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A novel 3-choice touchscreen task to examine spatial attention and orienting responses in rodents

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1 **1. A novel 3-choice touchscreen task to examine spatial attention and orienting responses in**
2 **rodents**

3

4 **2. Running title:** Touch-screen orienting task

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47 deactivation, Long-Evans rats.

48

49 **Abstract**

50 Mammalian orienting behaviour consists of coordinated movements of the eyes, head, pinnae,
51 vibrissae, or body to attend to an external stimulus. The present study aimed to develop a novel
52 operant task using a touch-screen system to measure spatial attention. In this task, rats were trained
53 to nose-poke a light stimulus presented in one of three locations. The stimulus was presented more
54 frequently in the center location to develop spatial attention bias towards the center stimulus.
55 Changes in orienting responses were detected by measuring the animals' response accuracy and
56 latency to stimuli at the lateral locations, following reversible unilateral chemogenetic inactivation
57 of the superior colliculus (SC). Additionally, spontaneous turning and rotation behaviour was
58 measured using an open field test (OFT). Our results show that right SC inactivation significantly
59 increased the whole body turn angle in the OFT, in line with previous literature that indicated an
60 ipsiversive orientating bias and the presence of contralateral neglect following unilateral SC
61 lesions. In the touch screen orienting task, unilateral SC inactivation significantly increased bias
62 towards the ipsilateral side, as measured by response frequency in various experimental conditions,
63 and a very large left-shift of a respective psychometric function. Our results demonstrate that this
64 novel touchscreen task is able to detect changes spatial attention and orienting responses, due to
65 e.g. experimental manipulations or injury, with very high sensitivity while taking advantage of the
66 touch screen technology that allows for high transferability of the task between labs and for open
67 source data sharing through mousebytes.ca.

68 **Significance**

69 Touch-screen rodent testing is a novel translational method of behavioural testing that is more
70 comparable to test batteries used in humans, such as the Cambridge Neuropsychological Test
71 Automated Battery (CANTAB). Its standardized approach in closed boxes allows for better
72 comparability of data between labs and for open source data sharing at the affiliated platform
73 “mousebytes.ca”. The goal of this study was to expand the toolbox for touch-screen boxes to
74 investigate orienting behavior and spatial attention. Unilateral reversible chemogenetic inhibition

75 of the SC revealed an ipsiversive orientating bias and the presence of neglect-like effects for
76 contralateral visual stimuli, demonstrating that this novel task is highly sensitive to detect
77 disruptions of spatial attention associated with psychiatric disorders, brain injury, or experimental
78 manipulations.

79

80 **Introduction**

81 Orienting behaviour in mammals consists of highly coordinated movements of the eyes, head,
82 pinnae, vibrissae, and body towards salient sensory stimuli. Sensory information relevant to spatial
83 orienting, such as visual stimulus location, is represented topographically in the superior colliculus
84 (SC) across a wide range of vertebrate species (Gaither and Stein, 1979; May, 2006). The SC is a
85 laminar midbrain structure that is critical for the generation of orienting behaviours, serving the
86 goal of aligning the sensory apparatus of an animal with objects of interest in the surrounding
87 environment. Consistent with the evolutionarily highly conserved nature of this structure, SC
88 lesions result in severe orienting impairments in a range of vertebrate species as diverse as tree
89 shrews (Casagrande and Diamond, 1974), cats (Sprague and Meikle, 1965), and nonhuman
90 primates (Schiller et al., 1987). Along the same line of evidence, electrical microstimulation of the
91 SC has revealed a topographic organization of orienting behaviours. In the rhesus macaque,
92 stimulation of rostral regions of the SC evokes small amplitude contraversive saccades (Robinson,
93 1972), while stimulation of the caudal SC evokes large amplitude contraversive saccades and head
94 movements (Corneil et al., 2002; Freedman et al., 1996; Robinson, 1972). Saccades are rapid eye
95 movements common to primate species that form a considerable part of their orienting behavior
96 by moving their fovea to the visual stimuli of interest. Although rodents do not possess a well-
97 defined fovea to produce the same kind of saccades, electrical stimulation of the SC in rodents has
98 evoked contraversive movements of the eyes and coordinated eye, head, pinnae, vibrissae and
99 whole-body circling (McHaffie and Stein, 1982; Northmore et al., 1988). Unilateral lesions of the
100 SC in rodents result in two classic changes in orienting behaviour: a tendency to circle in the
101 direction of the lesioned SC (ipsilesional circling), and an inability to localize relevant stimuli in
102 the hemifield contralateral to the lesioned SC (contralesional neglect; Dean et al., 1984; Duan et
103 al., 2015; Felsen and Mainen, 2008; Kirvel et al., 1974; Kopec et al., 2015; Schneider, 1969;
104 Sprague and Meikle, 1965; Tehovnik, 1989). The former deficit has been characterized as a failure
105 of motor implementation of orienting, while the latter has been ascribed to changes in spatial

106 attention, i.e. a failure of visual selection of relevant information akin to visual neglect (Krauzlis
107 et al., 2013; Sinnamon and Garcia, 1988). Contraversive neglect has typically been evaluated by
108 presenting animals with various visual stimuli along the edges of their visual field or in operant
109 testing paradigms that require orienting towards different stimuli in order to receive a reward
110 (Felsen and Mainen, 2008; Kopec et al., 2015; Schneider, 1975, 1967; Sinnamon and Garcia, 1988;
111 Stubblefield et al., 2013). More recently, touchscreen testing platforms have become available for
112 rodent studies in an attempt to increase translation from rodents to human experimental tasks.
113 Behavioural testing with touch screens is more directly comparable to test batteries used in
114 humans, such as the Cambridge Neuropsychological Test Automated Battery (CANTAB): it uses
115 the same types of stimulus materials (objects and locations on a computer screen), and the same
116 types of responses (responses directly to the stimuli on the screen using a touchscreen apparatus),
117 along with precise control of the timing and identity of visual stimuli (Bussey et al., 2006). Touch
118 screen systems are very versatile, offering flexible means of investigating visual and cognitive
119 function in rodents, and they allow to test behavior between labs in a standardized way (Bussey et
120 al., 1997b, 1997a, 1994).

121 So far, orienting behavior in rodents has not been studied within a touchscreen-based system. We
122 therefore aimed to develop a task to test for spatial attention and orienting responses in a touch
123 screen system and to validate the sensitivity of the system by inactivating the SC unilaterally in
124 rats. Instead of chronic lesions, pharmacological inhibition, or optogenetic approaches, we used
125 the expression of designer receptors exclusively activated by designer drugs (DREADDs; Burnett
126 and Krashes, 2016), allowing for counterbalanced within-subject comparisons with each animal
127 being its own baseline, without any restrictions in movement after injection of the designer drug
128 Clozapine-N-Oxide (CNO) or the vehicle.

129

130 **Materials and Methods**

131 *Animals and group overview*

132 Adult Long Evans rats, weighing 250–350g, were obtained from Charles River Canada (St.
133 Constant, QC, Canada). Animals were housed in pairs (except during recovery period after
134 surgery) at a temperature of $21 \pm 1^\circ\text{C}$ in a 12h light/dark cycle with lights on at 7a.m., and food
135 and water available *ad libitum*, except during the weeks encompassing touchscreen training and
136 testing, where the animals were food restricted. All animal procedures were approved by the the

137 University of Western Ontario's Animal Care Committee and followed the guidelines of the
138 Canadian Council on Animal Care. All efforts were made to minimize the number of animals used
139 and any discomfort resulting from surgical or behavioral procedures. Testing occurred during the
140 light part of the light/dark cycle.

