracortical stimulation of the somatosensory cortex is likely to be the best choice for investigating the possible role of the SC in relaying cortical input to DA neurons.

The present experimental design involves recording from, and modulating the activity of SC neurons responsive to cortical stimulation. To do this effectively, the distribution of corticotectal projections throughout the SC needs to be understood. The following sections provide a brief summary of the corticotectal projections of visual and auditory cortex and the issues that make them less suitable for investigating corticotectal input. It then focus in more depth on the somatosensory cortical projections.

1.6.3 Visual cortex

The SC is regarded as a primarily visual structure, and it receives significant input from cortical regions associated with visual processing. Broadly, the visual cortical areas of the rat can be divided into the primary (or striate visual cortex), and extrastriate areas (although the reference to striation is a misnomer, as rodent visual cortex lacks the striae of Gennari, which give visual striate cortex in primates its

name). These extrastriate areas consist of secondary and association cortices, and are spatial or functional transformations of primary visual cortex. Although several divisions and subdivisions of cortical regions have been suggested (e.g. Whitlock et al., 2008), a distinction will only be made here between primary visual cortex (Oc1, approximately area 17 in Krieg, 1946)) and the medial and lateral divisions of secondary visual cortex (Oc2M, Oc2L, areas 18a and 18 in Krieg, 1946).

The corticotectal projection of Oc1 is highly focal, strongly topographic and "the densest of any visual corticotectal projection (Harvey and Worthington, 1990)". Oc1 projects exclusively to superficial layers of the colliculus; layers Op and above (Harvey and Worthington, 1990; Coogan and Burkhalter, 1993). Projections from secondary visual areas terminate in deeper layers of the colliculus; layers SO and below. Projections from the medial subdivisions of Oc2 terminate primarily in two horizontal tiers, one in the middle of InG, and one on the border of InW and DpG (Harvey and Worthington, 1990; Coogan and Burkhalter, 1993). This pattern of projections raises problems for investigating the cortical input via SC by using disinhibition of the deeper layers. SuSC is responsive to visual stimuli in the anesthetised prep, and may also be responsive to V1 stimulation. Thus, the onset of sensitivity to stimulation with disinhibition of the SC cannot be used to establish the SC as a relay of distinct primary visual cortical input. The regions of primary and secondary visual cortex are very close, so even if secondary cortex is stimulated, this may activate primary cortex, which would make the respective contributions difficult to differentiate.

1.6.4 Auditory cortex

Division of the auditory cortex into primary and association areas, and delineation of association cortex into distinct regions, is the source of some dispute (Zilles et al., 1980; Romanski and LeDoux, 1993; Palomero-Gallagher and Zilles, 2004), and perhaps as a result there has been less focus on the corticotectal projections of auditory areas. Roger and Arnault (1989) made no mention of labelling in SC, but did report labelling in IC as a result of injection of anterograde tracers in auditory cortex, however, subsequent studies focussing on non-primary auditory regions have reported projections in the deep layers of the SC (Arnault and Roger, 1990; Kimura et al., 2004). The lack of a firm map of auditory cortical regions, and

the potentially sparse projection of auditory cortex to the SC makes it a less practical option for investigating cortical input to DA neurons via the SC.

1.6.5 Somatosensory cortex

Topographic maps in somatosensory cortex

The somatosensory cortex is dominated by a single somatotopic map in primary somatosensory cortex, although Brett-Green et al. (2004) describe several somatotopic representations within S2. The somatotopic map is likewise dominated by the representation of the head and whiskers to such an extent that Zilles et al. (1978) made a distinction between Pr1, and area of cortex that contains the representation of the head – which is dominated by the barrel field – HL (a hindlimb area), and FL (a forelimb area). However, the distinction was also functional, as Zilles et al. (1978) suggested that FL and HL exhibit characteristics of sensory and motor cortex. They also outlined an area designated Pr2, which lies ventral to Pr1, and corresponds to secondary somatosensory cortex.

Projection to the SC

Somatosensory cortex projects topographically to superior colliculus, primarily ipsilaterally. As with the somatotopic representation in the cortex, the somatosensory projection to the SC is dominated by projections from the barrel field. Wise and Jones (1977) demonstrated that the projection of somatosensory cortex extends to the lateral border of the superior colliculus. With the exception of the extreme anterior end of the colliculus, the projection does not extend to the medial extent of the SC. The projection is densest in the InG, but there was also lighter terminal labelling in InW. The distribution of the projection is topographic, with injections of the areas of somatosensory cortex corresponding to the face and head resulting in labelling in the anterior and lateral extent of SC, injections in the hindlimb areas resulting in labelling in posterolateral SC, and injections in the forelimb areas resulting in labelling in a small area of posterior SC.

Killackey and Erzurumlu (1981), disputed the claims of Wise and Jones, however. Killackey and Erzurumlu claimed that injections of retrograde tracer into SC produced labelling in broadly similar regions to Wise and Jones (1977), but that that labelling excluded the barrel field. However, examination of the figures suggests their demonstration of an absence of projection from S1Bf to SC seems to be based on an injection into a more caudal (and possibly dorsal) location in SC than Wise and Jones,

which is less likely to include vibrissal regions. Further, the area Killackey and Erzurumlu indicate as the barrel field on coronal section, which lacks retrograde labelling, also seems to be inaccurate. Upon examination of the gross anatomy of Killackey and Erzurumlu's coronal slide demonstrating an absence of labelling in S1Bf their slide is, in my estimation, from a position more rostrally in the brain than they suggest. This would further reduce the likelihood of labelling from an injection into caudal SC. Thus, despite the observations of Killackey and Erzurumlu (1981), both their study and the slides from Wise and Jones (1977) do show significant retrograde labelling in the barrel field when compared against the atlas of Paxinos and Watson (2004).

Several more recent studies into the precise connectivity of the barrels provide more evidence of a projection from the barrel field to SC. Injections of PHA-L (Mana and Chevalier, 2001) and fluorescent agents (Hoffer et al., 2005) demonstrated that projections from the barrel field terminate in small clusters in InG. An accidental injection in S1 by Harvey and Worthington (1990) when mapping visual cortex (presumably in the more caudal regions of S1) also revealed patchy labelling in ventral InG, with some labelling extending along the InG/DpW border. Studies in other rodent species provide further evidence of a projection from barrel field to SC. Aronoff et al. (2010) made injections that were largely contained within one barrel and the surrounding septa, which resulted in a few patches of labelling in intermediate layers of SC.

Although studies always report at least a broad topographic projection from somatosensory cortex, the precise projection of the barrel field is not always reported. Welker et al. (1988) reported that injection of PHA-L into a single barrel resulted in labelling throughout the mediolateral extent of InG, but the labelling was limited in a rostrocaudal direction (corresponding to the SC receptive fields of an arc of vibrissae). Injections into barrels in the same arc resulted in the same extensive mediolateral labelling at the same rostrocaudal point in the SC. Injections into barrels in different arcs in the same row resulted in multiple mediolateral stripes of labelling at different rostrocaudal points in the SC, which when cut sagittally could be seen as patches corresponding a row of vibrissae.

Although the above studies, which were investigating specific aspects of the barrel/septal corticotectal projection using modern techniques provide some

corroboration of the initial work of Wise and Jones (1977), the Wise and Jones paper is still the paper cited as evidence of a corticotectal projection of the barrel field, despite disagreements (e.g. Killackey and Erzurumlu (1981), Welker et al. (1988)). The absence of any full-scale systematic investigation of the projection of S1 and specifically the barrel field to SC using modern techniques is a source of potential investigation. A replication of Wise and Jones' work using modern anatomical methods is arguably overdue, although outside the scope of this project.

1.7 Whisker pathway as a useful investigatory tool

An investigation of the relative contributions of every region of sensory cortex is beyond the scope of this project. Instead, the contribution of sensory cortex in one modality will be examined. The comparatively weaker projection from auditory cortical regions to the SC suggests that this is probably the least viable option. There is a significant projection from visual cortical regions to the SC; however, the picture is complicated by different visual regions projecting to different regions of SC. In contrast, S1 provides a large area of cortex with a consistent projection to In/DpSC. Stimulation of S1 thus provides a more practical way of investigating whether cortical input can have an effect on DA neurons, and whether that route is via the same tectonigral projection from the intermediate and deep layers of the SC as visual sensory stimulation. The vibrissal system also has advantages in the form of its well defined, modular anatomy based around input from the whiskers, or vibrissae. The following section provides a brief description of the anatomy of the vibrissal system.

1.7.1 Trigeminal connectivity and anatomy

The trigeminal nerve, which carries vibrissal input, synapses onto neurons of the trigeminal nuclear complex (TNC). The TNC is a collection of nuclei that are the first processing stage for whisker input. The trigeminal nuclei are divided into several subnuclei. Although functional distinctions can be made between subnuclei and the neurons contained within them, the focus of this thesis is not to study the different effects of particular types of somatosensory stimuli, and so the trigeminal nuclei will be considered to be a homogeneous 'somatosensory nucleus'.

The TNC projects to a range of non-thalamic subcortical structures (ventral zona incerta and the anterior pretectal area (Jacquin et al., 1989; Veinante et al., 2000), the cerebellum, and the anterior pretectal area (Jacquin et al., 1989; Hallas and

Jacquin, 1990)). Perhaps most significant for the present work is its projection to SC. Pr5, Sp5o, Sp5i and Sp5c all project to the SC (Hallas and Jacquin, 1990; Veinante and Deschênes, 1999; Veinante et al., 2000). The trigeminal nucleus also has a significant projection to several thalamic nuclei, and from there onto somatosensory cortex (see Deschênes (2009) for a review). It is important to note, however, a specific aspect of trigeminal anatomy: the neurons projecting to the SC from the trigeminal nuclei receive input from multiple whiskers.

At various points along the pathway of whisker input, discrete cytoarchitectonic units can be distinguished, with each unit relating to a single whisker. These are known as barrels in the primary somatosensory cortex (Woolsey and Van der Loos, 1970) barreloids in the ventral posterior medial nucleus of the thalamus (VMP) (Van Der Loos, 1976), and barrelettes in the trigeminal nuclei (Ma and Woolsey, 1984; Ma, 1991). Barrelettes are not found throughout the trigeminal nuclei (Ma and Woolsey, 1984; Ma, 1991; Henderson and Jacquin, 1995; Deschênes, 2009), and the majority of trigeminal efferents that project to the SC originate in the subnuclei that lack barrelettes. There is a population of neurons projecting from Pr5, where barrelettes are present, to the SC. However, these neurons span several barrels, and respond to the stimulation of several whiskers equally well (Veinante and Deschênes, 1999). This may mean that the somatosensory input being relayed to the SC is less fine grained than the information that passes to the cortex via the thalamus, which would have implications for the discriminatory capabilities of any DA response relying on trigeminotectal input.

1.7.2 Differentiating direct and indirect somatosensory input

The division of trigeminal input between cortical and subcortical targets may provide an opportunity to investigate the respective contributions of direct sensory input to the SC, as well as indirect input via the sensory cortex. A detailed analysis of the SC response was provided by Cohen et al. (2008), who showed that the response of individual SC neurons to vibrissal deflection was composed of two short latency components of approximately 2-8 ms (Peak 1 component) and 9-25 ms (Peak 2 component) after deflection, then a longer period from 26-100 ms. More importantly, Cohen et al. (2008) demonstrated that the second component of the collicular response to vibrissal manipulation was the result of cortical input from the primary somatosensory cortex. Figure 1-6 shows a PSTH of a single SC neuron in response to

multiwhisker stimulation under control conditions (black), during application of a "small dose" of BMI (blue) and TTX (red) into the cortex. During BMI application, the response in the second component was enhanced, but suppressed by TTX application.

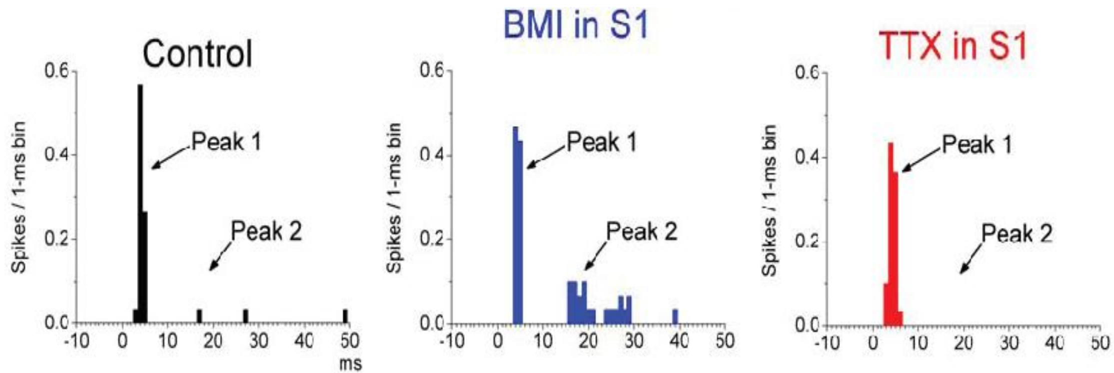


Figure 1-6 Effect of barrel cortex response enhancement and suppression on SC responses. Example of single neuron PSTH recorded in the SC during control conditions (black) BMI in the barrel cortex (blue) and TTX in the barrel cortex (red). From (Cohen et al., 2008).

Although the primary focus of this thesis is the effect of sensory input on the activity of DA neurons, the distinction between direct trigeminotectal and indirect trigemino-thalamocorticotectal components of the SC response, and the effect of intracollicular injections of bicuculline would also be of interest.

1.8 Rationale of identifying cortical input to DA neurons

Much is known about many aspects of the ascending DA systems, and the origin of short latency sensory information is beginning to be examined. However, less is understood about how longer latency elaborative information, which reward prediction may rely on, reaches DA neurons. Recent work (Hudgins et al., 2009) has suggested that the phasic DA response to sensory stimuli is made up of two components, one that is short latency, and does not discriminate between stimuli, and a second component, which can discriminate between some aspects of stimuli and indicate associated reward values.

Discrimination of complex stimulus properties requires the cortex, and it is possible that DA neurons receive cortical information at longer latencies. The superior colliculus is known to be a relay of direct sensory input to DA neurons, but it also receives input from a broad range of cortical areas, which may be the source of a presumed elaborative input to DA neurons. While it has been demonstrated that SC

can respond to more complex stimulus properties at a longer latency, it is not known whether the cortical information suggested to underlie this ability is able to influence DA neuron activity. If the SC is responsive to a greater range of sensory features after presumably cortically processed inputs, then this may provide a source of the information that allows DA neurons to discriminate between stimuli and signal presumed reward prediction at longer latencies.

1.9 Overview of the thesis

This thesis presents an investigation into whether, and by what route, cortical input affects the activity of DA neurons in SNc, and the interaction between cortical and subcortical input, using the vibrissal system as an investigatory tool. Chapter 3 presents the initial investigation into the capability of stimulation of S1Bf to affect the activity of SNc DA neurons after disinhibition of the SC. Chapter 4 confirms the SC as the route of S1Bf input to DA neurons by addressing questions raised by the previous research and the results of chapter 3. This is done by producing a response in DA neurons without disinhibiting the SC, then suppressing collicular activity with an intracollicular injection of muscimol. Chapter 5 uses stimulation of the trigeminal nucleus to investigate the relative contributions of direct subcortical and indirect cortical input to the SC, and consequently DA neuron response. Finally, the results are discussed in terms of the SC as a common relay for sensory and cortical input to SNc DA neurons, and the implications of these findings for our understanding the sensory capabilities and function of the phasic DA response are also discussed.

2 Materials and methods

2.1 Chapter summary

This chapter summarises the materials and methods common to the experiments presented in this thesis. The experiments involved recording multiunit activity in the SC and the activity of single DA neurons in response to direct electrical stimulation of the brain. Stimulation was performed before and during chemical manipulation of SC by a local injection made with a specially constructed combined cannula/recording electrode described below. Further specific details on experimental and analytic processes used in each experiment are given in the relevant experimental chapters.

2.2 Electrode and cannula construction

2.2.1 Construction of glass microelectrode

One to five glass fibres were inserted into a thick walled glass capillary tube (G-2, Narishige Scientific Instrument Lab, Tokyo, Japan), which was pulled to a point using a puller (Narishige Scientific Instrument Lab, Tokyo, Japan) to produce a pipette. The tips were broken against a glass rod under a microscopic guidance to a tip width of approximately 1-2.5 μm. The pipettes were then filled with 2 M sodium chloride and 2% pontamine sky blue (BDH Chemicals Ltd, Poole, England). Pipettes were used as electrodes if their in vitro impedances measured between 6-10 MΩ in 0.9 % saline at 10 kHz (Impedance tester: Winston Electronic Co. BL-100, San Francisco, USA).

2.2.2 Construction of multiunit electrode-cannula assembly

To inject chemical agents, a cannula was constructed by bevelling the tip of a short length of 30 ga stainless steel tubing, and soldering around it a sleeve of 23 ga tubing, leaving 2-3 mm of 23 ga exposed at the unbevelled end. A short sleeve of 30 ga polyethylene tubing was put around the exposed tip which, when the cannula was inserted into a long length of 23 ga polyethylene tubing. When the 23 ga polyethylene tubing was pushed onto the cannula, the sleeve of 30 ga would stretch over the 23 ga metal tubing, forming a tight seal (all gauges are needle gauge, also known as Stubs Iron Wire Gauge, or Bristol Wire Gauge). Figure 2-1 illustrates how the cannula was

constructed. A parylene-C-insulated tungsten microelectrode, 250 μm in diameter with an impedance of 1-2 MΩ in 2M saline at 10 kHz (A-M Systems Omc. Carlsborg, USA), was coupled to the cannula with heat shrink. The cannula was then held to the electrode with a combination of quick dry epoxy, a suture, or a short section of heat shrink such that the tip separation of electrode and cannula was <0.5 mm, with the electrode slightly forward of the cannula.

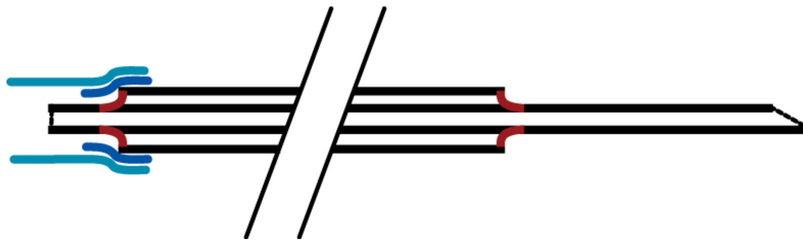


Figure 2-1 Figure 2-2 Diagram of cannula construction. 30 ga stainless steel tubing inside 23 ga tubing (both in black), soldered together (red). 30 ga polyethylene tubing (dark blue) 23 ga polyethylene tubing (connected to syringe pump)

2.3 Experimental design

This thesis presents an investigation into whether, and by what route, cortical and subcortical somatosensory input affects the activity of DA neurons in SNc, and the interaction between cortical and subcortical input, using the vibrissal system as an investigatory tool. The studies presented here used electrophysiological recording techniques to record the effect of electrical stimulation of the barrel cortex or trigeminal nuclei on collicular (multiunit) activity and DA (single unit) activity in substantia nigra pars compacta (SNc), both before and during chemical manipulation of the SC. To ensure that only collicular neuronal elements were manipulated, local microinjections of an excitatory substance, the $GABA_A$ receptor antagonist BMI, or an inhibitory substance, the $GABA_A$ receptor agonist muscimol, were used.

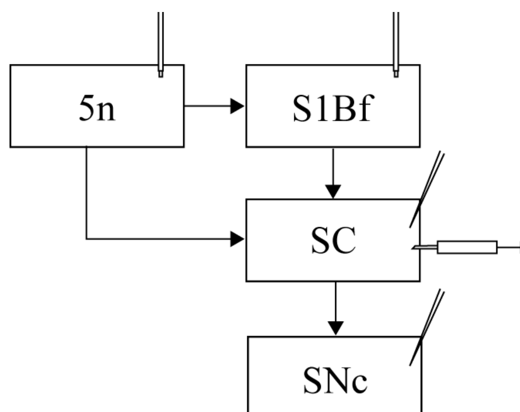


Figure 2-2 A simplified illustration of the structures involved in the investigation presented in this thesis: 5n – trigeminal nucleus, S1Bf – barrel field of the primary somatosensory cortex, SC – superior colliculus, SNc – substantia nigra pars compacta.

A schematic of the experimental procedures is shown in Figure 2-2. Icons represent experimental procedures used: simultaneous electrophysiological recording in the SC and SNc, direct electrical stimulation in 5n and S1Bf, and injection of modulating substances (BMI or muscimol) into the SC.

2.4 Subject preparation and surgical procedures

All aspects of the studies were performed with prior approval of the University of Sheffield ethics committee and the Home Office [section 5(4) of the Animals (Scientific Procedures) Act of 1986].

Animals were housed together with free access to food and water, in a room with a maintained temperature of 20-22 °C on a 12-hour light/dark cycle. Animals were anaesthetised with an intraperitoneal injection of urethane (ethyl carbamate; 1.25 g/kg as a 25 % aqueous solution). Supplemental doses of urethane (up to 10 %) were given on rare occasions when necessary. The depth of anaesthesia was assessed by a pinch to the toe of the hindpaw. When no leg retraction (pedal reflex) was observed, the animal's head was shaved and it was mounted into a stereotaxic frame (Kopf Instruments, Tujunga, USA), which held the skull level in the plane employed by the stereotaxic atlas of Paxinos and Watson (2004). The temperature of the rat was maintained at approximately 37 °C with a heating blanket. The animals were periodically surveyed for regular respiration and tested for areflexia.

A midline incision was made in the head, and the skin was reflected back. Anterior-posterior measurements were taken from bregma, while medial-lateral measurements were taken from the midline. The skull was thinned with an electric drill over target recording sites at distances relevant to bregma/midline reference, then the thinned skull was broken and removed with a bent 35 ga needle and tweezers under a binocular microscope to form a burr hole approximately 3-4 mm in diameter. Table 1 shows the coordinates for burr holes for each recording and stimulation site (See Figure 2-3A for a graphical illustration). Somatosensory cortex projects to ipsilateral SC, which projects to ipsilateral SNc. Projections from the trigeminal nucleus to the SC cross the midline. Consequently, burr holes for cortical stimulation were made ipsilateral to burr holes for SC recording, while trigeminal stimulation burr holes were made contralateral to SC burr holes. Burr holes for SNc were also made contralateral to burr holes for collicular recording to allow an unimpeded contralateral approach (Figure 2-3B). After the skull was removed, the dura was carefully removed.

Site of Recording	Anterior-posterior (mm)	Medial-lateral (mm)
Superior colliculus	-6.0	2.0
Substantia nigra pars compacta	-5.3	-3.0
Site of Stimulation		
Barrel cortex	-2.5	5.0
Trigeminal nucleus	-12.7	-2.7

Table 1 Coordinates of craniectomy. Anterior-posterior measurements relative to bregma, negative values posterior to bregma. Medial-lateral measurements relative to midline, negative values contralateral to SC site.

2.5 Implantation of electrodes

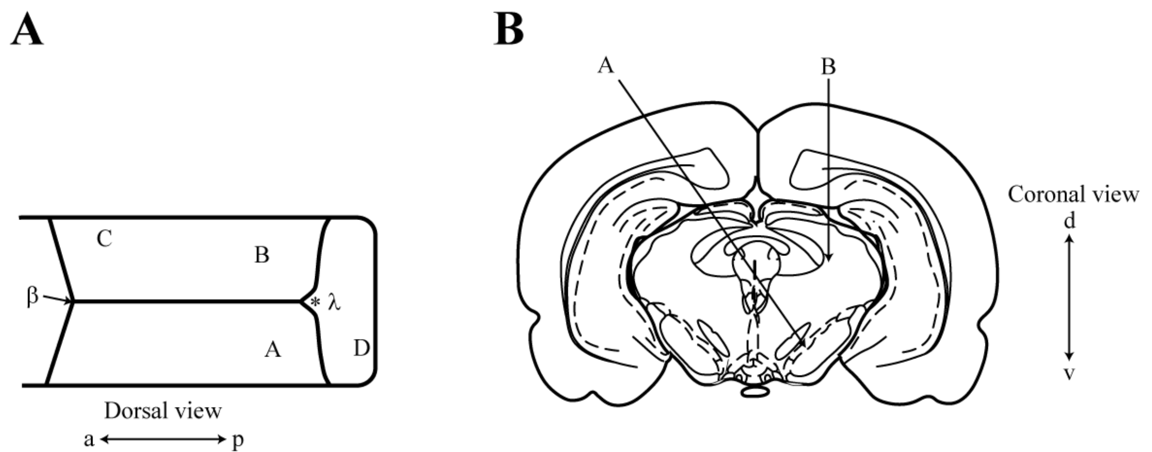


Figure 2-3 (A) Diagram of craniectomy sites. (B) Illustration of contralateral approach to SNc (B). (Abbreviations: A: SN entry point, B: SC entry point, C: barrel cortex stimulation entry point, D: trigeminal nuclei stimulation point, β: bregma, λ: lambda, a: anterior, p: posterior, d: dorsal, v: ventral.

Incoming signals were amplified and band-pass filtered (3 dB points 200 Hz-4 kHz for single unit, 400 Hz-16 kHz for multiunit). Some multiunit recordings were band-pass filtered at 1 Hz-16 kHz to allow low frequency EEG signals to be extracted by digital filters offline. Neuronal responses were displayed on an oscilloscope and played through an audio monitor. Recordings were digitised at 20 kHz and recorded direct to computer disc using a 1401+ data acquisition system (CED Systems, Cambridge, UK) connected to a PC running the CED Spike2 software.

After preparation of the subject, the cannula was flushed through and filled with distilled water. A small amount of air was drawn into the cannula, and then the cannula was backfilled with the relevant agent (BMI or muscimol). The coupled electrode/cannula assembly was lowered into the deep layers of the SC. The final position of the probe was determined by using the responsiveness of the superficial layers to visual stimuli under urethane anaesthesia. The eye contralateral to the

collicular recording was sutured open and artificial gel tears (Ciba Vision Viscotears, Duluth, USA) were applied to prevent the eye drying out. The probe was lowered though the colliculus and a multiunit recording was obtained concurrent with a whole field contralateral light flash (0.5 Hz, 10 ms). The probe was considered to be sufficiently within the deep layers after moving a further 1 mm ventral after the visual response could no longer be detected

Once the SC electrode/cannula had been positioned, the DA recording electrode was inserted. The recording coordinates given above were used to make a small mark on the cortical surface with a pipette filled with pontamine blue and broken to a tip size of approximately 3 µm. This point was used as the target entry point for the recording electrode. A micromanipulator was used to lower the electrode into SNc at a rate of 500 µm/s until approximately 2 mm above the area of interest, at which point the rate was reduced to 1.25 µm/sec until a DA neuron could be identified. To prevent the two electrodes coming into contact, and to avoid damaging SC, the DA recording electrode was inserted using a angle of 35° from vertical on the side contralateral to cortical stimulation, crossing the midline to record cells on the ipsilateral side. Finally, a stainless steel bipolar stimulating electrode (NEX-100, Rhodes Medical Instruments, Woodland Hills, CA) was placed vertically into S1Bf or into Sp5i.

2.5.1 Stimulus generation

For cortical and trigeminal stimulation, square wave pulses of 100 µs were produced using a Grass S48 Square Output Stimulator (Grass Technologies, West Warwick, RI, USA) and a stimulus isolation unit (PSIU6 Photoelectric Isolation Unit, Grass Technologies, RI, USA), or an in-house constructed stimulator. Whole field light flashes (0.5 Hz, 10 ms duration) were delivered from an orange LED positioned 5 mm from the eye.

2.6 Experimental procedures

2.6.1 Identification of putative dopamine cells

Single neurons recorded in the area of interest were detected by distinguishing the presence of spikes of electrophysiological activity above background noise, i.e. by discriminating action potentials. A threshold was selected on the amplifier's window discriminator such that spike activity triggered an event, and waveform averages of

the activity around the events were produced. Waveform averages were produced from the last 30-60 seconds of triggered activity to produce the action potential shape of the neuron. Putative DA neurons were identified primarily by showing a stereotypical biphasic or triphasic waveform, having baseline firing rate between 1 and 10 Hz, and spike onset-trough lengths of >1.0 ms. Single units were recorded between 8.2 mm and 9.5 mm below the contralateral entry point of the 35° angled trajectory. Once a suitable putative DA neuron had been identified, recording began.

2.6.2 Experimental procedure

When both probes were suitably positioned, baseline activity was recorded from both the SC and the SNc for a period of at least 60 s. At least 150 sweeps of either single pulse or at least 450 sweeps of pulse train stimulation were then applied to quantify the response at baseline. While the electrophysiological recordings continued, a pressure injection of a neuromodulatory agent was made into the SC. Injections of either the $GABA_A$ antagonist BMI (100 µg/ml (196.3 µM) made in saline, Sigma, St Louis, USA) or the $GABA_A$ agonist muscimol (200 µg/ml (1.75 mM) made in saline, Sigma, St Louis, USA) were made into the SC using a syringe pump (World Precision Instruments Inc, Saratosa, FL, USA), injecting 0.5 µl at a rate of 0.5 µl/min. At least 150 further stimulations were then applied to characterise the response over the time course of drug action, usually up to 450 stimulations for BMI injections, at least 450 stimulations for muscimol injections. For experiments involving BMI, time was allowed to ensure metabolisation of the drug and a return to baseline state in SC after a successful recording, usually around 20 minutes. Additional DA neurons in the SNc were then identified, and the process was repeated. Due to the prolonged effect of muscimol, only one injection was made per animal. At the termination of the experiment, the last recording track in SC was marked with a small electrolytic lesion (150 s duration 10 µA cathodal DC). Ejection of pontamine sky blue from the glass pipette marked the recording sites in the ventral midbrain (900-1500 s duration 27.4 µA cathodal DC).

2.7 Histological techniques

After the marking lesions, the animals were killed with a overdose of barbiturate and perfused transcardially with 400 ml of warmed saline (40 °C), followed by 500 ml of 4 % formaldehyde in phosphate buffer (pH 7.4). Brains were removed and

postfixed in 4 % paraformaldehyde at 4 °C. Serial coronal sections (30 μm) of the SN, SC, S1Bf and the brainstem were cut on a cryostat and collected in 0.1 M phosphate buffer (pH 7.4). Sections were divided into two series and processed separately for Nissl (cresyl violet), and double processed for c-Fos and TH immunohistochemistry with procedures described previously (Shehab et al., 1992). To reveal Fos-like immunoreactivity (FLI), free floating sections were washed with 0.1 M phosphate buffered saline (PBS) followed by 0.1M PBS containing 0.3 % Triton-X100 (PBS-TX) for 20 min and then processed according to the procedures of (Hsu et al., 1981) overnight, with agitation at room temperature. The primary polyclonal antibody (Autogen Bioclear) was diluted 1:15,000 in the PBS-TX with 1 % bovine albumin in saline (BSA) and 2 % normal horse serum. The following day, sections were washed with PBS-TX and incubated for 2 h with biotinylated goat anti-rabbit IgG (1:100, Vector Laboratories Inc., in PBS-TX with 2 % normal horse serum). After washes, sections were exposed (2 h) to the Elite Vectastain ABC reagent (Vector Laboratories Inc., 1:100 in PBS-TX). Immunoreactivity was revealed by reacting the sections with nickel enhanced diaminobenzidine for ~1 min (Adams, 1992). Finally, sections were washed in distilled water, dehydrated in graded alcohols, cleared in xylene and coverslipped with DPX. Similar procedures were used to reveal tyrosine hydroxylase immunoreactivity in a second series of sections. Tissue was incubated with a primary mouse monoclonal antibody (1:500 dilution, Boehringer Mannheim UK), raised against TH. The secondary antibody was horse anti-mouse IgG (1:1000, Vector Laboratories Inc.) and exposed to the Elite Vectastain ABC reagent (1:200, Vector Laboratories Inc.). Immunoreactivity was revealed by incubation with VIP (Vector Laboratories Inc.).

2.8 Data analysis

A combination of built in functions of Spike2, the Spike2 script language, and the R analysis language were used to perform analyses.

2.8.1 SC processing

The raw recording was processed prior to analysis to enable better measurement of the multi-unit activity. First, a waveform average triggered by stimulus onset was calculated and subtracted from the data to reduce the effect of the stimulus on the signal (see Figure 2-4A-D). The SC trace was then high-pass filtered with an FIR

digital filter (Spike2 function) at 1.5 kHz (transition gap 1.2 kHz, -3 dB point 1.069 kHz) to reduce the influence of LFP on the signal. A threshold was set at mean plus two standard deviations of the rectified waveform voltage. Data rising through this level was considered a spike, and was triggered as an event (Figure 2-5).

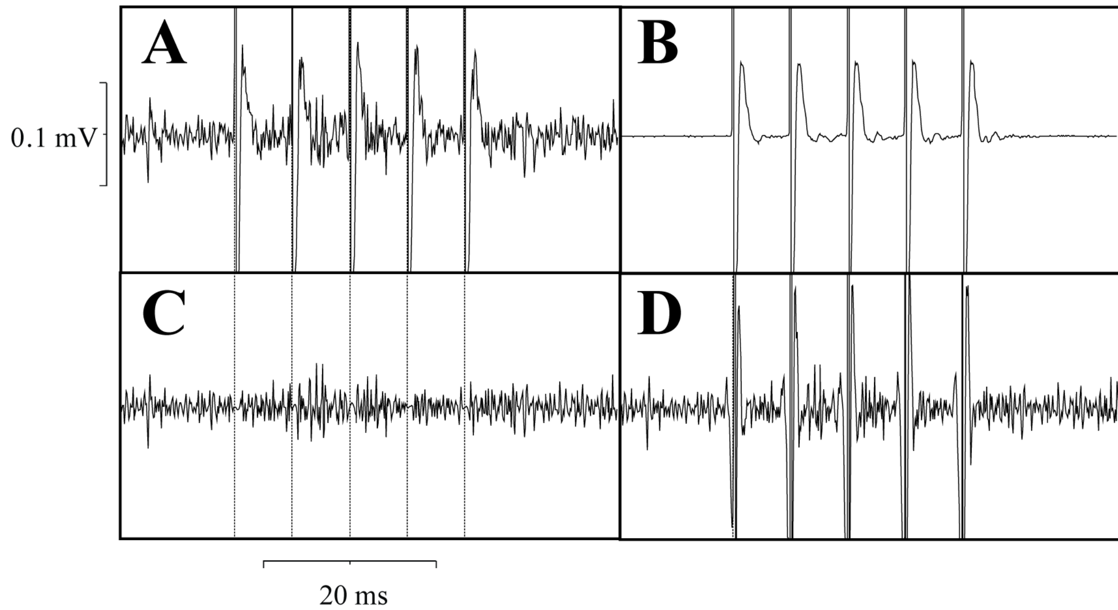


Figure 2-4 An example of waveform average subtraction on a response to a train of 5 pulses of cortical stimulation. A: A short section of raw SC waveform, B: A waveform average triggered by stimulation onset (1500 trials). C: Waveform from A with waveform average in C removed and filtered as described above. D: The same filter applied to the waveform in A. Vertical cursors indicate the time of each pulse in the train.

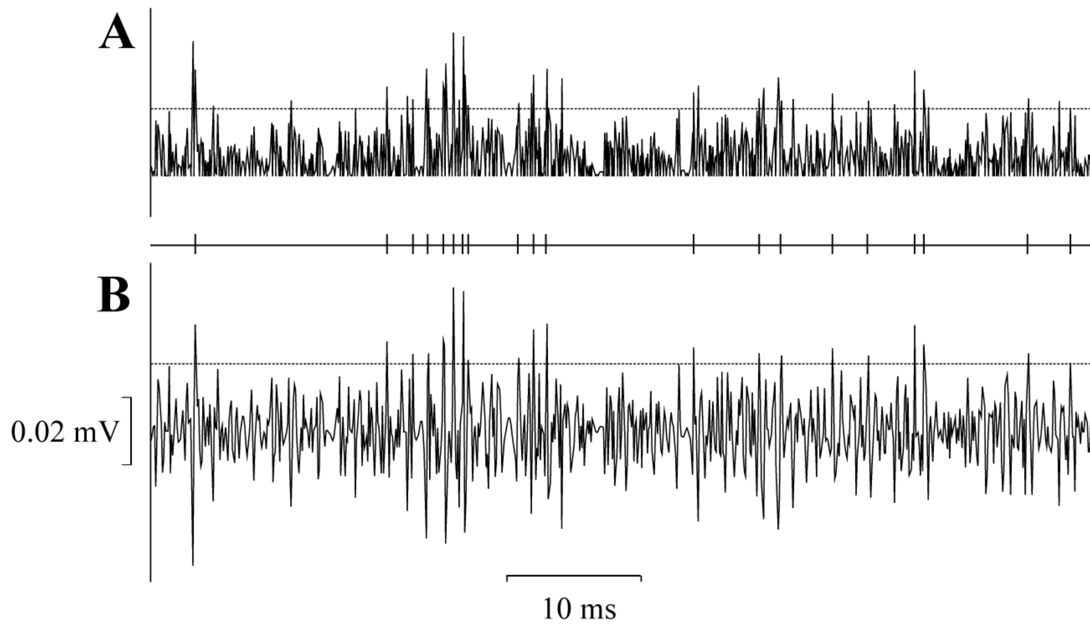


Figure 2-5 An example of event triggering in SC. A: A short section of processed SC waveform, fully rectified. The horizontal cursor is set at the mean plus two standard deviations. B: (Below) The threshold is applied to the unrectified waveform. Activity rising through the threshold was recorded as an event. Above: The event channel for the section of waveform.

