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Effect of methylphenidate on visual responses in the superior colliculus in the anaesthetised rat: role of cortical activation

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Abstract

The mechanism of action of psychostimulant drugs in the treatment of Attention Deficit Hyperactivity Disorder is still largely unknown, although recent evidence suggests one possibility is that the drugs affect the superior colliculus (SC). We have previously demonstrated that systemically administered d-amphetamine attenuates/abolishes visual responses to whole field light flashes in the superficial layers of the SC in anaesthetised rats, and the present study sought to extend this work to methylphenidate (MPH). Anaesthetised rats were administered MPH at a range of doses (or saline) and subjected to monocular whole field light flashes at two intensities, juxta-threshold and super-threshold. In contrast to d-amphetamine, systemic MPH produced an enhancement of visual activity at both intensities. Methylphenidate was also found to produce activation of the cortical EEG in anaesthetised rats. Furthermore, cortical activation induced by electrical stimulation of the pons was found enhance visual responses in superficial layers of the SC, and when MPH was paired with pontine-induced cortical activation, the response-enhancing effects of MPH were substantially attenuated. Taken together, the results suggest that the enhancement of visual responses in the superficial layers of the SC by MPH in the anaesthetised rat is an artefact of the drug's interaction with cortical arousal.

Key words: local field potential; multiunit activity; psychostimulants; cortical arousal

Introduction

It is well documented that methlphenidate (MPH) improves vigilance (Coons et al., 1981; Strauss et al, 1984; Peloquin & Klorman, 1986; Camp-Bruno & Herting, 1994; Hermens et al., 2007) and divided/focused attention (Hink et al, 1978) in normal humans and attentional performance in rats (Bizarro et al., 2004). Methylphenidate is also particularly effective at ameliorating the attentional difficulties in children and adults with Attention Deficit Hyperactivity Disorder (ADHD; Brown et al., 2005; Tucha et al., 2006). Therapeutic efficacy aside, MPH has clear abuse potential (e.g. Williams et al, 2004), and hence is far from ideal as a long-term treatment. However, the development of non-addictive pharmacotherapies for ADHD is hampered by two inter-related obstacles. Firstly, despite the proposal of several models of the pathophysiological changes underlying ADHD (reviewed for example by Himelstein et al., 2000), the neurobiology of ADHD is far from completely understood (Biederman, 2005). Secondly, possibly partly because of this pathophysiological uncertainty, the therapeutic mechanism of action of current psychostimulant medications in ADHD is also far from clear (Spencer et al., 2002).

A central component of the attentional problems in ADHD children is an increase in distractibility (Douglas, 1983; Thorley, 1984; see also DSM V, American Psychiatric Association, 2013) and MPH has been found to be effective against distractibility in ADHD patients (Weiss et al., 1971; Riordan et al., 1999; Brodeur & Pond, 2001; Pelham et al., 2011). Behavioural evidence suggests that distractibility is intimately linked with the midbrain superior colliculus (SC), a major component of the subcortical visual system that is highly conserved across species (Overton et al., 1985; May, 2005). Work in a range of species has shown that collicular lesions lead to a decrease in distractibility (rat: Goodale et al., 1978; cat: Sprague & Meikle, 1965; monkey: Milner et al. 1978). Conversely,

disconnecting the colliculus from the controlling influence of the prefrontal cortex leads to an increase in distractibility in humans (Gaymard et al., 2003).

Although it is still at present unclear how MPH effects attentional improvements, a logical possibility is that the drug acts to modulate sensory processing in the SC and thereby raise the threshold for distraction (Overton, 2008). The proximal effects of MPH are mediated by increasing synaptic levels of the monoamines dopamine (DA) and noradrenaline (NA; Kuczenski & Segal, 1997; Easton et al., 2007) and possibly serotonin (5-HT; Kuczenski et al., 1987). Evidence suggests SC itself receives extensive noradrenergic (Lindvall & Bjorklund, 1974; Weller et al., 1987) and serotonergic (Parent et al., 1981; Weller et al., 1987) innervation, as well as a limited dopaminergic input (Weller et al., 1987; Campbell et al., 1991). Noradrenergic and serotonergic afferents appear to preferentially target the superficial, exclusively visual layers (Parent et al., 1981; Wichmann & Starke, 1988).

We have recently shown that systemic d-amphetamine - another widely used treatment for ADHD (e.g. Brown et al., 2005; Arnsten, 2006), which is also effective against distractibility (e.g. Spencer et al., 2001) - dose-dependently decreases the responsiveness of cells in the superficial layers of the SC to visual stimuli, measured both by local field potentials (LFPs) and multiunit activity (MUA; Gowan et al., 2008). Similar results were obtained with intra-collicular administration, suggesting that the effects of systemic d-amphetamine on visual responses in the superficial layers of the SC appear to be produced, at least in part, by a local action in the colliculus (Gowan et al., 2008). Similar findings were also obtained in an animal model of ADHD – the New Zealand Genetically Hypertensive rat (Clements et al., 2014). Reducing the sensory responsiveness of the SC is consistent with the idea that d-amphetamine achieves its therapeutic effects on distractibility by modulating the activity of the colliculus, and hence by inference that the SC may be dysfunctional in ADHD

(Gowan et al., 2008; Overton, 2008). The present study was designed to extend this work to MPH by examining the effects of systemic MPH on visual responses in the superficial layers of the rat colliculus.

Materials and Methods

Study 1: Effects of methylphenidate on visual responses in the superficial layers of the rat superior colliculus.

Subjects

Data were obtained from 24 male Hooded Lister rats (bred in house), weighing 300-520 g at the time of testing. All procedures were carried out in accordance with the Principles of Laboratory Animal Care (NIH publication No. 86-23, revised 1985) and the Animals (Scientific Procedures) Act, 1986. Every effort was made to minimise suffering and reduce the number of animals used.

Surgical preparation

Animals were anaesthetised with urethane (ethyl carbonate; 1.25g/kg as a 25% aqueous solution; i.p.) and mounted in a stereotaxic frame (David Kopf Instruments, Tuajanga, CA) with the skull level. Body temperature was maintained at 37°C with a thermostatically controlled heating blanket. A unilateral craniotomy was performed (centred on 6.3 mm caudal to bregma; 2 mm lateral to midline) to allow access to the SC. The rat's right eye was sutured open and the cornea protected with Viscotears (Ciba Vision, Duluth, MN). Finally, the left femoral vein was cannulated for the later injection of MPH or saline.

Recording procedure

A single Parylene-C-insulated tungsten microelectrode (2 MΩ, A-M Systems Inc., Carlsborg, WA, USA) was introduced vertically into the cortex overlying the SC (6.3 mm caudal to bregma; 2 mm lateral to midline) and lowered until the SC was encountered (2.8-3.9 mm below dura). Extracellular low frequency (local field potential; LFP) and high frequency (multiunit activity; MUA) activity was amplified (gain 1000), band-pass filtered (LFP; 0.1-300 Hz, MUA; 300 Hz-10 kHz), digitized at 11 kHz and recorded directly onto computer disc using a 1401+ data acquisition system (Cambridge Electronic Design Systems [CED], Cambridge, UK), running CED data capture software (Spike 2).

Electrode position was assessed by determining the multiunit response to contralateral whole field light flashes (0.5 Hz, 50 ms duration, 22 LUX, 505 nm peak green LED: stimulus onset was jittered within a 50 ms window to reduce the effects of 50 Hz mains noise in averaged data). Using the characteristically vigorous visual response of the superficial layers of the SC as a positional cue (see Figure 1), the electrode was lowered until the tip was located in those layers. Once the electrode was correctly positioned, the animal was left to dark adapt for at least 25 min. Subsequently, responses to successive series of 150 light flashes (0.5 Hz, 10 ms duration) were assessed. Once the LFP response was stable, responses were recorded to 150 light flashes at a range of intensities in ascending order. Thereafter, for testing, an intensity was chosen which was juxta-threshold for animals in the low intensity group ('Lo intensity'; N=12; mean intensity 0.4 LUX), and an intensity was chosen which was clearly supra-threshold for animals in the high intensity group ('Hi intensity'; N=12; mean intensity 40.0 LUX). Having two intensities towards the extreme end of the range in this way was designed to take account of the fact that the drug's actions may be selective for part of the intensity range (Dommett et al., 2009). Following the selection of the test intensity, a final

baseline (pre-drug) set of responses to 210 light flashes were recorded. Delivered at a rate of 0.5 Hz, the baseline recording session lasted for 7 min.

