Cortical regulation of dopaminergic neurons: role of the midbrain superior colliculus

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Dopaminergic (DA) neurons respond to stimuli in a wide range of modalities, although the origin of the afferent sensory signals has only recently begun to emerge. In the case of vision, an important source of short latency sensory information seems to be the midbrain superior colliculus (SC). However, longer latency responses have been identified which are less compatible with the primitive perceptual capacities of the colliculus. Rather, they seem more in keeping with the processing capabilities of the cortex. Given that there are robust projections from the cortex to the SC, we examined whether cortical information could reach DA neurons via a relay in the colliculus. The somatosensory barrel cortex was stimulated electrically in the anaesthetised rat using either single pulses or pulse trains. Although single pulses produced small phasic activations in the colliculus, they did not elicit responses in the majority of DA neurons. However, following disinhibitory intracollicular injections of the GABA$_A$ antagonist bicuculline, collicular responses were substantially enhanced and previously unresponsive DA neurons now exhibited phasic excitations or inhibitions. Pulse trains applied to the cortex led to phasic changes (excitations to inhibitions) in the activity of DA neurons at baseline. These were blocked or attenuated by the intracollicular administration of the GABA$_A$ agonist muscimol. Taken together, the results indicate that the cortex can communicate with DA neurons via a relay in the SC. As a consequence, DA neuronal activity reflecting the unexpected occurrence of salient events and that signalling more complex stimulus properties may have a common origin.

Key words: Dopamine, Basal ganglia, Electrophysiology, Salience
INTRODUCTION

Dopamine-mediated transmission has been implicated in a number of human clinical disorders as well as in a wide range of normal brain functions. Typically, dopaminergic (DA) neurons exhibit a stereotyped short latency, short duration population response to unpredicted stimuli in a variety of modalities that are salient by virtue of their novelty, intensity or reward value (Freeman & Bunney 1987; Horvitz et al. 1997; Schultz 1998). In the case of vision, we have previously shown that visual information is provided to DA neurons by the midbrain superior colliculus (SC; Comoli et al. 2003; Dommett et al. 2005). The SC is a primitive subcortical visual structure with limited perceptual processing capabilities. Hence, visual neurons in the primate colliculus are sensitive to the onset, offset and movement of stimuli, but the majority are insensitive to shape, contrast, orientation, velocity and direction of movement (Schiller and Koerner, 1971; Goldberg and Wurtz, 1972). Furthermore, the SC does not receive an input from colour opponent cells in the retina (Schiller and Malpeli, 1977).

However, in spite of this limited processing capacity in the colliculus, DA neurons in the primate appear able to distinguish between complex visual stimuli, and can reflect the magnitude and probability of obtaining the reward with which they are associated (Fiorillo et al. 2003; Morris et al. 2004; Tobler et al. 2005; Nomoto et al. 2010). Closer analysis of these responses however suggests that the response of DA neurons to complex visual stimuli is actually biphasic. The truly discriminatory phase of the DA response has a peak latency around 80-100 ms after an earlier, more invariant response feature (Morris et al. 2004; Hudgins et al. 2009; Nomoto et al. 2010). Indeed, more generally, it has been proposed that short latency responses in DA neurons reflect the sensory intensity of the inducing stimulus whilst the later phases reflect motivational value (Fiorillo et al., 2013a). In relation to complex visual stimuli, given the rather primitive visual processing capabilities of the SC, the
natural assumption would be that the shorter latency component of the DA response is of
collicular origin whilst the longer latency discriminatory phase has its origin elsewhere in the
brain. Sophisticated visual processing required to discriminate between complex stimuli most
likely involves the visual cortex (Zeki, 1993; Thorpe and Fabre-Thorpe, 2001; Orban, 2008),
however cortical inputs to DA neurons are relatively sparse (Frankle et al. 2006; Watabe-
Uchida et al. 2012). In contrast, the cortical mantle projects heavily to the SC in a wide range
cortical projection to the SC suggests that the cortical information which may be signalled by
the later phase of the sensory response in DA neurons could arise via a relay in the SC.
Indeed, cortically-dependent responses in the SC are temporally delayed with respect to those
arising from more direct sensory activation (Cohen et al. 2008; White et al. 2009). The
possibility that the cortex can communicate with DA neurons via the SC was tested in the
current study by using electrical stimulation to directly activate the cortex, whilst recording
the activity of DA neurons in the presence of pharmacological manipulation of the SC.

For a number of reasons, the whisker barrel cortex was chosen as the locus of
stimulation in the present study: 1. Dopaminergic neurons are exquisitely sensitive to whisker
deflection in the awake rat (Freeman et al. 1985); 2. Inputs from the somatosensory cortex
terminate primarily in the intermediate layers of the SC (Wise and Jones, 1977), the lateral
aspect of which contain a concentration of neurons which project directly to DA neurons in
the ventral midbrain (Comoli et al, 2003); 3. Lateral intermediate layer neurons respond
vigorously to whisker deflection in the rat (Hemelt and Keller, 2007). Although the barrel
cortex constitutes an early stage of cortical sensory processing, it is clear from the research
literature that cells there encode complex stimulus characteristics and can do so adaptively
(e.g. Jacob et al., 2008; Diaz-Quesda and Maravall, 2008). Hence even this primary sensory
area is in a position to potentially communicate highly processed stimulus-related
information to DA neurons. To confirm that activation induced by electrical stimulation of the cortex was confined within the barrel region, optical imaging was performed to map the extent of the activation.
MATERIALS AND METHODS

All aspects of these studies were performed with Home Office approval under section 5(4) of the Animals (Scientific Procedures) Act 1986, and experimental protocols received prior approval from the Institutional Ethics Committees.

Electrophysiology

Recording and injection procedure. Twenty two male Hooded Lister rats (288-515 g) were anaesthetised with an intraperitoneal injection of urethane (ethyl carbonate; 1.25g/kg as a 25% aqueous solution; additional doses of 25 mg/kg were administered if required) and mounted in a stereotaxic frame with the skull level. Body temperature was maintained at 37°C with a thermostatically controlled heating blanket. Craniotomies were performed to allow unilateral access to the barrel cortex, SC and to DA neurons in the substantia nigra pars compacta (SNc), and a concentric bipolar electrode (NEX-100, Rhodes Medical Instruments, Woodland Hills, CA) was inserted into the former structure (1.6-3.3 mm posterior to bregma, 4.2-5.4 mm lateral to midline and 1.5-1.8 mm ventral to the brain surface).