2.8.2 SC analysis

Defining measures of activity

Following data collection and processing, the data were examined to see if the chemical manipulation had taken effect by comparing several measures of activity. The measures were defined as follows (letters correspond to Figure 2-6 An illustration of the measurements used to determine drug effects and response characteristics. A: A truncated series of events triggered from the beginning of a recording. Each short line represents an event, the two longer lines to the right indicate the onset of the first two stimulations of the file. B: An example rate histogram of the activity of the beginning of a recording (1 s bins) C: PSTH of stimulus related activity (1 ms bins) Black vertical dotted line indicates stimulus onset, white horizontal dotted line indicates mean background activity. Lighter blue portion of PSTH indicates post-stimulus activity above mean background firing used to calculate response magnitude. Lowercase letters on B and C correspond to measures of activity described above – (a) baseline activity, (b) background activity (c) response):

a) Baseline activity – a measure of the resting spontaneous activity. Calculated as the mean firing rate in the period at the start of the recording, preceding any stimulation or chemical manipulation
b) Background activity – a measure of the spontaneous activity during periods of stimulation. Calculated from a PSTH as the mean firing rate in the period before stimulation onset
c) Response magnitude – a measure of evoked activity. Calculated from the PSTH as the mean firing rate in the period following stimulation onset above background firing. The particular period is defined in each chapter. Note, that to produce a measure of evoked activity independent of changes in background activity, the mean background firing rate is subtracted from the mean firing rate in the period following stimulus onset.

PSTHs were constructed from pre-injection trials and post-injection trials to compare the effect of the injection, or from a series of blocks of trials to track the time-course of a measurement of activity. The pre-stimulation period used to calculate background activity was usually the 500 ms preceding stimulation. The post-stimulation period used to calculate response is defined in the methods section of each chapter. Response onset and offset were defined by the activity in a PSTH crossing thresholds determined from background activity, typically mean±2SD. Response duration was defined as the time between response onset and offset. Response amplitude was also measured, and defined to be the value of the largest bin in the response period, minus the mean background firing rate.

Defining effective injections and periods of effect – BMI

For the following section, “electrical stimulation” refers to either cortical or trigeminal stimulation as appropriate. “Stimuli” refers to both light flash, and cortical or trigeminal stimulation. For chapters 3 and 5, and effective injection of BMI was defined using the light flash as a positive control. The average response magnitude for sets of 10 stimulations was plotted over time, and an effective injection was defined as when there was an increase in response magnitude to the light flash rising above mean+2SD of the pre-injection response magnitude. In both chapters 3 and 5, there were no cases in which the there was a significant change in the SC response to electrical stimulation after an injection of BMI, but no change in the SC response to the light flash.

After determining whether the injection had been successful, the presence and duration of a period of significant change in SC response to electircal stimulation was determined in a similar way. The start of a period of significant increase was defined as two consecutive sets of 10 stimulations after BMI injection where the response magnitude exceeded a threshold of mean+2SD of the response magnitude of pre-BMI stimulations. The period of significant increase was defined as ending when two consecutive sets of 10 stimulations fell below the same threshold.

Defining effective injections and periods of effect - muscimol

In chapter 4, the background activity from 450 pre-injection trials and 450 post-injection trials were compared, and a significant change in the background activity after injection of muscimol was taken as indication of a successful injection. If no significant change occurred, then the record was excluded from further analysis. The

response based approach for defining successful injections differs from that used in chapter 3 due to the absence of any previously established criteria related to stimulus-evoked activity.

The background activity from 450 pre-injection trials and 450 post-injection trials were compared, and a significant change in the background activity after injection of muscimol was taken as indication of a successful injection. If no significant change occurred, then the record was excluded from further analysis. The change from a response based approach for defining successful injections differs from that used in chapters 3 and 5 due to the absence of any previously established criteria for determining muscimol effect related to stimulus-evoked activity.

Measuring activity

The trials in the pre-injection and post-injection periods were used to create peri-stimulus-time-histograms (PSTHs) for responses to the stimuli. The onset latency and duration of the response were measured. The onset latency was defined as bin counts exceeding mean±1.96 SD of the background activity. The response ended when bin counts returned to within the thresholds. In cases where the response was multiphasic, the end of the first phase was deemed to be at the beginning of two consecutive increasing bin counts marking the beginning of a second phase. The second phase ended when bin counts returned to within thresholds. The onset latency, duration, and magnitude of the first phase of the response were measured (Spike2 cursor functions). A bin size of 1 ms was used to achieve the fine time resolution needed to determine the precise latencies of SC response to stimuli.

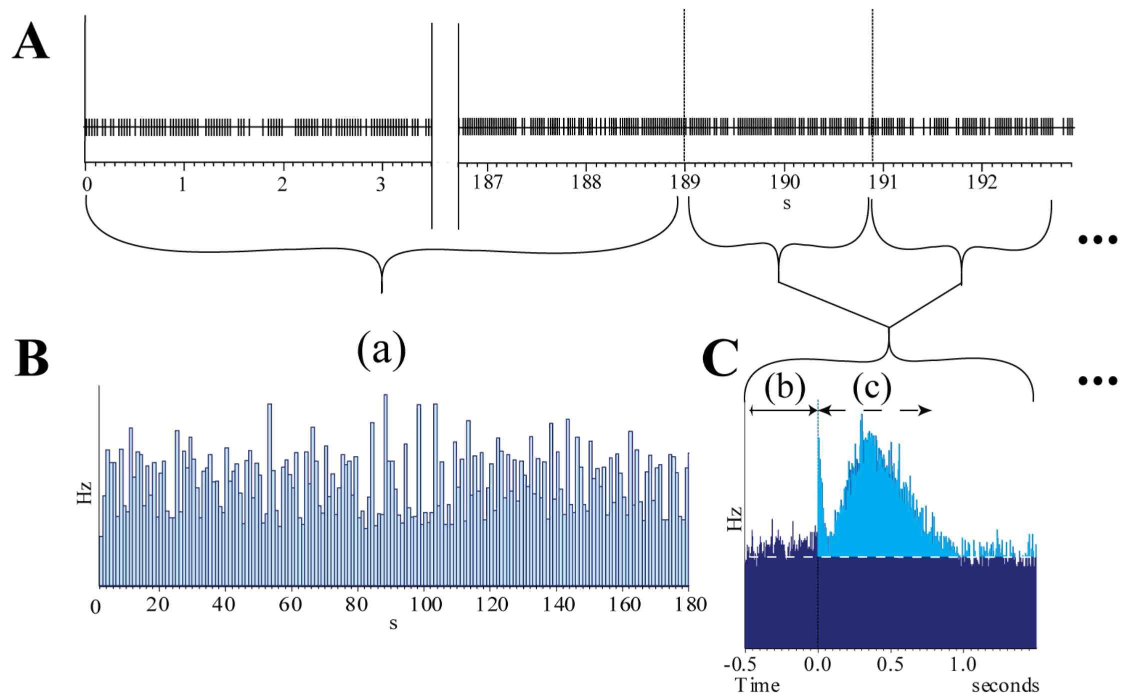


Figure 2-6 An illustration of the measurements used to determine drug effects and response characteristics. A: A truncated series of events triggered from the beginning of a recording. Each short line represents an event, the two longer lines to the right indicate the onset of the first two stimulations of the file. B: An example rate histogram of the activity of the beginning of a recording (1 s bins) C: PSTH of stimulus related activity (1 ms bins) Black vertical dotted line indicates stimulus onset, white horizontal dotted line indicates mean background activity. Lighter blue portion of PSTH indicates post-stimulus activity above mean background firing used to calculate response magnitude. Lowercase letters on B and C correspond to measures of activity described above – (a) baseline activity, (b) background activity (c) response magnitude.

2.8.3 DA processing

Stimulus triggered waveform averages were calculated and subtracted from the recording in the same manner as for SC recording. DA neuron action potentials were isolated from the background noise by using the Spike2 WaveMark function so that each action potential was represented by a single event. Activity that had triggered an event was checked and any event triggered by non-DA neuron activity was removed.

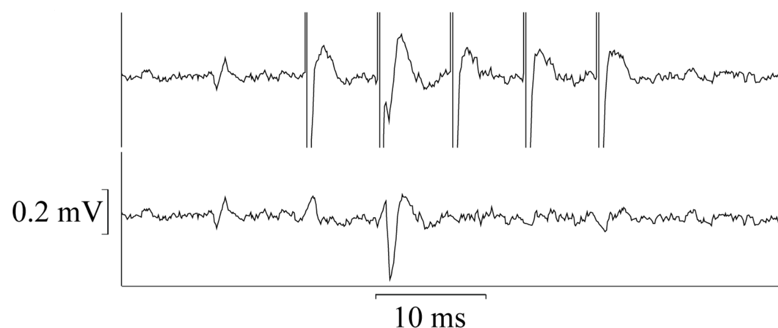


Figure 2-7 An illustration of waveform average subtraction revealing a DA neuron spike, previously disguised by the stimulus artefact. Top: Raw waveform. Bottom: Waveform with stimulus triggered waveform average subtracted.

2.8.4 DA neuron waveform measurement

The waveforms of nigral DA neurons recorded in SNc were determined from offline averaged records of discriminated action potentials. Spike onset-trough lengths were obtained as described previously for on-line DA neuron identification. Total spike durations were obtained by reading the time difference between cursors placed at the points where the averaged spike waveform exceeded and returned below 5% of the spike height (Spike2 software function).

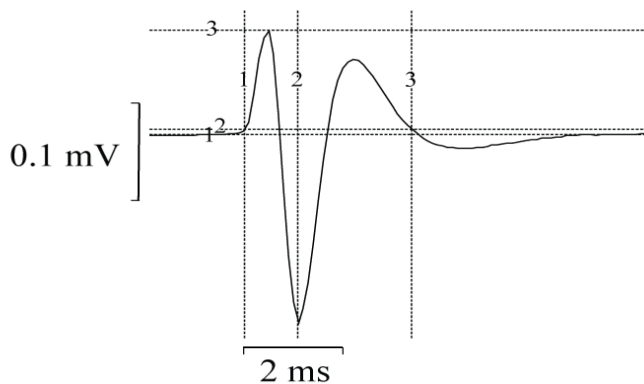


Figure 2-8 DA spike measurements. Spike onset (vertical cursor 1) to trough (vertical cursor 2) length, and total spike length (onset: vertical cursor 1, end: vertical cursor 3). Vertical cursors 1 and 3 positioned where the waveform crosses 5% of the spike height (horizontal cursor 2). Height is measured from mean pre-spike activity (horizontal cursor 1) to spike peak (horizontal cursor 3)

2.8.5 DA analysis

PTSHs were then created to assess responses in DA neurons to stimuli in pre-injection and post-injection trials. In chapters 3 and 5, post-injection measures were taken from the trials where there was evidence of BMI induced change in activity in SC. In contrast to the BMI experiments, injections of muscimol had an immediate, but gradually increasing effect, rather than the comparatively transient onset and wash-out of BMI. Therefore, rather than attempting to define criteria for selecting trials with an effect of muscimol, the last 450 trials of each recording were used for post-injection measures. Raster plots of the DA response for the period of collicular activation were examined by eye to see if there was a period where the response was particularly clear. If this was the case, then the number of trials was reduced. Measures of baseline and background activity, and response magnitude were made as shown in Figure 2-6 An

illustration of the measurements used to determine drug effects and response characteristics. A: A truncated series of events triggered from the beginning of a recording. Each short line represents an event, the two longer lines to the right indicate the onset of the first two stimulations of the file. B: An example rate histogram of the activity of the beginning of a recording (1 s bins) C: PSTH of stimulus related activity (1 ms bins) Black vertical dotted line indicates stimulus onset, white horizontal dotted line indicates mean background activity. Lighter blue portion of PSTH indicates post-stimulus activity above mean background firing used to calculate response magnitude. Lowercase letters on B and C correspond to measures of activity described above – (a) baseline activity, (b) background activity (c) response magnitude. The response period was defined as 20-260 ms after stimulation, to encompass the entirety of the DA response as described by Hudgins (2010). A neuron was defined as responsive if at least three consecutive bins within the response period exceeded mean±1.96SD thresholds. A 20 ms bin size was used to provide unambiguous onset and offset, and data were smoothed with a three period sliding average. The DA response was then characterised, measuring response latency, duration, amplitude and magnitude. Response onset was defined as the start of at least three consecutive bins within the response period. The response was considered to last until the start of two consecutive bins where activity returned to within the thresholds. The presence of a second phase was defined as at least three consecutive bins starting within 60 ms of the end of the response offset. Peak amplitude was also measured, and defined to be the value of the largest bin in the response period, minus the mean background firing rate. Peak latency was the start of the peak amplitude bin.

3 The effects of disinhibition of the superior colliculus on the responsiveness of dopaminergic neurons to stimulation of the barrel cortex

3.1 Chapter summary

It was suggested in the introduction of this thesis that cortical input may support the stimulus sensitive, longer latency phase of the DA response that has recently been demonstrated. As the SC has been established as a relay for early sensory input to DA neurons, and is also a target for extensive cortical projections, it may offer a route by which cortical input reaches DA neurons. The results of this study show that disinhibition of the SC is sufficient for intracortical stimulation of somatosensory barrel cortex to modulate DA neuron firing rates, which strongly suggests that the SC is a relay for cortical input to DA neurons.

3.2 Introduction

DA neurons typically exhibit spontaneous baseline activity of 1-9 spikes/s. As well as the baseline firing rate, DA neurons also exhibit bursts of typically 2-6 spikes with subsequent spikes in the burst decreasing in amplitude, increasing in duration and increasing in interspike interval (Grace and Bunney, 1983, 1984a). The effect of sensory stimuli on the activity of DA neurons can be measured as a tonic or phasic change (Schultz, 2007). Phasic changes in response to a single presentation of a stimulus are typically up to several hundred milliseconds in duration, and may be made up of a burst of spikes or a transient change in activity. Tonic changes, in contrast, are changes in activity measured on the scale of more than a few seconds, and may be associated with behavioural states. The following sections examine in more depth the responses of DA neurons to sensory stimuli in order to provide comparison for DA responses to cortical stimulation.

3.2.1 Tonic changes in DA activity in response to stimuli

Studies using awake behaving rats have demonstrated that DA neuron firing rate can be modulated by simple behavioural changes such as turning (Diana et al., 1989) and motivated behaviour such as lever pressing (Miller et al., 1981; Kosobud et al., 1994; Hyland et al., 2002). Other studies, using awake, restrained rats found that

visual and auditory stimulation, as well as aversive stimuli affected the subsequent firing rate of presumed DA neurons (Kiyatkin, 1988; Kiyatkin and Zhukov, 1988). Initially the neurons showed rapid changes, but these lasted for several seconds and were typically associated with behavioural and physiological changes in alertness. The majority of responsive neurons showed excitatory responses, although both excitatory and inhibitory responses were seen. The direction of responses within a given neuron to different stimuli was not always consistent. DA neurons showed excitatory and inhibitory responses after both aversive and non-aversive stimuli. Not all neurons were responsive. It has also been reported that tonic changes in DA neuron occur in response to repeated phasic visual, somatosensory and olfactory stimulation in the anaesthetised rat (Chiodo et al., 1980). However, this apparent tonic change might be the result of repeated phasic changes, as peristimulus histograms were not constructed, and neither were changes in firing rate measured on sufficiently short timescales at which to detect phasic changes. A similar picture emerges from research involving cats and primates. DA neuron activity is modulated during behavioural tasks in primates before and during motivated arm movements, but not associated with particular changes in EMG (Schultz and Romo, 1987, 1990). Likewise, DA neurons in cats typically show an increase during active exploration, although the changes are not associated the onset of movement or EMG activity (Steinfels et al., 1983b).

3.2.2 Phasic changes in DA activity in response non-noxious stimuli

The responses of DA neurons to stimuli have been extensively studied, however the distinction between phasic and tonic changes in DA activity is often difficult to make in some studies. For example, Kiyatkin and Zhukov (1988) recorded on-going activity in response to stimuli lasting for 500-1000 ms, rather than examining the response profile of repeated shorter duration stimuli. Thus, the presence of a phasic burst is hard to distinguish from longer duration changes. However, examination of the language and figures presented in the study reveals an initial rapid onset of activity, which is likely to represent a phasic burst.

Changes in DA neuron firing rate have been shown in response to external stimuli such as delivery or consumption of a reward (Miller et al., 1981; Kosobud et al., 1994; Hyland et al., 2002), but also non-rewarding sensory stimulation. In awake behaving rats, responses have been reported to visual (Miller et al., 1981; Freeman et

al., 1985; Kiyatkin and Zhukov, 1988; Hyland et al., 2002), somatosensory (Freeman and Bunney, 1987; Kiyatkin and Zhukov, 1988), auditory (Miller et al., 1981; Freeman et al., 1985; Freeman and Bunney, 1987; Kiyatkin and Zhukov, 1988; Kosobud et al., 1994), and olfactory stimulation (Roesch et al., 2007). However, although sensory stimuli commonly produce responses in DA neurons, the effect is neither consistent in terms of direction, nor ubiquitous. Although Freeman et al. (1985), Freeman and Bunney (1987) and Hyland et al. (2002) report increases in firing rate in response to sensory stimulation, Kosobud et al. (1994) reports a decrease, whilst Miller et al. (1981), Kiyatkin and Zhukov (1988), and Roesch et al. (2007) report both increases and decreases in the firing rate of some, but not all DA neurons.

The papers above found an effect of sensory stimuli on the activity of DA neurons in the awake rat. The firing properties of DA neurons under anaesthesia can be shown to be similar to those in awake rats; however, the phasic change in activity that characterises the DA neuron response to non-noxious sensory stimuli in awake rats is typically absent in the anaesthetised animal (Dommett et al., 2005; Tsai et al., 1980; Schultz and Romo, 1987).

3.2.3 Changes in DA activity in response to aversive stimuli

There has been comparatively little research into the effects of aversive stimuli on DA neuron responses in the awake rat. Kiyatkin and Zhukov (1988) and Kiyatkin (1988) reported that neurons presumed to be DA responded to a noxious pin prick or electrical skin stimulation on the tail. Again, as with non-noxious stimuli, not all of these presumed DA neurons responded to the stimuli, and responding neurons showed both increases and decreases in firing in response to the stimulus. Matsumoto and Hikosaka (2009) demonstrated both positive and negative responses in the monkey to aversive stimulus of an air puff, and the conditioned stimuli that predicted it, as well as reporting unresponsive neurons.

In contrast to study in the awake animal, there is more work on the effect of aversive stimuli on DA neuron in the anaesthetised rat. Tsai et al. (1980), which used tail pinch and immersion of the tail in hot (57°C) water as a noxious stimulus, and Ungless et al. (2004), which also used tail pinch, found that while not all DA neurons responded to noxious stimulation, all that did respond did so with an inhibition. However, studies by Maeda and Mogenson (1982), Mantz et al. (1989), Gao et al. (1990, 1996), and Brischoux et al. (2009) report both excitation and inhibition to

aversive stimuli of varying degrees. As with non-noxious sensory stimulation, not all cells are responsive. Schultz and Romo (1987) found intensely noxious stimuli to be the only effective stimulus for anaesthetised monkeys. Both excitatory and inhibitory responses were found, and the response in a given neuron was consistent for stimulation across the whole animal. Interestingly, given Schultz's current position on DA neuron function as indicating the value of a stimulus, he suggests that "the bilateral nontopographic nature of the responses does not support a role in precise stimulus recognition."

3.2.4 The SC as a blocked route of sensory input in the anaesthetised rat

Given the apparent absence of a phasic response in DA neurons to non-noxious sensory stimuli in the anaesthetised animal, it might be of interest to ask what phasic sensory stimuli have in common that distinguishes them from noxious stimuli. The answer, perhaps, lies in the SC. Dommett et al. (2005) demonstrated the SC and DA neurons were insensitive to sensory stimuli in the anaesthetised rat without disinhibition of the SC. Noxious stimuli, in contrast, may avoid the SC, and so be able to modulate DA neuron firing rates without disinhibiton (Coizet et al., 2006). The phasic response in DA neurons has recently been shown to be composed of two components, the first of which is insensitive to stimulus identity, and the second component, which can discriminate between stimuli. It was suggested in the introduction of this thesis that the SC is potentially the route of cortical input to DA neurons. If this is the case, the SC and DA neurons may be insensitive to cortical stimulation without disinhibition in a similar manner to sensory stimuli.

3.3 Experiment rationale

It has not yet been investigated whether the cortex (which may underlie the second component of DA neuron responses) can modulate the activity of DA neurons, and whether this input acts via the SC. The purpose of this study is therefore to establish whether the SC could be a relay for cortical somatosensory input to DA neurons in substantia nigra.

Activity of the intermediate and deep layers of the SC in response to visual stimuli is suppressed under urethane anaesthesia by $GABA_A$ mediated inhibition (Katsuta and Isa, 2003), which in turn suppresses the responsiveness of midbrain DA neurons (Dommett et al., 2005). Disinhibition can be induced by local injections of

pharmacological agents such as the GABA antagonist BMI into the SC (Katsuta and Isa, 2003). The projection from primary somatosensory cortex barrel field (S1Bf) terminates largely within the intermediate and deeper layers of the SC. Thus in this experiment the responses of the SC and nigral DA neurons to stimuli will be assessed both before and during a pharmacological disinhibition of the deep layers of SC.

The responses of DA neurons and multiunit activity of the SC were recorded throughout intracortical microstimulation of the primary sensory barrel field to determine the influence of cortical inputs on DA neurons, and whether that input operated via SC. Responses of both DA neurons and SC were recorded before, and in the presence of local disinhibition of SC by a pressure injection of BMI, to determine how the responsiveness of SC affects the influence of cortical input on DA firing rates.

3.4 Method

3.4.1 Experimental procedure

The experimental design is summarised in graphical form in Figure 3-1. The present study used simultaneous electrophysiological recording of SC (multiunit) activity and DA (single unit) activity in SNC, in response to electrical stimulation of S1Bf, both before (Figure 3-1a) and during (Figure 3-1b) chemical disinhibition of SC. To ensure only neuronal elements in the SC were disinhibited, local injections of an excitatory substance, the $GABA_A$ receptor antagonist BMI (Figure 3-1b, green microsyringe), were used.

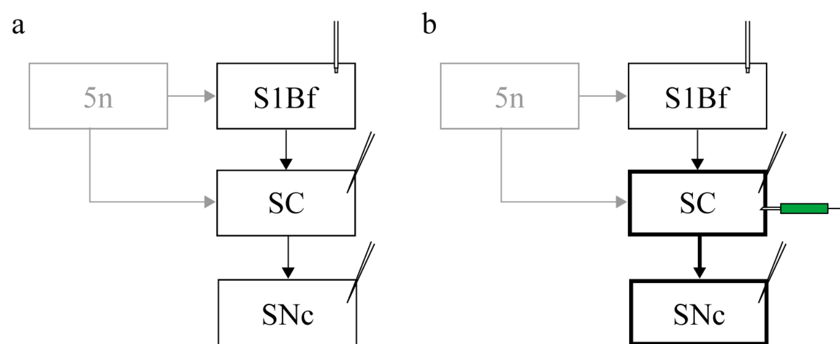


Figure 3-1 Schematic of the experimental design for this experiment.

The subject preparation, experimental procedure, histology, and statistical analysis have been previously described in the Methods chapter. Some sections have been repeated here, with further detail regarding this experiment where appropriate.

Data were obtained from 13 acutely prepared adult hooded Lister rats (325-515 g). The stimulating electrode was placed vertically into S1Bf (AP 1.8-2.56 ML 4.2-5.4) 1.5-1.8 mm below dura. The multiunit electrode/cannula (filled with BMI, 100 ng/µl saline; Sigma) was introduced vertically into the lateral intermediate layers of SC (AP 6.04-6.8 mm caudal to bregma; lateral 1.8-2.4 mm; dorsoventral 4.7-5.4 mm below dura). DA neurons were recorded from SNc (AP 4.6-6.04 mm caudal to bregma, lateral point of surface entry 2.2-4.4 mm. Single units were recorded between 8.3 mm and 9.7 mm below the contralateral entry point of the 35º angled trajectory.

The experimental procedure is described in chapter 2. Electrical stimulation consisted of single pulses of current to the barrel cortex (1 mA, 100 µs). The responses to cortical stimulation and the effects of SC activation were tested on 1-3 SNc neurons in a single subject. See chapter 2 for a description of the histological procedures used in the present chapter. Analyses were performed using the methods as described in chapter 2.

3.4.2 Data analysis

Differences between groups were assessed with Student's or Welch's t-tests for normally distributed data and Wilcox's tests for non-normal data. See the Methods chapter for more detail. For inferential tests, although the precise p values are given, the two-tailed significance threshold is taken as $p<0.05$, unless otherwise stated.

Data were collected, processed and analysed as described in the Methods chapter. Also, see Methods chapter for definitions of "baseline" and "background" activity, and "response magnitude" as used here.

Data were collected and processed as described in chapter 2. PTSHs were created for DA responses to the light flash and cortical stimulation for the block of pre-BMI trials, and for the trials where there was evidence of BMI induced change in activity in SC, as defined in chapter 2. In order for a DA neuron to be defined as responsive by the criteria described in chapter 2, there has to be a change in the firing rate that exceeds the natural variance in the firing rate of the cell. However, DA neurons may show reliable changes in firing rate in response to a stimulus that do not reach the thresholds described above. To test whether DA neurons showed responses that were not detected by the criteria described in chapter 2, a cumulative sum (CUSUM) method of analysing responses was used. The CUSUM has been "applied to peristimulus histograms to reveal small changes in the probability of spike

occurrences normally obscured by random fluctuations” (Ellaway, 1978). The precise application of CUSUM analysis used here is based on Tepper et al. (1995) and Ji and Shepard (2007). CUSUMs were generated from PSTHs of DA neuron responses with 1 ms bin widths. A change in firing was defined as a greater than 30% change in the slope of a linear fit line of 30 ms blocks of data. Onset latency and duration of responses were defined using the points of intersection between the fit lines of adjacent blocks of activity. If no significant deflection was detected within 260 ms of stimulation, then the neuron was deemed non-responsive. The end of the response period was selected as 260 ms after stimulation to encompass the entirety of the DA response as described by Hudgins (2010). When response onsets and offsets were determined from the CUSUM, they were applied to a PSTH with 20 ms bins to calculate peak amplitude and latency. The process is illustrated in Figure 3-2.

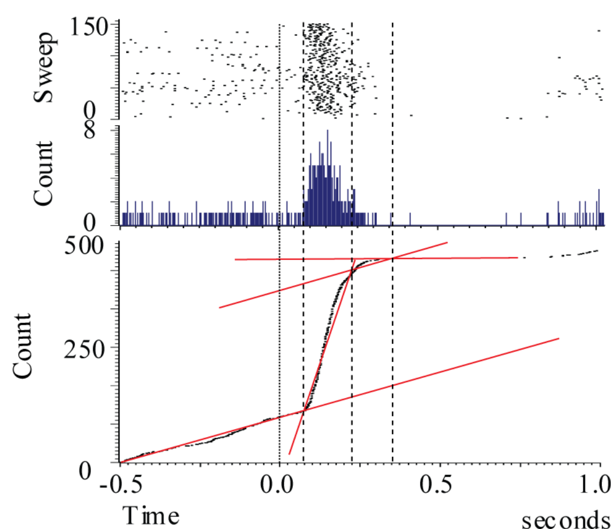


Figure 3-2 Illustration of the CUSUM method of determining response onset and duration. Top: A PSTH of the response is constructed using 1 ms bins. Bottom: A CUSUM plot is constructed by plotting a running total of the sum of the bins (black dots). Linear fits are plotted from the data (red lines), slopes exceeding ±30% of the slope of the fit line of prestimulus data indicates a significant change in the activity, and the two lines are plotted. The points at which the lines intersect defines the response onset/offset (dashed lines). These times can then be transferred back to the PSTH. Although the method was used to detect subthreshold responses, the process is demonstrated here on a large response for clarity.

3.4.3 Optical imaging spectroscopy procedure

In order to examine whether the activation produced by direct stimulation of the cortex was contained within the barrel field, optical imaging spectroscopy (OIS) was used to measure the spread of activation produced by stimulation, and to compare the

haemodynamic response to direct cortical stimulation to the response to whisker pad stimulation. The methods used here for OIS are similar to those used in Boorman et al. (2010).

Three Female Hooded Lister rats (230–330 g) were kept in a 12 h dark/light cycle at a temperature of 22°C, with food and water supplied ad libitum. Animals were anesthetized with an intra-peritoneal injection of urethane (1.25 g/kg), additional doses of 0.1 ml of urethane were administered if required. Atropine was also administered subcutaneously at 0.4 mg/kg to lessen mucous secretions during surgery. Temperature was maintained at 37°C using a homoeothermic blanket (Harvard Apparatus) through rectal temperature monitoring during surgery and experimental procedures. The animals were tracheotomized, allowing the animal to be artificially ventilated and end-tidal CO_2 to be recorded. Blood gas measurements and end-tidal CO_2 measurements were taken to allow correct adjustment of ventilator parameters to keep the animal within normal physiological limits. Both the left and right femoral arteries and veins were cannulated to allow the measurement of mean arterial blood pressure (MABP) and drug infusion. Phenylephrine was infused at 0.13– 0.26 mg/h to maintain MABP between 100 and 110 mmHg. Physiological parameters were continuously monitored and maintained within normal ranges [pO2 = 94.9 ± 2.9 (SE) mmHg; pCO2 = 32.1 ± 1.6 mmHg; arterial blood saturation = 97.8 ± 0.25% (mean ± SE)].

Platinum stimulation electrodes insulated to within 2 mm of the tip were inserted into the whisker pad. To ensure the majority of the whisker pad was stimulated, electrodes were inserted in a posterior direction between rows A/B and C/D of the left whisker pad of the rat. The animals were placed in a stereotaxic frame (Kopf Instruments). The skull overlying somatosensory cortex was thinned to translucency with a dental drill. The skull surface was cooled with saline during drilling. A circular plastic 'well' (20 mm diameter) was positioned over the thinned area of the skull and attached with dental cement. To reduce specularities reflecting from the skull surface the well was filled with saline. A small hole was punctured in the thinned skull and a bipolar stimulating electrode (NEX-100, Rhodes Instruments) was introduced perpendicular to the cortical surface and to a depth of 1500 µm.

A Dalsa 1M30P camera operating in 4 × 4 binning mode recorded the images with each pixel representing 75 × 75 µm of the object. The camera's quantum

efficiency was 28% at 500 nm. To generate spatial maps of cortical hemodynamic responses, 2D-OIS was performed using a Lambda DG-4 high-speed filter changer (Sutter Instrument Company, Novato, CA). The 4 wavelengths were specifically chosen as 2 pairs (495 ± 31 nm FWHM and 559 ± 16 nm FWHM; 575 ± 14 nm FWHM, and 587 ± 9 nm FWHM) such that each pair had a similar total absorption coefficient (therefore sampled the same tissue volume) but had absorption coefficients for oxyhemoglobin (HbO_2) and deoxyhemoglobin (Hbr) that were as different as possible to maximize signal-to-noise ratios. The frame rate of the camera was 32 Hz, which was synchronized to changes between the filters. This gave an effective frame rate of 8 Hz for each wavelength and corresponding frequency estimates of hemodynamic changes. Spectral analysis was based upon the path length scaling algorithm (PLSA) (Berwick et al., 2005, 2008). Briefly, the algorithm used modified Beer-Lambert Law with a path length correction factor. We estimated the concentration of haemoglobin in tissue at a concentration 104 μM based on previous measurements (Kennerley et al., 2005) and saturation was calculated on a pixel by pixel basis (Berwick et al., 2008). The spectral analysis produced 2D images over time, of HbO_2, Hbr, and total blood volume (Hbt). The effects of electrical stimulation of the barrel cortex and whisker pad on intrinsic signal haemodynamics were measured with 2D-OIS. The electrical stimulation parameters used for whisker pad stimulation were 1.2 mA, frequency 5Hz for 2 s. A 5 Hz stimulation frequency is known to result in the greatest magnitude of hemodynamic responses in the somatosensory cortex of the anesthetized rat preparation (Martin et al., 2006), without producing a change in MABP, partial pressure of CO_2 or heart rate. Each experiment consisted of 30 trials separated by an interval of 26 s. Electrical stimulation of the whisker pad was compared to 60 trials of direct intracortical stimulation with single pulses of 1 mA, separated by an interval of 26 s.

3.4.4 OIS Data analysis

Data analysis was performed using MATLAB (The Mathworks). The first stage of the statistical analysis was to determine the centre of an area of activation determined using the general liner model (GLM) SPM approach (Friston et al., 1991). The time series of each pixel was compared with a design matrix of a DC offset, and a square wave representing the hemodynamic response function. This allowed voxel-by-voxel calculation of activation z-scores. The spatial distribution of activation was

determined by plotting the region of action exceeding a z-score threshold, and the centre point of this area was determined using a MATLAB script written by Dr Luke Boorman and Dr Samuel Harris (see Figure 3-4).

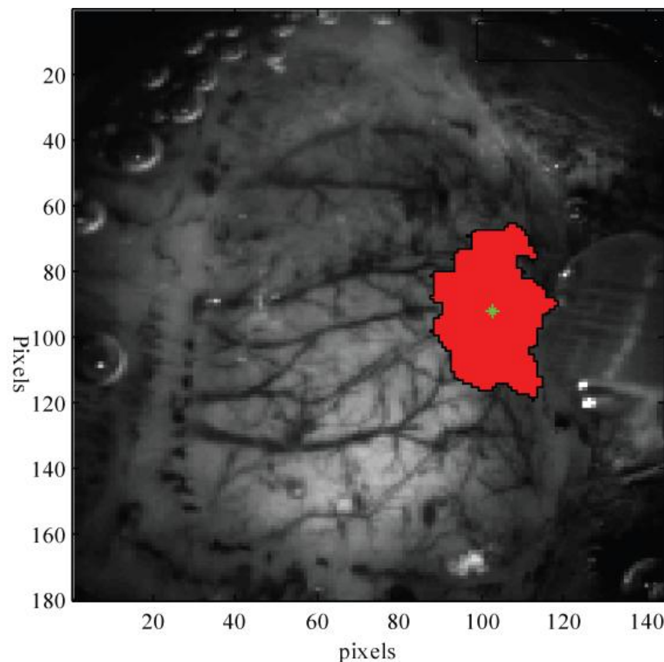


Figure 3-4 An illustration of the output of the analysis script, which determined the centre of a z-score thresholded area of activation (red). The centre is indicated by the green star. Activation has been plotted on a reference photograph of the thinned cranium. The midline suture is visible at the left edge of the figure. Lambda is toward the bottom of the figure, and bregma towards the top.

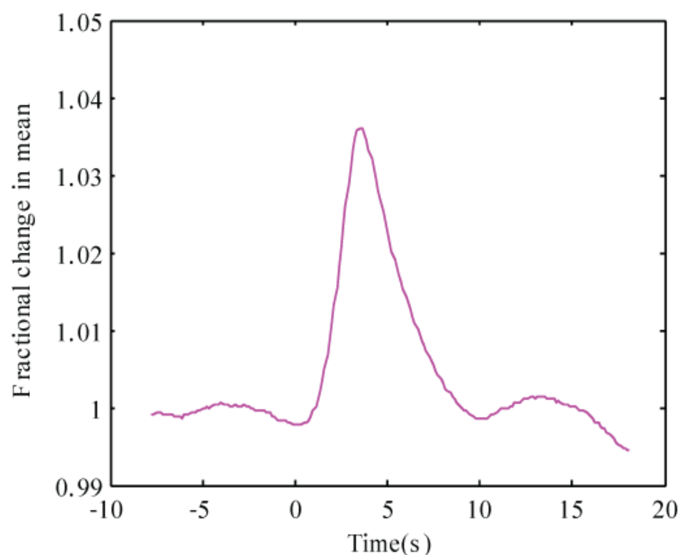


Figure 3-3 An example mean haemodynamic response to 30 trials of 2 s, 5 Hz, 1.2 mA electrical whisker pad stimulation for the z-score thresholded region indicated in Figure 3-4

Figure 3-3 illustrates a typical time course of a haemodynamic response. It shows the haemodynamic activity of the region shown in Figure 3-4 over time, plotted as a fractional change of the mean pre-stimulus activity. The centre point of the

spatial distribution was used for the second stage in the analysis – determining the spatial extent of the haemodynamic response. The area around the centre of activation was divided into a series of concentric circular regions.

