A multidisciplinary study of factors precluding native Japanese researchers from more frequent academic presentations in English, with special reference to the underlying neural substrates involved in language and cognitive function

（日本人研究者の英語での学術発表を妨げる要因の多分野解析：特に背後にある言語及び認知機能に関連する神経基盤について）

Greggory J. Wroblewski

Division of Neuroanatomy

Department of Neuroscience

Yamaguchi University Graduate School of Medicine, Japan

June 2017

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Abstract

In Japan, lack of contribution to the international academic literature in one's area of expertise is often blamed on the lack of English skills necessary to communicate the research. Nevertheless, despite a reported and conspicuous dearth of presentations by Japanese physician–researchers at international conferences (*i.e.*, in English), a comprehensive investigation to determine the causes of the phenomenon remained lacking until the present study. Here, in Part 1, we used a survey of 200 Japanese physician–researchers to identify factors that precluded more frequent participation in English-language-medium presentations. The results clearly indicated that lack of confidence in their ability to communicate their findings and field questions in English was the strongest precluding factor. Nevertheless, despite identifying a potential explanation for the lack of participation, the mechanisms responsible for the oral communication deficiency, whether differences between language types or brain differences, remain elusive. Correspondingly, in Part 2, we used functional near-infrared spectroscopy in native Japanese to compare neural correlates of phonemic fluency in both Japanese and English contexts. We found structural differences between the two languages as well as neural activity differences between "Higher" and "Lower" English proficiency individuals to be putative influencers of second language production. In the entire population, besides loci activated in the Japanese test, bilateral precentral channels were specifically recruited in the English test, and we interpreted precentral increases as the consequence of additional articulatory resource recruitment necessary for English pronunciation. When divided by English proficiency, the Higher group showed almost no increase in oxygenated hemoglobin in either language context in any of the brain areas surveyed, while participants in the Lower group showed numerous, widespread increases during both Japanese and English versions of the test. We interpreted this lack of activation in the Higher group as a possible advantage in non-verbal executive control. Finally, in a more detailed attempt to elucidate the neuroanatomy of cognition, in Part 3, we investigated distribution of huntingtin-associated protein 1 (HAP1) in rat brain through an immunohistochemical analysis of the retrosplenial and retrohippocampal areas, which lie at the very center of cognition and memory. HAP1-immunoreactive (-ir) cells were conspicuously present in four distinct zones in areas that support spatial navigation, memory and learning. Overall, our findings suggest that second language production/cognition is dependent upon but also reflects an interplay between the language-family types of the first and second language and brain differences in more highly-proficient individuals. Similarly, the neuroanatomical data on HAP1-ir cells may better determine the pathophysiological involvement of HAP1in the plausible center of cognitive function and serve as a base for elucidating the relevant cognitive processes involved in the future.

PART 1

Factors dissuading Japanese doctors from presenting more frequently at international conferences

1.Introduction

Today more than ever, scientific innovation and research is a worldwide endeavor with 218 countries producing over 1.5 million research papers in 2008 (Royal Society, 2011). And while research continues to be spread to more and more locations around the globe, collaboration across borders has continued to rise as well, with over 35% of international journal articles resulting from international collaboration compared to 25% only 15 years ago (Royal Society, 2011).

This increased collaboration brings with it a host of benefits. On a broad scale, it has a positive impact on the scientific community and the overall quality of science conducted, *e.g.*, granting increased exposure for important data and allowing access to new markets or population groups for research (Royal Society, 2011). Collaborating with colleagues from around the world can also be extremely beneficial to the individual researchers themselves in terms of career growth and the achieving of personal research objectives. One important vehicle for this collaboration is the oral presentation of research findings at international conferences during oral or poster presentations.

Oral presentations and the interactions with colleagues which follow, by their very nature, provide unique advantages for presenting one's research – thus facilitating collaboration – as well as opportunities for one's continued development in the field. These include enhanced ability to communicate one's research through the use of gestures, intonation, and other methods of non-verbal communication; the convenience of being able to answer questions or address concerns on the spot; opportunities for immediate feedback from the audience after the presentation (Graves, 2007); and chances to present data regardless of their stage in the development process (Dunn, 2008). Additionally, networking opportunities frequently present themselves after the presentation or during the rest of the conference when the presenter has a chance to mingle with the audience, make face-to-face contact with

potential collaborators – or even employers – and answer further questions in a more relaxed format (Dunn, 2008; Wallwork, 2010). Finally, conference presentations are evidence of an ongoing and active interest in research, and their inclusion can greatly enhance a CV and lead to career growth (Wallwork, 2010).

Japan continues to be a world leader in research and innovation according to a variety of indicators such as overseas patent registrations at the U.S. Patent Office (US Patent and Trademark Office, 2010) and percentage of GDP spent on research and development (R&D) (UNESCO Institute for Statistics Data Centre, 2007). More specifically, Japan was 3rd of 106 countries in number of papers published in clinical medicine journals over a ten-year span and 4th in terms of citation frequency (ScienceWatch, 2006). In light of the above, it would stand to reason that Japanese medical researchers should be highly visible advocating for their research at international meetings. Not only that, but a 2004 survey sent to over 3000 Japanese physicians regarding English education in Japanese medical schools (Kawagoe, 2004) indicated that nearly 75% of respondents thought that it was "important" or "extremely important" to feature English for Scientific Presentation courses in the medical curriculum. By implication, these results suggest that presentation of Japanese research to a broad audience via oral and poster presentations at international conferences is considered to be highly important by the Japanese medical establishment. However, the same survey showed a striking disconnect between theory and practice. When asked how often they presented their findings at international (*i.e.*, English-speaking) conferences, a quarter did so "only once every several years" and half "almost never" did.

While the number of physicians surveyed in the above study is only a fraction of the total number of physicians nationwide (295, 049) (Ministry of Health, Labor and Welfare, 2010), extrapolating these results to the greater population of doctors begs the question: Why do there appear to be so few presentations by Japanese medical researchers at international meetings despite the excellence of Japanese R&D as well as indicators suggesting the Japanese medical establishment places value on future medical students being prepared to do so?

A number of possible factors could account for the apparent low number of presentations by Japanese medical researchers, though no direct relationships have yet to be investigated or established in the current context. In terms of the current state of medical English education in Japan, research suggests that English Presentation Skills classes at the university level have historically been few and far between (Pribyl et al., 2001) and those that do are lacking on several fronts. For example, presentation skills texts in Japan have been

criticized by some for concentrating on the technological side of giving Powerpoint presentations while neglecting language features and rudimentary presentation skills (Miles, 2007). This comes despite the foundational assumption of skills training that students who lack adequate skills are anxious when faced by the prospect of giving a presentation (Phillips, 1991) and the fact that a year-long presentation skills course for sophomore English majors at a Japanese university was shown to significantly reduce public-speaking anxiety when using a systematic approach to developing a presentation theoretically linked to communication apprehension reduction techniques (Pribyl et al. 2001).

Historically the Japanese educational system is said to have underemphasized public speaking in general (Pribyl et al., 2001), and the act of speaking in front of an audience has been shown to be one of the most feared context-based apprehensions among Japanese (Nishida, 1988; Pribyl et al., 1998), even when done in a Japanese-speaking context. While this public speaking anxiety is not a uniquely Japanese phenomenon – American college students have been shown to demonstrate similar anxiety levels (Pribyl, et. al., 2001) – it is thought that the origins for said anxiety likely differ do to the fact that American students are presumed to have numerous opportunities to learn and practice presentation skills in both high school and college (Pribyl et. al, 2001), making the Japanese situation somewhat unique.

To compound matters, second language acquisition research on the whole has reported high levels of anxiety in individuals who must express themselves in a foreign language despite limited competence (McCroskey et al., 1985; Aida, 1994), and English teachers have further described Japanese learners as quiet and passive with particularly high degrees of anxiety (Doyon, 2000; Claro, 2007; Williams & Andrade, 2008).

In addition to anxiety, a lack of English language proficiency in general has been indicted in the competitive disadvantage on the global stage that Japanese scientific researchers have been said to face (Okamura, 2006; Fujii, 2007; Rodis et al., 2011). And while only anecdotal in nature, my own experiences as spouse of a Japanese physician–researcher and as an editor, translator, and friend to many of her colleagues lends credence to this assertion, especially in the context of the oral presentation. While listening to their experiences and witnessing them firsthand on several occasions, common themes have included difficulty understanding anything but "standard" English accents, occasional unintelligibly to listeners themselves, and perceptions of lack of vocabulary.

Although the preceding research hints at possible causes for a relative lack of presentations by Japanese medical researchers at international conferences, the question has yet to be addressed explicitly, and a detailed survey examining the factors that may preclude

them from doing so has yet to be conducted. Is it simply a matter of expense and time constraints, or are there affective factors at play? Are they reluctant for reasons related to English proficiency as anecdotal evidence might suggest? What perceptions seem to influence their behaviors in said context? Do responses differ according to gender, age grouping, or medical specialty?

Findings could have ramifications for English education at Japanese medical schools going forward, guide Japanese physician–researchers how to present their research more effectively, and/or provide suggestions for hospital and university administrators on how to provide maximum support for physician–researchers who want to collaborate with overseas colleagues through the presentation medium and subsequently make greater contributions to global scientific discourse in general. Accordingly, the current study centers around a questionnaire of 200 MDs from three separate facilities in Western Japan that was designed to help discern factors that influence Japanese physician–researchers' frequency of presentations to an international audience. Analysis of these factors can then potentially be used to inform medical English education in Japan going forward to better equip Japanese medical-researchers for the international stage, thus increasing their visibility and contributions at international conferences and seminars.

2. Materials and Methods

2.1 Participants

An anonymous survey on factors precluding more frequent presentations at international conferences was taken of medical doctors from the following facilities: Kokura Medical Center (KMC), Kitakyushu (N = 40), Kurume University School of Medicine and University Hospital (KU), Kurume (N = 118), and Shikoku Cancer Center (SCC), Matsuyama (N = 42). Selected background statistics for each facility (Kokura Medical Center, 2014; Kurume University Hospital, 2014; Shikoku Cancer Center, 2014) can be found in Table 1. These particular hospitals were chosen A) because of professional associations between staff members and the authors and B) to enable responses from diverse facility types (a general hospital, university hospital, and cancer center, respectively). All of the participants surveyed were medical doctors (MDs) or MD/PhDs. The survey itself was formulated in English (Figure 1) and then translated into Japanese for distribution (Figure 2) by one of the authors at each respective facility. At KMC and SCC, hard copies were printed and made available at

a monthly hospital-wide staff meeting. At KU, the questionnaire was forwarded via e-mail to all departments, and each department head was asked to distribute a hard copy questionnaire at his/her respective regular staff meeting if possible. Completed forms were collected at the end of each meeting.

2.2 Materials

The survey was divided into two sections. The first section deals with the number of presentation experiences and the existence of any previous English presentation skills training. The second section consisted of six items requiring "level of agreement" responses using a Likert 5-point scale. Survey items were based on implications from the existing literature, *e.g.* educational and cultural factors as well as mundane considerations such as travel expenses and workload that might affect the decision to attend and present at an international conference. An "Other" line allowed for open-ended responses to the question of precluding factors. Items for age, gender, and department were also included to allow for comparative analysis between groups.

2.3 Data analysis

For comparative analysis, data were analyzed according to A) respondent population overall, B) gender, C) age group (those under the median age and those above), and D) "department category." The department category groupings used were *surgical* (patient care including surgery, *e.g.* obstetrics & gynecology or orthopedics), *non-surgical* (patient care without performing surgery, *e.g.* internal medicine or psychiatry), and *lab work focus* (rarely seeing patients, *e.g.* physiology or hematology). Statistical analysis was performed via the chi-square test and results with $p < 0.05$ were deemed statistically significant.

3. Results

Selected data can be found in Table 1, and for simplicity's sake, the median age of 39.5 will be rounded to "40" from this point forward. Answers in the "G) Other (Please be specific)" field were translated into English and are shown in Figure 3. Particularly relevant findings are outlined below:

a) The majority of those surveyed had little to no experience presenting to an international audience, with 36% having never done so and 66% having presented 3 times or less. When asked if they had ever taken an English presentation skills-type

course in preparation for a career in research, only 6% responded in the affirmative. For both categories, there were no significant differences between groups.

b) When asked which factors discouraged more frequent delivery of poster or oral presentations at international conferences (see Figure 4), the lowest level of agreement was in response to the statement "I don't think presenting at international conferences is necessary/important," with only 6% showing any level of agreement.

c) As a whole, the greatest level of agreement was to the statement "I'm not confident in my ability to communicate/field questions in English," with 68% expressing some level of agreement and 34% strongly agreeing. There was also a significant difference by age group, with 82% of those under 40 expressing some level of agreement, but only 61% of those over 40 ($P = 0.003$).

d) For the general population, there was also a high level of agreement to the statement "Associated expenses (airfare, lodging, etc.) are too high (*i.e.* exceed research budgets)" (58%). There was a significant difference by gender, with 67% of males expressing some level of agreement compared to 31% of females ($P = 0.0003$), and those over 40 were more likely to agree than those under 40 (66% and 54%, respectively, $P = 0.04$).

Table 1: Selected background data and survey results.

a. Institutional statistics

Total doctors: KMC = 81, KU = 541, SCC = 90

2013 research output (MedicalOnline*): KMC – 32, KU – 403, SCC – 94

2013 research output (Pubmed**): KMC = 3, KU = 242, SCC = 26

b. Survey: General

Total respondents: 200

Respondents by gender: M = 145, F = 38, unspecified = 17

Age: average = 41.2, mean = 39.5

Respondents by category: surgical = 60, non-surgical = 85, basic research = 40, unspecified = 17

Number of career presentations: 0 = 36%, 1-3 = 30%, 4-6 = 16%, 7-9 = 7%, 10+ = 13%

Respondents having taken an English presentation skills course: 6%

c. Agreement with statements describing precluding factors (avg. out of 5)

I don't think presenting at international conferences is necessary / important: 1.7

I'm too busy with work and job responsibilities to attend such conferences: 3.1

Associated expenses (airfare, lodging, etc.) are too high (i.e. exceed research budgets): 3.5

I'm not confident in my ability to communicate my results / field questions in English: 3.8

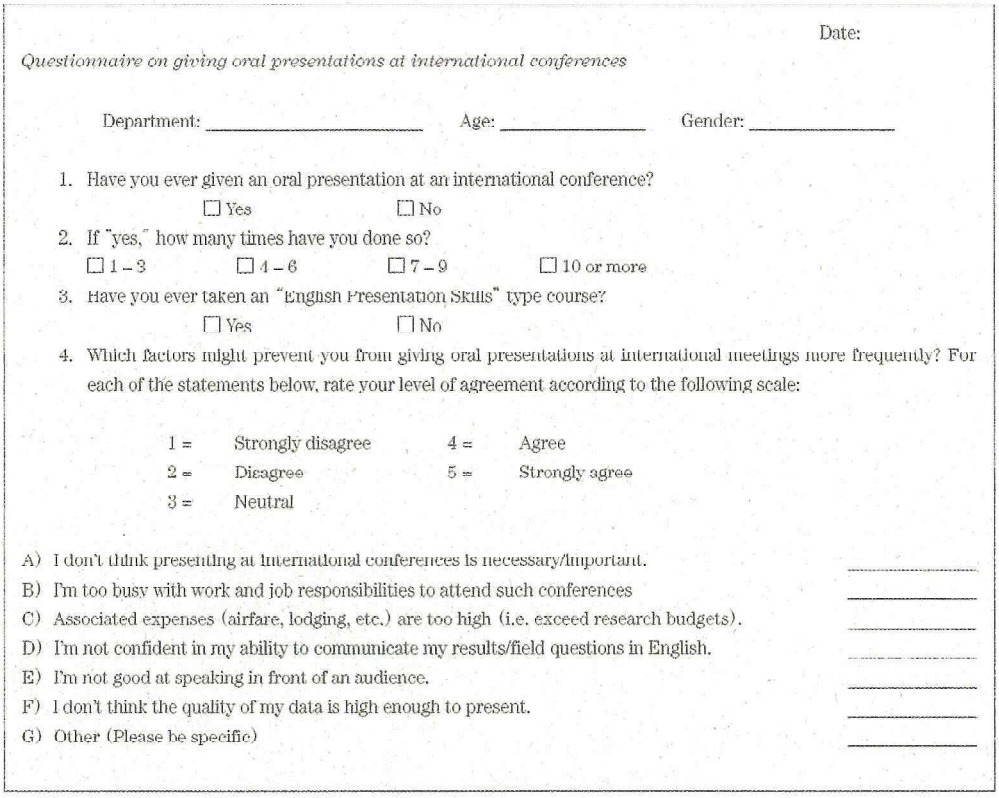
I'm not good at speaking in front of an audience: 2.8

I don't think the quality of my data is high enough to present: 3.1

* Includes both journal articles and conference abstracts in Japanese.

** Includes journal articles in English. Does not include conference abstracts.

Figure 1: English text which served as the basis for the translated Japanese questionnaire



Date:

Questionnaire on giving oral presentations at international conferences

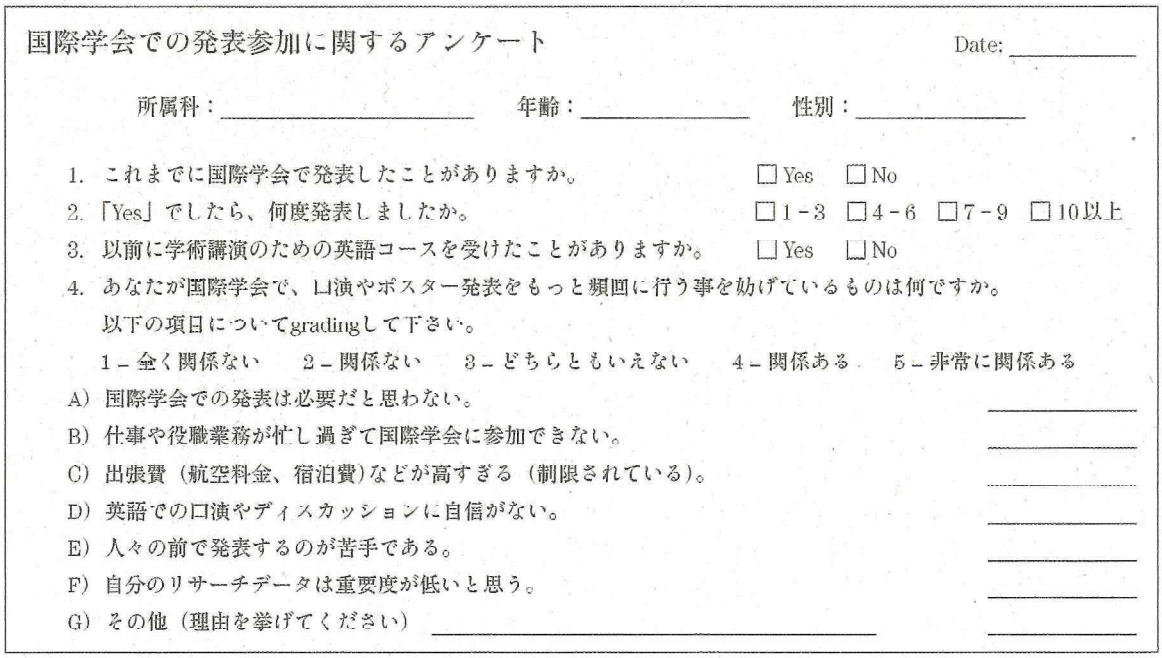
Department: _____ Age: _____ Gender: _____

1. Have you ever given an oral presentation at an international conference?
   ☐ Yes ☐ No
2. If "yes," how many times have you done so?
   ☐ 1 – 3 ☐ 4 – 6 ☐ 7 – 9 ☐ 10 or more
3. Have you ever taken an "English Presentation Skills" type course?
   ☐ Yes ☐ No
4. Which factors might prevent you from giving oral presentations at international meetings more frequently? For each of the statements below, rate your level of agreement according to the following scale:

   1 = Strongly disagree 4 = Agree
   2 = Disagree 5 = Strongly agree
   3 = Neutral

A) I don't think presenting at international conferences is necessary/important. _____
B) I'm too busy with work and job responsibilities to attend such conferences _____
C) Associated expenses (airfare, lodging, etc.) are too high (i.e. exceed research budgets). _____
D) I'm not confident in my ability to communicate my results/field questions in English. _____
E) I'm not good at speaking in front of an audience. _____
F) I don't think the quality of my data is high enough to present. _____
G) Other (Please be specific) _____

Figure 2: Japanese questionnaire as distributed to the participants



国際学会での発表参加に関するアンケート Date: _____

所属科：_____ 年齢：_____ 性別：_____

1. これまでに国際学会で発表したことがありますか。 ☐ Yes ☐ No
2. 「Yes」でしたら、何度発表しましたか。 ☐ 1 – 3 ☐ 4 – 6 ☐ 7 – 9 ☐ 10以上
3. 以前に学術講演のための英語コースを受けたことがありますか。 ☐ Yes ☐ No
4. あなたが国際学会で、口演やポスター発表をもっと頻回に行う事を妨げているものは何ですか。
   以下の項目についてgradingして下さい。
   1 – 全く関係ない 2 – 関係ない 3 – どちらともいえない 4 – 関係ある 5 – 非常に関係ある

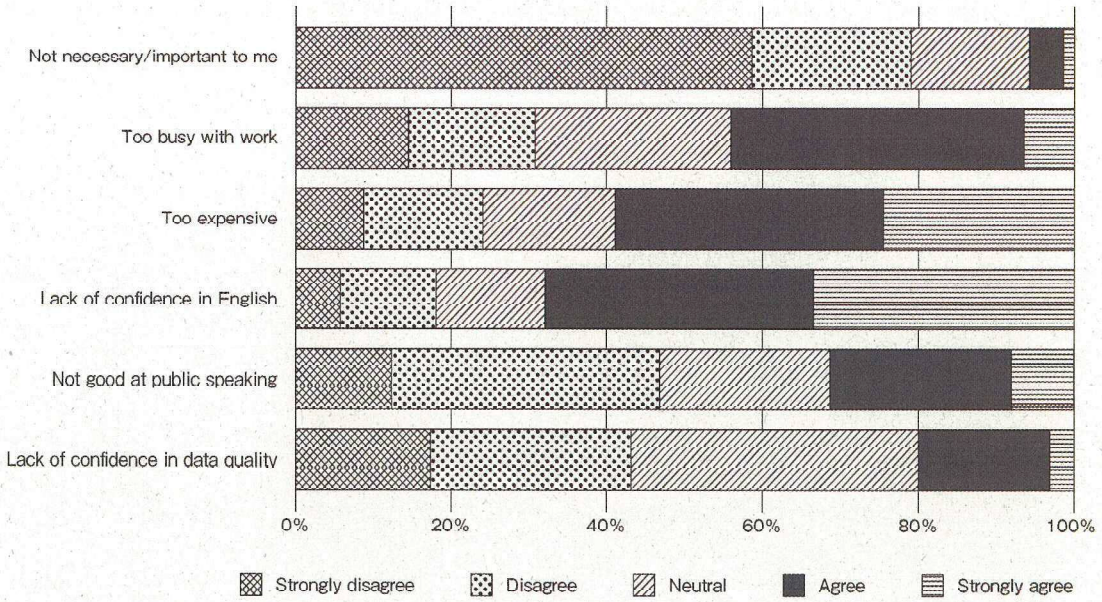
A) 国際学会での発表は必要だと思わない。 _____
B) 仕事や役職業務が忙し過ぎて国際学会に参加できない。 _____
C) 出張費（航空料金、宿泊費）などが高すぎる（制限されている）。 _____
D) 英語での口演やディスカッションに自信がない。 _____
E) 人々の前で発表するのが苦手である。 _____
F) 自分のリサーチデータは重要度が低いと思う。 _____
G) その他（理由を挙げてください） _____ _____

Figure 3: "Other" factors precluding more frequent oral presentations

- "Believe it or not, going to international meetings doesn't always come across in a positive light. I think sometimes it's perceived as nothing more than an opportunity to get away from the pressures of work and go sightseeing. For me, if going didn't have this kind of baggage attached to it, I'd probably try to go and present two or three times next year."
- "For the same expense, presenting at domestic meetings the same number of times is looked upon more favorably by your co-workers, other departments, and hospital administration."
- "I don't feel right leaving behind patients in the middle of ambulatory care, especially the bad ones, for the other doctors to have to tend to."
- "I don't have any chances to interact with foreign doctors on a regular basis."
- "I don't really have any opportunity to do so."
- "If given the chance, I'd like to present more often."
- "I haven't been accepted to present yet."
- "I know that it'd be much easier for me personally if my employer helped more with business trip expenses. I do think though that presenting at international meetings boils down to how motivated you are, but if you're not good at the sort of discussion with foreign doctors that's required, it's a real chore."
- "It is difficult for us Japanese to understand Indian doctors' talk at international meetings in Asia. However, we need to understand them because they will have a substantial power in the future."
- "Because of obligations with domestic conferences, it's hard to fit them into my schedule."
- "Leaving means saddling my co-workers with extra work."
- "My going entails more work for the people I leave behind."
- "Not only is the travel expensive, but so are the annual membership fees for professional associations."
- "Personal reasons"
- "The data I'd like to present just doesn't seem to be coming together."
- "There are already too many domestic conferences I have to attend."
- "Time and money constraints, etc."
- "To me, the current rate at which I present is good enough."
- "We have a shortage of staff for handling outpatient treatment."
- "With the economy being what it is, paying for sightseeing and eating out after the meetings is not as easy as it used to be."

