Title page

Noradrenergic administration improves cognitive flexibility even after glutamatergic damage in rat mediodorsal thalamus or thalamic nucleus reuniens

Jennifer J. Hamilton[1], Lisa Berriman[1], Shae Harrison-Best[1], John C. Dalrymple-Alford[1,2,3], Anna S. Mitchell[1,2]

1. Te Kura Mahi ā-Hirikapo | School of Psychology, Speech and Hearing, University of Canterbury, Christchurch, New Zealand

2. New Zealand Brain Research Institute, Christchurch, New Zealand

3. Department of Medicine, University of Otago, Christchurch, New Zealand

Word count: 7079

Number of Figures = 5

Abstract

Cognitive flexibility, switching behaviour responses to changing task demands, is classically attributed to the prefrontal cortex. Yet thalamocortical circuits involving the mediodorsal thalamus (MD) and thalamic nucleus reuniens (Re) are dysfunctional across a range of neurological conditions with cognitive flexibility deficits. Interventions involving thalamocortical interactions may offer therapeutic benefits. Here we examined the effects of MD or Re bilateral glutamatergic neurotoxic damage in rats on cognitive flexibility using the attentional set-shifting task. Rats must attend to a sensory dimension that reliably predicts reward (intradimensional shift, ID) followed by a shift in attention to a previously irrelevant sensory dimension when contingencies change (extradimensional shift, ED). We found MD rats required more trials to criterion in the ED, while Re rats showed significant impairments on the first of three ID subtasks (ID1) only. Both MD and Re rats required more trials to criterion to complete each subtask than Sham controls. Intraperitoneal noradrenaline (atipamezole 1mg/kg), given 30 minutes prior to the task reduced trials to criterion across all rats, improving cognitive flexibility even after thalamic damage. These findings demonstrate the influence MD and Re contribute to cognitive flexibility and support noradrenergic regulation of thalamocortical circuits as potential therapeutic targets for cognitive flexibility dysfunction.

Introduction

Both the mediodorsal thalamus (MD) and thalamic nucleus reuniens (Re) support cognitive flexibility, through different aspects of attention, learning, memory, and decision-making. In humans and animal models, the MD has been identified as essential for cognitive flexibility – the ability to keep track of current task demands and switch thoughts and responses rapidly when encountering changes in these demands. These MD functions reflect its reciprocal connectivity with the prefrontal cortex (PFC) and temporal lobes [1-10]. The Re has reciprocal connections with the medial PFC and ventral hippocampus and is also identified as influencing learning when cognitive flexibility is required [11-15]. Deficits in cognitive flexibility are hallmark symptoms in many neurological and neuropsychiatric conditions including strokes, dementia and Parkinson's disease, as well as anxiety disorders, major depression, obsessive compulsive disorder, and schizophrenia [3,7,8,16,17].

The attentional set-shifting task [18] provides an excellent way to compare the effects of MD and Re dysfunction on cognitive flexibility in rodents. Analogous attentional set-shifting tasks are used in monkeys [19-22] and humans [23-25]. In rats, the attentional set-shifting task first examines the ability to learn and maintain responding to one sensory dimension (either odour or touch using different digging media) across new stimulus pairings of odours (or digging media) with one of the pair reliably predicting reward, and thereby demonstrate the ability to show an intradimensional (ID) shift. The rat is then required to shift attention to the previously irrelevant sensory dimension (e.g., now digging media) for rewards, thereby making an extradimensional (ED) shift. Rats with damage to the medial PFC have deficits in the ED shift [18,26]. Monkeys and humans with PFC damage also have deficits in the ED shift, while rats with anterior thalamic damage or anterior cingulate cortex chemogenetic manipulations have deficits in ID shifts [27,28]. Thalamocortical interactions are also crucial for cognitive flexibility in the rat because permanent neuronal damage in the

MD that disrupts thalamocortical interactions causes ED shift deficits [5], while damage to the Re causes deficits in learning the ID shift [11].

Interestingly, noradrenergic (NA) efferents from the locus coeruleus innervate the MD, Re, in addition to pathways coursing to the medial PFC and hippocampus [29]. This NA innervation is dysfunctional in various neurological conditions, e.g., Parkinson's and Alzheimer's Disease and schizophrenia [30,31]. Administering NA agonists in humans and animal models can improve cognition including cognitive flexibility [30, 31]. In rats, improved cognitive flexibility was demonstrated after permanent damage to the medial PFC and treatment with intraperitoneal (i.p) injections of the NA agonist atipamezole (e.g., [26] while manipulations to the locus coeruleus or deafferentation of noradrenergic pathways in the medial PFC impairs cognitive flexibility (e.g., [32-34]. However, we do not know if deficits in cognitive flexibility after permanent damage in either MD or Re thalamic nuclei can be restored by administration of NA agonists. In neuropsychiatric disorders and most neurodegenerative conditions during the early to mid-phases, the PFC, MD, and Re are all dysfunctional, so understanding the benefits of NA agonists after thalamic dysfunction provides therapeutic insights for NA treatments to recover cognitive flexibility.

Here, we tested rats with permanent glutamatergic neurotoxic damage in MD or Re thalamic nuclei to identify cognitive flexibility deficits in the attentional set-shifting task (IDED). Given Re damage previously caused deficits in the ID shift [11], we assessed performance in three consecutive ID shifts to determine if Re supports learning and implementation of the attentional learning set strategy [13,35]. We found dissociable deficits with Re damage affecting the first ID shift only, and MD damage affecting the ED shift. Next, the rats were re-run on the IDED task with novel stimulus pairings for the two sensory dimensions and received intraperitoneal (i.p.) injections of a NA agonist, atipamezole, 30

minutes prior to testing. We found atipamezole improved cognitive flexibility in all rats including those with permanent MD or Re damage.

Materials and Methods

Animals

Twenty-four male Piebald Virol Glaxo cArc (PVGc) rats weighing between 315-415g at time of surgery were housed four per cage in a temperature (21±2°C) and humidity (50±5%) controlled holding room. Lights were on a reverse 12-hour light-dark cycle (lights on 8pm), with all behavioural testing completed in the dark (i.e., more active) cycle for the rats. The rats were on a controlled feeding schedule (between 12-18g/rat per day) to maintain 85% of their free-feeding body weight with *ad libitum* access to water in their home cage. Weekly cage cleaning occurred after the rat's behavioural training and testing for that given day. The University of Canterbury Animal Ethics Committee approved all housing and experimental procedures (2022/14R).

Neurosurgery to create permanent MD or Re thalamic damage

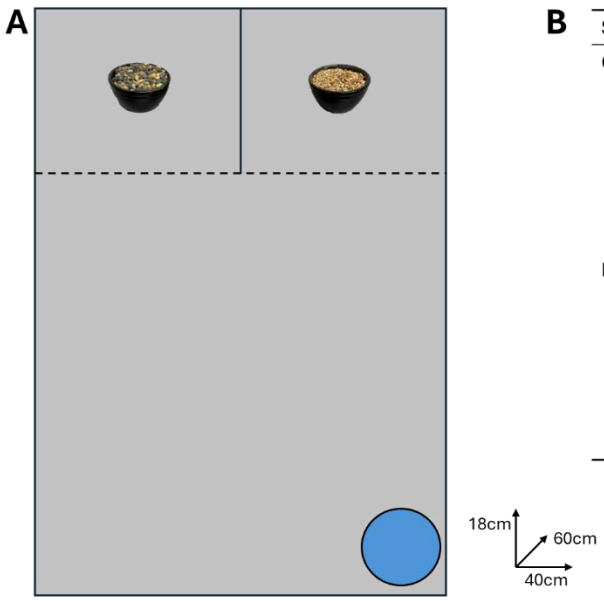
Rats were pseudorandom assigned to one of three groups (N= 8 MD; N = 8 Re; N=8 Sham). Rats were anaesthetised using isoflurane (4% induction; 2-2.5% maintenance), the shaved head was cleaned with Hibitane using aseptic technique and secured on a stereotaxic frame (Kopf, Tujunga, CA). The incisor bar was set to -5.5mm below interaural line to minimise syringe damage to the fornix. Methopt Forte eye drops (Aspen Pharm, NZ) were administered and a wet gauze pad applied above, but not touching the eyes to stop them drying out and minimise the impact of surgery lights. A midline incision was made, the skull exposed and stereotaxic coordinates made relative to bregma and lambda. To create the permanent damage in either the MD or Re, 0.12M N-methyl-d-aspartate (NMDA; Sigma, Castle Hill, NSW), a potent glutamate receptor agonist, was bilaterally infused with one

