NEUROSCIENCE
RESEARCH ARTICLE
T. M. Sandini et al. / Neuroscience 440 (2020) 230–238

NMDA Receptors in Visual and Olfactory Sensory Integration in Male Long Evans Rats: A Role for the Orbitofrontal Cortex

Thaísa M. Sandini, Wendie N. Marks,[†] Nimra B. Tahir Yuanyi Song, Quentin Greba and John G. Howland[*]

Department of Anatomy, Physiology, and Pharmacology, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5E5, Canada

Abstract—Sensory integration (SI) is a cognitive process whereby the brain uses unimodal or multimodal sensory features to create a comprehensive representation of the environment. Integration of sensory input is necessary to achieve a coherent perception of the environment, and to subsequently plan and coordinate action. The neural mechanisms mediating SI are poorly understood; however, recent studies suggest that the regulation of SI involves N-methyl-D-aspartate receptors (NMDARs) in orbitofrontal cortex (OFC). Thus, we tested this hypothesis directly in two experiments using object oddity tests that require SI for visual and olfactory stimuli. First, we blocked NMDARs with acute CPP treatment (i.p., 10 mg/kg) and tested rats in unimodal visual and olfactory SI tests, and respective control unimodal oddity tests that do not require SI. Second, we used intra-OFC infusions of AP5 (30 mM) to examine the role of NMDARs in the OFC in the oddity tests requiring SI. Systemic blockade of NMDARs impaired performance on the visual tests regardless of whether SI was required for determining oddity. In the olfactory tests, systemic treatment with CPP impaired the test requiring SI while sparing olfactory oddity, demonstrating a selective impairment in the olfactory SI. Intra-OFC blockade of NMDARs impaired olfactory SI, without effect on visual SI, demonstrating that intra-OFC NMDARs are essential for olfactory, but not visual SI. The present results are discussed in the context of the function of the OFC and its associated circuitry.

Key words: glutamate receptors, OFC, olfactory oddity, visual oddity, object, spontaneous exploration.

INTRODUCTION

Sensory integration (SI) is a process whereby the brain uses unimodal or multimodal sensory features to create a coherent representation of sensory stimuli in the environment (Stein and Stanford, 2008). Each sense conveys a unique perspective of the external world; because of this, proper processing of sensory information into a contextually rich representation of the environment is fundamental to cognition (Stein et al., 2014; Marks et al., 2018b). Indeed, without proper SI, behavioral responses appear disorganized, erratic, repetitive, or disconnected (Davis et al., 2001). In addition, impaired SI of unimodal stimuli has been observed during psychosis (Carter et al., 2017), as well as in schizophrenia (SZ) (Stone et al., 2011; Silverstein et al., 2012; Kiparizoska and Ikuta, 2017). Because SI is relevant to daily cognitive functions and is further involved in cognitive symptoms of psychiatric illness, enhancing our understanding of the neural bases of SI in both healthy and disease states is required (Stein et al., 2019).

Currently, the neural substrates of SI are poorly understood in part because few tasks are available to evaluate SI directly in rodent models. However, recent progress in developing rodent tests of SI have been made with object oddity tests (Cloke et al., 2016; Marks et al., 2018b). These tests involve allowing rats to explore five objects in an open field. The objects are composed of two identical pairs and a 5th 'odd' object. The objects can have features from the same or different sensory modalities, permitting assessment of unimodal SI and multimodal SI, respectively. In this test, all objects are presented at the same time and rats explore them for a 5 minute period. Rats tested under these conditions show an innate preference for exploration of the odd object (Cloke et al., 2016; Marks et al., 2018b). Thus, SI is assessed in just one phase with a minimal mnemonic demand and does not require a pre-training phase (Cloke et al., 2016; Marks et al., 2018a,b).

A large body of literature indicates that SI involves the collective interaction of numerous brain regions such as cortex, midbrain, and thalamus (Stein et al., 2009; Winters and Reid, 2010; Reid et al., 2014). Cytoarchitectural studies indicate the orbitofrontal cortex (OFC)

*Corresponding author. Address: Health Sciences Building, University of Saskatchewan, 107 Wiggins Rd, Saskatoon, SK, S7N 5E5, Canada.
E-mail address: john.howland@usask.ca (J. G. Howland).
[†] Current address: Department of Biochemistry and Molecular Biology, University of Calgary, Calgary, Alberta T2N 4N1, Canada.
Abbreviations: NMDARs, N-methyl-D-aspartate receptors; OFC, orbitofrontal cortex; OP, oddity preference; SI, sensory integration.

https://doi.org/10.1016/j.neuroscience.2020.05.041
0306-4522/

receives convergent visual, somatosensory, olfactory, and gustatory inputs in rats and in nonhuman primates (Ongur, 2000; Schuck et al., 2018). Because of this, the rat OFC seems to play an important role in systems supporting SI (Izquierdo, 2017). Individual OFC neurons have been identified in single-unit recordings that respond to olfactory and visual stimulation, either separately or in combination (Rolls and Baylis, 1994). Recent studies in rodents indicate that the OFC is crucial for unimodal SI, especially with olfactory stimuli (Marks et al., 2018b). Together, these data suggest that OFC is involved in SI regulation; however, more investigation is required to delineate the role of the rodent OFC in SI.

In the present report, we tested the potential contribution of N-methyl-D-aspartate glutamate receptors (NMDARs) in a series of oddity tasks that do and do not require SI of visual and olfactory stimuli. It is known that cognition in rodent models relies heavily on NMDARs (Floresco, 2013; Cadinu et al., 2018; Peyrovian et al., 2019). In OFC, NMDARs are expressed on pyramidal neurons (Thompson et al., 2016) and their blockade disrupts OFC function and consequently may impair the appropriate integration of relevant sensory stimuli (Homayoun and Moghaddam 2008; van Wingerden et al., 2012). Currently, limited information exists regarding the potential role of NMDARs in SI in rodent models. In one study, rats treated sub-chronically with ketamine showed impaired multimodal SI without unimodal SI impairments (Cloke et al., 2016). As the rats were tested ten days after their last treatment, the acute effects of NMDAR blockade could not be determined. Therefore, we tested the hypothesis that acute treatment with an NMDAR antagonist would impair SI. To this end, we employed systemic treatment with the competitive NMDAR antagonist CPP as well as intra-OFC infusions of AP5 to examine the role of NMDARs in that area during SI in object oddity.

EXPERIMENTAL PROCEDURES

Animals

Thirty two adult male Long Evans rats (300–400 g; Charles River Laboratories, Kingston, NY, USA) were pair housed if receiving systemic treatment and singly housed (after surgery) if receiving infusions in standard, ventilated polypropylene cages in a temperature controlled (21 °C) room with *ad libitum* access to food (Purina Rat Chow) and water. Animals were provided with a plastic tube in their home cage for environmental enrichment throughout the experiment. Behavioral tests were conducted during the light phase of a 12 h light–dark cycle (lights on a 7 am) between 1 and 4 pm. All procedures adhered to the guidelines of the Canadian Council on Animal Care and were approved by the University of Saskatchewan's Animal Research Ethics Board.