Responses in the "G) Other (Please be specific) field were translated into English and compiled.

Figure 4: Factors precluding more frequent presentations (N = 200)



4. Discussion

4.1 Prior experience and coursework

Prior experience items indicated that roughly two-thirds of those surveyed had presented at international conferences 3 times or less over the course of their entire careers. The relative lack of presentations agrees with the findings of a survey of nearly 3,000 doctors in which roughly three-quarters of respondents did so "only once every several years" or "almost never" did (Kawagoe, 2004). And while one might reasonably expect that younger, less experienced doctors be disproportionately represented in this category simply because their older colleagues had had more opportunities over the years, in the current study this was not the case. There was no significant difference between those above and below 40. As a preliminary finding, this seems to suggest that factors other than age were responsible for limiting presentations at international conferences.

The fact that only 6% of total respondents reported having taken an English presentation skills course in the past dovetails with observations by those such as Pribyl *et al.* (2001) suggesting a relative lack of English Presentation Skills university courses in general, and especially for medical researchers historically in Japan.This does, however, seem to be slowly changing as indicators exist that teaching presentation skills in English for Specific Purposes classes is on the increase at Japanese universities as educators recognize the need for applying those skills in both educational and workplace contexts (Ministry of Health, Labor, and Welfare, 2010).

4.2 Major precluding factors

4.2.1 Perceived importance to one's career

In the current survey, the lowest level of agreement was in response to the statement "I don't think presenting at international conferences is necessary/important," with only 6% expressing any agreement whatsoever. This finding seems to reflect the importance of presenting in the minds of the Japanese medical establishment at large, as demonstrated in a survey of nearly 3,000 doctors in which roughly three-quarters of them considered that including English for Scientific Presentation courses in medical school curricula was "important" or "extremely important" (Kawagoe, 2004). By implication, this most likely means that the application of said training at international meetings is also considered to be

valued highly by the Japanese medical establishment. However, as described in 4.1, roughly two-thirds of those surveyed in the current study had presented 3 times or less. At least for this sample, there appears to be a striking disconnect between theory and practice. Consequently, it seems unlikely that low participation frequency is a function of any perceived irrelevance in the minds of Japanese doctors.

4.2.2 Lack of confidence in English ability

While there have been some accounts suggesting Japanese doctors experience language-related anxiety when presenting their research in English (Guest, 2013), this appears to be the first study demonstrating how such a lack of confidence in a sizable and varied sample group could be the most significant factor when many of them decide whether or not to present. While the source of this hesitation is still unclear, one possibility is the basic framework of English education in Japanese medical schools. For example, Kawagoe's (2004) broad survey on the current state of English education in medical and nursing schools around Japan revealed that only around 20% of English study overall was spent on "speech/presentation" or "English conversation (medical)" These numbers seem comparatively small, especially in light of the fact that nearly one-third of class time was still being spent on general English conversation and listening skills work. Furthermore, according to the same study, shortages of English teachers in general and English-speaking foreign staff were reported, with almost half of those universities surveyed reporting a complete lack of field-specific English staff. And in contexts where they were indeed present, nearly 40% of staff were native Japanese speakers (Japanese L1) only (Kawagoe, 2004). These data suggest that many Japanese medical schools may lack the specialist staff necessary to prepare medical students to engage in data presentation and discussion in English with confidence.

While age did not seem to play a role in presentation frequency (see 4.1), comparative analysis did yield a significant difference by age group, with doctors under 40 being less confident than those over 40 in presenting data and fielding questions in English. This could be at least partially attributable to the fact that older doctors have likely been speaking English and engaging in public speaking longer, with the corresponding confidence and desensitization that often accompanies repetition. For this reason, it would seem even more crucial that medical students receive as much practice as possible in English presentation

before their careers truly begin and they become comparatively busy.

4.2.3 Economic, cultural, and sociological factors

While perceived lack of English skills may have been the strongest precluding factor, it was not the only one, and this multiplicity demonstrates the complex background that must be considered when examining the low participation rate of Japanese doctors in presentations at international conferences.

For example, a substantial number of total respondents expressed concern over the cost of attending and presenting at international conferences. According to one doctor, all three of the facilities surveyed provide some form of monetary assistance for travel expenses related to giving a presentation – whether through direct reimbursement or through individual research grants. However, when taking into account annual membership fees to the medical associations themselves, meals, and the requisite souvenirs for co-workers left behind, there can still be a significant out-of-pocket expenditure for the doctor involved (personal communication, June 4, 2014), possibly dissuading some from making such a trip.

In addition to a substantial concern expressed on the whole, there were also significant differences between groups. Interestingly, male doctors were more than twice as likely to report monetary concerns than females. In light of the strict gender roles that are said to still prevail in many Japanese families (Nakatani, 2006; North, 2009; Ministry of Health, Labor, and Welfare (2010), it is possible that female doctors who are married are more likely to belong to dual income households – and presumably less concerned with supplementing travel and conference costs out-of-pocket – than male doctors who are married. Also, the increasing age of marriage that has been reported for women in Japan in recent years (Nakatani, 2006; North, 2009) could also mean more expendable income for a longer period for single female doctors.

Those over 40 were also more inclined to worry about expense than their younger counterparts. While data is currently lacking, this could be attributed to the fact that doctors over 40 are more likely to be married and/or have children with the ensuing financial burden that entails, leaving less money to cover conference-related expenses that exceed their research budgets.

Perceptions of being too busy to prepare for and attend such conferences differed by gender, departmental category, and age, with males, surgeons, and those over 40 feeling comparatively constrained. First regarding a difference by gender, the aforementioned perception is at least partially substantiated in a recent study by Nakamura (2012) in which male physicians in Japan on average where shown to work roughly 4.5 hours longer per week than their female colleagues (47.5 and 43.0 per week, respectively). When considering differences by department category, one of those surveyed suggested that surgeons may indeed be busier than their colleagues, since multiple doctors are required to care for a single patient during surgeries that can often last hours (personal communication, March 20, 2013). Finally, regarding a difference by age, the discrepancy could be explained by the fact that the older the doctor, the more likely s/he is to be married and/or have children, limiting the amount of time after work available for writing abstracts, preparing slides, and so on.

Admittedly, economic, cultural, and sociological considerations are probably outside the purview of pedagogically-minded English for medical purposes (EMP) professionals. Nevertheless, these findings do demonstrate the complex background against which Japanese doctors have to make their decisions.

4.2.4. Other affective factors

While ranking lower than English proficiency, expense, and time considerations on the level-of-agreement scale, a number of respondents nonetheless agreed that both a lack of interesting data and public speaking itself were also concerns when it came to presenting more. First regarding the former, the level of agreement (22%) in itself is not overly striking, especially when compared to the aforementioned factors more commonly agreed with by participants. However, this seemingly low level of concern over inability to assemble worthwhile data, combined with the fact that there was no significant difference between department categories for this item, suggests that reticence to present internationally likely was not based simply on an inability to conduct research due to one department's relative emphasis on "research" over "patient care" compared to another. This result implies that, for the current study at least, one's department category is less responsible for dissuading would-be presenters than other factors.

Public-speaking anxiety in Japan has been well-documented, and the findings of the present study (31% agreement) dovetail with past research. Historically, the Japanese educational system is said to have underemphasized public speaking in general (Pribyl et al., 2001) and the act of speaking in front of an audience is thought to be one of the most feared context-based apprehensions in Japan, even when done in Japanese (Nishida, 1998; Pribyl et al., 1998). Specifically, said anxiety could be attributed to fewer opportunities to learn and practice presentation skills in high school and college than in countries like the U.S. (Pribyl et al., 2001). These studies as well as the current findings suggest that any attempt to increase the number of English presentations by Japanese physician–researchers should consider affective obstacles as well as linguistic.

5. Implications and conclusions

Since the sample size for the current study is admittedly small (N = 200) and each facility is representative of a distinct geographic location with its own unique circumstances, extrapolating to a national scale must be done cautiously. Additionally, though tracking age, the current survey made no provision for respondents' position title. Further research may benefit from comparative analysis between professors and assistant professors, doctors and senior doctors, etc. Finally, while just under half of the doctors at KMC and SCC took the survey, less than one-quarter did at KU. This is most likely due to the fact that distribution and collection at KU was conducted separately by dozens of department heads, all with varying responsibilities and varying levels of free time available for conducting a voluntary survey. For this reason, future questionnaires might benefit from expanded and effective distribution through web-based tools such as SoGoSurvey (2014) that can send e-mail invitations for an online survey from an imported list of e-mail addresses thus ensuring that each doctor receives an invitation and can make a personal choice of whether or not to participate pate in the survey. Regardless of its limitations, the major finding of this study – that lack of confidence in English seems to dissuade potential presenters from giving presentations at international conferences more than any other factor – has several implications for EMP professionals in Japan and curriculum planners at Japanese medical schools. Admittedly, changes to the basic framework of English language education in Japan or revised curricula can be seen as long-term goals at best. However, in light of the fact that

so few of those surveyed have had regular chances to give presentations, there are a few steps that any instructor who works with medical students or physician–researchers could use now to increase the experience and confidence level of one such learner:

a) As is the case here at our institution, graduate schools of medicine often employ graduate students or post-docs from outside of Japan who speak English as a second or foreign language and use it as a lingua franca while doing research. These researchers frequently present their findings in on-campus seminars or PhD dissertation defenses, and medical students at the same campus can be encouraged to attend their lectures. While the level of English will almost certainly be high, providing our students with the researcher's written work in advance may serve to activate schema to facilitate the comprehension process. Attendance at these events can be viewed as part of a slow acclimatization process to "presentation language" as well as the kinds of questions that are asked in an English oral presentation setting. As an added bonus, such foreign researchers can serve as role models who have demonstrated ability to advocate for their research successfully using English despite it not being their L1.

b) Since medical school students may have few real-world opportunities to present their research in English, EMP teachers and administrators should encourage or organize the formation of "English Journal Clubs" or similar outlets that meet once a week and simulate the experience of a biomedical presentation context in English. Besides providing further occasion to read journal articles in English and become familiar with their writing conventions, repeated attempts at presenting might also serve to further desensitize students to any generalized public-speaking anxiety. Even if students mainly participate during the first three years of their education while they are comparatively free, such an outlet would provide numerous opportunities for practice over a six-year program.

c) For those of us who serve as advisors to hospital clinical research departments or work with basic researchers, there are also ways to address this issue for those who have already begun their medical careers. For example, journal clubs likely already exist in some form in hospital departments or graduate schools of medicine, albeit in Japanese. Even if one weekly meeting per month was devoted to an English

presentation instead, opportunities to practice oral presentation in English would add up considerably over a doctor's career.

Presentations, and the personal interactions that follow, provide unique opportunities for a researcher. These include enhanced ability to communicate through the use of gestures, intonation, and other methods of non-verbal communication, the convenience of being able to answer questions or address concerns on the spot (Graves, 2007), opportunities for immediate feedback from the audience after the presentation, and chances to present data regardless of their stage in the development process (Dunn, 2008). Additionally, networking opportunities frequently present themselves after the presentation when the presenter has a chance to mingle with the audience, potential collaborators, or even potential employers. Finally, conference presentations are evidence of an ongoing and active interest in research, and their inclusion can greatly enhance a CV and lead to career growth (Wallwork, 2010). When taken into account together with Japan's relative lack of poster and oral presentations at international biomedical conferences despite world-class research, these factors should serve as strong motivation to improve the preparation of medical school students and doctors – both linguistically and affectively – for presenting their research findings orally to an expanded audience going forward.

While the current study (Part 1) was successful in identifying a potential explanation for the lack of participation, it does not concretely address the mechanisms or influences that may be responsible for the oral communication difficulty attributed to the Japanese physician–researchers surveyed. Though deficiencies in the Japanese educational system or cultural factors might indeed be the culprits as discussed above, these hypotheses are difficult to test in an empirical sense, and are therefore beyond the purview of the current study. Accordingly, in Part 2, we set out to test the influence of two parameters on second language production which are readily measurable and especially salient in the current context: the effects of both language-type and second language proficiency on the neural correlates of second language production. How are the neural correlates during a highly-constrained verbal task affected by the language used (*i.e.,* Japanese vs. English), and how do the brains of native Japanese who are highly-proficient English language learners differ from those of less proficient ones?

PART 2

Effects of task language and second language proficiency on the neural correlates of phonemic fluency in native Japanese speakers: a functional near-infrared spectroscopy study

1.Introduction

In countries where it is not the native language, English proficiency often represents a major expenditure of time and resources for students and professionals alike. In Japan, compulsory study extends from elementary to high school (Tahira, 2012; Tada, 2016) and high weight is placed on the English component of university entrance examinations (Ikegashira et al., 2009). Many degree programs require English courses for graduation, and English proficiency is believed to be highly correlated with analytical and logical processing abilities (Butler & Iino, 2005). Particularly in the sciences and academia, English level can also be a major contributor (or obstacle) to career growth: in Japan, lack of contribution to the international literature in one's area of expertise is often blamed on the lack of English skills necessary to communicate the research (Fujii, 2007; Okamura, 2006). Indeed, in our previous study (Wroblewski, et al., 2014; see Part 1), perception of English deficiency was the primary factor dissuading Japanese physician–researchers from presenting more frequently at international conferences. This perception is at least partially supported by data in which Japanese test-takers score near the bottom in international rankings of certain standardized English tests (Educational Testing Service, 2016a). While communicative competence in a foreign language admittedly depends upon a number of factors (see Alptekin, 2002 for a review), English pronunciation seems to present a unique challenge to native Japanese, with phonological differences between first (L1) and second (L2) languages making speech production especially taxing (see Ohata, 2004 for a review). However, despite noted dissimilarities between the two language systems and the increasing necessity of English speech production in a Japanese context, the neural processes underlying said differences are not well-established. Likewise, how varying degrees of L2 proficiency are reflected in neuroimaging during verbal tasks remains unclear.

Japanese and English belong to two distinct language families, mora-based and alphabet-syllable-based languages, respectively, and the cognitive processes underlying

certain word production tasks are thought to be influenced by the unique orthographical and phonological systems of the respective language type (see Dan et al., 2013 and Sumiyoshi et al., 2014 for reviews). Behaviorally, studies with schizophrenic patients have suggested that phonemic fluency task performance (PFT, also known as a "FAS test") (Strauss et al., 2006) may be language-dependent, with Japanese speakers displaying greater deficits than those using alphabet-syllable-based languages such as English (Suga et al., 2011; Sumiyoshi et al., 2014). Besides performance, the neural correlates underlying such tasks may also be language-dependent. Measuring cortical activation patterns during a PFT in healthy Japanese subjects and comparing them with previously published studies conducted using alphabet-syllable-based languages, Dan et al. (2013) reported Japanese-specific bilateral supramarginal activation.

Activation patterns during word production may also be influenced by the existence and extent of bilingualism. The last decade has seen a host of studies suggesting a "bilingual advantage" (see Bobb et al., 2013 for a review) in which important cognitive benefits are associated with bilingualism. Relative to monolinguals, bilinguals are reported to have advantages in tasks involving executive control, such as those requiring the need for the suppression of distracting information (Bialystok et al., 2006; Colzato et al., 2008) or conflict resolution (Bialystok et al.,2004; Carlson & Meltzoff, 2008; Costa et al., 2008). Said advantage may extend to verbal tasks as well, with bilinguals outperforming monolinguals on a PFT when vocabulary level was controlled for (Bialystok et al., 2008; Luo et al., 2010) and one report of high-vocabulary bilinguals outperforming both monolinguals and low-vocabulary bilinguals (Luo et al., 2010). Performance differences may also correspond to unique brain states, as suggested in studies involving the use of PFTs in combination with different neuroimaging modalities (*e.g.*, Kuwabara et al., 2006; Grogan et al., 2009).

PFTs are standard components in several neuropsychological batteries for assessing cognitive impairment in clinical conditions such as aphasia (Spreen & Benton, 1969; Benton et al., 1994), dementia (Katoh et al., 1991), and schizophrenia (Keefe et al., 2004). In addition, they have also been used to investigate basic cognitive processes such as executive control (*e.g.*, Luo et al., 2010; Fitzpatrick et al., 2013) and verbal abilities such as lexical knowledge and retrieval (*e.g.*, Federmeier et al., 2002; 2010). In recent days, these tasks have often been used in conjunction with neuroimaging to investigate the neural underpinnings of a variety of psychiatric disturbances (*e.g.*, Matsuo et al., 2000, 2007; Takizawa et al., 2008). Nevertheless, despite the PFT's status as a benchmark diagnostic tool and the previously-mentioned studies hinting at language- and proficiency-dependent activation, language

background is not currently screened for in most neuropsychological batteries that incorporate PFTs, and the applicability of their data across populations with disparate language backgrounds remains an important question.

Likewise, the effects of language of administration and extent of bilingualism on the test's underlying neural correlates are not completely understood. While Dan et al. (2013) attempted to identify differential neural mechanisms sub-serving the Japanese PFT, their conclusions were based on data from Japanese speakers that were contrasted with a literature review of studies from countries representing several different alphabet-syllable-based languages. The heterogeneity of the latter group might increase the probability that confounding variables such as culture, age, or socioeconomic status (SES) would account for differences with the Japanese cohort. While another study used only Chinese–English bilinguals to compare PFT activation patterns in two similarly disparate languages (Shi et al., 2014), data were limited to the frontal cortex and therefore unable to elucidate contributions from other cortical regions implicated in the task such as temporal (*e.g.*, Grogan et al., 2009; Birn et al., 2010; Tupak et al., 2012) or inferior parietal (Dan et al., 2013; Heinzel et al., 2013) areas.

Regarding "proficiency-dependent activation" during word production in unequal multilinguals, most research to this point seems to have focused on comparisons between one's more proficient and less proficient language(s) (*e.g.*, Vingerhoets et al., 2003; Briellmann et al., 2004; Shi et al., 2014;); *i.e.*, essentially, comparisons of L1 and L2 language activation patterns. In contrast, comparatively few studies have truly examined the neural correlates of varying L2 proficiency by using a "higher/lower" grouping paradigm in the same L2 and juxtaposing thusly. And those that have done so have not typically involved word generation: *e.g.*, word-reading (Stein et al., 2009), listening comprehension/discourse processing (Reiterer et al., 2005; 2009), or semantic or syntactic anomaly detection (Wartenburger et al., 2003; Ojima et al., 2005).

In response to the aforementioned gaps in the current literature, we set out to investigate language- and proficiency-dependent cortical activation during PFTs. To measure cortical activity, we chose functional near-infrared spectroscopy (fNIRS). NIRS is a relatively recent, non-invasive neuroimaging modality that uses near-infrared light (Boas et al., 2004; Strangman et al., 2002a) to monitor changes in oxygenated hemoglobin ([oxy-Hb]) in micro-blood vessels on the brain surface that serve as indicators of cortical activity (Hoshi et al., 2001; Strangman et al., 2002b; Obrig & Villinger, 2003). NIRS has a history of combined use with PFTs in healthy subjects (Herrmann et al., 2003; Kakimoto et al., 2009;

Schecklmann et al., 2010; Dan et al., 2013) and has numerous advantages over other neuroimaging techniques (see Dieler et al., 2012 for a review), including lower cost of operation, quick implementation, and more accurate approximation of real-life conditions (*e.g.*, data collection from a seated position). To minimize confounding variables such as socioeconomic status (SES) and culture cited by critics of the current bilingual advantage research (*e.g.*, Morton, 2015; Paap et al., 2016), all participants were native Japanese-speaking medical students at a single university – and thus largely homogenous in terms of age, education level, and native culture – who were rigorously screened by trained personnel for psychiatric disorders or neurological diseases. And because lack of objective measures of L2 proficiency has drawn criticism (Morton, 2015) in similar studies, we utilized the Test of English for International Communication (TOEIC) (Educational Testing Service, 2016b), which all subjects had previously taken as part of their curriculum.

Thus, our current objectives were to (1) compare functional changes in oxygenated hemoglobin during a PFT in both Japanese and English and (2) investigate the effects of L2 proficiency in a native-Japanese cohort. Because of the purported orthographical and phonological differences between language types mentioned above, we hypothesized that the Japanese task would elicit differential and more numerous cortical activations due to more effort-intensive word production. In addition, due to reduced cognitive burden on similar tasks thought to be inherent to bilinguals, we hypothesized that the higher English proficiency group would display less widespread activity during the English task.

2. Materials and methods

2.1 Participants

Thirty-seven right-handed, healthy volunteers (19 females, 18 males, mean age ±SD: 22.7 ±2.6 years, range: 20–33 years) participated in the study. All were native Japanese-speaking students at Yamaguchi University Faculty of Medicine, Japan. Each had taken the TOEIC Listening and Reading test at least once, and in cases of multiple sittings, most recent score was used for statistical analysis (mean score: 738.9 ±89.9, range: 620–980). Handedness was assessed by means of the Edinburgh Inventory (Oldfield, 1971). Participants were screened via questionnaire, and any with neurological illness, history of traumatic brain injury with loss of consciousness, alcohol or drug abuse or any physical illness such as hepatitis, brain tumor or epilepsy were excluded from the study. On the day of fNIRS measurement, subjects

were further screened for psychiatric illness in a structured interview by a senior psychiatrist (K.H.) using the Mini-International Neuropsychiatric Interview (MINI., Japanese version 5.0.0; Otsubo et al., 2003) and excluded if they had first- or second-degree relative(s) with a history of psychiatric disorders. Participants were also asked to complete the Beck Depression Inventory, second edition (BDI-II, Japanese version; Kojima et al., 2002), Social Adaptation Self-evaluation Scale (SASS, Japanese student version, Goto et al., 2005), State-Trait Anxiety Inventory (STAI - Form JYZ; Hidano, 2000), Two-factor Index of Social Position for determining socioeconomic status (SES) (Hollingshead, 1965), and the World Health Organization Quality of Life (WHOQOL 26, Japanese version; Tazaki & Nakane, 1997) survey. This study was approved by the Institutional Review Board of Yamaguchi University Hospital and written informed consent was obtained from all subjects after a complete description of the study was provided.