infusion in each hemisphere of the MD or the Re. For MD infusions, to ensure greater AP spread, one infusion was made more anteriorly in one hemisphere at AP -3.75mm from Bregma, and more posteriorly in the contralateral hemisphere, at AP -3.95mm from Bregma. Both MD infusions were made ±0.7mm from midline, at a depth of -5.70mm below dura. The infusion volume was 0.20µl NMDA at each MD infusion site at a rate of 0.04µl/min. For Re infusions, bilateral infusions were made at a 10-degree angle -2.3mm from Bregma, ±1.1mm from midline, at a depth of -7.0mm below dura. The infusion volume was 0.12µl NMDA at each Re infusion site at a rate of 0.04µl/min. For each MD or Re infusion, the syringe needle remained in situ at the end of each infusion for 4mins diffusion time. Sham infusion control surgeries used the same surgical procedures and stereotaxic coordinates, but the syringe needle was lowered to 1mm above each of the MD or Re infusion sites and no infusion was administered.

IDED attentional set-shifting task

Apparatus

The apparatus (Figure 1; 40cm x 60cm x 18cm) was placed on a 70cm high table. It contained three compartments made from opaque black Perspex: a large start compartment (40cm x 36cm) and two identical choice compartments (20cm x 24cm) to which access was controlled by clear Perspex doors (20cm x 20cm). Black ceramic digging bowls (7cm diameter x 3.5cm high) with wire mesh epoxy glued ~1cm inside the bottom of the bowl (for hiding an unreachable reward, see *Procedures*) were placed inside each of the two choice compartments. The bowls were filled with clean bedding sawdust scented with odours (herbs or spices), digging media, or a combination of odours and digging media (examples in Fig. 1B).



B

Stimuli	Example pairings	
Odour	O1 – turmeric	O2 – clove
	O3 – nutmeg	O4 – cumin
	O5 – dill	O6 – coriander
	O7 – cinnamon	O8 – ginger
	O9 – thyme	O10 – paprika
	O11 – all spice	O12 - cardamom
Digging media	M1 – fine gravel	M2 – coarse gravel
	M3 – fine shavings	M4 – coarse shavings
	M5 – coarse tea	M6 – fine tea
	M7 – fine sand	M8 – coarse sand
	M9 – pebbles	M10 - vermiculite
	M11 – raffia	M12 – thread

Figure 1. **A.** Schematic of the ID/ED attentional set-shifting apparatus. Access to two identical choice compartments was controlled by clear Perspex doors (dotted line). Blue circle = small glass jar to provide ad libitum water throughout testing. The black ceramic digging bowls were filled with a combination of odours and digging media. **B.** Stimulus pairings used in the IDED test sessions.

Preoperative dig training

Before surgery, rats maintained at 85% of their free-feeding weight were taught to dig in bowls filled with sawdust to retrieve one half of a Honey Cheerio (Uncle Toby's, Australia). The Honey Cheerio was initially placed on the top of the sawdust and gradually lowered until it was completely hidden from view in subsequent trials. The Honey Cheerio was rebaited (fully concealed) in each bowl (both choice compartments baited) until the rat made 6 retrievals from each choice compartment. It took up to 3 days and 6 consistent digs in each bowl for all rats to complete dig training.

IDED Testing Procedures

The IDED attention set-shifting task is self-paced and relies on the natural foraging behaviours of the rats. During the experiment, one of the two ceramic digging bowls is baited with the reward (Honey Cheerio) while the other one is unbaited, and the rat learns which

bowl is baited using either the types of digging media or odours as cues to determine the reward. A hidden reward is placed underneath the wire mesh in each digging bowl to reduce odour cues between baited and non-baited bowls.

At the beginning of each trial, the rat is held in the larger compartment and the clear Perspex panels are removed to allow the rat access to both of the two smaller choice compartments (Fig. 1A; correct choice counterbalanced across right / left). If an incorrect choice is made, this is recorded as an error, and for the first four trials only of any subtask, the rat is allowed access to the correct choice compartment to dig in the bowl to receive the reward before being returned to the larger compartment area and the Perspex panels blocking entry until the start of the next trial (~10-20 second inter trial interval).

For this set of experiments, the IDED task was run twice (IDED1 and IDED2). IDED1 was divided into two sessions comprised of a simple discrimination (SD) training session for the first session and on the next day (see *Postoperative training on the simple discrimination*), the full IDED attentional set-shifting test session. The full IDED attentional set-shifting test session comprised of 8 different subtasks: a novel SD; concurrent discrimination (CD); concurrent discrimination reversal (CDR); ID1; ID2; ID3; ED; ED reversal. The correct (rewarded) odour or digging media discriminations and the correct stimulus within pairs (see Figure 1B for stimuli used in IDED1 and IDED2) were counterbalanced across the subtasks and matched between different experimental groups. IDED2 used the same stimuli as IDED1 but the pairings and correct (rewarded) stimulus were shuffled so all pairings were novel to counter any effect of retained learning about a particular stimulus cue between the first (IDED1) and second (IDED2) runs of task. Counterbalancing of the rewarded stimulus dimension was also conducted during the SD subtask so that rats who received odour as the rewarded sensory dimension in the IDED1 SD subtask now had digging medium as the rewarded sensory dimension in the IDED2 SD

subtask. For IDED2, rats only ran in the full IDED attentional set-shifting test session comprised of the 8 different subtasks, that is, there was no repeat of the initial SD training session prior to running the full test session because the rats already knew how to distinguish between different stimuli in the bowls for rewards linked to specific cues.

Postoperative training on the simple discrimination

Rats completed two runs of postoperative testing IDED1 and IDED2 after recovery from surgery. The day before rats completed the full IDED1 session, each rat learnt a simple discrimination (SD) beginning with either odour (all spice versus cardamom in clean bedding sawdust) or digging media (pebbles versus vermiculite) counterbalanced across rats and groups. Rewarded stimuli within any pair was counterbalanced across the groups and rats were trained to a criterion of 6 consecutive correct trials or a maximum of 30 trials per subtask. The stimuli used in the training session were not used again until IDED2. Digging was defined as active digging or foraging with the paws or nose in the digging media, but sniffing or resting paws on the digging media was not counted as an active dig. This digging criterion was used throughout testing on the IDED attention set shifting task.

Postoperative testing on IDED attention set-shifting task

On the next day, the rat was given a series of eight subtasks, each subtask consisted of a series of two-choice trials with only one of the two-choice bowls baited, counterbalanced across side. The rat must learn each new two-choice discrimination to a performance criterion of 6 correct consecutive responses before moving onto the next subtask. The first subtask was a simple discrimination (SD), which is run per the training day but using a new pairing of either odour or digging media, and these pairings were counterbalanced across groups. If a rat was assigned the odour, they would run the odour-to-media trials for IDED1 and media-to-odour for IDED2. For example, in odour-to-media order of discriminations rats were initially

required to attend to the odour sensory dimension, then switched to digging media in the final two subtasks, ED and EDrev.

Following the SD subtask was the compound discrimination (CD) subtask where the same stimulus from the SD is rewarded, with the addition of a new irrelevant sensory dimension (i.e., if odour was learnt on the SD, a digging media pair were added but not required to be attended to, or if digging media was learnt as the sensory dimension on the SD, an odour pair was added but not required to be attended to). The CD subtask is followed by the CD reversal (CDrev) used the same stimuli pairings for the CD but the other stimulus in the pair, from the same sensory dimension is rewarded, with the previously rewarded stimulus now the non-rewarded stimulus. This reversal was followed by three separate intradimensional (ID1, ID2, ID3) shift subtasks. For the ID task, the sensory dimension used in the SD and CD subtasks was to be attended to, but new stimuli pairings were used for each of the three different IDs. As indicated in the Introduction, previous evidence showed that after damage to the Re, rats require more trials to complete the ID subtask (see [11]) suggesting a deficit in shifting the attentional set strategy after Re damage. So, in the current study, we included two additional ID subtasks, as described previously [35] to determine if damage to the Re disrupts shifting an attentional set strategy, as was identified after damage to the anterior thalamic nuclei [35].