Systemic drug treatment

(R)-CPP (±3-(2-carboxy-piperazine-4-yl)-propyl-1-phosphonic acid) (Tocris, Bioscience) was dissolved in physiological saline (0.9%) and injected (i.p. 1 ml/kg) at a dose of 10 mg/kg (Whitlock et al. 2006; Davies et al. 2017). A group of naïve rats was used for assessing the effects of NMDAR blockade on the visual and olfactory SI tests (n = 8) and another group of naïve rats was run for the control object oddity tests that do not require SI (n = 8). Both groups of rats received either saline or CPP 30 min before each of the visual and olfactory tests. The systemic experiment followed a within subjects' design in which consecutive tests were separated by a minimum of 48 h. Each rat received four tests counterbalanced for drug treatment. The experimental design for systemic treatment is illustrated in Fig. 1.

Surgery and intracranial drug treatment

A third group of naïve rats (n = 16) were implanted with guide cannula (23 Ga) bilaterally to target the ventral and lateral (VL)-OFC. During the procedure, rats were anesthetized with the inhalant anesthetic isoflurane (Janssen) and were treated with 5 mg/kg (s.c.) of Anafen (Merial Canada, Inc). After animals were positioned in a stereotaxic apparatus, the scalp was cut and retracted to expose the skull. Holes were drilled, and guide cannula was inserted bilaterally at the following coordinates VL-OFC (AP +3.50 mm; ML +2.30; DV −4.20 from bregma). The cannulae were secured to the skull using six jeweler screws and dental acrylic. The skin was then sutured, and an antiseptic cream (Hibatane® – Veterinary Ointment) was applied. Rats were singly housed post-operatively and recovered for 1 week prior to behavioral testing. The intracranial experiment followed a within subjects' design, in which consecutive infusion treatments were separated by at least 48 h. Each rat received four testing trials counterbalanced for drug treatment.

For intracranial infusions, stock solutions of D-2-amino-5-phosphonopentanoic acid (AP5) were prepared in physiological saline (0.9%) to yield concentrations of 30 mM (5.9 mg/mL). This concentration was chosen from previous studies demonstrating it was sufficient to impair rodent memory after infusion into cortex (Davies et al., 2017; Scott et al., 2019). Stock solutions were kept at −20 °C and used throughout the course of testing. Immediately before infusions, Hamilton syringes (10 μl) were connected to the infusion cannulae (30 Ga) via polyethylene tubing (PE50) loaded with drug or vehicle. Needles were lowered into the brain 1 mm past the end of the guide cannulae and infusions were conducted (infusion rate of 0.5 μl/min over 2 min for a total volume of 1 μl in each hemisphere; Davies et al., 2017). Infusion needles remained in place for 1 min after the infusion to allow diffusion of the drug. Rats were tested 15 min following brain infusions. Preceding to the infusions, rats were habituated to the infusion procedure for four days, in which all aspects of the infusion procedure were carried out, apart from drug or vehicle administration.

Behavioral testing

Apparatus and testing materials. Habituation and testing occurred in a 60 cm (l) × 60 cm (w) × 60 cm (h)

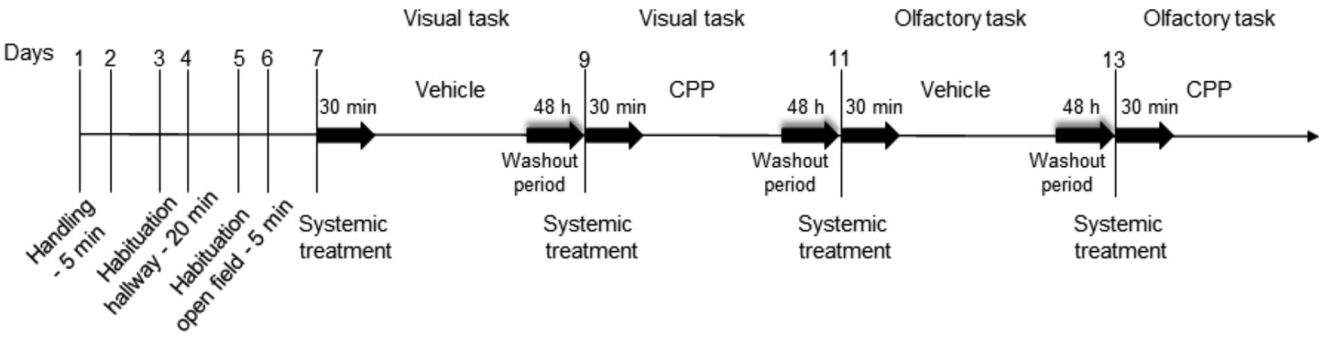

Fig. 1. Experimental design illustrates all steps for systemic treatment with vehicle (saline) or CPP (10 mg/kg) 30 min before the tests. Each rat received four testing trials counterbalanced for drug treatment, and all tests were separated by a minimum of 48 h. For intra-OFC study the experimental design applied was the same, except for surgery and the infusion habituation (see Experimental procedures for details). The rats received treatments with either vehicle (saline) or AP5 (30 mM) 15 min before testing. All treatments were separated by a minimum of 48 h.

open field constructed from white corrugated plastic (Fig. 2A). The testing stimuli were created using 250 mL glass canning jars filled with sand for stability. The oddity tests involved presentation of five objects with visual or olfactory features. Four versions of the oddity test were conducted: visual oddity, visual SI, olfactory oddity, and olfactory SI. In the control oddity tests, one sensory feature per object was presented (i.e., A/A, B/B and one odd (C) – Fig. 2C), whereas in the respective SI versions, two sensory patterns per object (i.e., AB/AB, CD/CD and an odd object (CB) – Fig. 2B) were presented. For each modality, two sets of different patterns were used. For the visual tests, printed patterns were affixed to the jars. For the olfactory tests, identical small plastic vials (25 mL) were filled with a powdered spice (lemon, onion, cloves, ginger, mustard, sage, basil, thyme, garlic, or cloves) and placed inside the glass canning jars to create distinct olfactory features used for the SI (Fig. 2D) and control oddity tests (Fig. 2E). Holes were drilled in the lids of the jars used for the olfactory test to allow for the odors to be accessible to the rats (Fig. 2F). Jars were affixed to the testing apparatus with Velcro to prevent them from tipping over during exploration.

