Complement component C4a binds to oxytocin and modulates plasma oxytocin concentrations and social behavior in male mice

（補体成分 C4a はオキシトシンに結合し、血漿中のオキシトシン濃度およびマウスの社会的行動を調節する）

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Complement component C4a binds to oxytocin and modulates plasma oxytocin concentrations and social behavior in male mice

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ABSTRACT

Oxytocin (OT) is a hormone with a short half-life that is released from the posterior pituitary gland into the bloodstream. It plays an important role in childbirth, breastfeeding, and social behavior in humans and animals. However, the endogenous OT system, including plasma OT pharmacokinetics, is not fully understood. In this study, we used a click chemistry probe to discover a novel OT-binding protein in human serum and identified C4a, a peptidase-cleaved fragment of complement component 4 (C4). A direct association between OT and C4a was also confirmed. Upon knocking out *Slp* gene, which is one of the two mouse *C4* genes, the level of free form of plasma OT was higher in *Slp* knockout ($Slp^{-/-}$) mice than in wild-type ($Slp^{+/+}$) mice after intraperitoneal OT injection. In addition, open-field tests revealed that social interactions were higher in $Slp^{-/-}$ mice than $Slp^{+/+}$ mice. An *in vitro* blood-brain barrier model showed that C4a neither inhibited nor accelerated receptor for advanced glycation end-product (RAGE)-dependent brain transport in OT. Our data validate the novel concept that C4a with OT-binding capacity can alter the dynamics of the free form of OT concentrations in the plasma, which may disturb the availability of OT to the brain, resulting in an interruption of OT-associated social behavior.

1. Introduction

Oxytocin (OT) is released from the posterior pituitary gland into the bloodstream and plays a pivotal role in uterine contractions during labor and milk ejection during breastfeeding in mammals. Recent studies have shown that administration of OT increases social interactions and behaviors in humans and animals by OT-binding to the OT receptor in the brain [1]. For this, OT should be transferred from the bloodstream to the brain through the blood-brain barrier (BBB). Our group has recently reported that OT can bind to the receptor for advanced glycation end-products (RAGE), suggesting that RAGE may function as the direct transporter of OT in brain vascular endothelial cells [2]. Based on this perspective, OT has therapeutic potential for conditions such as autism spectrum disorder, schizophrenia, posttraumatic stress disorder, anxiety, and depression [3]. However, the clinical effects of OT administration are not the same in all individuals, which may be due to variations in the endogenous OT system.

Prosocial behavior is essential for survival in diverse society [4]. During such social interactions, the OT system is activated [5]. Therefore, social behavior may be affected by variations in the endogenous OT system [6]. However, the biological basis for these effects remains unclear. Kosfeld et al. described the trust-enhancing effect of OT in

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economic studies [7], wherein an investor group transferred more money (monetary units) to potential trustees after intranasal OT administration. However, subsequent studies often failed to replicate this effect based on individual differences [8]. The failure to show the effect of OT in such experiments may be due to procedural differences and may reflect individual differences in peptide availability.

To address the puzzle of individual differences in the effects of OT, we hypothesized that such differences may reflect previously unidentified molecules that regulate the OT system. Specifically, we assumed that OT-binding proteins were present in blood. To assess this, we used a click chemistry probe to identify a novel OT-binding protein in human serum.

2. Materials & methods

2.1. Preparation of OT-immobilized beads, affinity purification, and amino acid sequencing

G9[propargylglycine]-substituted OT was synthesized to form an addressable synthetic epitope (Medical & Biological Laboratories, Tokyo, Japan) and immobilized on azide ferrite-glycidyl (FG) methacrylate beads (Tamagawa Seiki, Kanagawa, Japan) using click chemistry [9]. For affinity purification, OT-immobilized beads (0.2 mg) were equilibrated with buffer IB-A (20 mM HEPES [pH 7.9], 100 mM KCl, 0.1 % Triton X-100, and 10 % glycerol) and incubated with human sera for 2 h at 4 °C. The beads were washed with buffer IB-A and the bound proteins were eluted with Laemmli dye and subjected to SDS-PAGE. Proteins were stained with aqueous $AgNO_3$ and each strip, including the protein, was cut off and subjected to sequencing-grade modified trypsin (Promega, Madison, WI, USA). After in-gel digestion, peptide fragments from each strip were analyzed using a 4800 Plus MALDI TOF/TOF analyzer (AB Sciex, Framingham. MA, USA). The MS/MS data were evaluated by comparing amino acid substitutions and modifications to the NCBI database using the Paragon algorithm of ProteinPilot™ v2.0 software (AB Sciex).

2.2. Plate binding and competition assays

The binding of human recombinant C4a (HRP-1648, LD Biopharma, Inc.) to OT (4084-v, Peptide Ins. Inc.) or human esRAGE (endogenous secretory RAGE; an extracellular domain-containing soluble RAGE) [10] was assayed in 96-well plates, as reported previously [2]. OT (100 nM) or C4a (10 nM) was immobilized on the plates, and bound C4a or esRAGE was detected using anti-C4a or anti-human RAGE antibodies, respectively [2].

