Mediodorsal Thalamus Is Critical for Updating during Extradimensional Shifts But Not Reversals in the Attentional Set-Shifting Task

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Full title: Mediodorsal thalamus is critical for updating during extra-dimensional shifts but not reversals in the attentional set-shifting task

Abbreviated title: Mediodorsal thalamus and choice response strategy updating

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40 **Abstract**

41 Cognitive flexibility, attributed to frontal cortex, is vital for navigating the complexities of
42 everyday life. The mediodorsal thalamus (MD), interconnected to frontal cortex, may
43 influence cognitive flexibility. Here, male rats performed an attentional set-shifting task
44 measuring intra-dimensional and extra-dimensional shifts in sensory discriminations. MD
45 lesion rats needed more trials to learn the rewarded sensory dimension. However, once the
46 choice response strategy was established, learning further two-choice discriminations in the
47 same sensory dimension, and reversals of the reward contingencies in the same dimension,
48 were unimpaired. Critically though, MD lesion rats were impaired during the extra-
49 dimensional shift, when they must rapidly update the optimal choice response strategy.
50 Behavioral analyses showed MD lesion rats had significantly reduced correct within-trial
51 second choice responses. This evidence shows that transfer of information via the MD is
52 critical when rapid within-trial updates in established choice response strategies are required
53 after a rule change.

54
55 **Significance statement**

56 We demonstrate for the first time that rodent mediodorsal (MD) thalamus is a critical node
57 when choice response strategies need to change rapidly after a within-session rule change
58 during reward-guided learning. MD interactions with orbitofrontal cortex are critical for
59 value-based learning, while MD interactions with medial prefrontal cortex are critical for
60 rapid within-trial updating of optimal choice response rules. MD interactions with the
61 orbitofrontal cortex are not always necessary for reversal learning. These results indicate that
62 the MD also contributes to attentional set-shifting. Our evidence advocates for studies
63 investigating deficits in updating optimal choice response strategies due to potentially
64 disrupted cortico-thalamocortical transfer of information in people diagnosed with

65 Alzheimer's disease combined with more frontal pathology, Parkinson's disease, or
66 schizophrenia.
67

68 **Introduction**

69 Cognitive flexibility describes our ability to quickly and selectively switch our thoughts,
70 responses, or behavior to everyday dynamic situations. This capacity to rapidly update or
71 alter one's actions conveys evolutionary benefit and is key to survival (Koechlin et al., 2003;
72 Rougier et al., 2005). Cognitive flexibility shows marked changes or declines in
73 neurodegenerative disorders, like Parkinson's disease, in patients with ventral prefrontal
74 cortex (PFC) damage, and in neurodevelopmental diseases, like schizophrenia (Andrews et
75 al., 2006; Barch et al., 2009; Clark et al., 2004; Giraldo-Chica et al., 2018).

76 Cognitive flexibility is not unique to primates. Studies show that rats can readily
77 switch between attentional sets to optimise reward outcome (Birrell and Brown, 2000;
78 McAlonan and Brown, 2003; Newman and McGaughy, 2011). Typically, these studies have
79 focussed on various frontal cortex subregions. Yet, it is becoming increasingly clear that the
80 cortex does not function in isolation, but rather, relies heavily on subcortical and peripheral
81 inputs supplied by the thalamus (Sherman and Guillery, 2002; Guillery and Sherman, 2011;
82 Halassa and Kastner, 2017). The mediodorsal thalamus (MD) has a critical role in functions
83 of frontal cortex during higher order cognitive processes across mammalian species (Alcaraz
84 et al., 2018; Browning et al., 2015; Chakraborty et al., 2016; Chakraborty et al., 2019;
85 DeNicola et al., 2020; Ferguson and Gao, 2018; Miller et al., 2017; Mitchell and Dalrymple-
86 Alford, 2005; Mitchell et al., 2007a,b; Mitchell and Gaffan, 2008; Parnaudeau et al., 2013;
87 Pergola et al., 2018; Perry et al., 2021; Schmitt et al., 2017). This evidence highlights that the
88 MD and PFC are working in partnership, contributing different but complementary roles to
89 goal-directed behaviors (Mitchell, 2015; Perry et al., 2021).

90 In mammals, the MD is densely interconnected with areas of frontal cortex involved
91 in various aspects of cognitive flexibility. Recent work shows MD projections to frontal
92 cortex allow for the simultaneous sharing of information across multiple cortical regions.

93 This distributed pattern of frontal innervation by the MD appears to be especially true for the
 94 central (MDc) and medial (MDm) subdivisions, which have projections to the orbitofrontal
 95 cortex (OFC), medial PFC, and frontal association areas (Alcaraz et al., 2016; Dajani and
 96 Uddin, 2015; Groenwegen, 1988; Kuramoto et al., 2017; Perry et al., 2021). Although
 97 phylogenetic differences exist in the organisation of rodent and primate frontal cortex
 98 (Preuss, 1995), similar functional contributions to aspects of cognitive flexibility are
 99 observed in monkeys and rodents after perturbations to similar areas of agranular frontal
 100 cortex (Birrell and Brown, 2000; Bissonette et al., 2008; Dias et al., 1996; Dias et al., 1997;
 101 McAlonan and Brown, 2003). Given there are similarities in neuroanatomical connectivity
 102 between agranular frontal cortex and MD in rodents and primates, it seems that cognitive
 103 flexibility and rapid updating deficits observed in monkeys with excitotoxic MD lesions
 104 (Browning et al., 2015; Chakraborty et al., 2016; Mitchell et al., 2007a; Mitchell and Gaffan,
 105 2008) may also extend to rodents.

106 Thus, in the current study, rats with excitotoxic lesions to the MD or MD Sham
 107 controls were run on a well-established test of cognitive flexibility - the intra-dimensional
 108 (ID)/ extra-dimensional (ED) attentional set-shifting task (Birrell and Brown, 2000). To test
 109 this premise and investigate any deficits in choice responding, we used the standard 7-stage
 110 version comprising of multiple subtasks all conducted within a single testing session (Birrell
 111 and Brown, 2000; Chase et al., 2012). In rats, the ID/ED task has produced dissociable
 112 deficits after permanent cortical or limbic thalamic lesions, or neuropharmacological
 113 manipulations (e.g., Linley et al., 2016; McGaughy et al., 2008; Tait et al., 2014; Tait et al.,
 114 2018; Wright et al., 2015).

115 In the current experiment, we predicted MD lesion rats would show deficits during
 116 the initial sensory acquisition (simple discrimination, SD) and the ED shift. Both the SD and
 117 ED subtasks require rapid, within trial learning or updating of choice response strategies.