Baseline data acquisition was immediately followed by an intravenous injection of either MPH (0.4 mg/kg, in 0.1 ml/kg 0.9% saline; Hi intensity group, N=6; Lo intensity group, N=6), or saline (0.1 ml/kg; Hi intensity group, N=6; Lo intensity group, N=6) and the acquisition of a subsequent set of responses to 210 light flashes. This process was repeated four more times, with rising cumulative doses of MPH (0.8, 1.6, 3.2 and 6.4 mg/kg for the 2nd, 3rd, 4th and 5th injection respectively), or volumetrically equivalent saline injections.

2.4 Data analysis

Collicular recordings were analyzed offline using Spike 2, custom-made Excel macros (Peter Furness, Sheffield University) and SPSS (version 23; IBM, New York). All analyses were performed on averaged data where averages were constructed from the last 5 min of the 7 min recording period (150 stimulations) at each of the five doses, allowing time for the drug to 'wear on' at each dose. For LFP data, a waveform average was created (1 s duration, 0.1 s offset) for each dose. The waveform average was exported into a custom-made Excel macro and a response was deemed to have occurred if the voltage trace exceeded a pre-determined threshold after stimulus onset, but not before 20 ms post stimulus. The latter requirement was used to avoid any stimulus-related artifacts; collicular LFP responses to light flash stimuli in dark-adapted rats have been reported to have an average onset latency in excess of 27 ms (Dyer & Annau 1977; Gowan et al. 2008). The threshold for change was set at ±1.96 standard deviations from the mean baseline (i.e. within 95% confidence levels). This threshold was used to assess three parameters: onset latency, peak latency and peak-to-peak amplitude. Onset latency was obtained by recording the time after stimulus presentation (and at least 20 ms) at which the voltage trace exceeded the threshold, and peak

latency was defined as the time after stimulus presentation when the post-stimulus voltage excursion reached a maximum (or minimum, depending on the sign of the largest excursion). Finally, peak-to-peak amplitude was defined as the voltage difference between the maximum positive peak and the maximum negative peak in the response period defined by the significant deviation from baseline.

For the MUA, similar measures were utilised following initial extraction of 'spikes' from the high-frequency data by thresholding. Peri-stimulus time histograms (PSTHs; 1-ms bins, 1 s duration, 0.1 s offset) were constructed from the trial-by-trial spike counts and the 100 ms pre-stimulus period was defined as baseline activity. A light response was deemed to have occurred if, post stimulus, the activity rose above 1.96 standard deviations of the mean for at least 5 ms (5 consecutive bins), the first of which was labelled as the onset of a response. The duration was calculated by measuring when the response fell back to within the baseline levels for at least 10 ms (10 consecutive bins), the first of which was labelled as the end of the response. Duration was then given as the difference between onset latency and the response ending. The amplitude was recorded as the peak value of the response minus the mean baseline value.

Finally, a normalisation was carried out by dividing the recorded activity by the baseline response to allow later statistical analysis. LFP and MUA responses were analysed using mixed measures analyses of variance (ANOVA). Where Mauchly's test of sphericity was significant in the ANOVAs, the degrees of freedom were adjusted using Greenhouse–Geisser correction (Greenhouse & Geisser, 1959). Missing data points were filled by using group averages. In all cases, probabilities of $P<0.05$ were considered to be significant (two tailed).

Histology

Site reconstruction: At the end of the experiment, brains were removed and stored in 4% paraformaldehyde in phosphate buffer (pH 7.4) for 48 h. They were then transferred into 20% sucrose in 0.1 M phosphate buffer, where they remained for at least 48 hr, before being frozen (-18°C) and cut into 50 µm coronal sections using a cryostat. Sections were mounted on gelled slides and stained with cresyl violet. Recording sites were reconstructed with the aid of a stereotaxic atlas (Paxinos & Watson, 1997).

Results

Recording sites and baseline visual responses within the superficial layers of the superior colliculus

Considering all animals with collicular placements (in Study 1 and Study 2 below; N =36), as expected, recording sites in both studies were found to be located in the superficial layers of the SC (consisting of the stratum zonale [SZ], stratum griseum superficiale [SGS] and stratum opticum [SO]; Paxinos & Watson, 1997; Figure 1). Electrode track size and shape sometimes meant that it was difficult to precisely localise electrodes to the SGS vs SO by visual inspection alone. However, characteristics of the LFP responses recorded at baseline allowed a broad distinction to be made between locations in these layers in all cases. The superficial layer LFP response to wholefield light flashes was a complex phenomenon consisting of several distinct components (see Figure 1). In the majority of cases (25/36; 69.4%), the shortest latency LFP component consisted of a short duration positivity (presumably corresponding to components P1-P3 of the superficial layer visual response to flashed wholefield stimuli previously reported by Dyer and Annau, 1977; see Figure 1), followed by a longer duration, larger amplitude negativity (presumably corresponding to components N4-N5 of Dyer and Annau, 1977). The initial positivity was, in some cases

(7/25; 28.0%), preceded by a brief low amplitude negativity (N1; Figure 1). These characteristics are typical of electrode placements in the SGS (Dyer & Annau, 1977; Hetzler et al., 1981). Deeper placements (in SO) tend to be associated with a reversal of all major field potential components (Dyer & Annau, 1977; Sefton, 1969; Hirai & Okada, 1995) and hence the majority of animals in the present study were considered to have electrode placements in SGS (or possibly SZ). Those cases in which an early positivity was not apparent (N=11/36; 30.5%) were considered to have electrode placements in SO.

Effects of systemically administered methylphenidate on visual responses in the superficial layers of the superior colliculus

Systemically administered MPH produced a marginally significant increase in firing rate, i.e. the level of spontaneous activity before each stimulus (mixed measures ANOVA, factors DRUG [MPH and Saline], DOSE [0.5 mg/kg-8.0 mg/kg] and INTENSITY [Hi and Lo], main effect of DRUG $F(1,20)=4.29$, $p=0.052$). In contrast, as is evident from Figures 1-3, the drug produced a substantial enhancement of superficial layer visual responses. The peak amplitude of visually-evoked MUA in the MPH group showed an increase with respect to baseline at all doses of the drug in both the Lo and Hi light groups (main effect of DRUG $F(1,19)=9.97$, $p<0.01$), which was particularly apparent at the higher doses. There was a trend towards dose dependency in the Hi light group and at lower doses in the Lo light group, however this was not reflected statistically (factors DOSE x DRUG, $F(1.60,30.35)=3.05$, $p>0.05$; DOSE x DRUG x INTENSITY, $F(1.60,30.35)=2.14$, $p>0.05$). Indeed, although ≥80% of animals in both the Hi and Lo light groups had greater MUA enhancements at the highest MPH dose than at the lowest dose, and a small number of animals (around one quarter) showed evidence of dose dependency (see Figure 1), across the groups dose dependency was rather weak.

As with the peak amplitudes, MPH significantly increased the durations of visually-evoked MUA responses in the Lo light group (Figure 3), an effect that was much more pronounced than in the Hi light group (Figure 2; factors DRUG x INTENSITY: $F_{(1,19)}=4.39$, $p=0.05$). In contrast, MPH significantly reduced both the onset latency (main effect of DRUG $F_{(1,19)}=22.34$, $P<0.001$) and the peak latency (factors DRUG $F_{(1,19)}=5.36$, $p<0.05$), with the effect being greater at higher doses (factors DRUG x DOSE: $F_{(1.80,34.14)}=4.51$, $p<0.05$; Figures 2 and 3).

The effects of MPH on visually-evoked LFP responses in the superficial layers of the SC closely resembled the drug's effects on MUAs. As with MUA responses, MPH enhanced the amplitude of superficial layer responses, although this was only apparent in the Lo light group, and then only at higher doses (factors DOSE x DRUG x INTENSITY $F_{(2.38,45.24)}=3.09$, $p<0.05$; Figures 2 and 3). As with visually-evoked superficial layer MUA responses, MPH also reduced the onset latency (in the Lo light group) and the peak latency (in both groups; Figures 2 and 3), although this was not statistically significant (onset latency - DRUG x INTENSITY: $F_{(1,19)}=1.67$, $p>0.05$; peak latency – main effect of DRUG: $F_{(1,19)}=1.09$, $p>0.05$).