Object oddity test protocol. Following facility acclimatization, rats were handled 5 min/day for two consecutive days and then habituated in their home cages near the behavior testing room for two additional days. Finally, rats were habituated to the open fields (without objects) for two consecutive days (5 min/day). For each test, five objects (two pairs of identical objects and a 5th 'odd' object) were placed in the open field along one wall. The odd object position was counterbalanced between rats and tests; thus, the odd object appeared equally in all positions throughout the experiment. On a given test day, rats were given 5 min to freely explore the objects. The testing apparatus and jars were cleaned with 40% ethanol between rats. Recordings of the tests were taken with an overhead camera connected to a personal computer. Videos were manually scored using stop-watches after testing was complete. Exploration of the olfactory cues was scored when the rat sniffed the top of the jar lids within 2 cm or touched them with its nose. Visual cue exploration was considered when the rat directed its gaze to the front of the jars and was within 2 cm of them. Object exploration was quantified using an oddity preference score, which was calculated using the following formula: oddity preference = time spent exploring the odd object / total object exploration time (Bartko et al., 2007; Cloke et al., 2016; Marks et al., 2018b). This score is used to quantify preferential exploration of the odd object. For these tests, an oddity preference (OP) significantly greater than chance (0.20 as five objects were available to be explored) indicates an object oddity preference (Bartko et al., 2007; Cloke et al., 2016; Marks et al., 2018b).

Histology

After visual and olfactory tests, rats from the infusion experiment were sacrificed with isoflurane and transcardially perfused with saline followed by 4% paraformaldehyde (Electron Microscopy Sciences). Brains were removed and post-fixed in paraformaldehyde before being transferred to a 30% sucrose solution and sectioned on a sliding microtome. Cannula position and infusion sites were confirmed using as reference a rat brain atlas (Paxinos and Watson, 2007).

Statistical analysis

Data were analyzed using GraphPad Prism 7 and P values ≤ 0.05 were considered significant. All data are presented as a means ± SEM except for those found in Fig. 3D. In all figures, the dashed line represents the oddity preference corresponding to chance. Behavior was analyzed by taking the mean oddity preference score and total exploration for each condition in each experiment. Paired-sample t-tests was used to compare treatments in each experiment. One sample t-tests were also used to assess significant differences from chance performance (0.2) for each group. Normality was verified using the Shapiro-Wilk test. Data that did not reach normality were analyzed using a nonparametric Wilcoxon matched-pairs test and are presented as

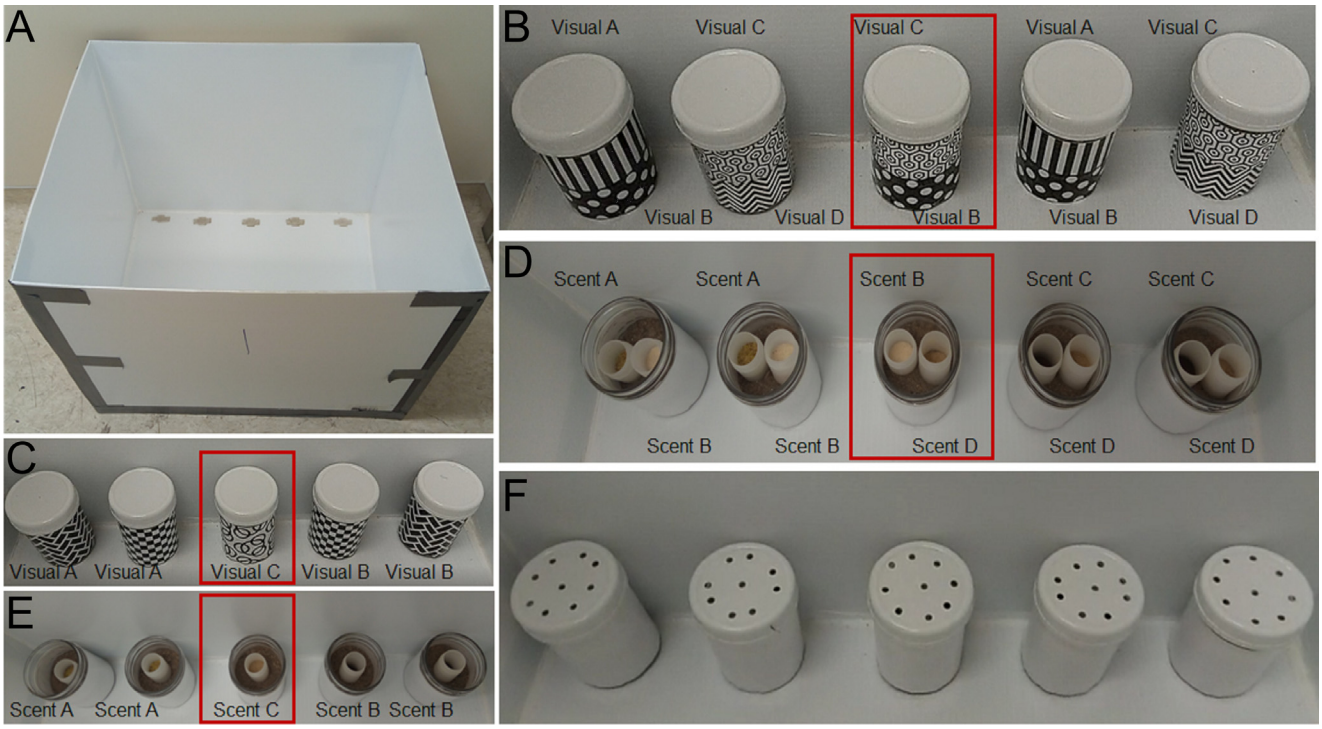

Fig. 2. Photographs of the open field **(A)** used for the oddity tests and representative stimuli used for each variant (visual and olfactory). Red highlight indicates the odd object in each test. **(B)** Patterns used for the visual test requiring SI; **(C)** patterns used for visual oddity test not requiring SI; **(D)** spices used for the olfactory test requiring SI; **(E)** spices used for the olfactory test not requiring SI. Panel **(F)** shows the holes drilled in the jar lids to allow for olfactory exploration. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

