Definition. Let f, g be G-isovariant maps. We call f and g isovariantly G-homotopic if there exists a G-isovariant map $H : X \times I \to Y$, called a G-isovariant homotopy, such that $H(-,0) = f$ and $H(-,1) = g$.

Let $[X,Y]_G^{\mathrm{isov}}$ denote the set of G-isovariant homotopy classes of G-isovariant maps.

By the definition of isovariance, we easily see the following.

(1) Let X and Y be free G-spaces. Then G-equivariance is equivalent to G-isovariance.
(2) If $f : X \to Y$ is an injective G-map, then f is G-isovariant.
(3) If there exists a G-isovariant map $f : X \to Y$, then $\mathrm{Iso}(X) \subset \mathrm{Iso}(Y)$, where $\mathrm{Iso}(X)$ is the set of isotropy subgroups of X.

Example 1.1. Let $X = G/H$ and $Y = G/K$.

(1) There exists a G-map $f : G/H \to G/K$ if and only if $(H) \leq (K)$, i.e., $H \leq aKa^{-1}$ for some $a \in G$.
(2) There exists a G-isovariant map $f : G/H \to G/K$ if and only if $(H) = (K)$. In this case, a G-isovariant map f is defined by $f(gH) = gaK$, $H = aKa^{-1}$.

2. Isovariant maps between representations

The following result says that isovariant maps between representations are essentially same as those between representation spheres.

Proposition 2.1. *Let V, W be (orthogonal) G-representations. The following are equivalent.*

(1) *There exists a G-isovariant map $f : V \to W$.*
(2) *There exists a G-isovariant map $f : V^{G^\perp} \to W^{G^\perp}$.*
(3) *There exists a G-isovariant map $f : S(V^{G^\perp}) \to S(W^{G^\perp})$.*

Here $V^{G^\perp}$ is the orthogonal complement of V^G in V. In particular, if $V^G = W^G = 0$, then there exists a G-isovariant map $f : V \to W$ if and only if $f : SV \to SW$.

Proof. (1) $\Rightarrow$ (2) $\Rightarrow$ (3) Composing the inclusion i and the projection p with $f : V \to W$, we have an isovariant map

$$\overline{f} : V^{G^\perp} \xrightarrow{i} V \xrightarrow{f} W \xrightarrow{p} W^{G^\perp}.$$

Composing the inclusion j and the normalization map with $\overline{f}$, we have an isovariant map

$$\overline{\overline{f}} : S(V^{G^\perp}) \xrightarrow{j} V^{G^\perp} \setminus \{0\} \xrightarrow{\overline{f}} W^{G^\perp} \setminus \{0\} \xrightarrow{\text{norm.}} S(W^{G^\perp}).$$

(1) $\Leftarrow$ (2) $\Leftarrow$ (3)

Let $g : S(V^{G^\perp}) \to S(W^{G^\perp})$ be an isovariant map. By the radial extension, we have an isovariant map

$$\tilde{g} : V^{G^\perp} \to W^{G^\perp}.$$

By adding the zero map to $\overline{g}$, we have an isovariant map

$$h := \tilde{g} \oplus 0 : V = V^{G^\perp} \oplus V^G \to W^{G^\perp} \oplus W^G = W.$$

□

By further arguments, we also obtain

Proposition 2.2. *When $V^G = W^G = 0$, there is a one-to-one correspondence*

$$[V, W]_G^{\mathrm{isov}} \cong [SV, SW]_G^{\mathrm{isov}}.$$

We here provide some examples. Let $G = C_n = \langle c \rangle$ be a cyclic group of order n, where c is a generator of C. Consider the irreducible representations of C. Let

$$U_k \ (= \mathbb{C}) \ (0 \leq k \leq n-1)$$

denote the irreducible representation with the linear action:

$$c \cdot z = \xi_n^k z \ (z \in U_k), \quad \xi_n = \exp(\frac{2\pi\sqrt{-1}}{n}).$$

Assume $n = pq$, where p, q are distinct primes and $G = C_{pq}$.

Example 2.3. If $(k, pq) = (l, pq) = 1$, then there exist a G-isovariant map $f : SU_k \to SU_l$.

In fact, fix s such that $ks \equiv 1 \bmod pq$. We define a map f by

$$f(z) = z^{sl}, \quad z \in SU_k.$$

Then one can check that

(1) f is G-equivariant,
(2) G acts freely on SU_k and SU_l.

Hence f is G-isovariant.

Further arguments show that the degree of maps classifies isovariant homotopy classes, and we have

$$[U_k, U_l]_{C_{pq}}^{\mathrm{isov}} \cong [SU_k, SU_l]_{C_{pq}}^{\mathrm{isov}} \cong \mathbb{Z},$$

and the representatives are given by

$$f_m(z) = z^{sl+mpq}, \quad z \in SU_k, \quad m \in \mathbb{Z}.$$

See [3], [4] for the detail.

Example 2.4. There do not exist isovariant maps $f : U_p \to U_q$ and $g : U_1 \to U_q$.

In fact, if $f : X \to Y$ is an isovariant map, then $\mathrm{Iso}\,(X) \subset \mathrm{Iso}\,(Y)$. However

$$\mathrm{Iso}\,(U_p) = \{C_p, G\} \not\subset \mathrm{Iso}\,(U_q) = \{C_q, G\}$$

and

$$\mathrm{Iso}\,(U_1) = \{1, G\} \not\subset \mathrm{Iso}\,(U_q) = \{C_q, G\}.$$

Example 2.5. There exists an isovariant map $f : U_1 \to U_p \oplus U_q$.

In fact there are isovariant maps

$$f_{\alpha,\beta} : SU_1 \to S(U_p \oplus U_q)$$

defined by

$$f_{\alpha,\beta}(z) = (z^{(1+\alpha q)p}, z^{(1+\beta p)q}), \quad \alpha, \beta \in \mathbb{Z},\ z \in SU_1.$$

These are isovariant maps since

$$G_{f_{\alpha,\beta}(z)} = G_{z^{(1+\alpha q)p}} \cap G_{z^{(1+\beta p)q}} = 1\ (z \in SU_1).$$

In this case, the multidegree classifies isovariant maps and one sees

$$[U_1, U_p \oplus U_q]^{\mathrm{isov}}_{C_{pq}} \cong [SU_1, S(U_p \oplus U_q)]^{\mathrm{isov}}_{C_{pq}} \cong \mathbb{Z} \oplus \mathbb{Z}.$$

See [3], [4] for the detail.

Example 2.6. There does not exist a G-isovariant map $f : U_1 \oplus U_1 \to U_p \oplus U_q$.

If there is an isovariant map, then the isovariant Borsuk-Ulam theorem stated in the next section shows

$$\begin{array}{ccc} \dim U_1 \oplus U_1 - \dim(U_1 \oplus U_1)^{C_p} & \leq & \dim U_p \oplus U_q - \dim(U_p \oplus U_q)^{C_p} \\ \| & & \| \\ 4 - 0 = 4 & & 4 - 2 = 2. \end{array}$$

This is a contradiction.

Remark. There is a G-map $f : S(U_1 \oplus U_1) \to S(U_p \oplus U_q)$. In fact there are G-maps $f_i : SU_1 \to SU_i$ defined by $f_i(z) = z^i$ for $i = p$ and q. Taking join of f_p and f_q, one obtains a G-map $f = f_p * f_q : S(U_1 \oplus U_1) \to S(U_p \oplus U_q)$.

Thus one can finally see

Proposition 2.7. *Let $G = C_{pq}$, and V, W G-representations. There exists a G-isovariant map $V \to W$ if and only if*

$$\begin{cases} \dim V - \dim V^H \leq \dim W - \dim W^H \\ \dim V^H - \dim V^G \leq \dim W^H - \dim W^G \end{cases}$$

for $H = C_p$, C_q.

See [2] for the detail.

Question (unsolved). How about C_n for an arbitrary n?

3. Borsuk-Ulam type theorem for isovariant maps

In this section we discuss a Borsuk-Ulam type theorem for isovariant maps, which provides non-existence results on isovariant maps as mentioned in the previous section.

The Borsuk-Ulam theorem due to Borsuk [1] is generalized in various ways (see [6], [7]). The following is one of them. Let C_p be a cyclic group of prime order p and assume that C_p acts freely on spheres S^m and S^n.