Figure 3-5 shows an illustration of the concentric circular regions plotted on a heat plot where each voxel represents the change in haemodynamic activity over the course of the response (i.e. integration of the response such as illustrated in Figure 3-3). The haemodynamic activity above pre-stimulus activity for each ring (activity by ring over time shown in Figure 3-6) was integrated, which provided a measure of the haemodynamic response at a given distance away from the centre of activation.

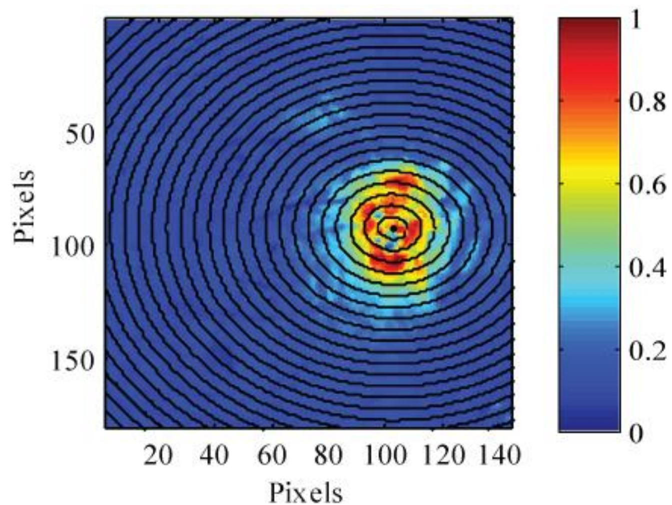


Figure 3-5 An illustration of the concentric circular regions used to quantify the haemodynamic response at increasing distances away from the centre of the area of activation. The circular regions are plotted on a heat plot showing the increase above the mean pre-stimulus haemodynamic activity for the entirety of the haemodynamic response, normalised to central ring.

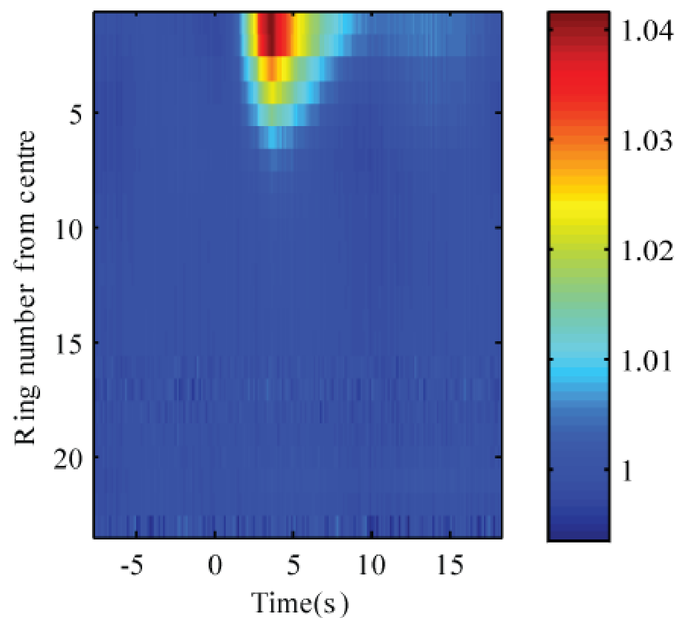


Figure 3-6 Haemodynamic responses as fractional change of mean activity over time by ring. The responses over time are similar to Figure 3-3, although changes in haemodynamic activity are represented as colour. Each row of the figure represents one concentric ring from Figure 3-5, with higher ring numbers further away from the centre of the region of activation.

The haemodynamic response in each ring was averaged across animals, and the average response for each ring was plotted for electrical whisker pad stimulation and cortical stimulation to produce a distance-decay curve.

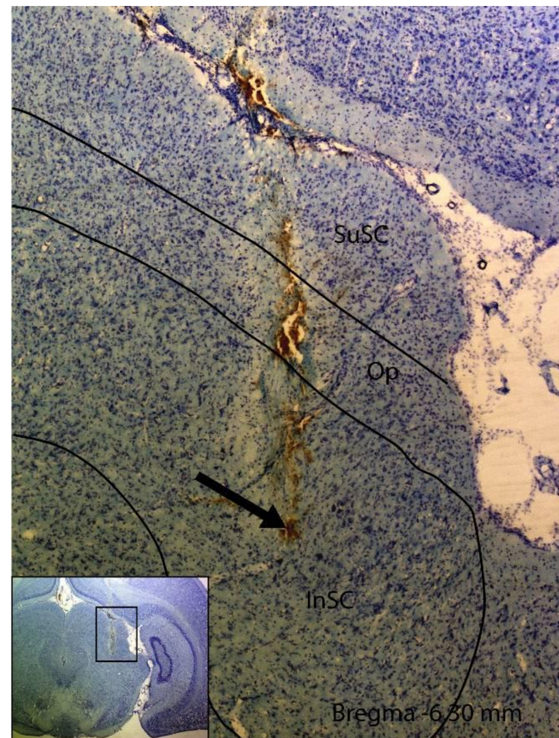
3.5 Results

3.5.1 Inclusion criteria

To be included in the analysis, putative DA neurons had to meet the following criteria: DA neuron recordings made in TH+ regions of the ventral midbrain, SC recordings were confirmed to have been made in intermediate or deep layers of SC, and the stimulation electrode was confirmed to have been placed within S1Bf without impinging on the underlying fibre tracts. A successful injection of BMI into the SC, as judged by the presence of a significant response to light flash stimulus in the SC (see chapter 2), was also required for inclusion of a neuron in the analysis. 24 DA neurons met these criteria. Out of those 24 DA neurons, 8 responded to cortical stimulation before the injection of BMI. These neurons were analysed separately.

Recording sites were taken as the centre of electrolytic lesion or the centre of an iontophoretic injection of Potamine blue dye. Stimulation sites were taken as the ventral extent of the electrode track. Examples of cresyl stained sections showing

Figure 3-7 Coronal section of the SC, processed for cresyl violet. Measurement relative to bregma indicates the location of the section. Arrow indicates electrolytic lesion at the recording/injection site. SuSC: superficial SC (zonal and superficial grey layers); Op: optic layer; InSC; intermediate layer (intermediate grey and white layers).



recording and stimulation sites are shown in Figure 3-7, Figure 3-8 and Figure 3-9.

There was no evidence of stimulation related tissue damage around the stimulation sites in S1Bf.

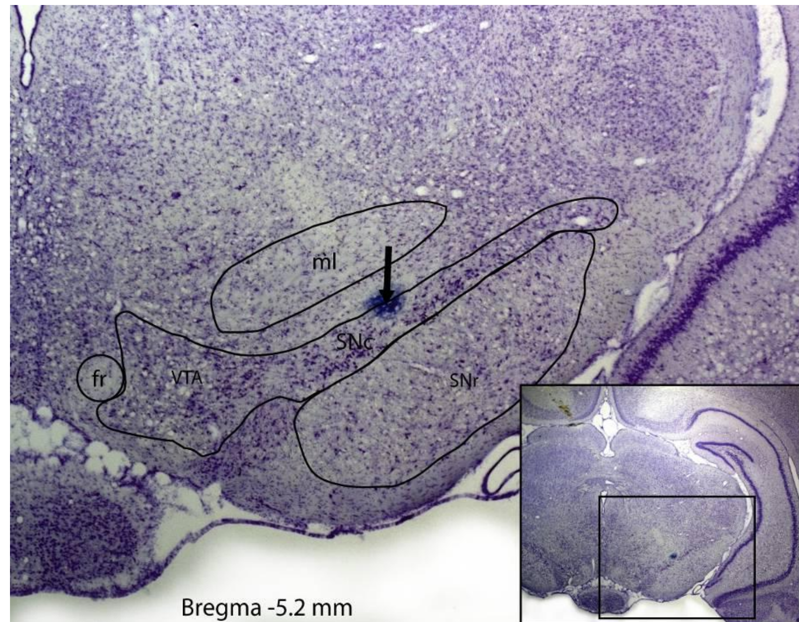


Figure 3-8 Coronal section of the SNc, processed for cresyl violet. Measurement relative to bregma indicates the location of the section. Arrow indicates ejection of pontamine blue dye at the recording site. SNc: substantia nigra pars compacta; SNr: substantia nigra pars reticulata; ml: medial lemniscus; VTA: ventral tegmental area; fr: fasciculus retroflexus.

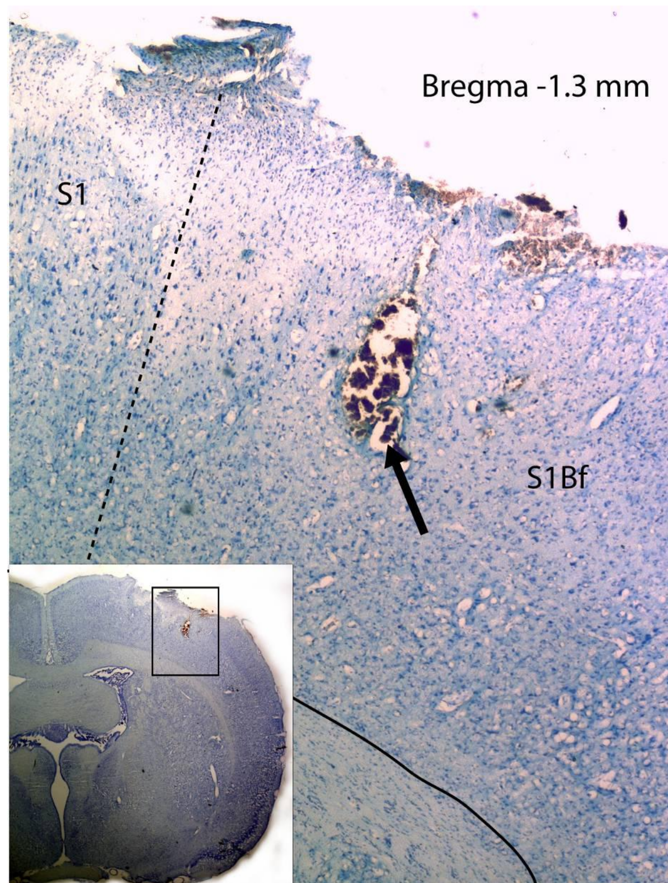


Figure 3-9 Coronal section of the somatosensory cortex, processed for cresyl violet. Measurement relative to bregma indicates the location of the section. Arrow indicates the approximate location of the tip of the stimulating electrode. S1: primary somatosensory cortex; S1Bf: primary somatosensory cortex – barrel field.

The recording location of the DA neurons included in the study, the recording and injection locations in the SC, and the stimulation sites in S1Bf are shown on modified diagrammatic sections from Paxinos and Watson (2004) in Figure 3-10 Reconstructed plots of recording sites in the midbrain on diagrams of coronal sections. Points indicate the approximate location of the DA cell., Figure 3-11, and Figure 3-12.

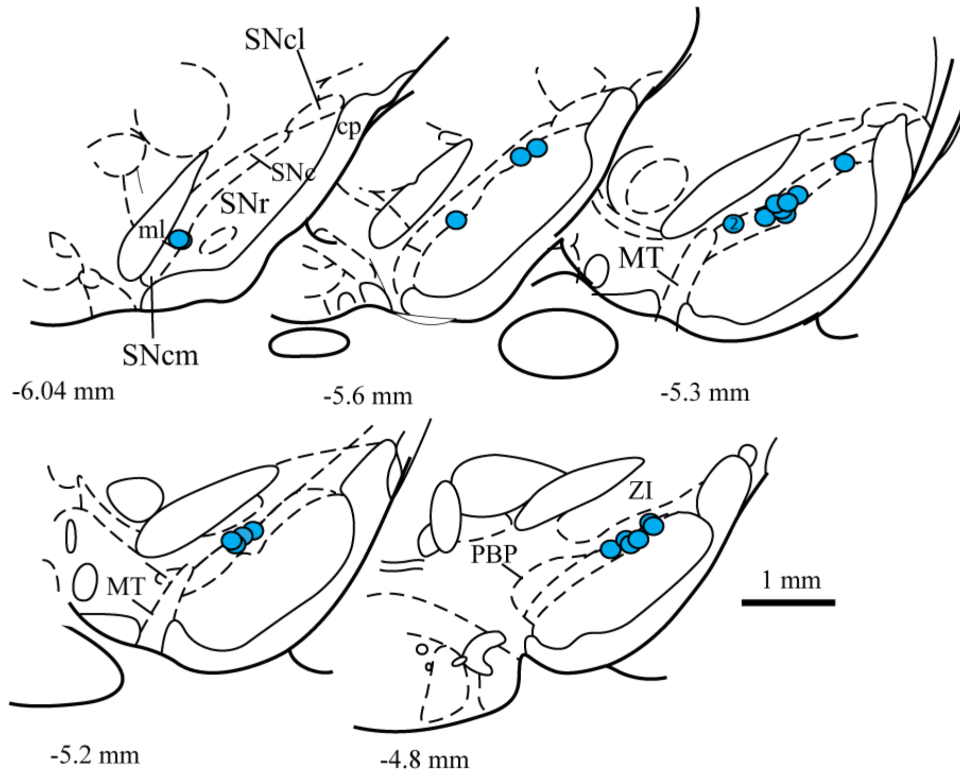


Figure 3-10 Reconstructed plots of recording sites in the midbrain on diagrams of coronal sections. Points indicate the approximate location of the DA cell. The point labelled 2 represents the location of two recorded DA neurons. SNc: substantia nigra pars compacta; SNcl: substantia nigra pars compacta, lateral part; SNcm: substantia nigra pars compacta, media part; SNr: substantia nigra pars reticulate; ml: medial lemniscus; cp: cerebral peduncle; MT: medial terminal nucleus of the accessory optic tract; PBP: parabrachial pigmented nucleus; ZI: zona incerta.

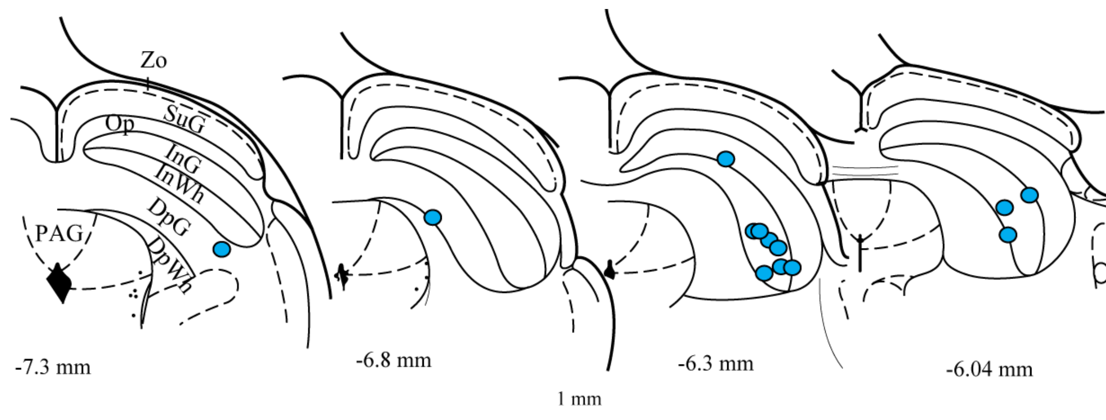


Figure 3-11 Reconstructed plots of recording sites in the midbrain on diagrams of coronal sections. Points indicate the tip position of the electrode-injector assembly. Measurements relative to bregma, and indicate the location of each section. Zo: zonal layer; SuG: superficial grey layer; Op: optic layer; InG: intermediate grey layer; InWh: intermediate white layer; DpG: deep grey layer; DpWh; deep white layer; PAG: periaqueductal grey.

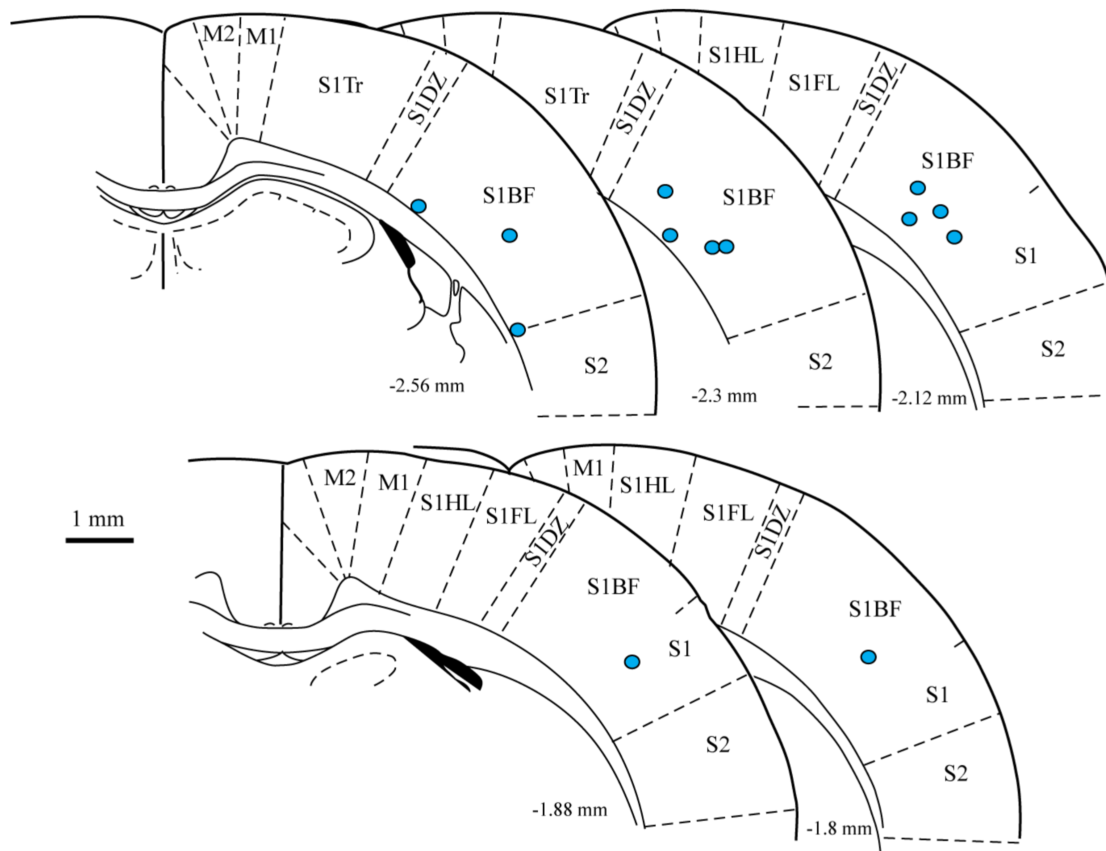


Figure 3-12 Reconstructed plots of stimulation sites in the cerebral cortex. Points indicate the tip position of the stimulation electrode. The exposed pole of the central electrode extends 500 um dorsally from the point indicated, followed by 500 um of insulated electrode, followed by a 500 um exposed section forming the surround electrode. Measurements relative to bregma, and indicate the location of each section. S1: primary somatosensory cortex; S1Bf: primary somatosensory cortex, barrel field; S1DZ: primary somatosensory cortex,

dysgranular region; S1FL: primary somatosensory cortex, forelimb region; S1HL: primary somatosensory cortex, hindlimb region; S1Tr: primary somatosensory cortex, trunk region; S2: secondary somatosensory cortex; M1: primary motor cortex; M2: secondary motor cortex.

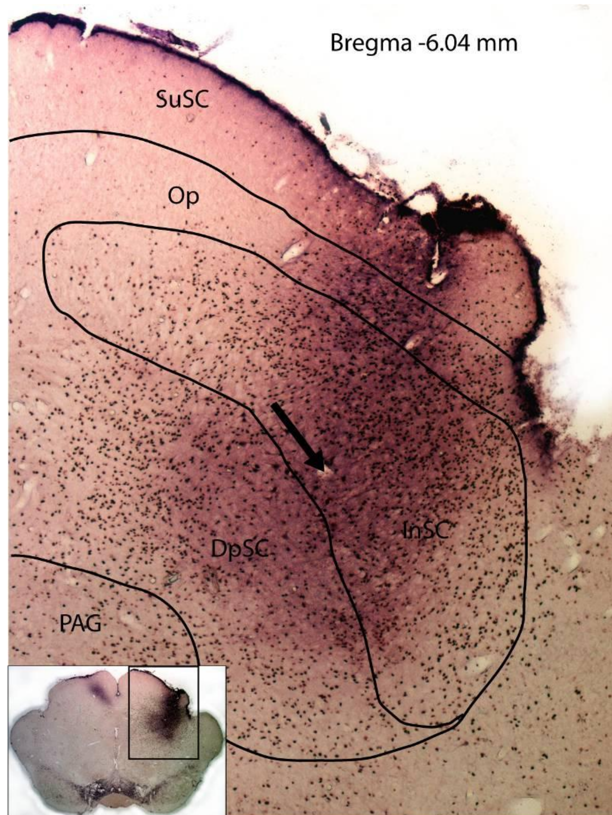


Figure 3-13 Coronal section of the SC processed for TH and c-fos. Section shows FLI (black dots) in SC as a result of neural activity induced by BMI injection. Measurement relative to bregma indicates the location of the section. Arrow indicates electrolytic lesion at the recording/injection site. SuSC: superficial layers of the SC (zonal layer and superficial grey layer); Op: optic layer; InSC: intermediate layers of the SC (intermediate grey and intermediate white layers); DpSC: deep layers of the SC (deep grey and deep white layers); PAG: periaqueductal grey.

Processing for c-fos and TH immunoreactivity was performed in all 13 animals. FLI indicates the expression of c-Fos a protein associated with neural activity (Herdegen and Leah, 1998), and would indicate the extent of the disinhibitory effect

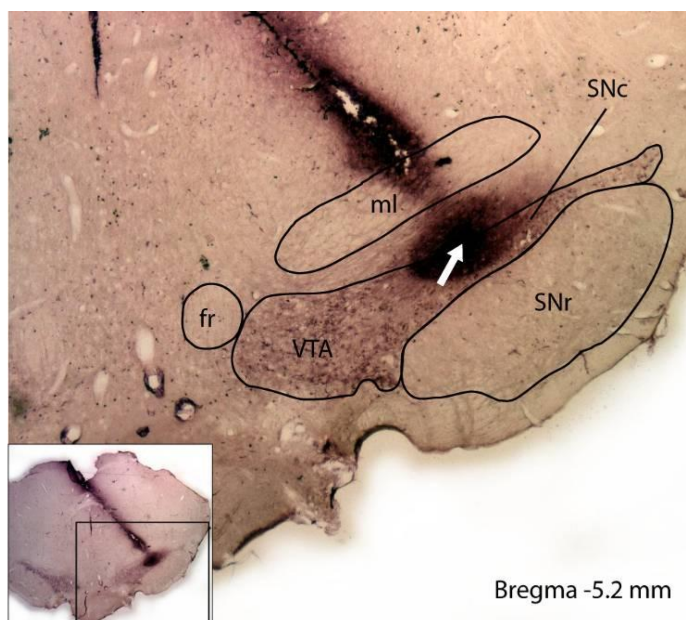


Figure 3-14 Coronal section of the SC processed for TH and c-fos. Section shows TH positive DA neurons (purple cells in SNc and VTA).

Measurement relative to bregma indicates the location of the section. Arrow indicates recording site.

SNc: substantia nigra pars compacta; SNr: substantia nigra pars reticulata; VTA: ventral tegmental area; ml: media lemniscus; fr: fasciculus troflexus.

of BMI. When injections were made within the intermediate and deep layers of the SC, FLI was largely contained within the SC (see Figure 3-13). This is supported by previous experiments using comparable protocols (Coizet et al., 2003).

Only recordings from putative DA neurons sited in TH+ regions of the midbrain were included for analysis. An example of TH immunoreactivity can be seen in Figure 3-14. The distribution of Fos-like immunoreactivity (FLI) was used as an indication of the spread of activation as a result of BMI injections.

3.5.2 Activity in the superior colliculus

To assess the effect of sensory stimulation on general SC activity without the presence of BMI, the mean background activity in the 500 ms before the light flashes in the block of pre-BMI stimulations was compared to the mean baseline activity in the 60-120 seconds before the start of any stimulation.

There was no significant effect of the stimulation on spontaneous activity (M_{base} = 227.9±11.4 Hz; $M_{prebkgd}$ = 223.2±13.4 Hz; $t = 1.23$, $df = 15$, $p > 0.05$). Across all 16 records, BMI had a significant positive effect on background collicular activity, ($M_{prebkgd}$ = 223.2±13.4 Hz; $M_{postbkgd}$ = 349.9±42.1 Hz; $t = -3.17$ $df = 15$, $p = 0.006$). Examination of the records shows that following injection of BMI, most records (12/16) showed at least a 10% increase in background activity. Two out of the remaining four showed at least a 10% decrease. However, there was nothing to indicate any difference between BMI injections causing an increase in spontaneous activity and those showing a decrease, and both increases and decreases in activity were seen in different recordings in the same animal.

Throughout the pre-BMI trials, there was no phasic response to the light in the intermediate and deep SC. There was, however, a short latency (onset latency: 2.6±0.4 ms, peak latency: 6.4±0.6 ms) short duration (17.9±1.6 ms) response to cortical stimulation (an example is shown in Figure 3-15). Mean peak amplitude above background firing rate was 1256.7±144.7 Hz.

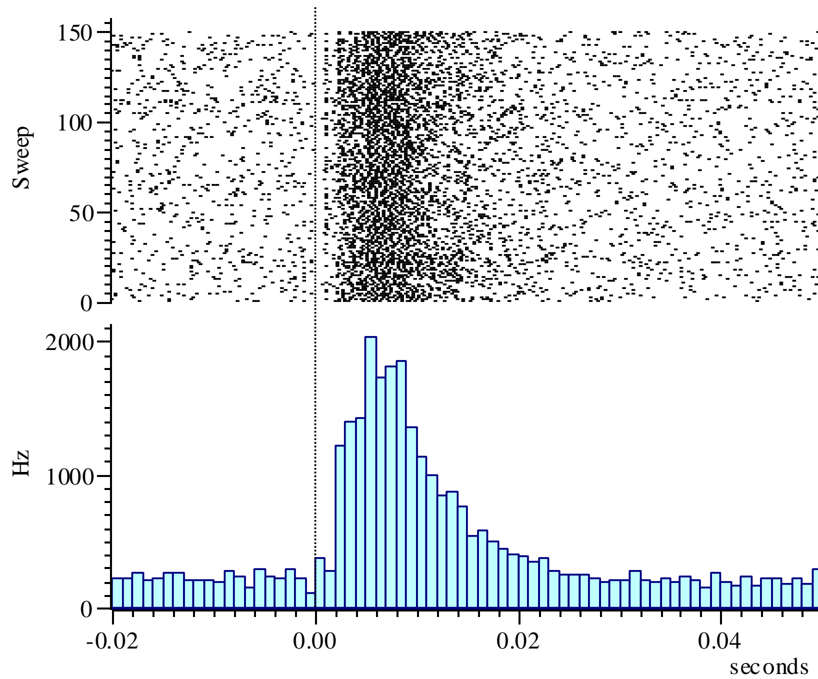


Figure 3-15 PSTH/raster plot of SC activity in response to a single 1 mA pulse stimulation to S1Bf. Stimulation occurs at 0.0 s.

Following intracollicular injection of BMI, a phasic excitatory response to the light flash was seen in all 16 records (onset latency: M = 49.1±3.4 ms; duration: M = 177.6±16.6 ms). Across all 16 records, BMI had a significant positive effect on the magnitude of collicular response in the 200 ms following cortical stimulation, (M_{premag} = 25.0±9.9 Hz; $M_{postmag}$ = 122.9±41.7 Hz; t = -2.63, df = 15, p = 0.019) and response duration (M_{predur} = 17.9±1.6 ms; $M_{postdur}$ = 64.1±16.0 ms; t = -3.06, df = 15, p = 0.009) but not peak amplitude (M_{preamp} = 1256.7±144.7 Hz; $M_{postamp}$ = 1230.75±98.63 Hz; t = 0.28, df = 15, p > 0.05). There was no significant change in onset latency ($M_{preonset}$ = 2.6±0.4 ms; $M_{postonset}$ = 2.8±0.5 ms; t = -0.62, df = 15, p > 0.05) or peak latency ($M_{preplat}$ = 6.4±0.6 ms; $M_{postplat}$ = 8.3±0.7; t = -2.05, df = 15, p > 0.05) of the collicular response to cortical stimulation following injection of BMI. Post-BMI SC responses to a light flash had significantly longer durations ($M_{lightdur}$ = 177.6±16.6; M_{ctxdur} = 64.1±16.0; t = 8.26, df = 15, p < 0.001) magnitudes ($M_{lightmag}$ = 308.5±47.5 Hz; M_{ctxmag} = 122.9±41.7; t = 7.85, df = 15, p < 0.001) onset latencies ($M_{lightonset}$ = 49.3±3.4 ms; $M_{ctxonset}$ = 2.8±0.5 ms; t = 14.83, df = 15, p < 0. 001) compared to post-BMI responses to cortical stimulation.

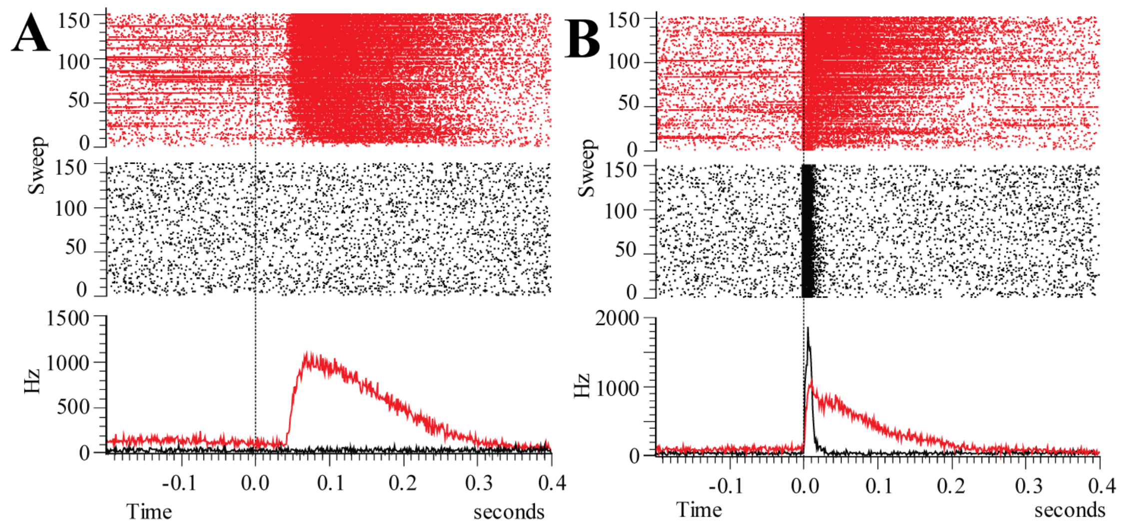


Figure 3-16 shows a typical response of the SC to light flash and cortical stimulation before, and after injection of BMI.

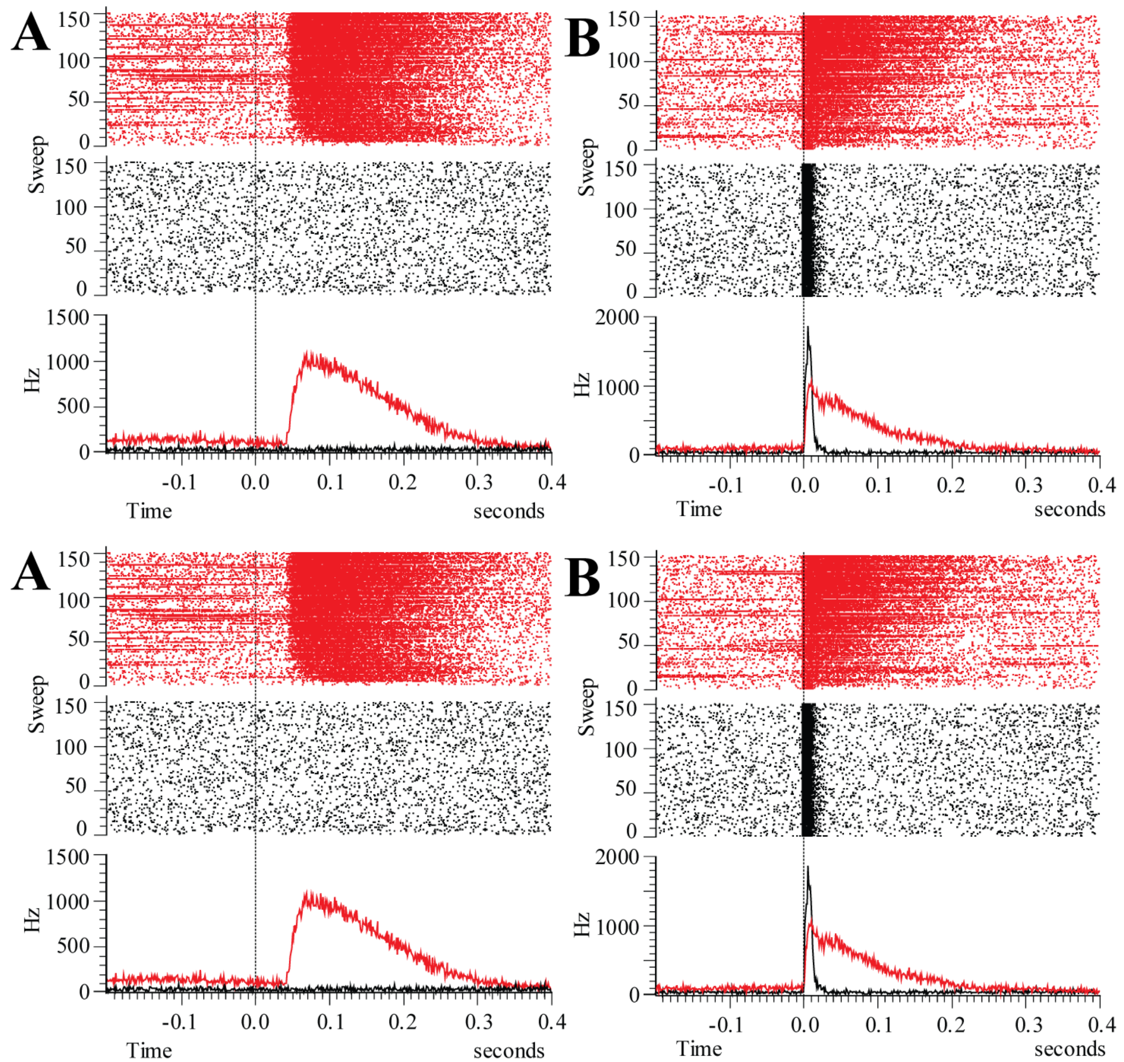


Figure 3-16 PSTH/raster plots of SC MUA in response to light flash (A) and cortical stimulation (B) before (black) and in the presence of a local microinjection of BMI (red). Vertical cursor indicates stimulus onset at 0.0 s.

3.5.3 Activity of DA cells unresponsive until BMI injection

There was no significant difference in the DA neuron activity in the baseline period and the background activity in pre-BMI trials (M_{base} = 3.4±0.5 Hz; M_{prebkg} = 3.5±0.4 Hz; t = -0.36, df = 15, p > 0.05). Across all 16 records, there was an increase in background DA neuron firing rate with BMI injection, but this did not reach significance, (M_{prebkg} = 3.5±0.4 Hz; $M_{postbkg}$ = 3.8±0.5 Hz; t = -2.11, df = 15, p > 0.05).

Overall, during periods of significant effect of BMI in SC, 14/16 (82.4%) DA neurons showed a significant response to the light flash. Of those 14, 9 also showed a response to cortical stimulation. On average, onset latencies of DA neuron responses reliably followed SC responses to both light flash (M_{SC} = 49.3±3.4 ms, *n* = 16; M_{DA} = 84.8±6.2 ms, *n* = 14; t = -5.44, df = 18.90, p < 0.001) and cortical stimulation (M_{SC} = 2.8±0.5 ms, *n* = 16; M_{DA} = 30.0±9.4 ms, *n* = 8; t = -2.89, df = 8.04, p = 0.020). Onset latencies of DA neuron responses to a light flash were significantly longer than those of DA neuron responses to cortical stimulation (M_{light} = 84.8±6.2 ms, *n* = 14; M_{ctx} = 30.0±9.4 ms, *n* = 8; t = 5.12, df = 14.67, p < 0.001). As onset latencies of SC responses to cortical stimulation were much shorter than SC responses to light, this may have an effect on DA response latencies. Examination of the onset latency of DA responses to light flash and cortical stimulation, minus the latency of the SC response to the same stimulus, showed there was no significant difference between the two stimuli (M_{light} = 40.3±7.2 ms, *n* = 14; M_{ctx} = 27.2±9.4 ms, *n* = 8; t = 1.10, df = 16.35, p > 0.05) (see Figure 3-17A).

