Interactions between medial prefrontal cortex and dorsomedial striatum are necessary for odor span capacity in rats: role of GluN2B-containing NMDA receptors

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Working memory is involved in the maintenance and manipulation of information essential for complex cognition. While the neural substrates underlying working memory capacity have been studied in humans, considerably less is known about the circuitry mediating working memory capacity in rodents. Therefore, the present experiments tested the involvement of medial prefrontal cortex (mPFC) and dorsal striatum (STR) in the odor span task (OST), a task proposed to assess working memory capacity in rodents. Initially, Long Evans rats were trained to dig in scented sand for food following a serial delayed nonmatching-to-sample rule. Temporary inactivation of dorsomedial (dm) STR significantly reduced span in well trained rats. Inactivation of mPFC or contralateral disconnection of the mPFC and dmSTR also reduced span. Infusing the GluN2B-containing NMDA receptor antagonist Ro 25-6981 into mPFC did not affect span; however, span was significantly reduced following bilateral Ro 25-6981 infusions into dmSTR or contralateral disconnection of mPFC (inactivation) and dmSTR (Ro 25-6981). These results suggest that span capacity in rats depends on GluN2B-containing NMDA receptor-dependent interactions between the mPFC and the dmSTR. Therefore, interventions targeting this circuit may improve the working memory capacity impairments in patients with schizophrenia, Alzheimer’s disease, and Parkinson’s disease.
Ragozzino 2014) to test whether functional interactions between areas are necessary for span.

Research examining the neurochemical modulation of working memory capacity in humans has focused on dopamine (Cools et al. 2008; Landau et al. 2009). We and others have shown that ionotropic glutamate receptors are involved in performance of the OST in rats. In particular, reduced and increased span capacity have been noted following either blockade of GluN2B-containing NMDA receptors with the antagonist Ro 25-6981 (Davies et al. 2013a) or genetically overexpressing GluN2B-containing NMDA receptors in the forebrain (Cui et al. 2011), respectively. Therefore, we also tested whether GluN2B-containing NMDA receptors in the mPFC and dorsal STR were involved in the OST.

Results

Training

Rats from all three experiments were initially trained to dig in a bowl of sand for a buried Froot Loop (dig training phase). This phase of training took an average of 5.13 d to complete (range = 3–9 d). After dig training, rats were trained for an average of 7.13 d (range = 3 to 12 d) in the nonmatching-to-sample task until they got 5/6 trials correct for three sessions. Rats were then trained on the OST for an average of 14.87 d (range, 11–23 d). Figure 1B displays the average spans obtained during the 7 training days before an error occurs. Span is calculated as the number of bowls on the platform for the last error free trial minus 1. Note that all bowls are moved around the platform before each new trial. (B) Mean odor spans during the 7 d of training prior to the first treatment for all rats in the three experiments (n = 24). Rats had significantly higher spans on days 4–7 than day 1 (see Results for details). (C) Infusions sites of fluorescent muscimol in the right hemisphere of the dmSTR (bottom left), and right hemisphere of the dlSTR (bottom right).

Odor span capacity depends on activation of GluN2B-containing NMDA receptors in the mPFC–dmSTR circuit

NMDA receptors containing GluN2B subunits have been implicated in the OST in two studies. Cui et al. (2011) showed increased odor span capacity in mice that overexpressed GluN2B-containing NMDA receptors in the forebrain. Previous research from our laboratory (Davies et al. 2013a) showed that blocking GluN2B-containing NMDA receptors with systemic injections of Ro25-6981 impaired span. Direct infusion of Ro 25-6981 into mPCF impaired odor span in the majority (6/7) of rats tested, although this effect failed to reach significance in that previous study (Davies et al. 2013a). Therefore, we assessed whether Ro 25-6981 infusions into mPFC or dmSTR affected odor span in a new sample of eight