For the last two subtasks, extradimensional (ED) shift followed by a reversal of this ED (EDrev), the rats had to rapidly switch their responses to the previously irrelevant non-rewarded sensory dimension due to a change in the current task demands. That is, until the end of the three ID shift subtasks, the rats were rewarded if they were digging in the correct bowl of new stimuli pairings linked to the initial sensory dimension (odour or digging media) they learnt from the SD subtask, while ignoring the irrelevant, non-rewarded sensory dimension that was introduced in the CD subtask. Now, for the ED shift, the rats had to learn

that they needed to attend to this previously irrelevant sensory dimension (based on negative feedback, i.e., the absence of reward) and learn which stimulus of the new pairing of the now relevant sensory dimension were rewarded. For example, if the rats had been rewarded for attending to the stimuli pairing linked to the odour sensory dimension throughout SD, CD, CDrev, ID1, ID2 and ID3, they had to switch to attend to the previously irrelevant sensory dimension, digging media, and learn to respond to the sensory dimension of touch while digging in the bowls to determine which of the digging media stimuli would reliably provide the reward. Similarly, if the rats had been attending to the sensory dimension of digging media, then they had to switch to attend to the sensory dimension of odour and respond to the different smell pairings and determine which stimuli reliably provided the reward. The last subtask, the EDrev, involves a reversal of the newly acquired reward contingencies related to the new stimuli pairing linked to the relevant ED sensory dimension.

Drug administration

Prior to running in the first IDED test session (IDED1), each rat received an intraperitoneal injection (i.p.) of saline (1ml/kg) and was placed back into a holding cage for 30 minutes. IDED1 was used to compare cognitive flexibility performance between the three experimental groups. This drug administration procedure was repeated prior to running in the second IDED test session (IDED2) but now with an i.p. injection of atipamezole (1mg/kg, Abcam; [26]) 30 minutes prior to testing. IDED2 was used to determine if a systemic increase in noradrenaline could mitigate any performance deficits in cognitive flexibility caused by the permanent damage to the MD or the Re, compared to Sham controls.

Open field exploration pre and post the atipamezole injection

For five minutes prior to the injection of atipamezole and running the IDED2 session, the rat was placed in a familiar grey Perspex box (60cm x 60cm x 30cm; 30cm above ground level) for 5mins to record baseline (no drug) activity. An overhead camera captured the rat's

behaviour for offline analysis. Upon completion of the IDED2 test session, each rat was returned to this open field for another 5mins to record drug activity at about 2-2.5 hours post the atipamezole injection (the delay varied according to the time it took each rat to complete the 8 subtasks in the IDED2 test session).

Histology

Perfusion and tissue collection

Following behavioural testing, rats were deeply anaesthetised with sodium pentobarbital (125 mg/kg) and perfused transcardially with saline and the brain fixed with paraformaldehyde (4% PFA in 0.1M phosphate buffer; PB). Brains were post-fixed in 4% PFA followed by a minimum of 48 hours in a long-term solution (30% glycerol in 0.1M PB). Coronal 40µm sections were collected using a freezing microtome (Bright Instruments, UK) and stored in cryo-protectant (30% glycerol, 30% ethylene glycol in 0.1M PB) at -20˚C until processed for immunohistochemistry. Coronal sections were collected consecutively in 10 cryovials for immunohistochemistry, with thalamic sections (between -1.08mm and -3.96mm from Bregma) from vials 1, 5, and 9 (i.e., every 160 microns) used to determine the loss of NeuN-stained neurons for verification of the damage.

NeuN thalamic damage verification

Per Hamilton & Dalrymple-Alford [36], sections throughout the thalamus were washed in 0.1M phosphate buffered saline with Triton-X (0.2%; PBSTx) before incubation in endogenous peroxidase blocking buffer for 30 minutes (1% hydrogen peroxide (H_2O_2), 50% methanol (CH_3OH) in 2% PBSTx). They were incubated overnight at 4˚C in anti-NeuN primary antibody (1:5000; monoclonal-Mouse Cat# MAB377; Millipore, California USA) in PBSTx with 1% normal goat serum (NGS; Life Technologies, NZ), followed by incubation in biotinylated goat anti-mouse secondary antibody (1:1000 Cat# BP-9200-50: Vector Laboratories, California USA) for 90min at room temperature in PBSTx and 1% NGS.

Sections were placed in ExtrAvidin (peroxidase conjugated; 1:1000; Sigma, NSW Australia), PBSTx and 1% NGS for 2 hours at room temperature. Sections were visualised using diaminobenzidine (DAB 0.05%; Sigma, in 0.01% H_2O_2 in Tris buffer; approximately 5 min). After mounting on gelatinised slides, sections were dried and slides were dehydrated through graded alcohol (70-100%), cleared in xylene and mounted with DPX (06522; Sigma Aldrich).

The number of Neu-N positive cells was used to determine loss of neurons. This used sections from both the left and right hemisphere for every one in four 40µm sections. NeuN-positive cell staining was photographed using the 5x objective on a Leica DM6 B upright microscope and DFC7000T camera (Leica Microsystems, Germany). Automated counts of the cells were obtained through ImageJ (image analysis software, National Institute of Health, NIH, USA). The area surrounding neurons in the thalamic region of interest was manually selected and the images were converted to 8-bit grey scale, background was subtracted (rolling = 40), converted to mask and the watershed function was applied, and all neuronal cells above threshold ('MaxEntropy' threshold, circularity 0.5-1.0) were counted. The detection threshold was the same for all sections. Automated NeuN-stained nuclei of cells were counted in all seven of the Sham rats to express average cell counts in both the MD and Re. Automated counts of NeuN-stained nuclei of spared neurons in either the MD or the Re NMDA infused rats were compared relative to the average count of NeuN cells observed in the Sham (no infusion) rats. Acceptable loss of neurons in the MD or Re were defined as reaching the criterion of at least 50% loss of cells in the respective thalamic region.

Statistical analysis

The criterion for completing each subtask was 6 correct consecutive trials. Repeated measures ANOVAs evaluated the number of trials to criterion for each rat for each the 8 subtasks (SD, CD, CDrev, ID1, ID2, ID3, ED, EDrev) with Group as the between subject factor (MD, Re or Sham group) for the IDED1 test session when saline i.p. was given prior to

testing. The same analysis was conducted for the IDED2 test session, when atipamezole was administered i.p. prior to testing. In the IDED1 test with saline, two rats (one from the MD group and one from the RE group) did not complete the EDrev subtask because they had spent 3 hours completing the first 7 subtasks. For the IDED1 ANOVA the first 7 subtasks were examined.

Due to the complex nature of the interaction between Subtask and Group, subsequent analyses were run to analyse MD or Re thalamic damage effects separately compared to Sham controls for each subtask. The 8 subtasks were divided further into four subtask clusters based on a priori hypotheses of expected deficits following MD or Re damage: Subtask cluster 1= SD, CD, CDrev; Subtask cluster 2= ID1, ID2, ID3; Subtask cluster 3= ID3, ED; and Subtask cluster 4 = ED. Subtask clusters 1, 2 and 3 were analysed using repeated measures ANOVAs (e.g., Subtask 1 [SD, CD, CDrev] x Group), with post-hoc analyses for relevant Subtask cluster x Group effects (2-way ANOVA). Subtask cluster 4 was analysed using a univariate ANOVA with pairwise comparisons for relevant Group effects. Further analyses were conducted for the Shift Cost, using a difference score (ED minus ID3) which compares the trials to criterion of ID3 with the trials to criterion for ED across the Groups. This Shift Cost analysis can provide insight into the behavioural performance of the rats when they need to switch their responses to the previously irrelevant sensory dimension. Additionally, four repeated measures ANOVAs compared trials to criterion for Subtask cluster 1 or Subtask cluster 2 or the ID-ED Shift Cost or the ED subtask across Drug Sessions (IDED1 (saline) x IDED2 (atipamezole)) x Group. Similarly, average latency to complete all trials for Drug Session (IDED1 vs IDED2) were compared using a repeated measures ANOVA (Drug Session x Group). Finally, a repeated measures ANOVA compared differences between Groups of second entry choice responses made for all subtasks combined as a measure of rapid adaptability in choice making.