Records were examined to see if there was any difference in duration between responses to the two modalities. There was no significant difference between the durations of DA neuron responses to each stimulus (M_{light} = 168.9±20.5 ms, *n* = 14; M_{ctx} = 148.8±25.6 ms, *n* = 8; t = 0.61, df = 15.45, p > 0.05). There were no significant differences between durations of DA neuron responses and the durations of the corresponding SC responses to light flash (M_{SC} = 192.8±16.5 ms; M_{DA} = 168.9±20.5 ms; t = 1.03, df = 13, p > 0.05, *n* = 14), or cortical stimulation (M_{SC} = 87.8±26.1 ms; M_{DA} = 148.8±25.6 ms; t = -1.69, df = 7, p > 0.05, *n* = 8) (see Figure 3-17A). There was no significant difference between absolute magnitudes of responses of DA neurons to each stimulus (M_{light} = 3.4±0.9 Hz, *n* = 14; M_{ctx} = 3.2±1.0, *n* = 8; t = 0.21,

df = 19.57, p > 0.05) (see Figure 3-17C), although there was still a significant difference between the corresponding response magnitudes ($M_{lightmag}$ = 339.1±48.5 Hz, n = 14; M_{ctxmag} = 156.2± 57.7 Hz, n = 8; t = 2.43, df = 16.06, p = 0.027) (see Figure 3-17B) and response durations ($M_{lightdur}$ = 192.8±16.5 ms, n = 14; M_{ctxdur} = 87.8±26.1, n = 8; t = 3.50, df = 11.51, p = 0.004) (see Figure 3-17A) in the SC.

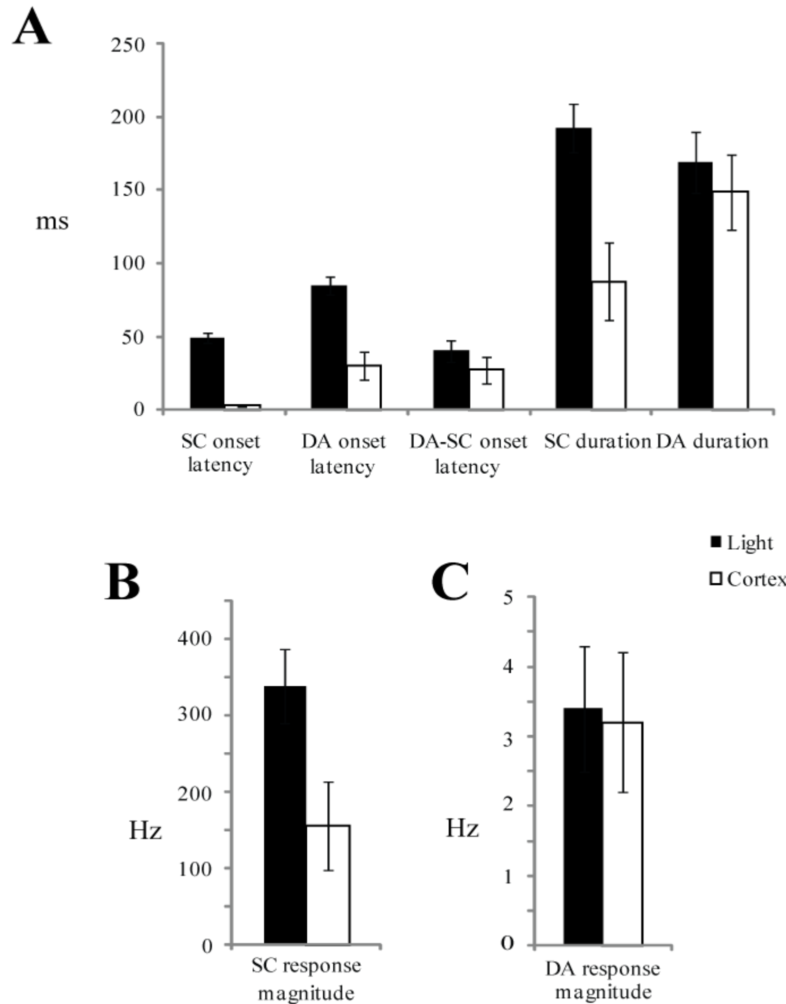


Figure 3-17 Comparisons of collicular and DA neuron response onset latencies and durations (A), collicular response magnitudes (B) and DA response magnitudes (C) to a light flash (black) and cortical stimulation (white).

3.5.4 Differentiating between inhibitory and excitatory responses

Out of all sixteen DA neurons, 50% (8/16) showed responses with an excitatory first component to the light flash, four of which also responded to cortical stimulation, all in the same direction (Figure 3-18, bottom). Thirty-seven point five percent (6/16) showed responses with an inhibitory first component, four of which also responded to cortical stimulation, all in the same direction (Figure 3-18 top). The remaining 12.5% (2/16) showed no significant response to either stimulus.

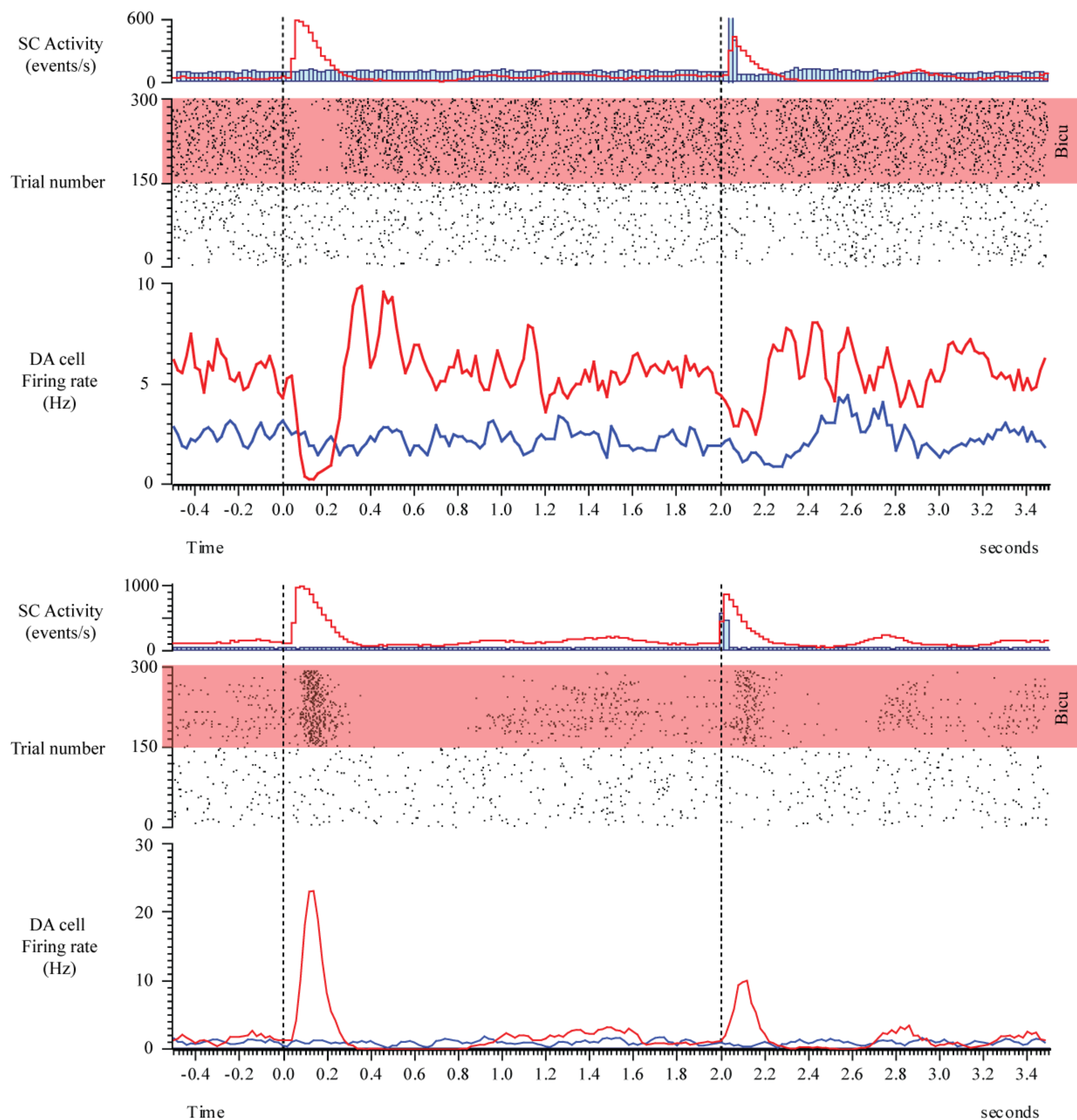


Figure 3-18 Raster plots of raw data and line plots of smoothed data from two DA neurons demonstrating excitatory (top) and inhibitory (bottom) responses to interleaved light flash (onset 0.0 s) and cortical stimulation (onset 2.0 s) before (un highlighted raster and blue line) and in the presence of (red highlighted raster and red line) local microinjections of BMI.

Records were examined to see if there were differences between DA neurons showing excitatory first phases and inhibitory first phases that might suggest the existence of separate sub-populations. Given that the first phase of responses to both stimuli in DA neurons that became responsive after BMI was in the same direction, DA neurons were categorised as excited and inhibited based on the first phase of their response to the light flash stimulus. Unpaired t-tests suggest that there is no significant difference between the baseline firing rates of cells which were excited or inhibited (M_{ex} = 3.1±0.7 Hz, n = 8; M_{in} = 3.6±0.7 Hz, n = 6; t = -0.48, df = 9.74, p >

0.05). There was a significant difference between action potential sizes as measured by the time from onset of the spike to the first trough (M_{ex} = 1.3±0.04 ms, n = 8; M_{in} = 1.5±0.04 ms, n = 6; t = 4.458, df = 9.64, p < 0.001). All cells met the criteria proposed by Ungless et al. (2004) of onset-trough measurement greater than 1.1 ms to safely exclude non-DA neurons. There was a significant difference in measurements of total spike widths between DA neuron showing responses with excitatory first phases and those showing responses with inhibitory first phases (M_{ex} = 4.0±0.08 ms, M_{in} = 4.9±0.1 ms, t = -5.46, df = 6.99 p < 0.01), although total spike widths were strongly associated with onset-trough measurements. Typical waveforms of excited, inhibited and unresponsive neurons are shown in Figure 3-19. Average spike shapes showed prominent initial segment spikes (Grace and Bunney, 1983) on 8/15 excited cells, but only 1/8 inhibited cells, and neither of the unresponsive cells.

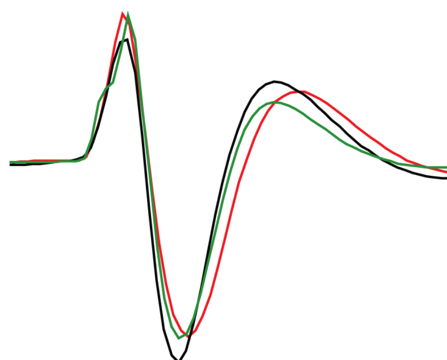


Figure 3-19 Waveform averages of typical spikes from DA neurons which showed excitatory (green) or inhibitory (red) responses to stimuli after injection of BMI, and a neuron which was unresponsive (black), aligned to spike onset.

DA neuron response characteristics were also examined to see if responses with excitatory first phases and inhibitory first phases might be the product of different inputs. There were no significant differences between DA neuron response onset latency ($M_{exlight}$ = 80.3±7.6 ms, *n* = 8; $M_{inlight}$ = 103.3±8.0 ms, *n* = 6; t = -2.08, df = 11.45, p > 0.05; M_{exctx} = 22.5±12.5 ms, *n* = 4; M_{inctx} = 40.0±17.8, *n* = 4; t = -0.80, df = 5.38, p > 0.05) or duration ($M_{exlight}$ = 136.8±19.6 ms, *n* = 8; $M_{inlight}$ = 221.7±34.5 ms, *n* = 6; t = -1.89, df = 8.14, p > 0.05; M_{exctx} = 150.0±37.2 ms, *n* = 4; M_{inctx} = 147.5±40.9 ms, *n* = 4; t = -0.05, df = 5.95, p > 0.05) to either stimulus.

3.5.5 Coincident spontaneous bursting in SC and DA

As well as stimulus evoked activity, injection of BMI could also produce spontaneous bursts of activity in the SC that were associated with increases in activity

in the DA neuron. Figure 3-20 shows an example of association between SC and DA neuron activity. Bursts of spikes in DA neurons (as defined by Grace and Bunney (1984a)) occurred alongside bursts of activity in the SC. Examination of the timing of bursts in a DA neuron (lower waveform, black arrows on Figure 3-20) showed that they followed an increase in the amplitude of SC activity (upper waveform), and a corresponding increase in SC firing rate (rate histogram).

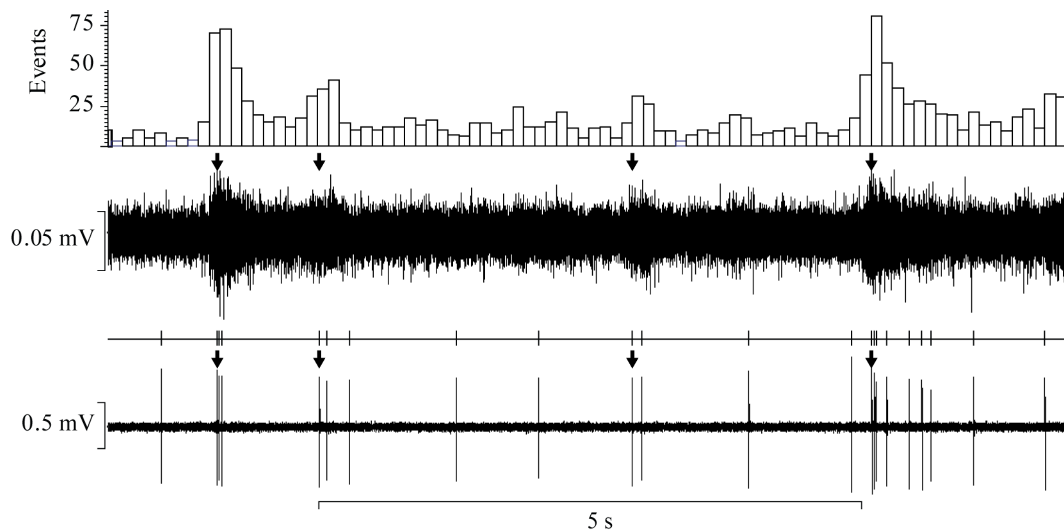


Figure 3-20 An example of spontaneous bursting in SC following intracollicular BMI injection, and associated activity in a DA neuron. From top to bottom: Rate histogram of SC activity (100 ms bins); SC electrophysiological recording; DA spike occurrence, with each vertical line representing one spike; DA neuron electrophysiological recording. Arrows indicate the onset of bursts in the DA neuron, as defined by (Grace and Bunney, 1984a), applied to both DA and SC traces.

3.5.6 Dopaminergic response to non-reinforced, familiar stimuli

A measure of phasic response magnitude was obtained by subtracting DA activity in the 500 ms before a stimulus from the activity in the period 20-260 ms after the stimulus. DA neurons typically habituate to repeated presentations of an unreinforced stimulus. If this response were to habituate, we would expect to see a decrease in the DA response as the stimuli become less effective at exciting or inhibiting the cell.

Figure 3-21 shows a typical time course of the response of the SC and a DA neuron a cortical and light flash stimulation. Rather than habituating, response magnitude for the SC and DA neurons to both light flash and cortical stimulation throughout the course of an experiment increases then returns to baseline in line with the effect of BMI.

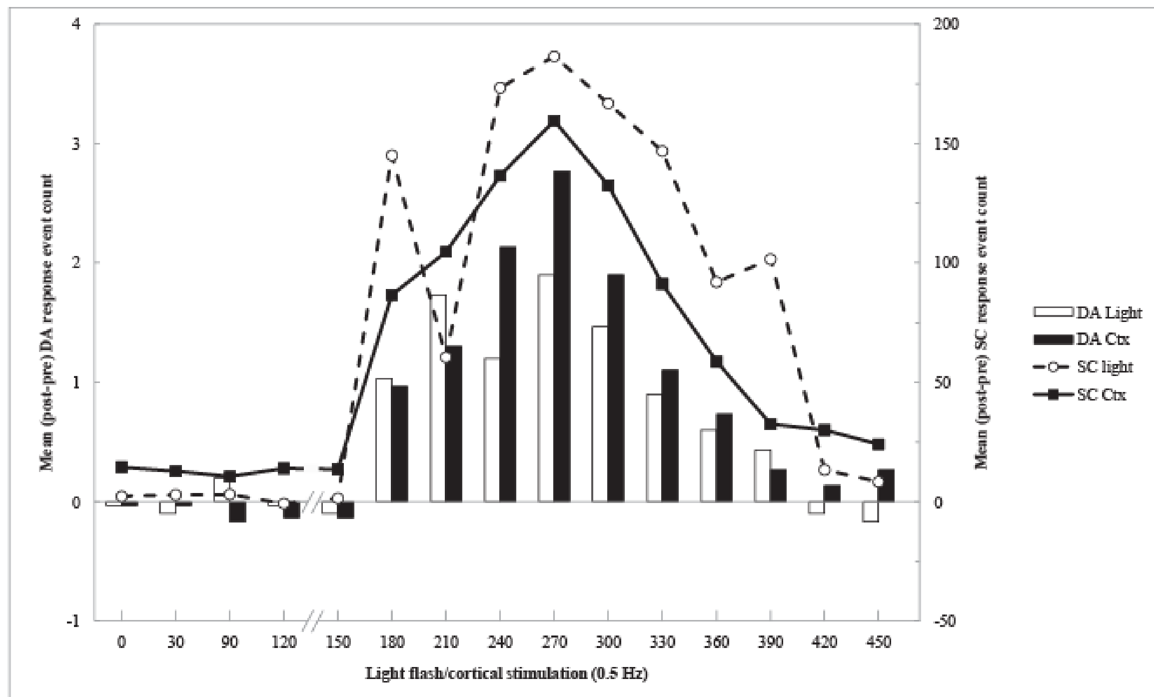


Figure 3-21 Response measured by activity above baseline of a DA neuron and SC across the timecourse of BMI effect.

3.5.7 Activity of DA neurons responding before BMI injection

DA neurons that responded to cortical stimulation before BMI injection were examined to see if they may represent a distinct subpopulation. There were no significant differences between pre-BMI responsive (n = 8) and pre-BMI unresponsive (n = 16) DA neurons on baseline firing rates (M_{resp} = 3.2±0.6; M_{unresp} = 3.4±0.5; t = -0.31, df = 14.18, p > 0.05), or onset-trough spike measurements (M_{resp} = 1.3±0.05 ms; M_{unresp} = 1.4±0.04 ms; t = -1.56, df = 17.08, p > 0.05). The characteristics of SC responses to cortical stimulation in pre-BMI responsive and pre-BMI unresponsive DA neurons were then compared to see if the response was the result of differences in SC responsiveness. There were no significant differences between records of pre-BMI responsive and pre-BMI unresponsive DA neurons on SC response magnitude (M_{resp} = 30.9±14.2 ms, n = 8; M_{unresp} = 25.0±9.9 ms, n = 16; t = 0.34, df = 13.92, p > 0.05), duration (M_{resp} = 18.6±3.8 ms, n = 8; M_{unresp} = 17.9±1.6 ms, n = 16; t = 0.17, df = 9.54, p > 0.05), or peak amplitude (M_{resp} = 1139.6±215.6 Hz, n = 8; M_{unresp} = 1256.7±144.7 Hz, n = 16; t = -0.45, df = 17.08, p > 0.05).

When compared to post-BMI DA neuron responses in neurons that did not respond before BMI (n = 10), pre-BMI DA neuron responses (n = 8) were

significantly shorter (M_{resp} = 78.8±11.7 ms; M_{unresp} = 148.8±22.9 ms; t = -2.49, df = 9.81, p = 0.033), but not significantly different in onset latency (M_{resp} = 56.9±14.8 ms; M_{unresp} = 31.3±9.5 ms; t = 1.41, df = 12.69, p > 0.05) or response magnitude (M_{resp} = 0.9±0.4 Hz; M_{unresp} = 2.8±1.0 Hz; t = -1.89, df = 8.85, p > 0.05).

3.5.8 Effect of interleaved stimulation on response

Throughout the course of recording, it was noticed that after injection of BMI, the activity in the SC preceding the stimulation affected the response to the stimulation. Figure 3-22A shows an example of such activity, where oscillatory activity in the SC preceding cortical stimulation (t = 0) was associated with less activity after the stimulation. The interaction between the two stimuli was tested experimentally by temporarily disabling one stimulus (i.e. presenting one stimulus approximately every four seconds, rather than every two seconds). Figure 3-22 shows an example of this, where turning off the light flash stimulation produced an increased response to cortical stimulation in the SC (not shown) and a corresponding increase in the DA neuron response.

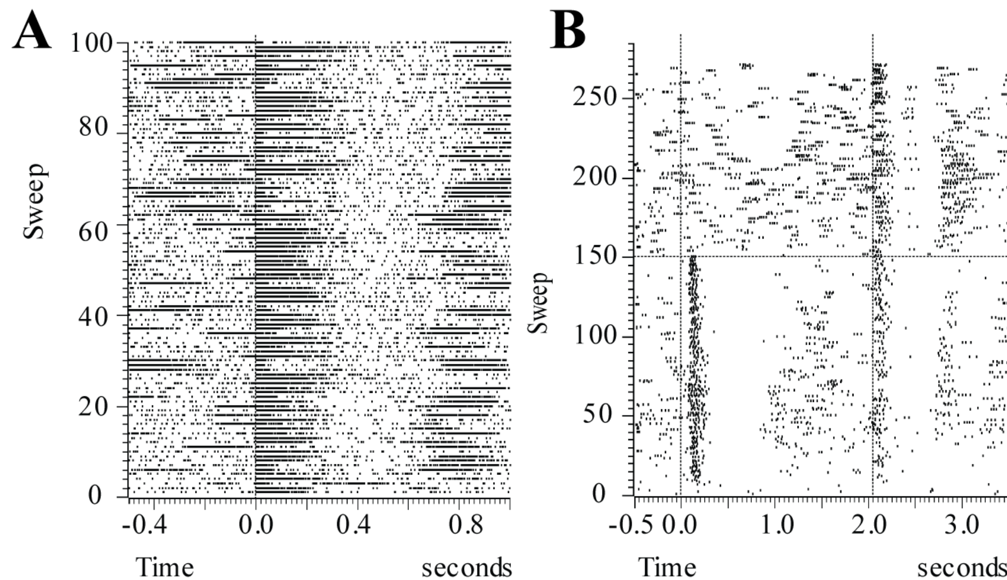


Figure 3-22 Demonstration of the effect of oscillatory activity in the period preceding stimulation disrupting the response to that stimulus (A), and the effect of presenting only one stimulus (B). Vertical cursors indicate the onset of stimulation – cortical stimulation in A, light flash (left) and cortical stimulation (right) in B. Horizontal cursor in B represents the point at which the light flash was disabled.

3.5.9 Optical Imaging

The change in haemodynamic response magnitude over distance was used to compare the effect of intracortical stimulation with electrical whisker pad stimulation.

Figure 3-23 shows the haemodynamic response over distance for intracortical stimulation and electrical whisker pad stimulation. Intracortical stimulation resulted in a lower peak response, but a more steady decay with distance than the response produced by electrical whisker pad stimulation. Single pulse electrical stimulation of the cortex produced a haemodynamic response with a similar spread to the haemodynamic response produced by whisker pad stimulation.

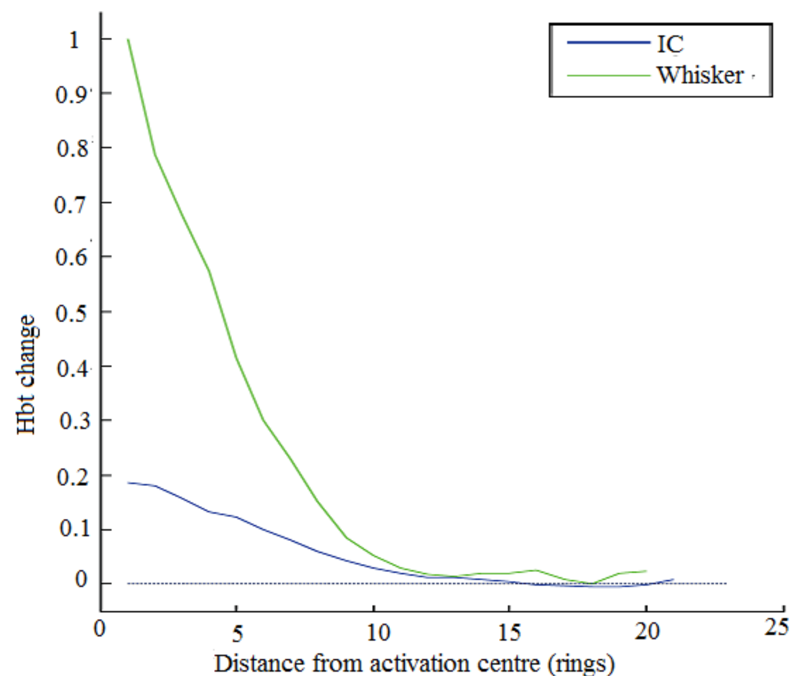


Figure 3-23 Mean haemodynamic response over distance from the centre of activation for intracortical stimulation (blue) and electrical whisker pad stimulation (green) (n=3), normalised to the peak response to whisker pad stimulation.

3.6 Discussion

3.6.1 Summary of findings

The current study indicates that the SC plays a role in relaying somatosensory cortical input to DA neurons in SNc. The findings suggest that local activation of the SC has the ability to modulate the firing rate of presumed DA neurons in SNc. Under urethane anaesthesia, electrical stimulation of somatosensory cortex with a single 1 mA 100 μs pulse produces a short latency, short duration response in the SC. A 10 ms

light flash produces no response. In the majority of DA cells, a response to cortical stimulation is usually absent; although in some cases a significant response can be seen. DA cells do not respond to the light flash. Removal of $GABA_A$ mediated inhibition by local microinjections of BIC in SC, as indicated by responsiveness to a whole field light flash, can increase the response to cortical stimulation. Light flash stimulation can then evoke a response in almost all DA neurons. The response of DA neurons is modulated in the same direction as the response to the light flash.

3.6.2 Discussion of findings

Responsiveness of SC and DA neurons to stimuli

It has demonstrated that under anaesthesia, responses of the SC and DA neurons are suppressed, but return when the suppression is lifted by an injection of BMI into SC (Dommett et al., 2005). In the unanaesthetised animal, responses in the SC and DA neurons habituate if the stimulus is predictable, or it is not associated with reinforcement that maintains its salience (Chalupa and Rhoades, 1977; Ljungberg et al., 1992), although this has not been found to be the case with responses to stimuli during intracollicular injections of BMI (Dommett et al., 2005).

In the current study, SC response to a light flash stimulus was suppressed by the effects of anaesthesia. In response to cortical stimulation, the SC showed a short latency short duration phasic excitation. After a successful local injection of BMI into the SC, all records showed a phasic excitation to the light. In most records, injection of BMI also increased the magnitude of the phasic response to cortical stimulation in the SC. In SNc, all DA neurons were insensitive to the light flash before an injection of BMI. While most DA neurons were similarly unresponsive to a single 1 mA pulse of cortical stimulation, some did show a small response. Following successful BMI injections, many DA neurons became responsive to cortical stimulation. The percentage of DA neurons responsive to a visual stimulus in the present study compares broadly well to studies using a similar paradigm (92% vs 85.7%, Dommett et al., 2005) and to studies with awake animals (81%, Strecker and Jacobs (1985); 75%, Horvitz et al. (1997); 75%, Schultz and Romo (1990). The response rate of DA neurons to cortical stimulation has not been established, and papers reporting the presence of responses in DA neurons to presentation of somatosensory stimuli (e.g. Freeman et al. (1985)) have not reported the proportion of responsive cells. However, the proportion of DA neurons that responds to auditory stimuli is similar to the

proportion that responds to visual stimulation (85%, Strecker and Jacobs (1985); 87% Horvitz et al. (1997)) suggesting that the proportion of responsive cells is consistent across modalities. Thus, it seems likely that a similar proportion of DA neurons will respond to somatosensory stimulation. In the present study, 63% of neurons responded to cortical stimulation, which is a similar, although slightly lower percentage than to sensory stimuli.

Direction of DA neuron responses

In the present study, DA neuron responses with both excitatory and inhibitory first phases were seen to both cortical stimulation and a light flash. The ratio of DA neurons showing excitatory first phases to those showing inhibitory first phases (slightly less than 2:1 for both cortical stimulation and light flash) is slightly higher than that found previously using a similar experimental paradigm (17:13 for visual stimulation, Dommett et al. (2005)), and slightly less than that found in some studies in awake animals (e.g. 16:7 for visual stimulation, 16:6 for Strecker and Jacobs (1985)) but notably lower than others (8:1 for visual stimulation, 11:2 for auditory stimulation, Horvitz et al. (1997); approximately 10:1 for combined visual/auditory stimulation, Schultz (1986)) The reason for this difference is not clear, although it should be noted that the lowest ratio of excited to inhibited cells was obtained in anaesthetised animals, while the highest ratio was obtained during a behaviourally motivated task, which may suggest some form of modulation of responsiveness related to behavioural state.

Response direction does not indicate separate populations of neurons

Previous investigation has suggested that a sub-population of VTA neurons exists, which responds with excitation to aversive stimuli. This group has been suggested to be a population of non-DA neurons, distinguishable by action potential width (Ungless et al., 2004), or a sub-population of DA neurons located in a restricted area of the VTA (Brischoux et al., 2009). The current study did not use an aversive stimulus, and both excitatory and inhibitory responses have been previously reported in VTA and SNc DA neurons to non-noxious sensory stimuli (Steinfels et al., 1983a, 1983b; Strecker and Jacobs, 1985; Schultz, 1986; Horvitz et al., 1997; Dommett et al., 2005). Nevertheless, the present data were examined to see if there were differences between DA neurons showing excitatory first phases and inhibitory first phases that might suggest the existence of sub-populations. A full consideration of the data from all chapters and their theoretical implications is given in the final chapter. All that will

be said here is that although the direction of the first phase of the responses of DA neurons after BMI was in the same direction to both cortical stimulation and light flash, DA neurons that were responsive before BMI occasionally showed responses to cortical stimulation in the opposite direction to the post-BMI response to light flash. The response to cortical stimulation either changed direction, or was absent after BMI. This alone suggests that a binary excited/inhibited distinction of SNc DA neurons is unwarranted.

Variation in stimulus evoked activity between animals

When comparing responses of SC and DA neurons across animals, differences could be seen in the magnitude and duration of responses to both cortical stimulation and whole field light flash. Whilst responses to visual and cortical stimuli both varied, responses to cortical stimulation varied more. This may be in part due to the nature of the stimulus. The light flash covered most of the contralateral visual field, and so is likely to have activated a large proportion of SC neurons fairly equally. The OIS data presented here suggest that electrical stimulation of barrel cortex produced a haemodynamic response across a most if not all of the barrel field. However, neural activation is likely to be restricted to a smaller area than haemodynamics suggest, and even if activation spread to the whole barrel field, it is possible that the region immediately around the electrode was excited to a greater extent than surrounding tissue. Thus peak cortical stimulation may have been focused on a more restricted region of SC, producing the variation in responses of neurons across the SC and SNc.

Interaction between multimodal stimulation

In a small number of experiments, only one stimulus was presented. The results showed that, on occasions where only one stimulus was presented at 0.25 Hz rather than the standard protocol of 0.5 Hz stimulation alternating between light flash and cortical stimulation, the SC phasic response to the stimulus increased. However, in several cases injections of BMI were made at a similar time. Thus, there were only a few cases in which the change in response as a result of less frequent unimodal stimulation could be dissociated from the course of effect of BMI. Figure 3-22 provides an example.

It has been reported by Rhoades (1980) that electrical stimulation of somatosensory cortex, which elicited a response in somatosensory neurons in deep SC, suppressed responses to stimulation of the cervical spinal cord, and to vibrissal

stimulation, in 30% of neurons. A similar pattern was found for a light flash and stimulation of the visual cortex and optic chiasm. The period of response suppression demonstrated by Rhoades only lasted for 50-200 ms after stimulation of 0.1-1.5 mA. While this might not immediately explain the interaction between stimuli 2 s apart in the present results, Rhoades (1980) showed that although stimulation at 0.8 mA produces outright suppression lasting 80 ms, there is attenuation for 200 ms. In this case, stimulation at higher current intensities, which produce longer periods of suppressions, might have attenuating effects at a much longer time scale.

Absence of habituation to repeated stimuli

In the awake animal, both SC and DA neurons habituate rapidly to unreinforced predictable stimuli (Wurtz and Albano, 1980; Schultz, 1998). The stimuli used here were spatially and largely temporally predictable. Both SC and DA neurons showed phasic responses to the light flash and cortical stimulation. This response did not habituate, but instead increased and decreased with the onset and offset of the effect of BMI on the SC. The absence of habituation supports the findings of previous electrophysiological studies with similar protocols (Dommett et al., 2005) and behavioural studies (Redgrave et al., 1981) which have shown that habituation can be blocked by disinhibition of the SC. It has been suggested that habituation in the SC to input from the optic nerve is the result of blocking of LTP induction in the superficial layers via a GABAergic mechanism (Hirai and Okada, 1993), which may explain the absence of habituation in the presence of the GABA antagonist BMI

Methodological considerations

Current spread from intracortical stimulation

Although the results strongly suggest that the SC is a relay of cortical input from whisker barrel cortex to DA neurons, it is important to consider methodological and theoretical issues that could affect this conclusion.

The intention of the intracortical stimulation was to activate the barrel field within primary somatosensory cortex. When using intracortical microstimulation of a restricted cortical area as a stimulus, it is important to determine the area of cortex the current pulse activates. The optical imaging data presented here show that the cortical haemodynamic response to 100 μs single pulse 1 mA stimulations is comparable in extent to electrical whisker pad stimulation. The electrical whisker pad stimulation parameters used here have been previously established to activate most, if not all of

the barrel field (Berwick et al., 2005), suggesting that cortical stimulation similarly activates a majority of the barrel field. The precise relationship of neurovascular coupling is still a source of investigation, and the extent haemodynamic response is not necessarily the same as current spread/extent of activated elements. Nevertheless, the results still support the assertion that the neuronal activation from the stimulation paradigm used here is contained within the barrel field. Using MRI Tolias et al. (2005) found that the haemodynamic response to cortical microstimulation measured by BOLD was larger than was expected by the figures for passive current diffusion given by Stoney Jr et al. (1968), suggesting that a haemodynamic response contained within the barrel field, as seen here, indicates that the activation was similarly constrained.

Interpretation of DA neurons responding pre-BMI

The research presented here strongly suggests that the activation of SC by cortical stimulation is the result of orthodromic activation, rather than an artefact resulting from antidromic activation. Activation of collicular efferents is then presumed to modulate the firing of DA neurons in SNc. However, some DA neurons were seen to respond before application of BMI to the SC. One possible explanation of this phenomenon is input reaching SNc via routes not involving the SC.

Somatosensory cortex projects broadly throughout the brain. However, there are few projections to structures with projections onwards to SNc that are likely candidates for alternate pathways. Somatosensory cortex projects extensively to ventral areas of the dorsolateral striatum (McGeorge and Faull, 1989; Alloway et al., 1999). These projections are overwhelmingly excitatory (Bellomo et al., 1998) and synapse onto medium spiny neurons, which project on to SN. There is a direct cortical projection to SNc and SNr, however, it appears in the rat to be restricted to the prefrontal cortex, with no projection from sensorimotor, or any other more caudal cortical region (Naito and Kita, 1994). Pedunculopontine tegmental nucleus (PPTg) also projects to DA neurons, and can produce both excitatory an inhibitory responses (Lokwan et al., 1999). However, the cortical input to PPTg seems to be limited to prefrontal cortex (Steininger et al., 1992), suggesting it is not a relay of S1Bf cortical input. Consequently, only a cortico-striato-nigral pathway seems able to provide input from S1Bf to DA neurons.

For the most part, the effect of cortical projections to the striatum is overwhelmingly excitatory (Bellomo et al., 1998). They typically synapse onto

GABAergic medium spiny neurons, which then project on to SN (Nitsch and Riesenberg, 1988), producing an inhibitory response. In the present study, DA cells could exhibit inhibitory or excitatory responses to somatosensory cortex stimulation. Cortico-striato-nigral projections could underlie both excitatory and inhibitory responses in DA neurons, as GABAergic MSNs project onto both DA neurons of SNc, and GABAergic neurons of SNr, which then project on in turn to DA neurons (Nitsch and Riesenberg, 1988). However, the absence of any significant difference in onset latency between excitatory and inhibitory responses, which might be expected with an extra synapse, and effect of disinhibiting the colliculus on DA responses suggests that this projection is also unlikely to be responsible for the present results.