2.2 Activation tasks

For each subject, relative changes in [oxy-Hb] were measured during two PFTs – one in Japanese (JPN) and one in English (ENG). The tasks were conducted separately but during the same sitting, one immediately after the other, with order alternated randomly. Preliminary instructions and practice exercises were provided in audio/video form in the corresponding language (*i.e.*, Japanese guidance during JPN, English during ENG). Each task included a 30-s pre-task baseline period, a 60-s word production period comprising three 20-s blocks, and a 70-s post-task baseline period. During the baseline periods, participants were asked to vocalize either the five Japanese or five English vowels in order and repeatedly. During the word production period, participants were instructed to produce as many words as possible for a given Japanese mora/English letter. Each word production period used three morae/letters (e.g., /a/, /ki/, /ha/ for JPN and /c/, /f/, /l/ for ENG), with three different sets alternated randomly. Words were recorded on a digital recorder, and repeats as well those words inflected for tense or number based on an earlier word were excluded when calculating total number of words as the measure of task performance.

2.3 Criteria for L2-proficiency groupings

Here, L2 proficiency was assessed through TOEIC score. The TOEIC Listening and Reading test has been used by nearly 14,000 organizations in around 150 countries as a common global standard for measuring English skills (Educational Testing Service, 2016b). Score reports (ranging from 10–990 points) are utilized by companies and educational institutions, and a minimum score is frequently set as a condition of employment or criterion for graduation, respectively. While the Reading and Listening test does not directly assess the other two skills (speaking and writing), studies have correlated Listening and Reading test scores with proficiency levels designated by the Common European Framework Reference (CEFR) for languages (Tannenbaum & Wylie, 2004, 2005, 2013; Educational Testing Service, 2011), whose score descriptors include detailed "can do" statements that also include speaking and writing ability. Therefore, it is possible to use the TOEIC Listening and Reading test as one measure of comprehensive English ability.

Participants were divided into two groups based on their TOEIC score: a "higher (HIGH)" group (N = 7) and a "lower (LOW)" group (N = 30), having scores of ≥ 785 and < 785, respectively. This (785) is the minimum "cut score" often set by institutes of higher education and international companies (Educational Testing Service, 2011) and also serves as the minimum score for the "Independent user – Vantage (B2)" designation when mapped onto the CEFR (Educational Testing Service, 2011; Tannenbaum & Wylie, 2013). Those who score between 785–900 are said to be "able to satisfy most requirements with language that is often, but not always, acceptable and effective," and those scoring above 900 have the "ability to communicate effectively in almost any situation." On the other hand, those scoring between 605–780 are said to have the "ability to satisfy routine social demands and limited work requirements" (Educational Testing Service Canada Inc., 2016). Altogether, in light of the above descriptors and the present participants' high average score, we chose 785 for proficiency grouping purposes.

2.4 fNIRS

Details of the fNIRS system have been described in our previous studies (*e.g.*, Matsubara et al., 2014; Watanuki et al., 2016). Briefly, we used a continuous wave NIRS system (ETG-4000, Hitachi Medical Co., Japan) to monitor relative changes in [oxy-Hb] concentration during activation tasks. As in previous studies (*e.g.*, Takizawa et al., 2008; Matsubara et al., 2014), frontal, temporal, and parietal areas were measured in a total of 52 channels (Figure 1)

and were anatomically identified by a virtual registration method with automated anatomical labelling (Tzourio-Mazoyer et al., 2002) which enables registration of fNIRS channel positions in the standard brain space (Tsuzuki et al., 2007).

2.5 Statistical analysis

2.5.1 Behavioral data

Performance results for JPN and ENG in the entire experimental population (N = 37) were compared via paired t-test (two-tailed). Performance and behavioral variables were also compared for the two TOEIC score groupings (HIGH and LOW): sex distribution was analyzed by Pearson Chi-square test and the remaining variables were compared via Mann-Whitney U test. We used SPSS Statistics version 20 for Windows (IBM, Chicago, IL) for all analyses. Differences were considered significant at $p < 0.05$.

2.5.2 fNIRS

We used mean [oxy-Hb] change during tasks as an outcome measure for statistical analyses, as [oxy-Hb] during fNIRS is considered to reflect the activation of gray matter in the brain (Sato et al., 2013). For in-group analyses, mean [oxy-Hb] for each channel was compared using a one sample t-test (two-tailed) against zero. All analyses were subjected to Bonferroni correction.

3. Results

3.1 Behavioral data

Behavioral data for the entire population as well as HIGH and LOW groups can be seen in Table 1. When measuring performance for the entire population, subjects generated significantly more words in the JPN task (mean 18.4 ±4.9) than in the ENG task (mean 16.6 ±4.1) ($p = 0.045$). For HIGH and LOW groups, apart from the expected significant difference in average TOEIC score, none of the other demographic, mental state, or behavioral categories demonstrated significant differences.

3.2 fNIRS data

3.2.1 Language-dependent effects (Japanese vs. English) on cortical activation patterns

When measuring the entire cohort (N = 37) (Figure 2), significant increases in [oxy-Hb] were found in 23 channels on the left hemisphere and 21 channels on the right for JPN (total = 44) and 25 channels (left) and 23 channels (right) during ENG (total = 48). For JPN (Figure 2A), channels with significantly increased [oxy-Hb] were found bilaterally throughout all the frontal areas, the superior and middle temporal areas, the postcentral area, and the supramarginal area, with significant increases in [oxy-Hb] in the precentral area on the left hemisphere only. In addition to areas in JPN, ENG (Figure 2B) also produced significant increases in the precentral area bilaterally. In total, six channels displayed task-specific increases. Only one channel demonstrated a JPN-specific increase (#3, corresponding to right middle frontal) while five channels did so during ENG: #2, #23 (right precentral), #4 (right middle frontal), #10 (left surpramarginal), and #31 (left postcentral).

3.2.2 L2-proficiency-dependent effects on cortical activation patterns

For $HIGH_{JPN}$ (HIGH group during the JPN task) (Fig. 3A), only one channel (#40, inferior frontal, triangular part) demonstrated significantly increased [oxy-Hb], while LOW_{JPN} (Fig. 3B) did so on a much more widespread basis: 18 channels on the left hemisphere and 19 channels on the right (Total = 37). Overall, these corresponded to the superior, middle and inferior frontal areas, superior and middle temporal areas, and supramarginal areas bilaterally, with right lateralization present in the pre- and postcentral areas. Channel 40 was the only one with increase in both groups during JPN. For $HIGH_{ENG}$ (Fig. 3C) also, there were no channels with significantly increased [oxy-Hb], but once again LOW_{ENG} (Fig. 3D) resulted in many instances: 24 channels on the left hemisphere and 21 channels on the right (Total = 45). For the LOW group, in addition to areas found during JPN, ENG resulted in significantly increased [oxy-Hb] bilaterally in both the pre- and postcentral areas.

Table 1: Demographic and behavioral data

	Total (N = 37)	"HIGH" TOEIC (N = 7)	"LOW" TOEIC (N = 30)	p-Value
Age (years)	22.68 (2.60)	22.71 (2.93)	22.67 (2.56)	0.719
Sex (M/F)	18/19	3/4	15/15	0.734
Years of education	15.46 (2.04)	16.14 (3.02)	15.30 (1.76)	0.556
TOEIC score	738.91 (89.92)	899.26 (60.58)	701.50 (40.28)	< 0.001
PFT performance				
English	16.60 (4.08)	19.14 (3.76)	16.00 (3.97)	0.160
Japanese	18.38 (4.91)	21.14 (4.95)	17.73 (4.75)	0.128
BDI	2.84 (3.18)	2.14 (2.79)	3.00 (3.28)	0.482
SASS	42.24 (6.30)	42.29 (4.92)	42.23 (6.65)	0.835
SES	13.30 (2.79)	15.00 (3.87)	12.90 (2.38)	0.227
STAI				
State	42.95 (5.50)	41.00 (5.83)	43.40 (5.42)	0.330
Trait	48.08 (4.63)	45.29 (4.86)	48.73 (4.41)	0.065
WHOQOL (overall)	8.30 (1.49)	8.29 (1.11)	8.30 (1.58)	0.719

All data are represented as means (SD). Sex distribution was analyzed by Chi-square test while all other group differences were compared via Mann-Whitney U test. Differences were considered significant at $p < 0.05$. Note that the significant difference in TOEIC score between groups is a direct result of the grouping system used. TOEIC, Test of English for International communication; PFT, phonemic fluency task; BDI, Beck Depression Inventory; SASS, Social Adjustment Scale, student version; SES, socioeconomic status; STAI, State-Trait Anxiety Inventory; WHOQOL, World Health Organization Quality of Life.

Table 2: Spatial profiles of channel with significantly increased [oxy-Hb] in phonemic fluency tasks in Japanese and English (N=37)

Channel	MNI coordinates *x, y, z*	Macroanatomical label	Broadmann Area
Left			
7	-30, 41, 44 (8)	Middle frontal G	9 – Dorsolateral prefrontal C
8	-46, 23, 44 (9)	Middle frontal G	9 – Dorsolateral prefrontal C
9	-57, -1, 43 (10)	Precentral G	6 – Pre-motor and supplementary motor C
10	**-63, -25, 42 (10)**	**Supramarginal G**	**2 – Primary somatosensory C**
16	2, 60, 32 (7)	Superior frontal G (medial part)	10 – Frontopolar A
18	-42, 42, 32 (8)	Middle frontal G	46 – Dosolateral prefrontal C
19	-55, 18, 31 (9)	Inferior frontal (triangular part) G	44 – Pars opercularis part of Broca's A
20	-64, -8, 31 (10)	Postcentral G	43 – Subcentral A
21	-66, -34, 30 (10)	Supramarginal G	2 – Primary somatosensory C
27	-13, 68, 20 (6)	Superior frontal G	10 – Frontopolar A
28	-35, 58, 20 (6)	Middle frontal G	46 – Dorsolateral prefrontal C
29	-52, 36, 19 (7)	Inferior frontal G (triangular part)	45 – Pars triangularis Broca's A
30	-62, 10, 20, (9)	Inferior frontal G (opercular part)	6 – Pre-motor and supplementary motor C
31	**-67, -17, 19 (10)**	**Postcentral G**	**22 – Superior temporal G**
37	2, 69, 8 (7)	Superior frontal G (medial part)	10 – Frontopolar A
38	-24, 68, 9 (5)	Superior frontal G	10 – Frontopolar A
39	-44, 53, 6 (6)	Middle frontal G	39 – 46 Dorsolateral prefrontal C
40	-57, 28, 7 (8)	Inferior frontal G (triangular part)	45 – Pars triangularis Broca's A
41	-63, -2, 6 (9)	Superior temporal G	48 – Retrosubicular A
42	-69, 27, 1 (9)	Middle temporal G	21 – Middle temporal G
48	-13, 72, -3 (4)	Middle frontal G (orbital part)	11 – Orbitofrontal A
49	-35, 63, -4 (4)	Middle frontal G (orbital part)	10 – Frontopolar A
50	-51, 45, -6 (5)	Inferior frontal G (orbital part)	46 – Dorsolateral prefrontal C
51	-57, 14, -8 (11)	Superior temporal pole	38 – Temporopolar A
52	-68, -11, 12 (7)	Middle temporal G	21 – Middle temporal G
Right			
1	65, -28, 42 (10)	Supramarginal G	40 – Supramarginal G part of Wernicke's A
2	**60, -3, 43 (10)**	**Precentral G**	**6 – Pre-motor and supplementary motorC**
3	*48, 23, 44 (8)*	*Middle frontal G*	*9 – Dorsolateral prefrontal C*
4	**33, 41, 44, (8)**	**Middle frontal G**	**9 – Dorsolateral prefrontal C**
11	67, -36, 30 (10)	Supramarginal G	40 – Supramarginal G part of Wernicke's A
12	66, -10, 31 (9)	Postcentral G	12 – Subcentral area
13	58, 16, 21 (9)	Inferior frontal G (opercular part)	44 – Pars opercularis part of Broca's A
14	44, 41, 32 (7)	Middle frontal G	46 – Dorsolateral prefrontal C
15	24, 58, 32 (7)	Superior frontal G	9 – Dorsolateral prefrontal C
22	68, -19, 18 (9)	Superior temporal G	22 – Superior temporal G
23	**64, 8, 20 (8)**	**Precentral G**	**6 – Pre-motor and supplementary motorC**
24	54, 36, 20 (7)	Inferior frontal G (triangular part)	45 – Pars triangularis Broca's A
25	37, 57, 20 (6)	Middle frontal G	46 – Dorsolateral prefrontal C
26	15, 68, 21 (6)	Superior frontal G	10 – Frontopolar A
32	71, -29, 2 (9)	Superior temporal G	21 – Middle temporal G
33	66, -4, 5 (9)	Superior temporal G	48 – Retrosubicular A
34	59, 27, 8 (8)	Inferior frontal G (triangular part)	45 – Pars triangularis Broca's A
35	47, 52, 7 (6)	Middle frontal G	46 – Dorsolateral prefrontal C
36	27, 68, 9 (5)	Superior frontal G	10 – Frontopolar A
43	69, -13, -10 (7)	Middle temporal G	21 – Middle temporal G
44	60, 11, -8 (9)	Superior temporal pole	38 – Temporopolar A
45	53, 43, -6 (5)	Inferior frontal G (orbital part)	46 – Dorsolateral prefrontal C
46	38, 63, -4 (4)	Middle frontal G (orbital part)	10 – Frontopolar A
47	15, 71, -3 (4)	Superior frontal G (orbital part)	11 – Orbitofrontal A

The most likely MNI coordintates are shown with standard deviation (SD) in mm. Macroanatomical and Brodmann estimations are based on AAL (Tzourio-Mazoyer et al., 2002) and MRIcro (Rorden & Brett, 2000), respectively, and macroanatomical labels with highest probability are listed for each channel. Channels written in plain text displayed significantly increased [oxy-Hb] during both JPN and ENG tasks. Channels indicated in bold did so only during the ENG, while italicized channels did so only during the JPN task. Due to the nearly equivalent probabilities for channel 9 (0.507463 for "Postcentral" and 0.492357 for "Precentral"), another macroanatomical atlas (LBPA40, Shattuck et al., 2008) was consulted to confirm that channel 9 most likely corresponds to "Precentral" (LBPA40 probability: 0.79). All channels were significant at $P<0.001$. Abbreviations; G, gyrus; C, cortex; A, area.

Table 3: Spatial profiles of channel with significantly increased [oxy-Hb] in phonemic fluency tasks for LOW group only (N=30)

Channel	MNI coordinates *x, y, z*	Macroanatomical label	Broadmann Area
Left			
8	-46, 23, 44 (9)	Middle frontal G	9 – Dorsolateral prefrontal C
9	**-57, -1, 43 (10)**	**Precentral G**	**6 – Pre-motor and supplementary motorC**
10	**-63, -25, 42 (10)**	**Supramarginal G**	**2 – Primary somatosensory C**
16	2, 60, 32 (7)	Superior frontal G (medial part)	10 – Frontopolar A
18	-42, 42, 32 (8)	Middle frontal G	46 – Dosolateral prefrontal C
19	-55, 18, 31 (9)	Inferior frontal (triangular part) G	44 – Pars opercularis part of Broca's A
20	-64, -8, 31 (10)	Postcentral G	43 – Subcentral A
21	-66, -34, 30 (10)	Supramarginal G	2 – Primary somatosensory C
27	-13, 68, 20 (6)	Superior frontal G	10 – Frontopolar A
28	-35, 58, 20 (6)	Middle frontal G	46 – Dorsolateral prefrontal C
29	-52, 36, 19 (7)	Inferior frontal G (triangular part)	45 – Pars triangularis Broca's A
30	**-62, 10, 20, (9)**	**Inferior frontal G (opercular part)**	**6 – Pre-motor and supplementary motorC**
31	**-67, -17, 19 (10)**	**Postcentral G**	**22 – Superior temporal G**
37	2, 69, 8 (7)	Superior frontal G (medial part)	10 – Frontopolar A
38	-24, 68, 9 (5)	Superior frontal G	10 – Frontopolar A
39	-44, 53, 6 (6)	Middle frontal G	46 – Dorsolateral prefrontal C
40	-57, 28, 7 (8)	Inferior frontal G (triangular part)	45 – Pars triangularis Broca's A
41	-63, -2, 6 (9)	Superior temporal G	48 – Retrosubicular A
42	-69, 27, 1 (9)	Middle temporal G	21 – Middle temporal G
48	**-13, 72, -3 (4)**	**Middle frontal G (orbital part)**	**11 – Orbitofrontal A**
49	-35, 63, -4 (4)	Middle frontal G (orbital part)	10 – Frontopolar A
50	-51, 45, -6 (5)	Inferior frontal G (orbital part)	46 – Dorsolateral prefrontal C
51	-57, 14, -8 (11)	Superior temporal pole	38 – Temporopolar A
52	-68, -11, 12 (7)	Middle temporal G	21 – Middle temporal G
Right			
1	65, -28, 42 (10)	Supramarginal G	40 – Supramarginal G part of Wernicke's A
2	*60, -3, 43 (10)*	*Precentral G*	*6 – Pre-motor and supplementary motor C*
4	33, 41, 44, (8)	Middle frontal G	9 – Dorsolateral prefrontal C
11	67, -36, 30 (10)	Supramarginal G	40 – Supramarginal G part of Wernicke's A
12	66, -10, 31 (9)	Postcentral G	12 – Subcentral area
13	58, 16, 21 (9)	Inferior frontal G (opercular part)	44 – Pars opercularis part of Broca's A
14	44, 41, 32 (7)	Middle frontal G	46 – Dorsolateral prefrontal C
22	**68, -19, 18 (9)**	**Superior temporal G**	**22 – Superior temporal G**
23	**64, 8, 20 (8)**	**Precentral G**	**6 – Pre-motor and supplementary motorC**
24	54, 36, 20 (7)	Inferior frontal G (triangular part)	45 – Pars triangularis Broca's A
25	37, 57, 20 (6)	Middle frontal G	46 – Dorsolateral prefrontal C
26	15, 68, 21 (6)	Superior frontal G	10 – Frontopolar A
32	71, -29, 2 (9)	Superior temporal G	21 – Middle temporal G
33	66, -4, 5 (9)	Superior temporal G	48 – Retrosubicular A
34	59, 27, 8 (8)	Inferior frontal G (triangular part)	45 – Pars triangularis Broca's A
35	47, 52, 7 (6)	Middle frontal G	46 – Dorsolateral prefrontal C
36	27, 68, 9 (5)	Superior frontal G	10 – Frontopolar A
43	69, -13, -10 (7)	Middle temporal G	21 – Middle temporal G
44	60, 11, -8 (9)	Superior temporal pole	38 – Temporopolar A
45	53, 43, -6 (5)	Inferior frontal G (orbital part)	46 – Dorsolateral prefrontal C
46	38, 63, -4 (4)	Middle frontal G (orbital part)	10 – Frontopolar A
47	**15, 71, -3 (4)**	**Superior frontal G (orbital part)**	**11 – Orbitofrontal A**

The most likely MNI coordinates are shown with standard deviation (SD) in mm. Macroanatomical and Brodmann estimations are based on AAL (Tzourio-Mazoyer et al., 2002) and MRIcro (Rorden & Brett, 2000), respectively, and macroanatomical labels with highest probability are listed for each channel. Channels written in plain text displayed significantly increased [oxy-Hb] during both JPN and ENG tasks. Channels indicated in bold did so only during the ENG, while italicized channels did so only during the JPN task. Due to the nearly equivalent probabilities for channel 9 (0.507463 for "Postcentral" and 0.492357 for "Precentral"), another macroanatomical atlas (LBPA40, Shattuck et al., 2008) was consulted to confirm that channel 9 most likely corresponds to "Precentral" (LBPA40 probability: 0.79). All channels were significant at $P<0.001$. Abbreviations; G, gyrus; C, cortex; A, area.

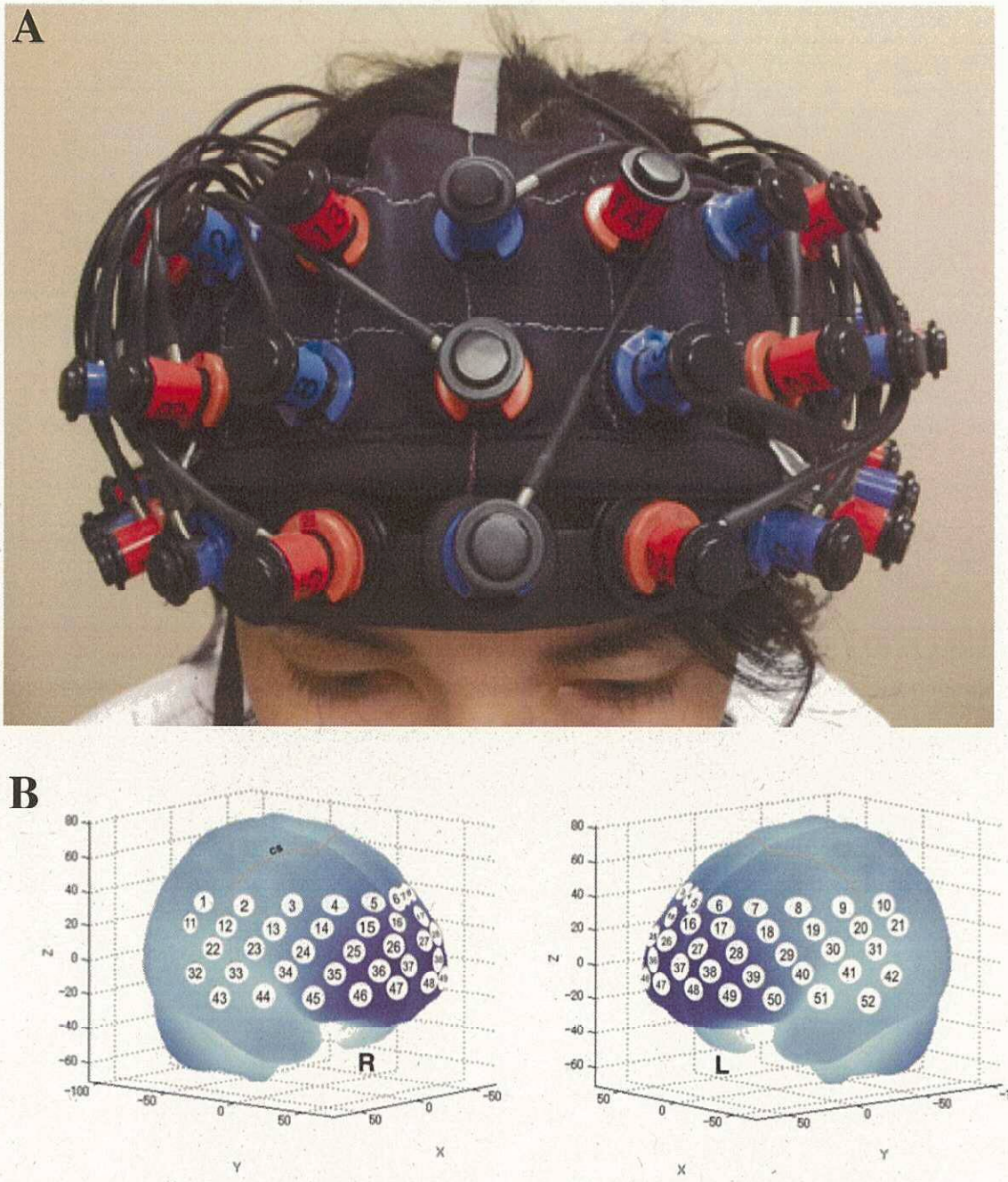


Figure 1: Location of probes in near-infrared spectroscopy (A): probe setting with 3 × 11 thermoplastic shells in a frontal view. (B): 2-D topographic map showing estimated cortical regions using the virtual registration method in right-anterior and left-anterior views. R, right; L. left; cs, central sulcus.