Results

Assessment of MD and Re damage

NeuN quantification led to the exclusion of two rats, one MD and one Re, due to sparing of over 50% of neurons in the target. One Sham control rat was excluded due to health concerns prior to completion of the experiment. Final group numbers were Sham controls, n=7, MD damage, n=7, Re damage, n=7. For rats included in the final analyses, the median MD damage was 57%, range 50-67% and the median Re damage was 60%, range 52-78% (see Fig. 2). In all cases, the alternate thalamic region (MD or Re) was spared with minimal neuronal loss (less than 10-20%). The central median thalamic region which lies between the MD and Re also showed minimal cell loss. Thalamic damage was consistently located in the medial portions of both the MD and Re in the respective groups.

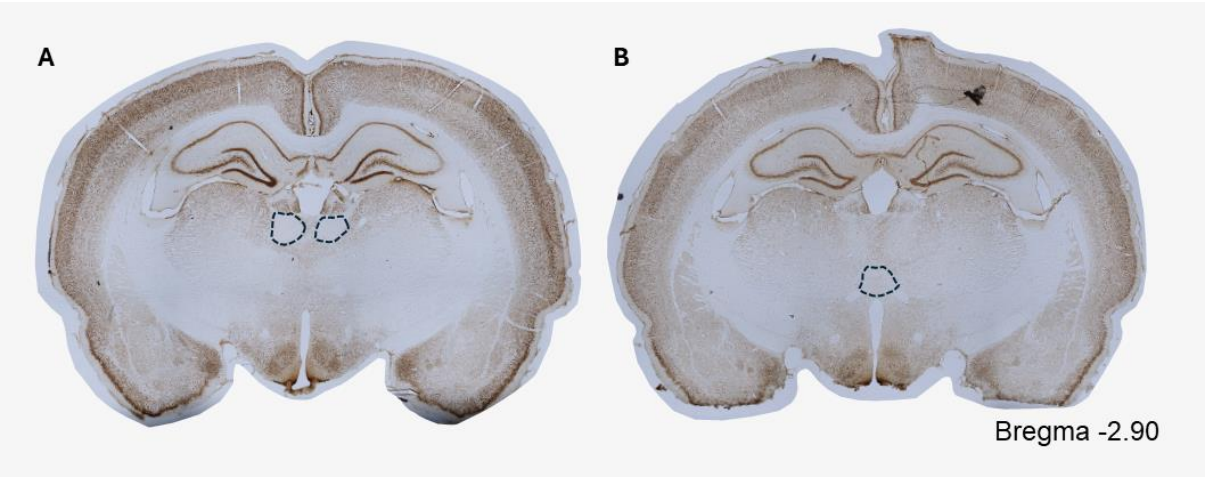


Figure 2. NeuN stained sections to identify the average NMDA neurotoxin damage (dotted lines) within the **A,** mediodorsal thalamus and **B,** the nucleus reuniens of the thalamus. Photomicrographs imaged at 2.5x at approximately -2.90mm from Bregma.

Group effects on overall trials to criterion across the IDED1 (saline) subtasks

To compare the number of Trials to criterion for the IDED1 test session, a repeated measures ANOVA with 7 subtasks (SD, CD, CDrev, ID1, ID2, ID3, ED) was conducted, which

revealed a significant Subtask x Group interaction ([order 4], $F(2,18) = 9.74$, $p = .00$). Rats with either MD (Sham vs MD, $p = .006$) or Re damage (Sham vs Re, $p = .003$; Fig. 3) required more trials to criterion than Sham control rats across these subtasks. Trials to criterion for MD or Re rats did not differ across these seven subtasks ($p = .69$). There was also a Group main effect, $F(2,18) = 7.28$, $p = .005$, and a significant Subtask main effect ([quad] $F(1,18) = 4.56$, $p = .04$). These interactions are further described below.

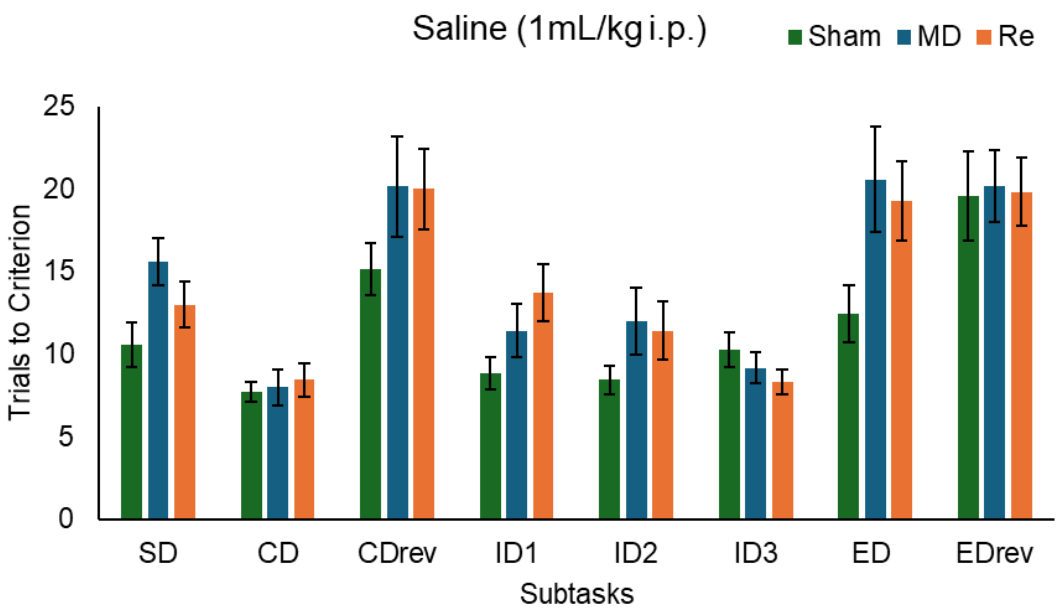


Figure 3. Mean (+/-SEM) for Trials to Criterion to complete each of the 8 subtasks for the three experimental groups (Sham control neurosurgery; NMDA infusions to the mediodorsal (MD) thalamic nuclei or NMDA infusions to the thalamic nucleus reuniens (Re) during the first test session of the intradimensional/ extradimensional attentional set-shifting task (IDED1) after saline (1mL/kg) intraperitoneal (i.p.) administration. MD (p= .006) or Re (p= .003) damage produced more trials to criterion than Sham controls. Re damage produced more trials to criterion in ID1 shift than Sham controls (p= .03). MD rats required more trials to criterion in the ED shift compared to Sham controls ($p < .03$). Abbreviations: CD = compound discrimination; CDrev = reversal of compound discrimination; ED =extradimensional shift; EDrev = reversal of extradimensional shift; ID1 = the

first intradimensional shift; ID2 = the second intradimensional shift; ID3 = the third intradimensional shift; SD = simple discrimination.

Group effects on the SD, CD, and CDrev subtask clusters on IDED1

The repeated measures ANOVA comparison of Group x SD, CD, CDrev subtask cluster revealed no main effect of Group, $F(2,18) = 3.28$, $p = .06$, although the MD and Re groups required more trials to criterion in each subtask. There was a main effect of Subtask cluster, $F(1,18) = 14.92$, $p = .001$, with all rats requiring more trials to complete the CD reversal. There was no Subtask cluster x Group interaction, $F(2,18) = 1.36$, $p = .28$.