3.6.3 Remaining questions

The current study suggests that the SC is a likely relay for cortical somatosensory input to DA neurons. However, its role is not confirmed by this study. Previous study has shown that stimulation of the SC, both electrical and chemical (including injections of BMI), can desynchronise cortical activity (Redgrave and Dean, 1985; Keay et al., 1988; Dean et al., 1991; Dringenberg et al., 2003). Desynchronisation may result in the cortex responding differently to stimulation, which might produce a different effect in DA neurons. Given this possibility, and the alternative pathways mentioned above, and the presence of responses in some neurons before injection of BMI in the SC, it is possible that the SC is not a relay of cortical input to DA neurons. This hypothesis could be tested by removing the input of the SC and examining its effect on the DA response. In some instances in this study, DA cells did respond to cortical stimulation before the injection of BMI into SC. It is possible that other stimulation parameters will be able to reliably drive DA neurons at 'baseline'. Activity in the SC could then be 'removed' by chemical suppression, and the effect on DA response to stimuli compared to baseline to confirm whether the SC is indeed a relay, and whether the sensitivity of DA neurons to stimulation is a specific effect of BMI injections in the SC.

4 The effects of collicular suppression by injection of muscimol on the responsiveness of dopaminergic neurons to stimulation of barrel cortex with pulse trains

4.1 Chapter summary

The previous chapter strongly suggested that DA neurons were responsive to cortical stimulation, and that this input was via the SC. The following chapter seeks to confirm these findings, and eliminate alternative explanations. Firstly, it describes how cortical stimulation could be adjusted to produce a response in DA neurons without disinhibition, and then discusses how this response might be supressed. The present study found that DA neurons respond to a lower intensity, high frequency pulse train in the naive animal, and that this response can be attenuated or eliminated by suppressing SC responses by injecting the $GABA_A$ antagonist muscimol. This adds additional supporting evidence to the assertion that the SC is a critical relay for cortical input to DA neurons.

4.2 Introduction

The work detailed in the previous chapter demonstrated that the majority of DA neurons were unresponsive to a single 1 mA, 100 μs pulse until the SC was disinhibited by injecting the $GABA_A$ antagonist BMI. The results suggest that the SC is a relay for cortical input to DA neurons. However, previous work has shown that injections of L-glutamate or BMI into the superior colliculus can cause cortical desynchronisation (Redgrave and Dean, 1985; Dean et al., 1991). Desynchronisation may change the response of the cortex to the direct electrical stimulation used in chapter 3. If cortical desynchronisation does have an effect on the cortical response to stimulation, then this may be the cause of the change in responses of DA neurons, rather than any specific effect of BMI on the SC. The possibility that the responsiveness of DA neurons to cortical stimulation is the result of cortical desynchronisation must be excluded in order to properly interpret the results. In the previous chapter, some DA neurons responded before BMI was injected. This strongly suggests that DA neuron responsiveness to cortical stimulation was not the result of BMI induced desynchronisation. The present experiment will develop this model further and establish whether responses can be reliably evoked in DA neurons

in the absence of BMI. The experiment will then go on to examine whether the SC is a critical relay for cortical input to DA neurons by examining whether the activity of SC affects the response in DA neurons.

4.2.1 Stimulation of the SC can induce cortical desynchronisation

Research into the role of the SC as a source of cortical arousal has suggested that intracollicular injections of L-glutamate or intracollicular stimulation can induce desynchronisation in urethane anaesthetised rats (Dean et al., 1991). Injection of BMI in sleeping rats also produces desynchronisation (Redgrave and Dean, 1985). There are several aspects of these studies by Redgrave, Dean and colleagues that make desynchronisation a less plausible explanation of the results of chapter 3 than collicular disinhibition. The present study uses injections of BMI into the deeper layers of the SC. Dean et al. (1991) found that injections of BMI were less likely to induce cortical desynchronisation than injections of L-glutamate. The injections of L-glutamate were more likely to induce cortical desynchronisation in sleeping rats than in urethane anaesthetised rats in Redgrave and Dean (1985), suggesting that anaesthesia reduced the ability of collicular activation to induce cortical desynchronisation. The doses of urethane used in Redgrave and Dean (1985) were much lower than the ones used in the present study (0.75 g/kg vs 1.25 g/kg). The larger dose of anaesthetic used in the present study may further reduce the chance of an injection of BMI producing cortical desynchronisation. Nevertheless, to be fully confident in the results, the possibility of a non-specific effect of BMI in the SC should still be excluded.

4.2.2 Producing a response in DA cells without BMI

In the previous chapter, some records showed a response in DA neurons before an injection of BMI had been made in the SC. If a set of stimulation parameters could be established that reliably produce a response in DA neurons in the naive animal, then this would be strong evidence that the responsiveness of DA neurons after an injection of BMI into the SC is not due to non-specific effects on cortical synchrony. If the suppression of collicular activity blocked or attenuated those responses in DA neurons, this would be further evidence for collicular relay of cortical information bound for DA neurons.

The parameters of the cortical stimulation that can be changed are: current intensity, pulse duration and number of pulses, and if a train of pulses is used, the

frequency of pulse. A full description of how changes to the configuration of electrical stimulation affect cortical neuronal activation is beyond the scope of this thesis, although see Tehovnik (1996) for a detailed review. Broadly, however, increasing the current intensity of a pulse increases the current density at a given distance from the electrode, which can also be seen as producing the same current density further from the electrode. Increasing the pulse duration increases the amount of charge transferred to the tissue. Alternatively, a train of pulses can be used to deliver the charge over a longer period of time, while allowing for the charge to dissipate between pulses.

The aim was to develop a set of stimulation parameters that activated a greater number of cortical neurons, on the assumption that more activated neurons would mean a greater likelihood of a response in DA neurons. The response in the SC produced by cortical stimulation is the result of depolarising presumably direct corticotectal cells. Although a greater number of neurons could be activated with a greater current or longer pulse duration (Tehovnik, 1996) this needs to be balanced against the risk of activating regions outside the barrel field, which would confound the interpretation of the results. Also, excessive currents have been shown to cause damage to cortical tissue (Asanuma and Arnold, 1975). Therefore, the decision was made not to increase the current of the pulse, but to use a high frequency train of pulses instead.

The particular train configuration was chosen to directly drive depolarisation of corticofugal neuron axons in a semi-naturalistic manner. A train of five pulses at 150 Hz was chosen, as this is similar to the firing pattern of intrinsically bursting (IB) cortical pyramidal neurons. Cortical neurons showing this distinct bursting pattern have been identified as a distinct population of tectally projecting neurons in the visual cortex (Kasper et al., 1994; Rumberger et al., 1998; Tsiola et al., 2003), and given the repetitive structure of cortical circuitry, this association between activity and anatomy may also apply in other sensory cortical areas. Before the study proper began, a pilot study was conducted to examine the stability of the response over time.

4.2.3 Suppressing SC activity

As well as choosing a set of stimulus parameters, a suitable method of suppressing SC activity is needed. Intracerebral injections of the $GABA_A$ receptor agonist muscimol are widely used in behavioural and electrophysiological studies to

examine the effect of reversibly inactivating a brain region (e.g. see Majchrzak and Di Scala (2000) for a review of the use of muscimol in studies of learning and memory). Muscimol is preferable to sodium channel blockers (e.g. tetrodotoxin) or local anaestheics (e.g. lidocaine) as they block electrical activity in both local neurons and fibres of passage (Hille, 1966, 1977; Ritchie, 1979). An injection of muscimol also provides rapid and long lasting effects, allowing for extensive investigation.

Muscimol also has particularly useful applications in the SC because of the extensive intrinsic and extrinsic GABAergic control of the SC: GABAergic neurons form up to fifty percent of the neurons in the superficial layers and one third of neurons in the deeper layers (Mize, 1992). Isa et al. (1998) demonstrated that tonic GABA suppresses glutamatergic connections between the optic tract and the superficial layers of the SC, as well as from the superficial to the intermediate layers. There are also similar local connections within deeper layers. This intrinsic circuitry is suggested to be a mechanism by which efferent cells of the deeper SC could associate, coordinate, or modulate their responses (Behan and Kime, 1996).

In the superficial layers, two circuits involving GABA receptors have been described (Binns and Salt, 1997; Binns, 1999), which were suggested to produce inhibitory surround (through presynaptic $GABA_A$ receptors) and habituation (through pre- and/or postsynaptic $GABA_B$ receptors). These circuits may provide a target for manipulation of activity in the SC. A disinhibitory circuit involving $GABA_{A-\rho}$ receptors has been described (Pasternack et al., 1999; Lee et al., 2001; Schmidt et al., 2001), which might have a counterproductive effect, as $GABA_{A-\rho}$ receptors are activated by low concentrations of muscimol (Schmidt et al., 2001). However, expression of the receptors within the SC is restricted to GABAergic interneurons in the SuG, so the likelihood of an effect is lessened. The SC also receives significant extrinsic GABAeric input from a variety of sources (Appell and Behan, 1990). The two most significant projections are the from SNr (Kaneda et al., 2008) and zona incerta, which contains “the largest number of non-nigral GABAergic afferents to the SC” (May et al., 1997). SNr and zona incerta GABAergic neurons from both SNr and zona incerta synapse onto cells in the InG layer, and exhibit high tonic firing rates that pause before the onset of saccades. (Chevalier et al., 1981a, 1981b; Hikosaka and Wurtz, 1985; Kim et al., 1992; Ma, 1996; Kaneda et al., 2008).

The evidence suggests that presumably GABAergic neurons form widespread networks within layers, and also between layers, particularly adjacent layers (Behan et al., 2002). Further, there are several extrinsic sources of GABAergic input that actively suppress the SC. The roles of GABAergic mechanisms in a range of inhibitory systems in SC make it an ideal target for suppressing the effect of cortical input to SC. Application of the $GABA_A$ agonist muscimol to SC should increase tonic inhibition in the intermediate and deep layers of SC through $GABA_A$ receptors, suppressing the activity of efferent cells.

4.2.4 Experiment rationale

In the previous study, disinhibition of the SC has been shown to be sufficient to produce a phasic response to cortical stimulation in DA neurons. However, the necessity of the SC in communication of cortical inputs to DA neurons is still unclear. The purpose of this study is to replicate the activation of DA neurons by cortical stimulation without disinhibition of the colliculus, and to establish whether responsiveness of the SC is a necessary condition for DA response to cortical stimulation by suppressing SC responses to cortical stimulation. Further, the manipulation of DA responses to cortical stimulation in the absence of disinhibition of the SC will allow alternative explanations of the results of chapter 3 to be ruled out.

4.3 Method

4.3.1 Experimental procedure

The experimental design is summarised in graphical form in Figure 3-1. The present study used simultaneous electrophysiological recording of SC (multiunit) activity and DA (single unit) activity in SNC, in response to electrical stimulation of S1Bf, both before (Figure 3-1a) and during (Figure 3-1b) chemical suppression of SC. To ensure only neuronal elements in the SC were suppressed, local injections of an excitatory substance, the $GABA_A$ receptor agonist muscimol (Figure 3-1b, red microsyringe), were used.

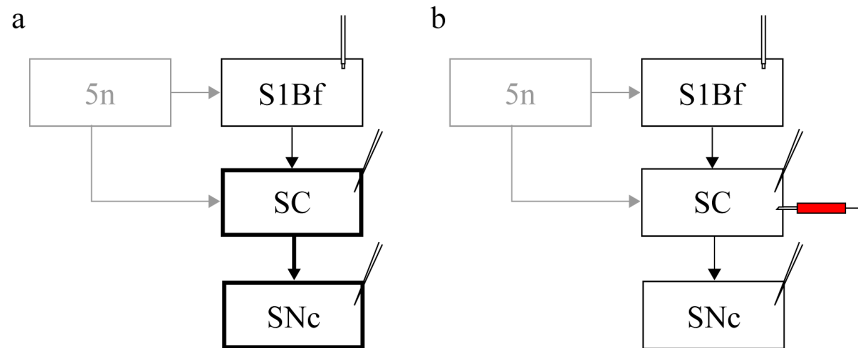


Figure 4-1 Schematic of the experimental design for this experiment.

The subject preparation, experimental procedure, histology, and statistical analysis have been previously described in the Methods chapter, and elaborated on in the previous experimental chapter. Some sections have been repeated here, with further detail regarding this experiment where appropriate.

Data were obtained from a total of 12 acutely prepared adult hooded Lister rats (288-480 g). The stimulating electrode was placed vertically into S1Bf (AP 1.6-3.14, ML 4.8-5.4) 1.5-1.8 mm below dura. The multiunit electrode/cannula (filled with muscimol where necessary,100 ng/µl saline; Sigma), was introduced vertically into the lateral intermediate layers of SC (AP 6.04-6.72 mm caudal to bregma; Lateral 1.6-2.6 mm; Dorsoventral 4.8-5.7 mm below dura). DA neurons were recorded from SNC (AP 4.8-5.3 mm caudal to bregma,lateral point of surface entry 2.5-4.0 mm).

The first section of this study did not involve muscimol, but instead investigated whether the response of the SC and DA cells to pulse train stimulation remained broadly stable over time. When both probes were suitably positioned, baseline activity was recorded from both the SC and the SNc for a period of at least 60 s. A block of 150 pulse train stimulations were then applied to establish the presence of a stable response. A single stimulation consisted of a train of 5 pulses of electrical stimulation of barrel cortex at 150 Hz (0.6-0.8 mA, 100 µs pulse width). Each train was separated by 2 s, jittered by 20 %. If the initial block showed a significant response in the DA neuron, then stimulation was continued for up to 45 minutes.

The second section of this study investigated whether the response of the SC and DA cells to pulse train stimulation was affected by the injection of muscimol into the SC, and followed the experimental procedure defined in chapter 2. Initially cells were stimulated at 0.6 mA. If there was no visible response on a PSTH of 150 trials, currents of 0.8 and 1.0 mA were tested. If no response could be seen at 1.0 mA, the

neuron was discarded as not sufficiently responsive. See Methods chapter for a description of the histological procedures used.

4.3.2 Data analysis

Data were analysed using Spike2 in-built functions, Spike2 scripts, and the R language (R Foundation of Statistical Computing, Vienna, Austria). Unless otherwise stated, paired t-tests were used to compare groups. Where data were non-normal ($p > 0.05$ for a Shapiro-Wilk normality test) they were transformed before analysis, typically log transformations. Summary statistics are reported as mean±SEM for normal data. For transformed non-normal data, mean and SEM are calculated using transformed data, then backtransformed for easier interpretation (i.e. $e^{mean(XT)\pm SEM(XT)}$ for log_e transformed data, where $mean(X_T)$ and $SEM(X_T)$ are the mean and SEM of the transformed data). As $e^{mean(XT)+SEM(XT)}$ and $e^{mean(XT)-SEM(XT)}$ would not be equal distances from $e^{mean(XT)}$, both backtransformed SEM limits are reported in the form mean, +SEM:-SEM. Where a single transformation cannot be applied to render data from both groups normal, then non-parametric tests are used. Summary statistics for groups analysed by non-parametric tests are reported as median, 1Q:3Q).

ECoG was recorded via a tinned wire placed on the frontal cortex. EEG recording was obtained from a broadband recording of SC activity. Both signals were low-pass filtered (32 dB, -3 dB point: 40 dB) and the dominant frequency band was determined, which was defined as the largest bin in an FFT (fast Fourier transform) with bin sizes of approximately 0.5 Hz. The dominant frequency was compared to the frequency bands described in Friedberg et al. (1999) to determine the depth of anaesthesia. FFTs were produced from the first 15 minutes of recording, the first fifteen minutes after muscimol injection, and the last fifteen minutes of each recording.

SC activity was recorded, processed and analysed as described in chapter 2. Following data collection and processing, the data were examined to see if muscimol had taken effect by comparing several measures of activity (see chapter 2 for definitions of baseline, background and response activity). PSTHs were constructed from pre-injection trials and post injection trials to compare the effect of the injection, or from a series of blocks of trials to track the time-course of a measurement of activity. The period used to calculate background activity was the 500 ms preceding the stimulus. The post-stimulation period was the 30 ms immediately following the

stimulus. PSTHs of SC activity evoked by cortical stimulation showed a peak of excitation that was sometimes followed by a rebound inhibition, and often a second, longer duration increase in activity. As the first peak is most likely to be the result of direct projection, a response period was calculated to cover only the first peak. Pre-muscimol trials on all records were examined, and a "first peak offset" was taken to be the point at which the activity fell below the background activity for two or more 1 ms bins. The mean offset point was 30 ms, and so response magnitude was measured using the period 0-30 ms after stimulus onset.

DA data were recorded and processed as described in chapter 2. Measures of baseline activity, background activity and response magnitude were taken to examine the effect of muscimol (see the methods chapter for a definition of these measurements). The response period was 20-260 ms after stimulus onset to encompass the entirety of the DA response as described by Hudgins (2010).

To examine whether muscimol increased or decreased response magnitudes regardless of response direction, absolute measures of response magnitude for 450 pre-injection trials and the last 450 post-injection trials were used. Five control experiments were also examined where no injection had taken place. The first 450 trials were compared to the last 450 trials of each recording. To examine the effect of muscimol over the course of the experiment, the response magnitudes were plotted over time. Response magnitudes were measured for blocks of 150 trials. The response magnitudes were standardised to the mean response magnitude for pre-muscimol blocks.

In the previous chapter, a response in a DA neuron to a single 1 mA current pulse was defined as deflections in the PSTH crossing a threshold of the mean pre-stimulus activity ±1.96 SD. Even though several cells in the present study succeeded in reaching this threshold, changes in the activity of several cells were detectable by eye but did not meet this threshold. This failure to cross the threshold was a combination large variance of pre-stimulus activity, and the tendency of responses to be in the form of several bins of low amplitude, and thus non-significant deflection, rather than the larger amplitude responses seen in the previous chapter. Consequently, the cumulative sum (CUSUM) method, described in chapter 3, was applied across all cells in the present chapter.

4.3.3 Optical imaging spectroscopy

In order to examine whether the activation produced by direct stimulation of the cortex was contained within the barrel field, optical imaging spectroscopy (OIS) was used to measure the spread of activation produced by our chosen stimulation parameters, and to compare the haemodynamic response to direct cortical stimulation to the response to whisker pad stimulation. Data were recorded from the same animals reported in the OIS experiments of chapter 3. The experimental methods are identical, except cortical stimulation consisted of 60 trials of direct intracortical stimulation with 150 Hz trains of five pulses at 0.6 mA, separated by an interval of 26 s. The analysis is also the same as that presented in chapter 3.

4.4 Results

4.4.1 Inclusion criteria

To be included in the analysis, putative DA neurons had to meet the same histological criteria as those in chapter 4, and also have a successful injection of muscimol into the SC, as judged by the presence of a significant decrease in the mean activity of SC. Nine DA neurons met these criteria.

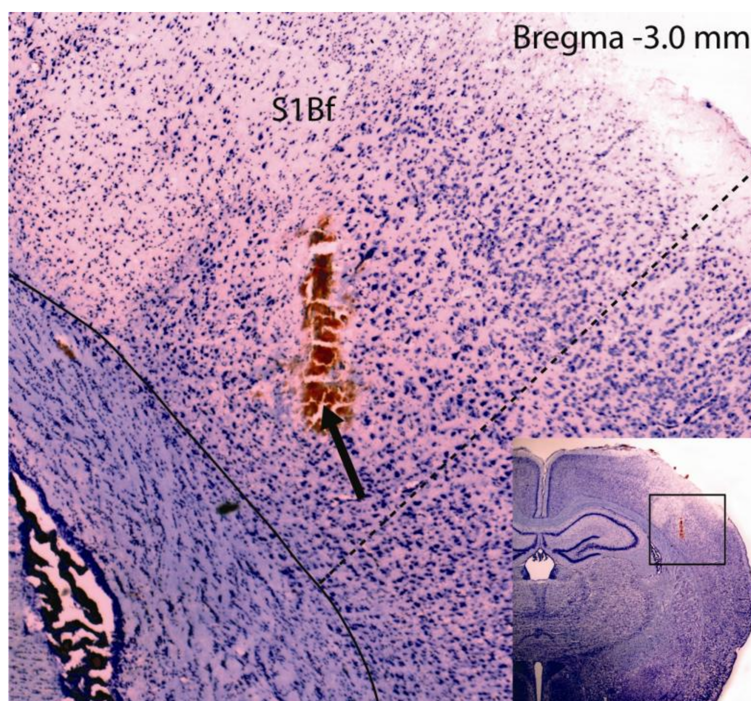


Figure 4-2 Coronal section of the somatosensory cortex, processed for cresyl violet. Measurement relative to bregma indicates the location of the section. Arrow indicates the approximate location of the tip of the stimulating electrode. S1Bf: primary somatosensory cortex, barrel field.

Recording sites were taken as the centre of electrolytic lesion or of the iontophoretic injection of Potamine Sky Blue dye. Examples can be seen in chapter 3.

Stimulation sites were taken as the ventral extent of the electrode track. An example is presented in Figure 4-2. There was no evidence of stimulation related tissue damage around the stimulation sites in S1Bf.

The recording locations of the DA neurons included in the study, the recording and injection locations in SC, and the stimulation sites in S1Bf are shown on diagrammatic sections from Paxinos and Watson (2004) in Figure 4-3, Figure 4-4, and Figure 4-5.

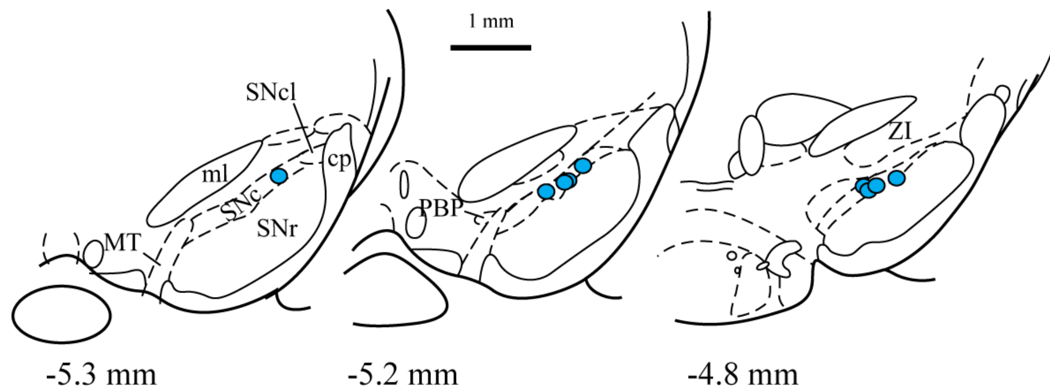


Figure 4-3 Reconstructed plots of recording sites in the midbrain on diagrams of coronal sections. Points indicate the approximate location of the DA cell. Measurements relative to bregma, and indicate the location of each section. Abbreviations as in chapter 3.

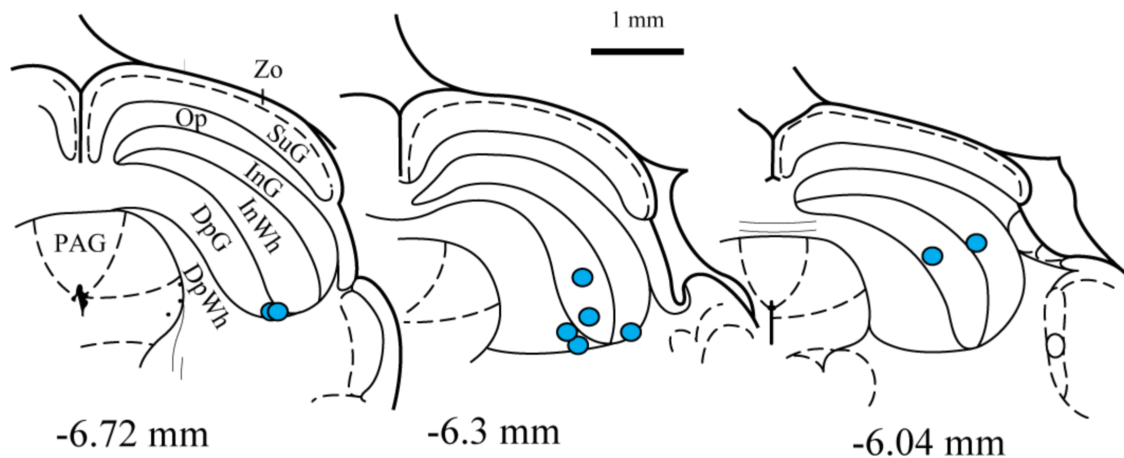


Figure 4-4 Reconstructed plots of recording sites in the midbrain on diagrams of coronal sections. Points indicate the tip position of the electrode-injector assembly. Measurements relative to bregma, and indicate the location of each section. Abbreviations as in chapter 3

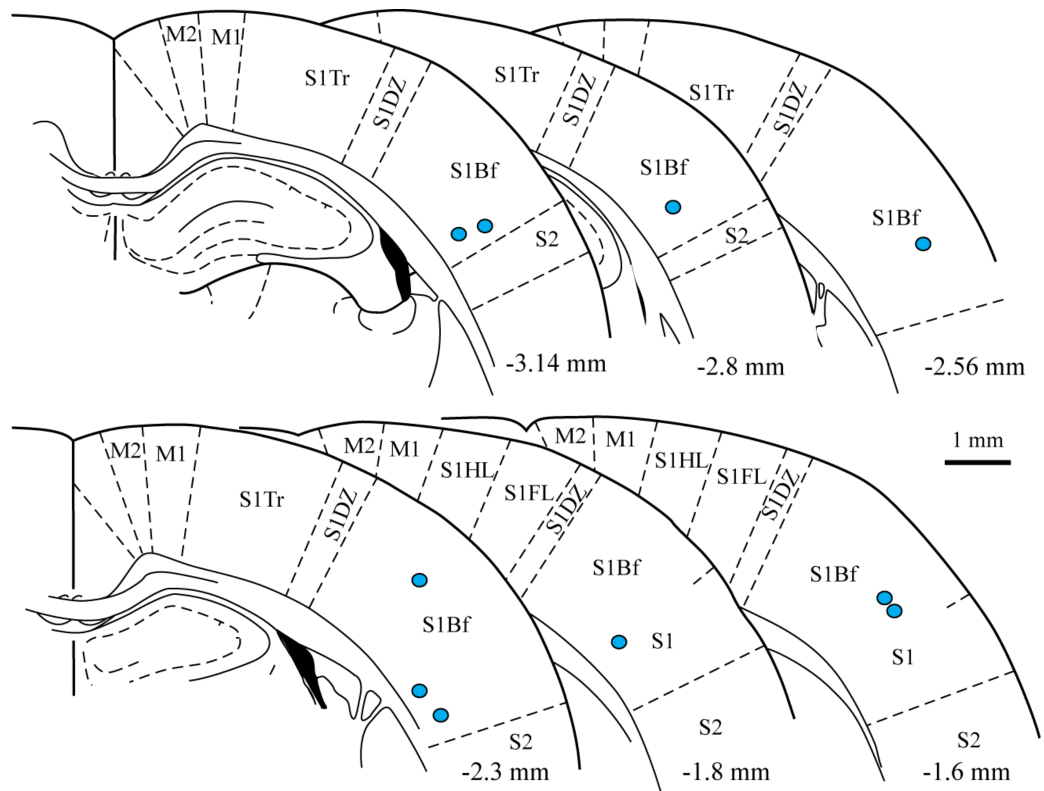


Figure 4-5 Reconstructed plots of stimulation sites in the cerebral cortex. Points indicate the tip position of the stimulation electrode. The exposed pole of the central electrode extends 500 um dorsally from the point indicated, followed by 500 um of insulated electrode, followed by a 500 um exposed section forming the surround electrode. Measurements relative to bregma, and indicate the location of each section. Abbreviations as in chapter 3

Processing for c-fos and TH immunoreactivity was performed in all 9 animals.An example of TH immunoreactivity can be seen in chapter 3. C-fos processing showed an absence of FLI, except at greater distances from the injection site (Figure 4-6).

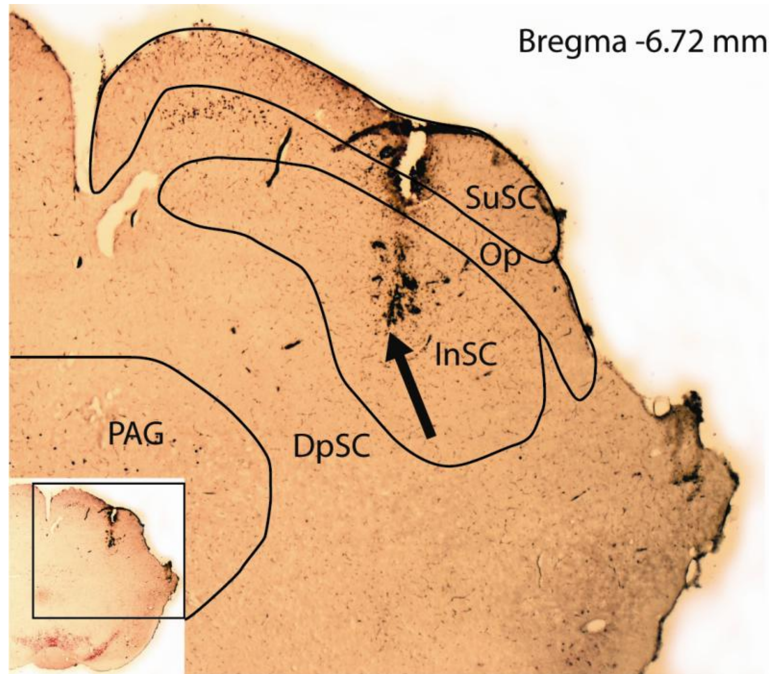


Figure 4-6 Coronal section of the SC processed for TH and c-fos. Section shows a lack of FLI (black dots) around the injection site. Measurement relative to bregma indicates the location of the section. Arrow indicates electrolytic lesion at the recording/injection site. SuSC: superficial layers of the SC (zonal layer and superficial grey layer); Op: optic layer; InSC: intermediate layers of the SC (intermediate grey and intermediate white layers); DpSC: deep layers of the SC (deep grey and deep white layers); PAG: periaqueductal grey.

4.4.2 Eliminating habituation as an alternative explanation

The effect of repeated stimulation on 5 DA neuron responses was tested without manipulation of the SC for between 750 and 1300 sweeps (approximately between 25 and 45 minutes of recording). There was no significant difference between DA neurons response magnitudes in the first 450 sweeps (M = 0.7±0.1 Hz) and last 450 (M = 0.7±0.1 Hz) sweeps of each recording (t = 1.624, df = 4 $p > 0.05$). An illustration of the consistent response of a neuron stimulated 1300 times is shown in Figure 4-7.

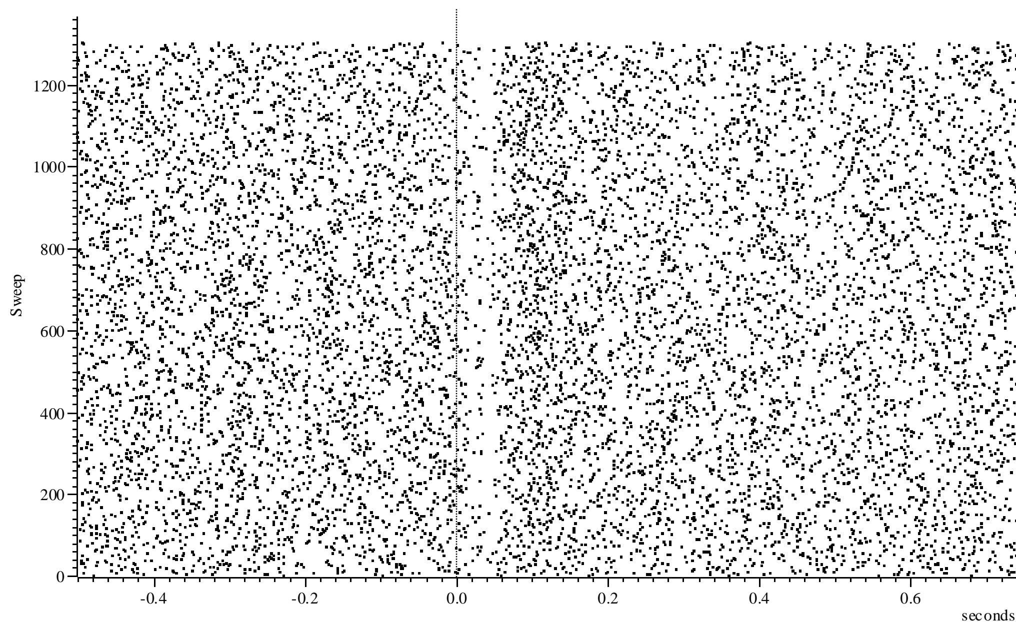


Figure 4-7 A raster plot of a DA neuron responding consistently to stimulation with 1300 pulse trains. Vertical cursor indicates stimulation onset.

4.4.3 Eliminating EEG change as an alternative explanation

A FFT was applied to all 9 records where muscimol was injected, the peak power frequency was determined as described in the methods section of this chapter, and the frequency was compared to the anaesthetic states of Friedberg et al. (1999). Throughout all 9 records where muscimol was injected, there was no change in the the dominant frequency band of EEG and ECoG recordings (mean peak power frequency across all 3 stages of recording (dominant frequency 1-1.5 Hz before stimulation, immediately after muscimol injection and at the end of recording – see Figure 4-8 for an example). Comparison of peak power frequency before and immediately after injection of muscimol, and at the end of the recording to the anaesthetic stages of Friedberg et al. (1999) suggested a stable anaesthetic state III-4 at all points.

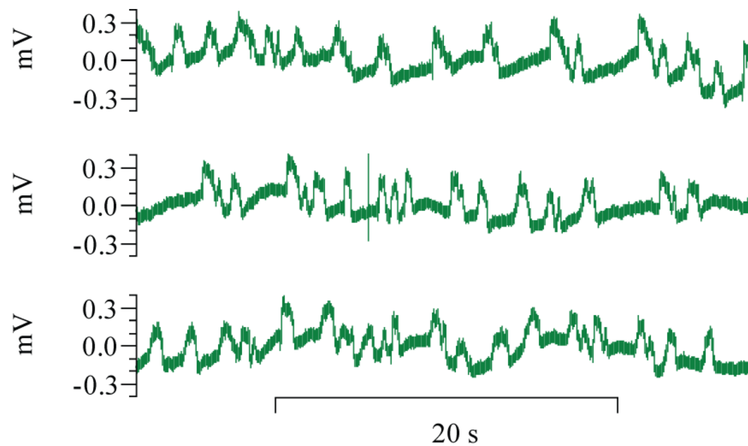


Figure 4-8 An example of EEG activity recorded before application of any stimulation (top) immediately following muscimol injection (middle) and 30 minutes after muscimol injection (bottom). There was no shift in depth of anaesthesia as measured by the dominant frequency.

4.4.4 Activity in the superior colliculus

To assess the effect of sensory stimulation on SC activity without the presence of muscimol, the baseline firing rate was compared to the background firing rate during pre-muscimol stimulation. A paired Wilcoxon signed rank test showed no significant difference in firing rate of SC when comparing baseline firing (395.2 Hz, 232.5:418.9 Hz) and background firing in pre-muscimol trials (367.9 Hz, 217.4:428.5 Hz; V=25, $p > 0.05$). Across all 9 records there was a significant difference between background firing rate in pre-muscimol trials (338.2±33.4 Hz) and post-muscimol trials (140.4±44.1 Hz; $t = 5.64$, $df = 8$, $p < 0.001$). Examination of the records shows that all 9 records showed a decrease in activity.

Throughout the pre-muscimol trials, there was a short latency (1.6±0.2 ms), short duration (34.0±2.5 ms) response to cortical stimulation. There was a significant difference in collicular response magnitude between pre-muscimol and post muscimol trials (prior to injection of muscimol: 870.2±220.2 Hz; after injection of muscimol: 597.0±232.9 Hz, $t = 4.68$, $df = 8$, $p = 0.001$). Response onset did not significantly change with application of muscimol (pre-muscimol median = 2 ms, 1 ms:2 ms; post muscimol median = 1.5 ms, 1 ms:2 ms, V=1, $p > 0.05$). There was a significant difference between pre-muscimol response duration (34.4±2.5 ms) and post-muscimol response duration (28.6±3.6 ms; $t = 2.34$, $df = 9$, $p = 0.052$). An example response in the SC can be seen in Figure 4-9.