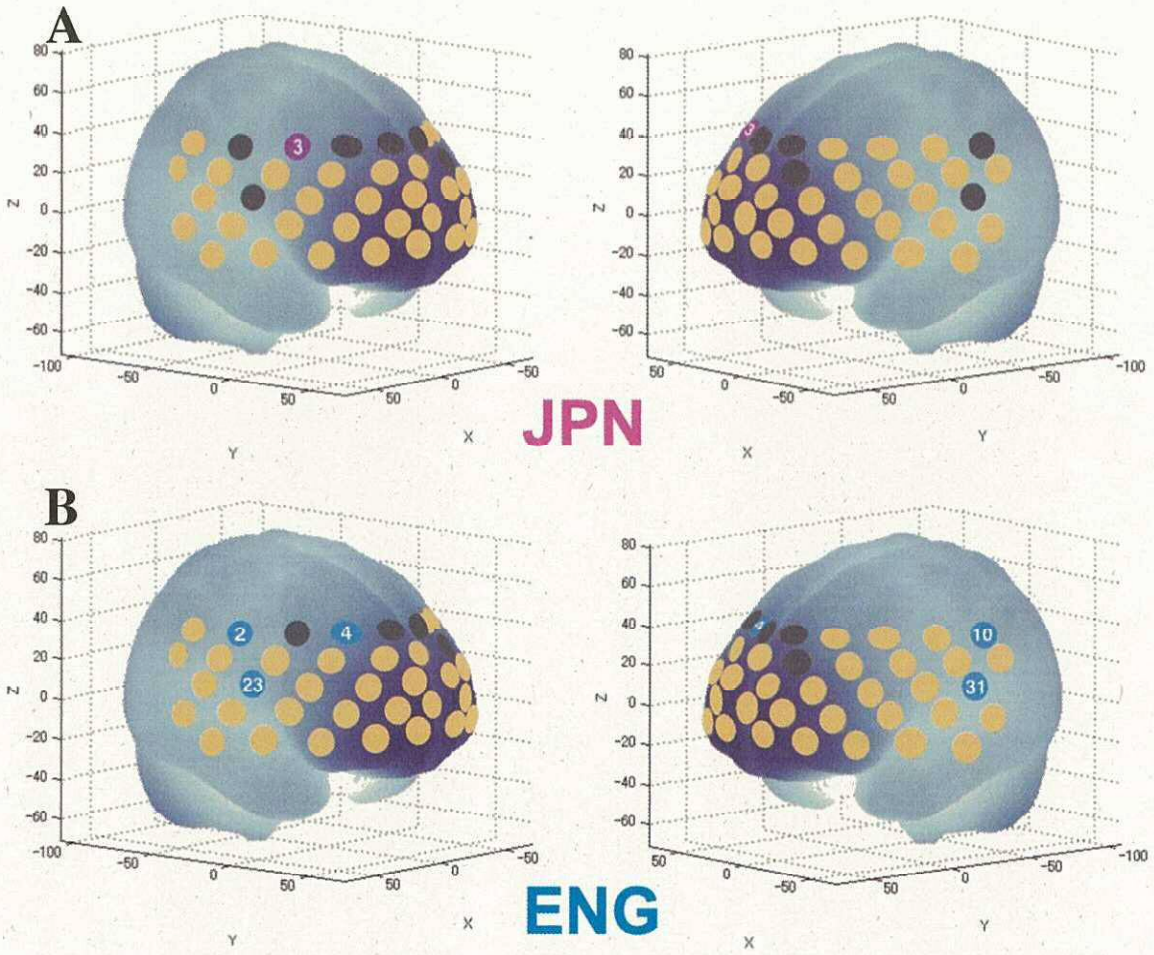


Figure 2: Results of fNIRS analyses during phonemic fluency tasks. Gray circles indicate channels without significantly increased [oxy-Hb], while orange circles indicate those with significant increases during both tasks. Purple circles in (A) represent channels with JPN-specific increases while blue circles in (B) indicate ENG-specific ones. Note that four additional channels were activated during the ENG task, and while precentral area increases during the JPN task were limited to the left hemisphere, the ENG task elicited increases on the right side (#2, 23) as well ($p < 0.001$, Bonferroni-corrected). R, right; L, left.

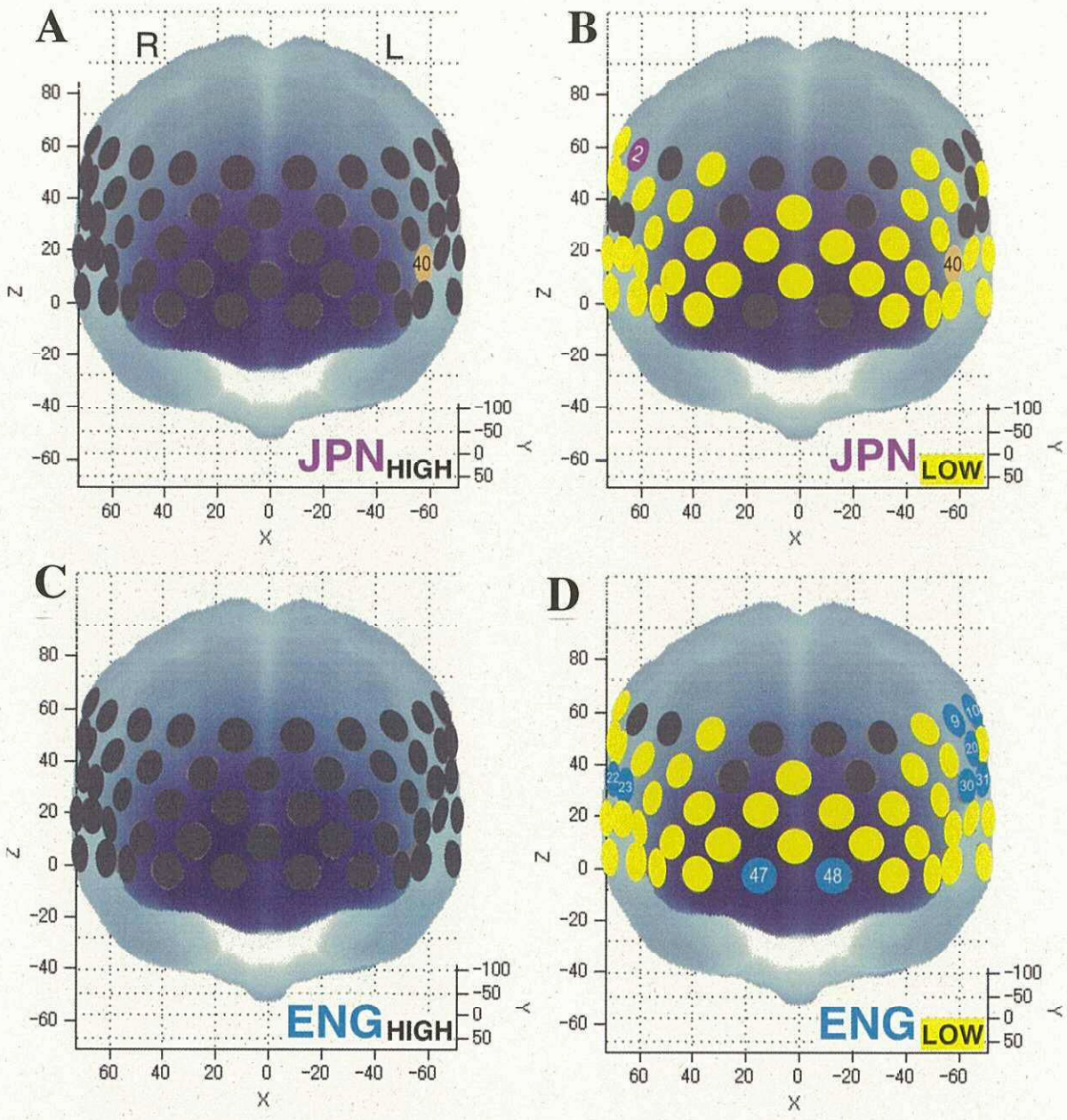


Figure 3: Comparison of channels with significantly increased [oxy-Hb] by proficiency group during JPN (A, B) and ENG (C, D) tasks. A, C represent the HIGH group while B, D represent the LOW. Gray circles indicate channels without significantly increased [oxy-Hb], while orange circles indicate those with significant increases in both groups. Yellow circles represent channels with significantly increased [oxy-Hb] in the LOW group only. Purple circles indicate JPN-specific channels while blue represent ENG-specific ones. Note the near complete absence of activations in the HIGH group, regardless of task language, and the widespread activations in the LOW group, regardless of task language ($p < 0.001$, Bonferroni- corrected). R, right; L. left.

4. Discussion

4.1 Differential activation patterns for L1 and L2 phonemic fluency tasks in native Japanese

The current study is the first attempt to compare the neural correlates underlying PFTs in the mora-based language, Japanese and the alphabet-syllable-based language, English via fNIRS at the same time in a single, healthy bilingual population of comparatively homogenous age, education level, SES, and native culture. Overall, both tasks invoked left superior lateralization, slightly more prominent in ENG. For JPN, observed activation patterns were very similar to those demonstrated by Dan et al. (2013) in their attempt to establish canonical reference data via fNIRS for verbal fluency tasks in healthy Japanese while here also demonstrating left lateralization and bilateral activation of the precentral and middle frontal areas, respectively.

In contrast, activation during ENG was more widespread than during JPN in the current study: exclusive right precentral activation was present, with additional channels in the left supramarginal, left postcentral, and right middle frontal areas also recruited to accomplish the L2 task. This is consistent with other studies that found increased cortical activity when verbal tasks were conducted in a bilingual or multilingual's less proficient language(s) (*e.g.*, Vingerhoets et al., 2003; Briellmann et al., 2004; Shi et al., 2014). Moreover, here subjects generated significantly fewer words on average during ENG. Our findings run somewhat contrary to the suggestion that PFTs in Japanese may require more intensive cognitive processing than analogous tasks in alphabet-syllable-based languages like English because of fundamental differences in their writing systems and, correspondingly, variations in the respective search strategies used to produce words (Sumiyoshi et al., 2004, 2014; Suga et al., 2011; Dan et al., 2013). Indeed, one fNIRS study (Dan et al., 2013) mentioned additional recruitment of the supramarginal area during the Japanese task due to the additional cognitive demand imposed by the orthographical and phonological features of the Japanese language. However, in the current study, both tasks demonstrated bilateral activation of the supramarginal area. Our contrasting findings may have resulted from methodological considerations, as participants in the current study were largely homogenous demographically and were stringently screened to mitigate any affects from psychiatric disorders. The lack of JPN-specific supramarginal activation, the presence of more widespread cortical recruitment observed during the ENG task overall, and the ENG performance deficit may collectively indicate that ENG was more cognitively demanding

than JPN instead of vice-versa in the current study. This suggests that the JPN task is not uniquely or inherently more difficult to perform than those in orthographically and phonologically dissimilar languages such as English.

The more widespread cortical recruitment during ENG above included ENG-specific bilateral activation of the precentral area. The precentral area in humans corresponds to the primary motor cortex, more than half of which is associated with motor activity of the tongue, lips, larynx, and hands (Patestas & Gartner, 2016). Lesions in sub-regions involving articulator movements can result in certain forms of dysarthria (Halpern & Goldfarb, 2013), and the precentral gyrus of the insula has been reported as the primary shared area of damage in chronic stroke patients demonstrating apraxia of speech (Dronkers, 1996). In addition, disruption of speech attributed to interference with the motor production of words can be induced by electrical stimulation of the ventral portion of the precentral gyrus (Petrides, 2014), and significant or differential precentral activation has been observed in speech disorders such as stuttering (*e.g.*, Braun et al., 1997; Toyomura et al., 2011).

Indeed, the precentral area has been implicated in a number of tasks involving the various sub-processes thought to underlie speech articulation: planning, motor programming, articulator movement initiation, and coordination of the timing and direction thereof (Hillis et al., 2004). In a study of patients with articulatory planning deficits, Dronkers (1996) suggested that the precentral gyrus may serve as a "preliminary processor to the initiation of articulatory movements," and after both overt and covert reading of words and pseudo-words resulted in precentral activation, Hagoort et al. (1999) concluded that activation during the silent task particularly was suggestive of internal articulatory preparation. Similarly, precentral activation has been observed occurring prior to vocalization in one syllable production task (Bouchard et al., 2013). Regarding initiation and regulation, the bilateral precentral gyrus is reported to correspond to an area of the motor/premotor network active in speech articulation, including that which Brown et al. (2008, 2009) localized as the primary motor area of the larynx and of the tongue. And correspondingly, bilateral activation has indeed been demonstrated in monosyllable or bi-syllable articulation (Riecker et al., 2000), tongue (Riecker et al., 2000; Bookheimer et al., 2000; Brown et al., 2008) and lip movement (Brown et al., 2008) contrasted with rest, as well as orolaryngeal motor tasks (Braun et al., 1997). Regarding bilinguals specifically, L2 speakers have demonstrated higher levels of activation in areas generally associated with the preparation and planning of motor activity – including the bilateral precentral gyrus – during a foreign language reading task (Rüschemeyer et al., 2005).

The precentral area has frequently demonstrated activation during PFTs in a number of studies and languages (*e.g.*, Heim et al., 2008; Birn et al., 2010; Heinzel et al., 2013), and in light of the important roles it plays in speech production seen above, it is not entirely surprising that bilateral activation was exclusive to ENG in the current study. Certain aspects of English pronunciation are said to be especially problematic for native Japanese (see Ohata, 2004 for a review). A disparity in available vowel sounds – 5 in Japanese and around 15 in English – suggests that Japanese would have difficulty producing English ones with no Japanese equivalent (Vance, 1987). Specifically, vowel distinctions made by a change in tongue positioning between the five "front" and five "back" vowels of English may be particularly problematic for Japanese, who are unaccustomed to making more than two distinctions in the front and back of the mouth (Ohata, 2004). In addition, the distinction between "lax" and "tense" vowel pairs, present in English and distinguished by the amount of muscle tension or movement in the mouth (Ladefoged, 1982), is absent in Japanese. Finally, the number of consonants available during Japanese speech are significantly less than those for English (Kenworthy, 1987; Avery & Ehrlich, 1992;). Collectively, these distinctions suggest a significant challenge in English pronunciation for native Japanese, implying the need for substantial articulatory resources to compensate during word generation.

Nevertheless, it still possible that ENG-dependent precentral activation in the current study instead reflected its role in cognitive functions unrelated to the articulatory role we hypothesized. For example, while there was no obvious occasion for it during the current tasks, precentral activation has been reported in studies involving bilingual voluntary language switching tasks (Abutalebi et al., 2008; Hernandez, 2009; de Bruin et al., 2014; De Baene et al., 2015). The precentral area has also been implicated in automatic speech tasks (Braun et al., 1997; Bookheimer et al., 2000; Riecker et al., 2000; Ma et al., 2014). However, in light of the established importance of the precentral area vis-à-vis the motor aspects of speech and unique motor challenges posed to Japanese speakers of English, it is reasonable that ENG-specific bilateral recruitment may have reflected an increased cognitive demand for resources relating to the preparation, initiation, and regulation of speech-related motor tasks during English word production.

4.2 L2-proficiency-dependent cortical activation during both ENG and JPN tasks

To the best of our knowledge, the current study is the first to use a "higher/lower" L2-grouping paradigm in combination with fNIRS to investigate proficiency-dependent effects

during a second language PFT. Furthermore, the current experimental design also permitted a novel investigation of L2-proficiency effects on cortical activation during an L1 PFT as well. Though the span of activations for the LOW group differed by task language, determination of language-dependent differential activation within a lower proficiency group was not one of the objectives of the current study and will not be discussed here.

While performance differences between groups did not reach significance for either task, activation patterns were strikingly different between the HIGH and LOW groupings for both tasks. First, while significant activations were observed in more than 85% of channels for LOW_{ENG}, they were completely and conspicuously absent for $HIGH_{ENG}$. In a few studies, L2-proficiency groupings have indeed demonstrated recruitment of distinct or additional cortical areas during language-related tasks. In an fMRI study of Italian–German bilinguals, the high proficiency group showed greater activation in a number of areas during a grammatical judgement task while both groups demonstrated differential activation in mutually exclusive areas under a semantic condition (Warternburger et al., 2003). Increased activity in the high group, which differed from the present results, may be attributable to the different neuroimaging modalities, activation tasks, and languages used. In contrast, in a similar semantic task using event-related brain potentials (ERP) in native Japanese speakers, the high (English) proficiency group, but not the low, demonstrated hemispheric asymmetry at anterior sites (Ojima et al., 2005). While differing from the current study methodologically, both of these results provide a general precedent for the current data, in which L2-proficiency-based grouping influenced the number and kind of brain areas activated during ENG.

More widespread or increased activation during PFTs has often been interpreted as reflecting increased difficulty or cognitive demand associated with one or more task variables, such as frequency of word beginnings (Dräger et al., 2004), the phonological or orthographical characteristics of the task language itself (Dan et al., 2013), or age-related cognitive decline (Heinzel et al., 2013). By implication then, complete lack of significant activation – as seen in $HIGH_{ENG}$ in the current study – would mean that said task may not be "demanding" enough to require certain cortical resources.

PFTs are thought to draw primarily upon two cognitive resources, language proficiency and executive control (Delis et al., 2001; Bialystok et al., 2008; Luo et al., 2010; Friesen et al., 2015). Regarding the former, both Bialystok et al. (2008) and Luo et al. (2010) demonstrated a phonemic fluency performance advantage for high proficiency bilinguals relative to low, and in both studies, L2 proficiency was measured as a combined function of

receptive and expressive vocabulary ability, with "high-" and a "low-vocabulary" groups. While vocabulary level was not expressly measured in the current study, Kanzaki (2010) demonstrated a moderate-to-strong positive correlation between TOEIC score and English vocabulary size, suggesting that our HIGH group was indeed likely able to draw upon greater vocabulary resources for the ENG task. And despite the lack of significant performance advantage in the HIGH group, the complete absence of significant activations – suggestive of increased cortical processing efficiency – could be at least partially explained by increased access to lexical resources in the task language.

Curiously, however, the disparity in number of significant activations between HIGH and LOW was not limited to ENG only. During the native language JPN task as well, only one channel was activated in the HIGH group, while more than 70% were activated in the LOW. Though increased English vocabulary size in the HIGH group could theoretically confer an advantage in cortical processing efficiency as observed during the current ENG task, it seems much less likely that a similar, nearly complete lack of activation in $HIGH_{JPN}$ would result from increased (Japanese) vocabulary size: all participants were native Japanese speakers without significant differences in age, years of education, or SES between proficiency groupings. The relative demographic homogeneity of the test population suggests that a substantial difference in usable Japanese vocabulary between groups is an unlikely explanation for the drastic activation difference also observed during JPN.

One possibility is that the near complete absence of significant activations by the HIGH group during the current PFTs – even in the L1, Japanese– may instead reflect the role of another cognitive resource implicated in their accomplishment: non-verbal executive control. Bilinguals have been reported to outperform monolinguals on non-verbal tasks such as those requiring conflict resolution and task-switching (see Kroll & Bialystok, 2013 for a review), and the "bilingual advantage" in such tasks is believed to originate from continuous practice controlling attention to two language systems during production and comprehension. This is thought to result in a more efficient executive control system in which fewer resources are necessary for monitoring or resolving conflict (Bialystok et al., 2009; Friesen et al., 2015). In addition to the more generalized, non-linguistic cognitive control tasks, executive control is also considered an important component of PFTs: generating words based on an initial Japanese mora or English letter often involves non-heuristic searches that place increased demands on effort-intensive executive control processes such as strategic organization and response inhibition (Strauss et al., 2006). Indeed, executive control functions such as conflict monitoring, response inhibition, search strategies, and working

memory have been implicated alongside vocabulary knowledge in the accomplishment of PFTs (Martin et al., 1994; Rosen & Engle, 1997; McDowd et al., 2011; Hurks, 2012). And superior task performance by (vocabulary-matched) bilinguals relative to monolinguals (Bialystok et al., 2008; Luo et al., 2010) and difference in slope of best-fitting curves during analysis of retrieval time-course functions in the latter study were interpreted as reflecting an enhanced executive control ability in bilinguals. In future studies, a similar analysis incorporated into the present methodological paradigm could help to dissociate the relative effects of language proficiency and executive function on PFT performance and underlying neural correlates.

As mentioned above, LOW_{JPN} recruited a broad swathe of brain areas that were nearly completely devoid of activation in $HIGH_{JPN}$. While correlates of executive control during PFTs are not yet clearly specified, there appears to be overlap between those implicated in phonemic fluency and those of non-linguistic executive control tasks. For example, frontal areas associated with performance in PFTs, such as the posterior opercular portion of the left inferior frontal gyrus (Paulesu et al., 1997), can also be recruited in cognitive control tasks not associated with language production (*e.g.*, task-switching, Yeung et al., 2006). In fact, task-switching is one of the most frequently-used paradigms for investigating the cognitive mechanisms underlying non-verbal executive control, and in a review of the task-switching literature, De Baene et al. (2015) described a "fronto-parietal network" sub-serving such tasks. Interestingly, the cortical areas of said network that were measurable using the current fNIRS modality – lateral prefrontal, premotor, and anterior parietal regions – overlapped substantially with those activated for LOW_{JPN} in the current study. This suggests that different executive control resource demands might explain the great disparity in activations observed between proficiency groups during the JPN (as well as the ENG) task(s).

While activation in the HIGH group was indeed almost completely absent for both tasks, a final possibility is that the HIGH group may have instead relied comparatively more on brain structures undetectable using the current methodology. In a meta-analysis of studies that used active task-based fMRI or PET to investigate neural networks sub-serving PFTs (Wagner et al., 2014), significant activations were also seen in the bilateral insula and anterior cingulate gyrus, the left thalamus, precuneus and putamen, and the right cerebellum, claustrum, and caudate head. As fNIRS is limited by its penetration depth of only a few centimeters (Dieler et al., 2012), deep brain structures cannot be measured non-invasively. In addition, the probe configuration used in the current study (3x11 fiber holder, Hitachi Medical Corp., Tokyo, Japan) does not allow for measurement of inferior temporal nor

posterior parietal regions. A neuroimaging modality such as fMRI would be necessary to address the contributions of the remaining cortical areas and deep brain structures in the current context.

4.3 Limitations

While the current study clearly demonstrates differential activation between languages and L2-proficiency groups, we should note certain limitations. First, the relatively small sample size, particularly in the HIGH group, may have limited statistical power.

Regarding proficiency, the minimum TOEIC score reported in the current study was still quite high. CEFR defines proficiency in three bands, "Basic User," "Independent User," and "Proficient User" (Council of Europe, 2001), and a score of 620 is still well above the cut score (550) corresponding to entrance to the Independent User band (Tannenbaum & Wylie, 2004, 2005, 2013; Educational Testing Service, 2011) and the average score of Japanese test-takers (513) (Educational Testing Service, 2016). For this reason and the highly-specific demographic background of the current participants, extrapolating the current results to the general population should be done cautiously. Also, while the $HIGH_{JPN}$ group may have demonstrated increased cortical processing efficiency, there was no way to dissociate the relative contributions of L1 proficiency and executive control. The inclusion of some objective measure of L1 vocabulary knowledge in a future study might further clarify whether the lack of significant activations observed in $HIGH_{JPN}$ truly reflected an increase in generalized executive control.

Finally, the stark lack of significant activations in the HIGH group was not accompanied by increased performance, and we must therefore exercise care before directly attributing the observed neural changes to an executive control "advantage." The direct mapping of a cognitive function to a discrete brain area in the absence of support from behavioral data – often referred to as the "misalignment" problem of bilingual advantage research – has been criticized by some groups (see García-Pentón et al., 2016; Paap et al., 2016 for reviews).