141 A total of 29 rats underwent either sham surgery or SC microinfusion of neuronal-specific
142 inhibitory DREADD viral vectors. After surgical recovery, rats were undisturbed for 2-3 weeks to
143 allow for sufficient DREADD expression. Following the recovery period, they were each tested in
144 an open field test, after systemic injection of either the DREADD activator CNO or vehicle, in a
145 counterbalanced experimental design with a 3-day washout period in between the two tests. After
146 this, rats underwent training in the touch screen task, which took approximately 1 month. Once
147 animals reached the training criteria, they were tested in the novel orienting task (see below)
148 following either CNO or vehicle. After behavioural testing, animals were perfused and brains were
149 dissected for immunohistochemical verification of DREADD expression.

150 4 out of 29 rats were tested in the OFT only and sacrificed afterwards to generally verify injection
151 coordinates and virus expression. Of the remaining 25 animals, 8 animals underwent sham surgery
152 and 17 were injected with DREADDs into the SC. After excluding miss-hits, 14 DREADD animals
153 (7 males, 7 females), and 8 sham animals (2 males, 8 females) were tested in both OFT and the
154 touch-screen task, with 3 additional males tested in OFT only.

155

156 *Surgery*

157 Animals were anesthetized with isoflurane, induced with a mixture of 5% isoflurane and 2 L/min
158 oxygen and maintained at 3% isoflurane with 1L/min oxygen. Meloxicam (1 mg/kg- SubQ) and
159 Baytril (10 mg/kg- IM) were administered pre-emptively. Meloxicam injection was repeated 24
160 hours after the first injection. Animals were secured in a stereotaxic frame and a midline incision
161 was made in the skin on top of the head. A unilateral burr hole (right side) was drilled at the
162 following coordinates from bregma: 1.8 mm medial/laterally, -6.1 caudally and 4.4 mm ventrally
163 (Paxinos and Watson, 2006). 0.7 μ l of the virus solution rAAV5/hSyn-hM4D(Gi)-mCherry
164 (Vector Core of the University of North Carolina at Chapel Hills, USA) was injected at a rate of
165 0.1 μ l/min using a blunt ended 1.0 μ l Hamilton syringe (Model 7001 KH SYR, Knurled Hub NDL,
166 25 gauges, 2.75 in, point style 3; Hamilton, Reno, NV, USA). The syringe rested for 1 min prior
167 to injection and 7 minutes post injection before slow retraction. In sham animals the needle was

168 introduced, but no injection was made. Silk suture was used to close the wound and rats were given
169 a 21-day recovery period to promote maximal expression of the DREADD protein before testing
170 began. Animals had free access to food and water throughout this waiting period. After OFT
171 testing, animals were food restricted and kept on 90 %t of their target body weight to ensure
172 motivation in the operant touch-screen task.

173

174 ***Behavior: Open-field test***

175 The open field was a square enclosure of 45.7 cm × 45.7 cm dimensions with surrounding walls
176 40.6 cm in height. Animals were administered an intraperitoneal injection of the DREADD ligand
177 CNO (Toronto Research Chemicals- C587520, Toronto, ON, CAN) at a dose of 3 mg/kg in 18%
178 Dimethyl Sulfoxide (DMSO) in saline, or vehicle (18% DMSO, in saline), 20 min prior to testing.
179 Animals were then placed in the open-field box and allowed to freely explore it for 20 min while
180 they were tracked using a webcam and ANYmaze software (Version 6.33, Stoelting, Wood Dale,
181 IL, USA). Default ANYmaze settings did not reliably track Long-Evans rats, in particular the
182 location of their head, because of their non-uniform coat making it difficult to distinguish between
183 the animal's entire body and the background. In order to enhance tracking, the brightness and
184 contrast of all OFT videos were adjusted by the same degree (reduce brightness, increase contrast),
185 and the 'Erase Lines' feature was used, as suggested by ANYmaze technical support, allowing to
186 reliably detect the animal's head and center of the body (see Movie 1). Total distance travelled,
187 total number of 360-degree rotations made in either direction (clockwise or anticlockwise),
188 percentage of 360-degree clockwise rotations, and the cumulative sum of all body and head turn
189 angles throughout the 20 minutes test period were analyzed. For the cumulative sum of turn angles,
190 clockwise turns were counted as positive and anticlockwise turns as negative values, so that a
191 positive cumulative sum indicates more clockwise than anticlockwise turns and vice versa. Turn
192 angle measures have an advantage over complete rotation measures in that they capture the entirety
193 of the animals' turning behaviour, whereas complete rotation measures only count fully completed
194 360-degree turns.

195

196 ***Behavior: 3-Choice orienting task***

197 After OFT testing, animals were trained to respond to a 3-choice orienting task in the touchscreen
198 testing platform (Bussey Saksida boxes, Campden Instruments Ltd. Loughborough, UK) to

199 measure orienting behaviour and potential signs of visual neglect. The task was adapted from the
200 5-choice serial reaction time task (5CSRTT) commonly used in rodent studies of attention (Bussey
201 et al., 2008; Mar et al., 2013, Fizet et al., 2016), and based on the method of double simultaneous
202 stimulation used to investigate visual selection deficits in human patients (de Haan and Karnath,
203 2012). The 3-choice orienting task used similar basic setup and training protocols as the standard
204 5-CSRTT, but animals were trained to specifically orient towards the center panel. Also, only the
205 leftmost, rightmost and center response panels were used (3-choice), in order to increase task
206 difficulty and sensitivity for the animals' orientation during stimulus presentation. Each response
207 panel was a 2.0-3.0 × 2.0-3.0 cm square with a spacing of 5 cm between the side and center panels,
208 and 1.5-2.0 cm away from the grid floor of the box.

209

210 *Pretraining*

211 Initial training consisted of four pre-training stages that acclimated the rats to the testing chamber
212 and taught them the basics of the task such as initiating trials and associating illuminated panel
213 touches with a sugar pellet reward (Mar et al., 2013). A touch is defined as poking the panel with
214 their nose which is detected by the touchscreen system through breakage of the infrared beams
215 along the touch screen panel. The first pre-training stage involved habituating the animals to the
216 testing chamber for 30 minutes, whereas the remaining pre-training stages progressively taught the
217 rats how to respond to a solid square light stimulus and start a new trial. Rats passed each of these
218 pre-training stage by completing 60 trials in under 60 minutes.

219

220 *General outline of the task during baseline training and testing sessions*

221 House light was turned off by default throughout all training and testing sessions. A session began
222 by delivering a sugar pellet to the food magazine and illumination of the food magazine to prime
223 responding and encourage the animal to initiate the first trial. The first trial started once the animal
224 poked its head into the food magazine (to collect the sugar pellet), followed by a delay interval of
225 5s, giving time for the animal to orient towards the screen. At the end of the delay interval, the
226 stimulus was presented for a set "stimulus duration" period, which was varied depending on the
227 stage of training, and this was followed by a "limited hold" period. If the animal correctly nose-
228 poked the panel where the stimulus was presented within the stimulus duration or limited hold, the
229 food magazine was illuminated and a sugar pellet was dropped there. During training sessions, the

230 limited hold was 5s from the start of stimulus presentation. During testing sessions, the limited
231 hold was removed and the animals could only respond during stimulus presentation, but the
232 stimulus duration was increased to reduce the attentional load of the task. Once the rat collected
233 the reward and exited the food magazine, an inter trial interval (ITI) of 5s began. After this ITI
234 period, the food magazine light was turned on again and the animal had to poke its head into the
235 food magazine in order to initiate the next trial. In cases where the animal responded incorrectly
236 by nose-poking a non-illuminated panel, or if the animal omitted a response, a time-out period of
237 5s followed that was signaled by turning on the house light. After the time-out period, the house
238 light went back to the normal off state and an ITI of 5s began. After that, the food magazine light
239 was turned on and the animal could initiate the next trial. Figure 1 outlines the timing of events in
240 the task at a stimulus duration of 5s for a representative correct (A), incorrect (B) and omission
241 (C) trial.