Group effects on ID1, ID2, ID3 subtask cluster on IDED1

The repeated measures ANOVA across the three ID subtask cluster showed a Group x ID Subtask cluster interaction with Re rats needing more trials to criterion to acquire ID1, $F(2,18) = 3.56$, $p = .05$ (see Fig. 3), compared to Sham control rats ($p = .03$). Across the remaining two ID subtasks, the Re rats' trials to criterion were similar to Sham controls (ID2 $p = .20$; ID3 $p = .14$). Further, Re rats demonstrated learning as indicated by less trials to criterion required across the completion of ID1, ID2, ID3, ($p = .008$). As expected, MD rats did not differ from Sham controls across the three ID subtask cluster ($p > .05$). There was no main effect of Group, $F(2,18) = 2.13$, $p = .14$ or ID subtask cluster, $F(1,2) = 3.98$, $p = .06$.

Group effects on the ID3-ED subtask cluster on IDED1

There was a significant Group x ID3-ED subtask cluster interaction, $F(2,18) = 3.69$, $p = .04$, which revealed MD rats required a greater number of trials to complete the ED compared to Sham ($p = .03$; see Fig. 3). Re rats did not differ to either Sham ($p = .06$) or MD rats ($p = .72$) on the ED subtask. All rats performed similarly on the ID3 (p's > .1). There was also a main effect of ID3-ED subtask cluster, $F(1,18) = 27.07$, $p < .001$, with all rats requiring more trials

to complete the ED subtask compared to the ID3. There was no Group main effect, $F(2,18) = 1.86$, $p = .18$.

Group effect on ED subtask on IDED1

While MD or Re damaged rats required more trials to criterion than Shams on the ED subtask, univariate ANOVA (Welch's test) showed only a non-significant trend for the Group main effect, $F(2,11.3) = 3.85$, $p = .053$.

Group effects for the Shift Cost (ED-ID3) on IDED1

The univariate ANOVA for the ED minus ID3 difference score showed a main effect of Group, $F(2,18) = 3.69$, $p = .04$ (see Fig. 4, saline), with both MD and Re rats showing a greater Shift Cost (larger difference score) than Sham rats (Sham vs MD $p = .02$, Sham vs Re $p = .03$, MD vs Re $p = .91$).

Group effects on the reversal subtasks (CDrev and EDrev) on IDED1

All rats that completed both reversals (Sham=7; MD=6; Re=6) required a similar number of trials to criterion for the CDrev and EDrev subtasks with no main effect of Group, $F(2,16) < 1.0$; no main effect of Subtask, $F(1,16) = 1.18$, $p = .29$, and no interaction, $F(2,16) < 1.0$.

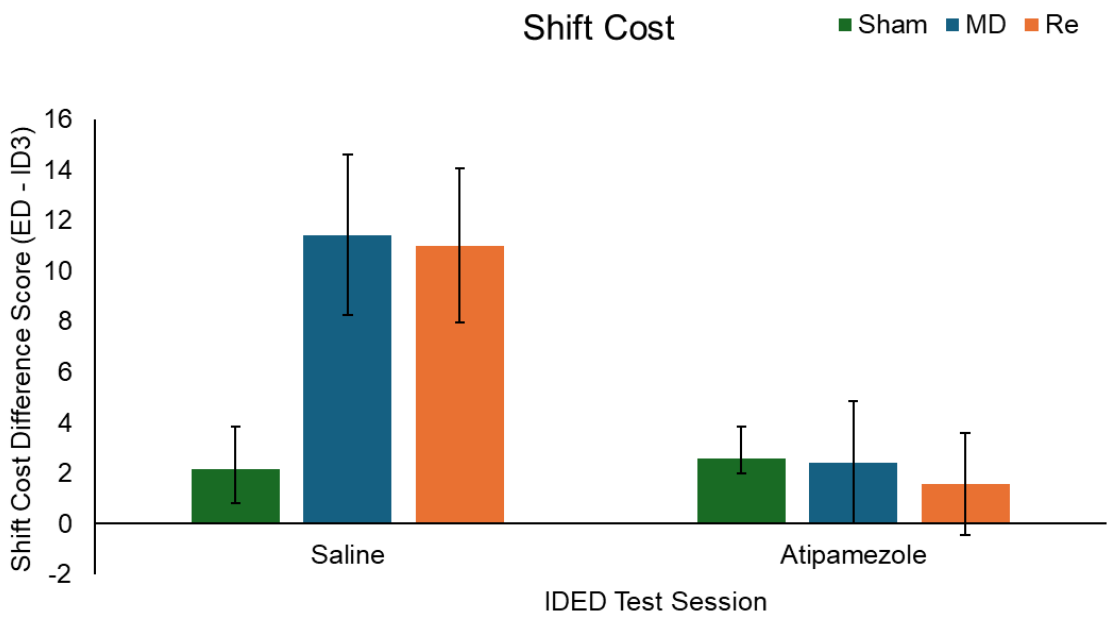


Figure 4. Mean (+/-SEM) Difference Score (ED - ID3) for trials to criterion for the three experimental groups (Sham control neurosurgery; NMDA infusions to the mediodorsal (MD) thalamic nuclei or NMDA infusions to the thalamic nucleus reuniens (Re) during the test sessions of the intradimensional/ extradimensional attentional set-shifting task after saline (IDED1, 1mL/kg) and atipamezole (IDED2, 1mg/kg) intraperitoneal (i.p.) administration.

Group effects of atipamezole on overall trials to criterion for IDED2 test session

The repeated measures ANOVA of trials to criterion for all 8 subtasks revealed a Group x Subtask interaction, $F(2,18) = 4.62$, $p = .04$; see Fig. 5), a main effect of Subtask, $F(1,18) = 6.07$, $p = .02$), but no main effect of Group, $F(2,18) = 2.72$, $p = .09$. The significant interactions are further described below.

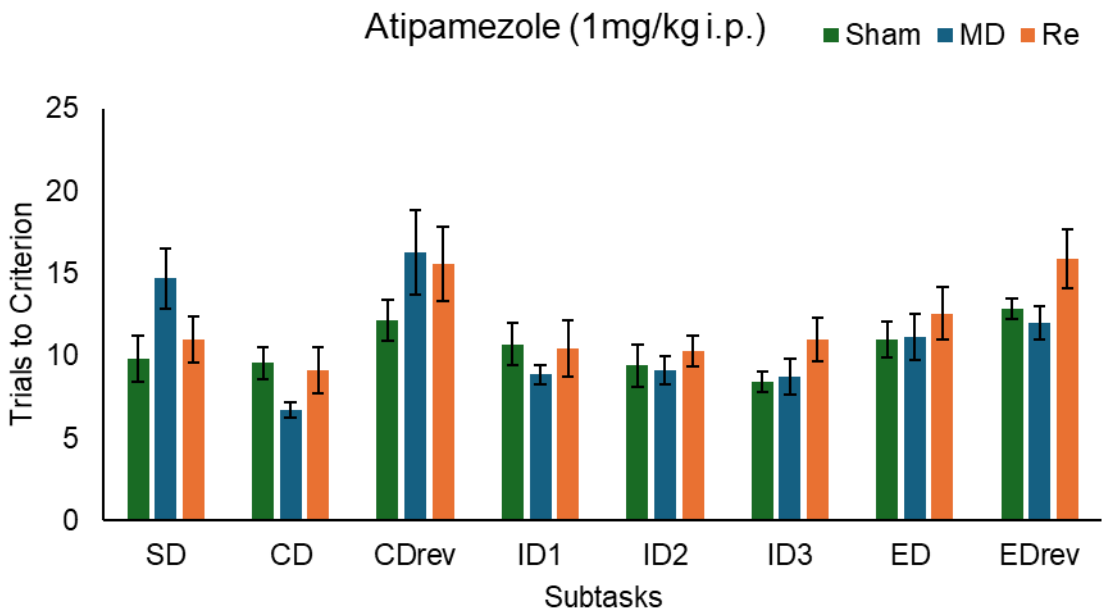


Figure 5. Mean (+/-SEM) for Trials to Criterion to complete each of the 8 subtasks for the three experimental groups (Sham control neurosurgery; NMDA infusions to the mediodorsal (MD) thalamic nuclei or NMDA infusions to the thalamic nucleus reuniens (Re) during the second test session of the intradimensional/ extradimensional attentional set-shifting task (IDED2) after atipamezole (1mL/kg) intraperitoneal (i.p.) administration. Atipamezole reduced the trials to criterion across all rats compared to saline, improving cognitive flexibility performance even after permanent MD or Re thalamic damage. For abbreviations of the subtasks, see Fig. 2.