5. Conclusion

Because of unique challenges reported in English speech production and questions about the effects of language background on the neural mechanisms underlying a widely-used tool for

the assessment of cognitive function, the current study examined language- and L2-proficiency-dependent effects on PFTs in Japanese–English bilinguals using fNIRS. There were two major findings: (1) in addition to areas activated during JPN, there was additional ENG-dependent bilateral recruitment of the precentral area, and (2) regardless of task language, the HIGH group demonstrated an almost complete lack of significant activations while there was widespread recruitment in the LOW. Here, we interpreted the former result as ENG necessitating recruitment of additional resources related to pronunciation, while the latter was provisionally attributed to a possible executive control advantage in the more highly-proficient group.

Regardless of their source, the clear presence of language- and L2-proficiency-dependent effects on phonemic fluency has important practical implications. From the viewpoint of foreign language education, an increased cognitive demand for articulatory resources during speech production might be mitigated by increasing the amount of focused phonetic training in the curriculum, especially in contexts like Japan where the productive aspects of oral communication are reported to be particularly troublesome. In the future, conducting a similar experiment, this time with native English-speaking students of Japanese, could investigate whether recruitment of bilateral precentral resources is inherent to any L2 speaker or specific to native Japanese speakers only. And a possible link between high L2 language proficiency and increased cortical efficiency in a native language PFT naturally begs an intriguing question: do (high) L2-proficiency-dependent effects extend to other cognitive tasks as well? An obvious and convenient extension of the current research would be to include other widely-used verbal fluency tasks in the current experimental paradigm. Finally, to mitigate potential confounding effects of bilingualism on PFT data when used in clinical settings, a "native language" field might be gradually included in demographic surveys accompanying PFTs in the future, and when possible, efforts could be made to administer the test in that language. Through the above considerations, the neural effects of bilingualism on speech production can be more thoroughly clarified and accounted for going forward.

Having originally set out to postulate a reason for the conspicuous dearth of presentations by Japanese physician–researchers at international conferences, we found clear evidence that lack of confidence vis-à-vis their English language competence was the strongest precluding factor (Part 1). Despite this striking finding, however, several questions remained: why does oral communication in English seem so difficult for native Japanese? What are the mechanisms responsible for "successful" English language production? In the

current study (Part 2), we found evidence that structural differences between the Japanese and English languages as well as brain differences (*e.g.*, task processing efficiency) between highly-proficient and less-proficient individuals may explain some of the reported difficulty in presenting in English, particularly with regards to English pronunciation.

Nevertheless, despite inroads being made into the identification of the neural correlates of second language production and proficiency, many brain areas at the core of cognition and memory have yet to be well-characterized neuroanatomically. In Part 3, we will examine the distribution patterns of huntingtin-associated protein 1 in the cognitively-important retrosplenial and retrohippocampal areas of the brain using immunohistrochemistry and light microscopy.

PART 3

Distribution of huntingtin-associated protein 1 (HAP1)-immunoreactive cells in the retrosplenial and retrohippocampal areas of adult male rat

1.Introduction

Huntingtin-associated protein 1 (HAP1) is a neural interactor of huntingtin (Htt), the causal gene product of Huntington's disease (HD) (MacDonald et al., 1993). HAP1 is mainly localized in distinct cytoplasmic inclusions or stigmoid bodies (STB) in the normal rodent brain (Li et al., 1998; Gutenkunst et al., 1998; Fujinaga et al., 2007, 2009), which were first observed as spherical-to-oval-shaped, non-membrane-bound inclusions (0.5 - 3 μm in diameter) of granular, fuzzy texture with low-to-moderate electron density (Shinoda et al., 1992, 1993). It has been reported that HAP1 is expressed abundantly in the hypothalamus and other limbic-associated regions, and physiological studies have suggested that HAP1 plays an important role in hypothalamic functions such as maintenance of neuronal survival (Li et al., 2003), regulation of food intake and body weight (Chan et al., 2002; Dragatsis et al., 2004; Sheng et al., 2006; Lin et al., 2010), and control of locomotor activity (Lin et al., 2010).

Regarding the other aspects of STB or HAP1 function, the STB/HAP1-abundant regions tend to be spared from degeneration in neurodegenerative diseases in general, while areas with little STB/HAP1, including the striatum, thalamus, cerebral neocortex, cerebellum and spinal motor neurons, are neurodegenerative targets not only in HD but also other neurodegenerative diseases. For example, STB/HAP1 can also bind to androgen receptor (AR) in a polyQ-length dependent manner, sequestering polyQ-expanded AR derived from spinal and bulbar muscular atrophy (SBMA) more strongly and suppressing SBMA-mutant-AR-induced apoptosis via inhibition of its nuclear translocation from the cytoplasm (Takeshita et al., 2006). In addition, STB/HAP1 can also interact with other expanded-polyQ gene products such as ataxin-3 in spinocerebellar ataxia (SCA) type 3 (Takeshita et al., 2011) and TATA-binding protein in SCA type 17 (Prigge and Schmidt, 2007), suppressing their nuclear translocation. Furthermore, apoptosis or neurodegeneration is facilitated in the hypothalamus of *Hap1*-KO mice (Li et al., 2003). This line of data indicates that HAP1 is not

a toxic enhancer of Htt mutants as was proposed in earlier studies (Li et al., 1996; Gutekunst et al., 1998) and rather strongly supports the "STB/HAP1 protection hypothesis" (Fujinaga et al., 2004) in which HAP1 is thought to raise the threshold of vulnerability for neurodegeneration, render increased stability to neurons, and consequently protect against apoptosis and cell death in several neurodegenerative diseases (Kamei et al., 2001; Koga et al., 2002; Li et al., 2003; Fujinaga et al., 2004; Metzger et al., 2008; Takeshita et al., 2006, 2011). In this context, as we consider the relationship between neurodegenerative diseases and deterioration of higher nervous activity (*e.g.*, memory or cognitive function), it becomes important to definitively clarify HAP1 expression in the hippocampus as well as retrosplenial and retrohippocampal areas. Previously, we found that HAP1-immunoreactive (-ir) cells are scattered throughout the hippocampus as the "sporadically lurking HAP1-ir (SLH) cells" (Islam et al., 2012), and our recent pilot study also reported the conspicuous presence of HAP1-expressing cells in the retrosplenial and retrohippocampal areas (Wroblewski et al., 2015). The detailed neuroanatomical distribution, however, has yet to be determined thoroughly.

In the current study, we set out to confirm expression of HAP1 by Western blotting and immunohistochemically clarify the distribution of HAP1-ir cells in the retrosplenial and retrohippocampal areas of the adult male rat brain using light and fluorescence microscopy. The data on HAP1-expressing cells in the plausible center of cognitive function should contribute to our ongoing understanding of the neuroanatomically-complicated brain region as well as the pathophysiological involvement of HAP1 in memory and/or cognitive function.

2. Materials and methods

2.1 Characterization of primary antibodies

Goat polyclonal anti-HAP1 antibody (R19) raised against a peptide mapping to the C-terminus of HAP1 of rat origin was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Rabbit polyclonal anti-HAP1 antibody (R12) was produced by immunization with GST-tagged $HAP^{170–433}$ in our previous study (Fujinaga et al., 2007). Mouse monoclonal antibody to neuronal nuclei (NeuN) (A60) raised against purified cell nuclei from mouse brain was purchased from Millipore (Billerica, MA, USA). Rabbit polyclonal antibody against glial fibrillary acidic protein (GFAP, G9269) developed using purified human brain GFAP was purchased from (Sigma-Aldritch Co., St. Louis, MO, USA). Mouse monoclonal antibody against oligodendrocyte lineage transcription factor 2 (Olig2, MABN50) made

using recombinant protein corresponding to human Olig2 as immunogen was obtained from Millipore. Rabbit polyclonal antibody against ionized calcium-binding adapter molecule 1 (Iba1, 019-19741) raised against a synthetic peptide corresponding to the C-terminus of Iba1 was purchased from Wako (Osaka, Japan). Mouse monoclonal anti-α tubulin with a microtubule derived from embryonic chicken brain as immunogen was purchased from Sigma-Aldritch Co.

2.2 Animals and tissue preparation

Wistar male (N = 30) rats (9 weeks old; Japan SLC Inc., Shizuoka, Japan) were used in this study. They were group housed (3 - 4 rats/cage) at a constant temperature (22℃) with a 12–12-h light–dark cycle (lights on 08:00–20:00) and provided food and water *ad libitum*. All experimental protocols were approved by the Committee on the Ethics of Animal Experimentation at Yamaguchi University School of Medicine and conducted according to the guidelines for Animal Research of Yamaguchi University School of Medicine and the Law (No. 105) and Notification (No. 6) of the Japanese Government. All efforts were made to minimize the number of rats used and their suffering. The animals were perfused transcardially with ice-cold saline, followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB; pH 7.4) under anesthesia with sodium pentobarbital (60–80 mg/kg i. p.). Brains were removed and postfixed for 24 h in the same fixative used for perfusion and then soaked in 0.1 M PB / 30% sucrose solution until they sank. After flash-freezing in powdered dry ice, the tissue was sectioned frontally at a thickness of 30 μm on a cryostat. Frozen sections were then stored in ice-cold 0.02 M sodium phosphate buffered solution (PBS; pH 7.4) containing 0.1% sodium azide and maintained at 4℃ until use.

2.3 Immunohistochemistry

2.3.1 Single immunoperoxidase staining

Immunohistochemistry was performed as previously described (Islam et al., 2012, 2017; Jahan et al., 2015). Sections were first pre-incubated at 4℃ for 2 h in PBS containing 10% normal donkey serum (NDS) and 0.3% Triton X-100. This was followed by bleaching in a mixture of 50% methanol and 1.5% hydrogen peroxide diluted with double-distilled water for 1 h at 4℃. Next, sections were washed three times for 10 min each with PBS containing 0.3% Triton X-100 and 0.05% NDS or normal goat serum (NGS) (PBST–NDS, PBST–NGS). Sections were then incubated with antibody against HAP1 (1:30000) in PBS

containing 1% NDS / 0.3% Triton X-100 for 5 days at room temperature. In the case of the preadsorption test, incubation of sections with primary antibody solution was preceded by separate incubation of HAP1 (R19) antibody and specific blocking peptide (Santa Cruz Biotechnology, Inc.) for 48 h at 4℃. Following primary antibody immunoreaction, sections were washed in PBST–NDS three times for 15 minutes each and then incubated overnight at 4℃ in PBS containing 1% NDS and biotinylated donkey anti-goat secondary antibody (Millipore; 1:500 diluted). The following day, sections were washed twice at 4℃ for 10 min with PBS containing 0.05% NDS and once for 10 min with PBS only. This was followed by a 3-h incubation at 20℃ with peroxidase-conjugated streptavidin (1:500 diluted in PBS; DakoCytomation, Glostrup, Denmark), after which sections were washed in 0.05 M Tris-HCl buffer (pH 7.6) three times for 10 min each. Sections were then colored in a violet-to-black color reaction using 0.02% 3,3'diaminobenzidine (DAB; Wako Pure Chemical Industries, Ltd., Osaka, Japan) and 0.6% nickel ammonium sulfate (Sigma-Aldritch, Tokyo, Japan) in 0.05 M Tris-HCl buffer containing 0.0008% hydrogen peroxide (nickel-enhanced DAB reaction) for 5 –20 minutes at room temperature. After washing in 0.05% Tris-HCl buffer and PBS, sections were mounted on glass slides using 0.6% gelatin solution, air-dried, dehydrated using a graded series of ethanol, immersed in xylenes, and finally embedded with Entellan Neu (Merck KGaA, Darmstadt, Germany).

2.3.2 Double-label immunofluorescence staining

Sections were blocked with PBS containing 10% NDS and 0.3% Triton X-100 for 3 h at 4℃. They were then washed twice for 10 min each in PBST-NDS at 4℃, followed by incubation for 5 days at 20℃ with goat anti-HAP1 antibody (1:10000) in PBS containing 1% NDS and 0.3% Triton X-100 in combination with one of the following antibodies : mouse anti-NeuN (1:500), rabbit anti-GFAP (1:1000), mouse anti-Olig2 (1:500), or rabbit anti-Iba1 (1:500). After washing with PBST-NDS three times for 10 min each at 4℃, sections were incubated with a mixture of Alexa Fluor 594-conjugated donkey anti-goat IgG and Alexa Fluor 488-conjugated donkey anti-rabbit or donkey anti-mouse IgG (Molecular Probes, Eugene, OR, USA; 1:500 diluted in PBS containing 1% NDS) overnight at 4℃. After washing with PBS three times for 15 minutes each at 4℃, sections were mounted on glass slides using 0.6%

gelatin solution, air-dried, and embedded with Fluoromount/Plus (Diagnostic Biosystems, Pleasanton, CA, USA). Slides were then stored in a light-tight slide box at 4℃ until use.

2.4 Western blotting

Western blotting was performed as described previously (Jahan et al., 2015; Islam et al., 2017). In brief, the whole brain, hypothalamus, and retrosplenial–retrohippocampal area (caudal tip of the cerebrum, isolated by making a cut perpendicular to the longitudinal axis near the rostral edge of the pons and separating the cortex from the midbrain/brainstem) were homogenized in radio immunoprecipitation buffer [25 mM Tris–HCl (pH 7.6), 150 mM NaCl, 5 mM EDTA, 1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS)] with 5 μl/ml of protease inhibitor cocktail (P8340; Sigma-Aldrich). Protein concentration for each sample was determined through the BCA Protein Assay method (Thermo Scientific, Waltham, MA, USA), and eighty micrograms of each protein was loaded, separated by 7.5% SDS-polyacrylamide gel electrophoresis, and then transferred onto a polyvinylidene difluoride membrane using a wet transfer apparatus. After being blocked for 1 h at room temperature with 5% skim milk in Tris-buffered saline with 0.1% Tween (TBST), the membrane was then incubated overnight at room temperature in the above blocking solution with either goat polyclonal anti-HAP1 (1:20000), rabbit polyclonal anti-HAP1 (1:20000), or mouse monoclonal anti-α tubulin (1:20000) antibodies. For the HAP1 preadsorption test, the diluted antibody was incubated at 4℃ overnight with the specific blocking peptide (Santa Cruz Biotechnology, Inc.). and after several washes in TBST, the membrane was incubated with horseradish peroxidase linked anti-goat (1:5000: SC-3851, Santa Cruz Biotechnology), anti-rabbit, or anti-mouse IgG (1:20000; GE Healthcare, Buckinghamshire, UK) antibody at room temperature for 2 h. After several additional washes in TBST, immunoreactive bands were visualized using enhanced chemiluminescense reagents (ECL select, GE Healthcare) and an Amersham Imager 600 (GE Healthcare).

2.5 Photomicrographs and line drawings

Photomicrographs were taken using an Eclipse E80i photomicroscope (Nikon) equipped with either a color digital USB 2.0 camera (Lumenera Corporation, Ottawa, Canada) or a USB 3.0 5M CMOS color camera (Sentech Corporation, Shinagawa, Japan) and processed via LuCam Capture (Lumenera), Imaging Tiling (Mitani Corporation, Tokyo, Japan), or Hybrid Measure

software (Inotech, Yokohama, Japan). For fluorescence images, single optical sections (1024 x 1024 pixels) were obtained by laser-scanning microscope (LSM510; Carl Zeiss, Jena, Germany). Image brightness and contrast were adjusted via Adobe Photoshop CS (Adobe Systems, Inc. San Jose, CA, USA), and no other modifications to the original images were made. HAP1-ir cell distribution data were transferred to computer, and line drawings of representative cortical levels and their areal boundaries were made using Adobe Photoshop CS.

2.6 Cytoarchitectonic analysis and terminology

Cytoarchitectonic examination was carried out using Nissl staining (cresyl violet, Merck Millipore) of sections neighboring or adjacent to those stained for HAP1. Nomenclature for the retrosplenial and retrohippocampal areas in the rat brain generally followed that of Paxinos and Watson (2009). However, for the retrosplenial subregions the terms "dysgranular and granular retrosplenial cortices (DRS and GRS)" were used. "Hippocampus" was used here for the two major components, the dentate gyrus (DG) and Ammon's horn. The subiculum has been categorized as one part of the "hippocampal formation" together with the DG and Ammon's horn (Angevine, 1965), while also being a considered as constituent of the retrohippocampal region (Taube et al., 1990). The subiculum is subdivided here into three parts in accordance with Paxinos and Watson (2009), and the terms "dorsal (DS) or ventral subiculum (VS)" were also adopted here. For the convoluted region of subiculum deep inside the postsubiculum (PoS)–presubiculum (PrS)–parasubiculum (PaS) area, we coined the term "central subiculum (CS)" instead of "ventral subiculum" (Swanson, 2004) or "transition area of the subiculum" (Paxinos and Watson, 2009). This is partly because the CS is located in a distinct region caudal to the convoluted hippocampal CA1 region and partly because all three parts have a transition area into the CA1 of Ammon's horn. The unified PoS–PrS–PaS area was termed the "subiculum-backing cortex (SBC)" as a distinct retrohippocampal part dorsally transiting the DS and retrosplenial cortex and ventrally transiting the VS and entorhinal cortex. Thus, the retrohippocampal area defined here includes the subiculum, the SBC, and the entorhinal and perirhinal cortices. Here, the terms "dorsal (MECd) and ventral parts (MECv) of the medial entorhinal cortex," "dorsal (PoSd) and ventral parts (PoSd) of the postsubiculum," and "lateral (LECl) and medial parts (MECl) of the lateral entrorhinal cortex" were also used for the sake of utility.

The term "granular retrosplenial superficial HAP1-ir (GRASH)" cells was used here to refer to the ones in layers II-III of the GRS where innumerable HAP-ir cells were clustered together. The "pericallosal zone" indicates the callosum-adjacent layers underneath the subiculum, SBC and layer VI of the cortices, which are regarded as a pericallosal continuum owing to the conventional corpus callosum turning around mesially at the entorhinal or parasubicular corner (ventral corner) and at the retrosplenial corner (dorsal corner) and continuing as an inverted callosum underlying the mesial retrosplenial cortex, mesial entorhinal cortex, SBC, and subiculum; the conventional and inverted callosums are conjoined to each other by both deeper interfaces and seem to be one callosal entity composed of its inner and outer leaves. In particular, many HAP1-ir cells were disseminated along the pericallosal zone in the VS, PaS and entorhinal cortex, and have been called here the "pericallosal HAP1-ir cells (PCH)." The entorhinal lamina dissecans also turns around mesially at the parasubicular or entorhinal corner and continues to another conspicuous lamina dissecans underlying the SBC, termed the "mesial lamina dissecans (MLD)" as a definite substrate distinct from the entorhinal lamina dissecans. The "intermediate entorhinal cortex (IEC)" was given to the gross-anatomically concaved cortex between the medial and lateral entorhinal cortices, in which tissue sections tends to have a well-stained layer II, thin cell-sparse zone between layers II-III, and continued layers III-VI with no clear cell-sparse zone.

3. Results

3.1 General expression of HAP1-immunoreaction in the retrosplenial and retrohippocampal areas

Both Western blotting and immunohistochemistry were used to analyze HAP1 expression in the retrosplenial and retrohippocampal areas. By Western blotting with either goat polyclonal anti-HAP1 antibody (R19) or rabbit polyclonal anti-HAP1antibody (R12) (Figure 1A), the two isoforms of HAP1 (approximately 85 and 75 kDa for HAP1B and HAP1A, respectively) were detected in the retrosplenial–retrohippocampal lysate, but expression for both was less than that of the hypothalamus (Figure 1A). Using R19, HAP1B stained much more strongly than HAP1A for all brain areas sampled, while using R12, similar levels of staining were demonstrated between them. Preincubation with blocking peptide against the primary antibody resulted in elimination of all the HAP1-ir bands from the blot. Distribution patterns

of HAP1-ir cells were similar for both antibodies (R12 and R19). In the preadsorption test, HAP1-immunostainings were eliminated in all areas (Figure 1C, E). Double label immunostainings were performed for HAP1 and GFAP (astrocyte marker), Olig2 (oligodendrocyte marker), Iba1 (microglial marker), or NeuN (neuronal nuclear antigen A60) in the retrosplenial cortex (Figure 2A) and PrS or PaS (Figure 2B). All the HAP-ir cells demonstrated clear NeuN immunoreactivity in their nuclei, while being negative for GFAP, Olig2 and Iba1, indicating that the HAP1-ir cells exhibited attributes of neurons but not of glial cells.

To clarify the detailed distribution of HAP1-ir cells in the retrosplenial and retrohippocampal areas, coronal sections spanning their entire rostrocaudal extent were stained via the single immunoperoxidase method and compared to adjacent Nissl sections (Figures 3-5). There were many sporadic HAP1-ir cells in the retrosplenial and retrohippocampal cortices. Some were intensely stained and had visible STBs in the cytoplasm (type 1), while other cells had diffuse staining in the cytoplasm without, or having undetectable, STBs (type 2). It has been reported that most HAP1-ir cells express both HAP1A and HAP1B and that the cells which express comparatively more of the HAP1A isoform form the immunoreactive STB in the cytoplasm (type1), while the ones expressing more HAP1B show diffuse immunoreaction in the cytoplasm (type 2) (Li et al., 1998; Fujinaga et al., 2007). It is, however, often difficult to determine solely by cytoplasmic immunoreaction whether the STB is present, particularly in strongly HAP1-ir cells and it is thus somewhat difficult to categorize HAP1-ir cells into either type 1 or 2. Nevertheless, the type 1 HAP1-ir cells were sporadically and widely distributed through the retrosplenial and retrohippocampal areas, being scattered across all layers but with a tendency of being slightly more numerous in layers III and V. In contrast, the type 2 HAP1-ir cells were weakly stained and particularly observed in layer II and the superior part of layer III. The HAP1-ir cells (types 1 and 2) were more densely disseminated along the lateral border of the retrohippocampal area (the ectorhinal and perirhinal cortices) and in the border between the DRS and neighboring visual cortices. In addition to the aforementioned sporadic cells, however, there were four other distinct groups of HAP1-ir cells (Figures 6-9): the first in the mesial surface of the retrosplenial cortex, the second spanning the deep layers of the PoS–PrS–PaS, the third in the deepest layer directly adjacent to the corpus callosum over the posteroventral part of the retrohippocampal area (subiculum, PaS and medial and lateral entorhinal cortices) and the fourth in the superficial layer of the medial entorhinal cortex at the transitional corner to the VS. They will be described as follows in order.

3.2 The granular-retrosplenial cortex-associated superficial HAP1-ir (GRASH) cells

In the retrosplenial cortex, a distinct HAP1-ir cell group was observed in the superficial layer of the mesial retrosplenial cortex. While faintly-to-weakly stained HAP1-ir cells in layer II and the superficial part of layer III were also seen in neighboring cortices including the visual cortices dorsally and the ectorhinal and temporal association cortices ventrally, the innumerable moderately-to-strongly HAP-ir cells without or with undetectable STBs (type 2 cells) were piled up in the GRS and formed a thick layer of densely-packed cells, the "granular-retrosplenial cortex-associated superficial HAP1-ir (GRASH)" cells (Figure 6B, C). The GRASH cells showed an abrupt discontinuation at the transition to the DRS. In addition, sporadic HAP-ir cells with STBs (type 1 cells) were intermingled in the cell-cluster of type 2 GRASH cells.

3.3 The mesial lamina dissecans-associated HAP1-ir (MLDH) cells

In the PoS–PrS–PaS continuum, heretofore referred to as the "subiculum-backing cortex (SBC)," the most distinct HAP1-ir cell group conspicuously formed a sharp line in coronal sections, which is associated with the "mesial lamina dissecans (MLD)" (Figure 6B, D). The MLD-associated HAP1-ir (MLDH) cell line was composed of intensely HAP1-ir type 1 cells spanning only a few cells in thickness. Morphologically, apical dendrites emanating from cell somata often clearly extended to more superficial layers towards the brain surface in a perpendicular fashion. The MLDH cells spanned the entire SBC in an uninterrupted manner, extending to bordering portions of the neighboring retrosplenial cortex dorsally and medial entorhinal cortex ventrally, and were gradually interspersed near the ventral and dorsal ends of the MLD. However, they never extended to the subiculum (Figure 7). The cells approached another distinct HAP1-ir cell group in the pericallosal zone (described later) at the dorsal and ventral turning corners of the corpus callosum, where the MLD is also apposed to the corpus callosum. The MLDH cells were arranged in probable layer V just beneath the MLD in most areas, while sometimes crossing over the MLD to the probable deep part of layer IV (particularly at the dorsal part). Their tangled relation to the MLD (Figure 8) makes their laminar demarcation difficult but suggests that they are present in layers IV/V and are intimately associated with MLD function in the SBC.