242

243 *Baseline center attention training*

244 After successful completion of pre-training stages, the baseline center attention training
245 commenced. In this stage, rats were trained to expect a center stimulus as the most likely stimulus,
246 and therefore orient towards it more than the left or right response panels. This was done by varying
247 the proportion of trials on which the stimulus was presented at each of the three locations (80%
248 center, 10% right and 10% left; see Fig. 2A). Trials were presented in a randomized order. Baseline
249 training difficulty was incrementally increased by shortening the duration of stimulus presentation.
250 Animals progressed to more difficult stages of baseline training when they reached criteria of >
251 80% total accuracy, < 20% omission rate and 100-150 trials completed in 60 minutes. Stimulus
252 duration decreased from 60s to 1.5s during 7 baseline stages (60s, 30s, 20s, 10s, 5s, 2.5s, 1.5s),
253 enforcing enhanced attention as the animal was mastering the task.

254

255 *Test sessions*

256 Once each rat passed the 7th substage of the baseline training (1.5s stimulus duration), they
257 advanced to the test sessions. In a few case (as can be seen in Figure 4), performance varied to
258 under 80%, accuracy or >20% omission after the initial session where the rats passed training
259 criteria. In this case, the rats were still considered test eligible, especially since the test session was
260 less difficult than training (5s stimulus duration in testing compared to 1.5s in training).

261 In test sessions, the central panel was illuminated on 75% of trials (center-only trials). The
262 remaining 25% of trials were divided equally into 5 double stimulus trial types: stimuli were
263 presented in both the leftmost and rightmost panels with the following stimulus onset asynchrony
264 (SOA) conditions: simultaneous left + right stimulus presentation; left stimulus followed by right
265 stimulus with either a 0.5 or 1s SOA; or right stimulus followed by left stimulus with either a 0.5
266 or 1s SOA (see Fig. 2B). Trials were presented in a randomized order. For all double-stimulus
267 trials, a correct response was defined as nose-poking either the left or the right panel after a
268 stimulus was presented, irrespective of which one appeared first, and the animal was rewarded
269 accordingly. An incorrect response in double-stimulus trials referred to when the animal nose-
270 poked the center panel (in which no stimulus was presented), or the leftmost or rightmost panels
271 before any stimulus was presented. Touching any other panel between the center and the sides was
272 recorded but neither rewarded nor punished. Trial types were presented in pseudorandomized
273 order. Performance of the full task involved completing 200 trials over 90 min (100 trials in 60
274 min, for an early cohort). The session was set to end once the animal finished all the trials or once
275 the time limit was reached. Each animal was tested twice with CNO and twice with vehicle, in a
276 counterbalanced order, 20 minutes following the injection (one early cohort was tested only once
277 for each condition). Tests were performed at least 3 days apart to ensure injection washout, and
278 rats were maintained on center attention baseline training between the test sessions. Data from
279 both sessions following the same injection was combined and used to calculate the respective
280 animal's performance parameters. The total number of completed trials, accuracy rate, and
281 omission rate were analyzed, as well as the number of responses made to each panel and the
282 respective latencies, separated for each trial type (center only or SOA trials), using the ABET II
283 Touch software (Lafayette Instruments, Lafayette IN, USA). All training and testing ABET II files
284 are provided with the manuscript as Extended Data 1. Touchscreen response rates were calculated
285 as a percentage of total trials where a response was made (excluding trials where the animals made
286 an omission) to avoid biasing the data with trials in which the animal was not facing the panels
287 (e.g. while grooming, eating), as was the case in a small fraction of total trials. In contrast, omission
288 rate was calculated as a percentage of all trials of a particular trial type presented in a session.

289

290 ***Immunohistochemistry***

291 To confirm the expression of the DREADDs in the SC, all animals were perfused with saline

292 followed by 4% paraformaldehyde (PFA) and brains were harvested, post-fixed in PFA for 1 h,
293 and stored in 30% sucrose at 4°C until completely sunken. Brains were sliced into 4 series of 40
294 µm coronal sections using a freezing microtome (Microm HM 560 M) and stored at -20°C in
295 cryoprotectant solution. One series of the sections was used for immunohistochemistry. Free
296 floating tissue sections were thoroughly washed in 0.1M phosphate-buffered saline (PBS) between
297 incubations and all incubations were performed at room temperature with gentle agitation. Sections
298 were blocked with 1% H₂O₂ in 0.1 M PBS for 10 minutes and pre-absorbed in PBS+ (0.1% bovine
299 serum albumin, 0.4% TritonX100 in PBS) followed by overnight incubation with rabbit anti
300 mCherry (Abcam Cat# ab167453, RRID: AB_2571870) in PBS+. Subsequently, the sections were
301 incubated with biotinylated goat anti-rabbit (Vector Labs Cat# BA-1000, RRID: AB_2313606) for
302 1 hour (1:500 in PBS) and by the avidin horseradish peroxidase for 1 hour (ABC-elite, 1:1000 in
303 PBS; Vector Laboratories, Burlingame, CA, USA). Finally, the peroxidase complex was
304 visualized by exposure for 10 minutes to a chromogen solution containing 0.02% 3,3'-
305 diaminobenzidine tetrahydrochloride. At the end of the staining protocol, sections were washed
306 thoroughly with 0.1 M PB, mounted onto plus-charged glass slides with 0.3% gelatin in distilled
307 water and cover-slipped with DPX mounting medium (EMD Millipore, USA). Imaging was
308 performed using a Nikon Eclipse Ni-U upright microscope with a DS-Qi2 high-definition color
309 camera and imaging software NIS Elements Color Camera (Nikon Instruments, Melville, NY,
310 USA).

311

312 *Statistical analysis*

313 All statistical analyses were carried out using SPSS (IBM).

314 *Testing for normality, outliers, homogeneity of variance, and sex:* Before conducting statistical
315 analyses, data were scanned for normality using the Shapiro-Wilk test, for outliers using box and
316 whisker plots, and for homogeneity of variance assumptions for relevant analyses. This was
317 conducted for each measure of interest in the OFT and touch screen 3-choice orienting task. For
318 data that did not exhibit a normal distribution according to the Shapiro-Wilk test ($p < 0.05$), we
319 conducted nonparametric Mann-Whitney or Friedman tests, instead of independent t-tests and one-
320 way repeated measures ANOVA (see below). For data that did not violate normality but violated
321 the assumption of homogeneity of variance, an adjusted p value for t-tests was used. Data of both

322 sexes were merged throughout the study, as there were no statistically significant differences
323 between sexes, except for overall locomotor activity. The introduction of difference scores for
324 each animal between CNO and vehicle trials normalized the data and eliminated the difference in
325 overall locomotion.

326

327 *Open field test:* A CNO-vehicle difference score was calculated for total distance travelled, total
328 rotations, the percentage of clockwise rotations, the head turn angle sum and the turn angle sum.
329 Independent t-tests were conducted for all measured except total distance travelled, comparing the
330 DREADD group with the sham group, and p values were chosen accordingly based on whether
331 the assumption of homogeneity of variance was met (only case is for turn angle sum). For the total
332 distance, a Mann-Whitney U test was performed.