Interim discussion

The major effect of MPH in Study 1 was to increase the amplitude of visually evoked activity in the superficial layers of the SC, as measured by both LFPs and MUA, as well as MUA response duration. These results with MPH are clearly very different to those with d-amphetamine, which dose-dependently decreases both the amplitude and duration of visual responses in the superficial layers of the SC, measured both by LFPs and MUA (Gowan et al., 2008: Clements et al., 2014). This difference could relate to differences in the

pharmacodynamics of the two drugs. Whilst the proximal effects of both MPH and d-amphetamine are mediated by DA and NA (Easton et al., 2007; Kuczenski & Segal, 1997), d-amphetamine also has significant effects on 5-HT (Holmes & Rutledge, 1976) at higher doses (Kuczenski & Segal, 1989), whereas MPH has little effect on 5-HT in some (Kuczenski & Segal, 1997) but not all (Kuczenski et al., 1987) areas receiving 5-HT innervation. Given that the effects of systemic d-amphetamine on visual responses in the SC are at least partly locally mediated (Gowan et al, 2008) and the effects of 5-HT are uniformly depressant in the SC (Kawai & Yamamoto, 1969; Straschill & Perwein, 1971), the lack of an inhibitory effect with MPH could result from a lack of interaction with 5-HT mediated transmission.

However, given the universally inhibitory effect of 5-HT in the SC, a lack of interaction between MPH and 5-HT mediated transmission would only explain a lack of an inhibitory effect on visual responses, not the presence of a facilitatory effect. The facilitatory effect of MPH on visual responses in the rat SC is made even more surprising by a recent study which reported that MPH reduced the amplitude of all three LFP components studied in the colliculus (two significantly) in the awake behaving rat (Hetzler et al., 2014). This difference however between the effects of MPH on visual responses in awake and anaesthetised animals may give a clue as to what is underlying MPH's facilitatory effects in the latter case. Studies have found that the administration of MPH under anaesthesia can affect EEG patterns, producing a shift in power to higher frequencies indicative of arousal (Solt et al., 2011; Chemali et al., 2012). Increased doses of anaesthetics are required for patients prescribed with MPH (Kasuga et al., 2008; Ririe et al., 1997), and effects of MPH on an awake EEG have been reported (Loo et al., 2004; Loo et al., 1999). In the present context, MPH-induced changes at the level of the cortex may potentially affect collicular activity via direct cortico-tectal projections (Harting et al., 1992; Comoli et al., 2012), or the

colliculus could be modulated by changes to other inputs associated with arousal, for example changes in the activity of cholinergic afferents from the pedunculopontine tegmental nucleus and lateral dorsal tegmental nucleus (Beninato & Spencer, 1986), which have an excitatory action on neurons in the superficial SC (Stubblefield et al., 2015). However, evidence for MPH-induced activation of the pedunculopontine tegmental nucleus/ lateral dorsal tegmental nucleus is unfortunately lacking, suggesting that activation via the direct cortico-tectal route is presently the favoured suggestion.

Cortical arousal has been found to enhance sub-cortical sensory responses in the rat, for example responses in the ventrobasal thalamus to stimulation of the medial lemniscus (Kanosue et al., 1986), and in the ventro-posterior medial thalamus to whisker stimulation (Castro-Alamancos & Oldford (2002). Interestingly, in both cases, animals were anaesthetised with urethane, as in the present study. Taken together, the fact that MPH has been reported to produce EEG patterns under anaesthesia that resemble an aroused state, and cortical arousal is associated with an enhancement of subcortical sensory responses, one likely conclusion is that MPH may be facilitating SC superficial layer visual responses via an action on the animal's arousal level under anaesthesia. As a consequence, we proceeded to investigate the possibility that cortical activation in the anaesthetised rat might underlie MPH's facilitatory effects in the SC.

Introduction (part 2)

In Study 1, MPH was found unexpectedly to have a facilitatory effect on visual responses in the superficial layers of the SC in the anaesthetised rat. In light of the evidence that MPH can produce EEG patterns under anaesthesia that resemble an aroused state (Solt et al., 2011; Chemali et al., 2012), and that cortical activation is associated with an enhancement of

some subcortical sensory responses (Kanosue et al., 1986; Castro-Alamancos & Oldford, 2002), we proceeded to investigate the possibility that changes in cortical arousal in the anaesthetised rat might underlie MPH's facilitatory effects on sensory processing in the SC. We addressed this in three ways: 1. By investigating the impact of increasing cortical activation in the anaesthetised rat on superficial layer visual responses in the SC; 2. By confirming that MPH does indeed induce cortical activation (in our hands); 3. By examining whether prior cortical activation blocks or attenuates the facilitatory effects of MPH on superficial layer visual responses in the SC.

In the studies below, general state changes of the cerebral cortex were manipulated by direct electrical stimulation of the brainstem reticular formation, at the level of the pons (Castro-Alamancos & Oldford, 2002; Castro-Alamancos, 2004). Such stimulation produces lower amplitude, fast activity in the cortex, which contrasts with the large-amplitude regular and slow activity typical of quiescent states (Moruzzi & Magoun, 1949), and also characteristic of the low-frequency, high-amplitude EEG of stage 3-III anesthesia (according to Kubicki [1968], scheme based on rat EEG, adapted from Guedel's [1920] work in humans), which is the typical cortical EEG state in rats under urethane anaesthesia (e.g. Clements et al., 2014). Electrical stimulation of the pons activates the reticular activating system, which in turn innervates the cortex via thalamic projections (Moruzzi & Magoun, 1949), producing an artificially induced high frequency, low amplitude EEG in an anaesthetised state.

Materials and Methods (part 2)

Study 2: Effects of methylphenidate on cortical activation and of cortical activation on visual responses in the superficial layers of the rat superior colliculus.

Subjects

Data were obtained from 24 male Hooded Lister rats (bred in house), weighing 226-530 g at the time of testing.

Preparation and recording

All surgical details were the same as in Study 1, except that a second craniotomy was performed (centred on 7.4 mm caudal to bregma; 4.5 mm lateral to midline) in some animals to allow access to the pontine reticular formation. In those animals, in addition to the tungsten electrode in the superficial layers of the SC, a concentric bipolar stimulating electrode (NEX-100, Rhodes Medical Instruments, Woodland Hills, CA) was inserted into the pons (7.4 mm caudal to bregma; 4.5 mm lateral to midline). The electrode penetration began at a DV of 6.0 mm and was advanced until stimulation (every 2 min; 2 s of 3 ms pulses delivered at 200 Hz, 100-200 µA) produced the strongest activation at the chosen anterior-posterior and mediolateral position. In all animals, an EEG electrode (2 mm diameter silver solder) was inserted through a drill hole to rest against the cortex, 1 mm anterior to bregma and 2 mm lateral to midline.

In the first set of animals (N=8), we examined the effect of cortical activation (via pontine stimulation) on visual responses (LFP and MUA) in the superficial layers of the SC. Once the electrode was correctly positioned, the animal was left to dark adapt for at least 25 min. Following dark adaptation, when the LFP response was stable, baseline EEG was recorded for 2 min, and then LFP and MUA responses were recorded to 150 light flashes at 5 intensities in ascending order (2.46, 8.69, 22.61, 56.36, 147.49 LUX) in the absence and then in the presence of pontine stimulation (every 2 min; 2 s of 3 ms pulses delivered at 200 Hz, 100-200 µA; EEG was recorded throughout).

After determining the effect of cortical activation on visual responses in the superficial layers of the SC, these animals and an additional 4 were used to explore the effect of MPH on visual responses in the superficial layers of the SC in the presence of cortical activation. Local field potential and MUA responses to successive series of 150 light flashes at a range of intensities were assessed in the presence of pontine stimulation (every 2 min; 2 s of 3 ms pulses delivered at 200 Hz, 100-200 µA; EEG was recorded throughout), after which an intensity was chosen which gave a response at approximately half maximum. A final baseline (pre-drug) set of responses were recorded to 150 light flashes at this intensity. Delivered at a rate of 0.5 Hz, the baseline recording session lasted for 5 min.

Baseline data acquisition was immediately followed by an intravenous injection of either MPH (0.4 mg/kg, in 0.1 ml/kg 0.9% saline; N=6), or saline (0.1 ml/kg, N=6) and the acquisition of a subsequent set of responses to 210 light flashes. This process was repeated four more times, with rising cumulative doses of MPH (0.8, 1.6, 3.2 and 6.4 mg/kg for the 2nd, 3rd, 4th and 5th injection respectively), or volumetrically equivalent saline injections.