| Figure 1. (A) Illustration of the OST. See text for details. Odors are indicated with letters. On subsequent trials for a given span, the bowl (black circle) that contains the novel odor is rewarded (+) while previously encountered bowl(s) are not (−). Bowls are added one at a time to the platform until an error occurs. Span is calculated as the number of bowls on the platform for the last error free trial minus 1. Note that all bowls are moved around the platform before each new trial. (B) Mean odor spans during the 7 d of training prior to the first treatment for all rats in the three experiments (n = 24). Rats had significantly higher spans on days 4–7 than day 1 (see Results for details). (C) Infusions sites of fluorescent muscimol in the right hemisphere of the dmSTR (top), left hemisphere of the dmSTR (bottom left), and right hemisphere of the dlSTR (bottom right). |
rats. Following Ro 25-6981 infusions in mPFC, span did not differ significantly in this sample with a mean span of 8.19 ± 1.42 odors, while vehicle treatment resulted in a mean span of 9.23 ± 1.65 (Fig. 4A, left side; $t(7) = 0.50$, $P = 0.63$). Latency to respond did not differ between treatments (Fig. 4A, right side; $t(7) = 0.57$, $P = 0.59$).

In contrast, bilateral infusions of Ro 25-6981 into dmSTR significantly impaired span without affecting response latency (Fig. 4B). Following blockade of GluN2B-containing NMDA receptors with Ro 25-6981, rats had an average span of 6.21 ± 0.89 odors while vehicle treated rats had an average span of 10.31 ± 1.64 odors ($t(7) = 2.87$, $P = 0.024$). Latency to respond did not differ between treatments (Ro 25-6981 = 4.95 ± 0.93 sec, vehicle = 5.15 ± 1.11 sec; $t(7) = 0.24$, $P = 0.82$).

To assess the role of glutamatergic mPFC inputs in activating postsynaptic GluN2B-containing NMDA receptors in dmSTR, we performed disconnections of the mPFC and dmSTR by unilaterally infusing the mPFC with Mus/Bac and the dmSTR of the opposite hemisphere with Ro 25-6981 as reported previously (Baker and Ragozzino 2014). Disconnection of mPFC (Mus/Bac) and dmSTR (Ro 25-6981) reduced span to 2.43 ± 0.56 odors, which was significantly lower than the vehicle treatment of 9.45 ± 0.85 (Fig. 4C; $t(6) = 7.44$, $P < 0.001$). Latency to dig did not differ between treatments; contralateral disconnection 2.97 sec ± 0.94 compared with vehicle 4.55 ± 0.80 ($t(6) = 1.56$, $P = 0.17$). A unilateral infusion into mPFC (Mus/Bac) and dmSTR (Ro 25-6981) was also performed to confirm the specificity of the disconnection procedure. Following unilateral infusions ipsilaterally into both sites, rats displayed a mean span of 6.88 ± 1.56, which was not significantly different from their vehicle treatment ($t(6) = 1.80$, $P = 0.12$). Figure 4D displays the representative infusion sites for the mPFC and dmSTR.

**Discussion**

To the best of our knowledge, the present experiments are the first to assess the role of a corticostriatal circuit in the OST in rats. Temporary inactivation of dmSTR and dlSTR reduced span in the OST (Figs 2, 3B), although the reduction was only significant.
following dmSTR inactivation. Previous studies have assessed the role of dorsal striatum in working memory using tasks in rodents without a capacity component (White 2009; Kang et al. 2013). Lesions of the medial aspect of the dorsal striatum impair performance of delayed alternation (Dunnett et al. 1999; Moussa et al. 2011) while small lesions centered in the middle of the dorsal striatum impair reference but not working memory in a radial arm maze task (Colombo et al. 1989; Packard and White 1990). Packard et al. (1989) failed to show a working memory impairment following dorsal striatum lesions. Upon further investigation, impairments following lesions were shown when rats were given food on the maze during adaptation, but no impairment was observed when adaptation was performed without food (Packard et al. 1992). These results suggest that the appetitive component of the OST may be necessary for the observed reduction in working memory capacity following the dmSTR and dlSTR inactivations. In our second experiment, blocking GluN2B-containing NMDA receptors in the dmSTR reduced span in the OST (Fig. 4B). This impairment is consistent with previous studies showing that blocking NMDA receptors in the dmSTR impairs working memory (Smith-Roe et al. 1999) and types of behavioral flexibility including reversal learning (Watson and Stanton 2009) and behavioral switching (Baker and Ragozzino 2014).