Theorem 3.1 (mod p Borsuk-Ulam theorem).
If there exists a C_p-map ($\Longleftrightarrow$ C_p-isovariant map) $f : S^m \to S^n$, then $m \leq n$, (or equivalently, if $m > n$, there does not exist a C_p-map $f : S^m \to S^n$).

Wasserman first studied the isovariant version of the Borsuk-Ulam theorem and introduced the notion of the Borsuk-Ulam group.

Definition (Wasserman). A compact Lie group G is called a *Borsuk-Ulam group (BUG)* if the following statement holds:

For any pair of G-representations V and W, if there is a G-isovariant map $f : V \to W$, then the Borsuk-Ulam inequality:

$$\dim V - \dim V^G \leq \dim W - \dim W^G$$

holds.

Proposition 3.2 ([8]). *C_p and S^1 are BUGs.*

The following are fundamental properties of Borsuk-Ulam groups.

Proposition 3.3 ([8]).

(1) *If $1 \to H \to G \to K \to 1$ is exact and H, K are BUGs, then G is also a BUG.*
(2) *A quotient group of a BUG is also a BUG.*

Question (unsolved). Is a subgroup of a BUG also a BUG?

Using this result repeatedly, we have

Corollary 3.4. *If*

$$1 = H_0 \lhd H_1 \lhd H_2 \lhd \cdots \lhd H_r = G$$

and H_i/H_{i-1} *are BUGs* $(1 \leq i \leq r)$, *then* G *is a BUG.*

We have the following.

Theorem 3.5 (Isovariant Borsuk-Ulam theorem). *Any solvable compact Lie group* G *is a BUG.*

Proof. As is well-known, G is solvable if and only if there exists a composition series

$$1 = H_0 \lhd H_1 \lhd H_2 \lhd \cdots \lhd H_r = G$$

such that $H_i/H_{i-1} = C_p$ or S^1. By Proposition 3.4, G is a BUG. □

So the next question is: how about non-solvable case? Wasserman also found non-solvable examples of BUGs using the prime condition.

Definition (Prime condition (PC)). (1) We say that a finite simple group G satisfies the prime condition (PC) if

$$\sum_{p|o(g)} \frac{1}{p} \leq 1$$

holds for any $g \in G$, where $o(g)$ is the order of g, and the sum is taken over all prime divisors of $o(g)$.

(2) We say that a finite group G satisfies (PC) if for a composition series

$$1 = H_0 \lhd H_1 \lhd H_2 \lhd \cdots \lhd H_r = G,$$

each simple H_i/H_{i-1} satisfies (PC) in the sense of (1).

Theorem 3.6 ([8]). *If a finite group* G *satisfies (PC), then* G *is a BUG.*

Remark. In the proof of [8], the fact that a cyclic group C is a BUG is used.

Example 3.7. Alternating groups $A_5, A_6, \ldots, A_{11}$ satisfy (PC), and hence BUGs. But A_n, $n \geq 12$, does not satisfy (PC). In fact A_n, $n \geq 12$, has an element of order $30 = 2 \cdot 3 \cdot 5$ and $1/2 + 1/3 + 1/5 = 31/30 > 1$.

Question (unsolved). Is A_n a BUG for $n \geq 12$?

Example 3.8. $PSL(2, p)$ satisfies (PC) for p: prime ≤ 53; hence a BUG. But $PSL(2, 59)$, $PSL(2, 61)$ do not satisfy (PC). Indeed there are infinitely many primes p such that $PSL(2, p)$ does not satisfy (PC).

4. A new family of Borsuk-Ulam groups

In this section G is a finite group. Let $\mathbb{F}_q$ be a finite field of order $q = p^r$, p: prime. Recall

$$\begin{aligned} PSL(2,q) &= SL(2,q)/\{\pm I\} \\ &= \{A \in M_2(\mathbb{F}_q) \mid \det A = 1\}/\{\pm I\}. \end{aligned}$$

Remark. $PSL(2,2^r) = SL(2,2^r)$.

Also recall:

(1) If $q = p^r \geq 4$, then $PSL(2,q)$ is simple. On the other hand $PSL(2,2) \cong S_3$ and $PSL(2,3) \cong A_4$, which are non-simple.

(2) $|PSL(2,q)| = \begin{cases} q(q-1)(q+1) & p = 2 \\ \frac{1}{2}q(q-1)(q+1) & p : \text{odd prime.} \end{cases}$

We introduce the Möbius condition in [5] and show the following.

Theorem 4.1 ([5]). *$PSL(2,q)$ is a BUG for any $q = p^r$.*

As a corollary,

Corollary 4.2. *$SL(2,q)$, $GL(2,q)$, $PGL(2,q)$ are BUGs.*

Proof. These are shown from the following exact sequences.

$$1 \to \{\pm I\} \to SL(2,q) \to PSL(2,q) \to 1$$

$$1 \to SL(2,q) \to GL(2,q) \xrightarrow{\det} \mathbb{F}_q^* \to 1$$

($F_q^* \cong C_{q-1}$)

$$PGL(2,q) = GL(2,q)/\text{center}$$

(center $= \{aI \mid a \in \mathbb{F}_q^*\} \cong \mathbb{F}_q^*$). □

As seen before, $PSL(2,59)$, $PSL(2,61)$ etc. do not satisfy (PC). Our result provides the first example to be a BUG not satisfying (PC).

Finally we announce the following result which will be proved in the forthcoming paper. Let $\mathrm{Syl}_p(G)$ denote a p-Sylow subgroup of G.

Theorem 4.3 (N-U). *If G satisfies one of the following conditions, then G is a BUG.*

(1) $\mathrm{Syl}_2(G)$ *is a cyclic group C_{2^r} of order 2^r.*

(2) $\mathrm{Syl}_2(G)$ *is a dihedral group D_{2^r} of oder 2^r ($r \geq 2$). As a convention, $D_4 = C_2 \times C_2$.*

(3) $\mathrm{Syl}_2(G)$ *is a generalized quaternion group Q_{2^r} of order 2^r ($r \geq 3$).*

(4) $\mathrm{Syl}_2(G)$ *is abelian and $\mathrm{Syl}_p(G)$ is cyclic for every odd prime p.*

Example 4.4.

(1) $PSL(2,q)$, q: odd, is an example of (2).

(2) $SL(2,q)$, q: odd, is an example of (3).

(3) $SL(2,2^r)$ is an example of (4).

(4) A finite group with periodic cohomology is an example of (1), (3) or (4).

For the proof, we use the fact that $PSL(2,q)$ is a BUG and several deep results of finite group theory.

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Homotopy Classification of Maps from a Closed Manifold to the Complement of a Subspace Arrangement

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Abstract. The Hopf classification theorem says that the degree of maps classifies the homotopy classes of maps from an m-dimensional closed manifold M to an m-sphere $\mathbb{S}^m$. In this paper, we give a generalization of the Hopf classification theorem using the multidegree; i.e., the multidegree of maps classifies the homotopy classes of maps from a connected closed manifold M of dimension $2(n-k)-1$ to the complement of a k-subspace arrangement of $\mathbb{C}^n$ for $0 \leq k < n$.

1. The multidegree

Throughout this paper, we assume that a manifold N is connected and has a CW structure. All maps between spaces are assumed to be continuous.

Although the notion of multidegree was first introduced in the equivariant setting [3, 4], the multidegree itself can be defined in the non-equivariant case. In this section we define the multidegree of maps from a closed manifold to the complement of a certain subspace arrangement.

We begin with some basic notation and terminology. Let $\mathcal{A}$ be a non-empty finite set of k-dimensional $\mathbb{C}$-vector subspaces of $\mathbb{C}^n$. We simply call it a *k-subspace arrangement* of $\mathbb{C}^n$. Let

$$N_{\mathcal{A}} = \bigcup_{U \in \mathcal{A}} U,$$

and set $M_{\mathcal{A}} = \mathbb{C}^n \setminus N_{\mathcal{A}}$ as the complement of $N_{\mathcal{A}}$ in $\mathbb{C}^n$, which is called the *complement of a k-subspace arrangement*. Let $\mathbb{S}^{2n-1}$ be the unit sphere of $\mathbb{C}^n$; namely,

$$\mathbb{S}^{2n-1} = \{(z_1, \ldots, z_n) \in \mathbb{C}^n \,|\, |z_1|^2 + \cdots + |z_n|^2 = 1\}.$$

We set $S_{\mathcal{A}} = \mathbb{S}^{2n-1} \cap M_{\mathcal{A}}$. We first show the following basic results.