In a second set of animals (N=18), we examined the effect of MPH on cortical EEG measures. EEG activity was amplified (gain 1000) and band-pass filtered (0.1-300 Hz; sampling rate 526 Hz). Baseline data acquisition (300 s) was immediately followed by an intravenous injection of either MPH (0.4 mg/kg, in 0.1 ml/kg 0.9% saline; MPH group: N=9), or saline (0.1 ml/kg; Saline group: N=9) and the acquisition of a subsequent a further 7 min of recording. This process was repeated three more times, with rising cumulative doses of MPH (0.8, 1.6, and 3.2 for the 2nd, 3rd and 4th injection respectively), or volumetrically equivalent saline injections.

Data analysis

Local field potentials and multiunit activity were analysed as in Study 1, with statistics this time including paired score and independent group t-tests. Spectral analysis was carried out using Spike 2. Mean spectral power for frequencies 0-32 Hz was identified for the different stimulus intensities/drug dosages as appropriate. To account for potential changes in overall power across injections, the power spectrum (0-32 Hz) was divided into two broad frequency bands, combining delta/theta (0-8 Hz), and alpha/beta (8-32 Hz) bands. Relative power of each frequency band (i.e. the power of a frequency band divided by the sum of all powers) was then identified for the different stimulus intensities/drug dosages. Values were normalised with respect to baseline for each cumulative dose to account for inter-subject variability in EEG activity. For the brainstem stimulation condition, the relative power of each frequency band pre- and post-stimulation was identified.

Histology

Site reconstruction: At the end of the experiment, brains were processed as in Study 1. As before, sections were mounted on gelled slides, stained with cresyl violet and recording sites were reconstructed with the aid of a stereotaxic atlas (Paxinos & Watson, 1997).

Results (part 2)

Direct electrical stimulation of the reticular formation produced the characteristic index of activation in the cortical EEG (Moruzzi & Magoun, 1949) by changing ongoing cortical activity from high-amplitude slow waves to lower amplitude, higher frequency signals (Figure 4). Stimulation produced a significant increase in the dominant frequency (from 0.77 ± 0.22 Hz to 1.59 ± 0.37 Hz, measured during the presentation of the lowest intensity light stimulation, $t[7]=2.4$, $p<0.05$). As light intensity increased, in the absence of reticular

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stimulation, onset and peak latency of the MUA and LFP visual responses increased, as did the amplitude and duration of the MUA response and the peak to peak amplitude of the LFP response (Figure 5). Reticular stimulation produced an enhancement of visual responses recorded in the superficial layers of the SC (Figures 4 and 5). Stimulation produced a significant increase in the amplitude ($F(1,14)=13.60$, $p<0.005$) and duration ($F(1,14)=8.97$, $p<0.05$) of visual stimulus-related MUA, although onset latency and peak latency were unaffected. Likewise, pontine stimulation produced a significant enhancement of the peak to peak amplitude of the visual LFP response ($F(1,14)=7.53$, $p<0.05$), an effect that was intensity dependent (factors STIM x INTENSITY, $F(2.57,36.03)=3.90$, $p<0.05$). The onset latency of the LFP response was also increased by the stimulation ($F(1,14)=7.28$, $p<0.05$), especially at lower intensities (factors STIM x INTENSITY, $F(4,56)=5.19$, $p=0.001$; Figure 5). Peak latency of the LFP response was unaffected.

Systemic MPH increased the higher frequency components of the EEG, as assessed by the relative power in the alpha/beta EEG bands (Figure 6). Animals administered MPH exhibited higher alpha/beta power across doses in comparison to saline treated animals, and this approached significance ($F(1,16)=4.03$, $p=0.06$), although there was no evidence of dose dependency as the interaction between (factors DOSE x CONDITION, $F(1.8,28.3)=0.50$, $p>0.5$). Although animals administered MPH exhibited higher alpha/beta power across doses in comparison to saline treated animals, substantial power (mean 83.9 +/− 4.3%) still remained in the delta/theta range in these animals at the highest dose of the drug.

In the presence of reticular stimulation, MPH slightly increased MUA peak latency and amplitude, and also LFP peak to peak amplitude (Figure 7). However, none of these effects were now statistically significant. Reticular stimulation also largely abolished the facilitatory effect of MPH on MUA duration (compare Figures 3 and 7). Importantly, comparing the

differences between saline and MPH MUA amplitude in the absence (Study 1; Lo light group) and presence of reticular stimulation (Study 2), the MPH-induced enhancement was significantly smaller in the presence of pontine stimulation ($t[6]=3.99$, $p<0.01$). The LFP peak to peak amplitude change was not significantly different however.

Overall discussion

Taken together, Study 1 and Study 2 sought to examine the effects of systemically administered MPH on visual responses in the superficial layers of the rat SC, and to begin to understand the means by which those effects were achieved. Across both studies, consistent with earlier work (e.g. Dyer & Annau, 1977), LFP responses in the superficial layers of the SC to wholefield light flashes were complex, multi-component phenomena. In the vast majority of cases, based on histological and electrophysiological criteria, electrode placements were localised to the SGS (or SZ).

The major effect of MPH in Study 1 was to increase the amplitude of visually evoked activity in the superficial layers of the SC, as measured by both LFPs and MUA, as well as MUA response duration. Given that LFPs represent (in large part) the sum of post-synaptic potentials (Mitzdorf, 1987), the results suggest that MPH enhances both the input to (synaptic activity) and output from (spike activity) sensory processing circuitry in the superficial layers. Although MPH did indeed facilitate visual responses in the superficial layers of the rat SC, its effects were somewhat different to simply altering the (apparent) brightness of the stimulus. As Figure 5 shows (control condition), brightening the light increased onset latency and peak latency (MUA and LFP), whereas the drug decreased onset and peak latency (MUA: Hi and Lo intensity groups; LFP: Hi intensity group). Both MPH and brightness did increase the amplitude of the visual responses (except for the LFP in the

Hi intensity group), although unlike brightness, the drug had little effect on response duration.

The facilitatory effects of MPH on visual responses in the superficial layers of the rat SC in Study 1 were somewhat unexpected, and were very different to those with d-amphetamine, which dose-dependently decreases the responsiveness of cells in the superficial layers of the SC to visual stimuli, measured both by LFPs and MUA (Gowan et al., 2008: Clements et al., 2014). In light of the evidence that MPH can produce EEG patterns under anaesthesia that resemble an aroused state (Solt et al., 2011; Chemali et al., 2012), and that cortical activation is associated with an enhancement of some subcortical sensory responses (Kanosue et al., 1986; Castro-Alamancos & Oldford, 2002), in Study 2 we investigated the possibility that changes in cortical arousal in the anaesthetised rat might underlie MPH's facilitatory effects on sensory processing in the SC.

We firstly demonstrated that increasing cortical activation in the anaesthetised rat enhances visual responses in the superficial layers of the SC. This is consistent with the demonstration that cortical arousal enhances sensory responses in the rat thalamus, for example responses in the ventrobasal thalamus to stimulation of the medial lemniscus (Kanosue et al., 1986), and in the ventro-posterior medial thalamus to whisker stimulation (Castro-Alamancos & Oldford (2002). In common with these studies, the enhancement in the present study was seen against a background of urethane anaesthesia. In our hands, reticular stimulation produced lower amplitude activity in the cortex, which contrasts with the large-amplitude regular and slow activity typical of stage 3-III anaesthesia (Kubicki, 1968), which is the typical cortical EEG state in rats under urethane anaesthesia (e.g. Clements et al., 2014). We also demonstrated that in our hands MPH administration moved cortical activity from high-amplitude slow waves towards lower amplitude, higher frequency signals characteristic of

arousal. In the presence of reticular stimulation-induced cortical activation, the drug no longer produced a statistically significant facilitation of superficial layer visual responses. The latter finding is supportive of the contention that drug-induced cortical activation plays a key role in the facilitatory effects of MPH, although it must be emphasised that our evidence for this is still rather circumstantial. It also has to be acknowledged that although cortical activation enhances visual responses in the superficial layers of the SC, MPH also affects both the onset and peak latencies of the responses, so the drug's effects in the SC go beyond the changes induced by cortical activation.