The involvement of mPFC in a variety of working memory tasks including delayed alternation (Kolb 1990; Baeg et al. 2003) and the delayed win-shift task on the radial arm maze (Floresco et al. 1997; Lapish et al. 2008) is well known (Holmes and Wellman 2009). Importantly, these tasks do not explicitly measure working memory capacity (Davchenko et al. 2013). Thus, the disruptive effects of mPFC inactivation on the OST (Fig. 3A) are noteworthy. Previous research with the OST has confirmed reduced spans in rats following infusions of Mus/Bac (Davies et al. 2013b) or the AMPA receptor antagonist CNQX (Davies et al. 2013a) into the mPFC; however, significant changes in response latencies complicated interpretation of these results (Davies et al. 2013a,b). In the present study, no significant changes in response latency following mPFC infusions of Mus/Bac were found. One possible reason for this discrepancy in the latency results is that Davies et al. (2013a,b) infused a higher volume (0.5 µL) of drug than in the present study (0.3 µL). Importantly, mPFC inactivation does not impair performance of an olfactory sensitivity test without a memory component (Davies et al. 2013b). Taken together, these results support the involvement of the rodent mPFC in working memory, and the OST in particular.

In our previous study (Davies et al. 2013a), blocking GluN2B-containing NMDA receptors in mPFC did not significantly alter OST performance, consistent with the present results (Fig. 4A). Interestingly, systemic injections of Ro26-6981 (10 mg/kg) impair span (Davies et al. 2013a) and overexpression of GluN2B-containing NMDA receptors in the forebrain and striatum (Cui et al. 2011) increase the spans of mice tested on the OST. Given the present results with targeted infusions, we propose that GluN2B-containing NMDA receptors in dmSTR, but not mPFC, are critical for the OST. However, caution should be taken when generalizing this interpretation to other working memory tasks as GluN2B-containing NMDA receptors in dorsolateral PFC are involved in the persistent firing exhibited by delay neurons during the delayed oculomotor response task in monkeys (Wang et al. 2013). We and others (Wang et al. 2013) have used the compound Ro 25-6981 which is 5000 times more selective for GluN2B subunits compared with GluN2A subunits. While we cannot eliminate the possibility that some GluN2A-containing NMDA receptors were also affected, it is highly likely that the effects observed on odor span were due to effects of Ro 25-6981 on GluN2B-containing NMDA receptors. In other studies, systemic administration of broad spectrum NMDA receptor antagonists including CPP (Davies et al. 2013b), MK-801 (acute treatment) (MacQueen et al. 2011; Galizio et al. 2013), and ketamine (repeated injections) (Rushforth et al. 2011) impairs performance of the OST. Whether these impairments are the result of reduced GluN2B-containing NMDA receptor activity in dmSTR will require further investigation.

The mPFC sends dense glutamatergic projections to the dmSTR (McGeorge and Faull 1989; Voorn et al. 2004) that are positioned to activate GluN2B-containing NMDA receptors on medium spiny projection neurons and interneurons (Standaert et al. 1994; Smeal et al. 2008). To test whether direct communication between these areas is critical for performance of the OST, we
performed contralateral disconnections of these regions. Unilateral inactivation of the mPFC combined with either inactivation (Fig. 3C) or Ro 25-6981 (Fig. 4C) into the contralateral dmSTR reduced span in the OST. Importantly, unilateral, ipsilateral infusions into both regions did not affect span. Taken together, these findings suggest that activity of glutamatergic projection neurons from mPFC to dmSTR enable performance of the OST by activating GluN2B-containing NMDA receptors in dmSTR.