2.3. Surface plasmon resonance (SPR) assay (Biacore)

The binding affinity of OT to recombinant C4a was analyzed using Biacore X100 (Cytiva) in a single-cycle affinity model [2]. The C4a was immobilized through amine coupling to a CM5 Biacore sensor chip (Cytiva) and the flow buffer utilized contained 10 mM HEPES (pH 7.4), 0.15 M NaCl, 3 mM Na-EDTA, and 0.005 % (v/v) surfactant P-20. Association and dissociation were measured at 25 °C at a flow rate of 30 μl/min. The sensor chips were regenerated by washing with 10 mM NaOH and 0.5 % (w/v) SDS. The KD was calculated from the *ka* and *kd* values using a trivalent analyte model.

2.4. Slp knockout mice

Slp-knockout mice were generated using ICR mice (Japan SLC, Inc., Shizuoka, Japan), and the CRISPR/Cas9 system was used as previously described [11]. Two crRNAs targeting the 5′-upstream region of exon 1 (TGAGCCCGGAAACCACGTGA) or intron 1 (GAAGACACTGGCGTTTGCGT) of the murine *Slp* gene (Ref Seq:NM_011413.2) were designed using the online sgRNA design tool available at https://sg.idtdna.com/site/order/designtool/index/CRISPR_CUSTOM and purchased from Integrated DNA Technologies (Coralville, IA, UAS). One of the three *Slp*-knockout ($Slp^{-/-}$) strains was used in the present study. PCR genotyping was performed using the forward primer (5′-AGAAGGGGAAAACAGGAGCA-3′) and reverse primer (5′-TGGCAAGCACATGTATTCGT-3′). All procedures were approved by the Ethical Committees for Medical Science and Animal Studies at the Kanazawa University Graduate School of Medical Sciences (ethics approval codes: AP-173824 and AP6-2589).

2.5. Collection of mouse blood samples

Blood samples (0.1–0.2 mL) were collected from $Slp^{+/+}$ and $Slp^{-/-}$ male mice through cardiac puncture. To obtain free OT, the samples were treated to remove interference-causing components using the acetonitrile protein precipitation (PPT) method as described previously [12].

2.6. Mouse C4 and C4a measurement

Complement component 4 (C4) and C4a levels in the plasma or serum from $Slp^{+/+}$ and $Slp^{-/-}$ male mice were measured using ELISA kits(Cloud Clone Corp., Katy, TX, USA; SEA888Mu and SEA389Mu, respectively).

2.7. Open-field test

$Slp^{+/+}$ and $Slp^{-/-}$ male mice were tested for locomotion and social behavior using an open-field test, as previously described [13]. Overall activity in the field was measured for 10 or 20 min using a digital video system and ANY-maze software (Stoelting Co., Wood Dale, IL, USA). The amount of time and distance traveled in the outer, inner, and central arenas were counted automatically. After each trial, the test chambers were cleaned with a damp towel and 1 % sodium hypochlorite, followed by 70 % alcohol [13].

2.8. In vitro blood-brain barrier (BBB) assay

We used an *in vitro* BBB model (MBT-24H; PharmaCo-Cell Co., Nagasaki, Japan) composed of rat brain pericytes, rat astrocytes, and monkey brain vascular endothelial cells [14]. *Trans*-endothelial electrical resistance (TEER), which primarily reflects the flux of sodium ions through the cell layers under culture conditions, was measured using an epithelial-volt-ohm meter and Endohm-6 chamber electrodes (World Precision Instruments, Sarasota, FL, USA). The apparent permeability constants (Papp) between the luminal and abluminal chambers were calculated using distribution ratios [14].

2.9. Statistics

Statistical analyses were performed using Prism software (GraphPad Prism 9.3.1) with a two-sided independent *t*-test to compare two groups, one-way analysis of variance (ANOVA) with Fisher's least significant difference post-hoc test to compare more than two groups, and two-way ANOVA with Fisher's least significant difference post-hoc test to compare two-factor study designs. Data are expressed as mean ± standard error of the mean (SEM), unless otherwise specified. For all tests, statistical significance was set at $P < 0.05$.

3. Results

3.1. Identification of serum OT-binding protein

Human serum proteins bound to the OT-immobilized magnetic beads (Fig. 1A) were isolated and subjected to gel electrophoresis (Fig. 1B). The amino acid sequences of each gel band of the peptide fragments