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Proposition 1.1. *Let $\mathcal{A}$ be a k-subspace arrangement of $\mathbb{C}^n$. Set $d = n - k$.*

(1) *$S_{\mathcal{A}}$ is a (strong) deformation retract of $M_{\mathcal{A}}$. In particular, the inclusion $i : S_{\mathcal{A}} \to M_{\mathcal{A}}$ induces isomorphisms*

$$H_*(S_{\mathcal{A}}; R) \cong H_*(M_{\mathcal{A}}; R) \quad \text{and} \quad \pi_*(S_{\mathcal{A}}) \cong \pi_*(M_{\mathcal{A}}).$$

(2) *$M_{\mathcal{A}}$ and $S_{\mathcal{A}}$ are $(2d-2)$-connected.*

(3) *There is the isomorphism*

$$\Psi = (\Psi_U) : H_{2d-1}(M_{\mathcal{A}}; R) \to \bigoplus_{U \in \mathcal{A}} H_{2d-1}(\mathbb{C}^n \setminus U; R) \cong \bigoplus_{U \in \mathcal{A}} R$$

induced by the inclusions $i_U : M_{\mathcal{A}} \to \mathbb{C}^n \setminus U$, where R is an arbitrary abelian group.

(4) *If $d \geq 2$, then $M_{\mathcal{A}}$ and $S_{\mathcal{A}}$ are simple spaces and*

$$\pi_{2d-1}(M_{\mathcal{A}}) \cong \pi_{2d-1}(S_{\mathcal{A}}) \cong \bigoplus_{U \in \mathcal{A}} \mathbb{Z}.$$

Here $H_q(-; R)$ means the q-th singular homology group with coefficients in R, and $\pi_q(-)$ means the q-th homotopy group.

Proof. (1) The map $r : M_{\mathcal{A}} \to S_{\mathcal{A}}$ defined by $r(x) = x/\|x\|$ gives a retraction and a homotopy $H : M_{\mathcal{A}} \times I \to M_{\mathcal{A}}$ between id and r is given by $H(x,t) = x/\|x\|^t$.

(2) Let $\alpha : \mathbb{S}^i \to M_{\mathcal{A}}$ be any continuous map for $0 \leq i \leq 2d-2$. Since α is null homotopic in $\mathbb{C}^n$, we take a homotopy $H : \mathbb{S}^i \times I \to \mathbb{C}^n$ between α and c a constant map into $M_{\mathcal{A}}$. Since

$$\dim \mathbb{S}^i \times I + \dim N_{\mathcal{A}} = i + 1 + 2k \leq 2n - 1 < \dim \mathbb{C}^n,$$

we can deform H such that the image of H is in $M_{\mathcal{A}}$ relative to $\mathbb{S}^i \times \{0, 1\}$. Therefore α is null homotopic in $M_{\mathcal{A}}$, and so $S_{\mathcal{A}}$ and $M_{\mathcal{A}}$ are $(2d-2)$-connected.

(3) We prove it by induction on $|\mathcal{A}|$ the number of elements in $\mathcal{A}$. When $|\mathcal{A}| = 1$, we see $M_{\mathcal{A}} = \mathbb{C}^n \setminus U \cong \mathbb{C}^k \times (\mathbb{C}^d \setminus \{0\})$, which is homotopy equivalent to $\mathbb{S}^{2d-1}$. Hence $H_{2d-1}(M_{\mathcal{A}}; R) \cong R$. Now let $\mathcal{A} = \{U_1, \ldots, U_r\}$, $r \geq 2$. Set $\mathcal{B} = \{U_1, \ldots, U_{r-1}\}$ and $M = \mathbb{C}^n \setminus \bigcup \mathcal{B}$. Suppose $H_{2d-1}(M; R) \cong \bigoplus_{U \in \mathcal{B}} R$. Set $N = \mathbb{C}^n \setminus U_r$; then $H_{2d-1}(N; R) \cong R$. Since $M_{\mathcal{A}} = M \cap N$, by the Mayer-Vietoris exact sequence, we have

$$\to H_{2d}(M \cup N) \to H_{2d-1}(M_{\mathcal{A}}) \to H_{2d-1}(M) \oplus H_{2d-1}(N) \to H_{2d-1}(M \cup N) \to$$

where the coefficient group is R. Since $M \cup N = \mathbb{C}^n \setminus \bigcap_{U \in \mathcal{A}} U$ and $\dim \bigcap_{U \in \mathcal{A}} U \leq 2(k-1)$, $M \cap N$ is at least $2d$-connected by a similar argument as (2). By the Hurewicz

theorem, we have $H_{2d}(M \cup N) = H_{2d-1}(M \cup N) = 0$. Thus one can see that

$$H_{2d-1}(M_{\mathcal{A}}; R) \cong H_{2d-1}(M) \oplus H_{2d-1}(N)$$
$$\cong \bigoplus_{i=1}^{r} H_{2d-1}(\mathbb{C}^n \setminus U_i; R) \cong \bigoplus_{U \in \mathcal{A}} R.$$

(4) If $d \geq 2$, then M is 1-connected by (2), and hence $M_{\mathcal{A}}$ is simple. By the Hurewicz theorem, we have

$$\pi_{2d-1}(M_{\mathcal{A}}) \cong H_{2d-1}(M_{\mathcal{A}}; \mathbb{Z}) \cong \bigoplus_{U \in \mathcal{A}} \mathbb{Z}.$$

□

Remark. If $d = 1$, $M_{\mathcal{A}}$ is not necessarily 1-simple; namely, $\pi_1(M_{\mathcal{A}})$ is not necessarily abelian. For example, let $\mathcal{A} = \{(z_1, z_2) \in \mathbb{C}^2 \mid z_1 z_2 (z_1 + z_2) = 0\}$. Then $M_{\mathcal{A}}$ is homotopy equivalent to $(S^1 \vee S^1) \times S^1$, see [5]; so $\pi_1(M_{\mathcal{A}}) \cong F_2 \times \mathbb{Z}$, where F_2 is the free group of rank 2.

We introduce the multidegree of a map from a $(2d-1)$-dimensional closed manifold N to $M_{\mathcal{A}}$ the complement of a k-subspace arrangement in $\mathbb{C}^n$, where $d = n - k$. Let $f : N \to M_{\mathcal{A}}$ be any continuous map. Assume that N is orientable and N is given an orientation by the fundamental class $[N]$ of N. On the other hand, $M_{\mathcal{A}}$ has an orientation coming from the standard orientation of $\mathbb{C}^n$. Thus we define the multidegree $\mathrm{mDeg}\, f$ of f by

$$\mathrm{mDeg}\, f = \Psi(f_*([N])) \in \bigoplus_{U \in \mathcal{A}} \mathbb{Z}.$$

Here $\Psi = (\Psi_U) : H_{2d-1}(M_{\mathcal{A}}; \mathbb{Z}) \to \bigoplus_{U \in \mathcal{A}} H_{2d-1}(\mathbb{C}^n \setminus U; \mathbb{Z}) = \bigoplus_{U \in \mathcal{A}} \mathbb{Z}$ is the isomorphism induced from the inclusions $i_U : M_{\mathcal{A}} \to \mathbb{C}^n \setminus U$.

Next we consider the case where X is non-orientable. Let $[N]_2 \in H_{2d-1}(X; \mathbb{Z}/2)$ denote the mod 2 fundamental class of N. We define the mod 2 multidegree $\mathrm{mDeg}_2 f$ of f by

$$\mathrm{mDeg}_2\, f = \Psi_2(f_*([N]_2)) \in \bigoplus_{U \in \mathcal{A}} \mathbb{Z}/2.$$

Here $\Psi_2 = (\Psi_{2,U}) : H_{2d-1}(M_{\mathcal{A}}; \mathbb{Z}/2) \to \bigoplus_{U \in \mathcal{A}} H_{2d-1}(\mathbb{C}^n \setminus U; \mathbb{Z}/2) = \bigoplus_{U \in \mathcal{A}} \mathbb{Z}/2$ is the isomorphism induced from the inclusions $i_U : M_{\mathcal{A}} \to \mathbb{C}^n \setminus U$. These multidegrees are homotopy invariants for maps.

Example 1.2. Let $\mathcal{A} = \{(z_1, z_2) \in \mathbb{C}^2 \mid z_1 z_2 = 0\}$. We define maps $f_{p,q} : \mathbb{S}^1 \to M_{\mathcal{A}}$ by $f_{p,q}(z) = (z^p, z^q)$. Then $\mathrm{mDeg}\, f_{p,q} = (p, q)$.