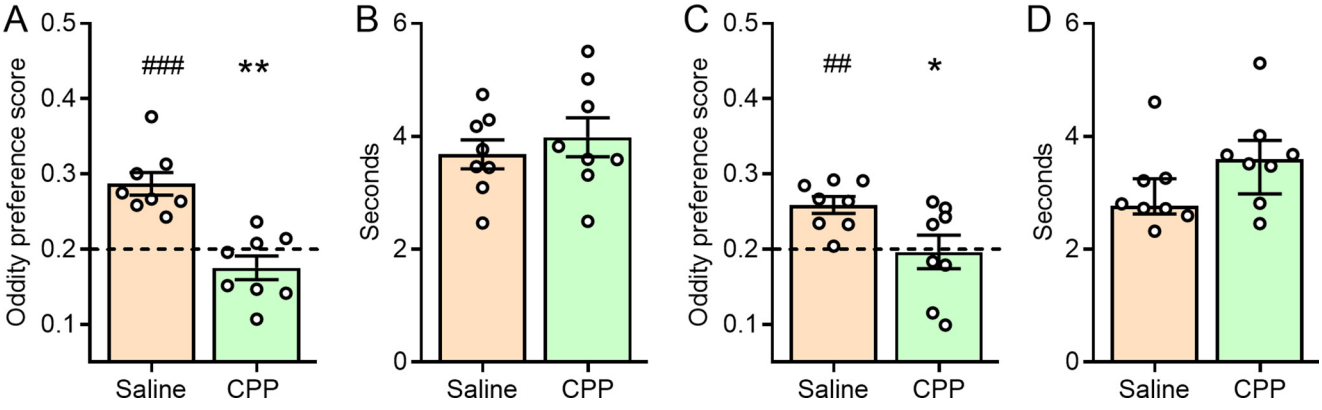

Fig. 3. Oddity preference scores from the visual test requiring SI and control visual oddity test that does not require SI following systemic CPP treatment in rats. Significant impairments in oddity preference score were found in systemic CPP treated rats in visual requiring SI (**$p < 0.01$; **A**) and in visual control (*$p < 0.05$; **C**) test that does not require SI *versus* vehicle treatment. Significant oddity preference above chance performance was found in vehicle (saline) treatment in both visual oddity tests (###$p < 0.001$; **A**, ##$p < 0.01$; **C**). Systemic CPP treatment had no effect on total object exploration time for either visual oddity test ($p > 0.05$; **B**; **D**). Statistical differences between treatment are indicated by asterisks (*) signs. Statistical differences above chance performance are indicated by pound (#) signs.

median with interquartile range (Fig. 3D). Multiple comparisons for each experiment were corrected using the Holm-Sidak method as implemented in GraphPad Prism. Effect size was provided with partial eta squared (η^2_p) which represents the total variability in each dependent variable that can be attributed to the independent variables. Small, medium, and large effect sizes are considered partial (η^2_p) values of 0.01, 0.06, and 0.14 respectively (Cohen 1988).

RESULTS

Systemic CPP administration impairs visual object oddity regardless of whether SI was required

Systemic administration of CPP significantly decreased oddity preference in the visual test requiring SI, when performance was compared to the saline testing day (Fig. 3A; $t_7 = 5.26$, $p = 0.0012$, $\eta^2_p = 0.798$). In addition, one-sample *t*-test showed that rats receiving

vehicle had an oddity preference significantly higher than chance for the visual test requiring SI (Fig. 3A; $t_7 = 5.79$, $p = 0.0007$, $\eta^2_p = 0.827$), whereas the oddity preference of CPP-treated rats did not differ significantly from chance (Fig. 3A; $t_7 = 1.57$, $p = 0.16$).

When we analyzed the data from the control visual oddity test that does not require SI, a paired-sample *t*-test showed that systemic CPP treatment significantly decreased oddity preference (Fig. 3C; $t_7 = 2.55$, $p = 0.0379$, $\eta^2_p = 0.482$), when performance was compared to the preferences of saline-injection. In addition, one-sample *t*-tests showed significantly oddity preference above chance performance when rats received vehicle treatment (Fig. 3C; $t_7 = 5.20$, $p < 0.0012$, $\eta^2_p = 0.795$), but not CPP (Fig. 3C; $t_7 = 0.16$, $p = 0.87$). Systemic CPP treatment had no effect on total exploration in visual oddity requiring SI (Fig. 3B; $t_7 = 0.81$, $p = 0.44$). Also, the Wilcoxon matched pairs nonparametric test showed that systemic CPP treatment had no effect on total exploration in the visual test that does not require SI (Fig. 3D; $W = 14$, $p = 0.38$). These results suggest that systemic treatment with an NMDAR antagonist impairs the visual control oddity test and the visual test requiring SI.

Systemic CPP administration selectively impairs olfactory object oddity when SI is required

Systemic administration of CPP significantly decreased oddity preference in the olfactory test requiring SI (Fig. 4A; $t_7 = 3.13$, $p = 0.017$, $\eta^2_p = 0.584$) when performance was compared to the preference following saline injection. In addition, one-sample *t*-tests showed that when rats received vehicle, their oddity preference was significantly higher than chance for the olfactory test requiring SI (Fig. 4A; $t_7 = 4.52$, $p = 0.0027$, $\eta^2_p = 0.745$), whereas the oddity preference did not differ significantly from chance following CPP (Fig. 4A; $t_7 = 0.18$, $p = 0.86$).

Systemic administration of CPP did not alter oddity preference in the olfactory test that does not require SI when performance was compared to that following saline injection (Fig. 4C; $t_7 = 0.15$, $p = 0.88$). One-sample *t*-tests showed that following either saline (Fig. 4C; $t_7 = 6.20$, $p < 0.0004$, $\eta^2_p = 0.846$) or CPP treatment (Fig. 4C; $t_7 = 2.81$, $p = 0.0258$, $\eta^2_p = 0.531$ – Fig. 4C), rats showed oddity preference above chance performance. Systemic treatment CPP had no effect on total exploration in the olfactory test requiring SI (Fig. 4B; $t_7 = 1.49$, $p = 0.17$) or in the control olfactory (Fig. 4D; $t_7 = 2.12$, $p = 0.07$) oddity test. These results demonstrated that systemic treatment with an NMDAR antagonist impairs the olfactory test requiring SI while sparing olfactory oddity.

Blockade of NMDA receptors in OFC impairs olfactory, but not visual, object oddity requiring SI

Intra-OFC AP5 treatment did not change the oddity preference in the visual test requiring SI (Fig. 5A; $t_{11} = 0.96$, $p = 0.35$) when performance was compared to the preferences following saline injection. One-sample *t*-tests showed that rats receiving vehicle (Fig. 5A; $t_{11} = 2.29$, $p = 0.0428$, $\eta^2_p = 0.32$) or AP5 (Fig. 5A; $t_{11} = 5.59$, $p = 0.0002$, $\eta^2_p = 0.74$) had an oddity preference significantly higher than chance performance for visual test requiring SI.

In contrast, a paired sample *t*-test showed that AP5 infusions into OFC significantly decreased oddity preference for olfactory SI-dependent oddity (Fig. 5C; $t_{11} = 3.12$, $p = 0.0097$, $\eta^2_p = 0.47$) when performance was compared to the preferences of saline-injected controls. One-sample *t*-tests comparing oddity preference showed significant oddity preference above chance when rats received vehicle (saline) treatment (Fig. 5C; $t_{11} = 3.47$, $p = 0.0052$, $\eta^2_p = 0.52$), whereas the oddity preference following AP5 treatment did not differ significantly from chance (Fig. 5C; $t_{11} = 0.56$, $p = 0.58$). Similar to systemic treatment with CPP, bilateral infusion of AP5 into the OFC did not affect the amount of time spent exploring the objects for visual (Fig. 5B; $t_{11} = 1.40$, $p = 0.18$) or olfactory (Fig. 5D; $t_{11} = 1.06$, $p = 0.30$) tests requiring SI. These results demonstrate that intra-OFC blockade of NMDARs did not affect the visual test requiring SI, while the olfactory test requiring SI was impaired. Thus, these results indicate that NMDARs into OFC are crucial for normal olfactory SI.