Therefore, impaired SD and ED performance would be consistent with the selective learning deficits observed in other rodent studies after MD perturbations (Chakraborty and Mitchell, 2013, for review; Courtiol et al., 2019; Ferguson et al., 2017; Hunt and Aggleton, 1998; Mitchell and Dalrymple-Alford, 2005; Perry et al., 2021). In addition, we predicted our MD lesion rats may show deficits during reversal subtasks. MD lesions in rodents can produce reversal learning deficits, although the evidence is mixed (see Chakraborty and Mitchell, 2013 for review). However, given the reciprocal direct MD connectivity with the OFC (Groenewegen, 1988; Krettek and Price, 1977; Ray and Price, 1992), and the selective deficits observed in rodents and monkeys with OFC manipulations performing the reversal subtasks during the ID/ED attentional set-shifting task (Dias et al., 1996, McAlonan and Brown, 2003), it remained an open question.

Materials and Methods

Animals: Twenty-five Lister hooded male rats weighing between 420 and 480 gm at time of surgery (Charles River, UK) were group housed in a temperature and humidity-controlled environment ($21 \pm 1^\circ\text{C}$). The housing and husbandry complied with the ARRIVE guidelines of the European Directive (2010/63/EU) for the care and use of laboratory animals. Testing was conducted in the light phase of a 12hr light/dark cycle (lights on at 7am). The rats were maintained on a controlled feeding schedule (20 g/rat/day) with water freely available in the home cage. All experimental procedures were performed in compliance with the United Kingdom Animals (Scientific Procedures) Act of 1986. A project license authorized all procedures after review by the university animal care and ethical review committee and Home Office Inspectorate.

Surgery: Rats were anesthetized by isoflurane and oxygen mix (4% induction, 1.8–2% maintenance), and given analgesia in subcutaneous injections of 0.05mg/kg buprenorphine

143 (Vetergesic; 0.3 mg/ml) and 1 mg/kg Metacam (Meloxicam; 5 mg/ml). Rats were secured in
144 a stereotaxic frame (Kopf) with atraumatic ear bars and the nose bar set to +3.3 mm to
145 achieve a level skull. A subcutaneous injection of 2 mg/kg bupivacaine (Marcaine; 2.5
146 mg/ml) was administered into the scalp in the location of the midline incision. Viscotears was
147 applied to keep the eyes moist. During surgery, each rat was placed on a heat pad and
148 covered in bubble wrap with an internal rectal thermometer probe to monitor and maintain
149 normal body temperature. Warmed sterile saline (1 ml/100 gm) was administered
150 subcutaneous into the scruff of the neck after one hour. A midline incision was performed.
151 Bregma and lambda coordinates were determined. A dental drill with a trephine head was
152 used to create a craniotomy over the midline. To maximize lesion accuracy, anterior and
153 posterior injection sites were calculated according to the bregma–lambda distance in each rat.
154 Coordinates were for anterior MD injection: anterior–posterior (AP) = – 0.395 mm, medial-
155 lateral (ML) = – 0.1 (avoiding the superior sagittal sinus, which was visible within the dura
156 inside the craniotomy) and dorsal-ventral (DV) = 0.56 mm (from dura), volume of
157 excitotoxin = 0.20 μ l; for posterior MD: AP = – 0.435 mm, ML = + 0.1 and DV = 0.57 mm
158 (from dura), volume of excitotoxin = 0.18 μ l. Fifteen rats (MD lesion) received 0.12M of
159 NMDA (N-methyl-D-Aspartate) dissolved in phosphate buffer (pH 7.20) in each hemisphere
160 from a 1 μ l-gauge Hamilton bevelled-tip syringe. The injections were performed manually,
161 taking 3 min each, and after injection the needle was left in situ for a further 3 min to allow
162 diffusion and to limit wicking of the NMDA. A further 10 rats (MD Sham controls) received
163 injections of sterile phosphate buffer instead of NMDA using the same injection coordinates.
164 Injections were given bilaterally in the MD. Upon completion of surgery, wounds were
165 sutured with Vicryl 4.0. Rats were housed singly during recovery for up to three days and
166 were then returned to their pre-surgery housing cohort. Postoperative oral analgesia, 1mg/kg
167 Metacam (Meloxicam, 1.5 mg/ml) dissolved and set in jelly was provided in individual bowls

168 to each rat daily for three days. Behavioral and physiological evidence showed that all rats
 169 recovered well, with normal eating and pre-surgery weights returning within 24h. Food
 170 regulation (20 gm/day/rat) started again 10d after recovery. Postoperative testing began at
 171 least 15d after surgery. The researcher was blind to the lesion group of each animal until all
 172 tests were completed.

173 **ID/ED Attentional Set Shifting paradigm**

174 **Apparatus:** The test chamber consisted of a modified Plexiglass home cage ($40 \times 70 \times 18$
 175 cm) with Perspex dividers separating the cage into a large start chamber (40×46 cm) and two
 176 identically sized (24×20 cm) choice chambers to which access was controlled by removable
 177 Perspex doors. The digging bowls were ceramic (internal diameter 7 cm, depth 4 cm) and
 178 were placed within the choice chambers. The bowls were filled with digging media of
 179 different textures, and the digging media were scented with different spices (see **Table 1** for
 180 examples). The odor or digging media discriminations, pairs of stimuli used, and the correct
 181 stimulus within pairs were counter-balanced across subtasks and matched between groups.
 182 The bowls were baited with Honey Nut Cheerios (Nestle®), each bowl contained a hidden
 183 (securely fixed under a metal grid) Honey Nut Cheerio to serve as an odor mask. During
 184 testing, only one of the bowls was baited and rats determined which bowl was baited using
 185 either the texture of the digging medium or the odor as cues. Prior to testing, rats were taught
 186 to dig in bowls filled with home-cage bedding material to retrieve one half of a Honey Nut
 187 Cheerio. The task was performed as previously described in Birrell and Brown (2000) and
 188 divided into two phases administered on two consecutive days.

189 **Preoperative and Postoperative Training Day:** On the day before testing, each rat learnt
 190 one simple two-choice discrimination (SD) using either of the two sensory dimensions: odor
 191 (mint vs oregano), or digging media (shredded paper vs cotton pads), to a criterion of six

192 consecutive correct trials. The rewarded odors or digging media were counterbalanced across
 193 the two groups, and these exemplars were not used again throughout testing. Digging was
 194 defined as active digging with both front paws or active foraging with the snout in the
 195 digging media. Sniffing or touching the media with the paws was not scored as a dig.

