A role of frontal association cortex in long-term object recognition memory of objects with complex features in rats

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Abbreviations: FrA, Frontal association cortex; IACUC, Animal Care and Use Committee; ORM, Object recognition memory; PRh, Perirhinal cortex; $RGS14_{414}$, Regulator of G protein signalling 14 of 414 amino acids; RRID, Research Resource Identifier. Mariam Masmudi-Martín and Manuel F. López-Aranda contributed equally to this work.

Abstract

Perirhinal cortex is a brain area that has been considered crucial for the object recognition memory (ORM). However, with the use of an ORM enhancer named $RGS14_{414}$ as gain-in-function tool, we show here that frontal association cortex and not the Perirhinal cortex is essential for the ORM of objects with complex features that consisted of detailed drawing on the object surface (complex ORM). An expression of $RGS14_{414}$, in rat brain frontal association cortex, induced the formation of long-term complex ORM, whereas the expression of the same memory enhancer in Perirhinal cortex failed to produce this effect. Instead, $RGS14_{414}$ expression in Perirhinal cortex caused the formation of ORM of objects with simple features that consisted of the shape of object (simple ORM).

Further, a selective elimination of frontal association cortex neurons by treatment with an immunotoxin Ox7-SAP completely abrogated the formation of complex ORM. Thus, our results suggest that frontal association cortex plays a key role in processing of a high-order recognition memory information in brain.

Keywords
complex features, episodic memory, frontal association cortex, object memory behaviour, object recognition, perirhinal cortex, recognition memory, simple features.

1. INTRODUCTION

Object recognition is one of the most studied examples of episodic memory, a kind of memory that is primarily affected in patients or individuals with memory dysfunction. Ventral stream circuits also known as "what" pathway, which runs from the visual cortex to regions of the medial temporal lobe, including the hippocampus, not only supports the object recognition process but also sustains the object memory functions (Khan et al., 2011). Therefore, object recognition memory (ORM) is thought to be processed through ventral pathway circuits, and within these interconnected brain areas of this pathway, perirhinal cortex (PRh) of medial temporal lobe is considered crucial for ORM (Brown & Aggleton, 2001; Murray & Richmond, 2001; Suzuki, 1996; Winters et al., 2008). Ablation of as well as lesions in the PRh cause dramatic deficits in the ability of rats (Ennaceur & Aggleton, 1997; Mumby & Pinel, 1994; Norman & Eacott, 2004), monkeys (Buckley & Gaffan, 1998; Buffalo et al., 2000; Hadfield et al., 2003; Meunier et al., 1993) and humans (Buffalo et al., 1998) to perform ORM tasks. The central role of PRh neurons in ORM was further confirmed by receptor antagonist infusions in rats (Balderas et al., 2013; Bartko et al., 2014) and monkeys (Tang et al., 1997) and by electrophysiological recordings in neuronal populations that appear to encode recognition memory in rats (Burke et al., 2012; Wan et al., 1999) and primates (Fahy et al., 1993; Hölscher et al., 2003). Although PRh is fundamental for object-related information processing and ORM, it has been shown that various aspects of ORM, such as associative and temporal order, require interaction with medial frontal cortical area (mFC) (Barker et al., 2007; Hannesson et al., 2004; Morici et al., 2015; Parker & Gaffan, 1998). Therefore, it was proposed that PRh recruits mFC only in condition when ORM task requires the integration of multiple components, whereas when ORM task has simply one component and requires low-order mnemonic functions, PRh not only is adequate but also is sufficient (Morici et al., 2015) (Warburton & Brown, 2015).