Histology

Fig. 5 panel E shows the approximate placements of the infusion needles. Only rats with placements in ventral and lateral OFC were accepted for statistical analysis ($n = 12$). Four rats had misplaced infusion sites that were either dorsal or ventral to the OFC; these four animals were excluded from all analysis.

DISCUSSION

Systemic CPP treatment significantly decreased oddity preference when rats were tested in the visual oddity tests either with or without an SI component. In contrast, systemic treatment with CPP impaired olfactory oddity requiring SI while leaving the control olfactory oddity test without SI intact. Intracranial infusions of AP5 into OFC impaired the olfactory oddity with SI test without affecting the visual oddity with SI test. Thus, the present findings provide evidence that NMDARs, particularly in the OFC, have a selective role in olfactory oddity tests requiring SI. No such selectivity is shown in the companion tests using visual stimuli.

In the present experiments, we modified some aspects of the tests used previously in Marks et al. (2018b). We used larger, printed patterns for the visual oddity tests that covered the jars (Fig. 2B, C). With this modification, variability in performance was reduced compared to the data generated in our previous study (Marks et al., 2018b). It is noteworthy that exploration times are low for the test, particularly the visual versions. However, as vehicle-treated rats consistently showed an oddity preference above chance in all the tests, we believe that our results are reliable. Importantly, we also added oddity tests without an SI component to test whether the judgement of oddity itself was impaired by the systemic

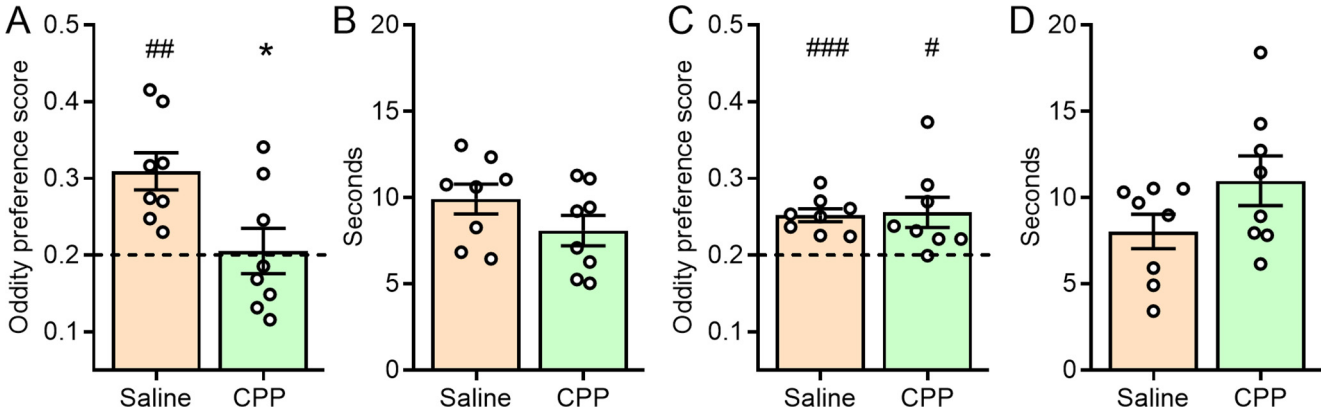

Fig. 4. Oddity preference scores from the olfactory test requiring SI and for olfactory control visual oddity test that does not require SI following systemic CPP treatment in rats. Significant impairments in oddity preference score were found in systemic CPP treated rats in olfactory test requiring SI (*$p < 0.05$; **A**) *versus* vehicle treatment. Significant oddity preference above chance performance was found following vehicle (saline) treatment in olfactory test requiring SI (##$p < 0.01$; **A**). Additionally, significant oddity preference above chance performance was found following vehicle (saline) treatment (###$p < 0.001$; **C**) as well as CPP treatment (#$p < 0.05$; **C**) for the olfactory test that does not require SI. Systemic CPP treatment had no effect on total object exploration time for either olfactory oddity test ($p > 0.05$; **B**; **D**). Statistical differences between treatment are indicated by asterisks (*) signs. Statistical differences above chance performance are indicated by pound (#) signs.