Extracellular single unit recordings were made from DA neurons using glass micro-electrodes with a tip diameter of approximately 1.0-2.5 µm (impedances 6-10 MΩ, measured at 135 Hz in 0.9% NaCl). Electrodes were filled with 2M saline and 2% Pontamine Sky Blue (BDH Chemicals Ltd., Poole, UK). After manufacture, the electrode was lowered to a position just dorsal to the SNc with a hydraulic microdrive (Model 650, David Kopf Instruments), ipsilateral to the cortical stimulating electrode. An angled (35°) contralateral approach (beginning 2.2-4.4 mm lateral to midline on the opposite side of the brain) was used to avoid collision with a second recording electrode inserted vertically into the SC (see below). All subsequent stages of the experiment were conducted in the dark.
Extracellular multiunit recordings were made from SC neurons ipsilateral to the DA recording and cortical stimulating electrodes using a parylene-C coated tungsten electrode (A-M Systems Omc., Carlsborg, USA) coupled to a 30 gauge stainless steel injector filled with either the GABA_\text{\(A\)}} receptor antagonist bicuculline methiodide (100 ng/µl in 0.9% saline, Sigma-Aldrich, Poole, UK) or the GABA_\text{\(A\)}} receptor agonist muscimol (200 ng/µl in saline, Sigma-Aldrich). Lateral separation between the electrode and the tip of the injector was 0.2-0.5 mm, with the electrode positioned 0.5 mm forward of the injector. Electrophysiological responses were determined while the electrode/injector assembly was lowered into the SC in the presence of a whole field light flash (0.5 Hz, 10 ms duration) from a yellow LED (30 LUX, 585-90 nm) positioned 5 mm from the contralateral eye, which was sutured open. Using the characteristically vigorous visual response of the superficial layers of the SC as a positional cue, the electrode was lowered into the lateral intermediate layers (6.0-6.8 mm caudal to bregma, 1.6-2.6 mm lateral to midline, 4.7-5.7 mm ventral to the brain surface). Intra-collicular microinjections were made (0.5 µl at a rate of 0.5 µl/min) via a 10 µl Hamilton syringe mounted on an infusion pump, connected to the injector by a length of plastic tubing.

Spike related potentials were amplified, band-pass filtered (-3 dB points: 200 Hz-4 kHz for single unit recordings, 400 Hz-16 kHz for multiunit recordings), digitised at 20 kHz and recorded directly onto computer disk using a 1401 Plus data acquisition system (Cambridge Electronic Design [CED] Systems, Cambridge, UK) running CED data capture software (Spike 2).

Once the SC electrode/injector had been positioned, the DA electrode was lowered until a putative DA neuron was identified on the basis of standard criteria (Grace and Bunney, 1983; Ungless et al. 2004): long duration (>2.0 ms) biphasic or triphasic action potential, with an initial phase exceeding >1.0 ms in length, a low firing rate (<10 Hz) and a firing
pattern that consisted of irregular single spikes or bursts. Dopaminergic neurons were encountered at 4.6-6.0 mm posterior to bregma, 1.8-3.2 mm lateral to midline and 8.0-9.7 mm ventral to the brain surface. Once encountered, the activity of the cell (and multiunit activity in the SC) was recorded during the application of electrical pulses to the cortex. Stimulation consisted of either single square wave pulses (0.1 ms, 1 mA) or trains of square wave pulses (5 pulses at 150 Hz, 0.1 ms each, 0.6-0.8 mA; designed to mimic the activity of tectally projecting intrinsically bursting pyramidal neurons in the cortex; see Kasper et al. 1994; Rumberger et al. 1998; Tsiola et al. 2003). The timing of the electrical stimuli was jittered by 20% around a mean frequency of 0.25 Hz (single pulses) or 0.5 Hz (pulse trains).

Single pulse electrical stimulation of the cortex frequently did not elicit changes in the activity of DA neurons at baseline. Hence, when single pulse stimuli were used, following a period of drug-free response determination (150 trials), a pressure injection of bicuculline was made into the SC to disinhibit the structure (N=13 animals): a paradigm which we have previously shown to convert previously unresponsive DA neurons into neurons which exhibit short latency responses to light flash stimuli (Dommett et al. 2005). Hence for comparison, in the single pulse condition, each pulse was preceded 2 s earlier by a wholefield light flash. Drug-free responses in DA neurons to cortical stimulation could be elicited more reliably by pulse trains. Therefore when pulse trains were used, following a period of drug-free response determination (150 trials), an injection of muscimol was made into the SC to inhibit the structure (N=9 animals). The demonstration that responses to cortical stimulation in DA neurons could be facilitated by intra-collicular bicuculline and blocked/attenuated by intra-collicular muscimol would provide convergent evidence that cortically induced activation of DA neurons occurred via a route of transmission which involves the SC. Electrical stimulation was applied to the cortex throughout the post-drug period, until either the effects of the drug wore off in the SC (in the case of bicuculline), the effect of the drug had reached
an asymptote (in the case of muscimol) or the DA neuron was lost. After a complete trial with bicuculline, which is relatively short acting in the SC (10-15 min; Dommett et al. 2005), further DA neurons were tested in the same way. Between 1 and 3 DA neurons were tested in a single subject. The effect of muscimol in the SC are much longer lasting than bicuculline (>90-120 min; Edeline et al. 2002), and so only a single DA neuron was tested in each subject in the muscimol experiments.

Histological processing. In each case, the final recording site of the DA electrode was marked with pontamine sky blue by passing a 27.5 µA cathodal current for 15-25 min using a constant current source (Fintronics Inc. Orange, CA). The recording/injection site in the SC was marked with a small electrolytic lesion (10 µA, 150 s, cathodal current) using the same constant current source. Animals were then killed with an overdose of barbiturate and perfused transcardially with 400 ml of warmed saline (40°C), followed by 400 ml of paraformaldehyde in phosphate buffer (PB, pH 7.4). Brains were removed and post-fixed overnight in 4% paraformaldehyde at 4°C, before being transferred into sucrose for 36 h. Serial coronal (30 µm) sections were cut in a cryostat. One series of sections was mounted on slides and processed with a Nissl stain (cresyl violet), while a second series was collected in 0.1 M PB and processed for tyrosine hydroxylase (TH), and in cases where bicuculline had been injected, c-fos immunohistochemistry, as previously described (Coizet et al. 2006).