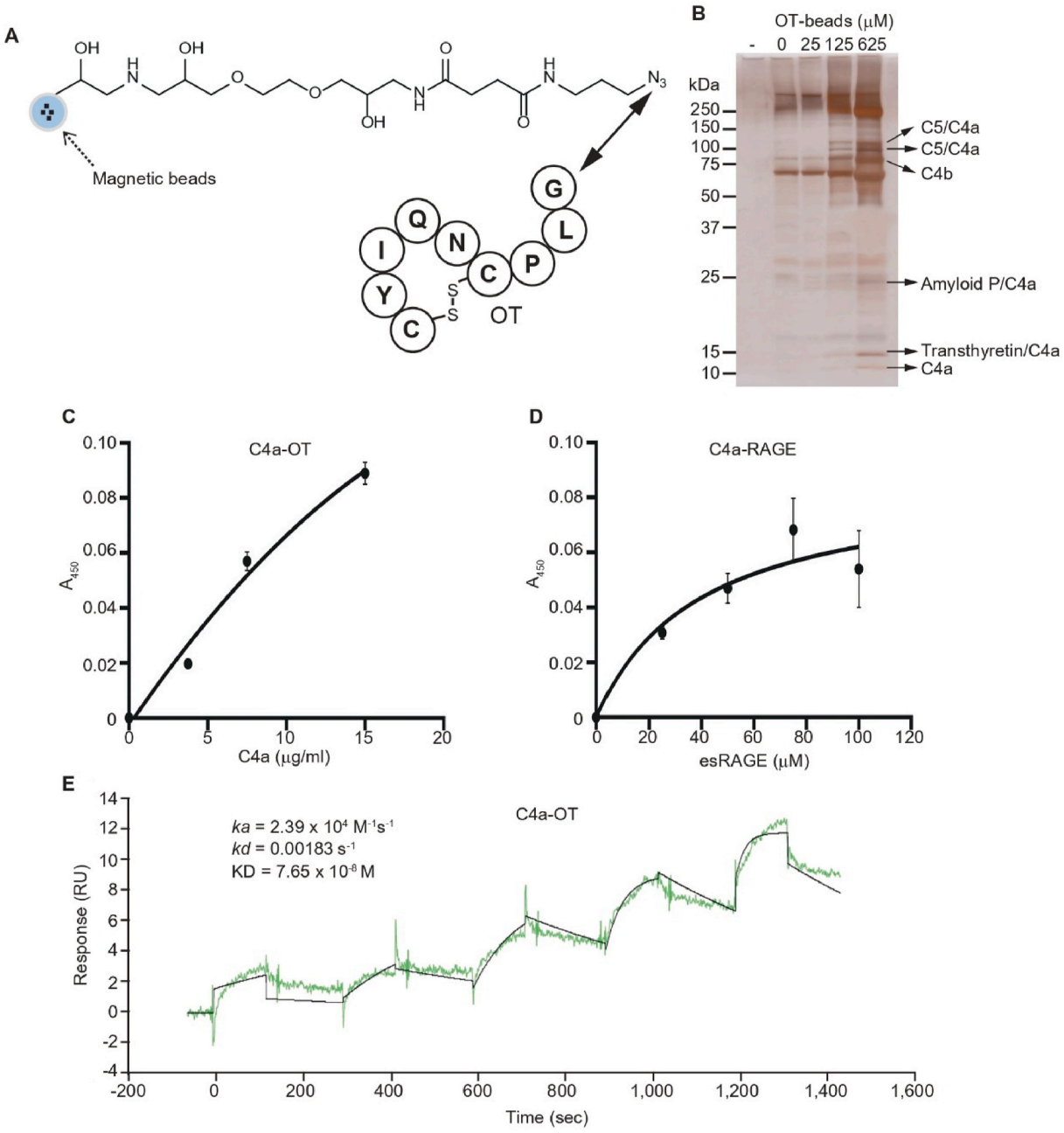

Fig. 1. Identification of oxytocin (OT)-binding proteins in human sera. **(A)** G9[propargylglycine]-substituted OT was synthesized and immobilized on azide ferrite-glycidyl (FG) methacrylate beads (Tamagawa Seiki, Kanagawa, Japan) using click chemistry [9]. (**B**) SDS-PAGE of OT-beads-associated proteins in human sera and $AgNO_3$ staining. OT-free beads (0) and 25, 125, and 625 μM of OT beads were used. Proteins were analyzed by TOF-MS/MS. (**C, D**) Plate binding assays. Dose-dependent binding measured between immobilized OT (100 nM) and 0–20 μg/mL of human recombinant C4a (**C**) and between immobilized C4a (10 nM) and of human recombinant esRAGE (endogenous secretory receptor for advanced glycation end-products as a representative of the extracellular domain of RAGE) (**D**) (n = 3). **(E)** Surface plasmon resonance (Biacore) analysis of OT (37, 111, 333, 1,000, and 2,000 nM) binding to human recombinant C4a immobilized to a CM5 Biacore sensor chip. The KD was calculated by utilizing *ka* and *kd* values by a trivalent analyte model.

were analyzed using time-of-flight mass spectrometry. The most likely annotated amino acid sequence was C4a, followed by C4b, C5, amyloid P, and transthyretin (Fig. 1B–Supplementary Table 1). C4a is a well-established anaphylatoxin in the complement system and is an activated (peptidase-cleaved) fragment of C4 with a molecular mass of 8,764 Da [15]. Although the *C4* gene is the most polymorphic among the complement components, leading to variability in plasma C4a levels in individuals [16], we focused mainly on C4a in this study.

To demonstrate direct physical binding, we used plate-binding assays and SPR analysis [2]. Dose-dependent binding was observed between immobilized synthetic OT and human C4a in solution (Fig. 1C). We examined the association between C4a and RAGE because RAGE is known to be an OT transporter. We found C4a-binding to the human extracellular domain of RAGE (an alternative splice variant of RAGE, esRAGE) [10] (Fig. 1D). Surface plasmon resonance methods showed concentration-dependent OT binding to immobilized recombinant C4a, with an apparent dissociation constant (KD) of 76.5 nM (Fig. 1E).