In contrast to PRh, the frontal association cortex (FrA) is located in the anterior part of the cerebral cortex just dorsal to orbital cortex and rostral to mFC (Laubach et al., 2018; Paxinos & Watson, 2014). mFC is a frontal brain structure that is formed by prelimbic, infralimbic and anterior cingulate cortices, and in rat brain, the site where FrA reside is distinct from the cortical structures that collectively form mFC (Schaeffer et al., 2020). The mFC, which is also referred as prefrontal cortex in the rat brain (Laubach et al., 2018; Schaeffer et al., 2020), is involved in working memory, executive functions and planning (Dalley et al., 2004; Kesner & Churchwell, 2011). In contrast, FrA integrates

complex perceptual information from sensory and motor cortices and relays to mFC through efferent synaptic connections (Nakayama et al., 2015; Zhang et al., 2016). Therefore, it has been thought that FrA functions as a relay station during learning of sensory and motor information. However, recent studies have revealed that FrA is involved in neural processes critical to associative fear learning and contributes to fear memory formation (Aime et al., 2020; Lai et al., 2012; Nakayama et al., 2015). In addition, this brain area also plays a role in the regulation of morphine-induced conditioned place preference (Chen et al., 2021; Li et al., 2008), in social stress (Shu & Xu, 2017) and in schizophrenia (Huang et al., 2021).

The processing of ORM associated to an object of one component but with simple feature (simple ORM), such as shape of the object, is expected to occur in the PRh (Navarro-Lobato et al., 2022). Nevertheless, the role of Rh in ORM of an object of one component but with complex feature (complex ORM), such as details of a drawing on the object surface, remains uncertain, and it is unknown whether other brain area is involved in this process or not. Therefore, in this study, we used $RGS14_{414}$ as a gain-in-function tool, to explore the role of PRh and FrA in the complex ORM. Our results demonstrate that FrA is essential for the complex ORM and PRh is not.

2 MATERIALS AND METHODS

2.1 Animals

Wistar Han rats (research resource identifier [RRID], RGD_2308816) obtained from Charles River were used for this study. All procedures were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC) of the University of Malaga. Protocols (CEUMA 32-2016-A and 7-2017-A) for performing the experiments were approved by the IACUC of University of Malaga. One to two rats were housed per cage, and they were housed in a temperature regulated (20 ± 2C) room under a 12 h light/dark cycle. Drinking water and food were available ad libitum. The animals were acclimatised to the room for at least 1 week before starting the experiments, which took place during the light phase. All possible measures are taken to maintain the good health and well-being of animals. Both departmental and veterinary staffs closely monitor animals and test routinely for the infection and diseases. Anaesthetics were used in accordance with the guidelines of the IACUC of the University of Malaga to minimize suffering of animals during the surgery as well as the sacrifice.

In this study, adult rats of 3 months of age were used for the experiments, and a total of 290 rats were used where 100 rats were for untreated group, 60 rats were for vehicle treated group, 70 rats were for $RGS14_{414}$ treated group and 60 rats for $RGS14_{414}$ + Ox7 group.

2.2 Lentivirus preparation

The cDNA of human RGS14 (GenBank accession number AY987041) was cloned into the pLenti6/Ubc/V5-DEST Gateway vector (catalogue number V49910; Thermo Fisher Scientific, Madrid, Spain), and RGS14 lentivirus was produced according to the

protocols of the ViraPower Lentiviral Expression System (Thermo Fisher Scientific, Madrid, Spain). Vehicle lentivirus (vehicle) was prepared by vector alone.

2.3 Lentivirus delivery

Rats were anesthetised with the sevoflurane system (induction at 5% sevoflurane +1 L/min O2 and maintenance at 2% sevoflurane + 0.4 L/min O2) and placed in a stereotaxic frame according to the coordinates obtained from Stereotaxic Coordinates of the Rat Brain by Paxinos and Watson (fourth edition) (Paxinos & Watson, 1998). The coordinates of the injection site in the FrA were AP + 4.70, ML ± 2.2 and DV -1.0; in PRh were AP -4.52, ML ± 6.7 and DV -4.75; and in parietal cortex were AP -4.30, ML ± 3.2 and DV -1.10. A total volume of 2 μL of $RGS14_{414}$ lentivirus from a stock titre of 2.3 x 107 TU/mL was injected bilaterally (1 μL in each hemisphere) through a 30-gauge stainless steel internal cannula. During surgery, animal body temperature was maintained with an electric blanket. After surgery, animals were treated daily for 5 days with local antibiotic (as Dermocan manufactured by Fatro; Barcelona, Spain) application on the incision and 150 μL intraperitoneal injection of Meloxicam analgesic (as Metacam 5 mg/mL manufactured by Boehringer Ingelheim, Barcelona, Spain) and then, they were left until the experiments started. Behavioural tests were performed 21 days after injection.