Reticular stimulation attenuated the facilitatory effects of MPH on visual responses in the superficial layers of the rat SC, but it did not unmask a depressive action of the drug – i.e. an action more in keeping with the depressive action of d-amphetamine of superficial layer visual responses. Or, indeed, one more in keeping with the depressive effect of MPH on superficial layer visual responses in the awake rat (Hetzler et al., 2014). The most likely explanation is that the level of cortical activation produced by MPH and reticular stimulation was incomplete. Although the relative power in the alpha/beta band was increased by the drug and stimulation, substantial power still remained at the lower frequencies in both cases. Were it possible to fully desynchronise the cortex in keeping with the alert, awake state (Moruzzi & Magoun, 1949), a depressive effect of MPH may well have been seen. Likewise, MPH might be expected to demonstrate depressive properties if injected directly into the colliculus, as this route of administration should circumvent the neural systems with which the drug interacts to produce cortical activation when given systemically.

The identity of those neural systems is at present unknown, although as we mentioned earlier, evidence for MPH-induced activation of excitatory pedunculopontine tegmental nucleus/lateral dorsal tegmental nucleus is lacking, although there is evidence of thalamic

activation (Marquand et al., 2012). Indeed, MPH activates many areas of the brain, although perhaps unsurprisingly various aspects of the basal ganglia form the major focus of drug-induced activity (Trinh et al., 2003). Hence it is possible that MPH produces cortical activation via several routes including direct thalamic activation and through an interaction with the well characterised cortical-basal ganglia re-entrant loops (e.g. Alexander et al., 1986). We then consider this cortical activation to have a direct excitatory impact on the colliculus via cortico-tectal afferents (see earlier). These effects are captured in diagram-matic form in Figure 8.

Finally, it has to be acknowledged that – like MPH - amphetamine has also been shown to desynchronise the cortical EEG (e.g. Bermudez Contreras et al., 2013), yet in the case of amphetamine, visual responses in the superficial layers are depressed by the drug. The reason for this may lie in the proximal effect of the two drugs. Whilst the proximal effects of both MPH and d-amphetamine are mediated by DA and NA (Easton et al., 2007; Kuczenski & Segal, 1997), d-amphetamine also has significant effects on 5-HT (Holmes & Rutledge, 1976) at higher doses (Kuczenski & Segal, 1989), whereas the effect of MPH on 5-HT is less clear Kuczenski & Segal, 1997; Kuczenski et al., 1987). Amphetamine's stronger effects on 5-HT mediated transmission, which is uniformly depressant in the SC (Kawai & Yamamoto, 1969; Straschill & Perwein, 1971), may be sufficient to combat and overcome any facilitatory effects stemming from cortical activation. From the point of view of the collicular hyper-responsiveness theory of distractibility in ADHD (see Introduction), amphetamine's ability to depress collicular sensory responses, as well as MPH's ability to do so in the awake preparation, continue to suggest that the SC may act as a primary therapeutic target in the disorder.

Conclusion

In contrast to d-amphetamine, systemic MPH produced an enhancement of visual activity in the superficial layers of the SC. Methylphenidate was also found to produce activation of the cortical EEG in anaesthetised rats. Furthermore, cortical activation induced by electrical stimulation of the pons was found enhance visual responses in superficial layers of the SC, and when MPH was paired with pontine-induced cortical activation, the response-enhancing effects of MPH were substantially attenuated. Taken together, the results suggest that the enhancement of visual responses in the superficial layers of the SC by MPH in the anaesthetised rat is an artefact of the drug's interaction with cortical arousal.

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Figures

Figure 1

Visual responses in the superficial layers if the superior colliculus to wholefield light flashes. Top: An example of an electrode track and recording site (asterisk) in the superficial layers. DL: Deep layers of the superior colliculus; MG: Medial geniculate; PAG: Periaqueductal gray; RSA: Retrosplenial agranular cortex; SGS/SO: Stratum grisium superficiale and stratum opticum (superficial layers of the superior colliculus); Bottom:

Modulation of visual responses in the superficial layers of the superior colliculus by methylphenidate in an animal exhibiting dose-dependent effects. Left: multiunit (MUA) responses and, right: local field potential (LFP) ~~in a representative animal~~. Data were averaged over blocks of 150 stimulations, corresponding to epochs of 5.0 min. Responses are shown at Baseline, and after 0.4 mg/kg (Dose 1), 0.8 mg/kg (Dose 2), 1.6 mg/kg (Dose 3), 3.2 mg/kg (Dose 4) and 6.4 mg/kg (Dose 5) of methylphenidate (i.v.). Stimulus onset was at time zero.

Figure 2

Effect of systemic methylphenidate ('MPH') or saline ('Saline') administration on visually evoked multiunit responses (left; MUA) and local field potentials (right; LFP) in the superficial layers of the superior colliculus in the high intensity light group. The Figure shows the normalised change (with respect to baseline) ± 1 SEM for the two groups for the following aspects of the evoked response: MUA: (A) Onset latency; (B) Peak latency; (C) Amplitude, (D) Duration; LFP: (A) Onset latency; (B) Peak latency; (C) Peak to peak amplitude. Data were averaged over blocks of 150 stimulations, corresponding to epochs of 5.0 min. Following a period of baseline data collection, animals received 5 injections of methylphenidate ([1] 0.4 mg/kg, [2] 0.8 mg/kg, [3] 1.6 mg/kg, [4] 3.2 mg/kg and [5] 6.4 mg/kg cumulative; iv) or volumetrically equivalent injections of saline.

Figure 3

Effect of systemic methylphenidate ('MPH') or saline ('Saline') administration on visually evoked multiunit responses (left; MUA) and local field potentials (right; LFP) in the superficial layers of the superior colliculus in the low intensity light group. The Figure shows the normalised change (with respect to baseline) ± 1 SEM for the two groups for the following aspects of the evoked response: MUA: (A) Onset latency; (B) Peak latency; (C)

Amplitude, (D) Duration; LFP: (A) Onset latency; (B) Peak latency; (C) Peak to peak amplitude. Data were averaged over blocks of 150 stimulations, corresponding to epochs of 5.0 min. Following a period of baseline data collection, animals received 5 injections of methylphenidate ([1] 0.4 mg/kg, [2] 0.8 mg/kg, [3] 1.6 mg/kg, [4] 3.2 mg/kg and [5] 6.4 mg/kg cumulative; iv) or volumetrically equivalent injections of saline.

Figure 4

Effect of electrical stimulation of the pons on visually evoked responses in the superficial layers of the superior colliculus in a representative animal. The left hand side shows two sample cortical EEG traces, the upper one in the absence of reticular stimulation and the lower one in the presence of reticular stimulation (showing the reduced amplitude and increased dominant frequency in the latter). Each EEG trace is accompanied to the right by peri-stimulus time interval histograms of multiunit activity (MUA) in response to 150 light flashes (147.49 LUX, 10 ms). Stimulus onset was at time zero (indicated by a vertical bar).

Figure 5

Effect of electrical stimulation of the pons on visually evoked multiunit responses (left, MUA) and local field potentials (right; LFP) in the superficial layers of the superior colliculus. Responses to 5 light intensities ([1] 2.46 LUX, [2] 8.69 LUX, [3] 22.61 LUX, [4] 56.36 LUX, [5] 147.49 LUX) were recorded in the absence and then in the presence of pontine stimulation. The Figure shows the normalised change (with respect to baseline) ± 1 SEM for the two conditions for the following aspects of the evoked response: MUA: (A) Onset latency; (B) Peak latency; (C) Amplitude, (D) Duration; LFP: (A) Onset latency; (B) Peak latency; (C) Peak to peak amplitude. Data were averaged over blocks of 150 stimulations, corresponding to epochs of 5.0 min.