2. A Hopf type theorem

Let X and Y are topological spaces. We denote by $[X, Y]$ the set of (unbased) homotopy classes of continuous maps from X to Y.

Classification of homotopy classes of maps or determination of the set $[X, Y]$ is a crucial problem in algebraic topology. Let X be a finite CW complex of dimension m and Y be an n-sphere $\mathbb{S}^n$. If $m < n$, then every continuous map $f : X \to \mathbb{S}^n$ is null homotopic; namely, $[X, \mathbb{S}^n]$ consists of one element. In our setting, we generalize this result as follows.

Theorem 2.1. *Let $M_{\mathcal{A}}$ be the complement of a k-subspace arrangement $\mathcal{A}$ in $\mathbb{C}^n$ and X a connected finite CW complex of dimension m less than $2d-1$, where $d = n-k$. Then every continuous map $f : X \to M_{\mathcal{A}}$ is null homotopic; namely, $[X, M_{\mathcal{A}}]$ consists of one element.*

Proof. Let $Z_{(i)}$ denote the i-skeleton of a relative CW complex $(X \times I, X \times \partial I)$ for $0 \leq i \leq m+1$. Set

$$H_{-1} := f \coprod c : Z_{(-1)} := X \times \partial I \to M_{\mathcal{A}},$$

where c is a constant map. We construct a map $H_i : Z_{(i)} \to M_{\mathcal{A}}$ extending H_{-1} inductively for $i \geq 0$. Suppose that there is a map $H_i : Z_{(i)} \to M_{\mathcal{A}}$. Note that $Z_{(i+1)}$ has a form $Z_{(i+1)} = Z_{(i)} \cup_j D_j^{i+1}$, where D_j^{i+1} are $(i+1)$-dimensional cells. Since $M_{\mathcal{A}}$ is $(2d-2)$-connected, $H_{ij} = H_i|_{\partial D_j^{i+1}} : \mathbb{S}^i \to M_{\mathcal{A}}$ extends to $\bar{H}_{ij} : D^{i+1} \to M_{\mathcal{A}}$ for $0 \leq i \leq m$. Thus we have a map

$$H_{i+1} = H_i \cup_j \bar{H}_{ij} : Y_{(i+1)} \to M_{\mathcal{A}}.$$

Consequently we have a homotopy $H : X \times I \to M_{\mathcal{A}}$ between f and c. □

H. Hopf [2] considered continuous maps from an n-dimensional closed manifold N to an n-sphere $\mathbb{S}^n$ and showed the so-called Hopf classification theorem below.

Theorem 2.2 (Hopf classification theorem)**.** *Let N be an n-dimensional closed manifold, where $n \geq 1$.*

(1) *If N is orientable, then the degree* $\deg f$ *of maps gives a bijection*

$$\deg : [N, \mathbb{S}^n] \to \mathbb{Z}.$$

(2) *If N is non-orientable, then the mod 2 degree* $\deg_2 f$ *of maps gives a bijection*

$$\deg_2 : [N, \mathbb{S}^n] \to \mathbb{Z}/2.$$

We generalize this result as follows.

Theorem 2.3. *Let $M_{\mathcal{A}}$ be the complement of a k-subspace arrangement $\mathcal{A}$ of $\mathbb{C}^n$ and N a closed manifold of dimension $2d-1$, where $d=n-k$. If $d=1$, then we assume that $\pi_1(M_{\mathcal{A}})$ is abelian.*

(1) *If N is orientable, then the multidegree* $\mathrm{mDeg}\, f$ *of maps gives a bijection*

$$\mathrm{mDeg} : [N, M_{\mathcal{A}}] \to \bigoplus_{U\in\mathcal{A}} \mathbb{Z}.$$

(2) *If N is non-orientable, then the mod 2 multidegree* $\mathrm{mDeg}_2 f$ *of maps gives a bijection*

$$\mathrm{mDeg}_2 : [N, M_{\mathcal{A}}] \to \bigoplus_{U\in\mathcal{A}} \mathbb{Z}/2.$$

Remark. By Proposition 1.1 (1), the inclusion $i : S_{\mathcal{A}} \to M_{\mathcal{A}}$ induces a bijection $[N, S_{\mathcal{A}}] \cong [N, M_{\mathcal{A}}]$. In particular, if $k=0$, then it follows that $S_{\mathcal{A}} = \mathbb{S}^{2n-1}$ and the multidegree coincides with the ordinary degree $\deg f$. Therefore we obtain the Hopf classification theorem in the case where n is odd.

To show the theorem, we recall obstruction theory, see [1] for the detail. Let X be an n-dimensional finite CW complex, and let $K(\pi, n)$, π is abelian, denote an Eilenberg-MacLane space; i. e., $\pi_n(K(\pi,n)) \cong \pi$ and $\pi_q(K(\pi,n)) = 0$ for $q \neq n$. By the universal coefficient theorem and the Hurewicz theorem, there are natural isomorphisms

$$H^n(K(\pi,n);\pi) \cong \mathrm{Hom}_{\mathbb{Z}}(H_n(K(\pi,n);\mathbb{Z}),\pi) \cong \mathrm{Hom}_{\mathbb{Z}}(\pi,\pi).$$

Let $\iota \in H^n(K(\pi,n);\pi)$ be the element, called the fundamental class of $K(\pi,n)$, corresponding to the identity of π. Then one can define a map

$$\bar{\phi} : [X, K(\pi,n)] \to H^n(X;\pi)$$

by $\bar{\phi}([f]) = f^*(\iota)$. On the other hand, using the obstruction class $\gamma(f,c)$ between f and c a constant map, one can define a map

$$\bar{\psi} : [X, K(\pi,n)] \to H^n(X;\pi)$$

by $\bar{\psi}([f]) = \gamma(f,c)$. A fundamental result of obstruction theory is the following.

Proposition 2.4 ([1, chapter 7])**.** *Both $\bar{\phi}$ and $\bar{\psi}$ are bijections and they coincide.*

Now we give the proof of Theorem 2.3. Since $M_{\mathcal{A}}$ is a smooth manifold, it has a CW structure. Attaching cells of dimension greater than $2d$ to $M_{\mathcal{A}}$, one can kill the

homotopy groups of $M_{\mathcal{A}}$ greater than $(2d-1)$-dimension and so one obtains a space which is $K(\pi, 2d-1)$. Consequently one may assume that $K(\pi, 2d-1)$ is a CW complex including $M_{\mathcal{A}}$ as a subcomplex and the relative dimension of a pair $(K(\pi, 2d-1), M_{\mathcal{A}})$ is $2d+1$. By the cellular approximation theorem, there is an isomorphism

$$i_* : \pi_{2d-1}(M_{\mathcal{A}}) \to \pi_{2d-1}(K(\pi, 2d-1)),$$

where i is the inclusion. We identify these homotopy groups and set

$$\pi = \pi_{2d-1}(M_{\mathcal{A}}) = \pi_{2d-1}(K(\pi, 2d-1)) \cong \bigoplus_{U \in \mathcal{A}} \mathbb{Z}.$$

Then there is a bijection

$$\bar{\phi} = \bar{\psi} : [N, K(\pi, 2d-1)] \to H^{2d-1}(N; \pi).$$

By the cellular approximation theorem, we also see that there is a bijection

$$i_* : [N, M_{\mathcal{A}}] \to [N, K(\pi, 2d-1)],$$

where $i : M_{\mathcal{A}} \to K(\pi, 2d-1)$ is the inclusion. In fact, for any map $\alpha : N \to K(\pi, 2d-1)$, there exists a map $\alpha' : N \to K(\pi, 2d-1)$ such that α' and α are homotopic and $\alpha'(N) \subset K(\pi, 2d-1)_{(2d-1)} = M_{\mathcal{A}}$. Hence $[\alpha'] \in [N, M_{\mathcal{A}}]$ and $i_*([\alpha']) = [\alpha]$. Next if α, $\beta : N \to M_{\mathcal{A}}$ are homotopic in $K(\pi, 2d-1)$, then the homotopy H between α and β is homotopic to a homotopy

$$H' : N \times I \to K(\pi, 2d-1)_{(2d)} = M_{\mathcal{A}} \subset K(\pi, 2d-1)$$

relative to $N \times \partial I$. Hence i_* is injective. Thus we obtain a bijection

$$\phi := i_* \circ \bar{\phi} : [N, M_{\mathcal{A}}] \to H^{2d-1}(N; \pi),$$

where $\pi = \pi_{2d-1}(M_{\mathcal{A}})$. In this case, there are natural isomorphisms

$$H^{2d-1}(M_{\mathcal{A}}; \pi) \cong \mathrm{Hom}_{\mathbb{Z}}(H_{2d-1}(M_{\mathcal{A}}; \mathbb{Z}), \pi) \cong \mathrm{Hom}_{\mathbb{Z}}(\pi, \pi).$$