2.4 Simple ORM test

The simple ORM test was performed as described previously (López-Aranda et al., 2009; Masmudi-Martín et al., 2019). Prior to the test, rats were handled for 8 min daily for five consecutive days and habituated to an open field apparatus (100 x 100 x 50 cm) for 12 min on the following 2 days. On the day of the experiment, for the object exposure session, the rats were placed in the same open field apparatus with two objects of identical shapes and were allowed to freely explore the objects for 3 min. After a delay from 15 min to 24 h, the ORM status of each animal was tested with one previously presented (familiar shape) object and a novel object with a different shape. The objects used in the study were plain bottles or containers of different shapes made of plastic or glass. The objects were similar in size to the rats such that the rats were unable to sit on or topple them. The objects were selected according to two criteria: (a) the rats did not show a preference between novel and familiar objects in preference test experiments and (b) after exposure to the objects for 3 min, the rats were unable to recall them after a delay of 24 h. The location of the novel object was changed randomly between the left and right sides. The open field apparatus and the objects were cleaned after each session. Objects shown previously were never presented to the same animal in any future sessions. Studies of each delay time shown in Figure 1 were done in different sessions with new set of objects. The sessions were video recorded. The exploration time was calculated from the video by two independent researchers without knowledge of the animal conditions and then was averaged. Exploration time was defined only as an animal was touching the object with its nose. Standing on or using the object as a support was not considered exploration. The average total exploration time of the objects (familiar + novel) during the simple ORM test session for the vehicle and $RGS14_{414}$ lentivirus-treated animals was 30.17 ± 2.68 and 29.74 ± 2.48 s, respectively.

Discrimination index (DI) data are presented in Figures 1a–d and 2a,b. The DI was calculated by dividing the time spent exploring the novel object by the total exploration time (familiar object + novel object). A DI equal to or less than 0.5 indicated that the animals were unable to retain object information in memory because they explored both the familiar and novel objects for equal amounts of time (50% for the familiar object and 50% for the novel object), whereas a DI above 0.66 indicated that the animals were able to successfully retain information about the object in memory because they spent more than 66% of the total time exploring novel objects and less than 34% of the time exploring familiar objects. The results presented in Figure 1a are after a delay of 15 min to 24 h, and those presented in other figures are after a delay of 24 h.

2.5 Complex ORM test

The complex ORM test was performed similar to as described above in simple ORM test except the objects either circle, square or star of 3 x 3 cm size all over the surface of the object. The space between each drawing was 2 cm. Therefore, for the object exposure session, the rats were placed in the open field apparatus with two objects with identical shapes and identical drawings and were allowed to freely explore the objects for 3 min. After a delay of 24 h, the memory status of each animal was tested with one previously presented (familiar drawing) object and a novel object with identical shape but with different drawing. The average total exploration time of vehicle and $RGS14_{414}$ lentivirus treated animals was 26.74 ± 2.36 and 27.13 ± 3.05 s, respectively.

2.6 Immunohistochemistry

Immunohistochemistry was performed as described previously (Khan et al., 1998; L_opez-Aranda et al., 2009). In brief, after termination of the behavioural tests, the rats were perfused transcardially with a fixative containing 4% paraformaldehyde, 1.37% L-lysine and 0.21% meta-periodate and cryoprotected with 30% sucrose. Sagittal brain sections with a thickness of 30 μm were incubated overnight at 4ºC with an affinity-purified rabbit anti-RGS14 antibody (1:30 dilution) that was prepared in our laboratory (López-Aranda et al., 2009). The sections were then incubated for 90 min with Alexa Fluor 488 goat antirabbit IgG (1:1000 dilution; A32731; Thermo Fisher Scientific; Madrid, Spain), and immunofluorescence labelling was detected by confocal microscopy.