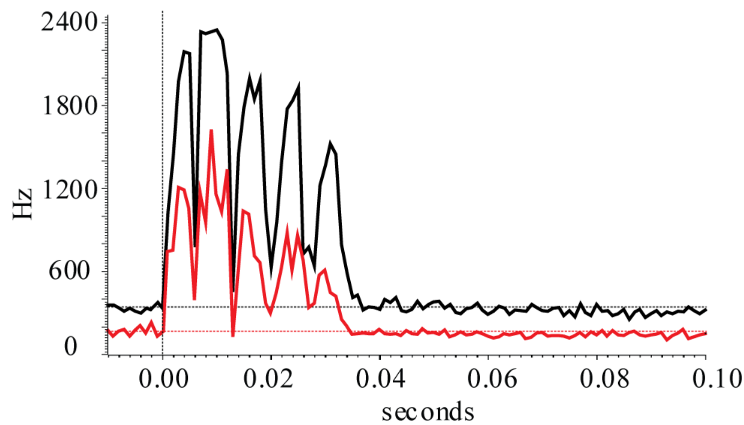


Figure 4-9 Graph of SC activity in response to a train of cortical pulse train stimulation before (black) and after (red) intracollicular injection of muscimol. Vertical cursor indicates the first pulse, black and red horizontal cursors indicate pre- and post-injection background firing rates.

To assess the response of SC over the course of the pulse train, the mean firing rate in the 6.5 ms after each pulse was measured, before and after injection of muscimol. Figure 4-9 shows an example of the effect of muscimol on the collicular response to cortical pulse train stimulation. A two-way within subjects ANOVA (IV: mean firing rate, DV: pulse number (5 levels), injection (2 levels)) revealed a significant effect on activity of the injection of muscimol ($F(1,8) = 43.54$, $p < 0.001$; mean pre-injection firing rate: 870.2 Hz, post-injection: 451 Hz), pulse number ($F(4,32) = 11.61$, $p < 0.001$; mean firing rates after each pulse – 1: 721 Hz, 2: 816 Hz, 3:614 Hz, 4:555 Hz, 5:524 Hz) and an interaction between muscimol and pulse number ($F(4,32) = 6.31$, $p < 0.001$; see Figure 4-10). In both pre- and post- muscimol trials, the activity after each pulse peaked with the second pulse, and then declined with each subsequent pulse. The activity after each pulse during pre-muscimol stimulation was consistently higher than the activity after the corresponding pulse during post muscimol stimulation. The difference between activity following corresponding pulses in pre- and post-muscimol trials was greatest for the activity after the second pulse. The same pattern was observed when the effect was considered as a relative change (reduction in activity for each pulse – 1: 28%, 2: 37%, 3: 35%, 4: 26%, 5: 24%).

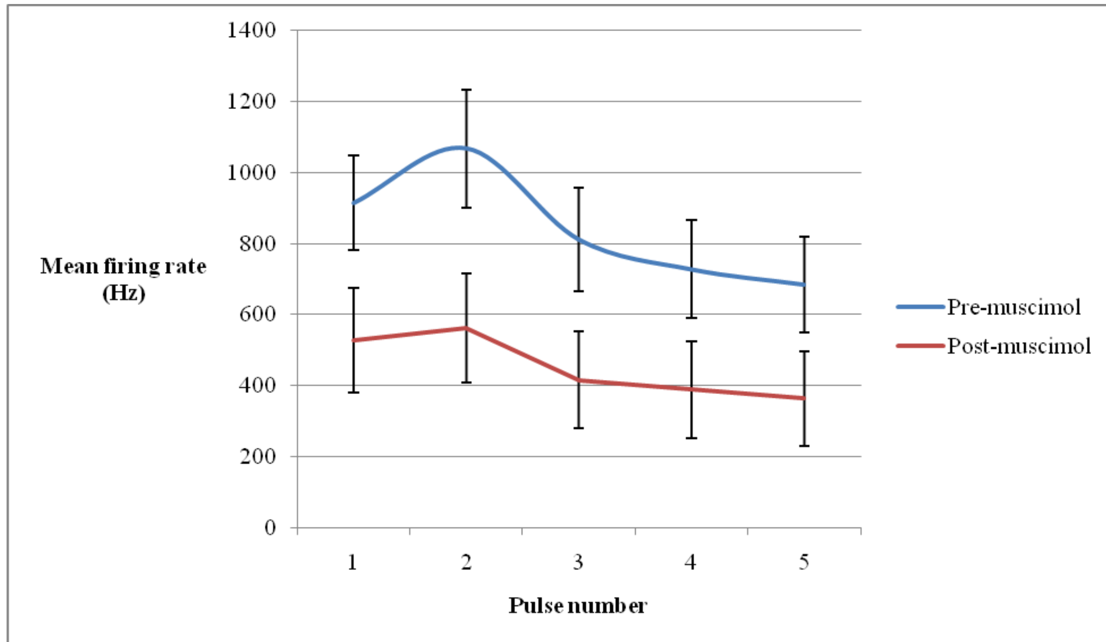


Figure 4-10 A breakdown of the response magnitude of each of the five pulses in the train (pulse number) for pre- (blue line) and post-muscimol (red line) trials. Error bars represent 1SEM.

4.4.5 Activity of DA cells

A paired t-test showed no significant difference in firing rate of DA when comparing baseline firing (M = 3.0±0.8 Hz) and mean background firing in pre-muscimol trials (M = 3.1±0.7 Hz; t = -0.51, df = 8, $p > 0.05$), nor between mean background firing in pre-muscimol trials (M = 3.1 ±0.7 Hz) and post-muscimol trials (M = 3.2±0.7 Hz; t = -0.41, df = 8, $p > 0.05$).

4.4.5.1.1 Stimulus evoked responses

In order to compare the change in response magnitude across both inhibited and excited cells, the absolute size of response magnitude was used. All 9 cells showed a significant response, as detected by the CUSUM method, starting in the response period (20-260 ms after stimulation) before an injection of muscimol (onset latency = 42 ms, 18:100 ms; duration = 82 ms, 50:237 ms; response magnitude: 0.6 Hz 0.3:0.8 Hz; response amplitude = 3.1 Hz, 1.4:3.6 Hz). Of these cells, 6 showed an excitatory response, two of which showed a second, inhibitory phase, and 3 showed an inhibitory response. There was no significant difference between excitatory and inhibitory DA neuron response onsets (Med_{ex} = 30 ms, 15:100 ms; Med_{in} = 79 ms, 48.5:160.0 ms; W = 12.5, $p > 0.05$), durations (Med_{ex} = 107 ms, 50:207 ms; Med_{in} =

68.8 ms, 50.5 ms:171.5 ms; W = 7, $p > 0.05$) response amplitudes (Med_{ex} = 3.3 Hz, 3.1:4.0 Hz; Med_{in} =1.4 Hz, 1.1:2.5 Hz; W = 5, $p > 0.05$).

Across all 9 cells, there was a significant difference between response magnitudes before (0.6 Hz, 0.3:0.8 Hz) and after (0.1 Hz, 0.1:1.1 Hz) muscimol injection (V = 41, p = 0.027). Absolute response magnitudes were plotted over blocks of 150 stimulations (each block lasting 5 minutes), with the response magnitude in each file standardised to its pre-muscimol mean response magnitude. The magnitude of DA neurons responses can be seen to decrease within the first few blocks, and continue to decrease over the course of the record (see Figure 4-11).

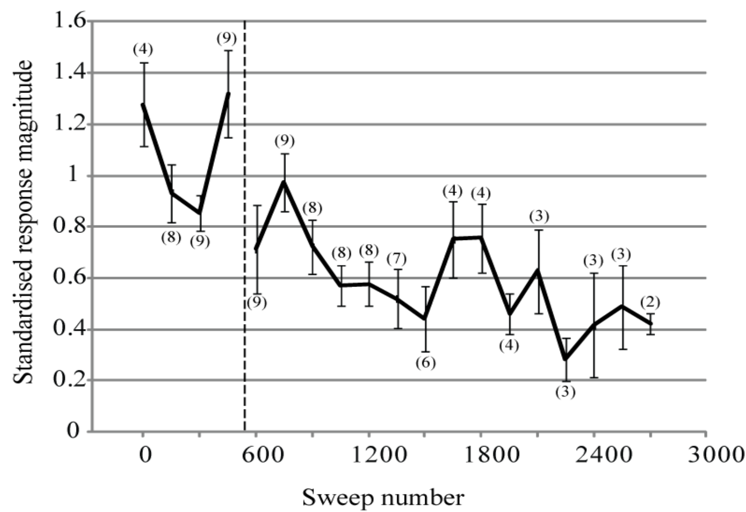


Figure 4-11 Demonstration of the effect of muscimol on standardised absolute response magnitudes averaged across all animals. Absolute response magnitudes for each animal were standardised to the mean response magnitude in their pre-muscimol trials. Error bars represent 1SEM. Numbers in brackets indicate number of animals contributing to each datapoint. Vertical cursor indicates muscimol injection.

After an intracollicular injection of muscimol, five cells ceased to show a detectable response to cortical stimulation. All five cells where the response was abolished showed excitatory responses before muscimol. In the four cells that remained responsive, there was no significant difference between pre- and post-muscimol measures of response onset latency (Med_{pre} = 48.5 ms, 15.5 ms:160.0 ms; Med_{post} = 119.0 ms, 57.0 ms:170.5 ms; V = 3, $p > 0.05$) duration (Med_{pre} = 152.5 ms, 50.5 ms:256.0 ms; Med_{post} = 138.0 ms, 80.0 ms:225.5 ms; V = 4, $p > 0.05$) or response magnitude (Med_{pre} = 1.3 Hz, 0.6:1.9 ms; Med_{post} = 1.2 Hz, 0.6:1.5 Hz; V = 10, $p > 0.05$).

Muscimol could have a differential effect on initial and second phases of DA responses. This was particularly prominent on one of the cells that showed an initial excitatory phase, which was followed by a second inhibitory phase. Although an injection of muscimol eradicated the initial phase, the second phase remained (see Figure 4-12).

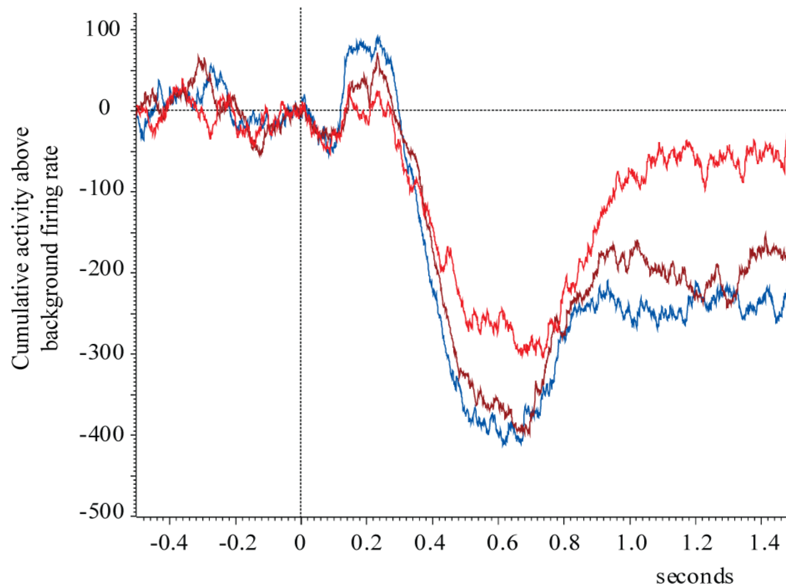


Figure 4-12 CUSUM showing the differential effect of intracollicular injection of muscimol on first and second phases of the response. Graph shows response before injection of muscimol (blue), immediately after injection of muscimol (dark red), and at by the end of the recording (light red). The initial excitatory response (indicated by a rising slope 100-150 ms after stimulus onset) is abolished after muscimol injection, while the inhibitory response (indicated by the prominent falling slope from 300 ms to approximately 500 ms after stimulus onset.

4.4.6 Topographic distribution of response directions

The nine DA neurons presented in this chapter show responses to cortical stimulation without any manipulation of the SC. Eight neurons in the previous chapter also showed responses in DA neurons before disinhibition of the SC. These neurons were all plotted together to see if there was any pattern of distribution of cells showing responses with excitatory and inhibitory first components.

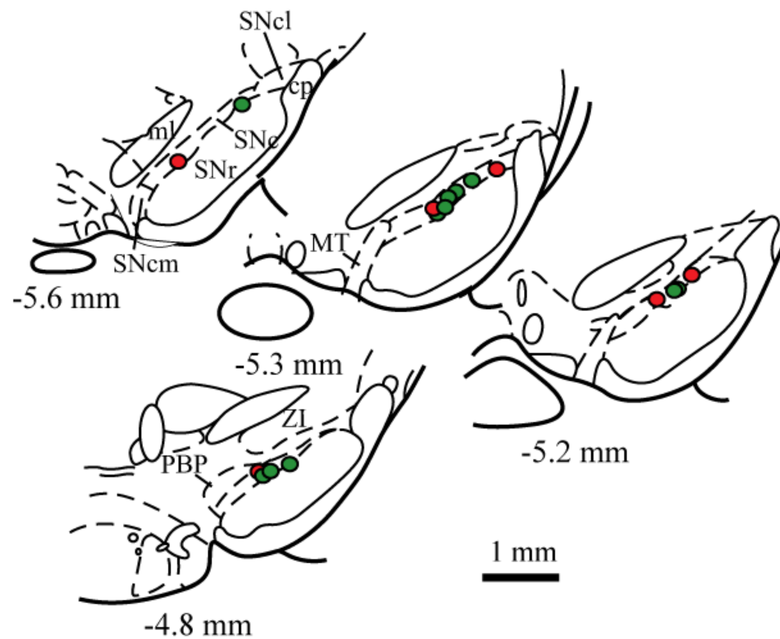


Figure 4-13 Reconstructed plots of recording sites in the midbrain on diagrams of coronal sections. Points indicate the approximate location of DA neurons showing responses with excitatory (green) and inhibitory (red) first components. Measurements relative to bregma indicate the location of the section. Abbreviations as in chapter 3.

Figure 4-13 Reconstructed plots of recording sites in the midbrain on diagrams of coronal sections. Points indicate the approximate location of DA neurons showing responses with excitatory (green) and inhibitory (red) first components. Measurements relative to bregma indicate the location of the section. Abbreviations as in chapter 3.shows the locations of DA neurons showing responses with excitatory (green) and inhibitory (red) first components. There was no clear relationship between recording location and response direction, with neurons showing inhibitory and excitatory phases being located at all rostro-caudal points, and toward the lateral extent of SNc, bordering on SNc lateralis. Although DA neurons were not recorded towards the border of SNc medial, DA neurons showing responses with excitatory first components were recorded as medially as those showing inhibitory first components.

4.4.7 Optical Imaging

The change in haemodynamic response with distance was used to compare the effect of intracortical stimulation with electrical whisker pad stimulation.

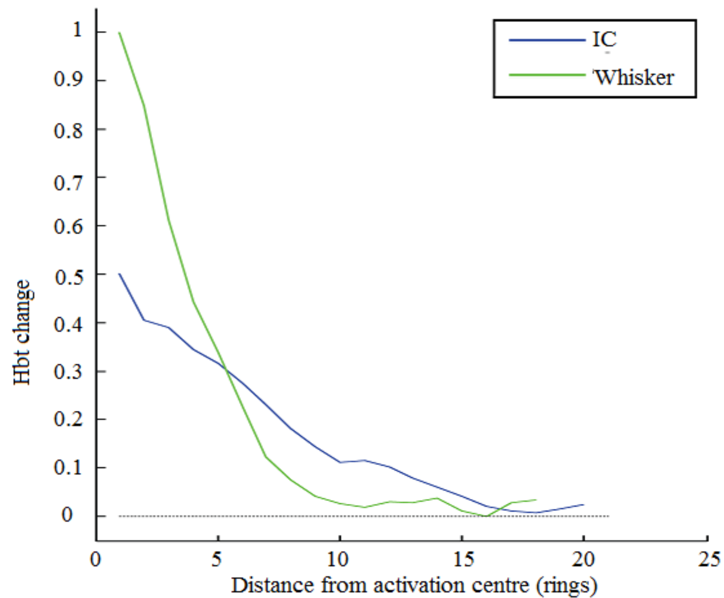


Figure 4-14 Mean haemodynamic response over distance from the centre of activation for intracortical stimulation (blue) and electrical whisker pad stimulation (green) (n=3)

Figure 4-14 Mean haemodynamic response over distance from the centre of activation for intracortical stimulation (blue) and electrical whisker pad stimulation (green) (n=3)shows the haemodynamic response over distance for intracortical stimulation and electrical whisker pad stimulation. Intracortical stimulation resulted in a lower peak response, but a more steady decay with distance than the response produced by electrical whisker pad stimulation. Pulse train electrical stimulation of the cortex produced a haemodynamic response with a similar spread to the haemodynamic response produced by whisker pad stimulation.

4.5 Discussion

4.5.1 Summary of findings

Under urethane anaesthesia, intracortical stimulation produces a short latency, short duration response in the SC, and a small response in the majority of DA cells. Local microinjections of muscimol into the SC decreased both the background and stimulus evoked activity of the SC. Injection of muscimol into the SC had no effect on the background firing rate of DA neurons, but the absolute magnitude of DA neuron responses to cortical stimulation significantly decreased after intracollicular injections of muscimol. These changes were neither the result of changes in cortical synchronisation, anaesthetic depth, or tissue damage. In conjunction with the findings

of the previous chapter, the current study confirmed the suggestion that the SC is a route for cortical input to DA neurons.

4.5.2 Discussion of findings

Averaging across all nine cells, muscimol significantly decreased the response magnitude of DA. There was an initial step change in the response, which strongly suggests that the change was due to the muscimol injection, and then the effect became progressively greater over the course of recording. After an injection of muscimol, five cells ceased to respond by the end of the recording. The remaining four cells continued to respond; however, three showed changes in their responses that could be considered a result of the suppressive effect of muscimol. The remaining DA neuron did not appear to be greatly affected; however, the sweeps used to determine the post-muscimol response of this neuron were comparatively soon after the muscimol injection, and so the muscimol may have had less time to diffuse throughout the SC to include the stimulated part of the SC (Edeline et al., 2002).

Examining the effect of muscimol on the response profile also suggests that apparent 'rebound' or 'oscillation' responses might need to be reinterpreted. Although some cells in chapter three showed cleared autocorrelative features – peaks and troughs following a particularly large response at intervals around the interspike interval – some cells in the present chapter showed what appeared to be a longer latency features. These later phases were differentially affected by muscimol injection in a way that suggested they were not simple rebound events. For example, two cells continued to show a longer latency phase even after intracollicular muscimol abolished the shorter latency phase. The fact that longer latency components of the response can persist even after the abolition of earlier components, suggests that some apparently autocorrelative features may in fact be the product of separate influences on the cell.

Comparison of the effects of pulse trains to single pulses on DA neurons

DA neurons showed a response to lower intensity cortical pulse train stimulation as they did to higher intensity single pulse stimulation. Comparison of the responses reveals similarities and differences. Responses were often small in magnitude compared to some of the post-BMI responses in chapter 3 but similar to those seen in pre-BMI responses. However, the durations of responses of DA neurons to pulse train stimulation were similar to those to a single pulse after intracollicular

BMI seen in chapter 3, suggesting that lower current pulse train stimulation produced smaller but longer duration responses in DA neurons. Responses of DA neurons to pulse train stimulation are of notably longer latency than to post-BMI responses to a single pulse. Responses latencies were closer to pre-BMI responses, but still noticeably different on average. However, examination of the onset latencies of each neuron suggests that there were similarities between single pulse and pulse train response latencies: four out of the eight cells had response latencies of <20 ms, much closer to the typical post-BMI responses to a single pulse. The remaining cells showing latencies of 79, 100, 194 and 241 ms. Although the former two onset latencies are within the range of what might be expected, the latter two latencies are substantially longer. Whether these responses represent a separate population of cells or a difference in some aspect of the stimulation is unclear.

Elimination of alternate explanations of results

At the beginning of this chapter, it was suggested that changes in responses throughout the course of the experiment might be an effect of a shift in anaesthetic depth on the responsiveness of neuronal populations. However, EEG and ECoG recording in the present study demonstrated that there was no shift in anaesthetic depth as a result of the injection of muscimol. Note that this was not due to a floor effect, as anaesthetic depth remained above stage IV. Further, given the dramatic shift in collicular activity and the step change in DA neuron responses following the injection, it seems unlikely there was a gradual drift of arousal level throughout the experiment.

Although the stimulation parameters used were selected with the risk of tissue damage in mind, tissue damage from repeated cortical stimulation was also suggested as a potential confound of the results of the present study. The results suggest that gradual tissue damage is not the cause of the change in response magnitude of DA cells, as most recordings showed an initial step change in response magnitude between the end of the pre-muscimol block and the start of the post muscimol block, suggesting that the muscimol was the cause of the decrease. Further, examination of the cresyl violet processed sections of the cortical stimulation sites also showed no evidence of tissue damage around the cortical electrode (for example, see Figure 4-2 Coronal section of the somatosensory cortex, processed for cresyl violet. Measurement relative to bregma indicates the location of the section. Arrow indicates

the approximate location of the tip of the stimulating electrode. S1Bf: primary somatosensory cortex, barrel field.). Finally, the potential for habituation of either the SC or DA responses to repeated cortical stimulation was suggested as a possible confound. Again, this possibility is discounted by the data presented here. While the response magnitude of DA neurons had shifted significantly by the end of the period of recording after an injection of muscimol, there was no comparable change in recordings where no injections were made. DA neurons recorded for up to 45 minutes, with 1300 presentations of trains of cortical stimulation, continued to show robust responses, while the response magnitude of DA neurons following an injection of muscimol had decreased notably by this point.

Effect of muscimol on SC activity

Prior to any injection of muscimol, trains of cortical stimulation produced a short latency, short duration response in the SC. While the latency is comparable to that of the single pulse of cortical stimulation from chapter 3, the duration is much longer. This is perhaps trivial, as the response to a longer duration stimulus would be expected to be longer in duration than the response to a shorter stimulus. However, comparison of the total response duration with the duration of a train of five pulses at 150 Hz shows that the response only lasted 9 ms after the end of the last pulse, much shorter than the response to a single pulse. As the interpulse interval for the pulse train used here (five pulses at 150 Hz) was 6.7 ms, the responses evoked by each pulse of the train likely overlapped. This suggestion is supported when the activity in SC between each pulse of the train is examined – the activity peaks after the second pulse, presumably representing the combination of the tail of the response to the first pulse and the beginning of the response of the second pulse. However, the activity after the third pulse, which occurs 13.3 ms after train onset, falls below that of even the first pulse, even though it might be assumed that the response to this pulse is combined with the tail of the response to the second pulse and the very tail of the response to the first pulse (if the duration of the response to each pulse is similar to that seen to a single pulse in chapter two). This may be the result of stimulus adaptation, a typical response to repeated high-frequency stimulation

Muscimol caused a significant and immediate decrease in background activity in SC, which remained largely constant throughout the recording. This rapid and prolonged suppression of activity following intracollicular muscimol is in line with

previous studies, e.g. Edeline et al. (2002) who reported locally recorded activity in the cerebral cortex fell to approximately 20% of pre-injection activity, and did not recover in the 2 h of subsequent recording. The dramatic suppression of activity in the SC is to be expected, as there is significant intrinsic GABAergic circuitry and extrinsic input. Injection of a $GABA_A$ agonist such as muscimol would mimic this tonic inhibition, and broadly inhibit neurons that are the usual target of GABA release.

As well as generally reducing the level of activity in the SC, intracollicular muscimol reduced the response evoked by cortical stimulation. There was a significant decrease in the response magnitude after injection of muscimol, over and above any change in background activity. When the activity following each pulse was considered separately, there was a significant difference in the effect of muscimol across pulse number. This further supports the interpretation that the measured decrease in evoked activity was due to a suppression of the evoked response, rather than a uniform decrease in activity.

Response direction does not indicate separate populations of neurons

As the DA neurons in the present study showed responses to cortical stimulation in the absence of injections of BMI in the SC, the responses of the DA neurons might be considered to be a solely a product of cortical stimulation, unconfounded by the effect of BMI, and so may provide a better idea of whether inhibited and excited DA neurons represent a topographically distinct subpopulation. The nine DA neurons in the present chapter were combined with the eight DA neurons from chapter 3 that showed responses to cortical stimulation in the absence of modulation of the activity of the SC to see if there was a difference in distribution between DA neurons showing responses with excitatory first components and those showing inhibitory first components. However, there was no clear difference in the distribution of response first component directions (see Figure 4-13 Reconstructed plots of recording sites in the midbrain on diagrams of coronal sections. Points indicate the approximate location of DA neurons showing responses with excitatory (green) and inhibitory (red) first components. Measurements relative to bregma indicate the location of the section. Abbreviations as in chapter 3.) suggesting that the difference in response was not the result of separate subpopulations.

Consideration of current spread from intracortical stimulation

Although the results strongly suggest that the SC is involved in relaying cortical input to DA neurons, it is important to consider theoretical and methodological issues that could affect this conclusion. As with chapter 3, the intention of the current study was to investigate the effect of modulation of the SC on input from the barrel field of the primary somatosensory cortex, and so it is important to confirm the activation was restricted to the barrel field. The optical imaging data presented here show that the cortical haemodynamic response to 100 μs 150 Hz 0.6 mA stimulations is comparable in extent to electrical whisker pad stimulation, and is confined within the barrel field.

4.5.3 Conclusion

The present chapter confirms the hypothesis that input to DA neurons to produce the phasic response to cortical stimulation is relayed by the SC. The DA response to cortical stimulation shows an initial step change with muscimol injection, and then gradually decreases after an injection of muscimol at a timescale that suggests it is necessary for muscimol to diffuse through a considerable portion of the SC to suppress responses in a given DA neuron. Although the experiments of the current and previous chapters are convincing evidence in the argument that somatosensory cortical input reaches DA neurons via the SC, and direct vibrissal input from the trigeminal nucleus also reaches the SC, the question still remains as to whether this is the source of subcortical somatosensory input to DA neurons, and how this input interacts with corticotectal input. These questions will be addressed in the following chapter.

5 The effects of collicular disinhibition on the responsiveness of dopaminergic neurons to trigeminal nucleus stimulation

5.1 Chapter summary

Chapters 3 and 4 demonstrated that input from the primary somatosensory cortex can modulate the activity of DA neurons via the SC. However, the somatosensory cortex is part of the vibrissal system, which originates in the trigeminal nucleus, a structure that also projects directly to the SC. This provides an opportunity to examine whether direct input from the trigeminal nucleus can modulate the activity of DA neurons via the SC, and whether the direct subcortical and indirect cortical inputs can be distinguished. The present chapter details findings that show that DA neurons respond to stimulation of the trigeminal nucleus, and injection of BMI into the SC modulates this response. The results suggest that the SC may act as a relay of somatosensory vibrissal input to DA neurons both directly from the trigeminal nucleus, and indirectly via a trigemino-thalamocorticotectal pathway, although discriminating them may require further study.

5.2 Introduction

The possibility of identifying and separating direct subcortical and indirect cortical input to DA neurons has been mentioned in the introductory chapter, and will be discussed further here. Although the primary focus of this project is to investigate the effect and route of cortical and subcortical sensory input to DA neurons, comparison of the response of the SC to trigeminal stimulation to its response to whisker deflection may provide information about how the stimulus is interpreted, which may then inform interpretations of its effect on DA responses.

5.2.1 Response characteristics of vibrissae sensitive SC neurons

Cohen et al. (2008) identified three components in the response of individual SC neurons to vibrissal deflection. The first component lasted from approximately 2-8 ms after deflection, the second last from 9-25 ms and the third from 26-100 ms. As was mentioned in the introduction, the first component was driven by direct trigeminotectal input, while the second component was driven by cortical input.

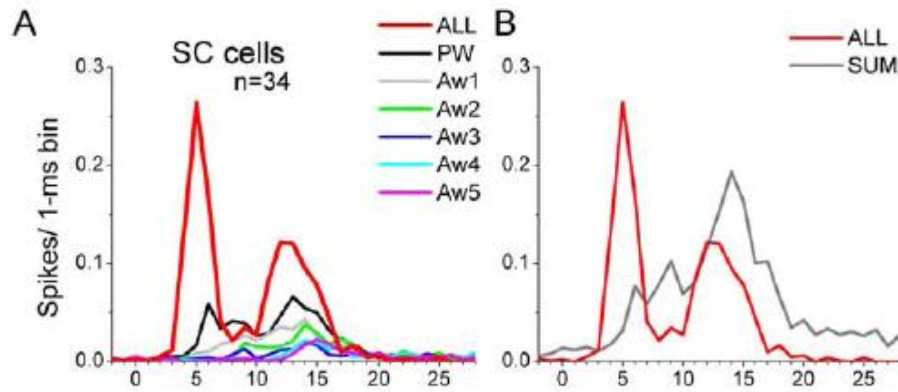


Figure 5-1 The effect of single and multiwhisker stimulation on single units in the SC. A Population PSTHs of SC responses evoked by single whisker stimulation of the principal whisker (PW, and five alternate whiskers (AW1-5), or by multiwhisker stimulation of these six whiskers (ALL). B Population PSTHs of the same multiwhisker response in A (ALL) compared with the sum of the single whisker responses in A (SUM). X-axis is time from whisker deflection onset in ms. From (Cohen et al., 2008).

SC neurons have broad receptive fields, responding most to deflection of a principle whisker, and also to deflection of 5 alternative whiskers with decreasing preference (Figure 5-1A, Cohen et al., 2008). Simultaneous deflection of all six whiskers of a neuron's receptive field produces a superadditive response in the first component, but not the second (see Figure 5-1B). Multiunit recording of SC activity in response to electrical whisker pad stimulation produces a response profile broadly similar to the response profile of single neuron responses to multiwhisker stimulation (Cohen and Castro-Alamancos, 2010). It is possible that direct electrical stimulation of the trigeminal nucleus may activate neurons related to multiple whiskers, and so the multiunit recording may show a similar response profile to the multiwhisker stimulation shown here, with similar numbers of events in first and second components, rather than the larger second components that characterise SC responses to single whisker stimulation.

Although the vibrissal somatosensory system is complex in terms of anatomy and physiology, the trigeminal nucleus is, in the most general terms, a relay of contact related signals from the vibrissae. Consequently, direct stimulation of the trigeminal nucleus may provide stimulation analogous to vibrissal stimulation stimulus, albeit a coarse resolution, potentially simultaneous 'whole field' stimulus. This stimulation, which is analogous to a whole field light flash, could be used to examine the response of DA neurons to somatosensory stimuli, and the route by which it arrives.

5.2.2 DA responses to trigeminal stimulation

The previous chapters showed that it was possible to drive DA neurons by direct electrical stimulation of the somatosensory cortex without disinhibiting the SCGiven the small size of the trigeminal nucleus, it is possible that a greater proportion of the trigeminal nucleus, and consequently a greater proportion of the SC would be activated by direct stimulation compared to stimulation of the barrel field. This might result in a greater number of preBMI responses that were seen in chapter 3.

As the trigeminal nucleus projects to the SC both directly and indirectly via the cortex, the effects of stimulating both pathways have to be considered. Given that direct cortical stimulation was not always successful in affecting DA neuron activity in the baseline state, activation of the indirect thalamocorticotectal projection by stimulating the trigeminal nucleus may be similarly ineffective. However, in a similar manner to which previously unresponsive DA neurons became sensitive to cortical stimulation after disinhibition of the SC, previously ineffective indirect cortical input may modulate the response of DA neurons to trigeminal stimulation after BMI injection. If the responses of the SC and DA neurons to trigeminal stimulation before and after BMI injection are examined, it might be possible to discriminate between the relative contributions of the direct trigeminotectal and indirect trigemino-thalamocortical input to the response.

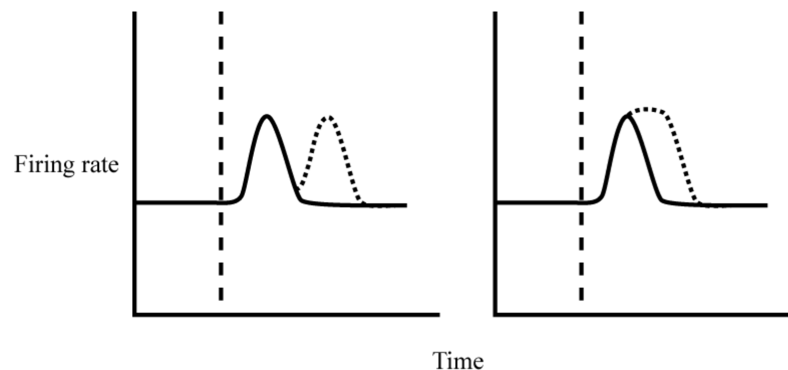


Figure 5-2 An illustration of the potential relative contributions of cortex (dotted line) and trigeminal nucleus (solid line) to DA neuron responses to trigeminal stimulation. The dashed line indicates stimulus onset. The cortex may produce a second peak in the response (left) or increase its duration (right).

As the thalamocortical projection is less direct than the trigeminal nucleus projection, we might expect to see different responses at different latencies if the pathways activate DA neurons separately or perhaps a longer response if the inputs

arrive more closely (see Figure 5-2). However, cortical response latencies to whisker deflection (~10 ms, Petersen (2007)), are not much longer than SC latencies (6-7 ms, Hemelt and Keller (2007), so the effect of indirect cortical input on the SC response to trigeminal stimulation might be difficult to distinguish. Instead, changes in peak latency or amplitude might be a better indication.

5.2.3 Experiment rationale

In chapter 3, disinhibition of the SC was shown to produce responsiveness of DA neurons to cortical stimulation, suggesting that cortical input reaches DA neurons via the SC. The following study will examine whether stimulation of the trigeminal nucleus produces responses in DA neurons, and as the trigeminal nucleus also projects to the SC, it will study whether the SC is involved in DA responses evoked by trigeminal stimulation by examining the effect of intracollicular injections of BMI. Further, if there are responses to trigeminal stimulation in DA neurons before any injection is made, then BMI may allow the respective contributions of direct and indirect (i.e. cortical) influences to be determined.

5.3 Method

5.3.1 Experimental procedure

The experimental design is summarised in graphical form in Figure 3-1. The present study used simultaneous electrophysiological recording of SC (multiunit) activity and DA (single unit) activity in SNc, in response to electrical stimulation of trigeminal nucleus, both before (Figure 3-1a) and during (Figure 3-1b) chemical disinhibition of SC. To ensure only neuronal elements in the SC were disinhibited, local injections of an excitatory substance, the $GABA_A$ receptor antagonist BMI (Figure 3-1b, green microsyringe), were used.

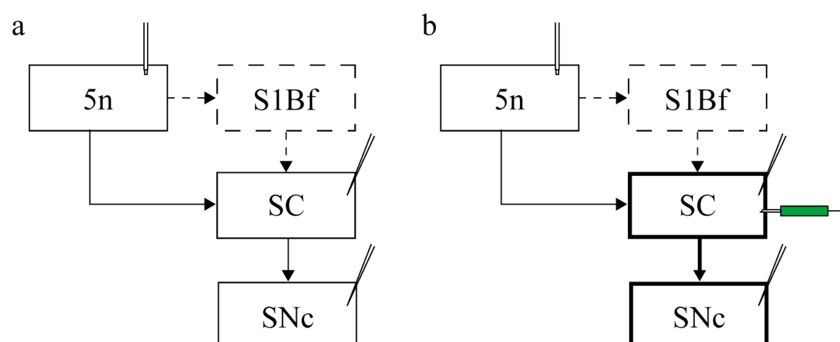


Figure 5-3 Schematic of the experimental design for this experiment.

The subject preparation, experimental procedure, histology, and statistical analysis have been previously described in the chapter 2, and elaborated on in chapters 3 and 4. Some sections have been repeated here, with further detail regarding this experiment where appropriate.

Data were obtained from 13 acutely prepared adult hooded Lister rats (315-440 g). To place the stimulating electrode in the trigeminal nucleus, the intra-aural line was used as a reference point for dorso-ventral and anterio-posterior coordinates, while the midline was used as the medio-lateral reference point. The stimulating electrode was placed at AP 2.6-3.3 mm caudal of inter-aural point, ML 2.2-3.0 mm from midline, 0.5-1.3 mm dorsal of inter-aural point. The multiunit electrode/cannula was introduced vertically into the lateral intermediate layers of SC (AP 6.04-7.64 mm caudal to bregma; lateral 1.5-2.2 mm; dorsoventral 3.9-5.2 mm below dura). The electrode/cannula tip separation was 0.2-0.5 mm. DA neurons were recorded from SNc (AP 5.3-6.04 mm caudal to bregma).

The experimental procedure is described in chapter 2, electrical stimulation consisted of single pulses current to the trigeminal nucleus (0.5 mA, 100 µs). The responses to trigeminal stimulation and the effects of SC disinhibition were tested on 1-2 SNc neurons in a single subject. See chapter 2 for a description of the histological procedures used in the present chapter. Analyses were performed using the methods as described in chapter 2.