Let $\nu \in H^{2d-1}(M_{\mathcal{A}}; \pi)$ be the element corresponding to the identity of π. Then we have

$$\phi([f]) = f^*(\nu) = \gamma(f, c) \in H^{2d-1}(N; \pi).$$

2.1. **The orientable case.** We first consider the orientable case. Then there is the isomorphism

$$\kappa : H^{2d-1}(N;\pi) \to \pi$$

defined by

$$\kappa(x) = \langle x, [N]\rangle,$$

where $[N] \in H_{2d-1}(N;\mathbb{Z})$ is the fundamental class of N. Thus we have

$$\kappa(f^*(\nu)) = \langle f^*(\nu), [N]\rangle = \langle \nu, f_*([N])\rangle = h^{-1}(f_*([N])) \in \pi,$$

where $h : \pi = \pi_{2d-1}(M_{\mathcal{A}}) \to H_{2d-1}(M_{\mathcal{A}};\mathbb{Z})$ is the Hurewicz isomorphism. Identifying π with $H_{2d-1}(M_{\mathcal{A}};\mathbb{Z}) = \bigoplus_{U\in\mathcal{A}} \mathbb{Z}$ via the Hurewicz isomorphism, we conclude

$$h(h^{-1}(f_*([N]))) = f_*([N]) = \mathrm{mDeg}\, f.$$

Thus $\mathrm{mDeg} : [N, M_{\mathcal{A}}] \to \bigoplus_{U\in\mathcal{A}} \mathbb{Z}$ is a bijection.

2.2. **The non-orientable case.** There are isomorphisms

$$r^* : H^{2d-1}(N;\pi) \to H^{2d-1}(N;\pi/2\pi)$$

and

$$\kappa_2 : H^{2d-1}(N;\pi/2\pi) \to \pi/2\pi,$$

where r is the mod 2 reduction, and κ_2 is the homomorphism defined by

$$\kappa_2(x) = \langle x, [N]_2\rangle$$

and $[N]_2 \in H_{2d-1}(N;\mathbb{Z}/2)$ is the mod 2 fundamental class of N. Indeed the reduction $r : \pi \to \pi/2\pi$ induces a surjection $r^{\#} : C^{2d-1}(N;\pi) \to C^{2d-1}(N;\pi/2\pi)$ and since the coboundary maps on $C^{2d-1}(N\ \pi)$ and $C^{2d-1}(N;\pi/2\pi)$ are zero, $C^{2d-1}(N;\pi)$ and $C^{2d-1}(N;\pi/2\pi)$ are cocycles. Therefore r^* is a surjection. On the other hand, $H^{2d-1}(N;\mathbb{Z})$ and $H^{2d-1}(N;\mathbb{Z}/2)$ are isomorphic to $\bigoplus_{U\in\mathcal{A}} \mathbb{Z}/2$ respectively. Hence r^* must be an isomorphism. By the universal coefficient theorem over $\mathbb{Z}/2$, we see that κ_2 is an isomorphism. Thus we have

$$\kappa_2(r(f^*(\nu))) = \langle r^*(f^*(\nu)), [N]_2\rangle = \langle r^*(\nu), f_*([N]_2)\rangle = h_2^{-1}(f_*([N])) \in \pi/2\pi,$$

where $h_2 : \pi = \pi_{2d-1}(M_{\mathcal{A}}) \otimes \mathbb{Z}/2 \to H_{2d-1}(M_{\mathcal{A}};\mathbb{Z}/2)$ is the mod 2 Hurewicz homomorphism. Identifying $\pi/2\pi$ with $H_{2d-1}(M_{\mathcal{A}};\mathbb{Z}/2) = \bigoplus_{U\in\mathcal{A}} \mathbb{Z}/2$ via the mod 2 Hurewicz isomorphism, we conclude

$$h_2(h_2^{-1}(f_*([N]_2))) = f_*([N]_2) = \mathrm{mDeg}_2\, f.$$

Thus $\mathrm{mDeg}_2 : [N, M_{\mathcal{A}}] \to \bigoplus_{U\in\mathcal{A}} \mathbb{Z}/2$ is a bijection. Thus the proof is complete. □

Example 2.5. Let $\mathcal{A} = \{ (z_1, z_2) \in \mathbb{C}^2 \mid z_1 z_2 = 0\}$. Then we have

$$[\mathbb{S}^1, M_{\mathcal{A}}] \cong \mathbb{Z} \oplus \mathbb{Z}$$

and the homotopy classes are represented by the maps $f_{p,q}$ defined in Example 1.2 for $(p, q) \in \mathbb{Z} \oplus \mathbb{Z}$.

3. Remarks on 1-dimensional case

If $d = 1$, then $\pi_1(M_{\mathcal{A}})$ is not necessarily abelian. In this section, we consider the case where $\pi_1(M_{\mathcal{A}})$ is non-abelian. Since $d = 1$, then the 1-dimensional closed manifold N must be a circle $\mathbb{S}^1$. We want to know the homotopy set $[\mathbb{S}^1, M_{\mathcal{A}}]$. As is well known, $[\mathbb{S}^1, M_{\mathcal{A}}]$ coincides with the orbit set of the $\pi_1(M_{\mathcal{A}})$-action on $[\mathbb{S}^1, M_{\mathcal{A}}]_0$ $(= \pi_1(M_{\mathcal{A}}))$; see for example [1]. Since the $\pi_1(M_{\mathcal{A}})$-action is the conjugate action on $\pi_1(M_{\mathcal{A}})$, we have $[\mathbb{S}^1, M_{\mathcal{A}}] \cong C(\pi_1(M_{\mathcal{A}}))$, where $C(\pi_1(M_{\mathcal{A}}))$ is the set of conjugacy classes of elements in $\pi_1(M_{\mathcal{A}})$.

Example 3.1. Let $\mathcal{A} = \{ (z_1, z_2) \in \mathbb{C}^2 \mid z_1 z_2 (z_1 + z_2) = 0\}$. Then $\pi_1(M_{\mathcal{A}}) \cong F_2 \times \mathbb{Z}$ as mentioned in section 1. In this case, $C(F_2 \times \mathbb{Z}) \cong C(F_2) \times \mathbb{Z}$ and hence $[\mathbb{S}^1, M_{\mathcal{A}}] \cong C(F_2) \times \mathbb{Z}$.

Finally we give a remark in the case where $M_{\mathcal{A}}$ is $K(\pi, 1)$ and N is an n-dimensional closed manifold N. If π is abelian, then we have

$$[N, M_{\mathcal{A}}] \cong H^1(N; \pi)$$

by obstruction theory. For example, let

$$\mathcal{A} = \{(z_1, \ldots, z_n) \in \mathbb{C}^n \mid z_1 \cdots z_n = 0\}.$$

Then

$$M_{\mathcal{A}} \cong (\mathbb{C} \setminus \{0\}) \times \cdots \times (\mathbb{C} \setminus \{0\}) \quad (n \text{ times}).$$

Hence $M_{\mathcal{A}}$ is $K(\mathbb{Z}^n, 1)$; in particular, we conclude

$$[N, M_{\mathcal{A}}] \cong H^1(N; \mathbb{Z}^n) \cong \bigoplus^n H^1(N; \mathbb{Z}).$$

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A Potential Neuroprotective Role of Apolipoprotein E-containing Lipoproteins through Low Density Lipoprotein Receptor-related Protein 1 in Normal Tension Glaucoma*

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Background: No effective treatment exists for normal tension glaucoma (NTG), which induces a significant loss of retinal ganglion cells (RGCs).
Results: Apolipoprotein E-containing lipoproteins (E-LPs) blocked Ca^{2+}-dependent apoptosis induced by glutamate in RGCs.
Conclusion: Administration of E-LPs protects RGCs from glutamate-induced degeneration *in vitro* and *in vivo*.
Significance: Protection from neuron death by E-LPs provides a novel strategy of treatment for NTG.