2.7 Ox7-SAP injection

The injection of Ox7-SAP (0.2 μg in 1 μl; IT-02; Advanced Targeting Systems, Carlsbad, California, USA) into the FrA was performed in a manner similar to that described in the lentivirus delivery section, using the coordinates AP + 4.70, ML ± 2.2, DV -1.0. The Ox7-SAP injections were performed simultaneously with the injections of $RGS14_{414}$ gene. The extent of damage in the brains of these animals was analysed after staining the brain sections with cresyl violet.

2.8 Cresyl violet staining

Brain sections of rats mounted on gelatin-coated slides were stained for 15 min with 1% cresyl violet. The sections were dehydrated and mounted for analysis under a microscope.

2.9 Statistics

The results were plotted, and statistical significance was evaluated using Prism 8. The data used to construct Figures 1 and 2 passed the normality test done by the Shapiro–Wilk normality test, and they showed p-values ranged from 0.071 to 0.972. The equality of group variances was tested by the Brown–Forsythe test, and the p-values ranged from 0.095 to 0.591. More than two group comparisons with single variables were analysed using one-way analysis of variance (ANOVA) with Tukey's post hoc test. For multiple group comparisons with more than one variable, two-way ANOVA with the Sidak's or Tukey's post hoc test was used. All data in the figures are presented as the mean ± SEM values.

3 RESULTS

Previously, we have shown that the overexpression of a gene corresponding to regulator of G protein signaling 14 of 414 amino acids ($RGS14_{414}$) protein in PRh caused an enhancement in the ORM (Masmudi-Martín et al., 2019; Navarro-Lobato et al., 2022). Therefore, we used here the overexpression of $RGS14_{414}$ as a strategy to induce long-term ORM. A lentivirus containing the $RGS14_{414}$ gene was delivered into the FrA and PRh of Wistar Han rats to induce expression of the $RGS14_{414}$ protein, and the memory status of these animals was evaluated 21 days after treatment by their performance on simple ORM and complex ORM tasks. We observed that when normal untreated 3-month-old rats were exposed to an object for 3 min, they were able to retain information about the objects with simple as well as complex features in memory after a delay of 15 or 30 min but not after a delay of 24 h (Figure 1a; two-way ANOVA, $F(2, 54) = 58.10$; 15 and 30 min versus 24 h, Tukey's post hoc test, $p < 0.0001$). However, rats treated with the $RGS14_{414}$ gene either in the FrA (RGS-FrA rats) or in the PRh (RGS-PRh rats) were able to retain the same information of an object with simple feature in memory after a delay of 24 h (Figure 1b; two-way ANOVA, $F(2, 54) = 59.08$; vehicle versus RGS14 in both the FrAand the Perirhinal cortex, Tukey's post hoc test, $p < 0.0001$). In contrast, when these animals were exposed to objects with complex features, RGS-FrA rats could hold the information of an object with complex feature 24 h after the object exposure (Figure 1c; two-way ANOVA, $F(2, 54) = 15.57$; vehicle versus RGS14 in FrA, Tukey's post hoc test, $p < 0.0001$); however, RGS-PRh rats failed to do so (Figure 1C; vehicle versus RGS14 in Perirhinal cortex, Tukey's post hoc test $p = 0.8332$). In contrary to $RGS14_{414}$ treatment, rats treated in FrA with either lentivirus vehicle (vehicle), saline solution or lentivirus containing RGS12, a protein that belongs to the same family as $RGS14_{414}$, did not produce any effect on both the simple and the complex ORM (supporting information Figure S1), and the performance of these rats in the test was similar to the performance of untreated rats (in Figure 1b,c). Furthermore, $RGS14_{414}$ treatment in parietal cortex, another brain area that is not involved in object recognition (Khan et al., 2011), produced no effect at all on both the simple ORM and the complex ORM (Figure 1d; two-way ANOVA, $F(1, 36) = 0.0256$;

vehicle versus RGS14, Sidak's post hoc test, $p > 0.8542$). These results suggest that $RGS14_{414}$ gene treatment in FrA area induced an enhancement in complex ORM, whereas this gene treatment in PRh did not. After termination of behavioural studies, the brains of RGS14 treated rats from Figure 1c were processed for the evaluation of the surface area affected by $RGS14_{414}$ lentivirus injection. A representative image of a sagittal brain section after immunostaining with antibodies specific for RGS14 and a depiction of immunolabelled sagittal serial brain sections in FrA are shown in Figure 1e,f, respectively. An analysis of serial brain sections of five animals indicated that the expression of $RGS14_{414}$ protein was limited to the FrA area (drawings in red colour in Figure 1e) in RGS-FrA rats and to the PRh area (drawings in red colour in supporting information Figure S2) in RGS-PRh rats.