Data analysis. The waveform characteristics of recorded DA neurons were determined off-line from averaged records of typically 60-90 digitised spikes/neuron. These averages were used to determine the width of the action potential according to the criteria of both Grace and Bunney (1983) and Ungless et al. (2004). Once the waveform characteristics of the recorded DA neurons had been assessed, analysis of the responses of these neurons and neurons in the
SC to the applied stimuli was facilitated by removing the artifact associated with electrical stimulation from the data records. To achieve this, a waveform average was constructed for the trials to be analysed, covering the period 1 ms before to 9 ms after the stimulation. This was then subtracted from the raw data trace. Action potentials of DA neurons were then isolated from background noise by using the WaveMark function in Spike 2 (CED), which marked the temporal position of each action potential as an event. In the case of the SC data, waveform averages were again constructed and subtracted from the raw data. The SC trace was then high-pass filtered with an FIR digital filter (Spike 2; CED; transition gap 1.2 kHz, -3 dB point: 1.069 kHz) and rectified. A spike threshold was set at two standard deviations (SDs) above the mean of the rectified voltage waveform. For pre- vs post-drug comparisons, the threshold value determined pre-drug was held over and applied post-drug to allow drug-induced changes in firing rate to be assessed.

Following this preprocessing, peristimulus time interval histograms (PSTHs) were constructed based on DA single unit and SC multiunit data, using a bin width of 20 ms and 1 ms respectively; these bin widths, tailored to the cell type, were used to extract quantitative aspects of cell behaviour which most closely matched estimates based on visual inspection) for the analysis period. PSTHs for DA single unit data were smoothed with a three point sliding average. Although smoothing combined with a 20 ms bin width attenuated the variability associated with the low firing rate in DA neurons, some degree of temporal precision in the measurement of responses in the cells had to be sacrificed to achieve this. PSTHs based on DA single unit and SC multiunit data were then analysed to determine the following response characteristics before and after an injection of bicuculline/muscimol: (i) Pre-stimulation baseline activity: the number of spikes per second occurring during the 500 ms prior to the light flash and/or cortical stimulation, divided by the number of trials; (ii) Post-stimulation baseline activity: the mean number of spikes per second occurring during the
200 ms following the light flash stimulation, divided by the number of trials; (iii) Response latency: defined as the time point at which deviations in activity post-light flash or electrical stimulation exceeded 1.96 standard deviations (SDs) of the baseline activity, sustained for three or more bins in the case of DA neurons (DA neurons were considered to be ‘responsive’ if at least 3 consecutive bins within a period spanning 20-260 ms following the stimulation exceeded the 1.96 SD threshold); (iv) Response duration: response offset was defined as the time point at which activity returned to within the threshold values post-light flash or electrical stimulation, sustained for two or more bins in the case of DA neurons - response duration was the difference in time between response latency (onset) and offset; (v) Response magnitude: the total number of spikes between response onset and offset, minus the pre-stimulation baseline activity for excitatory response components and subtracted from the baseline mean for inhibitory response components, divided by the number of trials.

When considering the effect of collicular bicuculline on the responses of DA neurons to electrical stimulation, given that the action of bicuculline in the SC is short lived (Dommett et al. 2005), DA and collicular neuronal activity was analysed for the period over which the drug affected SC activity. This period was defined as starting when the post-stimulation baseline activity exceeded a threshold of +2 SDs of pre-bicuculline levels for two consecutive sets of 10 stimulations after collicular bicuculline and was defined as ending when two consecutive sets of 10 stimulations fell below the same threshold. Since the effects of muscimol in the SC are much longer lasting (e.g. Edeline et al. 2002), the effects of this drug were determined for the whole post-drug period. Analysis focused on the later phases of the post drug period due to the slow diffusion rate of the drug (Edeline et al. 2002). Quantitative differences in pre- vs post-drug response parameters were assessed with Student’s or Welch’s t-tests. The latter was used if samples had unequal variance or sizes, and the Wilcoxon Signed Rank Test was used where data were non-normally distributed. Statistical tests were
confined to situations where group sizes exceeded 4 per condition. In all cases, the accepted significance level was \( p < 0.05 \), 2 tailed.

Two dimensional optical imaging spectroscopy (2D-OIS)

In order to assess the spread of cortical activation induced by the electrical stimulation protocols used in the current study, two dimensional optical imaging spectroscopy (2D-OIS) was used to obtain a two-dimensional activation map evoked by the stimulation, based upon changes in local tissue hemodynamics. The hemodynamic response evoked by direct electrical stimulation of the barrel cortex was compared to the hemodynamic response elicited by whisker pad stimulation.

Three additional female Hooded Lister rats (230–330 g) were anesthetized with an intraperitoneal injection of urethane (1.25 g/kg), and additional doses of 25 mg/kg were administered if required. Our methodology for 2D-OIS has been described in detail elsewhere (Boorman et al. 2011). Briefly, animals were tracheotomised, artificially ventilated and the left and right femoral arteries and veins were cannulated for measurement of mean arterial blood pressure (MABP) and for the infusion of phenylephrine. Physiological parameters were maintained within normal ranges. Platinum stimulating electrodes insulated to within 2 mm of the tip 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 with the skull level and the skull overlying somatosensory cortex was thinned to translucency. A circular plastic ‘well’ (20 mm diameter) was positioned over the thinned area, attached with dental cement and filled with saline. A small hole was drilled in the thinned skull above the barrel cortex and a bipolar stimulating electrode (NEX-100, Rhodes Instruments) was introduced perpendicularly to a depth of 1.5 mm.
For 2D-OIS, a Dalsa 1M30P camera recorded the images (with each pixel representing $75 \times 75 \, \mu m$) and a Lambda DG-4 high-speed filter changer (Sutter Instrument Company, Novato, CA) was used to sequentially expose the thinned cranial window to 4 different wavelengths of visible light. Spectral analysis was used to produce 2D images over time of oxyhemoglobin (HbO$_2$), deoxyhemoglobin (Hbr), and total blood volume (Hbt). The effects of 60 trials of direct electrical stimulation of the cortex were assessed using both single pulses and trains of pulses (each single pulse or train was separated from the next by 26 s), using the same stimulation parameters as were used in the electrophysiology experiments (single pulses: 0.1 ms, 1 mA; trains of pulses - 5 pulses at 150 Hz, 0.1 ms each, 0.6 mA). The hemodynamic changes evoked by electrical stimulation of the barrel cortex were compared to the effects of whisker pad stimulation at 5 Hz (1.2 mA for 16 s). A stimulation frequency of 5 Hz is known to result in the greatest magnitude of hemodynamic responses in the somatosensory cortex of the anesthetized rat (Martin et al. 2006).