Glaucoma is an optic neuropathy and the second major cause of blindness worldwide next to cataracts. The protection from retinal ganglion cell (RGC) loss, one of the main characteristics of glaucoma, would be a straightforward treatment for this disorder. However, the clinical application of neuroprotection has not, so far, been successful. Here, we report that apolipoprotein E-containing lipoproteins (E-LPs) protect primary cultured RGCs from Ca^{2+}-dependent, and mitochondrion-mediated, apoptosis induced by glutamate. Binding of E-LPs to the low density lipoprotein receptor-related protein 1 recruited the *N*-methyl-D-aspartate receptor, blocked intracellular Ca^{2+} elevation, and inactivated glycogen synthase kinase 3β, thereby inhibiting apoptosis. When compared with contralateral eyes treated with phosphate-buffered saline, intravitreal administration of E-LPs protected against RGC loss in glutamate aspartate transporter-deficient mice, a model of normal tension glaucoma that causes glaucomatous optic neuropathy without elevation of intraocular pressure. Although the presence of α2-macroglobulin, another ligand of the low density lipoprotein receptor-related protein 1, interfered with the neuroprotective effect of E-LPs against glutamate-induced neurotoxicity, the addition of E-LPs overcame the inhibitory effect of α2-macroglobulin. These findings may provide a potential therapeutic strategy for normal tension glaucoma by an LRP1-mediated pathway.

From global surveys, the second leading cause of blindness next to cataracts is glaucoma (1). Glaucoma is the major optic neuropathy and is characterized by significant death of retinal ganglion cells (RGCs)[2] (2). Although an elevated intraocular pressure greater than 21 mm Hg increases the risk of developing primary open-angle glaucoma, many glaucoma patients suffer from normal tension glaucoma (NTG), a subset of primary open-angle glaucoma, with a normal range of intraocular pressure (10–21 mm Hg). The occurrence of NTG varies worldwide. However, of Japanese adult patients with primary open-angle glaucoma, 92% were classified as having NTG (3). In addition, the proportion of glaucoma patients who had NTG (70%) was 4-fold higher than those with high intraocular pressure (17%) in a Japanese American clinical population (4). Moreover, it was also reported that all American Indian and Alaska Native patients with glaucoma had normal eye pressure (5). Although several factors appear to be associated with the development of this disorder (6), the cause of NTG has not been identified. Current clinical treatments for NTG, mostly controlling intraocular pressure, are very limited and unsatisfactory. Although the strategies for providing neuroprotection by Ca^{2+} channel blockers, neurotrophins, and inhibitors of the *N*-methyl-D-aspartate (NMDA) receptor (7–9) against RGC

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[2] The abbreviations used are: RGC, retinal ganglion cell; apo, apolipoprotein; E-LP, apolipoprotein E-containing lipoprotein; GLAST, glutamate aspartate transporter; GSK, glycogen synthase kinase; HBSS, Hanks' balanced salt solution; LRP1, low density lipoprotein receptor-related protein 1; NMDAR, NMDA receptor; NTG, normal tension glaucoma; Z, benzyloxycarbonyl; fluoromethyl ketone; EGFP, enhanced green fluorescent protein; Glu, glutamate; a2M, α2-macroglobulin.

degeneration have been recently considered, these treatments have not been clinically successful. Thus, a novel strategy of treatment for NTG is urgently needed.

It has been reported that nerve injury promotes the secretion of significant amounts of apolipoprotein E-containing lipoproteins (E-LPs) (10, 11). This response of glia to nerve injury has been suggested to provide support for the repair of neurons by supplying materials for the cells. We have reported that glia-derived E-LPs promote axon extension of RGCs mediated by receptor(s) of the low density lipoprotein (LDL) receptor family after axon injury (12). One multifunctional endocytotic and signaling receptor of this family is the LDL receptor-related protein 1 (LRP1). We have also demonstrated that E-LPs strongly protect RGCs from neurodegeneration elicited by withdrawal of trophic additives (brain-derived neurotrophic factor, ciliary neurotrophic factor, basic fibroblast growth factor, and other supplements) (13). This neuroprotection was initiated upon binding of E-LP to LRP1, which induced an intracellular signal involving phospholipase Cγ1, protein kinase Cδ, and glycogen synthase kinase 3β (GSK3β), without endocytosis of the E-LPs (14). Thus, we propose that E-LPs not only supply lipids but also can function as an endogenous neuroprotective factor for suppressing neurodegeneration by inducing intracellular signaling.

Here we provide a potential therapeutic strategy for NTG by intravitreal administration of E-LPs. E-LPs bind to LRP1, recruit NMDA receptors, and inhibit intracellular Ca^{2+} elevation in RGCs. The inhibition of Ca^{2+} elevation by E-LPs suppresses mitochondrion-mediated and caspase-dependent apoptosis in RGCs. In addition, treatment of E-LPs inactivates the proapoptotic kinase GSK3β *in vitro* and *in vivo*. A deficiency of the glutamate aspartate transporter (GLAST), a major glutamate transporter in the retina, in mice induces optic neuropathy without affecting intraocular pressure and exhibits many features similar to human NTG (15). Thus, GLAST-deficient mice are utilized as an animal model for NTG. In this study, intravitreal administration of E-LPs prevented RGC loss induced in GLAST-deficient mice. Although an increase of α2-macroglobulin, another endogenous ligand of LRP1, in vitreous humor of GLAST-deficient mice may interfere with the neuroprotective effect of E-LPs, exogenous administration of E-LPs overcomes this inhibition.

EXPERIMENTAL PROCEDURES

Materials—A rabbit polyclonal anti-LRP1 antibody (R2629) was generously provided by Dr. D. K. Strickland (University of Maryland School of Medicine, Baltimore, MD) (16). A colony of GLAST-deficient mice was established at Kumamoto University from mice obtained from Tokyo Medical and Dental University. All experimental procedures were approved by the Animal Care Committee of Kumamoto University.

Primary Culture of Retinal Ganglion Cells—Sprague-Dawley rats (2 days old) were used for primary culture of RGCs according to Barres *et al.* (17) with minor modifications (13, 14). Briefly, retinae were digested with papain (16.5 units/ml) for 30 min at 37 °C and then triturated in minimum essential medium (Invitrogen) with rabbit anti-rat macrophage antiserum (Accurate Chemical, Westbury, NY). The cell suspension was first incubated on a panning plate (150-mm Petri dish) coated with goat anti-rabbit IgG (Pierce Biotechnology) for 20 min at room temperature. Nonadherent cells were incubated for 35 min on a second panning plate (100-mm Petri dish) coated with goat anti-mouse IgMμ (Pierce) and mouse anti-Thy1.1 antibodies secreted from T11D7e2 cells (American Type Culture Collection, Manassas, VA). The plate was washed with phosphate-buffered saline (PBS), and then adherent RGCs were released by treatment with 0.125% trypsin for 10 min at 37 °C. Isolated RGCs were suspended in medium containing 1 mM glutamine, 5 μg/ml insulin, 60 μg/ml *N*-acetylcysteine, 62 ng/ml progesterone, 16 μg/ml putrescine, 40 ng/ml sodium selenite, 0.1 mg/ml bovine serum albumin, 40 ng/ml triiodothyronine, 0.1 mg/ml transferrin, 1 mM sodium pyruvate, 2% B-27 supplement (Invitrogen), 10 μM forskolin, 50 ng/ml brain-derived neurotrophic factor (PeproTech, Rocky Hill, NJ), 50 ng/ml ciliary neurotrophic factor (PeproTech), and 50 ng/ml basic fibroblast growth factor (PeproTech) in Neurobasal medium. Culture plates (96 wells) were coated with poly-D-lysine (Sigma) and laminin (Sigma). RGCs were plated at a density of 5,000 cells/well in 96-well plates, 5,000 cells/culture insert for μ-dishes (ibidi, Munich, Germany), or 15,000 cells/dish for compartmented cultures and were cultured for at least 10 days before experiments.