3.2. Interruption of dynamic plasma OT elevation by C4a in mice

A gene-knockout model was used to assess the function of C4a. Humans and mice have two genes encoding C4, and the mouse sex-limited protein gene *Slp* is a homolog of human *C4B* gene [17]. C4a is a cleaved fragment of C4 [16]. *Slp*$^{-/-}$ mice were created using the gene-editing CRISPR-Cas9 method (Fig. 2A) and gene deletion was confirmed by PCR genotyping (Fig. 2B). We then measured plasma C4a levels and found that the concentration in *Slp*$^{-/-}$ mice was approximately half that in wild-type (*Slp*$^{+/+}$) mice (Fig. 2C), as the other *C4* gene was not disrupted. Male and female *Slp*$^{-/-}$ mice grew normally without any apparent impairment (including locomotion) or body weight loss. The knockout mice were maintained by mating *Slp*$^{-/-}$ males and females.

We first measured OT concentrations in plasma with or without the acetonitrile protein precipitation method (extracted or unextracted, respectively) [12]. The OT concentrations in the extracted plasma samples represent the free form of plasma OT (ffpOT). Basal OT concentrations in unextracted plasma were 2,039 ± 592 pg/mL and 1,578 ± 263 pg/mL in *Slp*$^{+/+}$ and *Slp*$^{-/-}$ mice, respectively. Basal levels of extracted (ffpOT) and unextracted oxytocin (OT) did not differ significantly between *Slp*$^{+/+}$ and *Slp*$^{-/-}$ mice (Fig. 2D).

A clear difference was observed in the time course of ffpOT concentrations after intraperitoneal OT administration (30 ng/mouse) (Fig. 2E). In *Slp*$^{+/+}$ mice, ffpOT concentration transiently increased by 150.7 % at 10 min and then promptly decreased to the initial level. In sharp contrast, in *Slp*$^{-/-}$ mice, the ffpOT concentration started to increase at 10 min, reached a peak level at 30 min (339 %), and was maintained at a high level compared with the initial level until 60 min (228 %) following OT injection (Fig. 2E). These findings suggest that C4a regulates OT dynamics in mouse circulation.

3.3. Slp$^{-/-}$ mice display increased social interaction

As ffpOT dynamics altered in *Slp*$^{-/-}$ mice, we examined the social interactions using an open-field test. No differences between *Slp*$^{-/-}$ and *Slp*$^{+/+}$ mice were observed at the habituation and non-social target stages (Fig. 3A and B). During the social stage, *Slp*$^{-/-}$ mice spent significantly more time than *Slp*$^{+/+}$ mice in the inner area (Fig. 3C), resulting in less time spent in the outer arena by *Slp*$^{-/-}$ mice (Fig. 3D). More precisely, *Slp*$^{-/-}$ mice spent more time on the cage (center) in the inner area, as depicted by the moving traces for the two genotypes (77 s for *Slp*$^{+/+}$ vs. 109 s for *Slp*$^{-/-}$; Fig. 3E). These data suggest that *Slp*$^{-/-}$ male mice preferred to interact more directly with the caged male (social target) mice.