Data analysis. The first stage of the analysis was to determine the centre of an area of activation using the general a linear model (GLM) statistical parametric mapping (SPM) approach (Friston et al. 1991). The time series of each pixel was compared to a design matrix consisting 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 activation exceeding a z-score threshold. This centre point of this region was then used for the second stage in the analysis – determining the spatial spread of the hemodynamic response. The area around the centre of activation was divided into a series of concentric circular regions (approximate inter-ring distance = 0.25 mm). The change in total hemodynamic concentration for the pixels inside each concentric ring was integrated, providing a measure of the hemodynamic
response at a given distance away from the centre of activation. The hemodynamic response in each ring was then averaged across animals, and the average response for each ring was plotted for electrical whisker pad stimulation and cortical stimulation to produce distance-decay curves.
RESULTS

Transcortical spread of activation induced by cortical stimulation

2D OIS was used to assess the extent of spread of activation induced by the electrical stimulation parameters used to stimulate the barrel cortex in the present study. Single pulse electrical stimulation of the cortex produced a localised region of enhanced activity (Figure 1A,B), which peaked 2.2 ± 0.3 s after stimulus onset, and decreased rapidly both temporally and spatially (Figure 1C,D), having a radius at its peak of approximately 2.0 mm (Figure 1D). The response produced by single pulse stimulation had a smaller peak amplitude, but a similar extent of spread, to whisker pad stimulation (Figure 1D). The hemodynamic response elicited by a train of pulses had a similar extent of spread to that elicited by single pulses, but had a greater peak amplitude (Figure 1D). All stimulating electrode tips in the present study were located in the grey matter of the barrel cortex (Figure 2A), which occupies approximately 4.3 mm x 4.0 mm medio-laterally and rostro-caudally in these dimensions (Paxinos and Watson, 2004), hence activation is likely to have been largely confined to the barrel cortex in all animals. Significant inclusion of the underlying white matter is contraindicated by the absence of activation of adjacent areas of the cortex within the thinned window (Figure 1A).

Cortically evoked activation of the SC

As expected from our previous work (Dommett et al. 2005; Coizet et al. 2009), neurons in the SC deep layers (for brevity, we will refer to all layers below the superficial layers as ‘deep’ here and below) were unresponsive to whole field light flash stimuli in urethane anaesthetised animals (Figure 3A). However, single pulse electrical stimulation of the barrel cortex produced very short latency, short duration excitatory responses in the deep layers of the colliculus, with low temporal variability (Figure 3B; Table 2). Pulse trains also produced very
short latency, temporally stable excitatory responses in the colliculus (Figure 4A-C; Table 2),
which had significantly longer durations than those following single pulse stimulation
\( (t[13.8] = 3.4, p < 0.05) \), but similar onset latencies \( (W = 131.5, p > 0.05) \); measured with
respect to the first stimulus in the train). The excitatory response to pulse trains consisted of
discrete, progressively decrementing responses to each pulse in the train and response size
lessened across the train, such that the response to earlier stimuli in the train were larger than
those to later stimuli in the train (Figure 4C; \( t[8] = 2.81, p < 0.05 \); assessed by measuring the
activity in the 6.5 ms following the second and last pulse in the train).

Local injections of the GABA\(_A\) receptor antagonist bicuculline into the deep layers of
the SC (see Figure 4 for a typical recording/injection site in the colliculus and Figure 2B for
reconstructed plots of all sites) produced widespread disinhibition within the colliculus, as
evidenced by an increase in baseline firing rate (Tables 1 and 2) and extensive expression of
c-fos product, a marker for neuronal activation (Herrera and Robertson, 1996; see Figure 4).
Following intra-collicular bicuculline, collicular neurons exhibited a short-latency, phasic
excitation in response to the light flash (Figure 3A,C; Table 1; cf. Dommett et al., 2005;
Coizet et al., 2009). At the same time, there was a significant increase in the magnitude and
duration of the phasic response to single pulse stimulation of the cortex, although the onset
latency of the response did not change (Figure 3B,D; Table 2). The onset latency of the
response to light flash stimuli was significantly longer than that to cortical stimulation
(Tables 1 and 2; \( W = 576, p < 0.05 \)).

In contrast to the effects of bicuculline on collicular responses to cortical stimulation,
intra-collicular injection of the GABA\(_A\) agonist muscimol produced a significant reduction in
the magnitude of the responses to stimulus trains (Figure 5C; Table 2). Again, onset latency
did not significantly change with the application of muscimol, whereas there was a significant
reduction in baseline firing rate (Table 2).
Cortically evoked activation of DA neurons

All putative DA neurons sampled in the present study had firing rates (3.1 ± 0.3 Hz) and action potential waveform durations (total duration = 4.2 ± 0.1 ms) which met the criteria of Grace and Bunney (1983) and also met the waveform duration criterion of Ungless et al. (2004; initial duration = 1.4 ± 0.03 ms). These waveform characteristics have been shown to be reliable markers for DA neurons in the SNc (Brown et al., 2009; Ungless and Grace, 2012). Furthermore, in all cases the neurons were located in the TH-immunoreactive region of the ventral midbrain corresponding to the SNc (Lindvall and Bjorklund, 1974; see Figure 2C; Figure 4). All SNc DA neurons tested (N=24) were unresponsive to light flash stimuli in the absence of collicular disinhibition by bicuculline and the majority (16/24) were also unresponsive to single pulse electrical stimulation of the cortex. The remaining cells - which did not differ from the unresponsive cells in terms of their position in the SNc or stimulation site in the barrel cortex - showed short latency phasic responses which had either an initial excitatory component (4/8) or an initial inhibitory component (4/8). The onset latencies of these responses were significantly longer than the corresponding onset latencies in the colliculus (Table 2; t[7] = 3.71, p < 0.05).