3.4 The pericallosal HAP1-ir (PCH) cells

Large numbers of HAP1-ir cells were disseminated along the pericallosal zone in the retrosplenial and retrohippocampal areas. They were present underneath layer VI and adjacent to the corpus callosum, particularly concentrated in the ventral retrohippocampal area, including the VS, PaS, and medial and lateral entorhinal cortices. They were regarded as a pericallosal continuum and called here, as a whole, the "pericallosal HAP1-ir (PCH)" cells because the conventional corpus callosum turns around mesially at the retrosplenial corner (dorsal corner) and at the entorhinal or parasubicular corner (ventral corner), and continues as an inverted callosum (or alveus) underlying the subiculum, SBC, and mesial entorhinal and retrosplenial cortices. The PCH cells were distributed over not only the outer leaf of the corpus callosum (conventional callosum) in the lateral entorhinal and intermediate cortices but also the inner leaf (inversed callosum) in the MEC, PaS, and VS (Figures 6B, E). Furthermore, the PCH cells continued to the deepest layer adjacent to the alveus (inner leaf of the corpus callosum or inversed callosum) of the "central subiculum (CS)" (transition area of the subiculum in Paxinos and Watson, 2009). At the most caudal levels of the CS in the coronal plane, the PCH cells seemed embedded in the enlarged alveus.

3.5 The medial entorhinal-subicular corner-associated HAP1-ir cells (MESCH) cells

In a very limited number of rostral levels of the MEC, there was a small but distinct HAP1-ir cell group at the transitional corner of the MEC to the VS and were called here the "medial entorhinal-subicular corner-associated HAP-ir cells (MESCH)" cells (Figure 9B, C). They were mostly composed of type 2 HAP1-ir cells in superficial layers II-III over the MEC to the transitional VS at the turning corner. In the DG (Figure 9B, D), HAP1-ir cells were primarily of the type 1 variety, and they were mostly limited to the subgranular zone of the DG as was previously reported in the rostral hippocampal formation (Islam et al., 2012).

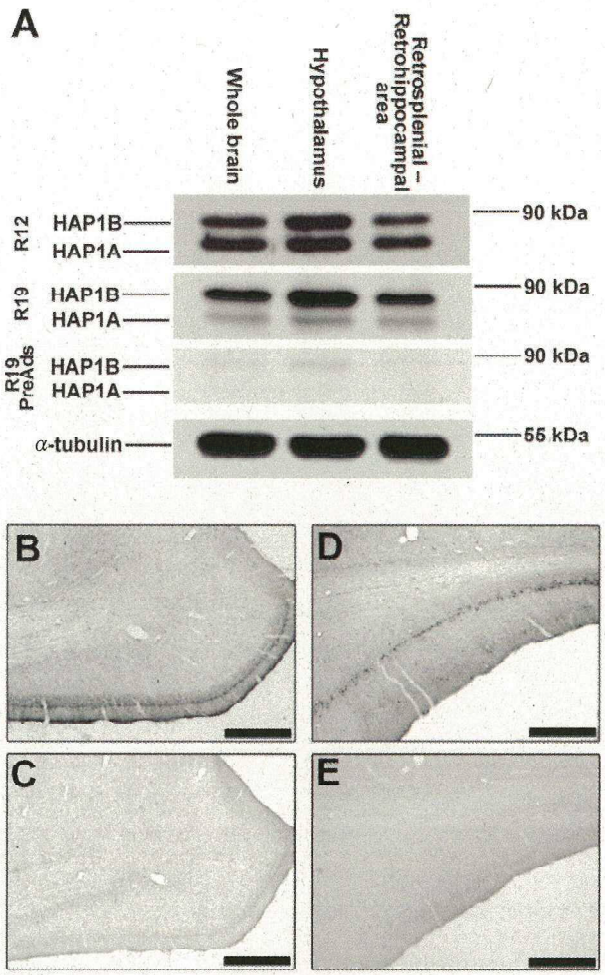


Figure 1: Western blotting and immunohistochemistry for huntingtin-associated protein 1 (HAP1) in adult male rat brain. (A) Western blot analysis using lysate from the whole brain and hypothalamus (controls) as well as the retrosplenial–retrohippocampal area. The blot shows protein bands of approximately 85 kDa and 75 kDa for HAP1B and HAP1A, respectively, using both R12 or R19 antibody, and 50 kDa for α-tubulin (loading control). Preadsorption of the R19 anti-HAP1 antibody with a blocking peptide resulted in elimination of the HAP1- positive bands. (B, D) Immunohistochemistry showing the presence of HAP1 immunoreactive (ir) cells in the retrosplenial cortex and postsubiculum, respectively. Preincubation with blocking peptide against R19 resulted in the disappearance of HAP1-immunoreaction in the corresponding cortices (C, E). Scale bars = 400 μm.

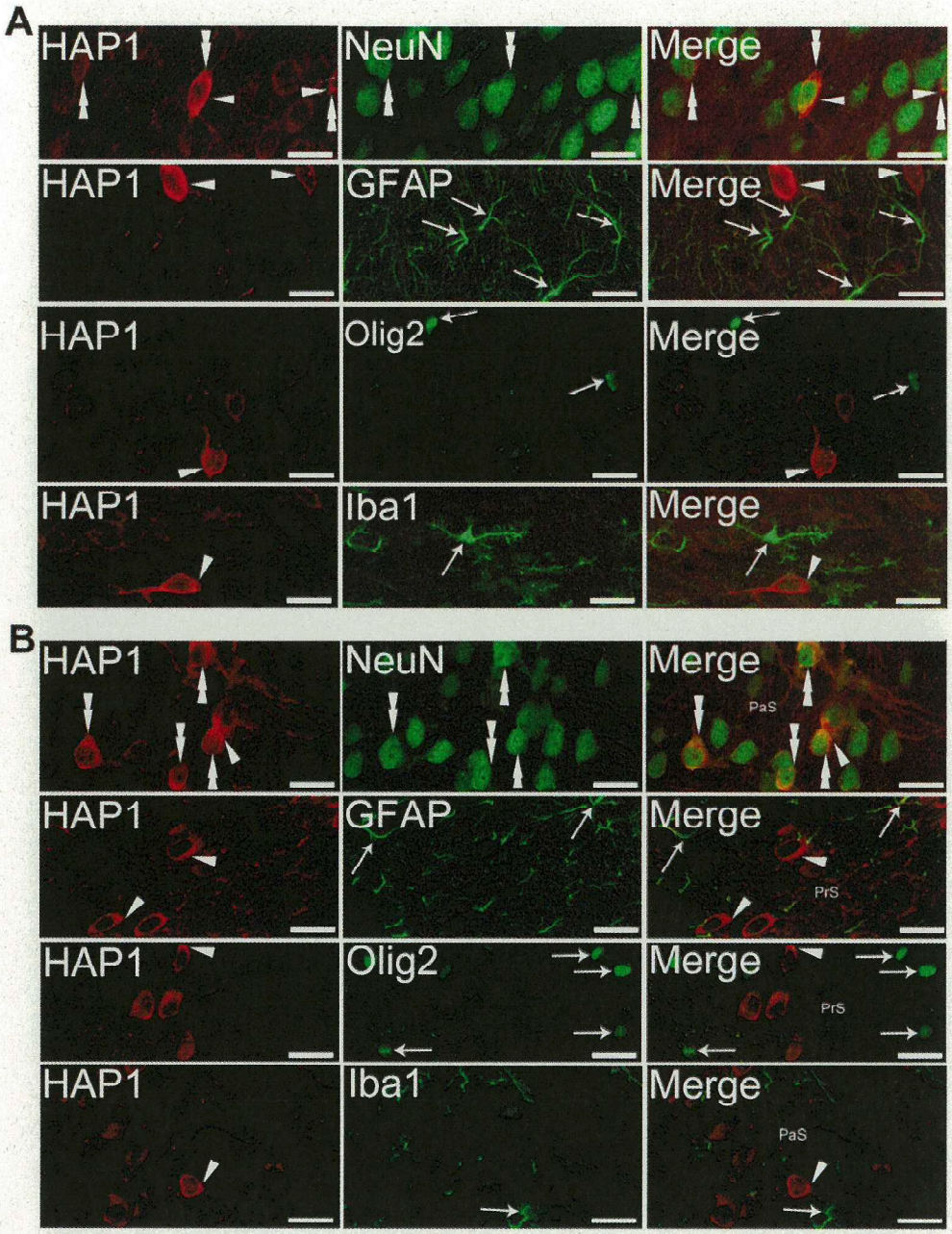


Figure 2: Photomicrographs showing double-label immunofluorescence staining with HAP1 and NeuN, GFAP, Olig2, or Iba1. (A) is taken from the retrosplenial cortex and (B) from the deep layers of the PrS or PaS. Single arrowheads indicate HAP1-ir STBs, double arrowheads those cells positive for both HAP1 and NeuN, and arrows those cells positive for only GFAP, Olig2, or Iba1. PrS, presubiculum; PaS, parasubiculum. Scale bars = 20 μm.

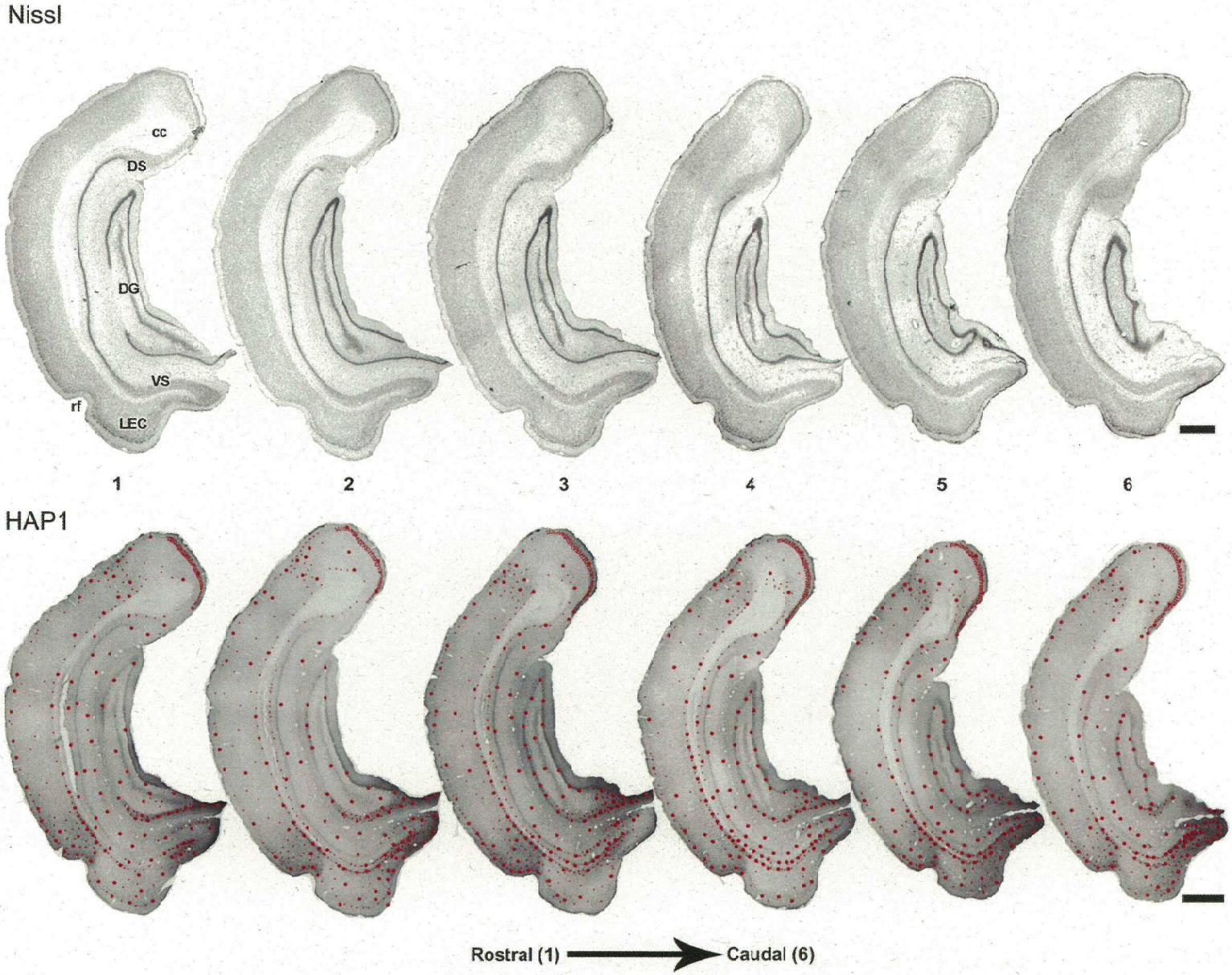


Figure 3: Photomicrographs of single-hemisphere coronal sections from an adult male rat showing HAP1-ir cells in the rostral retrosplenial–retrohippocampal area. Section 1 is the most rostral and section 6 the most caudal. Large red dots represent intensely-stained HAP1-ir cells with visible STBs (type 1), and small dots represent cells that stained diffusely and were devoid of visible STBs (type 2). Approximate stereotaxic reference points were determined by consulting an Atlas of the Rat Brain (Paxinos & Watson, 2009): 1 = Bregma -5.52 mm, 2 = -5.76 mm, 3 = -5.88 mm, 4 = -6.00 mm, 5 = -6.12 mm, 6 = -6.24 mm. cc, corpus callosum; DS, dorsal subiculum; DG, dentate gyrus; VS, ventral subiculum; LEC, lateral entorhinal cortex; rf, rhinal fissure. Scale bars = 1 mm.

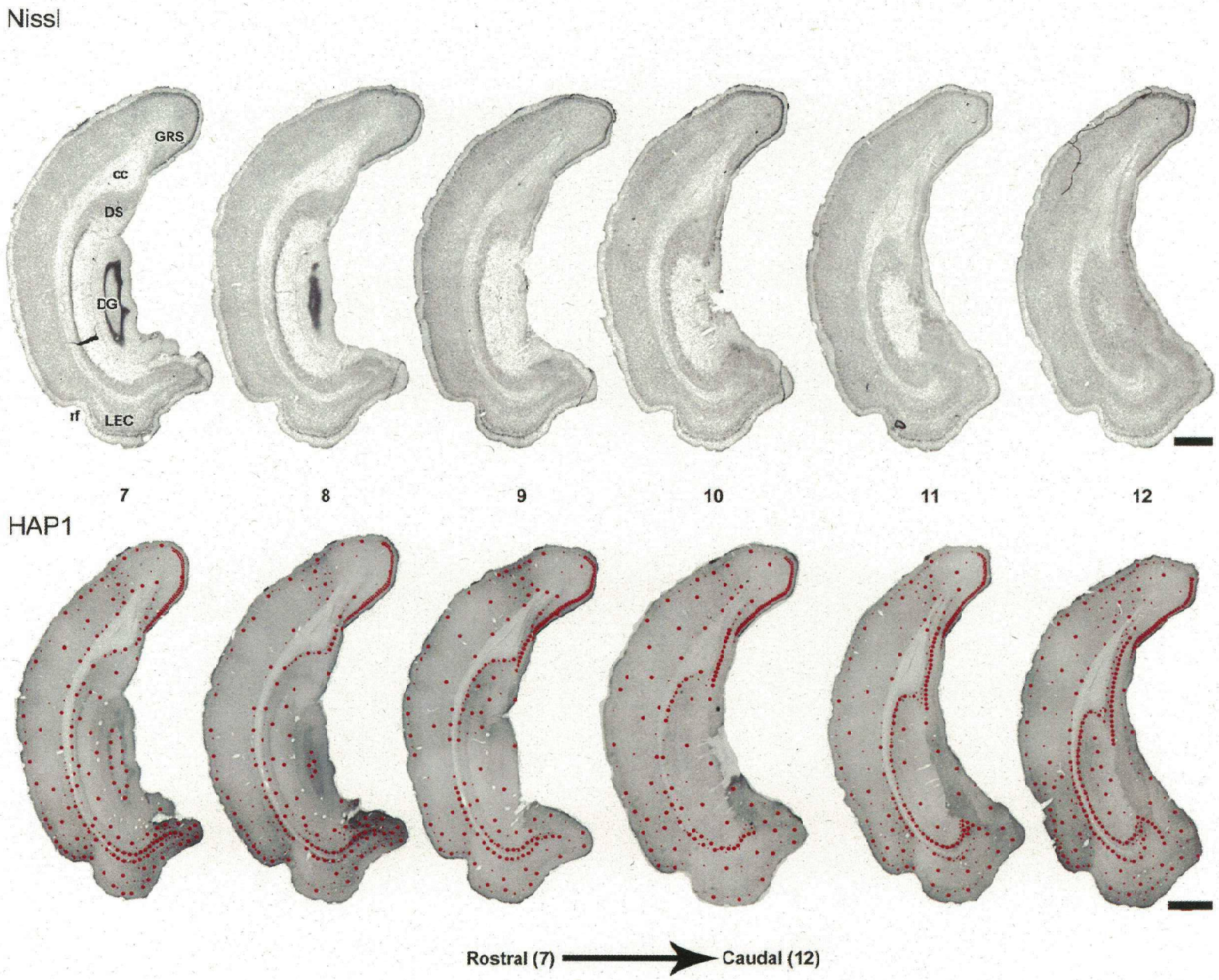


Figure 4: Photomicrographs of single-hemisphere coronal sections from an adult male rat showing HAP1-ir cells in the mid retrosplenial and retrohippocampal area. Section 7 is the most rostral and section 12 the most caudal. Large red dots represent intensely-stained HAP1-ir cells with visible STBs (type 1), and small dots represent cells that stained diffusely and were devoid of visible STBs (type 2). Approximate stereotaxic reference points were determined by consulting an Atlas of the Rat Brain (Paxinos & Watson, 2009). 7 = -6.36 mm, 8 = -6.48 mm, 9 = -6.60 mm, 10 = -6.84 mm, 11 = -6.96 mm, 12 = -7.08 mm. GRS, granular retrosplenial cortex. Scale bars = 1 mm.

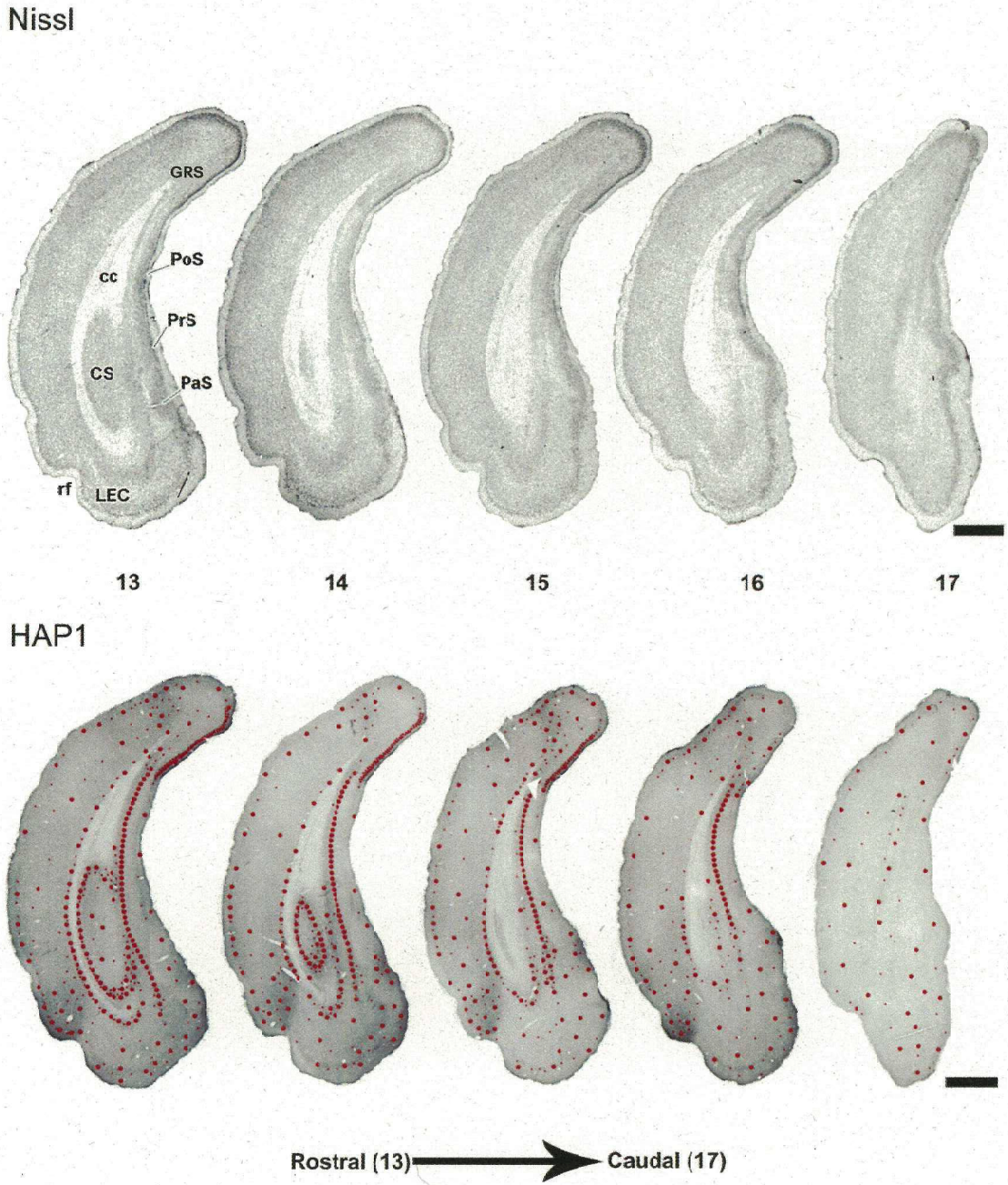


Figure 5: Photomicrographs of single-hemisphere coronal sections from an adult male rat showing HAP1-ir cells in the caudal retrosplenial and retrohippocampal areas. Section 13 is the most rostral and section 17 the most caudal. Large red dots represent intensely-stained HAP1-ir cells with visible STBs (type 1), and small dots represent cells that stained diffusely and were devoid of visible STBs (type 2). Approximate stereotaxic reference points were determined by consulting an Atlas of the Rat Brain (Paxinos & Watson, 2009). 13 = -7.44 mm, 14 = -7.56 mm, 15 = -7.68 mm, 16 = -7.80 mm, 17 = -8.16 mm. PoS, postsubiculum; PrS, presubiculum; PaS, parasubiculum; CS, central subiculum. Scale bars = 1 mm.