It should be noted that following our manipulations, rats still performed the task, albeit at a significantly reduced level (i.e., their spans were not 0). If memories for the odors were maintained in these areas, span should have been completely eliminated by the infusions. However, it is unknown where memory for the odors is maintained during the OST. In addition, partially preserved performance of the rats may be the result of the relatively small volume (0.3 µL) used for our infusions. Thus, some portion of the mPFC or dmSTR were likely unaffected by our infusions and could have supported a partial memory load. The infusions also increased latency to initiate digging in some cases, although these effects did not reach significance. Anecdotal observations made by the experimenters suggest that some rats took somewhat longer to initiate any movement on the testing platform following infusions while other rats sampled more bowls than controls before digging. In addition, as latencies were never decreased following manipulations, it is unlikely that the rats were impulsively digging in the first bowl they sampled on the platform. Future experiments will systematically assess the rats’ behavior following brain manipulations. Group spans also varied from 6 to 10 following vehicle infusions. Variability in performance is characteristic of the OST as noted previously for individual testing days (Dudchenko et al. 2000) and following prolonged training (Davies et al. 2013a). While this variability has the potential to complicate interpretation of the results, we note that in the present data set, Mus/Bac infusions into dmSTR were tested in two separate groups. A similar degree of impairment was noted in each case (Fig. 2A, span from 7 to 2; Fig. 3B, span from 11 to 5). Impairments in span following inactivation of mPFC have also been observed in the present data set and our previous work (Davies et al. 2013b). Thus, we are confident that the disruptive effects of mPFC and dmSTR inactivation on span are robust.

Presently, the specific contributions of the mPFC and dmSTR to performance of the OST are not well understood. Corticostriatal interactions are implicated in working memory in a number of tasks (Frank et al. 2001; McNab and Klingberg 2008; Baire et al. 2010; Wallace et al. 2011) and theories suggest that information is maintained in cortical circuits during the delay while the striatum may serve to “gate” or control the maintenance and updating of this information at the start of the delay via cortico–striato–thalamo-cortical loops (Alexander et al. 1986; Frank et al. 2001; Gruber et al. 2006). Electrophysiological recordings during the delayed oculo-motor response task in primates support these ideas as persistent neuronal activity in the dorsolateral PFC and caudate nucleus have been found during shorter delays of <4 sec (Schultz and Romo 1988; Hikosaka et al. 1989; Goldman-Rakic 1996; Kawagoe et al. 1998, Wang et al. 2013 but see also Lundqvist et al. 2016). Neurons recorded from rat dmSTR in a delayed nonmatching-to-position task with longer delays (up to 10 sec) show sequential patterns of activation over the course of the delay following initial responses to the sample stimulus early in the delay suggesting that the dmSTR may be involved in maintaining memory for the stimulus as well (Akhlaghpour et al. 2016). However, the OST has characteristics which distinguish it from simpler delayed response tasks. These include the longer retention interval (20–30 min for rats that reach spans greater than 10) and repeated exposure to the previously sampled stimuli in each “trial” of a given span. Thus, other maze based tasks such as the win-shift radial arm maze task, which puts trial-unique demands on memory over typical delays of 30 min, may engage corticostriatal circuits similarly to the OST. Recordings of neural ensembles from the mPFC of rats support its role in monitoring behavior and attending to actions (Lapiš et al. 2008). Thus, during the OST, neuronal activity in mPFC may enable rats to organize actions through glutamatergic afferents to the dmSTR which activate GluN2B-containing NMDA receptors. Notably, corticostriatal projections have a greater GluN2B-containing NMDA receptor component than thalamo-striatal projections (Smeal et al. 2008), blocking striatal NMDA receptors reduces spontaneous firing of medium spiny neurons in rats (Sandstrom and Rebec 2003; Pomata et al. 2008), and stimulation of medial prefrontal cortex induces short- and long-term patterns of synaptic plasticity in dorsal striatum (Galíñanes et al. 2011; Höhn et al. 2011). Thus, disruption of these processes by impeding the influence of mPFC inputs on dmSTR neurons may underlie the impairment of span we observed.