Figure 6

Effect of methylphenidate on cortical EEG. Animals received 4 injections of methyl-phenidate (MPH; [1] 0.4 mg/kg, [2] 0.8 mg/kg, [3] 1.6 mg/kg, and [4] 3.2 mg/kg cumulative; iv) or volumetrically equivalent injections of saline. The Figure shows the relative power (i.e. the power of the frequency band divided by the sum of all powers) in the alpha and beta bands (combined; 8-32 Hz) for animals administered saline or methylphenidate.
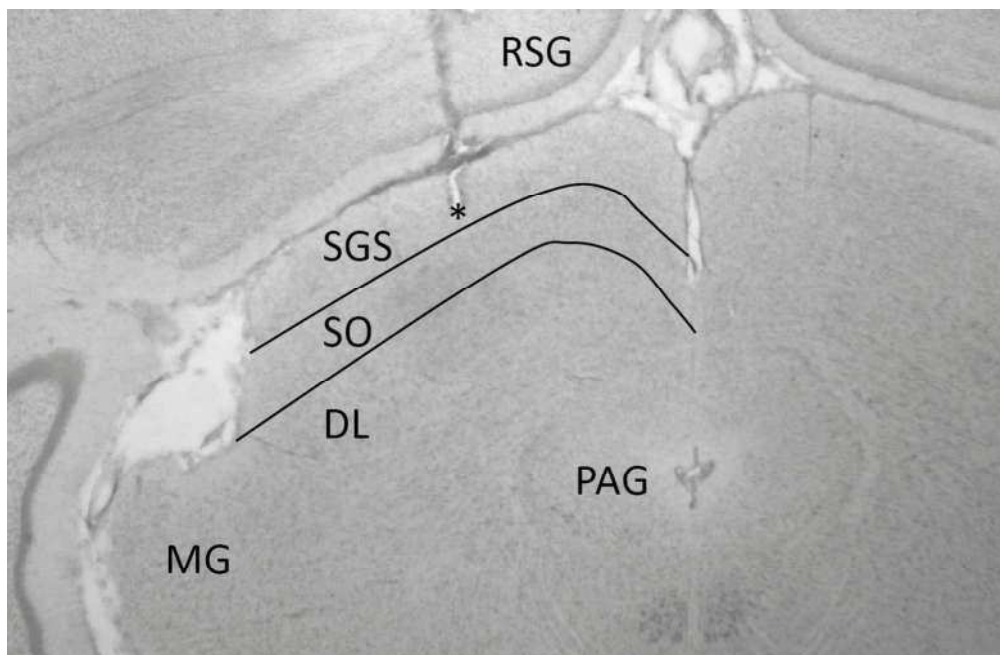
Figure 7

Effect of electrical stimulation of the pons on methylphenidate-induced modulation of visually evoked multiunit responses (left, MUA) and local field potentials (right; LFP) in the superficial layers of the superior colliculus. The Figure shows the normalised change (with respect to baseline) $\pm$ 1 SEM for animals administered methylphenidate (MPH) or saline in the presence of reticular stimulation (every 2 min; 2 s of 3 ms pulses delivered at 200 Hz, 100-200 μA) for the following aspects of the evoked response: MUA: (A) Onset latency; (B) Peak latency; (C) Amplitude, (D) Duration; LFP: (A) Onset latency; (B) Peak latency; (C) Peak to peak amplitude. Data were averaged over blocks of 150 stimulations, corresponding to epochs of 5.0 min. Following a period of baseline data collection, animals received 5 injections of methylphenidate ([1] 0.4 mg/kg, [2] 0.8 mg/kg, [3] 1.6 mg/kg, [4] 3.2 mg/kg and [5] 6.4 mg/kg cumulative; iv) or volumetrically equivalent injections of saline.
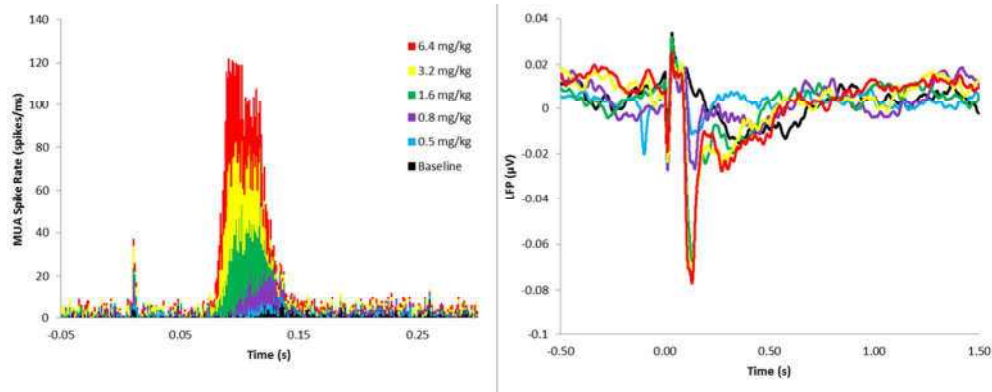
Figure 8

Summary scheme for the proposed interaction between methylphenidate (MPH) and visual processing in the superior colliculus (SC). (A) In the absence of MPH, visual stimuli activate cells in the superficial layers of the SC in the anaesthetised rat. However, (B) MPH activates elements in the thalamus directly or via an interaction with the basal ganglia, which

in turn activates the cortex, excitation from which is fed directly to the SC via cortico-tectal afferents, resulting in an enhanced response to visual stimuli.

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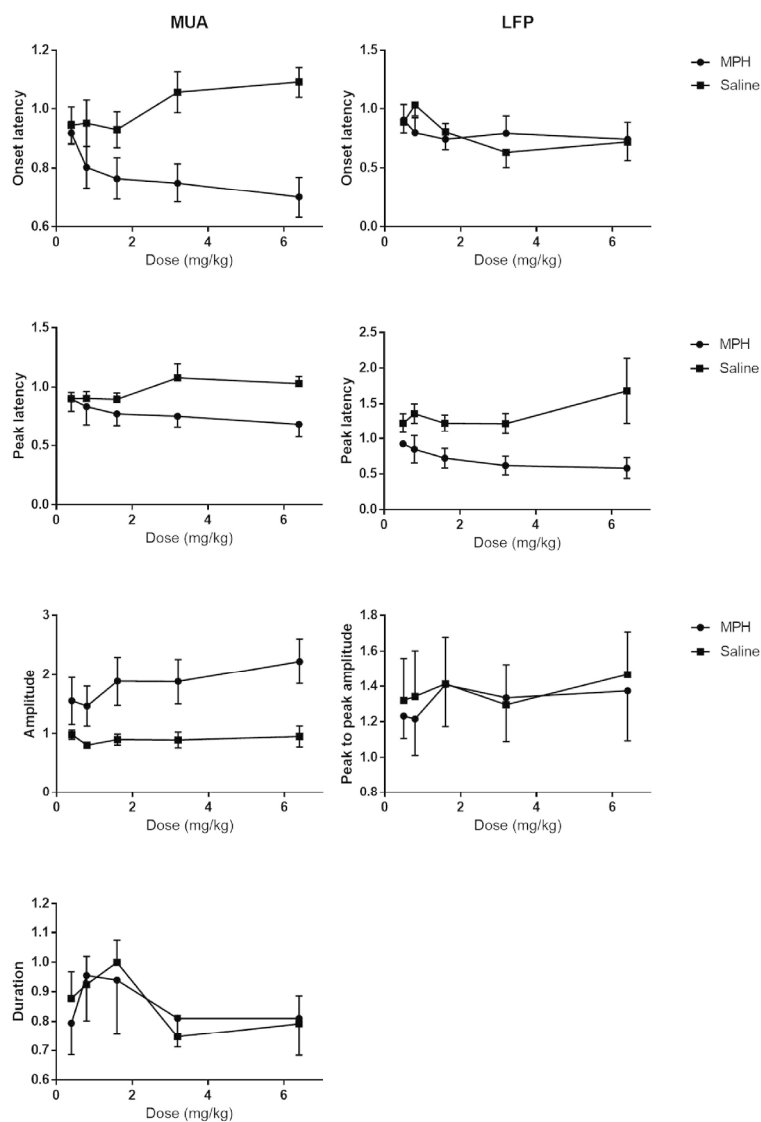41
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Visual responses in the superficial layers if the superior colliculus to wholefield light flashes. Top: An example of an electrode track and recording site (asterisk) in the superficial layers. DL: Deep layers of the superior colliculus; MG: Medial geniculate; PAG: Periaqueductal gray; RSA: Retrosplenial agranular cortex; SGS/SO: Stratum grisium superficiale and stratum opticum (superficial layers of the superior colliculus)....

Bottom: Modulation of visual responses in the superficial layers of the superior colliculus by methylphenidate in an animal exhibiting dose-dependent effects. Left: multiunit (MUA) responses and, right: local field potential (LFP) in a representative animal. Data were averaged over blocks of 150 stimulations, corresponding to epochs of 5.0 min. Responses are shown at Baseline, and after 0.4 mg/kg (Dose 1), 0.8 mg/kg (Dose 2), 1.6 mg/kg (Dose 3), 3.2 mg/kg (Dose 4) and 6.4 mg/kg (Dose 5) of methylphenidate (i.v.). Stimulus onset was at time zero.