Following intra-collicular bicuculline, the majority (22/24) of DA neurons exhibited a short-latency, phasic excitatory (14/22) or inhibitory (8/22) response to light flash stimuli (Figure 3A,C; cf. Dommett et al. 2005). Although there was no overall change in the baseline firing rate of DA neurons (Table 2), post-bicuculline, 9/16 of the cells which were previously unresponsive to single pulse cortical stimulation, now showed a short latency excitation (5/16) or inhibition (4/16; Figure 3B,D) following collicular disinhibition. Neurons that already responded in the absence of bicuculline reacted to the treatment by decreasing their response latency (n=3/8; pre, 50.0 ± 26.5 ms vs post, 21.7 ± 10.1 ms), becoming unresponsive (n=2/8) or changing the sign of their responses (n=3/8; see Figure 56A). As a
consequence of the ability to switch between response types, cells responding to cortical
stimulation with either an initial excitation or an initial inhibition do not appear to constitute
two separate populations of neurons (unlike DA neurons which respond to noxious/aversive
stimuli with excitations and inhibitions; Brischoux et al. 2009; Matsumoto and Hikosaka,
2009; but see Fiorillo et al., 2013b). This conclusion is supported by the fact that those DA
neurons which exhibited initial excitations to either single pulse electrical stimulation (pre-
drug), or pulse trains, did not differ from those exhibiting initial inhibitions in terms of
baseline activity (excited: 2.8 ± 0.6; inhibited: 3.1 ± 0.6; t[14.5] = 0.33, p > 0.05), or action
potential shape (the time from onset of the spike to the first trough, excited: 1.3 ± 0.1,
inhibited: 1.4 ± 0.1; t[14.2] = 1.14, p > 0.05). Likewise, there was no evidence of a systematic
bias in the location of excited and inhibited cells in the SNc (Figure 7).

Interestingly, after intracollicular bicuculline, all responsive DA neurons exhibited the
same sign response (initial excitation or initial inhibition) to both light flash stimuli and
cortical stimulation. As with the colliculus, the onset latency of responses to cortical
stimulation were significantly shorter than the onset latency to light flash stimuli (30.0 ms ±
9.4 [combined baseline responsive and newly responsive DA neurons] vs 86.5 ± 5.3 ms
respectively, t[13.4] = 5.21, p < 0.05). In addition, when stimulation in one modality (light or
electrical stimulation) temporarily ceased, there was an enhancement of the collicular
response to the remaining stimulus (Figure 6B,C). This enhanced response at the collicular
level was then reflected in the activity of DA neurons recorded at the same time, which also
exhibited an enhanced response (Figure 6B,C).

In contrast to the single pulse condition, all DA neurons tested (N=9) were responsive
to pulse trains applied to the cortex, with cells exhibiting short latency phasic excitations
(6/9) or inhibitions (3/9; Figure 4A,B). Again, the onset latencies of these responses were
significantly longer than the corresponding onset latencies in the colliculus (Table 3; t[8] =
Intracollicular muscimol had a profound effect on cortically induced responses in DA neurons. In the absence of muscimol, responses in DA neurons to trains of cortical stimuli were stable over time (Figure 5D). However, after intra-collicular muscimol administration, five DA neurons ceased to show a detectable response to cortical stimulation (Figure 4B). In the four cells that remained responsive, muscimol reduced the magnitude of the response Figure 5A; Table 3). Intracollicular muscimol did not affect the baseline firing rate of DA neurons ($t[5.2] = 0.74, p > 0.05$; Table 3).
DISCUSSION

Cortical stimulation and collicular responses

2D OIS showed that with stimulating electrodes in the barrel cortex, the parameters used for stimulation in the present study were likely to have activated an area of cortex which was largely confined to the barrel fields. Furthermore, electrical activation of the cortex elicited responses which on a macroscopic scale resembled those elicited by whisker stimulation itself, and hence although not naturalistic, such activation produces cortical driving, the parameters of which fall within those of the system’s normal behaviour. Furthermore, the lack of evidence for activation of areas of the cortex outside the barrel field suggests a lack of spread of current ventrally to the underlying striatum, which could have generated a change in collicular activity and DA activity via a striato-nigro-collicular route, and DA activity via a direct striato-nigral route.

Although, as in previous work (Comoli et al. 2003; Dommett et al. 2005; Coizet et al. 2009), the deep layers of the SC were unresponsive to whole field light flash stimuli in the absence of bicuculline, these same layers responded with a short latency, short duration phasic response to single pulse electrical stimulation of the barrel cortex. Single pulse electrical stimulation produced a single, short latency, temporally stable response in the deep layers of the SC, whereas pulse trains produced a more exaggerated response consisting of a short latency response following each pulse in the train. The response to single pulses was substantially enhanced following the intracollicular injection of bicuculline, and the responses to pulse trains substantially suppressed following intracollicular muscimol. The latency of these responses to cortical stimulation was significantly shorter than the visual responses following bicuculline. Short latency and low temporal variance are commonly used criteria for discriminating monosynaptic from polysynaptic events (e.g. Yoshimura and Jessell, 1989; Gil et al. 1999), suggesting the cortically-evoked responses are almost certainly
monosynaptic in nature. Indeed, the response latencies are very similar to the conduction
times in the corticotectal pathway from the primary somatosensory cortex in the rat (median
1.7 ms; Kassel, 1982). However, although monosynaptic in nature, there is still the unlikely
confounding possibility that they arise as a result of the antidromic activation of cells which
provide an orthodromic input to the SC, rather than via the direct activation of cortico-tectal
afferents.

Fortunately, only a limited range of structures provide inputs to both the barrel cortex
and the SC. The locus coeruleus, dorsal raphe nucleus and zona incerta all project to the SC
(Cadusseau and Roger, 1985; Beitz et al. 1986; Dauvergne et al. 2008) and the barrel cortex
(Lin et al. 1990; Kirifides et al. 2001; Lee et al. 2009), providing significant noradrenergic,
serotonergic and GABAergic input respectively. However, the locus coeruleus and dorsal
raphe both primarily target the superficial layers (Parent et al. 1981; Wichmann and Starke,
1988). Furthermore, the effect of noradrenaline, serotonin and GABA in the SC is primarily
inhibitory (Sato and Kayama, 1983; Nicolelis et al. 1992; Huang et al. 1993; Mooney et al.
1996; Tan et al. 1999). Hence, neither the locus coeruleus, dorsal raphe nor zona incerta are
likely to play a role in the generation of the short latency excitations in the SC we report
following barrel cortex stimulation. Instead, the responses most likely reflect the direct
orthodromic activation of inputs to the SC deep layers from the cortex.