Group effects of atipamezole on the SD, CD, and CDrev subtask cluster on IDED2

The repeated measures ANOVA revealed a Group x Subtask interaction, $F(2,18) = 4.77$, $p < .02$, which indicated that the MD rats required significantly more trials to criterion to learn the SD (MD vs Sham, $p = .04$), but once the SD was acquired the MD rats were not different to the Re or Sham control groups (CD and CDrev p's > .062). There was a main effect of Subtask, $F(1,18) = 23.71$, $p < .001$, with all rats requiring more trials to criterion to complete the CDrev. There was no main effect of Group, $F(2,18) = 1.56$, $p = .23$.

Group effects of atipamezole on the ID1, ID2, ID3 subtask clusters on IDED2

The repeated measures ANOVA of the ID1, ID2, ID3 subtask clusters revealed no main effect of Group, $F(2,18) = 2.19$, $p = .14$, no main effect of Subtask cluster, $F(1,18) < 1.0$, and no Group x Subtask cluster interaction, $F(2,18) = 7.73$, $p = .59$, suggesting that atipamezole had mitigated the ID1 learning deficit in the Re group of rats.

Group effects of atipamezole on ID3, ED subtask cluster on IDED2

The repeated measures ANOVA of the ID3, ED subtask clusters revealed a main effect of Group, $F(2,18) = 4.29$, $p = .03$ (see Fig. 5), but no main effect of Subtask cluster, $F(1,18) = 3.09$, $p = .09$, and no Group x Subtask cluster interaction, $F < 1.0$.

Group effects of atipamezole on ED subtask on IDED2

Univariate ANOVA for the ED subtask showed no main effect of Group, $F < 1.0$, with all rats requiring a similar number of trials to complete this ED shift, suggesting that atipamezole had mitigated ED shift performance.

Group effects of atipamezole on the Shift Cost (ID3 - ED) on IDED2

The univariate ANOVA for the ED minus ID3 difference score showed no main effect of Group, $F(2,18) = 0.06$, $p = .93$ (see Fig. 4, atipamezole), with all rats showing no, to minimal, shift cost.

Group effects of atipamezole on the CDrev and EDrev subtask cluster on IDED2

All rats had similar numbers of trials to criterion for the CDrev and EDrev subtasks, with no differences for Group, $F(2,18) = 1.44$, $p = .26$, or for Subtask, $F < 1.0$, and no Group x Subtask interaction, $F(2,18) = 1.69$, $p = .21$.

Overall Group Effects of the Drug Administration: Saline (IDED1) vs Atipamezole (IDED2)

The repeated measures ANOVA (Group x Drug Session [Saline (IDED1) vs Atipamezole (IDED2)] x Subtask [SD, CD, CDrev, ID1, ID2, ID3, ED, EDrev]) revealed that the Group main effect was still apparent with both MD or Re damaged groups of rats requiring more

trials to criterion compared to Sham control rats, $F(2,16) = 5.77$, $p = .01$; MD vs Sham, $p = .02$; Sham vs Re, $p = .006$; MD vs Re, $p = .57$. Notably, administration of atipamezole prior to the IDED2 test session significantly reduced the number of trials to criterion (Drug main effect, $F(1,16) = 28.35$, $p < .001$; saline (M) = 13.30, SD= 0.89; atipamezole (M) =11.09, SD= 0.77). However, there was no Group x Drug Session interaction, $F(2,16) = 3.01$, $p = .07$. The main effect of Subtask remained when combined across the two test sessions, $F(1,16) = 17.42$, $p < .001$.

Group effects of saline vs atipamezole on SD, CD, CDrev subtask cluster

A repeated measures ANOVA for the SD, CD and CDrev cluster to determine the Group effects for both saline and atipamezole IDED tests revealed a Group x Subtask interaction [order5], $F(2,16) = 6.04$, $p = .01$, with MD rats specifically requiring more trials to criterion to acquire the SD subtask irrespective of Drug Session compared to Sham control ($p = .004$) or Re ($p = .03$) rats. MD rats also required more trials to criterion for the CDrev subtask than Sham rats ($p = 0.04$). There was also a main effect of Group $F(2,18) = 4.43$, $p = .02$.

Group effects of saline vs atipamezole on ID1, ID2, ID3 subtask cluster

The repeated measures ANOVA revealed that despite the Re deficit following saline administration there are no group differences or interactions between the saline and atipamezole test sessions for the ID1, ID2 and ID3 subtasks (p's > .05), indicating that atipamezole improved cognitive performance.

Group effects of saline vs atipamezole on ED subtask

The repeated measures ANOVA across the IDED1 (saline) and IDED2 (atipamezole) test sessions on the ED subtask shows a significant effect of Drug Session $F(1,18) = 12.55$, $p = .002$, with atipamezole reducing the number of trials to criterion on the ED subtask compared to saline administration. There was no main effect of Group $F(2,18) = 2.87$, $p = .08$, and no Group x Drug session interaction $F(2,18) = 2.01$, $p = .16$.

Group effects of saline vs atipamezole on the Shift Cost (ID3-ED)

A repeated measures ANOVA across the IDED1 (saline) and IDED2 (atipamezole) test sessions for Shift Cost (ED minus ID3) shows a significant main effect of Drug Session, $F(1,18) = 10.05$, $p = .005$, with a decrease in Shift Cost following atipamezole injections compared with saline (see Fig. 4). This drug effect was consistent across all three groups (Group x Drug Session, $F(2,18) = 2.89$, $p = .08$), with no main effect of Group, $F(2,18) = 1.82$, $p = .19$. Pairwise comparisons showed that atipamezole did not alter responding on the ID3 subtask ($p = .85$), but it significantly reduced trials to criterion on the ED subtask ($p = .002$).

Group Average Latencies across all subtasks for saline vs atipamezole

The repeated measures ANOVA of average latencies to complete trials across all subtasks for the three experimental groups during the two Drug Sessions (Saline (IDED1) vs Atipamezole (IDED2)) revealed a main effect of Drug Session, $F(1,18) = 23.51$, $p < 0.001$, with the latency to respond reduced after atipamezole compared to saline, but there was no main effect of Group, $F < 1.0$ and no interaction, $F < 1.0$.

General activity (distance travelled) in the open field box

The repeated measures ANOVA of distance travelled in the open field box prior to the atipamezole administration and immediately on completion of the EDrev subtask showed a significant main effect Pre vs Post injection, $F(1,18) = 18.37$, $p < .001$, with an increase in distance travelled at the end of the EDrev compared with before atipamezole. However, there was no main effect of Group, $F < 1.0$, and no interaction, $F < 1.0$, indicating that the effect of atipamezole on distance travelled by all rats was similar regardless of Group.

Rapid adaptability across all subtasks (saline vs atipamezole)

To determine whether MD or Re damage altered inhibitory responding behaviours across the two drug conditions, the percentage of correct 2nd choice responses for all trials was

calculated (2nd choice correct trials/total correct trials). A repeated measures ANOVA (Group x Drug Session) indicated that neither MD nor RE damage impaired adaptability across either session (Group main effect, $F(1,18) = 2.34$, $p = 0.125$; Group x Drug Session interaction, $F < 1.0$. Adaptability scores did differ significantly across the two sessions, with a significantly lower score apparent following atipamezole administration (Drug Session main effect, $F(1,18) = 12.61$, $p = .002$. This could be due to the significantly reduced number of 2nd entries following atipamezole compared to saline, Drug Session main effect, $F(1,18) = 17.22$, $p < .001$; Saline M = 24.04, Atipamezole M = 12.67).