To test whether elimination of $RGS14_{414}$ expressing neurons in FrA can abolish the increase in formation of complex ORM seen after a treatment with $RGS14_{414}$ gene, we used Ox7-SAP, which is a saporin-based immunotoxin that causes selective eradication of neurons and does not affect other brain structures or passing nerve fibres (Nolan & Freeman, 2005; Traissard et al., 2007). Therefore, RGS-FrA rats were injected with Ox7-SAP in FrA and then they were subjected to test for the retention of information of an object with complex feature. We observed that Ox-SAP injection in RGS-FrA rats completely dissipated the enhancement in complex ORM (Figure 2a; one-way ANOVA, $F(3, 36) = 19.86$; RGS14 versus RGS14 + Ox7, Tukey's post hoc test, $p < 0.0001$), whereas in contrast to $RGS14_{414}$ treated rats, Ox7-SAP injection in vehicle treated rats produced no effect on their performance on complex ORM task (Figure 2a; vehicle versus vehicle + Ox7, Tukey's post hoc test, $p = 0.8766$). Next, we tested the effect of injection of Ox7-SAP on simple ORM in both RGS-FrA rats and RGSPRh
rats (Figure 2b). We found that the elimination of $RGS14_{414}$ expressing neurons by Ox7-SAP injection in both the FrA area of RGS-FrA rats and the PRh area of RGS-PRh rats abrogated the enhancement in simple ORM seen after the treatment with $RGS14_{414}$ (Figure 2b; one-way ANOVA, $F(7, 7) = 18.51$; RGS14 versus RGS14 + Ox7 in both the FrA and the perirhinal cortex, Tukey's post hoc test, $p < 0.0001$). Ox7-mediated lesions studies suggest that complex ORM is processed in FrA area neurons and that the simple ORM is processed in both FrA and PRh. After termination of behavioural studies, the brains of Ox7-SAP injected rats from Figure 2a were processed for the evaluation of the lesions. A representative image of a sagittal brain section after staining with cresyl violet and a drawing of lesions in serial brain sections in FrA are shown in Figure 2,c,d, respectively. An analysis of serial brain sections of five animals indicated that the lesions caused substantial damage in FrA area (drawings in sky blue in Figure 2d) and PRh area (drawings in sky blue in supporting information Figure S3).

4 DISCUSSION

Our findings show that FrA is crucial for the complex ORM and PRh, a brain area thought to be fundamental for ORM and does not take part in this process. Instead, PRh is involved in the formation of simple ORM. According to our observation, it has been shown that PRh is essential for the ORM that requires low-order mnemonic functions (Morici et al., 2015; Warburton & Brown, 2015), and the implication of PRh in simple