Dopaminergic responses to cortical stimulation

All of the presumed SNc DA neurons in the present study met the electrophysiological
identification criteria of Grace and Bunney (Grace and Bunney, 1983), and the more stringent
criterion suggested by Ungless et al. (Ungless et al., 2004). These waveform characteristics
have been shown to be reliable markers for DA neurons in the SNc (Brown et al., 2009;
Ungless and Grace, 2012). Likewise, all cells were located in the TH immunoreactive region
of the ventral midbrain, corresponding to the A9 DA cell group (Lindvall and Bjorklund, 1974). Non-DA neurons in this region of the brain account for only a small proportion (~20%; Matsuda et al., 1987) of the total. Nonetheless, in the absence of direct neurochemical identification, the DA nature of our neurons cannot be known with certainty. That said, for the reasons given above, it is probably safe to assume that the overwhelming majority our neurons were indeed DA.

All SNc DA neurons were unresponsive to light flash stimuli in the absence of local collicular disinhibition with bicuculline and the majority were unresponsive to single pulse electrical stimulation of the cortex. In contrast, all cells were responsive to pulse trains applied to the cortex, with cells exhibiting short latency phasic excitatory or inhibitory responses (as did those cells which were responsive to single pulse stimulation in the absence of bicuculline). In those cells which responded to cortical stimulation (single pulse or train) at baseline, the latency of responses in DA neurons was significantly longer than the response in the SC, consistent with the transmission of information from the SC to DA neurons in the SNc (see also de Lafuente and Romo, 2012). As in our previous work (Dommett et al. 2005), following collicular bicuculline, the majority of DA neurons showed a short-latency, phasic response (with an initial excitation or inhibition) to light flash stimuli. Although there was no overall change in the firing rate of DA neurons, post-bicuculline, 9/16 of the previously unresponsive cells now showed a short latency phasic response to the single pulse cortical stimulation. In those cells which exhibited phasic responses to trains of cortical stimuli, intra-collicular muscimol abolished the response in the majority of cells and in the remainder decreased the magnitude of the responses.

Neurons showed consistent responses to light flash stimuli and cortical stimulation, responding with either an initial excitation or initial inhibition to both. Insofar as these responses are driven the SC, this suggests that the same collicular neurons may be conveying...
activity related to both stimulation types. This conclusion is further supported by the fact that responses to the two stimulus types tended to interact at both the collicular and nigral level. It is known that collicular neurons can receive afferent inputs from both cortical and subcortical sources (the spinal cord and somatosensory cortex, Rhoades, 1980; the retina and visual cortex, McIlwain and Fields, 1970), and that non-contiguous activation of these afferents results in an inhibitory interaction between them (McIlwain and Fields, 1970; Rhoades, 1980). In a similar manner in the present study, collicular responses to light flash stimuli were suppressed when preceded two seconds earlier by electrical stimulation of the barrel cortex, and this suppression at the collicular level was also evident in simultaneously recorded DA neurons. These correlated changes imply that interactions between stimulus types at the single cell level in the colliculus are being passed on to DA neurons in the SNc by virtue of the fact that they share a common channel of communication.

Functional implications

Taken together, evidence that single pulse electrical stimulation of the (barrel) cortex produces responses in previously unresponsive DA neurons when the SC is disinhibited with bicuculline, and muscimol-induced inhibition of the SC blocks or attenuates responses in DA neurons elicited by pulse trains applied to the barrel cortex, strongly suggests that the cortex can transmit information to DA neurons via the SC. As a consequence, this route of information flow is a clear contender for the substrate which underlies the second phase of the response of DA neurons to complex visual stimuli reported by several authors in the monkey, which has a peak latency of around 160 ms (Morris et al. 2004; Hudgins et al. 2009; Nomoto et al. 2010). Somatosensory information reaches the somatosensory cortex more quickly than visual information reaches the visual cortex (somatosensory, ~10 ms; Di et al. 1990; Ahisser et al. 2001; Martin et al. 2006; Zhu et al. 2009; visual 30-50 ms; Maunsell and
Gibson, 1992; Thorpe and Fabre-Thorpe, 2001), and so cortically driven responses to somatosensory stimuli have the capacity to be even faster than those to visual stimuli. Given dopamine’s critical role in reinforcement learning (Wise, 2004), we have argued that the short latency of the dopamine signal is particularly well suited to reinforce the discovery of agency – those events in the world for which the agent is responsible (Redgrave and Gurney, 2006; Redgrave et al. 2008). According to this view, the advantage of having DA reinforcement occur before any behaviour evoked by the sensory event itself is that the record of behavioural output (motor efference copy) will remain uncontaminated by non-causal components of behaviour. This would greatly simplify the problem of assigning credit to appropriate causal components of immediately preceding behaviour (Izhekevitch, 2007).

Sensory cortical inputs to the DA systems – which have the potential to be modified by reward (e.g. Mogami and Tanaka, 2006; Hui et al. 2009; Frankó et al. 2010; Hickey et al. 2010; Serences and Saproo, 2010; Weil et al. 2010) - can be seen as a means by which the connection between an individual’s behaviour and its outcome is ‘stamped in’.