333 *Touch screen baseline performance:* For training data across days on the 3-choice orienting task,
334 pre-test baseline training days just prior to respective CNO or vehicle testing days were analyzed,
335 using only animals that had 2 CNO and 2 vehicle test days (4 pre-test days in total). Accuracy
336 percentage, omission percentage, reward collection latency, and correct response latency were
337 analyzed. After determining no difference in performance across pre-test days between DREADD
338 and sham animals, data of the two groups was merged.

339 Accuracy percentage, omission percentage and correct response latency were analyzed separately
340 for left stimulus trials, center stimulus trials, and right stimulus trials. Preliminary analysis using
341 repeated measures ANOVA or Friedman test revealed no significant difference between pre-test
342 days, after adjusting for multiple comparisons, in percentage accuracy, percentage omissions or
343 correct response latency, for any of the 3 trial types. Therefore, data from all 4 pre-test days were
344 combined and used to calculate overall trial type-specific percentage accuracy, percentage
345 omission and correct response latency. Then, the percentage accuracy, percentage omission and
346 correct response latency were compared between trial types using a one-way repeated measures
347 ANOVA or Friedman test, depending on whether normality and homogeneity of variance criteria
348 were met.

349 *Touch screen testing performance:* For center stimulus trials on the 3-choice orienting task testing
350 sessions, the CNO-vehicle difference score was calculated for the percentage left responses, center
351 responses, right responses, and omissions for each animal. Additionally, we calculated the CNO-

352 vehicle difference score for the center response latency. Independent t-tests or Mann-Whitney U
353 tests were conducted for each of these measures comparing the DREADD group with the sham
354 group.

355 Double stimulus trials were analyzed as described in Johnston et al. (2016). The proportion of
356 responses to the ipsiversive choice, the rightmost panel, were computed and plotted as a function
357 of SOA. Values were fitted with a logistic function and plotted for each individual animal, as well
358 as for each group using the average value of all animals in that group for each SOA. The midpoint
359 of this psychometric function represents the point of equal selection (PES), which is the SOA value
360 at which the proportion of rightward responses equals the proportion of leftward responses (0.5
361 proportion of right responses).

362 Curves were fitted and PES values were calculated only for animals that had more than 5 trials on
363 all SOAs in which they made a response, which led to the exclusion of 1 Sham animal and 5
364 DREADD animals. CNO effects were so strong on some DREADD animals that they responded
365 almost always to the right, so the PES could not be calculated (Fig. S6). In these cases the smallest
366 SOA value used to generate the fitted curve (SOA = -1) was used as a conservative estimate. A
367 two-way repeated measures ANOVA was performed with group (Sham vs DREADD) as a
368 between subject factor and injection (vehicle vs CNO) as a within subject factor.

369

370 **Results**

371 This study examined the effects of DREADD-induced transient deactivation of the right SC on
372 orienting behavior in Long Evans rats. The OFT was initially utilized to determine changes in
373 spontaneous turning and rotation behavior upon deactivating the right SC, followed by a novel
374 touchscreen-based 3-choice orienting task to detect any changes in spatial attention and orienting
375 behavior. At the end of data acquisition, immunohistochemistry was performed post-mortem to
376 confirm DREADD expression.

377

378 ***DREADD expression***

379 All animals underwent post-mortem immunohistochemistry to identify the regions with
380 DREADDs expression. No expression was observed in the sham operated animals. In four animals,
381 the injection was identified as missed-hit as no expression of the virus was observed in their right

382 SC. These animals were excluded from the study. In all the remaining animals, DREADDs were
383 uniformly expressed throughout a large portion of the right SC, from superficial layers down to
384 the deeper layers and from rostral to caudal sections (see Fig. 3 for a representative example). The
385 darker colors in Fig. 3A demonstrate areas with stronger expression of DREADDs. In some cases,
386 DREADD expression included fibres crossing the midline and invading the left SC, evident by
387 fiber-shaped staining in the region of the commissure, or a very low level of expression in deeper
388 layers of the contralateral SC as well the periaqueductal gray (PAG) area inferior to the SC.

389

390 ***Behavior: Open-field test***

391 The OFT was implemented to evaluate spontaneous orienting during exploratory locomotor
392 behavior. Ipsiversive (rightward or clockwise) body turns and head turns were analyzed. Head
393 turns were measured through the head turn angle and full 360-degree rotations, whereas body turns
394 were measured through the turn angle sm. Differences between behaviour after CNO and vehicle
395 injections (CNO-vehicle scores) were calculated for each animal and averaged for each group
396 (sham versus DREADD expressing animals).

397 We found no significant difference between sham and DREADD animals in CNO-vehicle scores
398 in locomotor activity measures. These included the total distance travelled (Figure 4A; $U = 72$, z
399 $= 0.233$, $p = 0.421$) and the total number of 360-degree rotations (Figure 4B; $t(23) = 0.054$, $p =$
400 0.479). However, CNO reduced overall locomotor activity in both groups of animals, as indicated
401 by a negative CNO-vehicle score for total distance and total rotations made throughout the 20-
402 minute test. In terms of head turning behavior, CNO-vehicle scores of percentage clockwise
403 rotations ($t(23) = 1.104$, $p = 0.161$; Fig. 4C) and head turn angle sum ($t(23) = 0.758$, $p = 0.229$;
404 Fig. 4D) were not significantly different between sham and DREADD animals (for more
405 information on turn measurements, see also extended figure 4-1). In contrast, DREADD animals
406 showed a significantly higher CNO-vehicle score for turn angle sum ($t(22.04) = 1.991$, $p = 0.029$,
407 Fig. 4E). Taken together, these results indicate that unilateral DREADD-induced inactivation of
408 the SC increased ipsiversive whole body turning as measured by the turn angle sum. In contrast,
409 neither head turning behavior, as measured by percentage clockwise rotations of the head and head
410 turn angle sum, nor the number of total rotations were significantly changed by SC inhibition in
411 these animals.

412

413 ***Behavior: 3-choice orienting task training***

414 Almost all rats progressed quickly through the pre-training and baseline training sessions described
415 in the methods section and figures 1 and 2. On average, rats took approximately 2 days to reach
416 passing criteria on each of the pretraining and baseline training stages, except the most difficult
417 training stage which on average took 3 days to learn.

418 The purpose of baseline center training sessions was to train the rats to orient towards the center
419 stimulus more than the flanking stimuli by using a higher proportion of center stimulus trials (80%
420 center, 10% left, 10% right). Given that the experimental design involves repeated testing of
421 animals, it was important that animals maintained a stable baseline performance between test
422 sessions, especially on pre-test days prior to their CNO or vehicle test sessions. The baseline
423 training was able to achieve this stable performance, as demonstrated by the lack of significant
424 change across 4 pre-test days in total accuracy ($\chi^2(3) = 5.333$, $p = 0.149$; Fig. 5A), indicating overall
425 stable performance, and reward collection latency ($F(3, 51) = 0.236$, $p = 0.871$; Fig 5C), indicating
426 no change in motivation. Interestingly, both omissions ($\chi^2(3) = 8.186$, $p = 0.042$; Fig 5B) and
427 average correct response latency ($F(3, 51) = 5.257$, $p = 0.003$; $p = 0.013$ for comparison between
428 day 1 and day 4 adjusted for multiple comparisons; Fig 5D) decreased over pre-test days, likely
429 due to the interim test sessions between these pretest days containing more rewarded trials and a
430 smaller chance of punishment. For information on progression through training stages, see also
431 extended figure 5-1.