196 **Preoperative and Postoperative Testing paradigm:** The following day, each rat was given
 197 a series of seven two-choice discriminations (subtasks) in the following order: a new simple
 198 discrimination (SD) using either of two sensory dimension (odor or digging media); a
 199 compound discrimination (CD) using the same rewarded sensory dimension and two-choice
 200 discrimination as the SD, combined with the other irrelevant sensory dimension; first reversal
 201 (REV1), in which the exemplars remained the same as in the CD but the correct (rewarded)
 202 and incorrect exemplars were reversed; intra-dimensional shift (ID), in which new exemplars
 203 were used, but the relevant sensory dimension remained the same as in the previous subtasks;
 204 second reversal (REV2), where exemplars in the ID remained the same but the reward
 205 contingencies of the two exemplars were reversed; extra-dimensional shift (ED), where new
 206 exemplars were used and the previously irrelevant sensory dimension becomes relevant; and
 207 a third reversal (REV3), where exemplars in the ED subtask remained the same but the
 208 reward contingencies of the two exemplars were reversed.

209 The task is self-paced and relies on the natural foraging behaviors of the rats. The first
 210 five two-choice discrimination subtasks (SD, CD, REV1, ID, REV2) must be solved by
 211 discriminating between exemplars from the same sensory dimension. In this stage of the task,
 212 the rat is rewarded for choices based on specific perceptual features of the stimuli, while
 213 ignoring other features that also distinguish the stimuli. After acquiring each set of two-
 214 choice discriminations to a performance criterion of six correct consecutive trials, the rats
 215 encounter a reversal of the reward contingencies associated with the exemplars (a reversal
 216 subtask: REV1 and REV2). For the final two subtasks (ED and REV3), exemplars from the

217 previously irrelevant sensory dimension become relevant. Therefore the rat must shift its
 218 attention (and adjust its choice response strategy) to the previously irrelevant sensory
 219 dimension and perceptual feature to receive reward (ED subtask). The attentional set-shifting
 220 cost is calculated by comparing trials to criterion during the intra-dimensional shift, where
 221 the sensory dimension is consistent with previous subtasks, and the extra-dimensional shift,
 222 where the now relevant sensory dimension had been previously ignored and thus requires a
 223 shift to optimise continuing to retrieve rewards.

224 **Odor detection test:** At the completion of ID/ ED testing, the olfactory abilities of the rat to
 225 smell the odor of the buried half-Honey Nut Cheerio were assessed. This task consisted of 10
 226 consecutive trials where the rat was exposed to bowls containing similar bedding. The rat was
 227 placed in the waiting area of the set-shifting apparatus. Similar bedding-filled bowls were
 228 placed, one in each of the choice chambers, only one bowl contained half a Honey Nut
 229 Cheerio at the bottom (pseudorandomly assigned to avoid the rat developing a response
 230 strategy). The barrier was raised, allowing the rat access to both bowls. The rat had to chose
 231 to dig into one bowl and had up to 15 minutes to make a digging response on each trial. If the
 232 rat dug in the correct bowl, the trial was recorded as correct and the rat was returned to the
 233 waiting area and the barrier lowered to block access to both choice chambers. If the rat dug in
 234 the incorrect bowl, the trial was marked as incorrect. The rat was permitted to continue to
 235 explore the incorrect bowl; the trial was terminated when the rat returned to the waiting area.
 236 In all cases, the ability of each rat to detect the reward location was no different to chance
 237 guessing.

238 **Perfusion and histology:** At the end of all experimental testing, rats were deeply
 239 anaesthetized with sodium pentobarbital (Euthanol 1.0 ml/rat, 200 mg/ml: Merial, Harlow,
 240 UK), and perfused transcardially with 0.9% (w/v) saline and 4% (w/v) paraformaldehyde in
 241 0.1M phosphate buffered saline (PBS). Brains were then removed and post-fixed overnight

242 (4°C), then incubated using 30% (w/v) sucrose in 0.1M PBS over 48h (4°C). Free floating
 243 sections (40 µm) were cut on a freezing microtome, the slices were then preserved in
 244 cryoprotectant (0.1M PBS containing 24% (v/v) glycerol and 24% (v/v) ethylene glycol) and
 245 stored at -20°C until required. MD thalamus lesion locations were histologically verified
 246 from cresyl violet stained brain sections.

247 **Lesion extent:** Photomicrographs of the Nissl-stained MD sections were captured using a
 248 camera mounted on a freestanding Leica DMR microscope (Leica Microsystems) using a
 249 1.6× objective so that the whole section could be visualised in the photomicrograph. MD
 250 volumes were measured using the NIH software ImageJ (<http://rsbweb.nih.gov/ij/>). In each
 251 rat, the intact MD volume from both the left and the right hemisphere was assessed. The final
 252 reading was calculated according to the Cavalieri principle (Regeur and Pakkenberg, 1989)
 253 and expressed as the percentage of MD Sham controls.

254 **Behavioral data analysis:** In the behavioral experiments, all rats performed a preoperative
 255 and postoperative session, and they completed the seven two-choice discrimination subtasks
 256 in an identical order. Data are expressed as mean and standard deviation. Preoperative and
 257 postoperative mean trials to criterion, shift cost, percent correct 2nd choice within trial
 258 responses, and latency to dig (seconds) were analysed using repeated measures ANOVAs
 259 with Stage (comprising the 7 subtasks) as the within-group repeated measure and
 260 Lesion_type (MD lesion or MD Sham control) as the between-group factor. For significant
 261 interactions, posthoc simple main effects analyses were performed using additional two-way
 262 ANOVAs for repeated measures (e.g., ID/ED shift) or independent t-tests for relevant
 263 subtasks (corrected for multiple comparisons). Univariate ANOVA was used to determine if
 264 the Shift between the two sensory dimensions between the MD lesion or MD Sham control

265 had an effect on the number of errors performed in the ED subtask. All statistical analyses
266 were calculated using SPSS 24 software. The significance level was set at $p < 0.05$.

267

268 **Results**

269 **MD lesion site**

270 Of the 25 adult rats involved in this experiment, 15 sustained MD lesions and 10 were MD
271 Sham controls (see Materials and Methods for details). All 15 MD lesion rats had extensive
272 excitotoxic (NMDA) lesion damage (see **Fig. 1**) within the medial and central subdivisions of
273 the MD, as intended. In all cases the lesion affected between 80–90% of the central and
274 medial MD, sparing only the lateral portions. The MD was consistently shrunken, and at the
275 lesion site there was significant cell loss. In all cases, the adjacent anterior thalamic nuclei
276 (ATN) were spared. In two cases, there was unilateral (right-sided) damage in the lateral
277 habenulae (LHb) and some damage to the central medial nucleus lying underneath the MD.