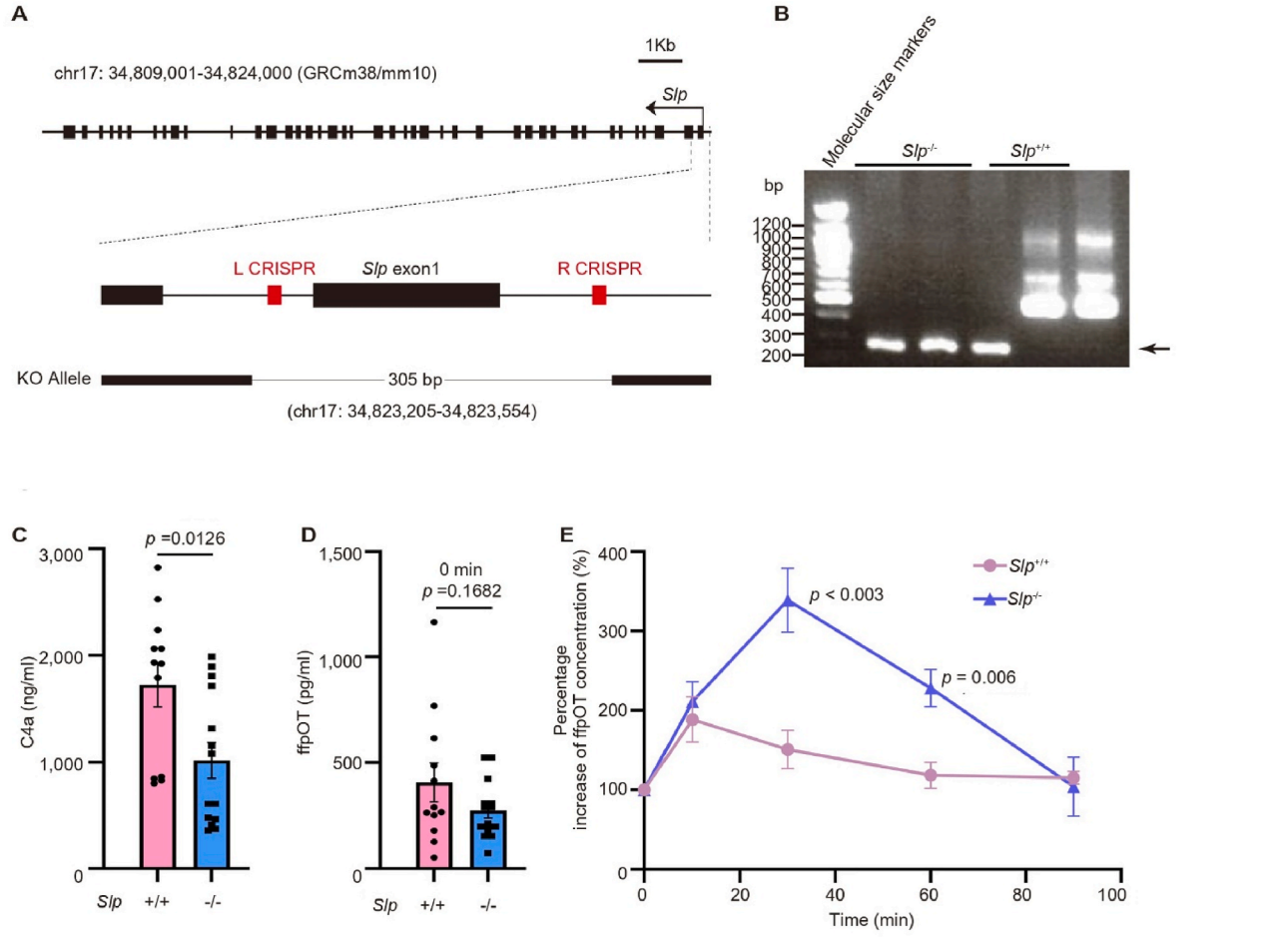

Fig. 2. Generation of the *sex limited protein* (*Slp*) knockout (*Slp*$^{-/-}$) mice and plasma OT dynamics. (**A**) A schematic representation of the genomic structure of *Slp*. Positions of crRNAs targeting the 5′-upstream region of exon 1 or intron 1 of *Slp* gene (Ref Seq:NM_011413.2). (**B**) PCR genotyping. PCR bands appear at 256 base pairs (arrow) from *Slp*$^{-/-}$ mice at 482 base pairs from *Slp*$^{+/+}$ mice. (**C**) Plasma C4a concentrations of *Slp*$^{+/+}$ and *Slp*$^{-/-}$ mice (n = 11 and 14, respectively). **(D)** OT concentrations (ffpOT) measured in protein extracted plasma from *Slp*$^{+/+}$ and *Slp*$^{-/-}$ mice using by the acetonitrile protein precipitation method (n = 12 and 14, respectively). Free form of plasma OT, ffpOT. (**E**) Time course of ffpOT concentrations in *Slp*$^{+/+}$ and *Slp*$^{-/-}$ male mice following intraperitoneal administration of OT (30 ng/mouse). *Slp*$^{+/+}$ vs *Slp*$^{-/-}$ mice at 30 min (each n = 17; $P < 0.05$); *Slp*$^{+/+}$ vs *Slp*$^{-/-}$ mice at 60 min (n = 12 vs 14, respectively; $P < 0.01$) after intraperitoneal administration of OT.