Discussion

The present study showed using the intradimensional extradimensional (IDED) task that as predicted permanent damage to the mediodorsal thalamus (MD) impairs the ED shift, as previously demonstrated [5], while permanent thalamic nucleus reuniens (Re) damage impairs the first ID shift, as previously demonstrated [11]. Subsequently, we demonstrated for the first time that atipamezole administered intraperitoneal 30 mins before re-running in the IDED task with novel stimulus pairings reduces these cognitive flexibility deficits. That is, across the attentional set-shifting task, noradrenaline administration robustly reduced the number of trials to criterion, irrespective of thalamic damage, indicating a global facilitation on task performance, rather than a selective rescue of damage related deficits. Consistent with this, some thalamic injury effects persisted across the saline vs atipamezole drug sessions, with MD or Re damaged rats still requiring more trials to criterion overall than Sham controls. Nonetheless, the noradrenergic benefits were most evident during the extradimensional (ED) shift and its reversal, aligning with previous evidence that atipamezole preferentially enhances ED shift cognitive flexibility on the attention set-shifting task [26, review 29].

Across the attention set-shifting task, noradrenaline administration produced robust effects on behavioural performance. Latencies to complete the trials was significantly reduced following noradrenaline compared with saline, reflecting faster and more decisive responding, consistent with evidence that noradrenaline enhances attentional focus and the efficiency of task-specific decision making [37]. This effect was not attributable to generalised hyperactivity, as locomotor activity measure in an open field was increased following noradrenaline, aligning with prior reports of noradrenaline's role in regulating arousal and effort allocation [26, 29]. Noradrenaline also reduced second-choice entries, suggesting a bias toward more decisive choice selection. Together, these findings indicate that noradrenergic modulation promotes rapid, efficient, and focussed decision-making across complex tasks.

The dissociable effects of MD and RE lesions across the attention set-shifting task are consistent with prior evidence, with distinct roles in decision-making and attention. The selective impairment of MD damaged rats during the ED shift, while sparing performance on the ID shift, aligns with evidence that the MD is critical for updating attentional set shifting to previously irrelevant stimuli and reconfiguring choice response strategies when task rules change unpredictably across different species, and for differing tasks that each assess cognitive flexibility [2, 5, 6]. The selective deficit in the ED shift also aligns with MD thalamocortical interactions to the medial PFC that when damaged produces selective deficits on the ED shift [18, 19, 22]. Additionally, this evidence mimics the effects of noradrenergic deafferentation within the medial PFC or damage to the dorsal noradrenergic bundle that innervates the medial PFC [32, 33] further implicating the importance of the noradrenergic system in cognitive flexibility [29].

In contrast, Re damaged rats showed a deficit during the first ID shift but performance comparable to Sham controls across the other two ID subtasks. This is consistent with

proposals that the Re supports early set formation and the integration of hippocampal-prefrontal information required for rule formation due to its thalamocortical interactions with these brain structures [11]. Together these findings support a functional dissociation in which MD primarily mediates updating of task-relevant choice dimensions, while Re contributes to the establishment and stabilisation of attentional sets.

Current understanding is that thalamocortical interactions between the MD or Re and mPFC are key for cognitive flexibility with each brain structure contributing to different components of the current task demands that constitute cognitive flexibility. Previous neuroimaging in healthy humans completing the Wisconsin Card Sorting task identified many brain structures support different aspects of the task. Changes in blood oxygenation level dependent activation occurred in the MD during processing of negative reward feedback [23]. One notable feature of our results is that baseline response latencies, saline IDED trials, were longer than typically reported in the literature. Previous studies that employ the attentional set-shifting task in rats have used other strains such as Long-Evans and Sprague-Dawley, which generally show faster response times and greater exploration in operant or digging-based tasks [18, 38]. In contrast, early research has shown that PVGc rats, as used in this experiment, are known to exhibit lower overall locomotion and more cautious behaviour [39, 40] which may have contributed to the slower response times observed. Despite these latency differences, the overall pattern of results aligns with previous reports. There were no lesion-related differences in average latency across the attention set-shifting task following saline administration suggesting similar task engagement across the groups. Noradrenaline consistently reduced these response latencies, demonstrating that the task remained sensitive to neuromodulatory effects. These findings highlight that strain differences can influence performance measures without affecting the underlying cognitive processes or

pharmacological responsiveness and emphasises the importance of strain characteristics when comparing behaviour across studies.

Limitations and Future Directions

An important limitation of this study is the reliance on systemic pharmacology, which does not isolate noradrenergic mechanisms at specific receptor subtypes or anatomical loci. Future work using regionally targeted manipulations will be required to dissect how noradrenergic signalling interacts with thalamocortical pathways during set shifting. Additionally, electrophysiological or imaging markers of thalamocortical communication during task performance could help clarify the dynamic contributions of MD and RE to rule learning and updating.

Data Availability Statement

Data will be available on request.

Author contributions

JJH, JCDA and ASM: funding, concept and design, writing and editing drafts, statistical analysis and interpretation; JJH, LB and SH-B: conducting experiment.

Funding

This research was supported by the Neurological Foundation NZ [2325 PRG]

Competing interests

The authors have nothing to disclose.

References

1. Browning, P.G., S. Chakraborty, and A.S. Mitchell, Evidence for mediodorsal thalamus and prefrontal cortex interactions during cognition in macaques. *Cerebral cortex,* 2015. 25(11): p. 4519–4534.

2. Chakraborty, S., et al., Critical role for the mediodorsal thalamus in permitting rapid reward-guided updating in stochastic reward environments. *Elife,* 2016. 5: p. e13588.

3. Hwang, K., et al., Neuropsychological evidence of multi-domain network hubs in the human thalamus. *Elife*, 2021. 10: p. e69480.

4. Mitchell, A.S., The mediodorsal thalamus as a higher order thalamic relay nucleus important for learning and decision-making. *Neuroscience & Biobehavioral Reviews*, 2015. 54: p. 76–88.

5. Ouhaz, Z., et al., Mediodorsal thalamus is critical for updating during extradimensional shifts but not reversals in the attentional set-shifting task. *Eneuro,* 2022. 9(2).

6. Suthaharan, P., et al., Lesions to the mediodorsal thalamus, but not orbitofrontal cortex, enhance volatility beliefs linked to paranoia. *Cell reports*, 2024. 43(6).

7. Wolff, M. and M.M. Halassa, The mediodorsal thalamus in executive control. *Neuron*, 2024. 112(6): p. 893–908.

8. Perry, B.A., E. Lomi, and A.S. Mitchell, Thalamocortical interactions in cognition and disease: The mediodorsal and anterior thalamic nuclei. *Neuroscience & Biobehavioral Reviews*, 2021. 130: p. 162–177.

9. Zhang, X., et al., Mediodorsal thalamus regulates task uncertainty to enable cognitive flexibility. *Nature Communications*, 2025. 16(1): p. 2640.

10. Rikhye, R.V., A. Gilra, and M.M. Halassa, Thalamic regulation of switching between cortical representations enables cognitive flexibility. *Nature neuroscience*, 2018. 21(12): p. 1753–1763.

11. Linley, S.B., M.M. Gallo, and R.P. Vertes, Lesions of the ventral midline thalamus produce deficits in reversal learning and attention on an odor texture set shifting task. *Brain research*, 2016. 1649: p. 110–122.

12. Rojas, A.K., S.B. Linley, and R.P. Vertes, Chemogenetic inactivation of the nucleus reuniens and its projections to the orbital cortex produce deficits on discrete measures of behavioral flexibility in the attentional set-shifting task. *Behavioural Brain Research*, 2024. 470: p. 115066.

13. Panzer, E., et al., In relentless pursuit of the white whale: A role for the ventral midline thalamus in behavioral flexibility and adaption? *Neuroscience & Biobehavioral Reviews*, 2024. 163: p. 105762.

14. Hoover, W.B. and R.P. Vertes, Collateral projections from nucleus reuniens of thalamus to hippocampus and medial prefrontal cortex in the rat: a single and double retrograde fluorescent labeling study. *Brain Structure and Function*, 2012. 217(2): p. 191–209.