If the SC is the conduit used by the sensory cortex to evoke short-latency phasic responses in DA neurons, the question arises as to why this differs from the manner in which the cerebral cortex makes contact with other basal ganglia input nuclei. Thus, with the evolutionary expansion of the cerebral cortex (Butler et al. 2011), direct, topographically organised connections were established with both the striatum (Selemon and Goldman-Rakic, 1985; Nambu, 2011), and the subthalamic nucleus (Nambu et al. 1997; Nambu, 2011). Why not also with DA neurons in the ventral midbrain, which receive comparatively few direct cortical inputs (Frankle et al. 2006; Watabe-Uchida et al. 2012)? One possibility could be that the topographically organised cortico-striatal and cortico-subthalamic connections are designed to convey bids for behavioural selection (Redgrave et al. 1999), the competitions between which are resolved (Gurney et al. 2001; Humphries et al. 2006; Prescott et al. 2006)
by selective disinhibition (Chevalier and Deniau 1990) within the basal ganglia’s re-entrant looped connectivity (Alexander et al. 1986). This highly topographic selection architecture may differ markedly from one that is designed to relay reinforcement signals widely throughout a selection network. Interactions within the striatum between the widely broadcast dopamine signal and more localised activity engendered by previously occurring successful bids for expression may be critical for reinforcement learning (Redgrave and Gurney, 2006; Redgrave et al. 2008). The advantage of having early cortical processing access the basal ganglia’s reinforcement mechanisms indirectly via the midbrain SC may be that the SC constitutes a useful point of convergence for many sources of sensory and non-sensory information (Edwards et al. 1986; May, 2006) which the cortex needs to take into account when reinforcing behavioural output. Alternatively, viewing the various afferent inputs to the colliculus more equally, the SC may be viewed as providing a final common nodal point for information destined for DA neurons. This may be the substrate which accounts for the wide range of non-noxious sensory stimuli which are able to produce responses in DA neurons at short latency in rats and other species. In rats, in addition to visual stimuli, auditory, whisker and non-whisker somatosensory stimuli all phasically activate DA neurons (Miller et al., 1981; Freeman and Bunney, 1987; Kiyatkin, 1988), and are all represented in the SC (McHaffie et al., 1989; Hemelt and Keller, 2007; Vachon-Presseau, et al, 2009). Given that the tectonigral projection is a conserved feature across rats, cats and monkeys (Comoli et al., 2003; McHaffie et al., 2006; May et al., 2008), a similar system-level organisation may be a widespread feature of the architecture for the regulation of DA neurons.
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GRANTS

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Figures and Tables

Figure 1
Cortical hemodynamic responses to direct electrical stimulation and electrical stimulation of the whisker pad. (A) *In vivo* camera image of the cortical surface vasculature viewed through a thinned cranial window. A small hole drilled through the thinned skull allowed a stimulating electrode (arrow) to be inserted into the barrel cortex. Single pulse stimulation (0.1 ms, 1 mA) produced a localised increase in blood volume (Hbt), as indicated by the blue shaded area (GLM model fit, z-score threshold at 50%). Scale bar, 1 mm; (B) Concentric rings centred on the geometrical midpoint of the area of activation were used to analyse the spread of activation (approximate inter-ring distance = 0.25 mm). The magnitude of the evoked hemodynamic response within each ring at the temporal peak of activation forms the basis for parts B-D of the figure. The scale bar represents the GLM model fit, as a z-score; (C) Temporal and spatial spread of activation following single pulse stimulation of the barrel cortex. The scale bar represents the percentage change in Hbt relative to baseline. Stimulation occurred at time zero; (D) Spatial spread of activation (mean change in Hbt) in the cortex induced by single pulse stimulation and pulse train stimulation (5 pulses at 150 Hz, 0.1 ms each, 0.6 mA) of the barrel cortex, and electrical stimulation of the whisker pad (5 Hz 1.2 mA for 16 s).

Figure 2
Reconstructed plots of stimulation, recording and injection sites: (A) Plots of stimulation sites in the cerebral cortex. Coloured circles indicate the position of the electrode tips in animals in which pulse trains were applied to the cortex (red) or single pulses were applied to the cortex (blue). S1: primary somatosensory cortex; S1BF: primary somatosensory cortex, barrel field;
S1DZ: primary somatosensory cortex, dysgranular region; S2: secondary somatosensory cortex; (B) Reconstructed plots of recording/injection sites in the superior colliculus. Coloured circles indicate the position of the recording/injection sites in animals in which muscimol was injected into the colliculus (red) or bicuculline was injected into the colliculus (blue). The layers are labelled as follows: 1. Zonal layer; 2. Superficial grey layer; 3. Optic layer; 4. Intermediate grey layer; 5. Intermediate white layer; 6. Deep grey layer; 7. Deep white layer; (C) Reconstructed plots of recording sites in the substantia nigra pars compacta (SNc). Coloured circles indicate the position of the recording sites in animals in which pulse trains were applied to the cortex (red) or single pulses were applied to the cortex (blue). The point marked with a star represents the location of two recorded dopaminergic neurons. The SNc is indicated by shading; m: substantia nigra medial part; l: substantia nigra lateral part; SNr: substantia nigra pars reticulata; In A-C, electrode positions are reconstructed onto coronal sections, the position of which is given relative to bregma.

Figure 3
Response of the superior colliculus and dopaminergic neurons to light flash stimuli and electrical stimulation of the barrel cortex. (A) Raster displays (top) and peri-stimulus time interval histograms (PSTHs; bottom) show that deep layer collicular neurons (SC) and a simultaneously recorded dopaminergic (DA) neuron in this animal were initially unresponsive to regular (0.5 Hz) wholefield light flash stimuli (VIS; vertical dotted line). After a collicular microinjection of bicuculline (VIS+BIC), both collicular neurons and the DA neuron were excited at short latency by visual stimulation. The PSTH for the DA neuron shows the three point smoothed change in firing rate from baseline (delta Hz); (B) Raster displays and PSTHs show that collicular neurons exhibited a short latency excitatory response to single pulse electrical stimulation of the barrel cortex (CTX; 0.1 ms, 1 mA 0.5
Hz; vertical dotted line), whereas (in common with the majority of DA neurons), a simultaneously recorded DA neuron in this animal was unresponsive. After a collicular microinjection of bicuculline (CTX+BIC), the collicular response to cortical stimulation was enhanced and the DA neuron was now excited at short latency by the stimulation. As well as short latency excursions, light flash stimuli (C) and single pulse electrical stimulation of the barrel cortex (D) could also induce short latency inhibitions in DA neurons post-bicuculline.

**Figure 4**

Location of the recording and injection sites in representative animals. Left hand side: A coronal section at high and low (inset) magnification, showing the superior colliculus processed using tyrosine hydroxylase (TH) and c-fos immunohistochemistry. The section shows fos-like immunolabeling (black dots) in the colliculus as a result of neural activity induced by an injection of bicuculline. An electrolytic lesion at the recording/injection site is indicated by an arrow. Right hand side: Coronal sections at high and low (insets) magnification, processed using TH immunohistochemistry (top) and cresyl violet (bottom). The top section shows TH immunolabeling (purple cells and processes) in the ventral midbrain and the electrode track and recording site (arrow) in the substantia nigra pars compacta (SNc). The site can be seen again as a blue dot (indicated by an arrow) in the bottom section. The position of all sections is given relative to bregma. Scale bars = 1 mm.