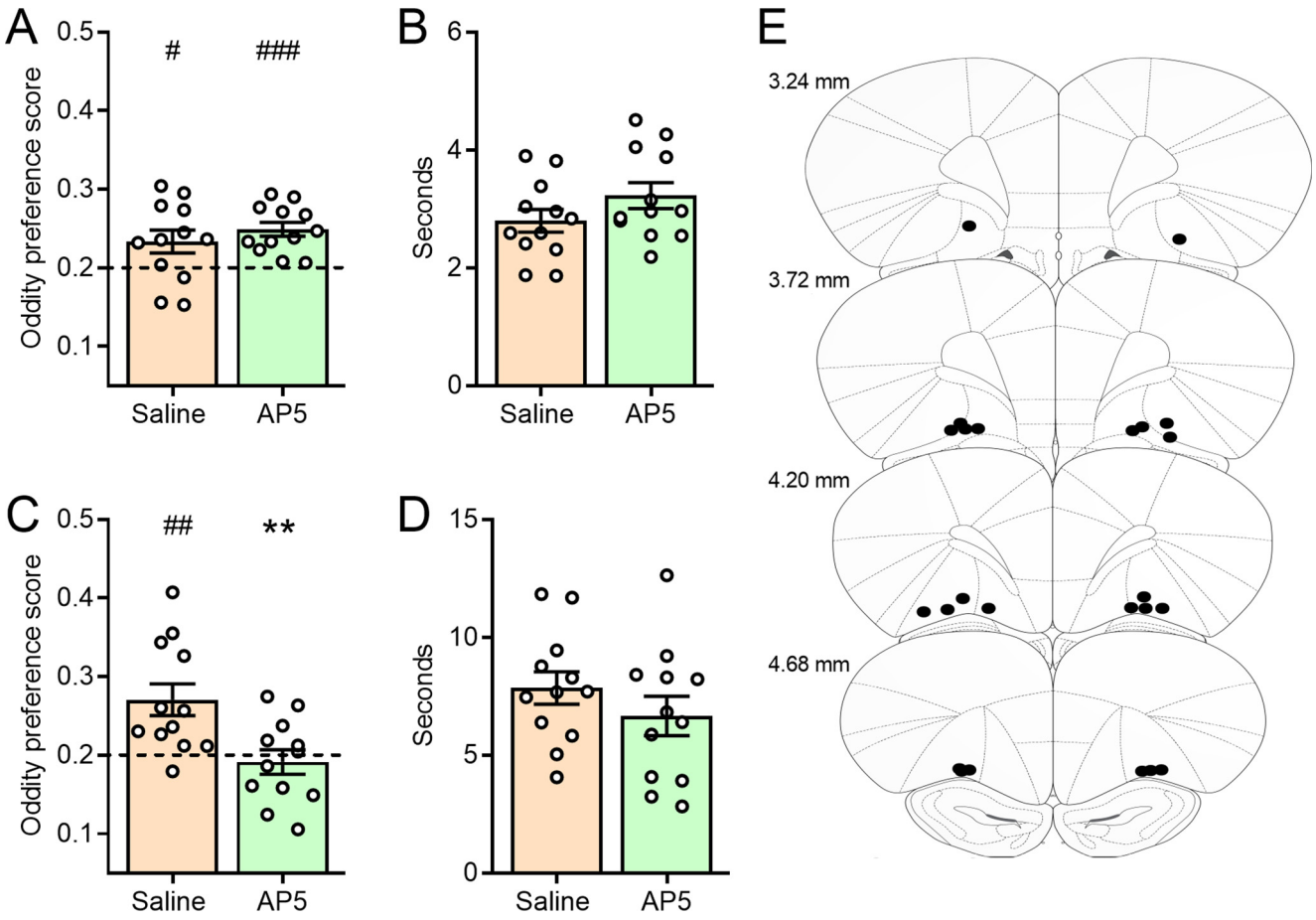

Fig. 5. Oddity preference scores from visual and olfactory tests requiring SI following intra-OFC infusion of AP5 in rats. AP5-treated rats showed oddity preference above chance performance in visual test requiring SI (###$p < 0.001$; **A**). Significant impairments in oddity preference were found following AP5 infusion in the olfactory test requiring SI (**$p < 0.01$; **C**) *versus* vehicle group. Significant oddity preference above chance performance was found following vehicle (saline) treatment in visual (#$p < 0.05$; **A**) and olfactory tests requiring SI (##$p < 0.01$; **C**). Bilateral infusion with AP5 into OFC had no effect on total object exploration time for visual or olfactory tests ($p > 0.05$; **B**–**D** respectively). Statistical differences between treatment are indicated by asterisks (*) signs. Statistical differences above chance performance are indicated by pound (#) signs. Panel **E** shows a schematic representation of the infusion needle tip placements of the infusions into ventrolateral (VL) OFC from all rats ($n = 12$). Black dots indicate infusion sites.

NMDAR manipulations made. Comparison of the results of these two tests allows us to dissociate the effects of our systemic NMDAR blockade on visual and olfactory oddity. In addition, as the olfactory test not requiring SI was unaffected by systemic NMDAR antagonist treatment, we believe that it is reasonable to conclude that NMDAR blockade limited to the OFC would not have impaired the oddity test without SI either.

Systemic CPP administration impairs visual oddity with or without SI, and olfactory oddity with SI

Our study provides evidence that NMDARs are important for both visual oddity tests. These effects could be the result of: (1) an inability of the rats to make an oddity judgement, or (2) an inability of the rats to discriminate the visual characteristics of the stimuli used in the tests (Winters et al., 2010; Bartko et al., 2011; Talpos et al., 2012). NMDARs are widely distributed in visual cortex (Aoki et al., 1994; Liu et al., 2015) and they play a role in integrating neural responses in supragranular layers of the rat primary visual cortex (Fukuda et al., 1998; Kurylo and Gazes, 2008). As a result, the hypothesis that blocking NMDARs impaired the normal visual cortex function and consequently the performance in visual object oddity tasks cannot be discarded. In contrast, we demonstrated that systemic CPP treatment selectively impaired the olfactory oddity test with SI while sparing olfactory oddity without SI. This suggests that oddity judgements for olfactory stimuli remain intact following NMDAR antagonism. This finding is in general agreement with previous researchers that has shown that rats treated with NMDAR antagonist had no difficulty in recognizing and discriminating odors, indicating intact olfaction (Staubli et al., 1989; Tarland and Brosda, 2018). When taken into consideration with the pattern of results for visual stimuli discussed above, this provides evidence that the deficits in the visual tasks are likely a result of a deficit in visual perception, and not the ‘‘oddity judgement process”, per se.

Few studies have directly assessed the effects of blocking NMDARs on SI in rodent models. Repeated treatment with ketamine failed to alter basic olfactory or visual SI in rats (Cloke et al., 2016); however, the acute effects of NMDAR blockade could not be determined as a ten day washout period followed the last ketamine injection in this study. Impaired performance in crossmodal and unimodal (tactile or visual) object recognition tests have been reported following repeated treatment with MK-801 or ketamine (Jacklin et al., 2012; Cloke et al., 2015). However, this impairment was observed when a 1-h retention delay was used. Thus, these impairments may relate to the mnemonic demands of testing and consequently confound the interpretation of a specific deficit in SI. Furthermore, rats were tested after a 7–10 day drug-free, washout period; thus, the acute effects of NMDAR blockade could not be determined.

Blockade of NMDARs in OFC impairs olfactory object oddity requiring SI

The rodent OFC contributes to an array of cognitive functions and there is growing evidence that OFC subdivisions (e.g., medial orbital area, ventral orbital area, ventrolateral orbital area) make distinct functional contributions to behavior (Stalnaker et al., 2015; Izquierdo, 2017; Murphy and Deutch, 2018; Constantinople et al., 2019; Hervig et al., 2019). Indeed, anatomical, mechanistic, and recording experiments demonstrate that medial regions are more related to affective regulation and cognitive control while lateral regions are more involved in SI (Rempel-Clower, 2007; Hoover and Vertes, 2011; Nogueira et al., 2017). However, few studies have investigated the function of the rodent ventrolateral (VL) OFC in object oddity tasks that require SI. Our intracranial infusions of AP5 into OFC impaired the olfactory oddity with SI test without affecting the visual object oddity with SI test. The role of the OFC in olfactory-dependent cognition is well documented in humans and rodents (Rolls, 2004; Reid et al., 2014; Cloke and Winters, 2015; Fagundo et al., 2015). Recently, VL-OFC infusions of the T-type calcium channel blocker Z944 impaired olfactory SI (Marks et al., 2018b). The results from the present study further suggest that the OFC and, in particular, NMDAR activity within the OFC, mediates the circuit involved in the olfactory oddity test requiring SI. However, the mechanisms underlying the involvement of NMDARs within the OFC in olfactory SI test are unknown. One consequence of blocking NMDARs would likely be alterations in gamma oscillations. Gamma oscillations are reduced in patients with schizophrenia during SI and working memory (Cho et al., 2006; Uhlhaas and Singer, 2010; Lewis et al., 2012), and abnormal gamma oscillations are also observed in pharmacological and genetic NMDAR hypofunction rodent models of SZ (Cunningham, 2006; McNally et al., 2013; Hiyoshi et al., 2014; Jadi et al., 2016). Others have shown that gamma oscillations appear in OFC during olfactory-dependent tasks (van Wingerden et al., 2010) suggesting that gamma oscillatory activity of the central olfactory system plays a functional role in conveying odor signals from the olfactory bulb to the OFC via the piriform cortex (Mori et al., 2013). These findings support the hypothesis that blocking NMDARs in the VL-OFC alter gamma oscillations and consequently the performance of olfactory tests requiring SI. Future *in vivo* electrophysiological experiments with NMDARs antagonists into OFC will help clarify the involvement of gamma oscillation in olfactory SI.