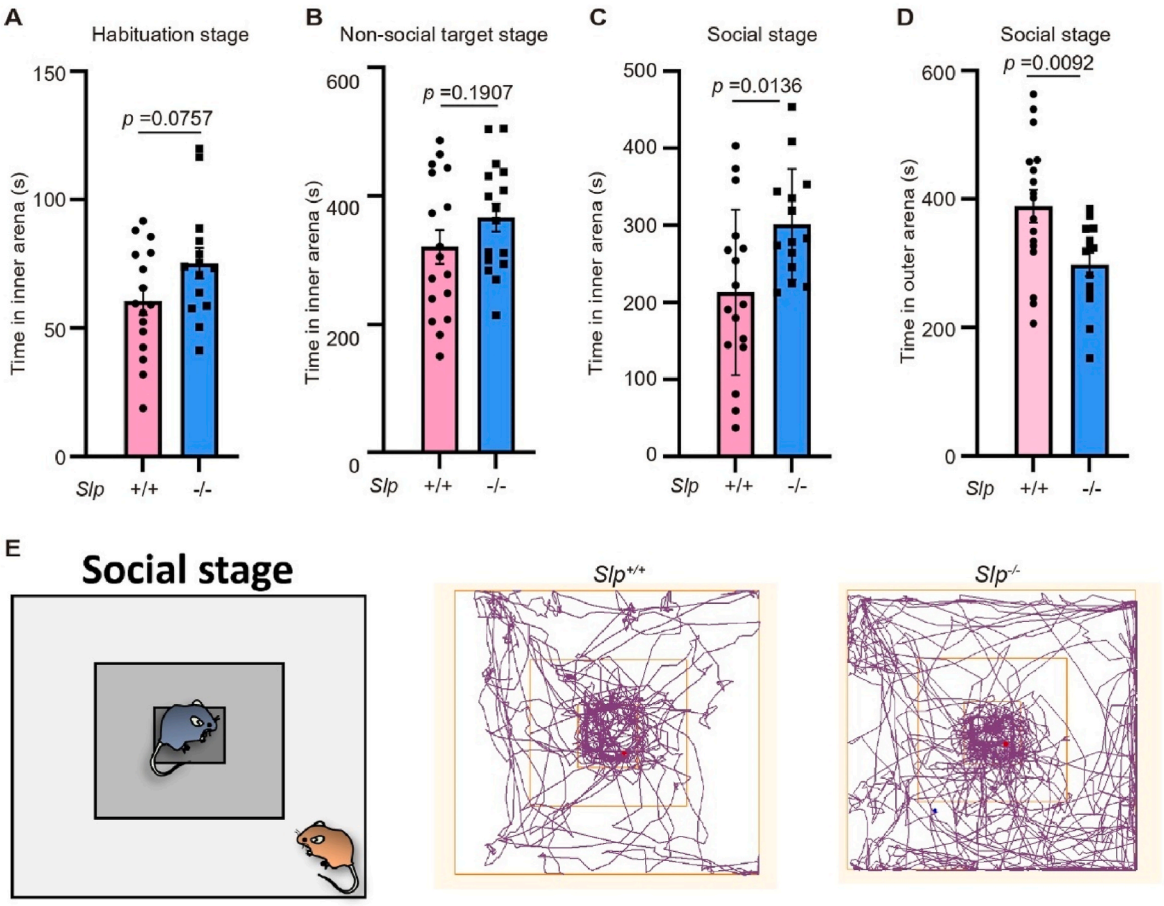

Fig. 3. The open field tests. (**A**) Time spent in the inner arena at the habituation. $Slp^{+/+}$ (n = 16) and $Slp^{-/-}$ (n = 17) male mice. (**B-D**) Non-social target [$Slp^{+/+}$ (n = 15) and $Slp^{-/-}$ (n = 17) male mice.] (**B**), social target stages and inner [$Slp^{+/+}$ (n = 14) and $Slp^{-/-}$ (n = 17) male mice.] (**C**) or outer arena [$Slp^{+/+}$ (n = 14) and $Slp^{-/-}$ (n = 17) male mice.] (**D**). (**E**) Small squares in the inside represent the inner arena which are not drawn in real test field. A non-living object (a small meshed mouse cage) or an unfamiliar mouse of the same sex was placed in the central zone. Representative moving traces of $Slp^{+/+}$ and $Slp^{-/-}$ male mice around the central social targe zones.

3.4. C4a has no or little effect on RAGE-dependent transport of OT

To further elucidate the function of C4a in the C4a-OT-RAGE complex, we tested whether C4a could facilitate RAGE-dependent OT transport using an *in vitro* BBB assay after evaluating tight junction formation (Fig. 4A) [2]. When OT (1.0 ng/mL, approximately 1.0 nM) plus C4a (0.1 μg/ml) were added to the blood side, OT concentrations in the abluminal (brain) side increased to 15.2 ± 0.8 pg/mL after 3 h, with an apparent permeability of $3.7 \pm 0.2 \times 10^{-6}$ cm/s (Fig. 4B and C); this OT transfer efficiency from the blood to the brain side was not significantly different from that without C4a. The movement of OT across this barrier did not differ with or without C4a treatment (Fig. 4B and C). RAGE-dependent OT transport was confirmed by RAGE knockdown in endothelial cells (Fig. 4B and C), which was consistent with previous data [2]. These findings suggested that OT transport by RAGE was neither facilitated nor suppressed by the presence of C4a, at least under the given culture conditions (Fig. 4D).

4. Discussion

In this study, we identified C4a, a cleaved fragment of the complement component C4, as an OT-binding protein in plasma for the first time. Relatively high-affinity binding (KD ~80 nM) was observed between C4a and OT (Fig. 1E). It is now evident that the OT-binding protein could be C4a (9 kDa) [16]. Thus far, a candidate with a molecular weight of approximately 10 kDa has been predicted in the blood [18]. We observed a dramatic rise in ffpOT concentrations 30 min after exogenous OT administration, accompanied by a ~50 % reduction in plasma C4a concentrations in $Slp^{-/-}$ mice, but only a mild elevation in ffpOT levels in $Slp^{+/+}$ mice (Fig. 2C and E). Therefore, plasma C4a may partially contribute to the formation of bound OT and is presumably inversely correlated with ffpOT concentration. OT is quickly eliminated with a very short half-life in the blood; therefore, we believe that this dynamic OT alteration affects OT availability in the brain and subsequent social behaviors. Further investigation is required due to the lack of significant differences in basal levels of extracted (ffpOT) and unextracted OT between $Slp^{-/-}$ and $Slp^{+/+}$ mice (Fig. 2C and D). Concerning that, it has been very recently reported that immunoglobulin G (IgG) is an OT-binding protein [19], with a serum concentration of ~12 mg/mL. This high plasma IgG concentration may contribute to further determination of basal plasma OT levels.