Figure 2
Effect of systemic methylphenidate ('MPH') or saline ('Saline') administration on visually evoked multiunit responses (left; MUA) and local field potentials (right; LFP) in the superficial layers of the superior colliculus in the high intensity light group. The Figure shows the normalised change (with respect to baseline) ± 1 SEM for the two groups for the following aspects of the evoked response: MUA: (A) Onset latency; (B) Peak latency; (C) Amplitude, (D) Duration; LFP: (A) Onset latency; (B) Peak latency; (C) Peak to peak amplitude. Data were averaged over blocks of 150 stimulations, corresponding to epochs of 5.0 min. Following a period of baseline data collection, animals received 5 injections of methylphenidate ([1] 0.4 mg/kg, [2] 0.8 mg/kg, [3] 1.6 mg/kg, [4] 3.2 mg/kg and [5] 6.4 mg/kg cumulative; iv) or volumetrically equivalent injections of saline.

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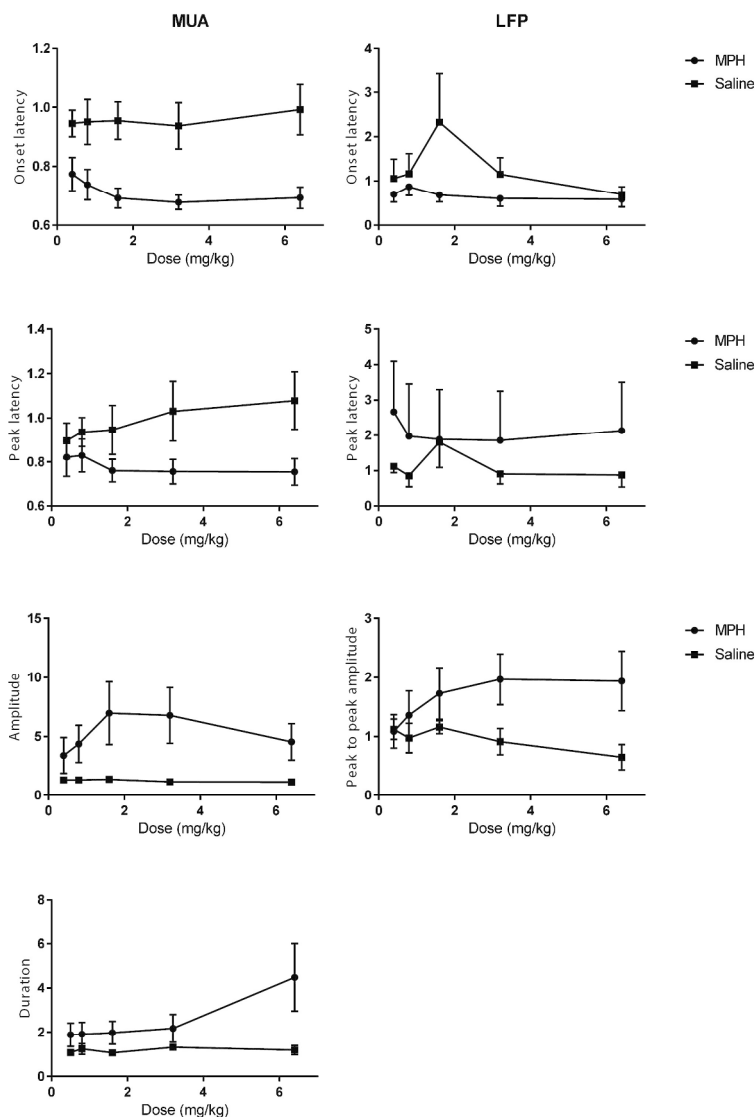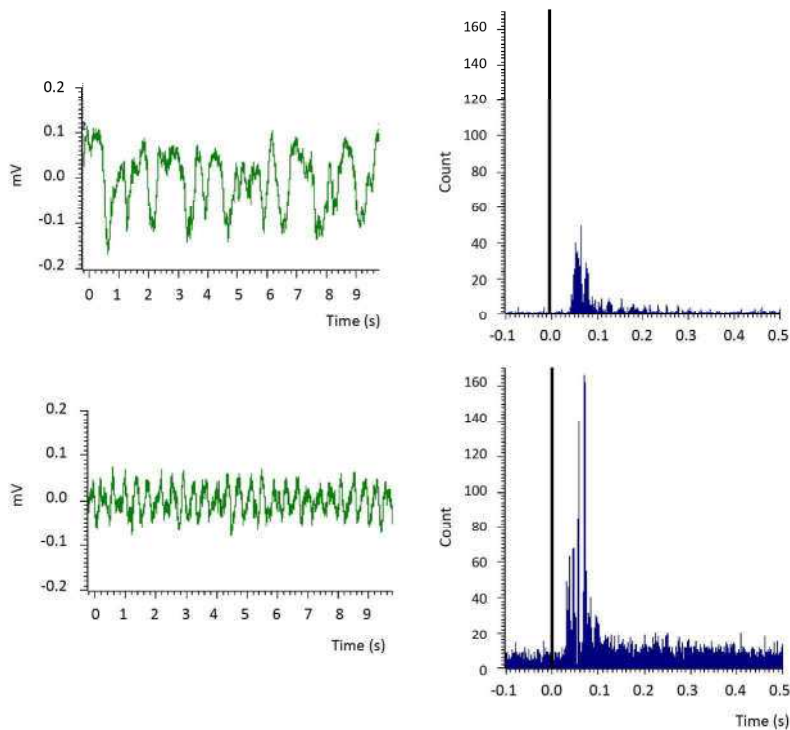48
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Figure 3
Effect of systemic methylphenidate ('MPH') or saline ('Saline') administration on visually evoked multiunit responses (left; MUA) and local field potentials (right; LFP) in the superficial layers of the superior colliculus in the low intensity light group. The Figure shows the normalised change (with respect to baseline) ± 1 SEM for the two groups for the following aspects of the evoked response: MUA: (A) Onset latency; (B) Peak latency; (C) Amplitude, (D) Duration; LFP: (A) Onset latency; (B) Peak latency; (C) Peak to peak amplitude. Data were averaged over blocks of 150 stimulations, corresponding to epochs of 5.0 min. Following a period of baseline data collection, animals received 5 injections of methylphenidate ([1] 0.4 mg/kg, [2] 0.8 mg/kg, [3] 1.6 mg/kg, [4] 3.2 mg/kg and [5] 6.4 mg/kg cumulative; iv) or volumetrically equivalent injections of saline.
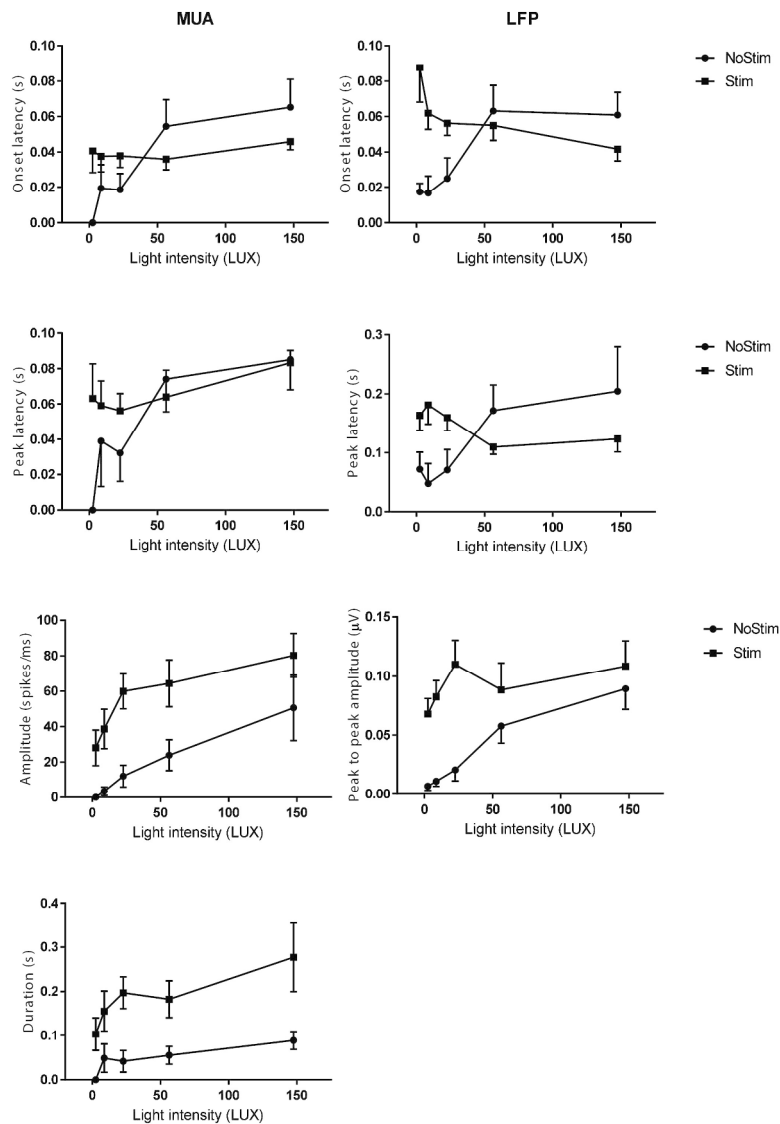
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Effect of electrical stimulation of the pons on visually evoked responses in the superficial layers of the superior colliculus in a representative animal. The left hand side shows two sample cortical EEG traces, the upper one in the absence of reticular stimulation and the lower one in the presence of reticular stimulation (showing the reduced amplitude and increased dominant frequency in the latter). Each EEG trace is accompanied to the right by peri-stimulus time interval histograms of multiunit activity (MUA) in response to 150 light flashes (147.49 LUX, 10 ms). Stimulus onset was at time zero (indicated by a vertical bar).