278

279 *Include Figure 1 here*

280

281 **The MD and attentional set-shifting**

282 Preoperatively, rats were pseudo-randomly assigned to either of two groups: MD lesion or
283 MD Sham control, based on the number of trials needed to reach the learning criterion (six
284 correct consecutive trials in nine trials) during the simple discrimination (SD) subtask.
285 Preoperative data from one MD Sham control rat had to be discarded. An independent
286 samples t-test confirmed there was no significant difference between the two groups during
287 preoperative learning of the SD, $t(22) = 0.095$, $p > 0.05$, with rats from both groups requiring
288 a similar number of trials to reach criterion, MD lesion (Mean = 9.67, S.D. = 2.44) and MD

289 Sham control (Mean = 9.78, S.D.= 3.31). Overall trials to criterion during the preoperative
 290 training session are presented in **Fig. 2A**.

291 Postoperatively, a repeated measures ANOVA with Stage (7 subtasks: SD, CD, REV1
 292 ID, REV2, ED, REV3) as the within-group factor and Lesion_type (MD lesion vs MD Sham
 293 control) as the between-group factor revealed a significant interaction, Stage $\times$ Lesion_type,
 294 $F(6,138) = 63.65$, $p < 0.001$, a significant main effect of Stage, $F(6,138) = 184.73$, $p < 0.001$,
 295 and a significant main effect of Lesion_type, $F(1,23) = 20.22$, $p < 0.001$ (see **Fig. 2B**). To
 296 explore the interaction effect, *posthoc* comparisons of the simple main effects of Stage
 297 (corrected for multiple comparisons, $p < 0.007$; $\alpha = 0.05$ divided by 7 tests) were
 298 computed. For the SD subtask, MD lesion rats required more trials to criterion ($M = 16.67$,
 299 S.D. = 1.23) compared with MD Sham controls ($M = 9.50$; S.D. = 2.07), indicating that the
 300 rats with the MD lesion were slower to acquire the new sensory discrimination. An
 301 independent samples t-test confirmed this difference was significant, $t(23) = 10.88$, $p < 0.001$.
 302 However, once the rats with MD lesion acquired the rewarded sensory dimension (to respond
 303 either to the digging media, or to the odor), they were unimpaired in subsequent subtasks that
 304 maintained the same rewarded sensory dimension, namely concurrent discrimination (CD):
 305 $t(23) = 0.00$, $p = 1.0$, and intra-dimensional shift (ID): $t(23) = 1.08$, $p < 0.291$. MD lesion rats
 306 were also not impaired in the reversals (REV1 and REV2) of the reward contingencies
 307 associated with the learned exemplars completed after the CD subtask, REV1: $t(23) = 2.31$, p
 308 < 0.030 , or after the ID subtask, REV2: $t(23) = 1.34$, $p < 0.192$, although, all rats were
 309 familiar with the reversal rule as they had completed the ID/ED task preoperatively (see
 310 Materials and Methods). **Extended Data Figure 2-1** shows the mean and 1st and 3rd quartile
 311 box plots with the smallest and largest numbers of postoperative trials to criterion (whiskers)
 312 for the individual rats during each of the subtasks.

313

314 *Include Figure 2 here*

315

316 For the extra-dimensional (ED) subtask, the rats had to shift their attention to the
 317 previously irrelevant (unrewarded) sensory dimension. Thus, given the rats had acquired the
 318 attentional set strategy, as expected, all rats required more trials to criterion to learn the ED
 319 subtask. MD Sham controls required ($M = 15.70$; $S.D. = 1.64$) more trials to learn the ED
 320 when compared to trials required to learn the ID ($M = 12.5$; $S.D. = 1.84$). Similarly, the MD
 321 lesion rats required more trials ($M = 23.47$, $S.D. = 2.07$) compared with trials to learn the ID
 322 ($M = 11.73$; $S.D. = 1.67$; see **Fig. 2B**). A repeated measures ANOVA computing the trials to
 323 criterion for these two subtasks (ID vs ED) showed a significant Stage x Lesion_type
 324 interaction, $F(1,23) = 99.96$, $p < 0.001$, a significant effect of Stage, $F(1,23) = 306.11$, p
 325 < 0.001 , and a significant effect of Lesion_type, $F(1,23) = 32.83$, $p < 0.001$ (see **Fig. 2B**). The
 326 interaction occurred as the MD lesion rats required significantly more trials to criterion to
 327 consistently implement a new choice strategy in order to learn which of two stimuli was
 328 rewarded from the previously ignored sensory dimension compared to the MD Sham
 329 controls, $t(23) = 10.00$, $p < 0.001$.

330 Interestingly, for REV3, the reversal subtask that occurred after the ED shift, MD
 331 lesion rats required fewer trials to criterion ($M = 12.13$, $S.D. = 1.25$) compared with the MD
 332 Sham controls ($M = 16.7$; $S.D. = 2.26$; see **Fig. 2B**). An independent samples t-test confirmed
 333 this difference was significant, $t(23) = 6.51$, $p < 0.001$, suggesting a facilitation in
 334 performance as a consequence of over-training experienced during the ED subtask (see
 335 discussion for interpretation).

336 The additional repeated measures ANOVA of Session (Preoperative vs Postoperative)
 337 shift cost (see **Fig. 3A**) between Lesion_type computed as the number of trials to criterion for
 338 the ED subtask minus the number of trials to criterion for the ID subtask for each session

339 showed a significant Session x Lesion_type interaction, $F(1,22) = 44.06$, $p < 0.001$, a
 340 significant effect of Session, $F(1,22) = 38.27$, $p < 0.001$, and a significant effect of
 341 Lesion_type, $F(1,22) = 15.46$, $p = 0.001$ (see **Fig. 3A**). The significant interaction occurred as
 342 there was a small shift cost for both groups during the preoperative test session. However,
 343 during the postoperative test session only the MD lesion rats showed a significant shift cost
 344 as a consequence of the task demands changing that required an adjustment to the previously
 345 well-established response strategy ($p < 0.001$). By contrast, the MD Sham controls showed a
 346 similar shift cost to their preoperative performance.

347

348 *Insert Figure 3 here*

349

350 **Within trial choice responses as a measure of rapid adaptability after MD lesion**

351 Given the observed deficits in the MD lesion group, we investigated the behavioral responses
 352 made in the MD lesion or MD Sham control rats. Given the task design and a criterion of six
 353 consecutive correct responses before switching to the next stage, overall during testing, the
 354 rats do not complete many trials. Nevertheless, we could determine whether the rats rapidly
 355 adapted their choice responses in the here and now (within trial choice responses) as
 356 measured by the number of correct 2nd choice within trial responses made, i.e. when the rat
 357 dug from the correct bowl only after visiting the incorrect bowl first but without digging in it,
 358 divided by the total number of correct responses (correct 1st and 2nd choice responses
 359 combined) for each session. During each subtask of the session, we recorded if the rat made a
 360 correct choice, either on the first attempt ('1st choice response'), or on the 2nd attempt ('2nd
 361 choice within trial response'). We did not include error trials in this analysis.