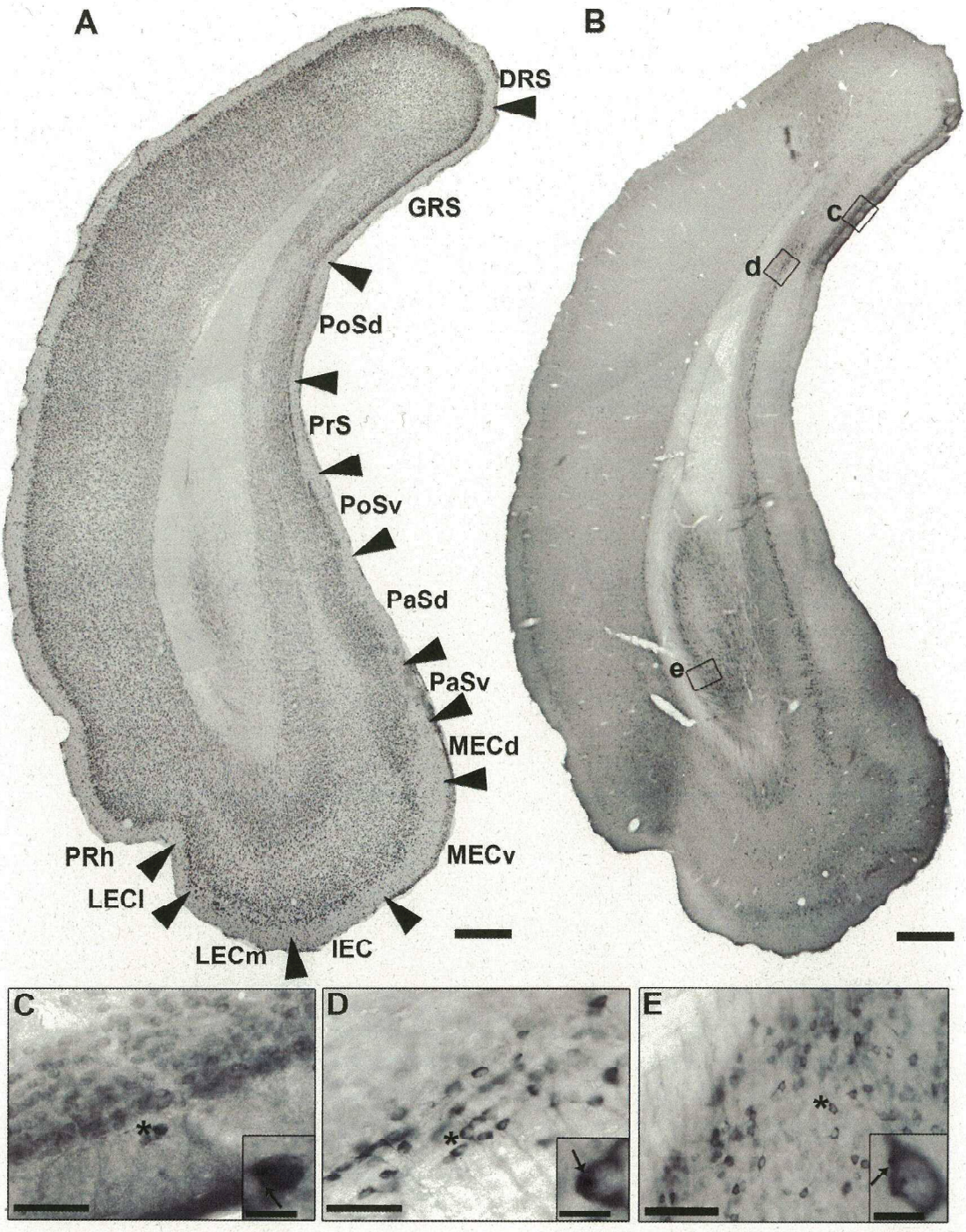


Figure 6: Enlargement of section 14 (B) and its adjacent Nissl section (A) from Figure 5, showing distinct HAP1-ir zones in the superficial layers of GRS (C), the deep layers of the PoS (D) and ventrally-neighboring cortices, and the layer closest to the corpus callosum (E). Arrowheads in **(A)** indicate boundaries between cortical areas. Boxes c, d, and e in **(B)** indicate the location of the enlargements in **(C)**, **(D)**, and **(E)**, respectively. Insets are the further enlargements of individual HAP1-ir cells indicated by asterisks in **(C-E)**. Arrows indicate HAP1-ir STBs. **(A)** and **(B)** are tiled images. DRS, retrosplenial dysgranular cortex; GRS, retrosplenial granular cortex; PoSd, dorsal postsubiculum; PrS, presubiculum; PoSv, ventral postsubiculum; PaSd, dorsal parasubiculum; PaSv, ventral parasubiculum; MECd, dorsal part of the medial entorhinal cortex; MECv, ventral part of the medial entorhinal cortex; IEC, intermediate entorhinal cortex; LECm, medial part of the lateral entorhinal cortex; lateral part of the lateral entorhinal cortex; PRh, perirhinal cortex. Scale bars = 565 µm in **(A)**, 500 µm in **(B)**, 50 µm in both **(C)** and **(D)**, 100 µm in **(E)**, and 10 µm for the insets in **(C-E)**

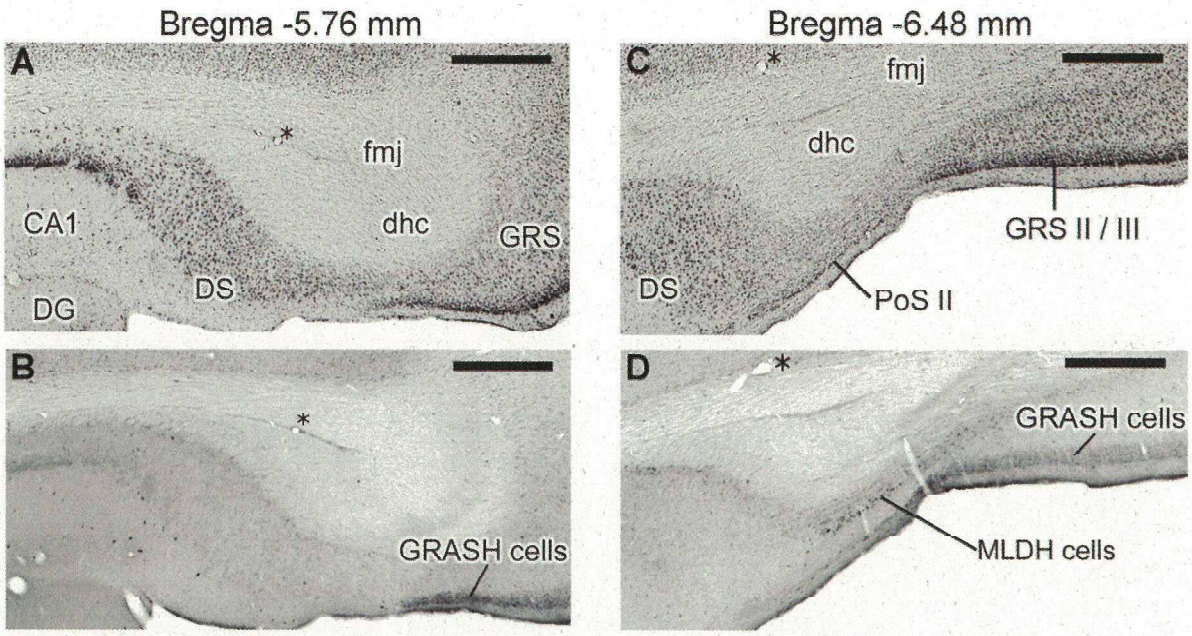


Figure 7: Photomicrographs comparing the distribution of HAP1-ir cells in the transition area between DS and GRS at two different rostrocaudal levels. (A) Nissl-stained section showing the DS–GRS transition area at Bregma -5.76 mm. (B) Distribution of HAP1-ir cells in the section adjacent to (A). (C) Nissl stained section showing the DS–PoS–GRS transition area at Bregma -6.48. (D) Distribution of HAP1-ir cells in the section adjacent to (C). CA1, CA1 of Ammon's horn; dhc, dorsal hippocampal commissure; fmj, forceps major of corpus callosum; GRASH cells, granular retrosplenial superficial HAP1-ir cells; MLDH cells, mesial lamina dissecans-associated HAP1-ir cells. Asterisks indicate the same blood vessel in adjacent sections. Scale bars = 300 μm in (A) and (C) and 240 μm in (B) and (D).

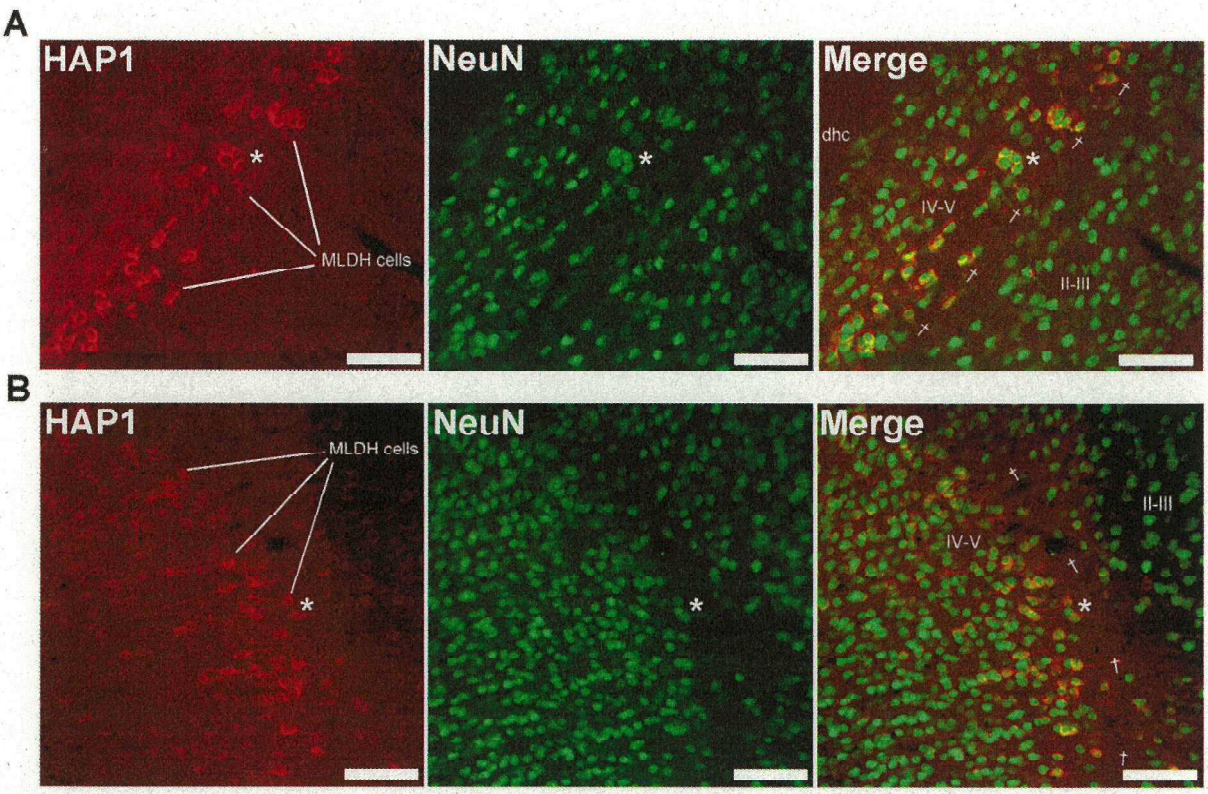


Figure 8: Double-label immunofluorescence staining of HAP1 and NeuN indicating the laminar distribution of MLDH cells in the PoS (A) and PaS (B). Asterisks indicate a landmark at the respective levels. Cross-marks indicate the cell-sparse lamina dissecans. Scale bars = 100 µm.

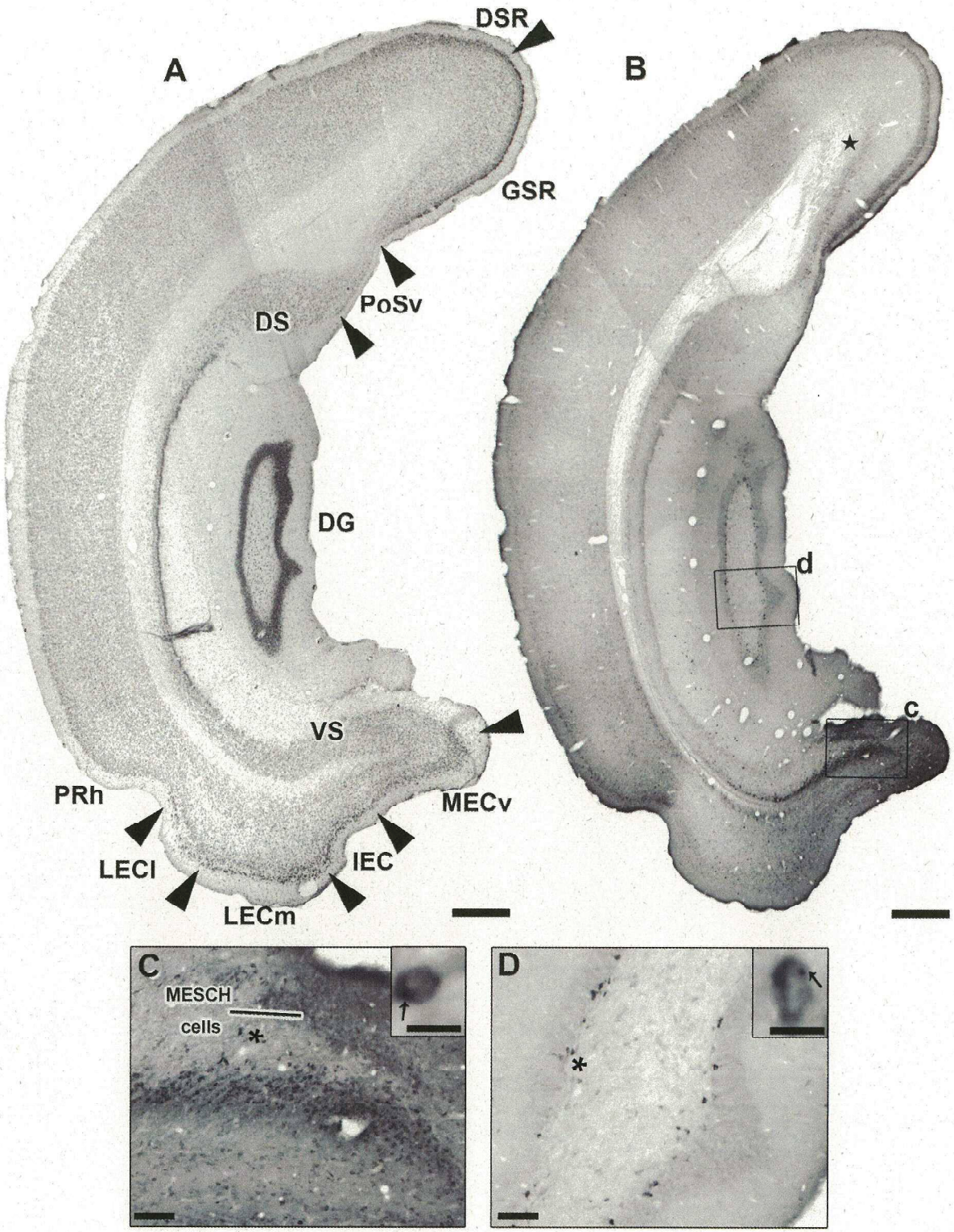


Figure 9: Enlargement of section 7 (B) and its adjacent Nissl section (A) from Figure 4, showing the distribution of HAP1-ir cells in the VS (C) and the DG (D). Arrowheads in (A) indicate boundaries between cortical areas. Boxes c and d in (B) indicate the location of the enlargements in (C) and (D), respectively. The star in (B) indicates the rostrodorsal extension of the HAP1-ir cells present in the deep layers of the PoSv. Insets are the further enlargements of individual HAP1-ir cells indicated by asterisks in (C) and (D). Arrows indicate HAP1-ir STBs. Scale bars = 565 µm in (A), 500 µm in (B), 100 µm in both (C) and (D) and 10 µm for the insets in (C) and (D).

4. Discussion

The present study has clarified the distribution of HAP-ir cells in major putative memory and cognitive centers: the retrosplenial and retrohippocampal areas of the adult male rat brain. They are largely classified into four groups, including the (1) granular-retrosplenial cortex-associated superficial HAP1-ir (GRASH) cells in the superficial layers (II-III) of the GRS, (2) the deep layers (IV-V) of the subiculum-backing cortex (SBC, including PoS, PrS, and PaS) associated with the mesial lamina dissecans (SBC-MLDH cells), (3) the pericallosal HAP1-ir (PCH) cells in the posteroventral part of the pericallosal layers, and (4) the medial entorhinal-subicular corner-associated HAP-ir cells (MESCH) cells.

An essential function of the hippocampus (here defined as the DG, Ammon's horn, and subiculum) is thought to be the binding together of objects in place and time (Cohen & Eichenbaum, 1993; Cohen et al., 1999; Eichenbaum, 2004, 2011; Davachi 2006), and the retrosplenial and retrohippocampal areas work in concert to provide the hippocampus with cortical input on the spatial and temporal context in which an object/event occurs (see Bucci & Robinson, 2014 for a review). Retrohippocampal areas, including the subiculum-backing cortex (SBC, including PoS, PrS, and PaS) as well as entorhinal and perirhinal cortices (Taube et al., 1990), share extensive reciprocal connections with the retrosplenial cortex (van Groen & Wyss, 1990a, 1992, 2003; Burwell & Amaral, 1998; Kobayashi & Amaral, 2003, 2007; Aggleton et al., 2012), and the relatedness of retrosplenial and retrohippocampal areas has led some to include the latter as a constituent of the retrohippocampal formation (Angevine, 1965; Kohler et al., 1981). Functionally-speaking, both retrosplenial and retrohippocampal areas have been found to harbor spatially-modulated neurons such as head direction, grid, and border cells involved in navigation and orientation in rats, especially in deep layers (Taube et al., 1990; Cho & Sharp, 2001; Sargolini et al., 2006; Boccara et al., 2010). Interestingly, cells in the deep layers of the PoS and PrS, plausibly corresponding to MLDH cells presented here, are known to project to the GRS, PaS, and MEC (Wyss & van Groen, 1992; Honda & Ishizuka, 2004; Honda et al., 2011), However, despite the fact that a detailed study of the area could potentially contribute to our understanding of how an animal conceptualizes and navigates its environment as well as forms memories, the retrosplenial and retrohippocampal and areas remain neuroanatomically under-investigated and under-characterized.

4.1 MLDH cells present in the subiculum-backing cortex but not in the subiculum

In the present study, a thin, continuous line of HAP1-ir neurons was found to span the entire length of the PoS–PrS–PaS, with minor dorsorostral and ventrocaudal extensions in the GRS and MECd, respectively. These cortical fields, along with subiculum proper, have often been considered subregions of a single entity, the "subicular complex" (*e.g.* van Groen & Wyss, 1990b; Wyss & van Groen, 1992; Ding, 2013). Recently, however, the relationship between these diverse areas has been deemed more etymological than functional or anatomical in light of certain differences (Witter & Amaral, 2004; O'Mara et al., 2009). For example, there has been general agreement that the subiculum is three-layered allocortex (Witter & Groenewegen, 1990; O'Mara et al., 2001; Witter & Amaral, 2004), though one recent study divided the subiculum into proximal and distal subfields, with the former suggested to consist of five layers based on the differential distribution of NOS- and PCP4-postive neurons (Ishihara & Fukuda, 2016). The PoS, PrS, and PaS, however, are each characterized by external (layers I–III) and internal cellular laminae (layers IV–VI) more similar to the entorhinal cortex or adjacent parts of the retrosplenial cortex (van Groen & Wyss, 1990b; Witter & Amaral, 2004). Connectivity-wise also, the subiculum is largely considered an output structure while the PoS, PrS, and PaS have higher degrees of reciprocity with their target regions (Witter & Amaral, 2004; Ding, 2013).

In the same way, HAP1-ir expression patterns in the current study support the assertion that PoS, PrS, and PaS should not necessarily be grouped together under the "subicular complex" terminological umbrella. The three can instead be categorized as constituents of the "subiculum-backing cortex (SBC)." While MLDH cells were conspicuously-absent in the subiculum, they spanned all three SBC fields. This neurochemical distinction, together with differences in cytoarchitecture and connectivity, suggest that SAC fields have more in common with each other than with subiculum proper, possibly necessitating a reconsideration of nomenclature for this important brain region.

4.2 HAP1-immunoreactivity as a marker for understanding the cytoarchitecture of the retrosplenial and retrohippocampal areas

Another significant finding of the current study was that HAP1-immunoreactivity allows for a more thorough understanding of this cytoarchitecturally-complex area of the rat brain. First, the distribution of MLDH cells described above serve as the first marker of the combined SBC. Second, a distinct group of HAP1-ir cells (GRASH cells) corresponded exclusively to the GRS, and more specifically, only the external laminae. While differential staining patterns between GRS and DRS using myelin-stained sections (Jones et al., 2005) or parvalbumin (Jones et al., 2005; Salaj et al., 2015) have been noted, boundaries were based largely on subtle changes in staining pattern across adjacent areas that both stained strongly in general. The discrete compartmentalization of HAP1-immunoreactivity via the GRASH cells provided an unambiguous and distinct contrast between strong and indeterminate staining in GRS and DRS, respectively. And while neurotensin-labelled varicosities (putative en passant synapses localized to fibers) have been reported to form a band corresponding to layer II-III of GRS only (Febvret et al., 1991), HAP1 – via GRASH cells – appears to be the first marker for GRS layer II-III that stains cell soma, not fibers. Consequently, HAP1's cytoplasmic staining pattern can enable precise and unambiguous colocalization analysis with other markers that also stain the soma.

HAP1's characteristic staining patterns provided utility as a marker in other applications as well. While differential staining for the GRS and PoS has been reported using AChE (van Groen &Wyss, 1990a, b) or calretinin and parvalbumin (Salaj et al., 2015), these observations were made primarily through fiber-staining, unlike HAP1 which primarily stained cell bodies as mentioned above. Similarly, HAP1-immunoreactivity was also a marker for determining the GRS/DS, DS/PoSv, and LEC/PRh boundaries as well.

4.3 Location of HAP1-ir cell zones suggest role in learning and memory

GRASH, MLDH, and PCH cells were localized to very discrete neuroanatomical locations: GRS superficial layers, SBC-MLD, and the areas just adjacent to the white matter, respectively. This compact compartmentalization of immunoreactivity, not only to certain cortical areas but to specific laminae within those divisions, suggest some role for HAP1-ir neurons related to the functional specificity of those locations.

In recent years, both the SBC and GRS have been recognized as sites for a variety of functionally-specialized, spatially-modulated cells involved in an animal's external

representation of space. Head direction cells fire whenever an animal faces a certain direction – regardless of its location or current activity – and while first reported in the PoS (Ranck, 1984; Taube et al., 1990; Taube, 2007), they have since also been observed in other areas including the GRS (Cho and Sharp, 2001) and the PaS (Boccara et al., 2010). Grid cells, which have been described as place-modulated neurons with periodically-spaced hexagonal firing fields spanning the animals's environment in a crystal-like manner (Boccara et al., 2010), were first identified in the medial entorhinal cortex (Hafting et al., 2005; Sargolini et al., 2006) but have been observed in the PrS and PaS as well (Boccara et al., 2010). A third type, border cells, signal specific geometric boundaries of the animal's surroundings. And while identified initially in the MEC (Solstad et al., 2008; Savelli et al., 2008), they have also been noted in the PrS and PaS (Boccara et al., 2010). The extensive intrinsic and extrinsic connectivity of SBC and the GRS have been well-documented (see Sugar et al., 2011 and Ding, 2013 for reviews), and the wide variety of spatially-modulated cells within the two brain areas suggest that these cortical areas contribute to a network of brain regions that create representations of an animal's environment. The fact that two distinct, highly-compartmentalized zones of HAP1-immunoreactivity (SBC-MLDH cells, and GRASH cells) are so conspicuously present there suggests a role for HAP1-ir neurons in one or more of these spatial navigation modalities.