In conclusion, the present study demonstrates that NMDARs are important for the olfactory oddity test requiring SI and their blockade also impairs visual oddity tests either with or without an SI component. Additionally, our data demonstrate that intracranial infusions of AP5 into OFC impaired the olfactory oddity with SI requirement without affecting the visual oddity. Thus, our findings provide evidence that NMDARs, particularly in the VL-OFC, have a selective role in olfactory oddity tests requiring SI. More studies investigating the role of NMDARs using rodent models related to neuropsychological disorders may help advance more specific and effective therapeutics for

treating SI impairments. Furthermore, future studies should focus on characterizing the neural substrates involved specially in visual oddity tests.

ACKNOWLEDGEMENTS

This research was supported by an operating grant from the Canadian Institutes of Health Research (#125984) and a Natural Sciences and Engineering Research Council of Canada Discovery Grant to JGH. TMS and WNM received fellowship funding from the Saskatchewan Health Research Foundation. NBT received a scholarship from the College of Medicine at the University of Saskatchewan. YS received a scholarship from the NSERC Undergraduate Student Research Award program.

REFERENCES

Aoki C, Venkatesan C, Go CG, Mong JA, Dawson TM (1994) Cellular and subcellular localization of NMDA-R1 subunit immunoreactivity in the visual cortex of adult and neonatal rats. J Neurosci 14:5202–5222.

Bartko SJ, Winters BD, Cowell RA, Saksida LM, Bussey TJ (2007) Perirhinal cortex resolves feature ambiguity in configural object recognition and perceptual oddity tasks. Learn Mem 14:821–832.

Bartko SJ, Vendrell I, Saksida LM, Bussey TJ (2011) A computer-automated touchscreen paired-associates learning (PAL) task for mice: Impairments following administration of scopolamine or dicyclomine and improvements following donepezil. Psychopharmacology 214:537–548.

Cadinu D, Grayson B, Podda G, Harte MK, Doostdar N, Neill JC (2018) NMDA receptor antagonist rodent models for cognition in schizophrenia and identification of novel drug treatments, an update. Neuropharmacology 142:41–62.

Carter O et al (2017) Sensory integration deficits support a dimensional view of psychosis and are not limited to schizophrenia. Transl Psychiatry 7 e1118.

Cho RY, Konecky RO, Carter CS (2006) Impairments in frontal cortical synchrony and cognitive control in schizophrenia. Proc Natl Acad Sci 103:19878–19883.

Cloke JM, Winters BD (2015) $\alpha 4\beta 2$ nicotinic receptor stimulation of the GABAergic system within the orbitofrontal cortex ameliorates the severe crossmodal object recognition impairment in ketamine-treated rats: Implications for cognitive dysfunction in schizophrenia. Neuropharmacology 90:42–52.

Cloke JM, Jacklin DL, Winters BD (2015) The neural bases of crossmodal object recognition in non-human primates and rodents: a review. Behav Brain Res 285:118–130.

Cloke JM, Nguyen R, Chung BY, Wasserman DI, De Lisio S, Kim JC, Bailey CD, Winters BD (2016) A novel multisensory integration task reveals robust deficits in rodent models of schizophrenia: converging evidence for remediation via nicotinic receptor stimulation of inhibitory transmission in the prefrontal cortex. J Neurosci 36:12570–12585.

Cohen J (1988). Statistical power analysis for the behavioral sciences, vol 2. New York: Academic Press.

Constantinople CM, Piet AT, Bibawi P, Akrami A, Kopec C, Brody CD (2019) Lateral orbitofrontal cortex promotes trial-by-trial learning of risky, but not spatial, biases. Elife 8 e49744.

Cunningham MO (2006) Region-specific reduction in entorhinal gamma oscillations and parvalbumin-immunoreactive neurons in animal models of psychiatric illness. J Neurosci 26:2767–2776.

Davies DA, Hurtubise JL, Greba Q, Howland JG (2017) Medial prefrontal cortex and dorsomedial striatum are necessary for the trial-unique, delayed nonmatching-to-location (TUNL) task in rats: Role of NMDA receptors. Learn Mem 24:262–266.

Davis AE, Gimenez AM, Therrien B (2001) Effects of entorhinal cortex lesions on sensory integration and spatial learning. Nurs Res 50:77–85.

Fagundo AB et al (2015) Modulation of higher-order olfaction components on executive functions in humans. PLoS One 10 e0130319.

Floresco SB (2013) Prefrontal NMDA receptors and cognition: working 2B remembered. Neuron 77:603–605.

Fukuda M, Hata Y, Ohshima M, Tsumoto T (1998) Role of NMDA receptors in the propagation of excitation in rat visual cortex as studied by optical imaging. Neurosci Res 31:9–21.

Hervig ME et al (2019) Dissociable and paradoxical roles of rat medial and lateral orbitofrontal cortex in visual serial reversal learning. Cereb Cortex (in press).

Hiyoshi T, Kambe D, Karasawa J, Chaki S (2014) Involvement of glutamatergic and GABAergic transmission in MK-801-increased gamma band oscillation power in rat cortical electroencephalograms. Neuroscience 280:262–274.

Homayoun H, Moghaddam B (2008) Orbitofrontal cortex neurons as a common target for classic and glutamatergic antipsychotic drugs. Proc Natl Acad Sci U S A 105:18041–18046.

Hoover WB, Vertes RP (2011) Projections of the medial orbital and ventral orbital cortex in the rat. J Comp Neurol 519:3766–3801.

Izquierdo A (2017) Functional heterogeneity within rat orbitofrontal cortex in reward learning and decision making. J Neurosci 37:10529–10540.

Jacklin DL, Goel A, Clementino KJ, Hall AWM, Talpos JC, Winters BD (2012) Severe cross-modal object recognition deficits in rats treated sub-chronically with nmda receptor antagonists are reversed by systemic nicotine: Implications for abnormal multisensory integration in schizophrenia. Neuropsychopharmacology 37:2322–2331.

Jadi MP, Behrens MM, Sejnowski TJ (2016) Abnormal gamma oscillations in N-Methyl-D-Aspartate receptor hypofunction models of schizophrenia. Biol Psychiatry 79:716–726.

Kiparizoska S, Ikuta T (2017) Disrupted olfactory integration in schizophrenia: functional connectivity study. Int J Neuropsychopharmacol 20:740–746.

Kurylo DD, Gazes Y (2008) Effects of Ketamine on perceptual grouping in rats. Physiol Behav 95:152–156.