5.4 Results

5.4.1 Inclusion criteria

To be included in the analysis, putative DA neurons had to meet same histological criteria as chapters 3 and 4, except the stimulation electrode had to have been confirmed to be within the trigeminal nuclear complex. At least 100 trials consisting of a light flash and trigeminal stimulation before and after a successful injection of BMI into SC were required for inclusion in the analysis. A successful injection of BMI into SC was judged by the presence of a significant response to light flash stimulus in SC. 17 DA neurons met these criteria

Recording sites were taken as the centre of electrolytic lesion or of the iontophoretic injection of Potamine Sky Blue dye. Examples can be seen in chapter 3. Stimulation sites were taken as the ventral extent of the electrode track. There was no evidence of stimulation related tissue damage around the stimulation sites in TNC (see Figure 5-4).

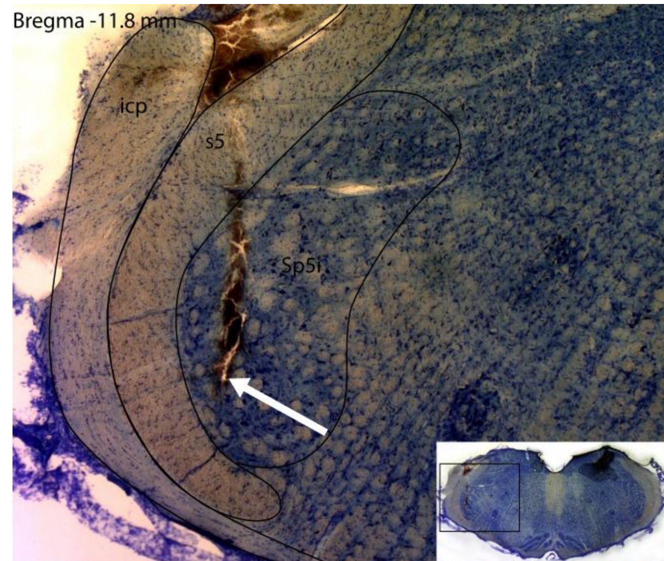


Figure 5-4 Coronal section of the brainstem, processed for cresyl violet. Arrow indicates location of the tip of the stimulating electrode. Sp5i: spinal trigeminal nucleus, interpolar part; s5: sensory root of the trigeminal nerve, icp: inferior cerebellar peduncle

The recording location of the DA neurons included in the study, the recording and injection locations in SC, and the stimulation sites in the TNC are shown in modified diagrammatic sections from Paxinos and Watson (2004) in Figure 5-5, Figure 5-6, and Figure 5-7. Recorded DA neurons (Figure 5-5) were located in a centrally located region of the SNc, which extended for approximately half of its rostro-caudal length. In terms of laterality, DA neurons were typically recorded towards the medial extent of SNc, bordering on the region designated SNcm (Paxinos and Watson, 2004). However, some DA neurons recorded rostrally extended towards the lateral extent of SNc. Recording and injection sites in the SC (Figure 5-6) extended over most of the rostro-caudal extent of the SC, although they avoided the extreme rostral and caudal poles. Sites were confined within the lateral intermediate and deep layers of the SC. Stimulation sites in the TNC (Figure 5-7) were found within the rostral half of Sp5i. Stimulation sites were typically in the ventral half of the subnucleus, but occasional sites were seen further dorsally.

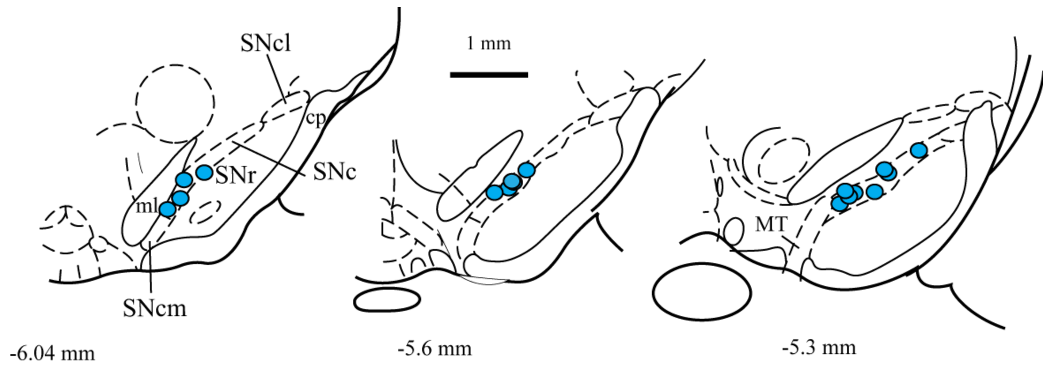


Figure 5-5 Reconstructed plots of recording sites in the midbrain on diagrams of coronal sections. Points indicate the approximate location recorded DA neurons. Measurements relative to bregma, and indicate the location of each section. Abbreviations as in chapter 3

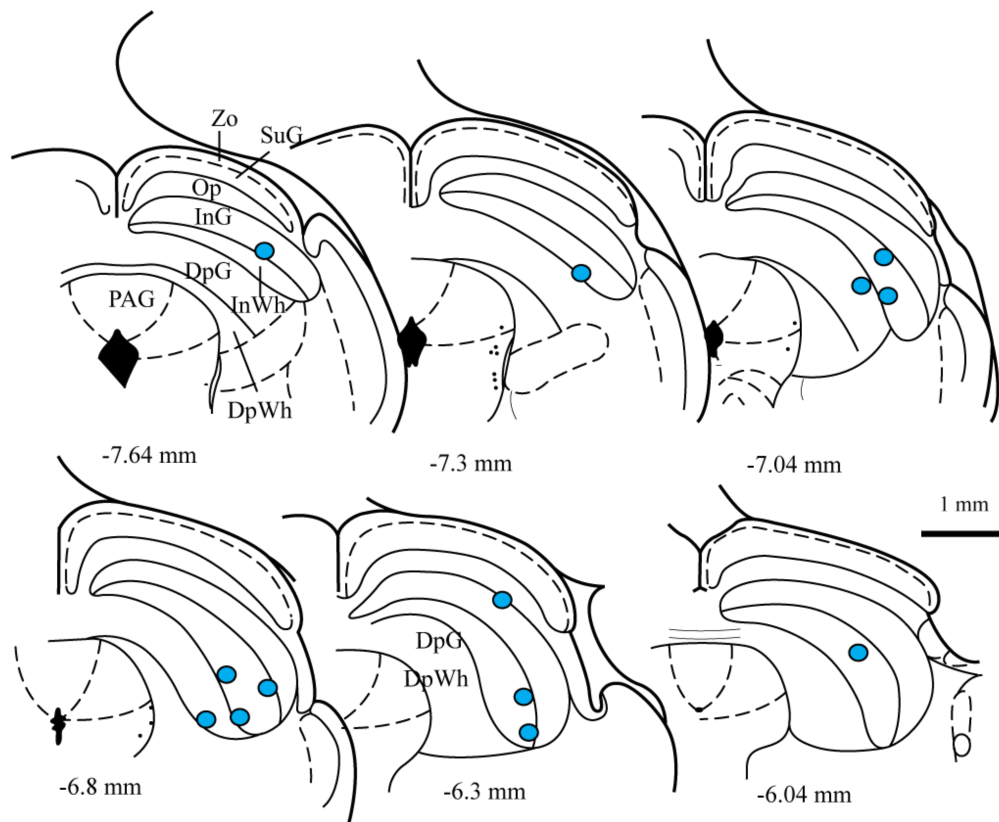


Figure 5-6 Reconstructed plots of recording sites in the midbrain on diagrams of coronal sections. Points indicate the tip position of the electrode-injector assembly. Measurements relative to bregma, and indicate the location of each section. Abbreviations as in chapter 3

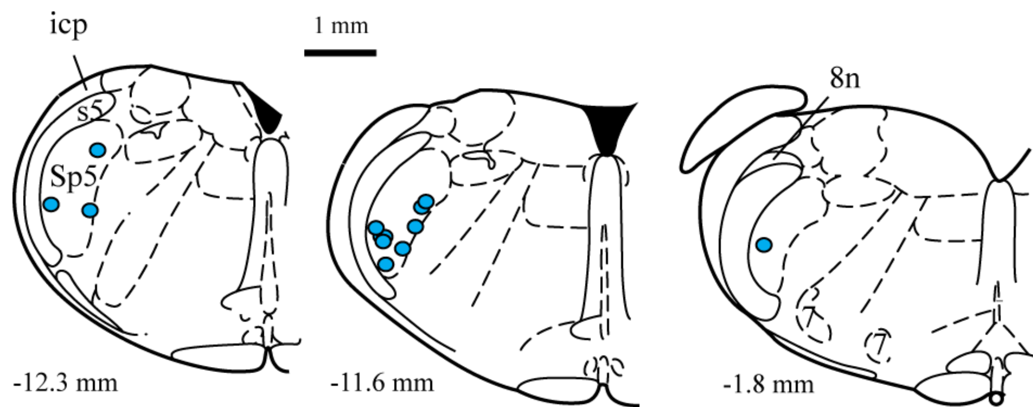


Figure 5-7 Reconstructed plots of stimulation sites in the trigeminal nucleus on diagrams of coronal sections. Points indicate the tip position of the stimulation electrode. The exposed pole of the central electrode extends 500 um dorsally from the point indicated, followed by 500 um of insulated electrode, followed by a 500 um exposed section forming the surround electrode. Measurements relative to bregma, and indicate the location of each section. Sp5: spinal trigeminal nucleus; s5t: sensory root of the trigeminal nerve; 7: facial nucleus, 8n: vestibulocochlear nerve; icp: inferior cerebellar peduncle.

Processing for c-fos and TH immunoreactivity was performed in all 13 animals. Only recordings from putative DA neurons sited in TH positive regions of the midbrain were included for analysis. An example of TH immunoreactivity can be seen in chapter 3. The distribution of Fos-like immunoreactivity (FLI) was used as an indication of the spread of activation as a result of BMI injections. An example of FLI is shown in chapter 3. FLI indicates the expression of c-Fos a protein associated with neural activity (Herdegen and Leah, 1998), and would indicate the extent of the disinhibitory effect of BMI. When injections were made within the intermediate and deep layers of the SC, FLI was largely contained within the SC. This is supported by previous experiments using comparable protocols (Coizet et al., 2003), and the results of chapter 3. Injections of BMI in different animals were centred on different locations within SC. Injection sites were categorised as medial/lateral and rostral/caudal.

5.4.2 Activity in the SC

To assess the effect of sensory stimulation on general SC activity without the presence of BMI, the mean background activity in the 500 ms before the light flashes in the block of pre-BMI stimulations was compared to the mean baseline activity in the 60-120 seconds before the start of any stimulation.

There was a significant increase in spontaneous activity during periods of stimulation before BMI injection (M_{base} = 207.0 Hz±8.1, Hz; M_{pre} = 232.1 Hz±11.8; t = -3.278, df = 16, p = 0.004). There was no significant difference between pre-BMI and postbicuculine background activity (M_{pre} = 231.1 Hz±11.8 Hz; M_{post} = 267.1 Hz±27.9 Hz; t = -1.316 df = 16, p > 0.05). Examination of the records shows that following injection of BMI, most records (11/17) showed an increase of at least 10% in the rate of triggered activity, while the other six showed at least a 10% decrease. However, there was nothing to indicate any difference between BMI injections causing an increase in spontaneous activity and those showing a decrease, and both increases and decreases in activity were seen in different recordings in the same animal.

Throughout the pre-BMI trials, there was no phasic response to the light in the intermediate and deep SC. There was, however, a short latency (onset latency: Median = 1 ms, 1 ms:1 ms, peak latency: M = 6.3±0.9 ms) short duration (M = 24.9±1.8 ms) response to trigeminal stimulation. Across the 200 ms response period, the mean amplitude above background activity of the peak of the response was 1309.9±101.0 Hz. Figure 5-8 shows an example of the SC to single pulse stimulation.

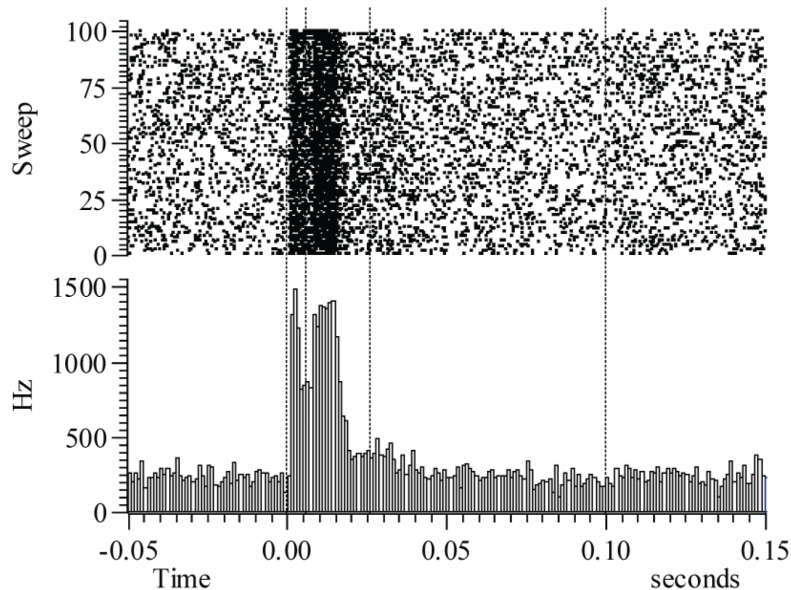


Figure 5-8 PSTH/raster plot of SC activity in response to a single 0.5 mA pulse of stimulation to Sp5i before BMI. Vertical cursor at t = 0 indicates the timing of the pulse. Subsequent cursors indicate the start of each components 1, 2 and 3 (see text for details) Horizontal cursor indicates mean pre-stimulus firing rate.

SC responses to trigeminal stimulation often showed two distinct peaks, the first

starting in the first 2 ms after stimulus onset, the second usually starting around 8 ms. Two records did not show two clear peaks, although this was due to activity in the period usually covered by two separate peaks merging into one, rather than the absence of a response in one of the periods. As stated above, the mean duration of the response was 24.9 ms. This corresponds closely to the two largest components of the SC neuron responses to whisker manipulation reported by Cohen et al. (2008) and Cohen and Castro-Alamancos, (2010). They divided the responses into two short latency components, with the first covering 2-8 ms after stimulation, and the second 9-25 ms, and a long latency component, covering 26-100 ms. Similar divisions were applied to the current data, although as the divisions were derived from whisker manipulation and the present data were derived from trigeminal nucleus stimulation, the responses presented here may be of shorter latency. Cohen et al. (2008) showed that cells in the spinal trigeminal nucleus responded to whisker manipulation at an average latency of 2 ms. Thus, the onset measurements of the components used here were shifted 2 ms – component 1 (C1) starting at 0 ms, component 2 (C2) starting at 7 ms, and component 3 (C3) starting at 24 ms. The response magnitudes (mean firing rate in each component, minus the mean background firing rate) of each of these components were measured. The results are shown in Figure 5-9 (left). On average, the response magnitude of C1 was greater than C2 (M_{C1} = 682.9±77.4 Hz; M_{C2} = 592.2±51.2 Hz), although five records showed the opposite pattern. There was little response in C3 (M_{C3} = 12.7±11.8 Hz).

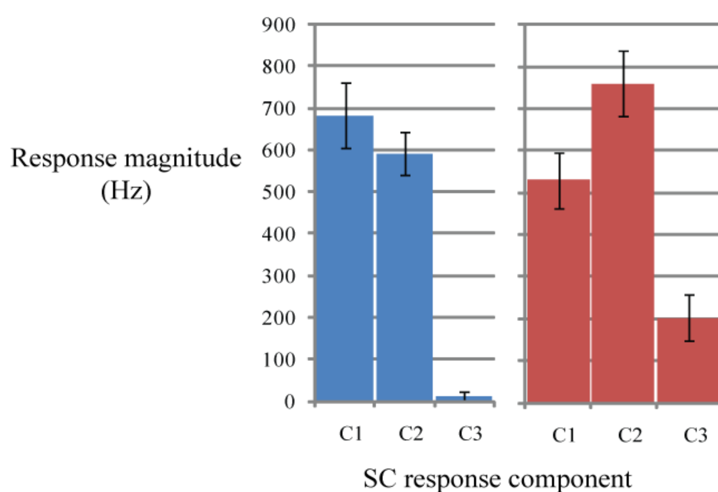


Figure 5-9 Response magnitudes of each component of the SC response to trigeminal stimulation, before (left) and after (right) intracollicular BMI injection.

After BMI injection, a phasic excitatory response to the light flash was seen in all 17 records (onset latency: M = 51.9±3.3 ms; duration: M = 128.5±15.8 ms), which

was taken as indication of a successful injection. Across all 17 records, considering the whole 200 ms response period, there was a significant increase in the magnitude of response to trigeminal nucleus stimulation after injection of BMI, (activity above background: M_{pre} = 94.4±11.8 Hz; M_{post} = 203.2±34.3 Hz; t = -3.308, df = 16, p = 0.004; see Figure 5-10). Injection of BMI resulted in a significant increase in onset latency ($median_{pre}$ = 1 ms, 1 ms:1 ms; $median_{post}$ = 1 ms, 1 ms:2 ms; V = 4, p = 0.042), peak latency ($median_{pre}$ = 8 ms, 2 ms:9 ms; $median_{post}$ = 9 ms, 9 ms:11 ms; V = 10.5, p = 0.015), and duration of the response to trigeminal stimulation ($median_{pre}$ = 26 ms, 19 ms:29 ms; $median_{post}$ = 50 ms, 30 ms:126 ms, V = 3, p = 0.001), but there was no change in the peak amplitude (M_{pre} = 1309.9±101.0 Hz; M_{post} = 1208.7±86.6 Hz; t = 1.793, df = 16, p > 0.05). Post-BMI SC responses to a light flash had significantly longer durations ($M_{lightdur}$ = 128.5±15.8; $M_{trigdur}$ = 77.9±14.4; t = 4.03, df = 16, p < 0.001) and onset latencies ($median_{lightonset}$ = 50 ms, 41 ms:59 ms; $median_{trigonset}$ = 1 ms, 1 ms:2 ms; V = 153, p < 0. 001) compared to post-BMI responses to trigeminal stimulation, but not significantly different response magnitudes ($M_{lightmag}$ = 200.6±30.2 Hz; $M_{trigmag}$ = 203.2±34.3 Hz; t = -.09, df = 16, p > 0.05). Figure 5-10 shows a typical response of the SC to light flash and cortical stimulation before, and after injection of BMI.

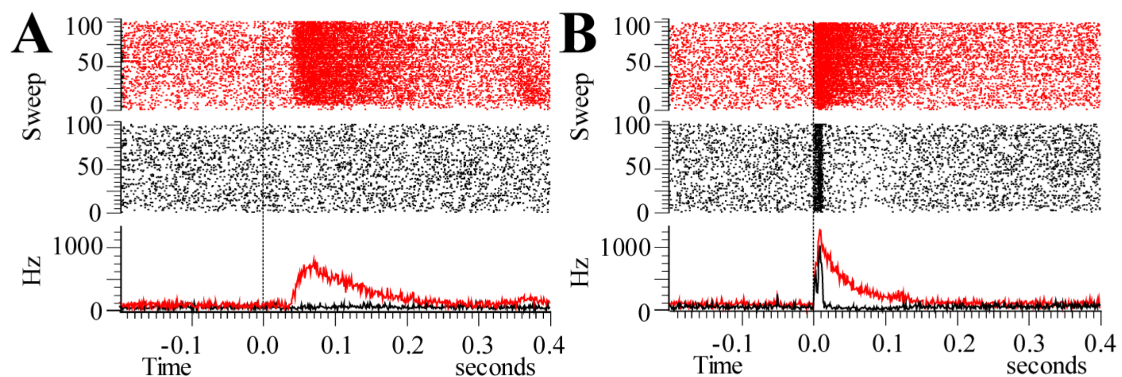


Figure 5-10 Plots of SC MUA in response to light flash (A) and trigeminal stimulation (B) before (black) and in the presence of (red) local microinjections of BMI. Stimulus onset at 0.0 s.

Although measuring across the whole 200 ms response period showed a consistent increase in response magnitude, there was a differential effect across the components of the SC response to trigeminal stimulation. To assess the response of SC over each component, the response magnitude of C1, C2 and C3 were measured, before and after injection of BMI. A two-way within subjects ANOVA (IV: response magnitude, DV: component number (3 levels), injection (2 levels)) revealed a

significant difference between response magnitudes in each component across both conditions ($F(2,32) = 53.51$, $p < 0.001$; response magnitudes in each component – C1: 606.1 Hz, C2: 675.5 Hz, C3: 107.8 Hz) and an interaction between BMI and component ($F(2,32) = 15.26$, $p < 0.001$; see Figure 5-9), but no main effect of BMI across all components ($F(1,16) = 3.65$, $p > 0.05$; response magnitudes in each condition – pre: 429.2 Hz, post: 497.0 Hz). When the components of the response were compared, the response magnitude of C2 on average was now greater than C1 ($M_{C1} = 529.3 \pm 65.0$ Hz; $M_{C2} = 758.9 \pm 77.5$ Hz), and there had been an increase in C3 ($M_{C3pre} = 12.7 \pm 11.8$ Hz; $M_{C3post} = 202.9 \pm 53.3$ Hz).

5.4.2.1 Activity of DA neurons

To assess the effect of sensory stimulation on general DA activity without the presence of BMI, the mean background activity of pre-BMI stimulations was compared to the level of baseline activity. There was no significant effect of stimulation on spontaneous firing rate ($M_{base} = 3.6 \pm 0.4$ Hz; $M_{pre} = 3.5 \pm 0.4$ Hz; $t=0.605$, $df=16$, $p > 0.05$). Across all 17 records, there was no significant effect of BMI injection on spontaneous activity ($M_{pre} = 3.5 \pm 0.4$ Hz; $M_{post} = 3.4 \pm 0.4$ Hz; $t=0.411$ $df=16$, $p > 0.05$). Examination of the records shows that following injection of BMI, 4/17 records showed an increase of at least 10% in the rate of triggered activity, while 6/17 showed at least a 10% decrease.

Prior to BMI injection, all but one DA neuron (94.1%) showed a response to trigeminal stimulation (onset latency: 32.8±5.8 ms, duration: 205.9±41.2 ms, absolute response magnitude: 2.5±0.5 Hz). No DA neurons responded to the light flash. On average, onset latencies of DA neuron responses to trigeminal stimulation reliably followed SC responses ($median_{SC} = 1$ ms, 1 ms:1 ms, $n = 17$; $median_{DA} = 20$ ms, 20 ms:40 ms, $n = 16$; $W = 0$, $p < 0.001$).

During periods of significant effect of BMI in SC, all but one DA neuron (94.1%) showed a response to trigeminal stimulation, and 1/17 (64.7%) DA neurons showed a significant response to the light flash. The DA neuron that did not respond to trigeminal stimulation did respond to the light flash. On average, onset latencies of DA neuron responses reliably followed SC responses to both light flash ($M_{SC} = 51.9 \pm 3.3$ ms, $n = 17$; $M_{DA} = 89.1 \pm 14.0$ ms, $n = 11$; $t = -2.71$, $df = 11.24$, $p = 0.020$) and trigeminal stimulation ($Median_{SC} = 1$ ms, 1 ms:2 ms; $n = 17$; $Median_{DA} = 20$ ms 15 ms:40 ms, $n = 16$; $W = 1$, $p < 0.001$). Onset latencies of DA neuron responses to a

light flash were significantly longer than those of DA neuron responses to trigeminal stimulation (M_{light} = 89.1±14.0 ms, n = 11; M_{trig} = 30.6±7.5 ms, n = 16; t = 3.82, df = 16.33, p = 0.001). As onset latencies of SC responses to trigeminal stimulation were much shorter than SC responses to light, this may have an effect on DA response latencies. Examination of the onset latency of DA neuron responses to light flash and trigeminal stimulation, minus the latency of the SC response to the same stimulus, showed there was no significant difference between the two stimuli (M_{light} = 41.1±13.6 ms, n = 11; M_{trig} = 28.9±7.5 ms, n = 16; t = 0.81, df = 16.62, p > 0.05) (see Figure 5-10).

Records were examined to see if there was any difference in duration between responses to the two modalities. There was no significant difference between the durations of DA neuron responses to each stimulus (M_{light} = 196.4±46.3 ms, n = 11; M_{trig} = 171.9±29.0 ms, n = 16; t = 0.46, df = 18.25, p > 0.05). There was no significant differences between durations of DA neuron responses and the durations of the corresponding SC responses to light flash (M_{SC} = 142.9±18.8 ms; M_{DA} = 196.4±44.1 ms; t = -1.13, df = 10, p > 0.05, n = 11), however, there was a significant difference between the durations of DA neuron responses and the corresponding SC responses trigeminal stimulation (M_{SC} = 68.0±11.2 ms; M_{DA} = 171.9±29.0 ms; t = -3.27, df = 15, p = 0.005, n = 16) (see Figure 5-11A). There was no significant difference between absolute magnitudes of responses of DA neurons to each stimulus (M_{light} = 2.1±0.7 Hz, n = 11; M_{trig} = 2.2±0.4, n = 6; t = -0.16, df = 18.17, p >0 .05) (see Figure 5-11C), corresponding response magnitudes ($M_{lightmag}$ = 234.2±39.5 Hz, n = 10; M_{ctxmag} = 184.8± 30.8 Hz, n = 8; t = 0.99, df = 20.76, p > 0.05) (see Figure 5-11B). There was a significant difference between the durations of responses in the SC corresponding to responsive DA neurons ($M_{lightdur}$ = 142.9±18.8 ms, n = 10; $M_{trigdur}$ = 68.0±11.2 ms, n = 16; t = 3.43, df = 16.92, p = 0.003) (see Figure 5-11A).

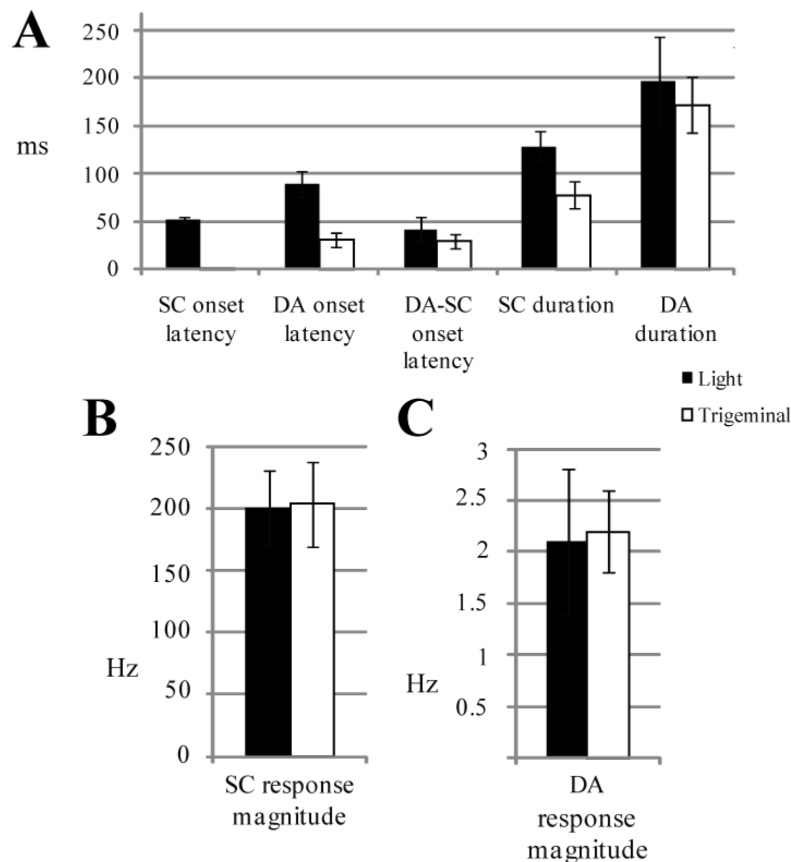


Figure 5-11 Comparisons of collicular and DA neuron response onset latencies and durations (A), collicular response magnitudes (B) and DA response magnitudes (C) to a light flash (blue) and trigeminal stimulation (red).

Records were examined to see if there were differences between DA neurons showing excitatory first phases and inhibitory first phases that might suggest the existence of different inputs or separate sub-populations underlying inhibitory and excitatory responses. Chapter 3 found some inconsistency between the response direction of pre-BMI responding DA neurons and their post-BMI response. This was also seen in the present study, so pre- and post-BMI DA neuron responses to trigeminal stimulation were compared separately. Out of the 16/17 DA neurons that responded to trigeminal stimulation before BMI injection, 5/17 showed responses with excitatory first components, and 10 showed responses with inhibitory first components. There were no significant differences between the characteristics of DA neurons showing responses with excitatory first components and those showing inhibitory first components in baseline firing rate (M_{ex} = 3.0±1.1, n = 5; M_{in} = 4.1±0.4, n = 10; t = -0.09, = df = 6.05, $p > 0.05$) onset-trough action potential measurement (M_{ex} = 1.5±0.1 ms, n = 5; M_{in} = 1.5±0.07 ms, n = 10; t = 0.50, df = 8.45, $p > 0.05$), or on measures of onset latency (M_{ex} = 35.8±15.4 ms, n = 5; M_{in} = 31.0±4.8 ms, n = 10; t

= -0.32, df = 6.18, $p > 0.05$), response duration (M_{ex} = 144.2±47.7 ms, n = 5; M_{in} = 243.0±58.0 ms, n = 10; t = -1.32, df = 13.87, $p > 0.05$) or absolute response magnitude (M_{ex} = 2.9±1.1 Hz, n = 5; M_{in} = 2.2±0.4, n = 10; t = -0.85, df = 5.82, $p > 0.05$). Out of the 16/17 DA neurons responsive to trigeminal stimulation after BMI injection, 9/17 (52.9%) showed responses with initial excitatory components, while 7/17 (41.2%) showed responses with initial inhibitory components. All DA neuron responses to the light flash showed initial excitatory components, so no comparison was made. There were no significant differences between DA neurons showing responses with excitatory first components and those showing inhibitory first components in baseline firing rate (M_{ex} = 3.3±0.8, n = 9; M_{in} = 3.9±0.4, n = 7; t = -0.61, = df = 12.24, $p > 0.05$), onset-trough action potential measurement (M_{ex} = 1.6±0.08 ms, n = 9; M_{in} = 1.5±0.09 ms, n = 7; t = 0.67, df = 12.49, $p > 0.05$), or on measures of onset latency (M_{ex} = 34.4±14.3 ms, n = 9; M_{in} = 25.7±3.7 ms, n = 7; t = 0.63, df = 9.02, $p > 0.05$), response duration (M_{ex} = 207.8±47.3 ms, n = 9; M_{in} = 125.7±18.4 ms, n = 7; t = 1.61, df = 10.28, $p > 0.05$) or absolute response magnitude (M_{ex} = 2.9±0.7 Hz, n = 9; M_{in} = 1.4±0.4, n = 7; t = 2.06, df = 12.08, $p > 0.05$).

5.4.3 BMI differentially modulates DA neuron multiphasic responses

When the responses of DA neurons to trigeminal stimulation were examined, it was noticed that there was often a clear short latency (~20 ms), short duration (~60 ms) response (Figure 5-12A-D, blue lines). In some cases, this short duration was particularly large in amplitude; either a total suppression (Figure 5-12A, D, blue lines), or a large amplitude peak (Figure 5-12B, C, blue lines). This could be followed by a longer latency (~80 ms), longer duration (~150 ms) response, either in the opposite direction (Figure 5-12A, B, blue lines), or the same direction, but usually distinguishable through its smaller amplitude (Figure 5-12C, blue line), although in some cases two separate phases were not visible (Figure 5-12D, blue line). The duration of these two phases closely matches the duration of the stimulus insensitive and stimulus sensitive components of the DA response described by (Hudgins, 2010). Thus, DA neurons were examined by separating the response into two components. The position of the components was shifted ±20 ms depending on the onset of the short latency phase of each DA neuron response. All 17 DA neurons showed a > 0.5 Hz change from background firing rate in the short latency component of the response, and 15/17 also showed a > 0.5 Hz change in the later component. Six of the

short latency components were excitatory, four of which were followed by later components, three of which were in also excitatory. Out of the eleven DA neurons showing inhibitory first components in their response, all eleven were followed by later components, eight of which were also inhibitory. The absolute response magnitudes of the short latency and longer latency components of the DA response were 3.1±0.5 Hz and 3.0±0.6 Hz respectively.

Following intracollicular injection of BMI, a differential effect on each of the components on some records made the presence of separate components in the DA response more obvious. 15/17 DA neurons continued to show > 0.5 Hz changes from background firing in the short latency component. Both of the DA neurons that ceased to respond showed inhibitory short latency components before BMI, one showed an inhibitory longer latency component, the other showed an excitatory longer latency component. Following BMI injection, the excitatory longer latency phase in on DA neuronwas largely unaffected, but the inhibitory longer latency component in the other was now a small (1.1 Hz) excitatory response. None of the shorter latency components of DA neurons that continued to respond after BMI changed direction. 15/17 DA neurons also showed > 0.5 Hz changes from background firing in the longer latency component of their response, however, the two DA neurons without longer latency components post-BMI were not the same as those prior to BMI. The post-BMI longer latency components in 5 DA neurons were in the opposite direction to their pre-BMI longer latency components. The absolute response magnitudes of the short latency and longer latency components of the DA response were 2.9±0.6 Hz and 2.4±0.5 Hz respectively.

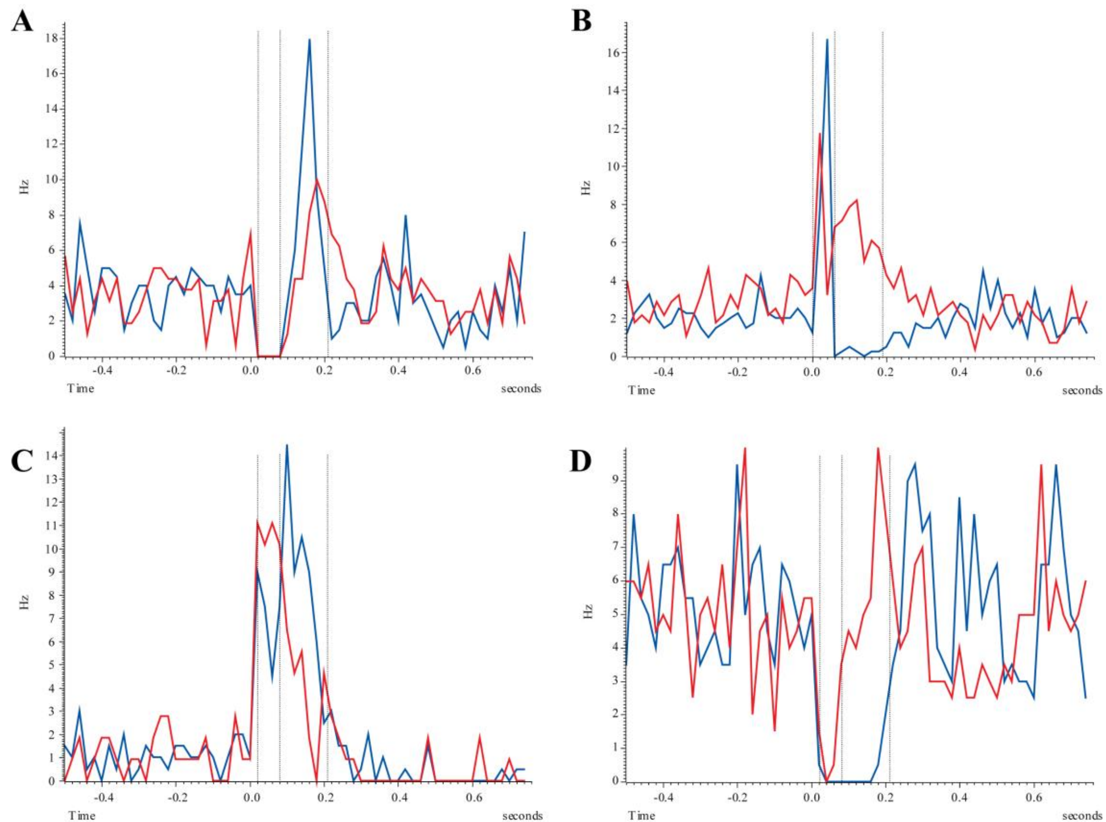


Figure 5-12 Illustration of the differential effect of BMI on the DA response to trigeminal stimulation. Figures show the response of a DA neuron to trigeminal stimulation before (blue) and after (red) intracollicular BMI. Cursors on each figure indicate, from left to right, start of C1 (0-20 ms), start of C2 (60-80 ms), end of C2 (210-230 ms). See text for a description of the changes. C1 responses may remain unaffected (A) be suppressed (B,D) or enhanced (C). C2 responses could also be suppressed (A) enhanced (C) or even change direction (B, D).