362 A repeated measures ANOVA of total percent correct 2nd choice within trial
 363 responses was conducted with Session (Preoperative vs Postoperative) as the repeated
 364 measure $\times$ Lesion_type revealed a significant interaction of Session $\times$ Lesion_type, $F(1,21)$
 365 $= 9.40$, $p = 0.006$, a significant main effect of Session, $F(1,21) = 57.06$, $p < 0.001$, and a
 366 significant main effect of Lesion_type, $F(1,21) = 7.18$, $p = 0.014$ (see **Fig. 3B**). To
 367 investigate the interaction effect, *posthoc* comparisons of the simple main effects showed that
 368 the MD lesion rats ($M = 28.18$, $SD = 7.61$) made fewer correct 2nd choice within trial
 369 responses compared to the MD Sham controls ($M = 35.23$, $SD = 8.46$). This deficit suggests
 370 the MD lesion rats had a diminished ability to rapidly bind together their previous choice
 371 about what stimuli are worth sampling in order to update their choice response strategy
 372 within the trial, rather than a deficit in perseverative responding, or an inability to respond to
 373 negative feedback.

374 In addition, we analyzed the rats' errors made reaching criterion during the ED
 375 subtask, as a consequence of whether they experienced a sensory dimension shift from odor
 376 to digging medium or digging medium to odor, which indicated that the increase in errors
 377 occurs irrespective of the direction of the attentional shift. The univariate ANOVA with
 378 Lesion_type and Sensory Shift as the between-subject factors and errors to criterion for the
 379 ED subtask as the dependent measure revealed a main effect of Lesion_type, $F(1, 21) =$
 380 106.03 , $p < 0.001$, but no main effect of Sensory Shift, $F(1, 21) = 3.84$, $p = 0.063$ and no
 381 interaction effect, $F(1, 21) = 0.19$, $p = 0.666$.

383 **Latency to dig changes**

384 Latency to dig (in seconds) for error trials and correct trials were also computed for each rat
 385 in preoperative (see **Fig. 4A; 4C**) and postoperative (see **Fig. 4B; 4D**) analyses. Separate
 386 repeated measures ANOVAs were used to assess the latency to dig for the preoperative or

387 postoperative sessions for either error trials or correct trials with Stage (7 subtasks: SD, CD,
 388 REV1 ID, REV2, ED, REV3) as the within-group factor and Lesion-type (MD lesion vs MD
 389 Sham control) as the between-group factor.

390 For error trials (**Fig 4C**), the repeated measures ANOVA for the preoperative session
 391 revealed a significant main effect of Stage, $F(1,21) = 23.99$, $p < 0.001$, but no effect of lesion,
 392 and no interaction, F 's < 1.0 . This result indicates there was no difference in response times
 393 between the pseudo-randomly assigned groups (MD lesion and MD Sham controls)
 394 preoperatively and that all rats responded quicker on errors trials as they moved through
 395 subsequent subtasks within the session.

396 In contrast, the repeated measures ANOVA of latency to dig during postoperative
 397 error trials (**Fig. 4D**) revealed a significant interaction of Stage $\times$ Lesion_type, $F(6,132) =$
 398 26.81 , $p < 0.001$, a significant main effect of Stage, $F(6,132) = 22.45$, $p < 0.001$, and a
 399 significant main effect of Lesion-type, $F(1,22) = 57.15$, $p < 0.001$. To investigate the
 400 interaction effect, *posthoc* comparisons of the simple main effects (corrected for multiple
 401 comparisons) were computed. Postoperatively, MD lesion rats responded slower on error
 402 trials during the SD subtask, $t(22) = 10.09$, $p < 0.001$, the CD subtask, $t(22) = 7.65$, $p < 0.001$,
 403 and the REV1 subtask, $t(22) = 4.44$, $p < 0.001$. However, for the final reversal subtask, REV3,
 404 completed after the ED shift, MD lesion rats were quicker on the error trials than MD Sham
 405 controls, $t(22) = 3.53$, $p = 0.002$. This result in combination with the reduced trials to
 406 criterion required to complete this subtask (see **Fig. 2A**) suggests that the MD lesion rats may
 407 have experienced overtraining due to the number of additional trials to criterion required to
 408 adapt their choice responding in the ED subtask (Dhawan et al., 2019). For correct trials,
 409 repeated measures ANOVAs for the preoperative or postoperative session showed no
 410 significant main effects and no significant interaction (p 's > 0.05).

411

412 *Insert Figure 4 here*

413

414 **4.0 Discussion**

415 This set of experiments investigated the active influence of rat mediodorsal thalamus (MD) in
416 cognitive flexibility. More specifically, we examined whether rats with excitotoxic lesions of
417 the central and medial MD were impaired in performing an attentional set-shifting task that
418 measures intra-dimensional (ID) and extra-dimensional (ED) shifts in attention to sensory
419 discriminations (digging media or odors). Our new results indicate that rats with MD lesions
420 required more trials to criterion to learn the optimal response strategy during the initial
421 simple discrimination (SD). Although, once this stable response strategy had been learnt, MD
422 lesion rats were able to attain an attentional set strategy as they acquired further two-choice
423 discriminations involving the same sensory dimension during the CD and ID shifts, and
424 during reversals (REV1 and REV2) of the reward contingencies associated to the exemplars.
425 However, when the relevant sensory dimension was switched, for the ED subtask, the MD
426 lesion rats were markedly impaired and required many more trials to update their choices
427 using a new response strategy, as measured by the significant ED shift cost. Further, for
428 REV3, the third reversal performed after the ED shift, the MD lesion rats showed facilitated
429 learning, suggesting they had received ‘overtraining’ as a consequence of completing many
430 more trials to reach criterion in the ED subtask (see below). The analyses of behavioral
431 responses indicated that the MD lesion rats made significantly fewer correct 2nd choice
432 within-trial responses suggesting that information transfer via the MD is critical for the rapid,
433 within-trial monitoring and updating of an optimal choice response. Interestingly, similar
434 updating deficits in choice responding have been previously reported in rhesus macaques
435 with magnocellular MD excitotoxic lesions (Chakraborty et al., 2016).

436 *Learning and updating*

437 These behavioral data accord somewhat with previous studies conducted in different
 438 species. For example, marmosets with lateral PFC lesions (Dias et al., 1996) or rats with
 439 medial PFC lesions (equivalent frontal regions; Birrell and Brown, 2000) are also impaired at
 440 the ED subtask, showing a similar ED shift cost as our MD lesion rats. However, unlike our
 441 MD lesion rats, these marmosets and rats did not need more trials to reach criterion during
 442 the SD subtask. Instead, the MD lesion rats' learning deficit during the SD subtask accords
 443 with rats that had anterior thalamic nuclei (ATN) lesions, who similarly required more trials
 444 to acquire the SD subtask (Wright et al., 2015), although these ATN lesion rats were not
 445 impaired during the ED subtask. Instead, and in contrast to our MD lesion rats, rats with ATN
 446 lesions continued to be impaired during the CD subtask and subsequent ID shifts (Wright et
 447 al., 2015) indicating that they never properly learnt the discrimination rule, which suggests an
 448 intact ATN (a brain structure adjacent to the MD but with markedly different cortico-
 449 thalamo-cortical connectivity) is important for supporting the formation of an attentional set
 450 strategy.