The present results confirm roles for the dmSTR and mPFC in the OST. Results of the disconnection experiments support the hypothesis that glutamatergic projections from mPFC to dmSTR activate GluN2B-containing NMDA receptors in the dmSTR to enable odor span. As the OST is one of the few tasks thought to involve the capacity component of working memory in rodents, these results may inform the development of therapies to improve the working memory capacity impairments in disorders such as schizophrenia, Alzheimer’s disease, and Parkinson’s disease (Barch and Smith 2008; Dudchenko et al. 2013). Assessments of potential therapies in rodent models of brain disorders that involve impaired odor span may prove particularly fruitful in this regard (Murray et al. 2017).

Materials and Methods

Animals

Three groups (total n = 24) of adult male Long Evans rats (265–415 g; Charles River, Quebec, Canada) were tested in the experiments using a within subjects design. The rats were individually housed in clear plastic cages in a colony room on a 12 h light–dark cycle (lights on at 07:00) with ad libitum access to water. Except for several days after arrival and surgery, rats were food restricted to maintain 85% of their free feeding weight. Experiments were conducted in accordance with the standards of the Canadian Council on Animal Care and were approved by the University of Saskatchewan Animal Research Ethics Board.

Apparatus

Training and testing occurred on a 91.5 cm² platform covered with black corrugated plastic with a 2.5 cm tall border on the outer edge. The platform was fastened to a metal frame with casters attached and stood 95 cm above the floor. It was surrounded by a beige curtain to block visual cues in the room. Velcro was used to fasten white porcelain bowls (4.5 cm high, 9 cm in diameter) to the platform and stop the rats from spilling the sand. Pieces of Velcro were equally spaced along the edge of the platform (one piece in each corner and five additional pieces on each side) and the bowls for a given trial were placed randomly on the pieces of Velcro.

Odors

Premium Play Sand (Quikrete Cement and Concrete Products) was sifted to remove rocks and then odors (0.5 g of a single dried spice) were mixed into the sand (100 g per bowl). The odor and sand mixtures were stored in separate Ziploc bags when not in use and new batches of sand and odors were freshly mixed every 7 d. Twenty-four different scents were used in the experiments: allspice, anise seed, basil, caraway, celery seed, cinnamon, cloves (0.1 g), cocoa, coffee, cumin, dill, fennel seed, garlic, ginger, lemon and herb, marjoram, mustard powder, nutmeg, onion powder, etc.
orange, oregano, paprika, sage, and thyme. Spices were purchased from a local grocery store. The odors used each day were selected using a random list generator and rats were regularly exposed to all odors. Bowls containing scented sand were placed on the platform as needed for each trial.

**Training on the odor span task**

Previously published protocols were followed closely (Davies et al. 2013a,b; Murray et al. 2017). Behavioral training was conducted during the light phase with the experimenter blind to the treatments administered. Rats were handled three times for 3 min before dig training. Dig Training/Shaping. First, rats were trained to dig for a food reward (Kellogg’s Froot Loops) in a bowl filled with 100 g of unscented sand. Rats were placed opposite to a bowl on the platform for three separate phases. In the first phase, the food reward was positioned on top of the sand, in the second phase the food reward was incompletely buried, and in the third phase, the food reward was fully buried. Rats were trained until they would reliably dig for the food reward regardless of the bowl’s location on the platform.

Odor nonmatching-to-sample. Once the rats consistently dug in unscented bowls, they moved onto the nonmatching-to-sample task. In the sample phase of a trial, the rat was presented with a scented bowl of sand randomly positioned on the platform. After the rat dug and consumed the food reward, it was removed from the platform and placed behind a curtain to obscure its view of the platform. The experimenter then positioned the bowl at a random location on the opposite end of the platform and added a second bowl with a different odor to the platform. In the choice phase of the trial, the rat was placed on the platform opposite to both bowls and then allowed access to both bowls. A food reward was only in the bowl with the novel odor for that trial. A choice was scored if the rat dug or placed its paws or nose on the sand and an error was scored if the rats chose the previously rewarded bowl. The rats performed six nonmatching-to-sample trials each day until they chose the novel odor on five of the six trials for 3 d.