432 In order to investigate the orienting bias produced by baseline training in more detail, we analyzed
433 trial-type-specific responses. For this purpose, all trials from pre-test days were combined after
434 confirming that trial-type-specific measures of interest did not significantly differ between the
435 different pre-test days (adjusted for multiple comparisons). Trial-type-specific responses during
436 pre-test days showed a strong center bias, with higher accuracy on center trials compared to left or
437 right trials (main trial type effect: $\chi^2(2) = 24.437$, $p < 0.001$; multiple comparisons with adjusted p
438 values shown on Fig. 6A) and a shorter correct response latency on center trials compared to left
439 or right trials (main trial type effect: $\chi^2(2) = 24.089$, $p < 0.001$; multiple comparisons with adjusted
440 p values shown on Fig. 6C). Interestingly, while omission percentage on left trials was significantly
441 higher than center trials, omission percentage on right trials was comparable to center trials (main
442 trial type effect: $F(2, 42) = 5.569$, $p = 0.007$; comparison between left and right trial types adjusted
443 for multiple comparisons shown on Fig. 6B). Overall, the 3-choice orienting task training

444 successfully produced a strong center bias, as also easily observed in a representative video taken
445 during one of the baseline training days on stage 7 (see movie 2).

446 ***Behavior: 3-choice orienting task testing***

447 The testing protocol was comprised of 75% baseline center trials to retain the animals' center bias,
448 and 25% double stimulus side trials with varying SOAs to test the orienting preference to varying
449 degrees following CNO or vehicle injections (see Fig. 2B). Separate analyses were conducted for
450 baseline center-only trials and double-stimulus trials.

451 *Baseline center-only trials:* Response choice indicates whether the animal made a left, center or
452 right response, even though only center choices were rewarded, and the side responses were
453 punished. As in previous analysis, CNO-vehicle difference scores were computed for the
454 percentage of left responses, center responses, right responses and omissions. In addition, the
455 difference for latency to respond correctly to the center was calculated. There was a significant
456 difference in CNO-vehicle scores between sham and DREADD animals for the percentage of
457 center responses ($U = 83$, $z = 1.834$, $p = 0.035$; Fig. 7B), percentage omissions ($U = 29$, $z = -1.834$,
458 $p = 0.035$; Fig. 7D), and center correct response latency ($t(20) = 1.865$, $p = 0.039$; Fig. 7E).
459 Specifically, DREADD animals, but not sham animals, showed lower percentage center responses
460 and higher scores in percentage omissions and correct response latency after CNO injections
461 compared to vehicle controls. There was also a trend for an increase in CNO-vehicle scores of
462 percentage right responses ($U = 33$, $z = -1.572$, $p = 0.064$; Fig. 7C), but no effect on the percentage
463 of left responses ($U = 69$, $z = 0.948$, $p = 0.201$; Fig. 7A). Overall, this shows that CNO slightly
464 reduced the center bias in DREADD but not sham animals, causing them to respond less
465 accurately, more slowly, and with a higher chance of omissions when faced with a center trial.

466

467 *Double-stimulus trials with varying SOAs:* The goal of double-stimulus trials was to assess the
468 extent of rightward bias produced by unilateral SC inactivation. On the psychometric function, a
469 rightward bias would manifest as a leftward shift and smaller PES values. A weak rightward bias
470 would show a left shift at negative SOAs only, whereas a strong rightward bias would show a left
471 shift at all SOA values.

472 In the sham group, CNO had no effect on the proportion of right responses, as can be seen by the
473 curves generated from the average of all sham animals at each SOA (Figure 8A, for individual

474 animals, see extended figure 8-1). In contrast, CNO injection in DREADD expressing animals
475 shifted the fitted curve for the proportion of right responses significantly to the left (Figure 8B, for
476 individual animals, see extended figure 8-2), indicating a strong rightward bias. A two-way
477 ANOVA conducted with respective PES values supported this rightward bias (Figures 8C & 8D).
478 There was a significant interaction between group and injection ($F(1,4) = 6.422, p = 0.024$). Post-
479 hoc Bonferroni tests adjusted for multiple comparisons revealed a significant decrease in
480 DREADD PES following CNO compared to vehicle ($p = 0.003$), and no change in sham PES
481 following CNO compared to vehicle ($p = 0.787$).

482

483 **Discussion**

484 In this present study we developed a touchscreen-based experimental approach to measure spatial
485 attention and orienting responses in rodents. Given the well-established role of the SC in orienting
486 behavior of rodents, we validated the novel task through unilateral SC inactivation using
487 DREADDs. Our results show that the touchscreen 3-choice orienting task was highly sensitive to
488 DREADD-induced SC inactivation, providing a variety of measures to assess orienting bias.

489

490 *DREADD expression*

491 In most of the animals, DREADDs were expressed throughout a large portion of the right SC, from
492 superficial layers down to the deeper layers (see Fig. 3). In some animals, we also observed a low
493 level of expression in deeper layers of the left SC. However, the strong imbalance of expression
494 between the left and right SC would still be expected to result in behavioral changes associated
495 with the stronger deactivation of the right side, as supported by our behavioural results. Minor
496 expression was also observed in periaqueductal gray (PAG) area in some animals. In a study by
497 Geula and Asdourian (1984), muscimol injections were made into the periaqueductal gray (PAG)
498 area in addition to the SC, to observe the impact on animal's circling and bodily asymmetry. They
499 only observed significant measures of bodily asymmetry and no significant effect on circling
500 (Geula and Asdourian, 1981). Since we did not observe any bodily asymmetry, the observed
501 orienting changes in the present study are likely associated with the strong DREADDs expression
502 in the right SC.

503

504 *Open Field analysis*

505 We first analyzed OFT behaviour to detect general alterations in the animals' spontaneous
506 locomotor behavior after deactivation of the right SC. We found that CNO increased clockwise
507 body turning behaviour, but not head turning behaviour or the percentage of 360-degree clockwise
508 rotations in DREADD animals. The observation of a higher ipsiversive body turn angle is
509 consistent with a deficit in orienting to the contralateral visual field as a consequence of unilateral
510 SC deactivation and the ipsilesional circling behavior that has previously been reported in lesion
511 studies of the SC (Di Chiara et al., 1982; Geula and Asdourian, 1981; Kirvel et al., 1974;
512 Northmore et al., 1988; Tehovnik, 1989). Our experimental approach also provides a method of
513 optimizing video tracking for Long-Evans rats to aid in the reliable automated detection of the
514 head, which is more difficult for these hooded rats compared to other strains with a uniform coat
515 color. Our findings clearly demonstrate the importance of accurately defining and quantifying
516 measures of turn and rotations, as inhibition of the SC in this study did not induce changes in
517 turning and rotation measures that only use the orientation of the animals' heads.

518 *SC manipulations impact orienting responses*

519 Various studies reported that damage to the SC impaired the ability to perform orienting
520 movements towards visual stimuli presented in the hemifield contralateral to the lesioned SC,
521 particularly those presented in the periphery (Schneider, 1975, Dean et al., 1984, Goodale and
522 Murison, 1975). The balance of neuronal activity between the two SCs has been proposed to play
523 a role in selection and initiation of the direction of the motor output (Carello and Krauzlis, 2004;
524 Horwitz, 2004). Both of these task-related processes are important for performance on our 3-choice
525 orienting task, as well as for other tasks typically analyzed after SC inactivation. Accordingly,
526 unilateral optogenetic stimulation of the SC revealed that the excitation of SC neurons resulted in
527 contraversive movements, while their inhibition caused an ipsiversive bias (Stubblefield et al.,
528 2013). Pharmacological studies have also consistently supported these findings: Dean et al. (1988)
529 examined the effect of unilateral excitation of SC neurons in rats using pharmacological
530 manipulations and reported contraversive head movements. Other studies reported postural
531 asymmetry following unilateral pharmacological deactivation of the SC in rats (Geula and
532 Asdourian, 1981; Imperato and Di Chiara, 1981). We did not observe any noticeable changes in
533 the rats' posture upon CNO administration in any of our experiments. However, studies reporting
534 bodily asymmetry often used the GABA agonist muscimol for SC inhibition, which might be
535 responsible for postural asymmetries observed, since manipulation of GABAergic mechanisms

536 has previously been reported to cause postural asymmetry and muscular rigidity in rats (Turski et
537 al., 1984). The DREADDS used in this study affected all cell types. Indeed, more recent studies
538 that applied optogenetic techniques to unilaterally deactivate the SC did also not report on any
539 postural asymmetry (Kopeck et al., 2015; Stubblefield et al., 2013). Taken together, our findings
540 were consistent with previous literature demonstrating deficits in contraversive orienting following
541 unilateral SC lesions or unilateral pharmacological or optogenetic manipulations of SC activity.
542 Compared to optogenetics, DREADDs provided a less restrictive technique for exploring orienting
543 behaviour, as there was no need to tether the animal's head which may limit the range of their
544 orienting behavior.