451 As already indicated, with more trials, our rats with MD lesions were able to learn the
 452 SD subtask to criterion and apply the optimal rule during subsequent subtasks. For the SD
 453 subtask, trials revert back to presenting only one sensory dimension and two stimulus
 454 exemplars. Other studies also confirm MD lesion rats are impaired in acquisition of an initial
 455 task rule (e.g. Hunt and Aggleton, 1998). Other rodent studies causing temporary
 456 perturbations to the MD also show impaired discrimination learning (Ferguson and Gao,
 457 2018; Courtiol et al., 2019). Thus these results from across species reinforce the notion that
 458 the MD is not simply mimicking the PFC (DeNicola et al., 2020; Miller et al., 2017; Mitchell
 459 et al., 2007; Mitchell and Gaffan 2008; Mitchell et al., 2014). Consequently the influence of
 460 other cortico-cortical interactions that also indirectly transfer information to the cortex via the

MD and other thalamic structures, e.g. those PFC interactions with limbic structures in the temporal lobes and sensory association areas are also critical during learning sensory discriminations (Banjeree et al., 2020; Bueno-Junior et al., 2018; Chakraborty et al., 2019; Floresco and Grace, 2003; Mitchell, 2015; Pelekanos et al., 2020).

However, during the ED subtask, our MD lesion rats were markedly impaired at rapidly adapting this now well-established choice response strategy when they had to attend to the previously irrelevant (non-rewarded) sensory dimension. The demands on cognitive flexibility, response inhibition, and adapting behavioral responses after negative feedback are increased when the animals must make an ED shift (Dhwana et al., 2019). The increased effort involved in adapting to these changes in attention is measured by a shift cost, and it is expected that more trials to criterion are required during learning the ED than during learning the ID shift. While the ID/ED shift cost was higher for both groups (Fig. 2B), it was significantly increased for the MD lesion rats compared to the MD Sham controls. As indicated, increased shift costs have also been observed in marmosets and rats with lesions to the comparative PFC regions (Dias et al., 1996; Birrell and Brown, 2000). Surprisingly though, rats with damage to the nucleus reuniens (Re), another thalamic structure located near the MD, which interconnects the medial PFC and hippocampus (Hoover and Vertes, 2007; Hoover and Vertes, 2011; Vertes et al., 2015) do not cause deficits in the ED subtask or produce a significant ED shift cost. Instead, rats with NRe damage are impaired at acquiring the attentional set strategy, similar to rats with ATN lesions (Linley et al., 2016). Thus, our MD thalamus results are unique.

Evidence from studies in mice suggests the MD supports the frontal cortex to sustain intra-cortical attentional control without transferring categorical information about a particular task rule (Schmitt et al., 2017), or that medial PFC-MD projections are important for behavioral flexibility but not task engagement (Marton et al., 2018; Nakayama et al.,

2018). Further, the MD has been shown to have a critical role in maintaining the balance between excitation and inhibition in dorsomedial PFC via its influence on interneurons (Anastasiades et al., 2021; Ferguson and Gao, 2018) and pyramidal neurons (Collins et al., 2018; Delevich et al., 2015). Our results are supportive of these circuit level interactions, although our deficits indicate the influence of MD on frontal circuits is specific to certain aspects of the attentional set-shifting task, e.g. linked to the ED shift and learning the optimal choice response strategy, but not to the ID shift or completing reversals. However, our study involved permanent MD lesions, which are likely to have caused some adaptations across the network, while the studies in mice likely involve short-term changes as temporary inactivation of the MD was employed. Nevertheless, it may be proposed that after MD perturbations, while the animal is still able to detect a change in the task demands (i.e. they are responsive to negative feedback), they are impaired at rapidly adapting their behavior and coordinating correct choice responses within a trial after a change to the already established choice response strategy (Block et al., 2007; Hunt and Aggleton, 1998; Chakraborty et al., 2016). Interestingly though, in both these rodent studies (Block et al., 2007; Hunt and Aggleton, 1998), the learning deficits were attributed to increased perseverative responding. Others have also observed increased perseverative responding after MD perturbations (Ferguson and Gao, 2018). In contrast, our current rodent work and previous work in rhesus macaques has shown an intact MDmc is necessary for rapid reward guided learning of complex discriminations (Mitchell et al., 2007a; Chakraborty et al., 2016; Chakraborty et al., 2019), although the deficits linked to learning in monkeys are attributed to increased response switching (Mitchell et al., 2007a; Chakraborty et al., 2016). These congruent results across species after MD damage suggest that cortical information transfer via the MD is particularly important when rapid, within a trial changes in choice response strategies linked to establishing a new rule are required.

511 *Reversal learning*

512 For the reversal subtasks (REV1 and REV2), somewhat surprisingly, our MD lesion
 513 rats were unimpaired during reversals in the reward contingencies. Rats and marmosets with
 514 comparable medial PFC damage are also not impaired in reversal learning subtasks (Dias et
 515 al., 1996; Birrell and Brown, 2000). Instead, OFC perturbations cause deficits in the reversal
 516 subtasks, while leaving performance intact during the SD and ED subtasks of the ID/ED task
 517 (Dias et al., 1997; McAlonan and Brown, 2003; Chase et al., 2012). These observations
 518 accord with a large body of research indicating that separate mammalian PFC subregions
 519 differentially contribute to learning, memory, and other cognitive functions (e.g. Clark et al,
 520 2004; Rudebeck et al., 2008; for review, see Dalley, Cardinal and Robbins, 2004; Uylings,
 521 Groenewegen and Kolb, 2003; Izquierdo et al., 2017).