**Odor span task (Fig. 1A)**

After reaching criterion on the nonmatching-to-sample task, rats were introduced to the OST. Trials were run as described for the nonmatching-to-sample task except that bowls with novel odors (for that trial) were added to the platform and previous bowls remained until the rat made an error (i.e., dug in any of the bowls except the novel one) which resulted in the trial ending. Previously presented bowls were randomly repositioned before each new bowl was added to the platform. Thus, rats could not use spatial cues to guide their choice. The span for a given trial was scored as the number of odor bowls correctly chosen minus one. The latency to dig on each trial was recorded by the experimenter with a stopwatch and averaged for all trials run on a given day. Each rat performed 1–3 “spans” per day (rats with high spans performed 1 span while rats with medium spans performed 2 spans, and rats with low spans performed 3 spans) with a break between spans occurring while other rats were tested. Averaging the spans reduced variance in our sample, as reported with other memory tests (Winters and Reid 2010). The mean of all spans for a given day is reported in the figures. Once a mean span of 7 or higher was reached for two training days, surgery was performed on the rats.

**Probe sessions**

To determine whether the rats were using the sample odors to solve the task, two types of probe sessions were conducted. The first probe session was implemented to test if the odor of the food reward guided behavior. In this probe session (three bowls on the platform: span of 2), rats were presented with bowls as described in the OST with the exception that the food reward was absent from all bowls. If the rat made a correct choice, the experimenter placed a food reward on top of the sand in the correct bowl. The second probe was implemented to test if the rats marked the bowls when they examined them. During this probe session (two bowls on the platform: span 1), all of the bowls and sand were replaced with new bowls and new sand that contained the same odors. If rats were marking the bowls, performance would be impaired on these trials. The rats’ performance was 100% accurate during the first probe with food reward absent, and 96% accurate (since one rat made an error) during the second probe of switching the bowls and sand (data not shown). The rat that made an error in the second probe was retested the next day on the same probe twice and got both trials correct.

**Surgery and infusions**

Rats were anesthetized with isoflurane and prepared for surgery using previously reported procedures (Davies et al. 2013a,b). Rats from each squad were implanted with guide cannulae (23 Ga) bilaterally to target two of the following three brain areas: mPFC (AP + 3.00 mm; ML ± 0.70 mm; DV −3.20 mm from bregma), dmSTR (AP + 0.80 mm; ML ± 2.20 mm; DV −3.40 mm), or dlSTR (AP + 0.80 mm; ML ± 3.60 mm DV −3.40 mm). Obdurators (0.033 cm diameter stainless steel wire) were placed into the cannula to prevent obstruction. Following surgery, rats were allowed to recover for at least a week before training resumed. Rats were habituated to the infusion procedure on three separate days during the week before infusions were administered. Bilateral infusions were performed by inserting custom made needles (30 Ga stainless steel tubing) linked via PE-50 tubing to an infusion pump (PHD 2000, Harvard Apparatus) 1 mm past the end of the cannula. Drugs were infused over 1 min while the infusion needles remained in place for an additional minute after the infusion to allow diffusion of the drug. Rats were tested on the OST 15 min following brain infusions. On treatment days, rats were tested for ~30 min (3 spans) without a break between spans.

**Experiment 1: Bilateral inactivation of dmSTR or dlSTR.** Rats (n = 9) for this experiment had cannulae implanted over the dmSTR and dlSTR. Infusion needles were inserted into one area bilaterally and either the GABA receptor agonists muscimol (Abcam) and baclofen (Mus/Bac; Abcam) or vehicle (PBS) was delivered to the dmSTR or dlSTR. The agonists were dissolved separately in PBS at a concentration of 500 ng/µl and then mixed together before infusion (McFarland and Kalivas 2001; St Onge and Floresco 2010; Davies et al. 2013b; Sangha et al. 2014).