Lewis DA, Curley AA, Glausier JR, Volk DW (2012) Cortical parvalbumin interneurons and cognitive dysfunction in schizophrenia. Trends Neurosci 35:57–67.

Liu H et al (2015) The distinct role of NR2B subunit in the enhancement of visual plasticity in adulthood. Mol Brain 8:49.

Marks WN, Parker ME, Zabder NK, Greba Q, Snutch TP, Howland JG (2018b) T-type calcium channels in the orbitofrontal cortex mediate sensory integration as measured using a spontaneous oddity task in rats. Learn Mem 25:317–324.

Marks WN, Parker ME, Howland JG (2018a) Variants of the spontaneous recognition procedure assessing multisensory integration reveal behavioral alterations in rodent models of psychiatric and neurological disorders. In: Ennaceur A, Silva MAS, editors. Handbook of behavioral neuroscience, vol 27. Elsevier: Academic Press. p. 125–137.

McNally JM, McCarley RW, Brown RE (2013) Chronic ketamine reduces the peak frequency of gamma oscillations in mouse prefrontal cortex ex vivo. Front Psychiatry 4:106.

Mori K, Manabe H, Narikiyo K, Onisawa N (2013) Olfactory consciousness and gamma oscillation couplings across the olfactory bulb, olfactory cortex, and orbitofrontal cortex. Front Psychol 4:743.

Murphy MJM, Deutch AY (2018) Organization of afferents to the orbitofrontal cortex in the rat. J Comp Neurol 526:1498–1526.

Nogueira R, Abolafia JM, Drugowitsch J, Balaguer-Ballester E, Sanchez-Vives MV, Moreno-Bote R (2017) Lateral orbitofrontal cortex anticipates choices and integrates prior with current information. Nat Commun 8:14823.

Ongur D (2000) The organization of networks within the orbital and medial prefrontal cortex of rats, monkeys and humans. Cereb Cortex 10:206–219.

Paxinos G, Watson C (2007) The rat brain in stereotaxic coordinates. sixth ed. New York, NY: Elsevier Acad Press.
Peyrovian B, Rosenblat JD, Pan Z, Iacobucci M, Brietzke E, McIntyre RS (2019) The glycine site of NMDA receptors: a target for cognitive enhancement in psychiatric disorders. Prog Neuro-Psychopharmacology Biol Psychiatry 92:387–404.
Reid JM, Jacklin DL, Winters BD (2014) Delineating prefrontal cortex region contributions to crossmodal object recognition in rats. Cereb Cortex 24:2108–2119.
Rempel-Clower NL (2007) Role of orbitofrontal cortex connections in emotion. Ann N Y Acad Sci 1121:72–78.
Rolls ET (2004) The functions of the orbitofrontal cortex. Brain Cogn 55:11–29.
Rolls ET, Baylis LL (1994) Gustatory, olfactory, and visual convergence within the primate orbitofrontal cortex. J Neurosci 14:5437–5452.
Schuck NW, Wilson R, Niv Y (2018) A state representation for reinforcement learning and decision-making in the orbitofrontal cortex. In: Morris R, Bornstein A, Shenhav A, editors. Goal-directed decision making. Elsevier. p. 259–278.
Scott GA, Roebuck AJ, Greba Q, Howland JG (2019) Performance of the trial-unique, delayed non-matching-to-location (TUNL) task depends on AMPA/Kainate, but not NMDA, ionotropic glutamate receptors in the rat posterior parietal cortex. Neurobiol Learn Mem 159:16–23.
Silverstein SM, Keane BP, Barch DM, Carter CS, Gold JM, Kovács I, MacDonald A, Ragland JD, Strauss ME (2012) Optimization and validation of a visual integration test for schizophrenia research. Schizophr Bull 38:125–134.
Stalnaker TA, Cooch NK, Schoenbaum G (2015) What the orbitofrontal cortex does not do. Nat Neurosci 18:620–627.
Staubli U, Thibault O, DiLorenzo M, Lynch G (1989) Antagonism of NMDA receptors impairs acquisition but not retention of olfactory memory. Behav Neurosci 103:54–60.
Stein BE, Stanford TR (2008) Multisensory integration: Current issues from the perspective of the single neuron. Nat Rev Neurosci 9:255–266.
Stein BE, Stanford TR, Rowland BA (2009) The neural basis of multisensory integration in the midbrain: Its organization and maturation. Hear Res 258:4–15.
Stein BE, Stanford TR, Rowland BA (2014) Development of multisensory integration from the perspective of the individual neuron. Nat Rev Neurosci 15:520–535.
Stein BE, Stanford TR, Rowland BA (2019) Multisensory integration and the society for neuroscience: then and now. J Neurosci 40:3–11.
Stone DB, Urrea LJ, Aine CJ, Bustillo JR, Clark VP, Stephen JM (2011) Unisensory processing and multisensory integration in schizophrenia: a high-density electrical mapping study. Neuropsychologia 49:3178–3187.
Talpos JC, Fletcher AC, Circelli C, Tricklebank MD, Dix SL (2012) The pharmacological sensitivity of a touchscreen-based visual discrimination task in the rat using simple and perceptually challenging stimuli. Psychopharmacology 221:437–449.
Tarland E, Brosda J (2018) Male rats treated with subchronic PCP show intact olfaction and enhanced interest for a social odour in the olfactory habituation/dishabituation test. Behav Brain Res 345:13–20.
Thompson JL, Yang J, Lau B, Liu S, Baimel C, Kerr LE, Liu F, Borgland SL (2016) Age-dependent d1–d2 receptor coactivation in the lateral orbitofrontal cortex potentiates NMDA receptors and facilitates cognitive flexibility. Cereb Cortex 26:4524–4539.
Uhlhaas PJ, Singer W (2010) Abnormal neural oscillations and synchrony in schizophrenia. Nat Rev Neurosci 11:100–113.
van Wingerden M, Vinck M, Lankelma JV, Pennartz CMA (2010) Learning-associated gamma-band phase-locking of action-outcome selective neurons in orbitofrontal cortex. J Neurosci 30:10025–10038.
van Wingerden M, Vinck M, Tijms V, Ferreira IR, Jonker AJ, Pennartz CM (2012) NMDA receptors control cue-outcome selectivity and plasticity of orbitofrontal firing patterns during associative stimulus-reward learning. Neuron 76:813–825.
Whitlock JR, Heynen AJ, Shuler MG, Bear MF (2006) Learning induces long-term potentiation in the hippocampus. Science 313:1093–1097.
Winters BD, Bartko SJ, Saksida LM, Bussey TJ (2010) Muscimol, AP5, or scopolamine infused into perirhinal cortex impairs two-choice visual discrimination learning in rats. Neurobiol Learn Mem 93:221–228.
Winters BD, Reid JM (2010) A distributed cortical representation underlies crossmodal object recognition in rats. J Neurosci 30:6253–6261.

(Received 28 January 2020, Accepted 23 May 2020)
(Available online 1 June 2020)