545

546 *The importance of a centre bias*

547 Training on the novel 3-choice orienting task was successful in developing a center-orienting
548 preference, evident from higher accuracy and shorter response latency on center trials compared
549 to left or right trials during the test sessions. Accuracy and reward collection latency were
550 consistent across all pre-test days, indicating that animals returned to approximately the same
551 baseline orienting bias and motivation before each test session. In the varying SOA trials during
552 test sessions, DREADD but not sham rats showed a substantial left shift in their rightward response
553 psychometric function following CNO injection. This indicated a rightward bias which was also
554 shown by a significant decrease in PES in DREADD animals post-CNO compared to vehicle. The
555 rightward bias was rather extreme in some cases, causing animals to respond to the right on all
556 trials in all SOAs, even when they were primed to respond towards the left side in -0.5 and -1 SOA
557 trials, indicating an ipsiversive orienting bias and contraversive neglect after CNO administration.
558 Even in analysis looking at baseline center-only trials, a rightward orienting bias following CNO
559 could be detected during test sessions. Animals were still very accurate on these center-only trials
560 (center response rate of > 90%), but there was a significant decrease in CNO-vehicle score of
561 percentage center responses, whereby animals chose the center choice less frequently and tended
562 towards choosing the right choice more frequently instead, even though only the center choice was
563 illuminated and choosing the non-illuminated right panel was punished. Similar findings were
564 reported by Sinnamon and Garcia (1988) using an operant orienting task with 2 lateral choices and
565 one center choice, however, their results also showed a neglect to center positions after unilateral
566 lesions of the SC, probably due to a lack of enforcing and maintaining a centre orientation bias.

567 Without this bias, it is difficult to interpret the behavioural data, as the animal might simply shift
568 its body posture and attention towards the preferred side.

569

570 *Benefit of the touchscreen system*

571 The touchscreen-based orienting task is a purely visual method that provided great advantages in
572 terms of precise control of the stimulus duration and location. Compared to tasks that require
573 animals to exert pressure to push a door or a lever, our orienting task benefits from detecting the
574 nose-poke via infrared beam without requiring any pressure, providing a more direct association
575 with orienting behavior (Bussey et al., 2008). Cook et al. (2004) found that rats learned a visual
576 discrimination much faster when they were required to nose-poke the stimuli on a touchscreen as
577 opposed to pushing a lever underneath the stimuli, likely due to differences in the spatial contiguity
578 of stimuli and responses (Cook et al., 2004). Spatial contiguity between stimulus and response is
579 crucial when orienting towards the stimulus is the main goal of the task, and it allows stimulus
580 location to be varied without interfering with the nature of the task or learning speed. The novel
581 task presented here can be easily adapted to studies of orienting response to various directions,
582 since stimuli can be presented anywhere on the touchscreen, or response to stimuli of varying
583 shapes, sizes and salience (brightness). Based on our findings, training rats on this orienting task
584 can be achieved in a relatively short period of time, reaching baseline training of the highest
585 difficulty level (stimulus duration of 1.5s) in less than 3 weeks on average. Similar to all other
586 touchscreen-based tasks, the 3-choice orienting task is highly automated and easy to run and task
587 parameters such as stimulus duration, timeout duration or the amount of reward can easily be
588 modified if needed. Although we did not perform video analysis in our touchscreen experiments,
589 the systems is equipped with video recorders, which can be used in combination with software like
590 ANY-maze to track the location of the animal's head at specific times during the task. Given the
591 broad utilization of touchscreen-based platforms for cognitive testing, this task offers the ability
592 to test orienting behaviour across labs under identical experimental paradigms, benefiting from the
593 numerous advantages these touchscreen systems provide (Mar et al., 2013). Touchscreen-based
594 platforms can also be combined with other techniques such as optogenetics or electrophysiology,
595 to manipulate or record neural activity at precise time points during a testing session (for example,
596 just before a response is made or right after a trial is initiated), and this can be used to more
597 accurately study the role of structures implicated in spatial attention and orienting behaviour

598 (Bussey et al., 2008, 1994; Horner et al., 2013).

599

600 *Limitations*

601 A limitation of the present study was the small number of testing sessions performed per animal,
602 which made it difficult to get an accurate representation of the responses to left stimuli, especially
603 regards to the extremely low number of leftward responses after CNO injection. For example, for
604 trials with an SOA of +1, very few animals responded towards the left at all, which made it difficult
605 to analyze measures such as left response latency given the low number of trials and animals.
606 Similarly, rightward responses in negative SOA trials and side responses in center-only trials were
607 relatively infrequent. Future studies should aim to test each animal multiple times or in longer test
608 sessions, until enough trials have been performed to accurately represent all possible response
609 scenarios. Repeated testing in this task is quite feasible, as we show that baseline performance is
610 stable across test sessions and pre-test days. Furthermore, the utilization of transient inactivation
611 methods such as DREADDs allows the possibility of including more testing sessions without
612 having post-lesion recovery time as a confounding factor.

613

614 *Conclusion*

615 In summary, the novel 3-choice orienting paradigm implemented in the present study has been
616 shown to be highly sensitive to neglect-like orienting deficits that resulted from DREADDs-
617 induced unilateral deactivation of the SC. This study provides a foundation for the application of
618 a standardized behavioral tests in a variety of research questions centered on alteration of orienting
619 behavior.

620

621 **References**

622

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746
747
748
749 **Figure Legends**

750
751 **Figure 1.** Timeline of events in the touchscreen-based orienting task during baseline training with
752 a difficulty level of 5s of stimulus presentation.
753 The house light is turned off by default and a sugar pellet (reward) is provided to prime the session,
754 along with illumination of the food magazine. A trial begins as soon as the animal enters the food

755 magazine (0 s), after which the food magazine light is switched off. Then, the stimulus is presented
756 after 5s and the animal has to make a response (nose poke) within a 5s limited hold of stimulus
757 presentation. (A) In a correct trial the animal has correctly poked the illuminated panel and is
758 rewarded immediately. The house light remains off throughout the trial. (B) In an incorrect trial
759 the animal has poked a non-illuminated panel and receives no reward. The house light turns on
760 immediately after the incorrect response for a period of 5s to signal a timeout and discourage the
761 animal for an inappropriate behavior. (C) In an omission trial the animal has not poked any panel
762 at all and thus is not rewarded. House light turns on for 5s right after the limited hold of stimulus
763 presentation (5s) to signal a timeout. For all trials: the next trial begins after a 5s intertrial interval
764 (ITI) with illumination of the food magazine. Note that whenever a sugar pellet (reward) is
765 dispensed, it is accompanied by illumination of the food magazine light, which is then switched
766 off once the animal collects the reward.