**Experiment 2: Bilateral inactivation of mPFC, bilateral inactivation of dmSTR, or disconnection of mPFC and dmSTR.** The same infusion method was used as for experiment 1. Seven rats were tested following three treatments: bilateral mPFC inactivation, bilateral dmSTR inactivation, and contralateral disconnection (unilateral infusions in mPFC and dmSTR of opposite hemispheres). The disconnection procedure was used to block transmission of information within the mPFC-dmSTR pathway in each hemisphere. This procedure has been used to define the route of serial information transfer between different brain regions in a number of tasks (Floresco et al. 1997; Hannesson et al. 2004; Baker and Ragozzino 2014).

**Experiment 3: Role of GluN2B-containing NMDA receptors in mPFC and dmSTR in the OST.** GluN2B-containing NMDA receptors were selectively targeted with Ro 25-6981. Eight rats were tested in a counterbalanced order following either vehicle (12% DMSO; 88% PBS) or Ro 25-6981 (2.5 µg/0.5 µL) (Zhang et al. 2008; Brigman et al. 2013; Davies et al. 2013a), delivered bilaterally to mPFC or dmSTR. In the contralateral disconnection treatment, Mus/Bac was infused into the mPFC and Ro 25-6981 was infused into the dmSTR. One rat was not tested in the disconnection experiment (bilateral or ipsilateral) as it failed to perform the task reliably on training days. Postmortem examination of its brain revealed evidence of an infection in the mPFC.

**Histology**

After testing on the OST was complete, rats were sacrificed with isoflurane and perfused with saline. Brains were removed and postfixed in a 10% formalin-10% sucrose solution. Brains were sectioned on a sliding microtome and infusion sites were determined using standard protocols with reference to a rat brain atlas (Paxinos and Watson 1997).
Fluorescent muscimol infusions

In order to assess the spread of muscimol in the mPFC and dorsal STR following infusions, two rats were anesthetized and fluorescent muscimol (BODIPY TMR-X Conjugate, Life Technologies) (Allen et al. 2008) was infused using the stereotoxic coordinates described above. One rat was infused unilaterally into the mPFC while the other had unilateral infusions into the dmSTR and dSTR of different hemispheres. Sixty minutes following the infusions, the rats were anesthetized with isoflurane, perfused with saline, and brain slices (200 µm) were cut with a vibratome. Images (Fig. 1C) of the fluorescent muscimol conjugate were captured using a Zeiss Discovery V8 stereo microscope equipped with a 16-bit, 1344 x 1024 ORCA-R2 CCD camera (C10600-108B, Hamamatsu) cooled to ~35°C. BODIPY TMR-X muscimol conjugate was excited using a filter (HQ 535/50) Schott KL 1600 wide spectrum LED light source and epifluorescence filtered with an ET 605/70 installed just prior to the camera.

Data analysis

Odor spans and latencies to choose the bowls were manually recorded during testing and entered into Microsoft Excel (2010) and Statistical Package for the Social Sciences (SPSS version 19) for analysis. All descriptive values are reported as means ± standard error of the mean. Comparisons were performed using paired t-tests and repeated measures analysis of variance with Tukey’s post hoc tests where appropriate. Statistical tests were considered significant if P values were < 0.05.

Acknowledgments

This work was supported by a Discovery Grant from the Natural Sciences and Engineering Research Council of Canada (NSERC) and Operating Grant from the Canadian Institutes for Health Research to J.G.H. J.G.H. is a Canadian Institutes of Health Research New Investigator. D.A.D. received funding from a Saskatchewan Innovation & Opportunity Scholarship and an NSERC Postgraduate Scholarship. J.K.C. received funding from the Branch Out Neurological Foundation.

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