Isolation of Glia-derived E-LPs, Plasma High Density Lipoproteins, and Reconstituted E-LPs—Glia were isolated from the cerebral cortex of 2-day-old Sprague-Dawley rats, digested with 0.25% trypsin, and cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. The glial cultures were enriched in astrocytes (>80%) (14). Glia were cultured for 3 days in the same medium used for RGCs but without forskolin, brain-derived neurotrophic factor, ciliary neurotrophic factor, and basic fibroblast growth factor. This culture medium was centrifuged for 10 min at 1,000 × *g*, and the supernatant is defined as glia-conditioned medium. Mouse or rat plasma high density lipoproteins (HDL) were isolated from blood collected from the abdominal aorta of C57BL/6J mice, apoE-deficient mice or Sprague-Dawley rats, as indicated. Reconstituted E-LPs were prepared as described previously (13) and contained 1-palmitoyl-2-oleoyl-glycerophosphocholine (Sigma), cholesterol (Sigma), and recombinant human apoE (Wako, Osaka, Japan) at a molar ratio of 100:10:1 or 100:0:1 as indicated. Briefly, 2.17 mg of 1-palmitoyl-2-oleoyl-glycerophosphocholine, with or without 0.11 mg of cholesterol, were dissolved in chloroform and then evaporated under nitrogen gas. Four hundred μl of 10 mM Tris-HCl (pH 7.4) containing 0.9% NaCl were added and incubated for 1 h on ice. One hundred μl of 15 mg/ml sodium cholate were added. The mixture was incubated for 2 h on ice, mixed with 1 mg of recombinant apoE3 or apoE4, and incubated for 1 h on ice. Bio-Beads (100 mg; Bio-Rad) were added to the mixture, rotated for 3 h at 4 °C, and filtered to remove beads. The mixture contained reconstituted lipoproteins. Glia-conditioned medium, plasma, or reconstituted lipoproteins were centrifuged in a SRP28SA1 rotor (Hitachi, Tokyo, Japan) at 100,000 × *g* for 72 h at 4 °C on a discontinuous sucrose gradient consisting of the following solutions: 3 ml of density 1.30 g/ml, 3 ml of density 1.2 g/ml, 3 ml of density 1.1 g/ml, and 6 ml of density 1.006 g/ml. Ten fractions (1.5 ml) were collected from the top of the gradient and immunoblotted for

apoE as described below. Fractions containing apoE were concentrated using an Amicon Ultra filter (UFC905008, Millipore, Bedford, MA). The amount of lipoproteins was adjusted for cholesterol concentration (2 μg/ml) for glia-derived E-LPs and HDL or for protein concentration (100 ng/ml) for reconstituted lipoproteins. The cholesterol and protein concentrations of lipoproteins were measured by a LabAssay cholesterol kit (Wako) and BCA protein assay kit (Thermo Fisher Scientific), respectively. α2-Macroglobulin (Sigma) was activated by treatment with 100 mM methylamine for 1 h at room temperature (18).

Immunoblotting—Immunoblotting was performed as described previously (13, 14). Proteins were separated by SDS-polyacrylamide gel electrophoresis, transferred to polyvinylidene difluoride membranes, and probed with primary and peroxidase-conjugated secondary antibodies. Immunoreactive proteins were visualized with SuperSignal West Pico or Dura (Thermo Fisher Scientific). The following primary antibodies were used: mouse anti-β-actin (a5441, Sigma), goat anti-albumin (A90-134A, Bethyl Laboratories, Montgomery, TX), goat anti-α2-macroglobulin (M5649, Sigma), goat anti-human apoE (k74190g, Meridian Life Science, Inc., Cincinnati, OH), goat anti-mouse apoE (sc-6384, Santa Cruz Biotechnology), rabbit anti-protein kinase Cδ (2058, Cell Signaling Technology, Danvers, MA), rabbit anti-GSK3β and phospho-Ser-9-GSK3β (9315 and 9336S, Cell Signaling Technology), goat anti-Brn-3a (sc-31984, Santa Cruz Biotechnology), rabbit anti-LRP1 (2703-1, Epitomics, Burlingame, CA), mouse anti-LRP1 (545503, R&D Systems, Minneapolis, MN), rabbit anti-phospholipase Cγ1 (sc-81, Santa Cruz Biotechnology), rabbit anti-NMDAR2A (AB1555P, Millipore), and mouse anti-NMDAR2B (610416, BD Biosciences).

Immunocytochemistry—Cultured RGCs were fixed in acetone for 10 min at −20 °C and then blocked with 1% bovine serum albumin and 5% goat serum in PBS for 1 h at room temperature. The cells were incubated with rabbit anti-LRP1 (Epitomics), mouse anti-NMDAR2B (32-0700, Invitrogen), or mouse anti-cytochrome *c* (556432, BD Biosciences) in PBS containing 5% goat serum for 1 h at room temperature, washed three times with PBS, and then incubated with Alexa Fluor 488-conjugated goat anti-rabbit IgG (Invitrogen), Alexa Fluor 488-conjugated goat anti-mouse IgG (Invitrogen), or Alexa Fluor 594-conjugated goat anti-mouse IgG (Invitrogen) for 1 h at room temperature. For staining of mitochondria, RGCs were incubated with 2 nM MitoTracker Red CMXRos (Invitrogen) for 30 min 1 day before the experiment. Fluorescence images were taken with an Olympus IX71 microscope or FV500 confocal microscope.

Apoptosis of RGCs—Primary cultured RGCs were washed twice (15-min incubation at 37 °C) with Hanks' balanced salt solution (HBSS; Invitrogen) containing 2.4 mM $CaCl_2$, 20 mM HEPES without magnesium. Magnesium was omitted from the washing solution to avoid blocking the NMDA receptor (19). Subsequently, RGCs were incubated ± 300 μM glutamate and 10 μM glycine, a co-activator of the NMDA receptor, in HBSS containing 2.4 mM $CaCl_2$, 20 mM HEPES without magnesium for 2 h at 37 °C. After control (HBSS containing 2.4 mM $CaCl_2$, 20 mM HEPES without magnesium) or glutamate treatment, RGCs were cultured in the same medium without forskolin, brain-derived neurotrophic factor, ciliary neurotrophic factor, and basic fibroblast growth factor for 22 h at 37 °C. For detection of apoptosis with Hoechst 33342 (Dojindo, Kumamoto, Japan), RGCs were incubated with 1 μg/ml Hoechst 33342 for 15 min. Fluorescent images (six images/well) were randomly taken using an IX71 fluorescence microscope. For each treatment, at least 12 images/two wells in 96-well plates were obtained. Fragmented or shrunken nuclei stained with Hoechst dye were counted as apoptotic neurons, and round/smooth nuclei were counted as healthy neurons. More than 300 neurons were blindly counted for each treatment. For detection of apoptosis with annexin V-EGFP apoptosis detection kit containing annexin V-EGFP, propidium iodide, and binding buffer, the manufacturer's instructions (Medical & Biological Laboratories Co., Ltd., Nagoya, Japan) were followed. Annexin V-EGFP and propidium iodide are membrane-impermeable reagents. During early stages of apoptosis, phosphatidylserine becomes exposed on the outer leaflet of the plasma membrane and is accessible to annexin V. Propidium iodide stains nuclei of necrotic cells and also end stage apoptotic cells. Healthy cells are not stained with either reagent.

Intracellular Ca^{2+}—RGCs were incubated with 3 μM Fluo-8 acetoxymethyl ester (AAT Bioquest, Sunnyvale, CA) for 30 min at 37 °C. The cells were washed twice (15-min incubation each) with HBSS containing 2.4 mM $CaCl_2$, 20 mM HEPES without magnesium and then administered 300 μM glutamate and 10 μM glycine. Fluorescent images were acquired every 500 ms using an ORCA-R2 digital CCD camera (Hamamatsu Photonics, Hamamatsu, Japan) and analyzed by the MetaFluor fluorescence ratio imaging software (Molecular Devices, Sunnyvale, CA).

RNA Silencing of Protein Kinase Cδ—Negative control small interfering RNA (siRNA) (300 nM) (Accell non-targeting pool, Thermo Fisher Scientific) or siRNA specific for protein kinase Cδ (Accell SMARTpool, E-080142-00-0005, Thermo Fisher Scientific) was added to culture medium as indicated by the manufacturer and then incubated with RGCs for 6 days. The knockdown by negative control or protein kinase Cδ siRNA was confirmed by immunoblotting.