15. Varela, C., et al., Anatomical substrates for direct interactions between hippocampus, medial prefrontal cortex, and the thalamic nucleus reuniens. *Brain Structure and Function*, 2014. 219(3): p. 911–929.

16. Pergola, G., et al., The regulatory role of the human mediodorsal thalamus. *Trends in cognitive sciences*, 2018. 22(11): p. 1011–1025.

17. Lange, F., C. Seer, and B. Kopp, Cognitive flexibility in neurological disorders: Cognitive components and event-related potentials. *Neuroscience & Biobehavioral Reviews*, 2017. 83: p. 496–507.

18. Birrell, J.M. and V.J. Brown, Medial frontal cortex mediates perceptual attentional set shifting in the rat. *Journal of Neuroscience*, 2000. 20(11): p. 4320–4324.

19. Dias, R., T. Robbins, and A. Roberts, Primate analogue of the Wisconsin Card Sorting Test: effects of excitotoxic lesions of the prefrontal cortex in the marmoset. *Behavioral neuroscience*, 1996. 110(5): p. 872.

20. Dias, R., T.W. Robbins, and A.C. Roberts, Dissociation in prefrontal cortex of affective and attentional shifts. *Nature*, 1996. 380(6569): p. 69–72.

21. Nakahara, K., et al., Functional MRI of macaque monkeys performing a cognitive set-shifting task. *Science*, 2002. 295(5559): p. 1532–1536.

22. Brown, V.J. and D.S. Tait, Attentional set-shifting across species. *Current Topics in Behavioral Neuroscience*, 2016. 28(11): p. 363-395.

23. Monchi, O., et al., Wisconsin Card Sorting revisited: distinct neural circuits participating in different stages of the task identified by event-related functional magnetic resonance imaging. *Journal of Neuroscience*, 2001. 21(19): p. 7733–7741.

24. Buchsbaum, B.R., et al., Meta-analysis of neuroimaging studies of the Wisconsin Card-Sorting task and component processes. *Human brain mapping*, 2005. 25(1): p. 35–45.

25. Nyhus, E. and F. Barceló, The Wisconsin Card Sorting Test and the cognitive assessment of prefrontal executive functions: a critical update. *Brain and cognition*, 2009. 71(3): p. 437–451.

26. Lapiz, M. and D. Morilak, Noradrenergic modulation of cognitive function in rat medial prefrontal cortex as measured by attentional set shifting capability. *Neuroscience*, 2006. 137(3): p. 1039–1049.

27. Owen, A.M., et al., Extra-dimensional versus intra-dimensional set shifting performance following frontal lobe excisions, temporal lobe excisions or amygdalo-hippocampectomy in man. *Neuropsychologia*, 1991. 29(10): p. 993–1006.

28. Bubb, E.J., et al., Chemogenetics reveal an anterior cingulate–thalamic pathway for attending to task-relevant information. *Cerebral Cortex*, 2021. 31(4): p. 2169–2186.

29. Poe, G.R., et al., Locus coeruleus: a new look at the blue spot. *Nature Reviews Neuroscience*, 2020. 21(11): p. 644–659.

30. Holland, N., T.W. Robbins, and J.B. Rowe, The role of noradrenaline in cognition and cognitive disorders. *Brain*, 2021. 144(8): p. 2243–2256.

31. Marien, M.R., F.C. Colpaert, and A.C. Rosenquist, Noradrenergic mechanisms in neurodegenerative diseases: a theory. *Brain Research Reviews*, 2004. 45(1): p. 38–78.

32. Tait, D.S., et al., Lesions of the dorsal noradrenergic bundle impair attentional set-shifting in the rat. *European Journal of Neuroscience*, 2007. 25(12): p. 3719–3724.

33. McGaughy, J., R.S. Ross, and H. Eichenbaum, Noradrenergic, but not cholinergic, deafferentation of prefrontal cortex impairs attentional set-shifting. *Neuroscience*, 2008. 153(1): p. 63–71.

34. Janitzky, K., et al., Optogenetic silencing of locus coeruleus activity in mice impairs cognitive flexibility in an attentional set-shifting task. *Frontiers in Behavioral Neuroscience*, 2015. 9: p. 286.

35. Wright, N.F., et al., A critical role for the anterior thalamus in directing attention to task-relevant stimuli. *Journal of Neuroscience*, 2015. 35(14): p. 5480–5488.

36. Hamilton, J.J. and J.C. Dalrymple-Alford, Anterior thalamic nuclei: A critical substrate for non-spatial paired-associate memory in rats. *European Journal of Neuroscience*, 2022. 56(7): p. 5014–5032.

37. Aston-Jones, G. and J.D. Cohen, An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. *Annual Review of Neuroscience*, 2005. 28(1): p. 403–450.

38. Chudasama, Y. and T.W. Robbins, Dissociable contributions of the orbitofrontal and infralimbic cortex to pavlovian autoshaping and discrimination reversal learning: further evidence for the functional heterogeneity of the rodent frontal cortex. *Journal of Neuroscience*, 2003. 23(25): p. 8771–8780.

39. Broadhurst, P., Determinants of emotionality in the rat: II. Strain differences. *Journal of Comparative and Physiological Psychology*, 1958. 51(1): p. 55.

40. Schmitt, U. and C. Hiemke, Strain differences in open-field and elevated plus-maze behavior of rats without and with pretest handling. *Pharmacology Biochemistry and Behavior*, 1998. 59(4): p. 807–811.

Figure legends

Figure 5. **A.** Schematic of the ID/ED attentional set-shifting apparatus. Access to two identical choice compartments was controlled by clear Perspex doors (dotted line). Blue circle = small glass jar to provide ad libitum water throughout testing. The black ceramic digging bowls were filled with a combination of odours and digging media. **B.** Stimulus pairings used in the IDED test sessions.

Figure 6. NeuN stained sections to identify the average NMDA neurotoxin damage (dotted lines) within the **A,** mediodorsal thalamus and **B,** the nucleus reuniens of the thalamus. Photomicrographs imaged at 2.5x at approximately -2.90mm from Bregma.

Figure 7. Mean (+/-SEM) for Trials to Criterion to complete each of the 8 subtasks for the three experimental groups (Sham control neurosurgery; NMDA infusions to the mediodorsal (MD) thalamic nuclei or NMDA infusions to the thalamic nucleus reuniens (Re) during the first test session of the intradimensional/ extradimensional attentional set-shifting task (IDED1) after saline (1mL/kg) intraperitoneal (i.p.) administration. MD (p= .006) or Re (p= .003) damage produced more trials to criterion than Sham controls. Re damage produced more trials to criterion in ID1 shift than Sham controls (p= .03). MD rats required more trials to criterion in the ED shift compared to Sham controls ($p < .03$). Abbreviations: CD = compound discrimination; CDrev = reversal of compound discrimination; ED =extradimensional shift; EDrev = reversal of extradimensional shift; ID1 = the first intradimensional shift; ID2 = the second intradimensional shift; ID3 = the third intradimensional shift; SD = simple discrimination.

Figure 8. Mean (+/-SEM) Difference Score (ED - ID3) for trials to criterion for the three experimental groups (Sham control neurosurgery; NMDA infusions to the mediodorsal (MD) thalamic nuclei or NMDA infusions to the thalamic nucleus reuniens (Re) during the test sessions of the

intradimensional/ extradimensional attentional set-shifting task after saline (IDED1, 1mL/kg) and atipamezole (IDED2, 1mg/kg) intraperitoneal (i.p.) administration.

Figure 5. Mean (+/-SEM) for Trials to Criterion to complete each of the 8 subtasks for the three experimental groups (Sham control neurosurgery; NMDA infusions to the mediodorsal (MD) thalamic nuclei or NMDA infusions to the thalamic nucleus reuniens (Re) during the second test session of the intradimensional/ extradimensional attentional set-shifting task (IDED2) after atipamezole (1mL/kg) intraperitoneal (i.p.) administration. Atipamezole reduced the trials to criterion across all rats compared to saline, improving cognitive flexibility performance even after permanent MD or Re thalamic damage. For abbreviations of the subtasks, see Fig. 2.