ORM where low-order mnemonic functions are required has also been shown in studies using the $RGS14_{414}$ (Masmudi-Martín et al., 2019; Navarro-Lobato et al., 2022). However, in ORM where high-order mnemonic functions are required, such as in associative and temporal order, PRh interacts with mFC to carry out processing of such information in brain (Morici et al., 2015; Warburton & Brown, 2015), and the significant impairments in these high-order ORM after either PRh lesions or PRh inactivation suggest that PRh actively participates in this process (Barker et al., 2007; Hannesson et al., 2004). Therefore, considering that PRh is engaged in both the low-order and high-order ORM, it was surprising to see that PRh is not involved in the formation of complex ORM and FrA, a brain area that is thought to function as sensorial and motor information relay centre and is responsible for the formation of complex ORM in brain. Thus, FrA seems to be an important brain area that is crucial in processing of a high-order brain functions, such as complex ORM. In line to this concept, it has been shown that the inactivation of FrA impairs memory consolidation of an auditory fear conditioning in mice, and the dendritic spine remodelling of FrA neurons is sensitive to paired sensory stimuli that produce associative memory (Lai et al., 2012). Furthermore, a phosphorylation of GluN2B subunits of N-methyl-D-aspartate (NMDA) receptors in the FrA is essential for morphineinduced conditioned place preference in mice (Chen et al., 2021), a protein synthesis in FrA neurons during associative learning of contextual fear conditioning supports formation of fear memory (Nakayama et al., 2015), a selective activation of parvalbumin-expressing interneurons in FrA ameliorates synaptic and behavioural deficits in animal models of schizophrenia (Huang et al., 2021), a cue-elicited drug craving represses extracellular signal-regulated protein kinase (ERK) activation in FrA (Li et al., 2008) and a chronic stress alters spine dynamics and increases connectivity in FrA neural circuits (Shu & Xu, 2017). Altogether, these findings suggest that FrA engages in broad range of critical brain functions and possess dynamic neural circuits that makes this brain area apt for memory-related information processing and memory formation, for dynamic synaptic connectivity and for memory-related behavioural expression.

Recently, we have shown that $RGS14_{414}$ mediates its effect through increase in local neuronal structural plasticity (Navarro-Lobato et al., 2022). $RGS14_{414}$ treatment in the rat brain caused dendritic neuronal arborization and an almost twofold increase in the total number of dendritic spines in pyramidal neurons. Pyramidal neurons are structures that innervate other brain regions and that primarily carry efferent synapses for synaptic communications between brain area (Spruston, 2008). An increase in dendritic spines of such scale is expected to cause synaptic remodelling in FrA neuronal circuit networks. Therefore, this increase in FrA neuronal circuitry could promote more efficient memory-related information processing and facilitate memory formation in $RGS14_{414}$-treated rats. Consistent with this concept, memory formation has been shown to be critically associated with synaptic remodelling evoked by the increase in synapse number (Bailey et al., 2015; Lamprecht & LeDoux, 2004). Furthermore, findings of the spine remodeling in FrA pyramidal neurons induced by fear memory (Lai et al., 2012), the change in FrA dendritic spines in memory related to chronic stress (Shu & Xu, 2017) and the rapid spine elimination in FrA during auditory-cued fear conditioning (Zhou et al., 2020) further

support the idea that memory formation in FrA is sensitive to local synaptic remodelling. In conclusion, our results demonstrate that FrA and not the PRh is involved in the long-term complex ORM.

AUTHOR CONTRIBUTIONS

Z. U. K. developed the overall research concept and the project. M. M. and M. F. L. designed the experiments. M. M., M. F. L., and I. N. performed the experiments. M. M. and Z. U. K. wrote the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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FIGURES