522 MD is reciprocally connected to the OFC and medial PFC (Groenewegen, 1988;
 523 Preuss, 1995; Ray and Price, 1992). Thus this double dissociation in deficits between these
 524 reciprocally interconnected MD-OFC and MD-medial PFC brain regions needs to be
 525 reconciled. First, it must be noted that the MD rats had experienced all of the subtasks (and
 526 thus were familiar with the concept of reversal learning and that it can appear in the task
 527 structure) during their preoperative test session, which may have reduced or eliminated the
 528 reversal learning deficit (Costa et al., 2015; Jang et al., 2015). Further, for the MD, while
 529 previous evidence of reversal learning deficits are reported for some tasks after MD
 530 perturbations (Block et al., 2007; Chudasama et al., 2001; Ferguson and Gao, 2018; Hunt and
 531 Aggleton, 1998; Ostlund and Balliène, 2008; Parnaudeau et al., 2013, 2015), the evidence is
 532 mixed (Beracochea et al., 1989; Alcaraz et al., 2018; Fresno et al., 2019). These mixed
 533 effects may be the consequence of differences in task structure and the way that the reversal
 534 is introduced. Other factors, including the extent of disruption caused to thalamic structures
 535 adjacent to the MD may also be a factor (Mitchell and Chakraborty, 2013; Wolff and Vann,

2019). Indeed, one recent study has identified that another thalamic structure nearby to the MD - the submedial thalamus - may contribute a role in reversal learning, instead of the MD (Fresno et al., 2019). Consequently, our current results and this above evidence highlights that the information transfer between OFC and MD is not always critical for reversal learning per se. Taken together, it is clear that the MD is not simply mimicking the behavioral deficits observed after PFC damage, and instead shows that the MD and different subregions of PFC are supporting different aspects of cognitive flexibility. At least in monkeys, OFC-MDmc interactions are critical for adaptive, value based decision-making (Browning et al., 2015; Izquierdo and Murray, 2010; Mitchell et al., 2007b), while in rodent studies, the OFC-striatal part of the cortico-striatal-thalamic neural circuits have been implicating in reversal learning (Gremel and Costa, 2013; Izquierdo et al., 2017; Nakayama et al 2018; Yang et al., 2020; Yin et al., 2005) or OFC-submedial thalamus circuits (Fresno et al., 2019). Other OFC-striatal-thalamic and OFC-thalamic interactions are potentially more involved in supporting reversal learning. In addition, thalamic inputs from the intralaminar thalamic nuclei and motor thalamus to the striatum contribute a selective role in inhibitory control and behavioral flexibility (e.g. Saund et al., 2017).

Intriguingly, our additional analyses used to investigate the types of behavioral deficits occurring after MD lesions showed that our rats made fewer correct 2nd choice within-trial responses. Given that our MD lesion rats required more trials to criterion to learn the SD and ED subtasks, this change in behavioral responses suggests that without the MD thalamus, our rats could not rapidly adapt their choice responses as the trial was progressing (within-trial) after the changed task demands. However, this reduced correct 2nd choice within-trial responding does not suggest the rats adopted perseverative responding (they would have potentially been impaired in reversal learning if they had) or were not learning about the stimulus and associated rewards. We can conclude this because, after acquiring the

561 ED shift, the MD lesion rats required fewer trials to criterion to learn the final reversal
 562 subtask, REV3. This facilitation of learning during the final reversal subtask suggests that the
 563 MD lesion rats had experienced ‘overtraining’ on all of the stimulus features associated with
 564 each sensory dimension as they required more trials to learn the ED subtask (Dhawan et al.,
 565 2019). It is well established that overtraining rats during discrimination learning can
 566 eliminate any reversal learning deficits (Reid, 1953; Lovejoy, 1966; Nilsson et al., 2015 for
 567 review). As Pearce and Mackintosh (2010) indicate, until learning is fully consolidated, all
 568 stimulus features continue to be attended to and this seemed to be so for our rats, even with
 569 the MD lesion. Thus, we propose, in accord with others, that through experiencing these extra
 570 trials during the ED subtask, the MD lesion group may have developed a greater
 571 understanding of the rewarded and unrewarded stimulus dimensions, thus increasing the
 572 salience of the predictive cues and reducing/eliminating the number of factors that can lead to
 573 an error. In essence, these extra trials in the ED subtask helped our MD lesion rats to slowly
 574 learn the optimal response strategy so when the final reversal was encountered, they were
 575 readily able to continue to implement this optimal response strategy for the sensory
 576 dimension while simply reversing the two stimulus exemplars (something they were already
 577 well-practiced at doing). An MD lesion does not render an animal or human densely amnesic
 578 or cause them to be insensitive to the receipt of rewards (Mitchell, 2015). Instead, we are
 579 proposing that after the loss of the MD, an animal or human is unable to rapidly learn a new
 580 choice response strategy or rapidly update a well-established optimal one.

581 However, in the Birrell and Brown (2000) study, rats with medial PFC lesions did not
 582 show a facilitation of learning during the final reversal subtask after showing a similar ED
 583 shift cost to our MD lesion rats. We cannot be certain why medial PFC lesion rats also did
 584 not show a facilitation of learning in the final reversal subtask, although exploring whether
 585 they experience overtraining is worthy of further investigation. What is known is that the

586 medial PFC is proposed to contribute to learning via supporting the processing of error
587 feedback related to adapting stimulus-action selection. For example, during instrumental
588 learning, medial PFC lesions in rats contribute to the acquisition but not the expression of
589 goal-directed actions (Ostlund and Balleine, 2005).

590 There may be other explanations for the SD and ED impairments observed in our MD
591 lesion rats. With the ID/ ED task, it is important to establish that the animals understand the
592 relevant sensory dimension that is rewarded across the related subtasks within the session.
593 Fortunately, in our animals, this transfer of knowledge was evident as the MD lesion rats
594 showed reliable learning in the subtasks, CD, REV1, ID and REV2, that required the
595 implementation of choice responses (follow the same rule) to the same sensory dimension as
596 the SD to receive reward. Further, both Sham controls and MD lesion rats showed similar
597 numbers of trials to criterion during the REV1 and REV2 subtask, which indicates that they
598 did not favor one feature of the stimulus dimension more than the other or that they were
599 insensitive to negative feedback. However, we only used male rats in this current study so the
600 results might not transfer to female rats. Additionally, the MD is a subcortical node in the
601 olfactory neural circuitry that also includes the OFC (Courtiol et al., 2015; Veldhuizen et al.,
602 2020). However, evidence collected from rodents with MD perturbations or humans with
603 strokes affecting the MD indicates that changes to the MD do not impair olfactory
604 discriminations (Courtiol et al., 2019; Tham et al., 2011). Moreover, our MD lesion rats
605 showed similar levels of olfactory discriminations as the MD Sham controls (see Material
606 and Methods for further details).

607 Finally, the diffuse influence of the MD thalamocortical inputs to several frontal
608 cortex structures (Alcaraz et al., 2016; Kuramoto et al., 2017; Mitchell, 2015; Wolff and
609 Vann, 2019) supports previous findings implicating medial PFC-MD connectivity in flexible
610 behaviors (Marton et al., 2018; Nakayama et al., 2018). Furthermore, the MD along with the

OFC provides additional evidence for the contribution of these areas in supporting online, ‘here and now’ reward-guided learning and decision-making (Gardner et al., 2017; Gardner et al., 2019; Schoenbaum et al., 2011; Wallis, 2007). Intact OFC connectivity is essential to the animals’ ability to perform reward-guided learning in order that other interconnected neural networks, likely including cortico-cortical, cortico-striatal, and cortico-thalamo-cortical connectivity, can determine the optimal choice responses and implement the appropriate actions (Browning et al 2015; Chakraborty et al 2016; Nakayama et al 2018; Perry et al., 2021; Rushworth et al., 2011).