767

768 **Figure 2.** Description of the 3-choice orienting protocol.

769 (A) After completing all the pre-training steps, the rats undergo baseline attention training, where
770 they learn to expect the center stimulus trial as the most likely trial type, by having the centre
771 stimulus appear in 80 % of the trials while the left and right stimuli each appear in only 10 % of
772 the trials. Animals are rewarded with one sugar pellet upon correctly nose-poking the illuminated
773 panel and punished with a timeout and inversion of the house light for poking a non-illuminated
774 panel. The duration of the stimulus presentation is decreased from 30 sec to 1.5 sec through 7
775 stages of baseline training to increase task difficulty. (B) Upon passing the criteria, rats perform
776 the testing session after injections of CNO or vehicle. Testing sessions include baseline trials to
777 reinforce the basic strategy the rats must use, in addition to ambiguous trials where flanking stimuli
778 are presented with varying stimulus onset asynchronies (SOA) to test their orienting bias at various
779 difficulties. 75 % of the test trials are centre-only trials where only the centre stimulus is presented
780 for 5s. The remaining 25% are double-stimulus trials where the left and right stimuli appear either
781 simultaneously or with a delay (0.5s or 1s) with left stimulus preceding the right or vice versa.

782

783 **Figure 3.** DREADD expression in the superior colliculus (SC) across all DREADD-expressing
784 animals. Expression is displayed across six slices, from Bregma -5.52 mm as the most rostral, to
785 Bregma -6.60 mm as the most caudal slice. The color gradient on the SC represents the number of
786 animals that had DREADD expression at each subregion of the SC: the darker the gradient at a
787 subregion, the higher the number of animals that had DREADD expression in that subregion. Each
788 schematic slice is accompanied by an immunohistochemistry photo of the same slice from a
789 representative animal. The images of the slices were taken from The Rat Brain Atlas in Stereotaxic
790 Coordinates from Paxinos and Watson (2006).

791

792 **Figure 4.** Body turning but not head turning behavior is increased following CNO in DREADD
793 but not sham animals.

794 Rats spontaneously explored an enclosed arena 20 minutes after injection of vehicle or CNO. A
795 difference score was calculated for each measure of interest between CNO and vehicle for each

796 animal, and the sham and DREADD groups were compared using independent t-tests or Mann-
797 Whitney U test (#). (A) There was no difference in the CNO-vehicle score of total distance
798 travelled. (B) No difference in total 360-degree rotations. (C) No difference in percentage
799 clockwise 360-degree rotations. (D) No difference in the sum of all head turn angles made during
800 the 20-minute test (clockwise head turns were denoted as positive and anticlockwise head turns as
801 negative). (E) There was a significant increase in the CNO-vehicle score of the turn angle sum
802 (clockwise body turns were denoted as positive and anticlockwise body turns as negative). All
803 values shown are mean \pm SEM, with individual dots representing individual animals. *
804 significant effect with $p < 0.05$, \$ denotes adjusted p value based on lack of homogeneity of
805 variance. For more information on how turn angles were measured, please see extended figure 4-
806 1.

807
808 **Figure 5.** Performance and motivation are stable across pre-test days, but animals omit fewer trials
809 and respond more quickly by the last pre-test day.

810 Animals underwent baseline training and were then tested up to 4 times. These graphs show
811 performance parameters on the days prior to testing days for both sham and DREADD animals
812 combined, which was analyzed using repeated measures ANOVA or nonparametric Friedman test
813 (#). (A) Overall accuracy did not change across pre-test days. (B) Percentage omission
814 significantly decreased across pre-test days. (C) Reward collection latency was unchanged across
815 pre-test days, indicating stable performance and motivation across pre-test days. (D) Correct
816 response latency significantly decreased across pre-test days, adjusted p refers to comparison
817 between day 1 and day 4, which was adjusted for multiple comparisons. These results indicate that
818 by undergoing test sessions which include more reward and less punishment, animals learned to
819 omit fewer trials and respond more quickly. All values shown are mean \pm SEM, with light grey
820 lines representing individual animals. * significant effect with $p < 0.05$. For more information on
821 progression through all training stages, please see extended figure 5-1.

822
823 **Figure 6.** Animals show strong center bias on pre-test days.

824 Data from all pre-test days were combined to analyze trial-type-specific performance using
825 repeated measures ANOVA or nonparametric Friedman test (#). All p values shown are adjusted
826 for multiple comparisons. (A) Animals were more accurate in response to centre trials. (B) The
827 omission rate was higher to left side trials than to centre trials. (C) Correct responses to centre
828 trials were faster than to side trials. All values shown are mean \pm SEM, with individual dots
829 representing individual animals. * significant effect with $p < 0.05$.

830
831 **Figure 7.** CNO reduces center bias in DREADD but not sham animals during testing.

832 Test sessions included 75% center trials, similar to the ones shown during baseline training. A
833 CNO-vehicle score was calculated for each parameter of interest. Depending on the distribution,
834 an independent t-test or Mann-Whitney U tests (#) were conducted to compare sham and
835 DREADD animals. (A) Left responses were unchanged. (B) There was a significant decrease in
836 the percentage of correct center responses and (C) a trend to an increase in rightward responses.

837 (D) There was also a significant increase in the percentage of omissions in DREADD animals. (E)
838 A reduction in center bias can also be seen by an increase in the time it took DREADD animals to
839 respond correctly to the center following CNO. All values shown are mean \pm SEM, with
840 individual dots representing individual animals. * significant effect with $p < 0.05$

841

842 **Figure 8.** CNO produces a strong rightward bias in DREADD animals.

843 (A-B) During test sessions, SOA trials were used to assess the extent of rightward bias produced
844 by CNO in DREADD animals. Circles in A and B indicate the average proportion of right
845 responses at that particular SOA for the entire group with either vehicle (blue) or CNO (red). A
846 logistic function was fitted to the proportion of rightward responses for each animal, as well as for
847 the whole group. DREADD animals, but not sham animals, showed a left shift of their response
848 profile after CNO injection. (C) The point of equal selection (0.5 right responses, PES) was
849 extrapolated from each individual animal's curve following either vehicle or CNO injection. A
850 value of -1 was used as a conservative estimate of PES for animals where the logistic function
851 revealed an extreme rightward bias that could not be extrapolated to a PES (see figures S6). A two-
852 way ANOVA was conducted to compare the PES within each group, which showed a significant
853 decrease in PES in the DREADD group, but no change in the sham group. Values are mean \pm
854 SEM, with individual dots representing individual animals. * significant effect with $p < 0.05$. For
855 more information of each single animal's performance, please see extended figure 8-1 for sham
856 animals, and extended figure 8-2 for DREADD animals.

857

858 **Figure 4_1.** Turn measure examples

859 Visual depiction of turn angle measures recorded by Anymaze software. A) Turn Angle: A vector
860 of movement from one position of the animal's center point to the next is created. For each vector,
861 the angle between it and the previous vector is calculated with anti-clockwise movement being
862 negative and clockwise movement being positive (Anymaze). B) Head Turn Angle: For each
863 position of the animal's head, a vector is created from the animal's center point to the head. The
864 angle between this vector and the same vector for the previous position of the animal's head is
865 calculated, with anti-clockwise movement being negative and clockwise movement being positive.
866 C) Example calculation from successive head turns: Panels are numbered from left to right as C1,
867 C2, C3, C4. C1 to C2 head turn angle = +75; C2 to C3 head turn angle = +45; C3 to C4 head turn
868 angle = -35. Turn Angle Sum is the sum of all turn angles, measured as in panel A or B, in a
869 particular time period (e.g the Head Turn Angle Sum for time period C1-C4 = $75 + 45 - 35 = +85$).
870 To complete a full rotation, an animal must make consecutive turns that add up (cumulative turn
871 angle) to 360 degrees. Reversal (turning towards the opposite direction) at any point before the
872 cumulative angle reaches 360 degrees resets the cumulative angle to 0 and starts adding up
873 successive turns to the opposite direction (e.g considering panels C1-C4, the cumulative angle
874 counted towards a full rotation is -35 because the animal made a reversal, despite the head turn
875 angle sum being +85). Because of the effects of the reversal, we believe the turn angle sum is a
876 more accurate representation of turning behaviour than complete 360-degree rotations.