C4 and its related glycopeptides play principal roles in the immune system and inflammatory processes [20]. High C4A copy numbers in neurons are associated with schizophrenia [21], while low copy numbers in astrocytes are linked to Alzheimer's disease [22]. In addition, deficiency of C4, and thereby C4a, is strongly associated with the development of autoimmune diseases [23]. Our findings suggest a new function of C4a in the blood.

Using an *in vitro* BBB assay [2], we observed that C4a had little direct effect on endothelial RAGE-dependent OT transport from the blood to the brain (Fig. 4B and C). In this assay, OT and C4a were co-incubated and the OT transfer efficiency over 3 h was evaluated. Although we

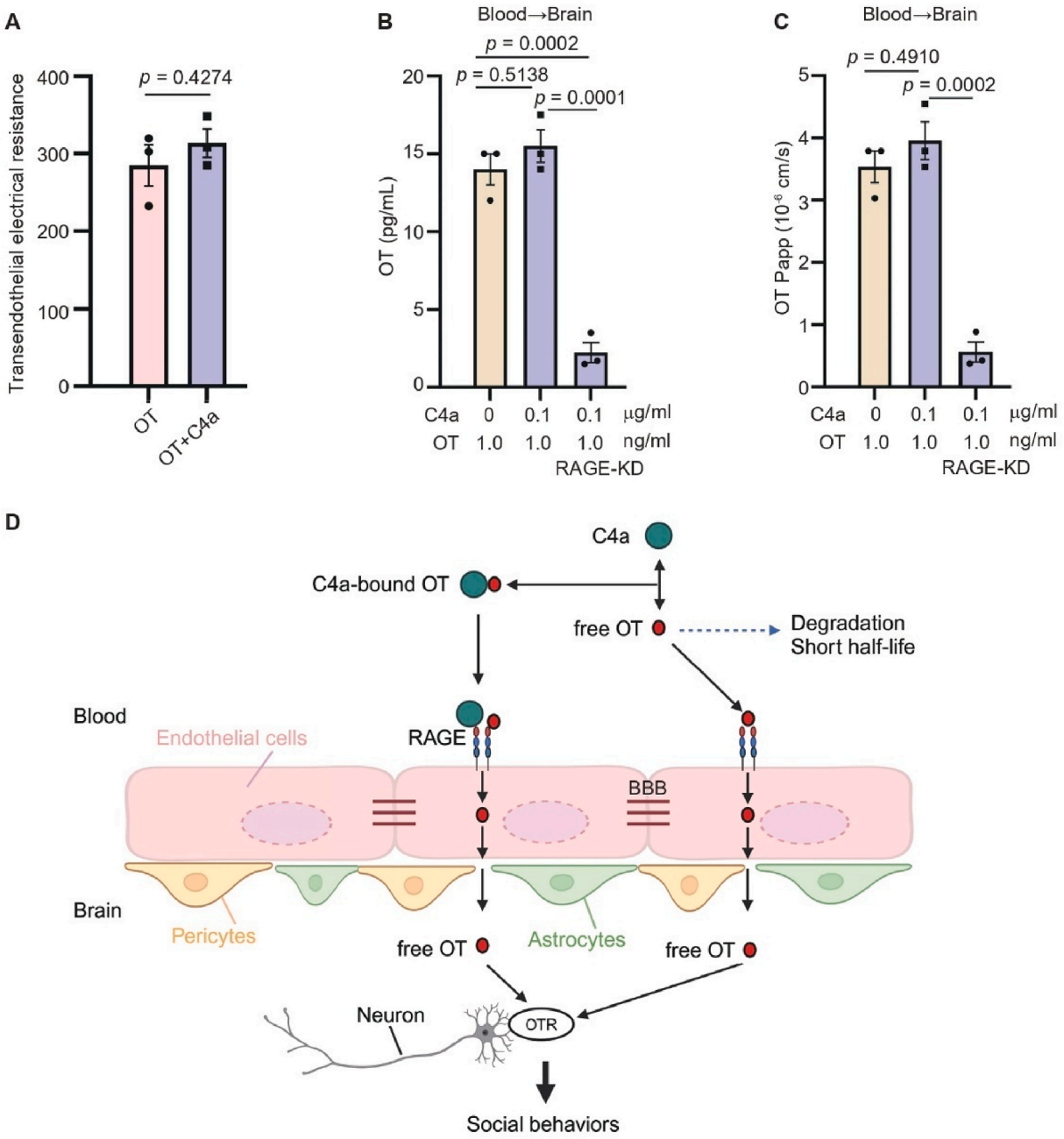

Fig. 4. Effects of C4a on RAGE-dependent transport of OT through the *in vitro* BBB. **(A)** *Trans*-endothelial electrical resistance (TEER) (Ωcm^2). OT (1.0 ng/mL) alone or OT (1.0 ng/mL) plus C4a (0.1 μg/mL) were added to the upper (luminal, blood side) chamber of the BBB system (each n = 3). (**B**) OT concentrations in the lower (abluminal, brain side) chamber after 3 h incubation (each n = 3). **(C)** The apparent permeability constants (Papp) (each n = 3). Endothelial RAGE was knocked down (RAGE-KD) by the shRNA method [2]. (**D**) Scheme depicting the equilibrium state of C4a, free OT and C4a-OT in the blood. Free OT would be transferred across the BBB by RAGE and activate OT receptor (OTR)-expressing neurons in the brain, possibly leading to increased social interactions.

did not check for dynamic alterations in OT transfer in the BBB assay, we assumed that overall OT transport from the blood to the brain would not be affected by the presence of C4a. We believe that dynamic increases in ffpOT after exogenous or endogenous OT supply in the blood play a key role in OT transfer and availability in the brain.