The ID/ ED task is analogous to the Wisconsin Card Sorting Test (WCST) in humans. In healthy humans performing the WCST during neuroimaging, the MD is activated during responding to negative feedback after the choice has been executed (Monchi et al., 2001). Unfortunately, thus far, humans with stroke damage in the MD have cognitive deficits that are clinically very poorly defined (Pergola et al., 2018). However, people diagnosed with Alzheimer’s disease (AD), Parkinson’s disease (PD), or schizophrenia show impaired attentional set-shifting performance (e.g. Barch et al., 2009; Monchi et al., 2004; Owen et al, 1993). Neuroimaging and post-mortem studies show marked changes in the MD and/ or ATN in these diseases (Hornberger et al., 2012; Ouhaz et al., 2018; Pergola et al., 2015; Perry et al., 2018). Our current evidence advocates for studies investigating cortico-thalamocortical transfer of information in people diagnosed with AD combined with more frontal pathology, or PD, or schizophrenia.

To conclude, excitotoxic damage to the rodent central and medial MD selectively increased the trials to criterion on the ED subtask, producing a shift cost. This selective performance deficit is similar to monkeys with lateral PFC inactivation (Dias et al., 1996; Dias et al., 1997) and rats with medial PFC lesions (Birrell and Brown, 2000). Further, this deficit after MD lesions contrasts with monkeys or rats with perturbations to the OFC, who

636 are selectively impaired on reversal learning, but not on ED shifts (Chase et al., 2012; Dias et
637 al., 1997; McAlonan and Brown, 2003). As evidenced, the frontal cortex is critically involved
638 in value-based decision-making and reward-guided learning (Miller, 2000; Rushworth et al.
639 2011; Wallis, 2007). However, cortico-thalamocortical connections also contribute a role
640 (Browning et al. 2015; Chakraborty et al., 2016; Izqueirdo and Murray, 2010; Mitchell et al.,
641 2014; Pelekanos et al., 2020). In rodents, the MD and the medial PFC together appear crucial
642 for binding reward information and behavior (Bradfield et al., 2013; Corbit et al., 2003), as
643 inhibition of dorsomedial PFC-projecting MD neurons results in rats having difficulties with
644 tracking changes in action-outcome contingencies (Alcaraz et al., 2018). In the current study,
645 we show that rat medial and central MD are critical for rapidly updating an optimal choice
646 response strategy. That is, when the MD is damaged, there is a diminished ability to rapidly
647 learn (within a trial) a choice response strategy, as well as update a well-established one as
648 task demands change. Behaviorally, our MD lesion rats made less correct 2nd choice within-
649 trial responses, suggesting they could not rapidly alter their choice response strategy when
650 they encountered the unrewarded stimuli on a given trial.

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937 **Figure Legends**

938

939 **Figure 1. A.** Photomicrographs of the thalamus in a MD Sham control rat (top) and a MD
940 lesion rat (bottom). **B.** A series of coronal schematics throughout the mediodorsal thalamus
941 showing the area of cell loss in the MD lesion group. Numbers refer to the distance from
942 bregma (Paxinos and Watson, 2008). Abbreviations: 3V: third ventricle; dLGN: dorsal lateral
943 geniculate nucleus; LHb: lateral habenula; LD: laterodorsal thalamus; MD: mediodorsal
944 thalamus; MHb: medial habenula; PV: paraventricular nucleus; VPM: ventral posterior
945 medial thalamus.

946

947 **Figure 2.** Mean (+SEM) trials to criterion data for the attentional set-shifting task. **(A)**
948 Preoperative test session. **(B)** Postoperative test session. MD lesion rats were significantly
949 slower to learn the SD and the ED subtasks compared to MD Sham controls ($*p < 0.001$). MD
950 Sham control rats, like MD lesion rats, needed more trials to learn the ED, as expected.
951 However, the MD lesion rats required significantly fewer trials to criterion to learn REV3,
952 the reversal occurring immediately after the ED, compared to the MD Sham controls ($*p$
953 < 0.001). Also see the **Extended Data Figure 2-1** showing boxplots of the mean trials to
954 criterion for individual rats during the postoperative session of the attentional set-shifting
955 task.

956

957 **Extended Data Figure 2-1** showing boxplots of the mean trials to criterion for individual
958 rats during the postoperative session of the attentional set-shifting task. MD lesion rats took
959 significantly more trials to learn the SD and the ED subtasks compared to MD Sham controls
960 ($*p < 0.001$). MD Sham control rats also needed more trials to learn the ED, as expected. The
961 MD lesion rats required significantly fewer trials to learn REV3, the reversal occurring

962 immediately after the ED, compared to the MD Sham controls ($*p < 0.001$). Box shows 1st
 963 and 3rd quartile and whiskers are the minimum and maximum trials to criterion for individual
 964 rats.

965

966

967 **Figure 3.** (A) Mean (+SEM) trials to criterion shift cost data for the ED stage for
 968 preoperative (Pre) and postoperative (Post) testing sessions. The shift cost is computed as the
 969 number of trials to criterion in the ED minus the number of trials to criterion in the ID. Both
 970 groups of rats showed a shift cost during the ED, when they had to learn to attend to the
 971 previously irrelevant dimension. However, the shift cost for the MD lesion rats in the
 972 postoperative test session was significantly different to the MD Sham controls. (B) Mean
 973 (+SEM) percent correct 2nd choice within trial responses made during the preoperative and
 974 postoperative performance sessions of the attentional set-shifting task. MD lesion rats made
 975 fewer correct 2nd choice within trial responses than MD Sham controls ($*p's = 0.001$).

976

977 **Figure 4.** Mean (+SEM) latencies to dig (secs) for MD Sham controls and MD lesion rats
 978 during each subtask of the ID/ED attentional set shifting task (A) Preoperative correct trials.
 979 (B) Postoperative correct trials. (C) Preoperative error trials. (D) Postoperative error trials ($**$
 980 $p < 0.001$; $*p = 0.002$).

Table 1. Sensory dimensions (odors and digging media) and stimulus features (O1–O6; M1–M6) used in the different subtasks of the ID/ED attentional set-shifting task.

Odors		Digging media	
O1 - Cinnamon	O2 - Ginger	M1 – Coarse tea	M2 – Fine tea
O3 - Sage	O4 - Paprika	M3 - Sand	M4 - Grit
O5 - Turmeric	O6 - Cloves	M5 - Coarse shavings	M6 – Fine shavings