877

878 **Figure 5_1.** Time in training Stages

879 Rats progress quickly through all training stages in the 5-choice orienting task. Pre-training stages
880 (Habituation to Must Initiate) acclimate rats to the chambers and them in a stepwise manner to
881 associate nose poking stimuli on the screen with reward. All rats passed quickly through pre-
882 training stages, taking at most 4 days to reach criterion and an average of about 5 days to finish
883 all of pre-training. Baseline training stages involve training the rats to orient towards the center
884 panel, with a stimulus being shown 10% in the left panel, 80% in the center panel, and 10% in the
885 right panel. To pass, rats must achieve an overall accuracy of 80% with fewer than 20% omitted
886 trials. Rats progressed quickly through Baseline 1 (60 s stimulus duration) to Baseline 4 (10 s
887 stimulus duration). Even on the most difficult stage, rats took an average of 3 days to reach
888 criterion and just under 12 days to complete all Baseline stages.

889

890 **Figure 8_1.** No rightward bias in individual sham animals

891 Sham animals do not show a rightward bias in double stimulus trials following CNO injection.
892 Individual psychometric curves for sham animals showing their performance on double stimulus
893 trials within the SOA range -1 to +1 following either vehicle (blue) or CNO (red) injections. A
894 rightward bias would manifest as a leftward shift in the psychometric curve, indicating a higher
895 proportion of rightward responses. As can be seen by the individual curves, Sham animals did not
896 exhibit a rightward bias following CNO injection compared to vehicle.

897

898 **Figure 8_2.** Rightwards bias in individual DREADD animals

899 DREADD animals show a rightward bias in double stimulus trials following CNO injection.
900 Individual psychometric curves for DREADD animals showing their performance on double
901 stimulus trials within the SOA range -1 to +1 following either vehicle (blue) or CNO (red)
902 injections. Animals are numbered left to right, top to bottom (row 1: animals 1-4; row 2: animals
903 5-8, row 3: animal 9). A rightward bias would manifest as a leftward shift in the psychometric
904 curve, indicating a higher proportion of rightward responses. Several rats showed extremely strong
905 rightward bias, as indicated by almost exclusive rightward responses regardless of SOA (animals
906 3, 5, 8, and 9). Other animals showed a more modest rightward bias (animals 2, 4, and 6) and only
907 2 rats showed no rightward bias following CNO (animals 1 and 7)

908

909 **Movie 1 Legend**

910 ANYmaze video tracking with adjusted settings consistently track the head and body position.
911 Video sample from the open field test, showing ANYmaze tracking using the adjusted settings
912 described in the methods section. The video sample shows consistent tracking of the animal's head
913 (green) and center (orange) positions, which are used to calculate turning and rotation measures as
914 described in the methods section and supplemental figures.

915

916 **Movie 2 Legend**

917 Baseline center training leads to a strong center bias. Video sample from the touch screen orienting
918 task baseline training. The video sample shows the animal orienting towards the center before the

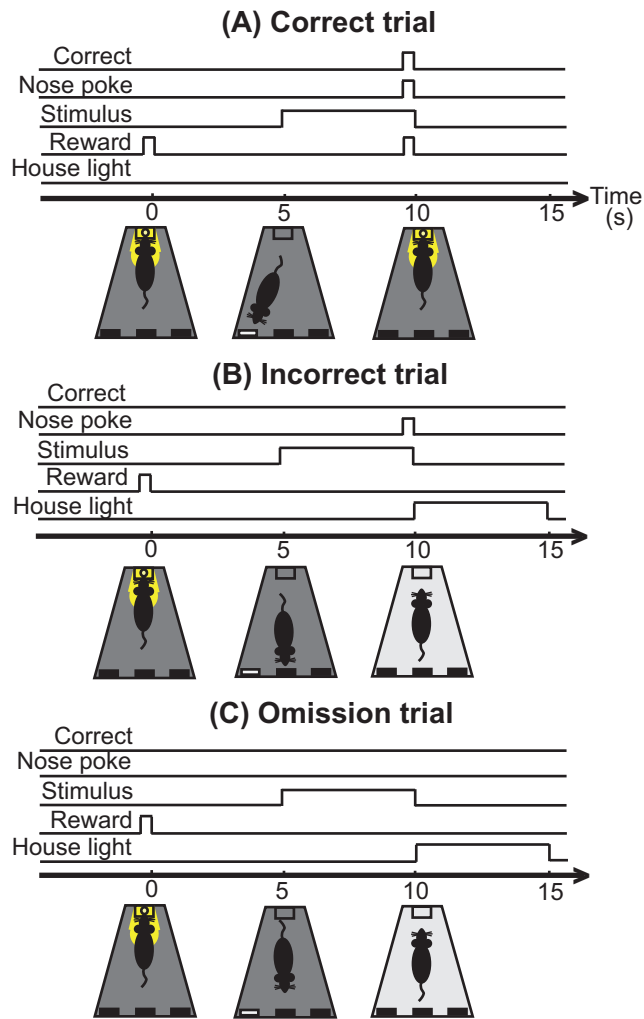
919 stimulus is shown and correctly responding to the center stimulus and receiving a reward (00:00-
920 00:02 for orienting before a stimulus is shown, 00:02-00:04 for responding to the stimulus). A
921 similar sequence of events can also be seen in the following three trials (00:12-00:15, 00:28-00:31,
922 00:40-00:44). Following a correct response, the animal collects its reward and initiates a new trial.
923 The last trial in the video shows an incorrect side trial, where the stimulus was shown on the side,
924 but the animal's center bias made it incorrectly choose the center (00:51-00:54).

925

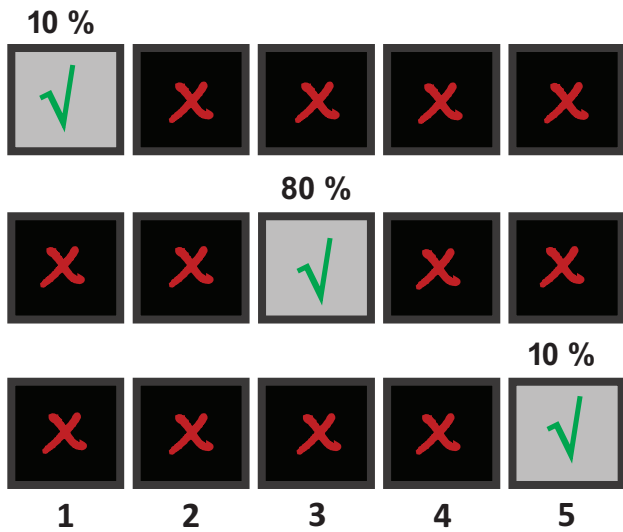
926 **Extended Data 1 Legend**



927 ABET II Touch software (Lafayette Instruments, Lafayette IN, USA)

928



(A) Baseline training session



-  Correct choice
Rewarded with 1 sugar pellet
-  Incorrect choice
Punished with a timeout

(B) Testing session