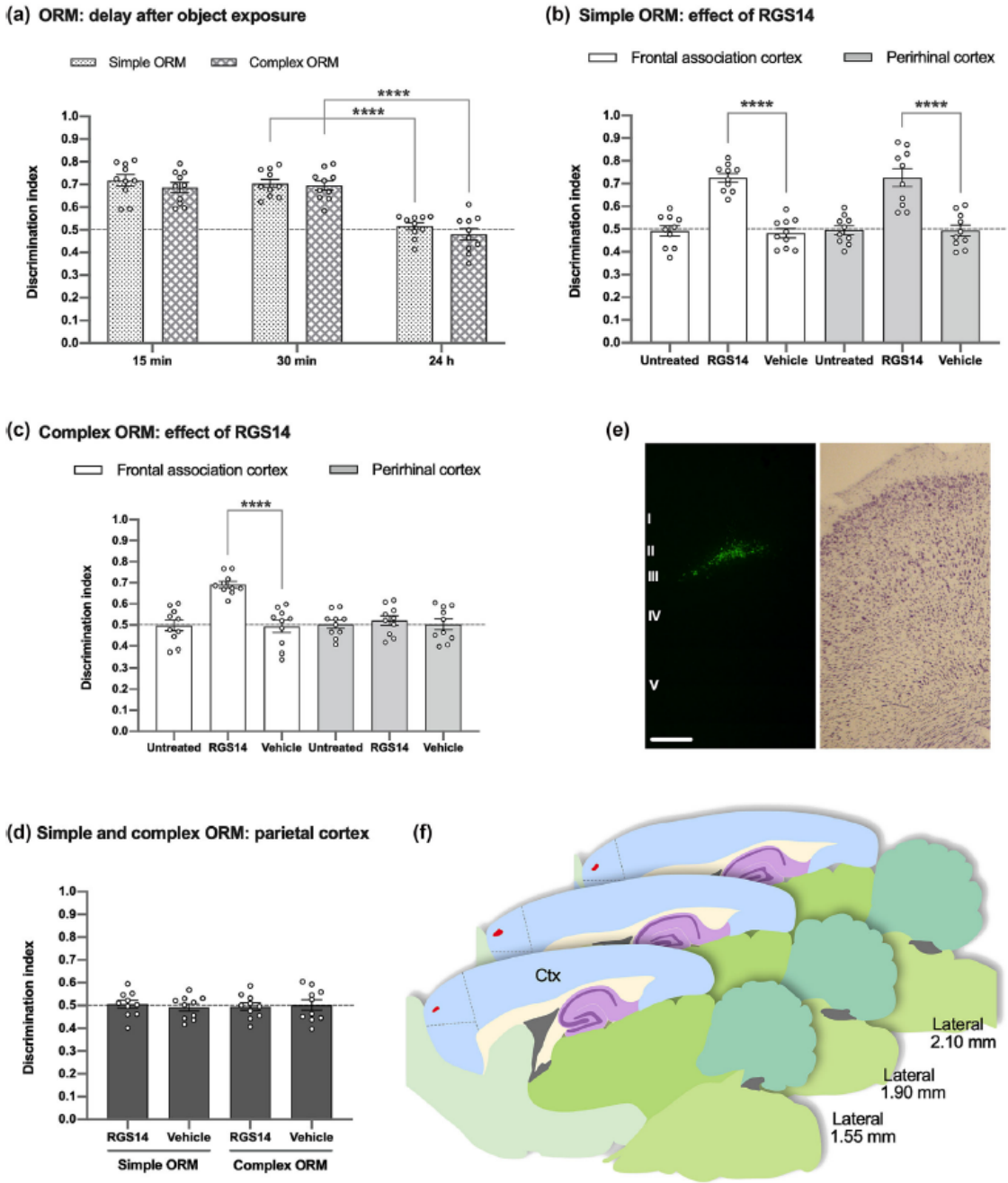

FIGURE 1 Activation of FrA (frontal association cortex) induces the formation of long-term simple and complex object recognition memory (ORM). (a) After exposure to an object with simple or complex features, 3-month-old normal untreated rats were able to retain of both type of objects information in memory for 15 and 30 min; however, they were unable to retain such information after 24 h. (b) RGS14414 gene treatment either in FrA or in PRh (perirhinal cortex) in these rats induced the formation of long-term simple ORM that was observed after 24 h, whereas vehicle treatment induced no effect. (c) In contrast to simple ORM, RGS14414 caused formation of longterm complex ORM only when the treatment was done in FrA and not when in PRh. (d) RGS14414 gene treatment

in parietal cortex, a brain area unrelated to ORM, showed no effect on both the simple and complex ORM. In (e), the left image shows the immunolabelled RGS14414 protein in green, and the right image shows the cresyl violet-stained brain section of the same area. Cortical layers (I–V) are indicated in the left image, for which the scale bar is 200 μm. (f) Represents a depiction of maximum expansion of the RGS14414 protein in FrA after serial brain sections analysis from five rats (red colour). Dotted lines across panels a to d indicate the threshold at which (0.5 discrimination index [DI] and below) the animals were unable to retain object information in memory and n = 10 (unfilled circle). **** (two-way ANOVA with Tukey's post hoc test, $p < 0.0001$).