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Effect of electrical stimulation of the pons on visually evoked multiunit responses (left, MUA) and local field potentials (right; LFP) in the superficial layers of the superior colliculus. Responses to 5 light intensities ([1] 2.46 LUX, [2] 8.69 LUX, [3] 22.61 LUX, [4] 56.36 LUX, [5] 147.49 LUX) were recorded in the absence and then in the presence of pontine stimulation. The Figure shows the normalised change (with respect to baseline) ± 1 SEM for the two conditions for the following aspects of the evoked response: MUA: (A) Onset latency; (B) Peak latency; (C) Amplitude, (D) Duration; LFP: (A) Onset latency; (B) Peak latency; (C) Peak to peak amplitude. Data were averaged over blocks of 150 stimulations, corresponding to epochs of 5.0 min.
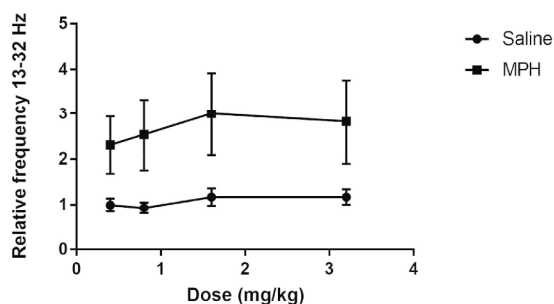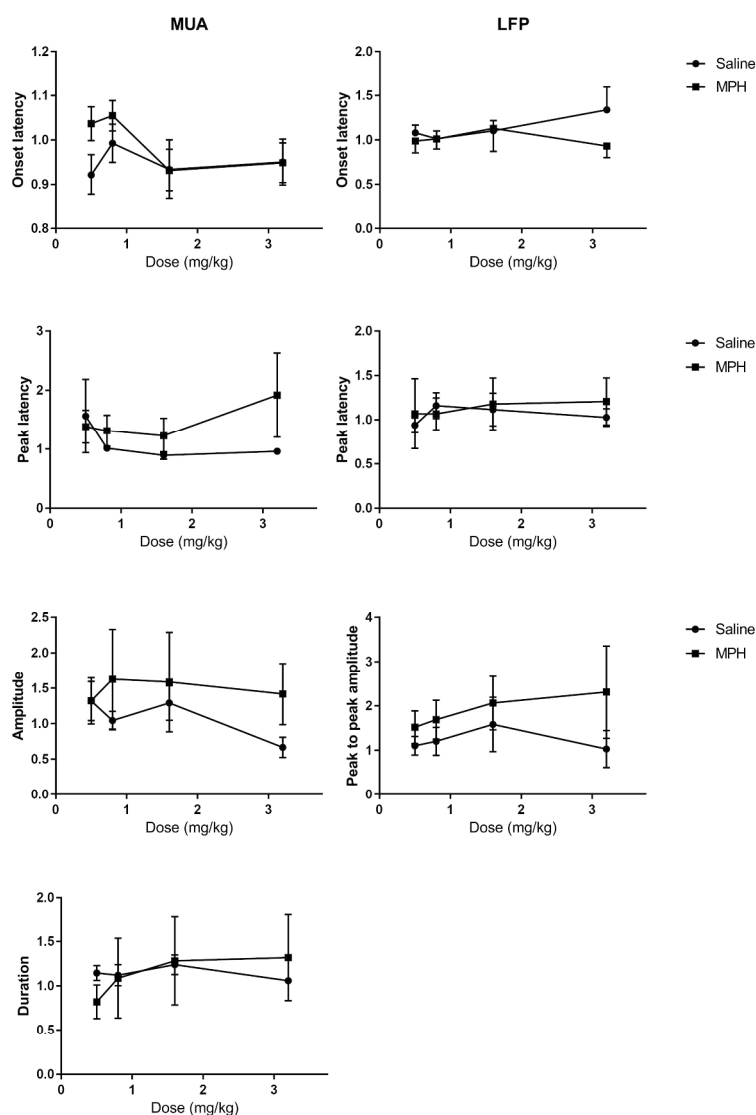
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Figure 6
Effect of methylphenidate on cortical EEG. Animals received 4 injections of methyl-phenidate (MPH; [1] 0.4 mg/kg, [2] 0.8 mg/kg, [3] 1.6 mg/kg, and [4] 3.2 mg/kg cumulative; iv) or volumetrically equivalent injections of saline. The Figure shows the relative power (i.e. the power of the frequency band divided by the sum of all powers) in the alpha and beta bands (combined; 8-32 Hz) for animals administered saline or methylphenidate.
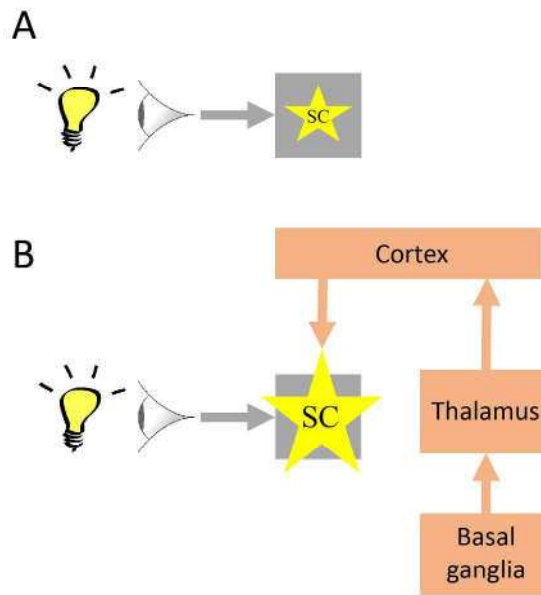
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Effect of electrical stimulation of the pons on methylphenidate-induced modulation of visually evoked multiunit responses (left, MUA) and local field potentials (right; LFP) in the superficial layers of the superior colliculus. The Figure shows the normalised change (with respect to baseline) ± 1 SEM for animals administered methylphenidate (MPH) or saline in the presence of reticular stimulation (every 2 min; 2 s of 3 ms pulses delivered at 200 Hz, 100-200 μA) for the following aspects of the evoked response: MUA: (A) Onset latency; (B) Peak latency; (C) Amplitude, (D) Duration; LFP: (A) Onset latency; (B) Peak latency; (C) Peak to peak amplitude. Data were averaged over blocks of 150 stimulations, corresponding to epochs of 5.0 min. Following a period of baseline data collection, animals received 5 injections of methylphenidate ([1] 0.4 mg/kg, [2] 0.8 mg/kg, [3] 1.6 mg/kg, [4] 3.2 mg/kg and [5] 6.4 mg/kg cumulative; iv) or volumetrically equivalent injections of saline.

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Summary scheme for the proposed interaction between methylphenidate (MPH) and visual processing in the superior colliculus (SC). (A) In the absence of MPH, visual stimuli activate cells in the superficial layers of the SC in the anaesthetised rat. However, (B) MPH activates elements in the thalamus directly or via an interaction with the basal ganglia, which in turn activates the cortex, excitation from which is fed directly to the SC via cortico-tectal afferents, resulting in an enhanced response to visual stimuli.

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