SuSC: superficial layers of the SC (zonal layer, superficial grey layer and optic layer); DpSC: deep layers of the SC (intermediate grey and intermediate white layers, deep grey white layers); PAG: periaqueductal grey; SNr: substantia nigra pars reticulata; VTA: ventral tegmental area; ml: media lemniscus; fr: fasciculus retroflexus.
**Figure 5**

Intra-collicular muscimol administration suppressed collicular and dopaminergic responses to cortical stimulation. (A) Raster displays (top) and PSTHs (bottom) show that collicular neurons (SC) in this animal exhibited a short latency excitatory response to pulse trains applied to the barrel cortex (CTX; 0.1 ms, 0.6 mA; vertical dotted line). Likewise, a simultaneously recorded dopaminergic (DA) neuron showed a short latency excitatory response to the pulse trains. After a collicular microinjection of muscimol (CTX+MUS), the collicular response to cortical stimulation was attenuated as was the response of the DA neuron. The PSTH for the DA neuron shows the three point smoothed change in firing rate from baseline (delta Hz); (B) As well as excitations, pulse trains applied to the barrel cortex could induce short latency inhibitions in DA neurons. In the example shown here, intra-collicular muscimol eliminated the DA neuron’s response to cortical stimulation. (C) Trains of electrical stimuli applied to the barrel cortex produced excitatory responses to each pulse in the train (black trace). Intra-collicular administration of muscimol reduced baseline activity and depressed the responses to stimulation (red trace); (D) Raster display (top) and peri-stimulus histogram (PSTH; bottom) of a representative case showing that electrical stimulation of the barrel cortex with pulse trains (5 pulses at 150 Hz, 0.1 ms each, 0.6 mA) produced temporally stable responses in DA neurons.

**Figure 6**

Responses of dopaminergic neurons to electrical stimulation of the barrel cortex are labile and interact with responses to visual stimulation. (A) Raster plots (top; middle) and a peri-stimulus histogram (PSTH; bottom) of a dopaminergic (DA) neuron which showed a short latency inhibitory response to single pulse cortical stimulation of the barrel cortex (0.1 ms 1.0 mA 0.5 Hz) in the absence of intra-collicular bicuculline (middle), which then switched to a
short latency excitatory response in the presence of bicuculline (top). The PSTH for the DA
neuron shows the three point smoothed change in firing rate from baseline (delta Hz); (B)
Top: Raster plot and PSTH of deep layer collicular responses to whole field light flashes in
the presence of intra-collicular bicuculline. On trials in black, flashes were preceded 2 s
earlier by electrical stimulation of the barrel cortex whilst on trials in red, only visual
stimulation was used. Bottom: Raster plot and PSTH of responses in a simultaneously
recorded DA neuron. In the presence of cortical stimulation, the response of the DA neuron
to visual stimulation in this animal was weaker than when visual stimulation was delivered
alone; (C) Top: Raster plot and PSTH of deep layer collicular responses to single pulse
electrical stimulation of the barrel cortex in the presence of intra-collicular bicuculline. On
trials in black, electrical stimulation of the barrel cortex was preceded 2 s earlier by whole
field light flashes whilst on trials in red, only electrical stimulation was used. Bottom: Raster
plot and PSTH of responses in a simultaneously recorded DA neuron. In the presence of
visual stimulation, the response of the DA neuron to cortical stimulation in this animal was
weaker than when stimulation was delivered alone.

Figure 7
Reconstructed plots of recording sites of dopaminergic neurons showing excitatory or
inhibitory initial response components. Coloured circles indicate the location of
dopaminergic neurons in the substantia nigra (SNC) showing excitatory (green) or inhibitory
(red) initial components in response to cortical stimulation (either single pulses or pulse
trains) at baseline. Recording positions are reconstructed onto coronal sections, the position
of which is given relative to bregma. The SNC is indicated by shading; m: substantia nigra
medial part; l: substantia nigra lateral part; SNr: substantia nigra pars reticulata.
Table 1

Effects of intra-collicular bicuculine on collicular and dopaminergic responses to light flash stimuli

Parameters (baseline firing rate, response latency, response duration and response magnitude, defined as the mean number of spikes per stimulus between response onset and response offset) of multi-unit responses measured in the superior colliculus (SC) and single unit responses measured in dopaminergic (DA) neurons in response to light flash stimuli, before and after intra-collicular injections of bicuculine. # p < 0.01 with respect to pre-drug.

Table 2

Effects of intra-collicular bicuculine on collicular and dopaminergic responses to cortical stimulation using single pulses

Parameters (baseline firing rate, response latency, response duration and response magnitude, defined as the mean number of spikes per stimulus between response onset and response offset) of multi-unit responses measured in the superior colliculus (SC) and single unit responses measured in dopaminergic (DA) neurons to electrical stimulation of the barrel cortex using single pulses. In the case of DA neuron responses, cells have been sub-divided into two groups: cells which were unresponsive [DA(1)] and cells which were responsive [DA(2)] to cortical stimulation in the absence of bicuculine. * p < 0.05; # p < 0.01; § p < 0.001 with respect to pre-drug.
Table 3

Effects of intra-collicular bicuculine on collicular and dopaminergic responses to cortical stimulation using pulse trains

Parameters (baseline firing rate, response latency, response duration and response magnitude, defined as the mean number of spikes per stimulus between response onset and response offset) of multi-unit responses measured in the superior colliculus (SC) and single unit responses measured in dopaminergic (DA) neurons to electrical stimulation of the barrel cortex using pulse trains. In the case of DA neuron responses, cells have been sub-divided into two groups: cells which were unresponsive [DA(1)] and cells which were still responsive [DA(2)] to cortical stimulation in the presence of muscimol. * p < 0.05; # p < 0.01; § p < 0.001 with respect to pre-drug.
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<th>DA SINGLE UNIT</th>
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<td>Magnitude</td>
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<tr>
<td></td>
<td>SC MULTI-UNIT</td>
<td>DA(1) SINGLE UNIT</td>
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<tr>
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<tr>
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