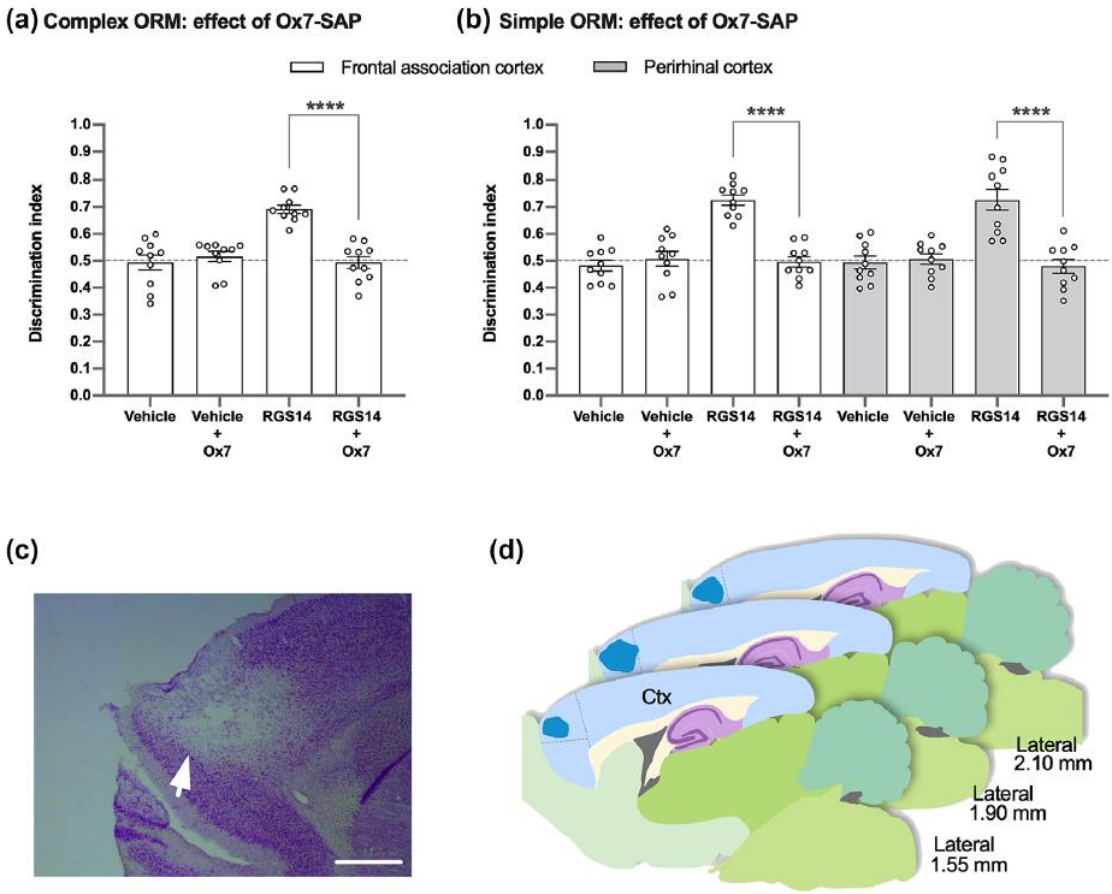

FIGURE 2 Elimination of frontal association cortex (FrA) neurons by immunotoxin Ox7-SAP caused complete loss in the formation of simple and complex object recognition memory (ORM). (a) An Ox7-SAP injection in FrA of RGS14-treated rats (RGS14 + Ox7) abrogated the formation of long-term complex ORM; however, Ox7-SAP injection in FrA of vehicle-treated rats (vehicle + Ox7) produced no effect and the performance of these rats was similar to the vehicle-treated rats. (B) Ox7-SAP injection in FrA or perirhinal cortex (PRh) of their respective RGS14-treated rats caused complete loss in the formation of simple ORM. (c) An example of a cresyl violet-stained brain section showing the lesions produced by Ox7-SAP immunotoxin injection to the FrA (arrow). The scale bar is 1000 μm. (d) A depiction of sagittal brain sections obtained after analysis of serial sections from five FrA-lesioned animals provides a view of the maximum affected area (sky blue colour). Ctx is the cortex. Dotted lines across panels in a and b indicate the threshold at which (0.5 discrimination index [DI] and below) the animals were unable to retain object information in memory and n = 10 (unfilled circle). **** (one-way ANOVA with Tukey's post hoc test, $p < 0.0001$).