It was suggested that the stimulus insensitive component may be driven by purely subcortical sensory input from the SC, while the later phase may be cortically mediated. With this in mind, the two components of the responses of DA neurons were compared to the components of the SC response, which are also suggested to be separately subcortically and cortically mediated (Cohen et al., 2008). The combination of inhibitory and excitatory responses in short and longer latency components of the DA response, and the changes in magnitude and direction of each component make a numeric analysis too complex to draw any conclusions. However, although the complex combination of SC and DA response changes in the present data do not allow for a clear pattern of effect to be extracted, they do establish the existence of separate clear response components in both the SC and DA neuron response, and a differential effect of intracollicular BMI on each component.

5.4.4 Dopaminergic response to familiar, non-rewarded stimuli

If the response to a non-reinforced stimulus were to habituate with familiarity, we would expect to see a decrease in the DA response as the stimuli become less effective at exciting or inhibiting the cell. Figure 5-13 shows an example experiment, where rather than habituating, response magnitude for DA cells to both light flash and trigeminal stimulation throughout the course of an experiment increases then returns to baseline in line with the effect of BMI on SC.

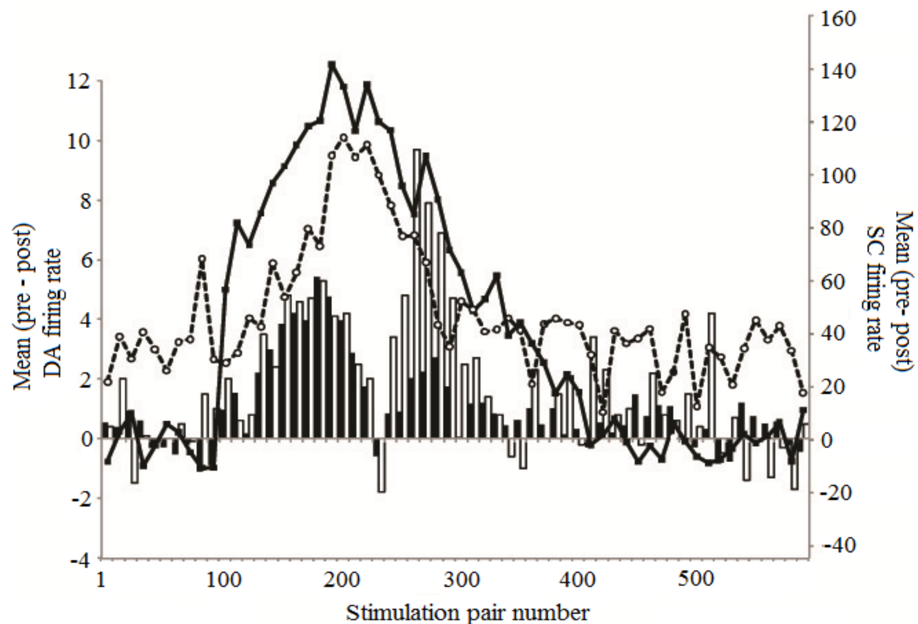


Figure 5-13 Response measured by activity above baseline of a DA neuron and SC across the timecourse of BMI effect.

5.4.5 Effect of interleaved stimulation on response

In chapter 3, it was observed that that after injection of BMI, the activity in the SC preceding the stimulation affected the activity in response to the stimulation. Light responses in the SC in the present chapter were generally of smaller magnitude (200.6±30.2 Hz) than those in chapter 3 (339.1±49.5 Hz). Although records typically did not show oscillatory activity following each stimulation, the effect could still be seen on some records. There were no instances in the present chapter where one stimulus was presented alone, so a comparable effect to that seen in chapter 3 could not be observed.

5.5 Discussion

5.5.1 Summary of findings

The current study indicates that the SC plays a role in relaying somatosensory trigeminal input to DA neurons in SNc. The findings suggest that local activation of SC has the ability to modulate the firing rate of presumed DA neurons in SNc. Under urethane anaesthesia, electrical stimulation of the trigeminal nucleus with a single 0.5 mA 100 µs pulse produces a short latency, short duration response in SC. A 10 ms light flash produces no response. Almost all DA cells showed significant responses to trigeminal nucleus stimulation before the disinhibition of SC. Both collicular and DA neuron responses were shown to be composed of multiple components. No DA cells responded to the light flash. Following removal of $GABA_A$ mediated inhibition by local microinjections of BIC in SC, light flash stimulation can evoke a response in the majority of DA neurons. When measured across the whole response period, the response to trigeminal stimulation in SC increases in magnitude, although closer examination reveals differential increase and decrease of the different components. Similarly, DA neuron responses can increase or decrease in size, or change direction (i.e. from inhibitory to excitatory), however, the response appears to be composed of several differentially affected components.

5.5.2 Discussion of findings

Responsiveness of SC and DA neurons to stimuli

In the current study, SC responses to a light flash stimulus were suppressed by the effects of anaesthesia. In response to trigeminal stimulation, the SC showed a short latency short duration phasic excitation. After a local injection of BMI into the SC, all records showed a phasic excitation to the light, and in most records the magnitude of the phasic response in the SC to trigeminal stimulation increased. All DA neurons were unresponsive to the light flash before BMI. In contrast to chapter 3, where most DA neurons were similarly unresponsive to single pulse cortical stimulation, the majority of DA neurons showed a significant response to single pulse trigeminal nucleus stimulation before injection of BMI. The topographic alignment between stimulation site and DA neuron location was suggested as a potential factor in the results of chapter 3, but it is unlikely that the majority of neurons in this study received focal stimulation selectively in the area of trigeminal nucleus corresponding to their receptive field. What is more likely is that given the trigeminal nucleus is a much smaller structure than S1Bf, a greater proportion of the somatotopic map in the trigeminal nucleus was stimulated, or that the volume of tissue receiving sufficient

current density to drive the neurons covered a greater number of receptive fields. As with the results of the previous chapters, the direction of the first phase of DA neuron responses was not necessarily consistent before and after injection of BMI, suggesting that there is not a firm distinction to be made between two subpopulations of differently responding DA neurons.

Contribution of the indirect, cortical input to the SC

The studies presented in the previous chapter attempted to investigate the contribution of cortical input to the later, stimulus sensitive phase of DA neuron responses (Hudgins, 2010). As the trigeminal nucleus provides input directly to the SC (Killackey and Erzurumlu, 1981) as well as indirectly via the barrel cortex (Wise and Jones, 1977; Killackey and Erzurumlu, 1981), it was suggested that comparison of the pre-BMI and post-BMI responses in the SC and DA neurons could be compared to infer the contribution of indirect cortical input. However, trigeminal stimulation appeared to produce direct and indirect responses in both the SC and DA neurons prior to BMI injection. Pre-BMI responses in the SC were composed of two components, similar in duration and latency to those described by Cohen et al. (2008) in response to whisker deflection. The second phase of the responses described by Cohen et al. (2008) were shown to be cortically mediated, while the first component was the product of direct trigeminal input. Thus, it seems reasonable to conclude that the similar responses seen in the present study are also the product of separate direct trigeminal and indirect cortical inputs.

DA neuron responses to trigeminal stimulation also showed two distinct phases (Figure 5-12). The latency and duration of these phases appeared to match the stimulus insensitive and stimulus sensitive components described by Hudgins (2010). It is suggested in this thesis that while the stimulus insensitive component could be the product of subcortical sensory input, the longer latency, stimulus sensitive component of the DA neuron response may be supported by cortical input via the SC. Following injection of BMI into the SC, the initial component of DA neuron responses to trigeminal stimulation was modulated in some cases, but remained largely unaffected. In contrast, the longer latency component showed substantial modification. In some cases, longer latency components of the DA neuron response after injection of BMI were in the opposite direction to the pre-BMI response.

That the initial component of the DA neuron response is largely robust in the face of BMI modulation of the SC response suggests that it is supported by direct trigeminotectal input. Given that the trigeminal nucleus is a small structure, it is possible that most, if not the entire vibrissal field was stimulated simultaneously, providing a simultaneous and consistent input across the SC. Thus, disinhibition via suppression of the GABAergic mechanisms of the SC (Binns and Salt, 1997) might be expected to have little effect. In contrast, trigeminothalamocortical input adds two levels at which the input from trigeminal stimulation could be modified (trigemonothalamic and thalamocortical synapses), which might affect the eventual input to the SC. Although the input to SC from the barrel cortex produced by trigeminal stimulation might not necessarily resemble normal vibrissal input, it is the result of normal synaptic processes, with their associated interneurons, rather than direct, simultaneous depolarisation. Therefore, it is not unreasonable to suggest that this input would be less robust to chemical manipulation. Although it was difficult to discern a clear pattern of association between collicular and DA neuron responses, and the effect of BMI, there did seem to be a broad effect by which changes in the later components of the SC responses were associated with changes in the later phase of the DA neuron response.

Response rates to each stimulus

Some neurons in the present study only responded to stimulation in one modality. In chapter 3, all unimodal responses were to the light flash; all DA neurons that responded to cortical stimulation also responded to light flash, but not vice versa. I suggested that this difference in responsiveness may be due to the more focal effect of cortical stimulation compared to whole field light flash – the locus of cortical stimulation might occasionally 'miss' the area of effect of BMI, or the region of SC that projects to the DA neuron being recorded from. In the present study, all DA neurons that responded to light flash also responded to trigeminal stimulation, but not vice versa. Given that all but one DA neurons responded to trigeminal stimulation before injection of BMI, the 'focus' of the stimuli might explain these results: the stimulus electrode was inserted across whisker columns in the trigeminal nucleus – potentially delivering a stimulus more successfully whole field than the light flash.

The current-distance relationships given by Tehovnik (1996) suggest that the current intensity and pulse duration used here would activate even the lowest

threshold neurons up to a maximum of 0.5 mm away from the electrode. Given that this would cover virtually all of the medio-lateral extent and a majority of the dorso-ventral extent of Sp5i, it is very likely that trigeminal activation would activate a large proportion of the SC. Having said that, this explanation relies on a focal topographic projection from the SC to DA neurons to enable cortical stimulation to "miss" the relevant region of SC. Although there is some topography in the tectonigral projection, it is not very focal (Comoli et al., 2003). In fact, some studies (Schultz and Romo, 1987) have commented on the consistency of response of DA neurons to stimulation of different somatotopic locations. The increased number of responsive cells could, however, be the result of activation of a greater proportion of trigeminal nucleus neurons, which produce a greater/broader activation in SC, which is more likely to result in modulation of DA neuron activity, without the need for any somatotopic map or alignment.

Lack of discriminable subpopulations of inhibited and excited DA neurons

As was mentioned in chapters 3 and 4, previous research has suggested the existence of two differentially responding sub-populations of VTA DA neurons, the presence of both inhibitory and excitatory DA responses to non-noxious stimuli (Steinfels et al., 1983a, 1983b; Strecker and Jacobs, 1985; Schultz, 1986; Horvitz et al., 1997; Dommett et al., 2005) and the existence of potentially inhibitory and excitatory tectonigral inputs (Comoli et al., 2003) indicating that the present results are unlikely to be explained by separate subpopulations. Examination of the data suggests that this is the likely to be the case. As with the DA neurons responding pre-BMI in chapter 3, it was found that a single neuron could display both excitatory and inhibitory responses to the same stimulus; all of the DA neurons in the present chapter showed a response before an injection of BMI, which in some cases differed in direction to the post-injection response. Further, the differences in neuron properties found in chapter 3, such as larger action potential size for inhibited DA neurons, were not found in the present study. A full discussion of the results and their implications is made in the final chapter.

In the awake animal, both SC and DA neurons habituate rapidly to unreinforced predictable stimuli (Wurtz and Albano, 1980; Schultz, 1998). The stimuli used here were spatially and largely temporally predictable. Both SC and DA neurons showed phasic responses to the light flash and trigeminal nucleus stimulation. However, the

response in SC and DA neurons did not habituate, but instead increased and decreased with the onset and offset of the effect of BMI on SC. The absence of habituation supports the findings of previous electrophysiological studies with similar paradigms (Dommett et al., 2005) and by behavioural studies (Redgrave et al., 1981) which have shown that habituation can be blocked by disinhibition of SC.

5.5.3 Final conclusions

While the results of the previous studies suggest a role of cortical input in the modulation of DA neuron activity, the present study attempts to extend the understanding of the source of subcortical sensory input. The results provide some indication of the route that information from the trigeminal nucleus nuclei take to DA neurons, however, the experiment is only preliminary. There are many different neuronal types in the trigeminal nucleus, which the present experiment did not discriminate between. Even if only vibrissal related cells are considered, the trigeminal nuclei contain neurons whose activity encodes different aspects of touch (contact, pressure, detach, contact-detach), phases of whisking, and combination whisking-touch neurons (Szwed et al., 2003). However, the intention of the present study was merely to activate trigeminal nucleus efferent pathways, rather than to provide naturalistic input.

The effect of a disinhibitory injection of BMI into SC suggests that it plays a role in the communication of somatosensory input to DA neurons. However, its precise role is not yet determined.

6 Discussion

6.1 Chapter summary

The previous chapters presented the experimental and theoretical background behind the studies in this thesis, and described and discussed the results. The present chapter begins by addressing the major experimental assumptions and findings in relation to previous research, including disinhibition of the SC as a means of establishing functional connectivity, the possibility of a common pathway for sensory and cortical input to DA neurons, and the presence or lack of a fixed distinction between DA neurons showing inhibitory and excitatory responses to stimuli. The chapter continues by discussing the functional implications of the results for the phasic DA signal, and by proposing an extension of an existing theory as an explanation of the role of the DA signal in learning. It concludes by suggesting future research that could extend the breadth and depth of the current findings, including further electrophysiological study, anatomical research, and possibly applications of optogenetic techniques.

6.2 Discussion of results

Summary of results

The aim of the work presented here was to investigate the origin of the afferent input to SNc DA neurons that might underlie the ability for DA neurons to show differential responses at longer latencies to stimuli associated with different reward probabilities. The results presented in chapter three demonstrated that in the anaesthetised rat, the majority of DA neurons are unresponsive to direct electrical stimulation of the somatosensory cortex. However, disinhibition of the SC with BMI increases its response to cortical stimulation, which in turn increases the likelihood that a previously unresponsive DA neuron will respond to cortical stimulation. This led to the conclusion that cortical information was capable of reaching DA neurons, and that the SC was likely to be a relay for this information. However, alternative explanations for the results remained a possibility. The results of the fourth chapter thus sought to confirm whether the SC was a relay for cortical information and eliminate these alternative explanations. It first showed that it is possible to reliably evoke responses in DA neurons with cortical stimulation without disinhibiting the SC,

and then it was demonstrated that an injection of the $GABA_A$ agonist muscimol in the SC can produce a dramatic change in collicular activity, and reduce or abolish the response of DA neurons to cortical stimulation, without changes in ECoG activity. This provided confirmatory evidence that the SC is a relay for information from somatosensory cortex to DA neurons. The results presented in the fifth chapter suggest that the SC is also involved in the direct transmission of trigeminal information to DA neurons. The differential effect of BMI on the different components of the SC and DA neuron response to trigeminal system reinforced the interpretation that these components represent the product of different inputs.

Confirming functional connectivity via disinhibition of the SC

The results presented here demonstrate that cortical and trigeminal activation is capable of phasically influencing the activity of DA neurons and that cortical information almost certainly reaches DA neurons via the SC. The effect of BMI on the SC could be tracked by plotting the activity over time. A clear onset and washout could often be seen, and the responses of DA neurons to cortical stimulation and a light flash often followed the same course as the responses in the SC. Bursts of activity in the SC after injections of BMI were sometimes associated with bursts of spikes in DA neurons (see chapter 3, and Coizet et al. (2003), suggesting that the activity of the two were linked. The effect of BMI is unlikely to be the result of diffusion to other structures. Previous studies using the expression of c-fos product (Dommett et al., 2005) have demonstrated that injections of BMI into the deeper layers of the SC using the same methods as the studies presented in this thesis remain largely within the confines of the SC. Immunohistochemical processing for FLI in the present studies confirmed that the effect of BMI was similarly contained.

Chapters 3 and 4 strongly suggest that the SC is a relay of cortical input to DA neurons. Although a similarly strong case for the SC relaying direct trigeminal input to DA neurons cannot be made without further studies, a case can still be made. As with the cortical stimulation, the responses of DA neurons to trigeminal stimulation over the course of the effect of BMI often followed that of the SC. Like the barrel cortex, the trigeminal nucleus also has significant projections to the SC. As the SC responds to, and directs gaze-shifts to, non-visual stimuli, including somatosensory stimuli (Grobstein, 1988; Dean et al., 1989; King, 2004; Boehnke and Munoz, 2008; Felsen and Mainen, 2008), it is reasonable to suggest that it relays short latency

vibrissal input from the trigeminal nucleus in a similar manner to its role as a relay of direct visual input from the retinal ganglion. Trigeminal stimulation produced responses in the SC that resembled those produced by vibrissal deflection, which consist of a subcortical and a cortically mediated component (Cohen et al., 2008). There was often an association between changes in the early and later components of the SC response, and the early and later components of the DA response. However, as responses in the later component of the SC response and the DA response were present both before and after injection of BMI, the extent to which trigeminal and cortical input contributes to each component is difficult to judge. On the basis of the present experiments, it is safe to say that the SC has a role in trigeminal influence on DA neuron activity, and that a case can be made for it as a relay of trigeminal input, but confirmation of this, and a decomposition of the direct and indirect cortical contribution to the response requires further study. Some potential avenues of investigation are suggested toward the end of this chapter.

The tectonigral pathway as a common route of input to DA neurons

If visual (light flash) stimulation, cortical stimulation and trigeminal nucleus stimulation share the same pathway from the SC to DA neurons, it might be expected that the difference in onset latencies between SC and DA responses would be similar with all stimuli, the rationale being that the pathway would have a similar conduction velocity. This was borne out by the results, which showed that there was a small, but non-significant difference between DA neuron onset latencies minus SC response onset latencies to light flash stimulus (~40 ms), cortical stimulation, and trigeminal stimulation (~30 ms).

The evidence that the response in DA neurons produced by cortical stimulation shares the tecto-nigral pathway by which visual information reaches DA neurons is stronger than for trigeminal stimulation. Firstly, the results presented in chapter 4 suggest that cortical information to DA neurons is relayed by the SC, whereas this is presently just an assumption for trigeminal stimulation, albeit a well justified one given the functional anatomy involved. More importantly was evidence that collicular activity driven by one stimulus could interact with the other. As the activity recorded in the SC was multiunit, it cannot be directly confirmed that the same population of neurons was responding to both light flash stimulation and cortical stimulation. However, there is indirect evidence. When the SC was disinhibited, SC responses to

both light flash and cortical stimulation were typically characterised by an initial peak, then one or more peaks and troughs. When activity from a preceding stimulus reached the period before the onset of the next stimulus, if could affect the size of the response to that stimulus; greater activity in the period before stimulus onset was associated with lower activity (i.e. a smaller response) in the post-stimulus period, while less pre-stimulus activity was associated with greater post-stimulus activity. Further, in some instances where cortical stimulation or the light flash was presented in isolation, the collicular response to the remaining stimulus, and the corresponding DA neuron response increased in magnitude. This interaction between collicular and DA responses to each stimulus suggests that visual input and cortical somatosensory input to the SC shares the same pathway onward to DA neurons.

A cortical influence on collicular input to DA neurons

Previous study has sought to rule out the cortex as being necessary (Comoli et al., 2003) or sufficient (Dommett et al., 2005) for sensory responses in DA neurons. Comoli et al. (2003) showed that local field potential (LFP) responses to a light flash were seen in the SC and SNc following visual cortex aspiration, demonstrating that the cortex was not necessary. However, aspiration of the visual cortex did affect LFP responses in the SC and SNc that were present even in the absence of disinhibition of the SC, suggesting that there was cortical influence. The present results demonstrate that disinhibition of the SC is usually necessary for cortical stimulation to activate DA neurons. Consequently, application of BMI to the cortical surface without concurrent 'unblocking' of the SC by disinhibition (as in Dommett et al. (2005)) is unlikely to produce responses in DA neurons to a sensory stimulus. Application of BMI to the visual cortex and injection into the SC may well produce an effect of the DA response to light flash versus injection of BMI into the SC alone.

Inhibited and excited DA neurons are not discriminable subpopulations

Previous investigation has suggested that a sub-population of VTA neurons exists, which responds with excitation to aversive stimuli. This group has been suggested to be a population of non-DA neurons, distinguishable by action potential width (Ungless et al., 2004), or a sub-population of DA neurons located in a restricted area of the VTA (Brischoux et al., 2009). Although:

- the current study did not use aversive stimulus

- the current study focused on SNc, so the VTA DA neurons of Brischoux et al. (2009) won't be considered
- excitatory and inhibitory responses have been previously reported in VTA and SNc DA neurons to non-noxious sensory stimuli (Steinfels et al., 1983a, 1983b; Strecker and Jacobs, 1985; Schultz, 1986; Horvitz et al., 1997; Dommett et al., 2005), where no distinction was made, or where a distinction was tested, no was found
- the tectonigral pathway may have excitatory and inhibitory effects on DA neurons, and so the differences in response direction may be the result of inputs rather than differences in the DA neurons

the present data were examined to see if, on average, there were differences between DA neurons classified as excitatory and inhibitory first phases.

First, it must be noted that a significant proportion of the earlier studies that looked for subgroups of DA neurons focused on VTA DA neurons. It might be harder to distinguish between subgroups of DA neurons in the SNc, if they exist. DA neurons in the SNc recorded here showed broad (range: 3.6-5.3 ms), triphasic spikes with positive going initial components, and low (<10 Hz) firing rates (see examples in chapter 3), and were clearly distinguishable from presumed GABAergic neurons, showing narrow, biphasic spikes with negative going initial components, and high (often >50 Hz) firing rates. In contrast, Dommett et al. (2005) presents an example DA neuron with a spike width close to the bottom end of the range seen in the present studies, but classifies VTA and SNc DA neurons as having spikes of >2.0 ms, suggesting the existence of DA neurons with narrower spikes.

Both excitatory and inhibitory responses were seen in DA neurons to light flash, cortical stimulation and trigeminal nucleus stimulation. Examination of the response characteristics suggested that there was no difference between the DA neuron responses with an excitatory first component and those showing an inhibitory first phase to the light flash, cortical stimulation, or trigeminal stimulation. Examination of the baseline firing rates of the DA neurons also showed no differences, which is supported by the findings of previous research (Dommett et al., 2005). A significant difference was seen in chapter 4 in the onset-trough measurement of average DA neuron spikes, with DA neurons showing responses with inhibitory first components having broader spikes than those showing responses with excitatory first components.

Although the analysis of chapter 3 found that DA neurons showing inhibitory responses had larger action potentials, chapter 5 found no significant difference. Combined with the findings of Dommett et al. (2005), which found no difference between action potential sizes of excited and inhibited cells, the reliability of this distinction may be questionable.

One of the arguments against the existence of separate populations put forward in chapter 3 was the presence of responses in one direction, which went on to respond in a different direction after BMI. This unusual response profile may not be merely an artefact of the effect of BMI – previous studies have reported occurrences of DA neurons changing response over a period of stimulation without any other manipulation (Tong et al., 1996). Although the nature of the change is not mentioned, the response is described as “labile” (p.198), suggesting a change more drastic than gradual habituation for example. Although it is not commonly reported, there are also reports of DA neurons that respond in opposite directions to stimuli of different modalities. Most dramatically, Strecker and Jacobs (1985) reported that out of 24 DA neurons that responded to a visual stimulus, an auditory stimulus, or both, 11 neurons responded with an excitation to one stimulus, but an inhibition to the other. This shows that at least some DA neurons cannot be classified as exclusively showing excitatory or inhibitory responses, nor is the direction of response to a stimulus necessarily fixed.

6.3 Broader functional implications

Conflicting findings about the DA response

DA neurons typically respond to unexpected sensory stimuli with a short latency, short duration increase in firing rate (Schultz et al., 1997). The SC has been established as the source of input about unexpected visual stimuli to DA neurons (Dommett et al., 2005). Redgrave et al. (1999) pointed out that the sensory responses in DA neurons must be based on pre-gaze shift sensory processing. In mammals, an unexpected sensory stimulus typically elicits a saccade to bring the stimulus onto the fovea, which provides a broader and more detailed input to cortical systems (Thorpe and Fabre-Thorpe, 2001; Rousselet et al., 2004). Saccadic latency is usually in the range of 150-200 ms (Hikosaka and Wurtz, 1983; Jay and Sparks, 1987). As the latencies of phasic DA responses are typically around 100 ms (Schultz, 1998) such detailed input would not be available to DA neurons. Consequently, the precise

identity of a stimulus that triggered the DA response would not be available at the timescale of DA neuron responses. The possibility of evaluation of a stimulus post-identification affecting DA neuron responses is even less likely given the duration of DA responses. Nevertheless, DA neurons can respond differentially to different stimuli, and signal aspects of their reward value (Fiorillo et al., 2003; Tobler et al., 2005). Hopefully the present thesis goes some way to providing a way for these findings to be reconciled.

A possible solution

It has been shown that the DA response comprises an initial short latency phase, followed by a second phase at a longer latency – around 150-200 ms (Hudgins et al., 2009; Joshua et al., 2009; Bromberg-Martin et al., 2010; Nomoto et al., 2010). The magnitude of the initial phase is apparently independent of the value associated with the stimulus, while stimuli associated with different reward probabilities appear to be able to produce different responses in the second phase. Cortical input is suitably placed to explain these differences. The projection of somatosensory cortex (and other areas of sensory cortex) to the SC is well known, and as a result the ability to evoke a response in the SC by stimulating the somatosensory cortex is not surprising. The ability of somatosensory cortex to evoke a response in DA neurons via the SC presents a mechanism through which DA neuron responses, driven by SC input, could be modified, and unites research indicating the SC as a relay for sensory input to DA neurons with evidence suggesting responses requiring perceptual capabilities in DA neurons previously thought to be beyond the SC (Boehnke and Munoz, 2008). Note that the second phase of the response would still be primarily, if not wholly presaccadic. This would necessarily limit the sensory capabilities of DA neurons to the capabilities of those structures that could provide presaccadic input. While this excludes the kind of high resolution and high level information provided by cortical processing of foveal input, it does not exclude all cortical input. The eye is not blind outside the fovea, and the cortex receives extrafoveal input. This extrafoveal input might support discrimination between stimuli at latencies that are longer than the onset of the initial phase of the DA response, but still are presaccadic.

Limitations of the solution

However, while this provides a potential explanation for studies showing that DA neurons can respond differentially to stimuli at presaccadic latencies, it still does

not completely rehabilitate the reward prediction error hypothesis. The presaccadic information provided to DA neurons about higher level sensory features, even if supplemented by cortical input, relies on the initial stages of processing of extrafoveal input, and is likely to be relatively coarse. It has recently been demonstrated that the SC is capable of responding differentially to, and driving saccades to, stimuli that are differently coloured but identical in luminance (White et al., 2009; White and Munoz, 2011). As the SC does not receive input from the colour sensitive cells of the retina (Schiller and Malpeli, 1977), the authors suggested that these responses are likely to be driven by cortical input. This suggestion is supported by the delay between luminance related and chromatic related responses in the SC, which implies that chromatic responses traverse different pathways (White et al., 2009). However, this capability of the SC to respond to chromatic stimuli is limited. White et al. (2009) showed that SC neurons are very broadly tuned, and respond to a wide range of colours. This lack of selectivity of SC neurons supported by White and Munoz (2011), who showed that there was an increase in erroneous saccades (i.e. saccades to a distractor) when targets and distractors were similarly coloured. Given the lack of selectivity and potential for errors in presaccadic processing, it seems likely that any response in DA neurons driven by this input would be similarly unreliable. Such a system would not be suitable to reliably indicate stimulus identity and value at presaccadic latencies.

Implications of a shared tectonigral pathway

The possibility that cortical and subcortical inputs of different modalities share a common route to modulate the activity of DA neurons has implications for the interpretation of the function of the DA response. It was suggested earlier in this chapter that the pre-saccadic cortical input available to the SC and consequently DA neurons at the latencies of the DA response is limited, such that identification and valuation of a particular stimulus would be difficult. If DA neurons receive input from SC neurons that respond to multimodal cortical and subcortical input without discrimination, then the likelihood of the function of the DA response being one that involves the fine-grained discrimination of stimuli becomes even smaller.

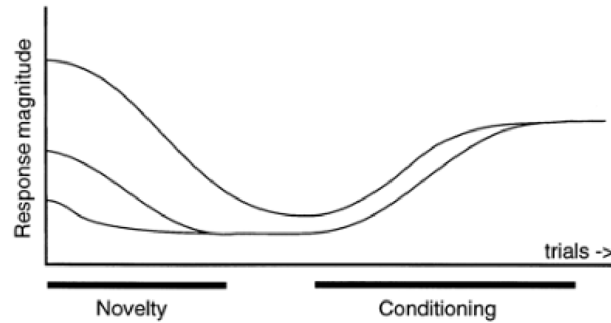


Figure 6-1 From Schultz (1998): "Time courses of activations of DA neurons to novel, alerting, and conditioned stimuli. Activations after novel stimuli decrease with repeated exposure over consecutive trials. Their magnitude depends on the physical salience of stimuli as stronger stimuli induce higher activations that occasionally exceed those after conditioned stimuli. Particularly salient stimuli continue to activate DA neurons with limited magnitude even after losing their novelty without being paired with primary rewards."

If modulation of cortical input to the SC is the mechanism by which responses in DA neurons indicating value are produced, then the shared pathway to DA neurons means it is unlikely that the value related response is providing a reward signal. This point is illustrated particularly well by Schultz himself (see Figure 6-1). Intense, novel sensory stimuli can produce responses with magnitudes exceeding those of a response to a stimulus associated with reward, and can continue to activate DA neurons without being paired with primary rewards. If DA neurons cannot distinguish between intense, but unrewarded stimuli, and stimuli associated with a reward, or between intense, unrewarded stimuli that continue to produce a limited magnitude response after repeated presentation, and stimuli associated with smaller reward values, which would also produce smaller magnitude responses, then their responses cannot signal reward value distinct from unrewarded stimulation if they produce a similar magnitude response.

The ability of the cortical input to modulate DA neuron activity through the SC provides a mechanism by which the responses of the SC and DA neurons to stimuli previously associated with rewards could be modified. However, given the lack of selectivity of cortically mediated collicular responses (White et al., 2009; White and Munoz, 2011), it seems unlikely cortical input could support precise stimulus identification and evaluation. Indeed, previous research has shown that DA neurons are largely insensitive to high spatial frequency stimuli associated with different reward probabilities presented at fixation point (Hudgins, 2010), where a stimulus could be precisely identified. Rather, it is suggested here that cortical information

might bias the response of the SC according to the presumed identity of the stimulus and its associated reward value. However, it is also suggested here that although DA neuron responses may be modulated by reward, they do not necessarily signal reward. Schultz (1998) noted that intense sensory stimuli may produce larger responses in DA neurons than stimuli associated with reward. Consequently, it seems unlikely that the DA response communicates intense sensory stimuli as distinct from stimuli evoking a large response due to association with reward. A theory of phasic DA neuron response function is proposed, where DA provides a 'time stamp' to enable the animal to determine potential behavioural causes of an unexpected event, as suggested by Redgrave et al. (2008). However, in contrast to Redgrave et al. (2008), it is suggested that this time stamp signal is modulated in the natural environment according to a best guess of the identity of the stimulus and its salience. It is speculated that this may serve to form a stronger connection, or form a connection more quickly, between context and behaviour, and stimuli of particular interest.

6.4 Alternative/further experiments

The present results provide support for the suggestion that sensory cortex is a likely source of input to modulate the DA response, and establishes the SC as a common relay for direct visual input and cortical input. It also extends the role of the SC as a relay for sensory input from subcortical structures from just vision to include somatosensation. However, these results are by no means an end point, and several further directions of research could prove informative.

It was mentioned earlier in this chapter that the precise role of the SC in trigeminal input to DA neurons could not be established in the same way as cortical input based on the experiments performed here. As trigeminal stimulation reliably evokes responses in DA neurons in the absence of BMI, as did the pulse train cortical stimulation used in chapter 4, it is ideally placed to use a similar method of suppressing collicular activity using muscimol, or a similar inhibitory agent. Further, once the role of the SC in trigeminal input to DA neurons is established, the respective putative contributions of direct trigemino-tectal input and trigemino-thalamo-cortico-tectal input could be investigated further. In the introduction to chapter 5, it was suggested that intracollicular BMI might result in a second phases in the DA response as a cortical input comes into play. However, the responses of the SC to trigeminal stimulation showed both the first component, produced by direct

trigeminal input, and the second component, produced by cortical input, described by Cohen et al. (2008) prior to BMI injection. Separate components were also clearly visible in the responses of DA neurons, which were differentially modulated by BMI injection. The presence of both direct and indirect components in both collicular and DA neuron response provides an ideal opportunity to better understand the contribution of cortical input to collicular and DA responses to trigeminal stimulation. Further study might look at the responses before and in the presence of intracollicular BMI, both before and after manipulations that suppress or enhance the contribution of corticotectal input in a similar manner to Cohen et al. (2008). In this way, the relative impact of direct and indirect input to the SC on the DA response can be separated.

The development of optogenetics (Deisseroth, 2011) allows for investigation into the problems examined here in finer detail, with more control, and potentially more validity. First, if neurons that project to the SC from the trigeminal nucleus (Killackey and Erzurumlu, 1981) and the barrel cortex (Wise and Jones, 1977) can be selectively targeted, then direct electrical stimulation of the barrel cortex and the trigeminal nucleus (which potentially depolarises several populations of neuron, which may have competing functions, Diamond et al., 2008), could be replaced with a stimulation more likely to activate select populations of neurons in a predictable manner. The risk of activation of the SC or other structures by alternate pathways or antidromic activation would be reduced if the fibre optic was located within the SC, thereby stimulating the terminal fibres of labelled neurons from the structure of interest. As optogenetics also allows for the same neurons to be both depolarised and hyperpolarised (Zhang et al., 2007), not only could muscimol be replaced as an inhibitory agent in the present studies, but the effect of inhibition and disinhibition of the SC could be investigated in the same animal at short timescales.

The present study suggested that the interference between cortical and sensory stimuli on the activity of the SC indicated that at least some of the population of neurons being recorded from were responsive to both stimuli, whether or not this is the case could be examined by studying the effects of the stimuli used here on the activity of a single SC neuron. It has been previously demonstrated that single SC neurons receive both cortical and subcortical information about whisker deflection (Cohen et al., 2008), and that individual SC neurons can show multisensory responses (Meredith and Stein, 1986). Single unit recording would enable the respective inputs

of sensory cortex and subcortical inputs in the same modality and other modalities to be investigated.

The results presented here (particularly the temporal consistency with which DA neuron responses follow responses in the SC for cortical and trigeminal stimulation, and in response to a light flash) were interpreted to suggest that the neurons in the SC which were directly activated by cortical and trigeminal stimulation formed part of the direct tecto-nigral pathway (Comoli et al., 2003). A combination of anatomical techniques could be used to confirm this hypothesis. Bearing in mind the caveats associated with direct electrical stimulation expressed in chapter 4, prolonged stimulation of the tectonigral pathway could be used to induce c-fos expression in neurons in the SC that receive projections from the barrel field (Dragunow and Faull, 1989). An injection of a retrograde anatomical tracer in the SNc, such as cholera toxin subunit b (CTb), would label SC neurons that were part of the tecto-nigral pathway (Comoli et al., 2003). If these techniques were combined in the same animal, any incidence of double labelling of fos like immunoreactivity and CTb would suggest that corticotectal neurons synapse directly onto neurons of the tecto-nigral pathway.

6.5 Final conclusions

A better understanding of the function of the phasic response of DA is important for our understanding of the reward valuation and learning mechanisms of the brain. A deeper knowledge of the DA system in a broader sense might also help is better understand the causes behind pathologies such as Parkinson's disease, and perhaps help develop more selective treatment. The results presented in this thesis extend the role of the SC from the primary relay of visual input to DA neurons at short latency to include somatosensory input. Given its multimodal nature (Meredith and Stein, 1986), the SC may well yet prove to be a relay for input in other sensory modalities. More importantly however, this study unites the apparently contradictory data showing that DA neurons can respond differentially to different stimuli, and the lack of selectivity to visual stimuli provided by the SC. Cortical input to the SC offers an explanation for recent discoveries (Morris et al., 2004; Hudgins, 2010) suggesting that DA neurons can differentiate between some stimuli at longer latencies. However, although this may also explain the results of experiments supporting the reward prediction error hypothesis, it seems unlikely that the phasic DA response supports a solely reward based function. Instead, it is suggested that the DA response to sensory stimuli

represents a signal of the occurrence of salient stimuli, which, although it can be sensitised by reward, cannot discriminate input from the SC triggered by reward from that triggered by non-reward stimuli, and is thus unlikely to communicate a reward value signal based function.

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