We also found that social interaction was higher in $Slp^{-/-}$ mice than $Slp^{+/+}$ mice using an open-field test (Fig. 3C and D). This finding suggests that the effect of C4a was not limited to ffpOT dynamics, but it also affected the brain and mouse social behavior. Further studies are required to determine whether C4a in the brain directly affects neuronal activity. Based on the results of the current study, it is highly likely that C4a modulates the free form of OT dynamics in the plasma and subsequent OT transport through the BBB and brain OT availability, contributing to the promotion of sociability and prosocial behavior (Fig. 4D). Further investigation is required to examine whether C4a levels contribute to individual variations in prosocial behavior.

CRediT authorship contribution statement

Yasuhiko Yamamoto: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **Anpei Zhang:** Formal analysis, Investigation, Writing – review & editing. **Teruko Yuhi:** Investigation. **Ai Harashima:** Investigation. **Anna A. Shabalova:** Investigation. **Emina Nishizawa:** Investigation. **Michiru Moriya:**

Investigation. **Seiichi Munesue:** Investigation. **Yu Oshima:** Investigation. **Maria Gerasimenko:** Investigation. **Kazumi Furuhara:** Investigation. **Chiharu Tsuji:** Investigation. **Shigeru Yokoyama:** Investigation. **Shinichi Horike:** Investigation. **Jumpei Terakawa:** Investigation. **Takiko Daikoku:** Investigation. **Takumi Nishiuchi:** Investigation. **Katsuya Sakai:** Investigation. **Haruhiro Higashida:** Conceptualization, Writing – original draft, Writing – review & editing.

Data availability

The datasets used and/or analysis during the current study are available from the corresponding author on reasonable request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2025.152004.

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Supplementary Table 1

Supplementary table 1				
Band No.	Accession No.	Protein Name	Score (Unused)	Coverage (%)
1	gi|62089266	Fibronectin 1 variant, partial	76.96	44.3
~250 kDa	gi|32187679	apolipoprotein B (including Ag(x) antigen)	9.15	4.3
	gi|7671645	dJ34F7.4 (complement component 4A)	8.7	12.4
2	gi|807066348	fibronectin isoform 11 preproprotein [Homo sapiens]	31.36	26.1
~150 kDa	gi|58194075	unnamed protein product [Homo sapiens]	24.18	26
	gi|929654753	C4A variant protein [Homo sapiens]	11.68	18.2
3	gi|58194075	unnamed protein product [Homo sapiens]	33.25	26.6
~120 kDa	gi|953514538	complement C5 isoform 2 [Homo sapiens]	8.48	5.8
	gi|929654753	C4A variant protein [Homo sapiens]	6.43	11
4	gi|953514538	complement C5 isoform 2 [Homo sapiens]	12.74	13.3
~100 kDa	gi|58194075	unnamed protein product [Homo sapiens]	6.74	12.1
	gi|51476364	hypothetical protein [Homo sapiens]	3.08	8.6
5	gi|338858017	complement C4-B-like preproprotein [Homo sapiens]	16.26	21.2
~80 kDa	gi|57999350	unnamed protein product [Homo sapiens]	13.85	47
	gi|736249	plasma gelsolin [Homo sapiens]	6.24	24
6	gi|88853069	vitronectin precursor [Homo sapiens]	20.02	29.7
~60 kDa	gi|6013427	serum albumin precursor [Homo sapiens]	14.79	33.7
	gi|929654753	C4A variant protein [Homo sapiens]	13.19	11.6
7	gi|357993639	unnamed protein product [Homo sapiens]	15.76	34.3
~50 kDa	gi|929654753	C4A variant protein [Homo sapiens]	14.98	11.8
	gi|910749465	prothrombin isoform 2 [Homo sapiens]	8.87	17
8	gi|929654753	C4A variant protein [Homo sapiens]	17.95	11.5
~25 kDa	gi|730704	RecName: Full=Serum amyloid P-component	10.24	35.4
	gi|87298828	complement C1q subcomponent subunit B precursor [Homo sapiens]	3.68	11.5
9	gi|929654753	C4A variant protein [Homo sapiens]	3.71	7.3
~18 kDa	gi|7331218	keratin 1 [Homo sapiens]	2.72	15.5
10	gi|847738	transthyretin precursor [Homo sapiens]	11.53	69.4
~15 kDa				
11	gi|7331218	keratin 1 [Homo sapiens]	5.1	16.3
~10 kDa	gi|929654753	C4A variant protein [Homo sapiens]	3.2	3.6
	gi|55956899	keratin, type I cytoskeletal 9 [Homo sapiens]	3.16	11.4