Compartmented Culture of RGCs—RGCs in compartmented cultures were prepared as described previously (12, 20). Distal axons and cell bodies/dendrites/proximal axons of the primary neurons can be separately maintained with different media in compartmented cultures. A Teflon divider, which creates three compartments, was applied to the μ-dish (ibidi) with silicone grease. RGCs were plated in the center compartment in RGC culture medium to which were added 25 ng/ml brain-derived neurotrophic factor and 25 ng/ml ciliary neurotrophic factor. The side compartments were supplied with the same medium that also contained 75 ng/ml brain-derived neurotrophic factor, 25 ng/ml ciliary neurotrophic factor, and 50 ng/ml basic fibroblast growth factor. Axons of RGCs crossed under the silicone grease into the side compartments within 5 days. Prior to the experiments, RGCs were cultured for at least 14 days.

Intravitreal Injection of E-LPs and Collection of Vitreous Humor—*Glast*$^{+/+}$, *Glast*$^{+/-}$, or *Glast*$^{-/-}$ mice (3 weeks old) were anesthetized by intraperitoneal injection of 50 mg/kg of

sodium pentobarbital. For intravitreal injection, the vitreous of one eye was injected with 1 μl of E-LPs (1.5 μg of protein/ml) or HDL (30 μg of cholesterol/ml), and the other eye was injected with the same volume of PBS through a 33-gauge needle (Terumo, Tokyo, Japan) connected to a Hamilton syringe (Bonaduz, Switzerland). This procedure was performed under a stereomicroscope (SZX7, Olympus) so that the lens and retina were not injured. For collection of vitreous humor, the same set of needles and syringes was used as for intravitreal injection. Each sample of vitreous humor for immunoblotting was combined from 10 eyes.

Histological Studies of Retinae—Eyes from 3- and 6-week-old mice were enucleated and fixed with Super Fix (KY-500, Kurabo, Osaka) overnight at 4 °C, and the cornea and lens were removed. Retinae with sclera were embedded in paraffin. Sequentially, 4-μm paraffin sections of retina were cut through the optic nerve and stained with hematoxylin and eosin. The number of cells in the ganglion cell layer was counted from one end through the optic nerve to the other end on the retinal section. Ten sections, more than 2,000 cells, were counted in each retina.

Co-immunoprecipitation—Co-immunoprecipitation was performed according to May *et al.* (21). RGC lysates were prepared in lysis buffer containing 10 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, and 1% Triton X-100 with Complete EDTA-free protease inhibitor mixture (Roche Diagnostics, Mannheim, Germany) and PhosSTOP phosphatase inhibitor (Roche Diagnostics). The lysate was passed 15 times through a 22-gauge needle and centrifuged at 15,000 × *g* for 15 min at 4 °C. The supernatant was precleared with 40 μl of 50% equilibrated protein G-Sepharose (GE Healthcare, Buckinghamshire, UK) for 1 h at 4 °C, and then the Sepharose beads were removed by centrifugation. Rabbit anti-LRP1 (Epitomics) or rabbit anti-NR2B antibody (AB1557, Millipore) was added, and the lysate was rotated for 12 h at 4 °C. Forty μl of 50% equilibrated protein G-Sepharose were added to the lysate and rotated for 1 h at 4 °C. The Sepharose beads were washed three times with lysis buffer containing 0.1% Triton X-100. For immunoblotting, 30 μl of sample buffer were added, and the beads were boiled for 5 min. The supernatant was subjected to SDS-PAGE and immunoblotting.

Statistical Analysis—Statistical analyses were performed using one-way analysis of variance followed by Bonferroni's multiple comparison. A confidence level of >95% was considered significant ($p < 0.05$).

RESULTS

Glutamate-induced Apoptosis in Retinal Ganglion Cells—Glutamate is a major excitatory neurotransmitter and also acts as an excitatory neurotoxin in acute and chronic central nervous system disorders such as cerebral ischemia, amyotrophic lateral sclerosis, Alzheimer disease and glaucoma (15, 22, 23). However, Ullian *et al.* (24) reported that RGCs were not vulnerable to NMDA excitotoxicity. Thus, we determined whether or not glutamate induced neurotoxicity in RGCs. As shown in Fig. 1*A*, 300 μM glutamate induced fragmentation or shrinkage of nuclei stained with Hoechst dye (a marker of apoptosis) in RGCs in the presence of 10 μM glycine (*Glu*: glutamate plus

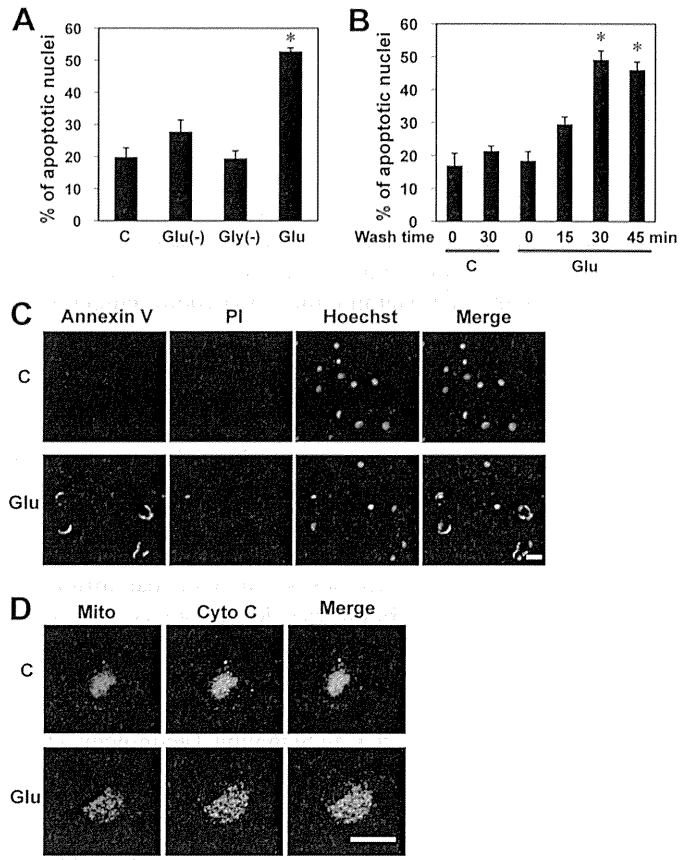

FIGURE 1. **Glutamate with glycine induces apoptosis in RGCs.** *A*, fragmented or shrunken nuclei in RGCs were detected by Hoechst staining 24 h after control (*C*; HBSS) or treatment with glutamate alone (*Glu(−)*; 300 μM glutamate), glycine alone (*Gly(−)*; 10 μM glycine), or glutamate + glycine (*Glu*; 300 μM glutamate + 10 μM glycine). Data are means ± S.E. from 4 independent experiments. *, $p < 0.001$ for control *versus* Glu. *B*, fragmented or shrunken nuclei were detected by Hoechst staining 24 h after control (HBSS) or glutamate + glycine treatment (*Glu*; 300 μM glutamate plus 10 μM glycine) with 0, 15 (one 15-min wash), 30 (two 15-min washes), or 45 min (three 15-min washes) of washing by HBSS. *C*, fluorescence images of RGCs stained with annexin V-EGFP, propidium iodide (*PI*), and Hoechst 12 h after control (HBSS) or Glu treatment (300 μM glutamate + 10 μM glycine). *Scale bar*, 20 μm. *D*, RGCs stained with 2 nM MitoTracker Red (*Mito*) were immunostained with anti-cytochrome *c* (*Cyto C*) 12 h after control or Glu treatment. *Scale bar*, 20 μm.

glycine), but neither component alone (*Glu(−)*, glutamate alone; *Gly(−)*, glycine alone) induced neurotoxicity. Thus, to induce neurotoxicity, glycine was added with glutamate as co-activator in the following experiments. Washing of the RGCs for 30 min (two 15-min washes) was required for induction of glutamate-induced neurotoxicity, but a longer washing time (45 min: three 15-min washes) did not cause further neurotoxicity (Fig. 1*B*), nor did a higher concentration of glutamate (1 mM) (data not shown). Exposure of phosphatidylserine on the cell surface after glutamate treatment was monitored as another marker of apoptosis. Annexin V-EGFP-positive and propidium iodide-negative RGCs (*i.e.* apoptotic RGCs) were observed 12 h after glutamate treatment but not without glutamate (Fig. 1*C*). Moreover, glutamate induced cytochrome *c* release from mitochondria (an additional marker of apoptosis), as indicated by less overlap of cytochrome *c* and MitoTracker Red (*i.e.* mitochondria) in fluorescence images in the presence of glutamate when compared with control (Fig. 1*D*). These