The current study found also that GRASH cells were present in the GRS but not the DRS. In the rat, different patterns of connectivity between the retrosplenial fields (Van Groen and Wyss, 1990a,1992, 2003) may imply functional distinctions as well. While such reports have been limited, Vann and Aggleton (2005) found that rats with DRS lesions were less reliant on distal visual cues to control performance of a working memory task in the radial-arm maze, a change in strategy that would be consistent with anatomical data showing that the DRS is the primary recipient of visual inputs to the rat GRS (van Groen & Wyss, 1992). Pothuzien et al. (2009) recently demonstrated that the GRS contributes to spatial learning and navigation when it is based on both internal and external cues (light and dark), while the DRS is more selectively involved when distal visual cues control performance (light only) in a radial-arm maze task. Thus, GRS may serve spatial learning and memory requiring self-generated movement information and consequently be less sensitive to lighting changes. The GRASH cells, which are exclusive to GRS, may serve performance during this cue-dependent spatial navigation by being more stable against apoptosis through putative HAP1 protectivity.

Additionally, we found that the MLDH cells of the SBC, were, by definition associated with the SBC's deep layers. Taube et al. (1990) first noted that head direction cells were more prevalent in deeper cortical layers than in superficial in the PoS, and this observation was reinforced by Boccara et al. (2010) vis-a-vis head direction, grid, and border cells in the SBC. Their preferential distribution in deeper layers – where MLDH cells were present in or just deep to the MLD – suggests a role for HAP1-ir neurons in deep layer processing of spatial information, possibly raising the threshold for such neurons to neurodegeneration.

Finally, the PCH cells were located just adjacent to the corpus callosum. Their placement suggests a role in regeneration in which neurons might be more stable to apoptosis due to putative HAP1 protectivity. While still unclear, these cells, as well GRASH and MLDH cells, could play important functional roles apart from the aforementioned hypothetical role in memory and learning. Their role in behavior and pathophysiology as well as those of the MESCH cells, investigated through the use of HAP1 transgenic mice, will be an important research goal going forward.

5. Conclusion

HAP1-ir cells in the retrosplenial and retrohippocampal areas, though distributed sporadically across all sub-areas, were conspiculously present as four distinct groups of cells. The first, GRASH cells, were limited to the superficial layers of the retrosplenial granular cortex – but not dysgranular – and consisted mainly of diffusely-staining type 2 cells. The second, present mainly in the cell-sparse zone spanning the PoS–PrS–PaS continuum or SBC, consisted of a thin layer of type 1 "MLDH cells." The third group – "PCH cells" – consisted of moderately-to-darkly-stained cells just adjacent to the corpus callosum. And the fourth main group, the "MESCH cells," were mostly composed of type 2 HAP1-ir cells in superficial layers II-III from the medial entorhinal cortex to the turning corner at the transitional ventral subiculum. The HAP1-ir cells, which were primarily compartmentalized in areas that support spatial navigation, memory and learning, should be more stable against apoptosis in neurodegenerative disease owing to putative HAP1 protectivity. Additionally, HAP1 staining presents itself as an extremely useful tool for differentiating the laminar and areal borders of the neuroanatomically-complicated retrosplenial and retrohippocampal areas. The neuroanatomical data on HAP1-expressing cells in the plausible center of cognitive function should provide a base for future structural and behavioral studies to better

understand the pathophysiological involvement of HAP1 in memory and/or cognitive function.

PART 4

Comprehensive conclusion for Parts 1, 2, and 3

The present study originated from a single set of observations I developed over several years as an English teacher and as a regular advisor and consultant to a Japanese MD/PhD (my wife) and her collaborators and colleagues: despite the high quality of their research and the tremendous importance of doing so, why do so many Japanese physician–researchers struggle so mightily with the act of sharing their research to international audiences via poster or oral presentation? How can their reported lack of participation be mitigated? What are the core issues at the heart of the problem? This comprehensive, multidisciplinary study, conducted in three parts, was conceived to help shed light on this question.

While anecdotal evidence suggested that the major precluding factor in more frequent participation centered on their complicated relationship to their second language (*i.e.*, English), until now no one had ever actually queried Japanese physician–researchers in a systematic fashion to determine which, of a number of factors, was most responsible for their comparatively low presentation frequency. Was it a matter of busyness or travel expense? Were their other cultural factors at play? Our survey of 200 respondents confirmed our suspicions: not only was lack of confidence in English oral communication skills a factor precluding more frequent participation, it was the *strongest* factor. However, despite drawing attention to an important social issue and serving as a legitimate justifier for things like increased medical English education in Japanese medical schools, our survey study was limited in its ability to answer the pressing question of "Why?" in a more empirical, brain-based context. Because of prior research which hinted that the phonological and orthographical characteristics of a language itself may play a role in second language speech production as well as reports of neural differences in the brains of monolinguals and bilinguals, we chose to examine the issue more closely in young, native Japanese using a phonemic verbal fluency test in combination with neuroimaging.

Using fNIRS, a convenient form of neuroimaging with inherent advantages over other modalities, we tested Japanese medical students who had received English training during their previous academic careers and possessed an objective measure of English proficiency

via TOEIC score. We found that, when comparing results of a phonemic fluency test given in Japanese to those resulting from the English version of the test, there was English-specific bilateral recruitment of the precentral area. Also, when participants were divided by TOEIC score into "Lower" and "Higher" English proficiency groups, the Higher proficiency group performed the task more efficiently and with comparatively little effort, whether in Japanese or English. With the former being attributed to compensatory recruitment of articulation / pronunciation resources when speaking in English and the latter perhaps reflecting a "bilingual advantage" in non-verbal executive function, results from Part 2 of our overall investigation suggested that English speech production by native Japanese is dependent on at least two factors: the structures of the languages themselves and neural differences in the brains of highly-proficient individuals; it is, however, not yet clear whether said neural differences are the cause of higher proficiency or simply reflect it.

Undoubtedly, the results of our fNIRS study are extremely useful when attempting to answer to answer the question set forth at the very beginning our investigation ("Why do Japanese physician–researchers tend to present infrequently at English-language-medium conferences?" as well as that arrived at the conclusion of Part 1: "What is it about the English language presentation context that makes performance so challenging?" And we hope to use a similar methodology in the future to compare the present results with those of native English speakers who also speak Japanese as a second language. However, understanding of the intricacies of cognition also require a detailed investigation of its neuroanatomical substrates, and hence, we continued (and continue) to explore an important brain region at the seat of memory and cognition: the retrosplenial and retrohippocampal areas. Because of noted expression of HAP1 protein in this region, we used its distribution patterns in rat brain in an immunohistochemical investigation of the aforementioned areas. HAP1-immunoreactive cells were conspiculously present in four distinct HAP1-ir zones and were primarily compartmentalized in areas that support spatial navigation, memory and learning, and in the future, the neuroanatomical data on HAP1-expressing cells will serve as a base to better understand the pathophysiological involvement of HAP1 in the plausible center of cognitive function. The present investigation is striking proof that an integrated approach to understanding social phenomena such as the behavior Japanese physician–researchers through a multidisciplinary approach can shed considerable light on a complex issue.

ACKNOWLEDGEMENTS

I would like to express my most heartfelt gratitude and feelings of indebtedness to Professor Koh Shinoda for the opportunity to conduct doctoral research in his laboratory and under his supervision: my sincerest thanks for his planning, advice, collaboration, resources, teaching, and guidance during the entire doctoral program, as well as during preparation of all associated manuscripts and this thesis. I would also like to express my immense gratitude to the following members of *Ni-kaibo* for their daily support, collaboration, technical expertise, and guidance: Dr. Ryutaro Fujinaga, Dr. Akie Yanai, Dr. Keiji Kokubu, Dr. Md. Nabiul Islam, and Dr. Mir Rubayet Jahan as well as Mr. Jun Oba, Mr. Chikahisa Matuso, and Ms. Mika Takahashi. Also, many thanks to all members of the Division of Neuropsychitary at Yamaguchi University Graduate School of Medicine, especially co-authors of the manuscript that served as the basis for Part 2: Professor Yoshifumi Watanabe, Dr. Koji Matsuo, Dr Keiko Hirata, Dr. Toshio Matsubara, and Dr. Kenichiro Harada. Likewise, I want to sincerely thank all participants who volunteered their time and energy as participants for the studies in Parts 1 and 2. Finally, I would like to acknowledge the technical expertise and assistance of the Institute for Biomedical Research and Education, Yamaguchi University Science Research Center. This work was partly supported by support from JSPS KAKENHI grant numbers 25293045, 16H05118 (KS), and 15K09832 (KM).

REFERENCES

Abutalebi J, Annoni J-M, Zimine I, Pegna AJ, Seghier ML, Lee-Jahnke H, ... Khateb A. (2008) Language control and lexical competition in bilinguals: An event-related fMRI study. *Cerebral Cortex 18*(7):1496–1505.

Aggleton JP, Wright NF, Vann SD, Saunders RC (2012) Medial temporal lobe projections to the retrosplenial cortex of the macaque monkey. *Hippocampus*, *22*(9), 1883–1900.

Aida Y (1994) Examination of Horwitz, Horwitz, and Cope's construct of foreign language anxiety: The case of students of Japanese. Modern Language Journal 78(2):155-168.

Alptekin C (2002) Towards intercultural communicative competence in ELT. *ELT Journal*, *56*(1):57–64.

Angevine Jr. JB (1975) Development of the Hippocampal Region. In R. L. Isaacson & K. H. Pribram (Eds.), *The Hippocampus: Volume 1: Structure and Development* (pp. 61–94). Plenum Press.

Avery P, Ehrlich S (1992) *Teaching American English pronunciation*. Oxford: Oxford University Press.

Benton AL, Hamsher K, de S. Sivan AB (1994) *Multilingual Aphasia Examination*. Iowa City, IA: AJA Associates.

Bialystok E, Craik FIM, Green DW, Gollan TH (2009) Bilingual minds. *Psychological Science in the Public Interest 10*(3): 89–129.

Bialystok E, Craik FIM, Klein R, Viswanathan M (2004) Bilingualism, aging, and cognitive control: Evidence from the Simon task. *Psychology and Aging 19*:290–303.

Bialystok E, Craik FIM, Luk G (2008) Lexical access in bilinguals: Effects of vocabulary size and executive control. *Journal of Neurolinguistics 21*(6):522–538.

Bialystok E, Craik FIM, Ruocco AC (2006) Dual-modality monitoring in a classification task: The effects of bilingualism and ageing. *The Quarterly Journal of Experimental Psychology 59*(11):1968–1983.

Birn RM, Kenworthy L, Case L, Caravella R, Jones TB, Bandettini PA, Martin A. (2010). Neural systems supporting lexical search guided by letter and semantic category cues: A self-paced overt response fMRI study of verbal fluency. *NeuroImage 49*(1), 1099–1107.

Boas DA, Dale AM, Franceschini MA (2004) Diffuse optical imaging of brain activation:

Approaches to optimizing image sensitivity, resolution, and accuracy. *NeuroImage 23 Supplement 1*, S275–S288.

Bobb S, Wodniecka Z, Kroll J. (Eds.) (2013) What bilinguals tell us about cognitive control [Special issue]. *Journal of Cognitive Psychology 25*(5).

Boccara CN, Sargolini F, Thoresen VH, Solstad T, Witter MP, Moser EI, Moser MB (2010) Grid cells in pre- and parasubiculum. *Nature Neuroscience*, *13*(8), 987–994.

Bookheimer SY, Zeffiro TA, Blaxton TA, Gaillard W, Theodore WH (2000) Activation of language cortex with automatic speech tasks. *Neurology*, *55*(8), 1151–1157.

Bouchard KE, Mesgarani N, Johnson K, Chang EF (2013) Functional organization of human sensorimotor cortex for speech articulation. *Nature 495*(7441):327–332.

Braun AR, Varga M, Stager S, Schulz G, Selbie S, Maisog JM, ... Ludlow CL (1997) Altered patterns of cerebral activity during speech and language production in developmental stuttering: An H2(15)O positron emission tomography study. *Brain*, *120*(5):761–784.

Briellmann RS, Saling MM, Connell AB, Waites AB, Abbott DF, Jackson GD (2004). A high-field functional MRI study of quadri-lingual subjects. *Brain and Language:89*(3), 531–542.

Brown S, Laird AR, Pfordresher PQ, Thelen SM, Turkeltaub P, Liotti M (2009) The somatotopy of speech: Phonation and articulation in the human motor cortex. *Brain and Cognition 70*(1):31–41.

Brown S, Ngan E, Liotti M (2008) A larynx area in the human motor cortex. *Cerebral Cortex*, *18*(4): 837–845.

Bucci DJ, Robinson S (2014) Toward a conceptualization of retrohippocampal contributions to learning and memory. *Neurobiology of Learning and Memory*, *116*, 197–207.

Burwell RD, Amaral DG (1998) Cortical afferents of the perirhinal, postrhinal, and entorhinal cortices of the rat. *The Journal of Comparative Neurology*, *398*(2), 179–205.

Butler YG, Iino M (2005) Current Japanese reforms in English language education: The 2003 "action plan." *Language Policy 4*(1), 25–45.

Carlson SM, Meltzoff AN (2008) Bilingual experience and executive functioning in young children. *Developmental Science*, *11*(2):282–298.

Chan EY, Nasir J, Gutekunst CA, Coleman S, Maclean A, MaasA, ... Hayden, MR (2002) Targeted disruption of Huntingtin-associated protein-1 (Hap1) results in postnatal death due to depressed feeding behavior. *Human Molecular Genetics, 11*(8), 945–959.

Cho J, Sharp PE (2001) Head direction, place, and movement correlates for cells in the rat retrosplenial cortex. *Behavioral Neuroscience, 115*(1), 3.

Claro J (2007) Interaction in the Japanese classroom: Moving toward common ground. In K. Bradford Watts, T. Muller, M. Swanson (Eds.), JALT 2007 Conference Proceedings (pp. 208-218). Tokyo: Japan Association of Language Teachers. Retrieved January, 2013 from http://jaltpublications.org/archive/proceedings/2007/E083.pdf

Cohen NJ, Eichenbaum H (1993) *Memory, Amnesia, and the Hippocampal System*. MIT Press.

Cohen, NJ, Ryan J, Hunt C, Romine L, Wszalek T, Nash C (1999) Hippocampal system and declarative (relational) memory: Summarizing the data from functional neuroimaging studies. *Hippocampus, 9*(1), 83–98.

Colzato LS, Bajo MT, van den Wildenberg W, Paolieri D, Nieuwenhuis S, La Heij W, Hommel, B (2008) How does bilingualism improve executive control? A comparison of active and reactive inhibition mechanisms. *Journal of Experimental Psychology: Learning, Memory, and Cognition 34*(2): 302–312.

Costa A, Hernández M, Sebastián-Gallés N (2008) Bilingualism aids conflict resolution: Evidence from the ANT task. *Cognition 106*(1):59–86.

Council of Europe (2001) *Common European Framework of Reference for Languages: Learning, Teaching, Assessment*. Cambridge: Cambridge University Press.

Dan H, Dan I, Sano T, Kyutoku Y, Oguro K, Yokota H, ... Watanabe E (2013) Language-specific cortical activation patterns for verbal fluency tasks in Japanese as assessed by multichannel functional near-infrared spectroscopy. *Brain and Language 126*(2): 208–216.

Davachi L (2006) Item, context and relational episodic encoding in humans. *Current Opinion in Neurobiology, 16*(6), 693–700.

De Baene W, Duyck W, Brass M, Carreiras M (2015). Brain circuit for cognitive control is shared by task and language switching. *Journal of Cognitive Neuroscience 27*(9):1752–1765.

de Bruin A, Roelofs A, Dijkstra T, FitzPatrick I (2014) Domain-general inhibition areas of the brain are involved in language switching: FMRI evidence from trilingual

speakers. *NeuroImage 90*:348–359.

Delis D, Kaplan E, Kramer J (2001) *Delis-Kaplan Executive Function Scale*. San Antonio, TX: The Psychological Corporation.

Dieler AC, Tupak SV, Fallgatter AJ (2012) Functional near-infrared spectroscopy for the assessment of speech related tasks. *Brain and Language 121*(2):90–109.

Ding, SL (2013) Comparative anatomy of the prosubiculum, subiculum, presubiculum, postsubiculum, and parasubiculum in human, monkey, and rodent: Comparative Neuroanatomy of the Subicular Cortices. *Journal of Comparative Neurology*, *521*(18), 4145–4162.

Doyon P (2000) Shyness in the Japanese EFL class: Why it is a problem, what it is, what causes it, and what to do about it? The Language Teacher 24(1):11-16.

Dragatsis I, Zeitlin S, Dietrich P (2004) Huntingtin-associated protein 1 (Hap1) mutant mice bypassing the early postnatal lethality are neuroanatomically normal and fertile but display growth retardation. *Human Molecular Genetics*, *13*(24), 3115–3125.

Dräger B, Jansen A, Bruchmann S, Förster AF, Pleger B, Zwitserlood P, Knecht S (2004) How does the brain accommodate to increased task difficulty in word finding? A functional MRI study. *NeuroImage 23*(3):1152–1160.

Dronkers NF (1996) A new brain region for coordinating speech articulation. *Nature 384*(6605):159–161.

Dunn K (2008) Why It's Important For You To Present Your Data at Scientific Conferences. Washington, D.C.: American Psychological Association. Retrieved November, 2012 from http://www.apa.org/science/about/psa/2007/11/student-council-1.aspx

Educational Testing Service (2011) Correlation table: TOEIC Listening and Reading scores descriptors and the CEFR levels. Retrieved October, 2016 from https://www.etsglobal.org/Global/Eng/content/download/768/12037/version/7/file/TOEIC+L%26R+Descriptors-MAR089-LR.pdf.

Educational Testing Service (2016a) 2015 Report on test takers worldwide: the TOEIC Listening and Reading test. Retrieved October, 2016 from https://www.ets.org/s/toeic/pdf/ww_data_report_unlweb.pdf.

Educational Testing Service (2016b) About the TOEIC Listening and Reading test. Retrieved October, 2016 from https://www.ets.org/toeic/listening_reading/about.

Educational Testing Service Canada Inc (2016) Use your TOEIC score on your resume. Retrieved October, 2016 from

http://www.etscanada.ca/students/toeic_score_resume.php.

Eichenbaum H (2004) Hippocampus: cognitive processes and neural representations that underlie declarative memory. *Neuron*, *44*(1), 109–120.

Eichenbaum H (2011) *The Cognitive Neuroscience of Memory: An Introduction*. Oxford University Press, USA.

Febvret A, Berger B, Gaspar P, Verney C (1991) Further indication that distinct dopaminergic subsets project to the rat cerebral cortex: lack of colocalization with neurotensin in the superficial dopaminergic fields of the anterior cingulate, motor, retrosplenial and visual cortices. *Brain Research*, *547*(1), 55–61.

Federmeier KD, Kutas M, Schul R (2010). Age-related and individual differences in the use of prediction during language comprehension. *Brain and Language 115*(3):149–161.

Federmeier, KD, McLennan DB, de Ochoa E, Kutas, M (2002) The impact of semantic memory organization and sentence context information on spoken language processing by younger and older adults: An ERP study. *Psychophysiology 39*(2):133–146.

Fitzpatrick S, Gilbert S, Serpell L (2013) Systematic Review: Are overweight and obese individuals impaired on behavioural tasks of executive functioning? *Neuropsychology Review 23*(2):138–156.

Friesen DC, Luo L, Luk G, Bialystok E (2015) Proficiency and control in verbal fluency performance across the lifespan for monolinguals and bilinguals. *Language, Cognition and Neuroscience 30*(3):238–250.

Fujii Y (2007) Making the most of search engines for Japanese to English translation: Benefits and challenges. Asian EFL Journal 23: 1-36.

Fujinaga R, Kawano J, Matsuzaki Y, Kamei K, Yanai A, Sheng Z, ... Shinoda K. (2004). Neuroanatomical distribution of huntingtin-associated protein 1-mRNA in the male mouse brain. *The Journal of Comparative Neurology*, *478*(1), 88–109.

Fujinaga R, Takeshita Y, Uozumi K, Yanai A, Yoshioka K, Kokubu K, Shinoda K (2009) Microtubule-dependent formation of the stigmoid body as a cytoplasmic inclusion distinct from pathological aggresomes. *Histochemistry and Cell Biology*, *132*(3), 305–318.

Fujinaga R, Yanai A, Nakatsuka H, Yoshida K, Takeshita Y, Uozumi K, ... Shinoda K (2007) Anti-human placental antigen complex X-P2 (hPAX-P2) anti-serum recognizes C-terminus of huntingtin-associated protein 1A common to 1B as a

determinant marker for the stigmoid body. *Histochemistry and Cell Biology*, *128*(4), 335–348.

García-Pentón L, Fernández García Y, Costello B, Duñabeitia JA, Carreiras M (2016) "Hazy" or "jumbled"? Putting together the pieces of the bilingual puzzle. *Language, Cognition and Neuroscience 31*(3):353–360.

Goto M, Ueda N, Yoshimura R, Kihara S, Kaji K, Yamada Y, ... Nakamura J (2005) Reliability and validity of the Japanese version of the Social Adaptation Self-evaluation Scale (SASS). *Seishin Igaku 47*(5):483–489 (in Japanese).

Graves R. (2007). Oral presentations: Advice and tips. University of Western Ontario. Retrieved October 24, 2012 from http://publish.uwo.ca/~rgraves3/oralpres.pdf

Grogan A, Green DW, Ali N, Crinion JT, Price CJ (2009) Structural correlates of semantic and phonemic fluency ability in first and second Languages *Cerebral Cortex 19*(11):2690–2698.

Guest M (2013) Japanese doctors at international conferences: Why the worry? Journal of Medical English Education 12(3):47-55.

Gutekunst CA, Li SH, Yi H, Ferrante RJ, Li XJ, Hersch SM (1998) The cellular and subcellular localization of huntingtin-associated protein 1 (HAP1): comparison with huntingtin in rat and human. *The Journal of Neuroscience*, *18*(19), 7674–7686.

Hafting T, Fyhn M, Molden S, Moser MB, Moser EI (2005) Microstructure of a spatial map in the entorhinal cortex. *Nature*, *436*(7052), 801–806.

Hagoort P, Indefrey P, Brown C, Herzog H, Steinmetz H, Seitz RJ (1999) The neural circuitry involved in the reading of German words and pseudowords: a PET study. *Journal of Cognitive Neuroscience 11*(4):383–398.

Halpern H, Goldfarb RM (2013) *Language and motor speech disorders in adults*. Burlington, MA: Jones & Bartlett Publishers.

Heim S, Eickhoff SB, Amunts K (2008) Specialisation in Broca's region for semantic, phonological, and syntactic fluency? *NeuroImage 40*(3):1362–1368.

Heinzel S, Metzger FG, Ehlis A-C, Korell R, Alboji A, Haeussinger FB, ... Fallgatter AJ (2013) Aging-related cortical reorganization of verbal fluency processing: a functional near-infrared spectroscopy study. *Neurobiology of Aging 34*(2):439–450.

Hernandez AE (2009) Language switching in the bilingual brain: What's next? *Brain and Language 109*(2–3):133–140.

Herrmann MJ, Ehlis AC, Fallgatter AJ (2003) Frontal activation during a verbal-fluency task

as measured by near-infrared spectroscopy. *Brain Research Bulletin 61*(1):51–56.

Hidano T, Fukuhara M, Iwawaki M, Soga S, Spielberger CD (2000) *New STAI Manual State-Trait Anxiety Inventory-Form JYZ*. Tokyo: Jitsumu Kyouiku Press (in Japanese).

Hillis AE, Work M, Barker PB, Jacobs MA, Breese EL, Maurer K (2004) Re-examining the brain regions crucial for orchestrating speech articulation. *Brain 127*(7): 1479–1487.

Hollingshead AB (1965) *Two-factor index of social position*. New Haven, CT: Yale University Press.

Honda Y, Furuta T, Kaneko T, Shibata H, Sasaki H (2011) Patterns of axonal collateralization of single layer V cortical projection neurons in the rat presubiculum. *The Journal of Comparative Neurology*, *519*(7), 1395–1412.

Honda Y, Ishizuka N (2004) Organization of connectivity of the rat presubiculum: I. Efferent projections to the medial entorhinal cortex. *The Journal of Comparative Neurology*, *473*(4), 463–484.

Hoshi Y, Kobayashi N, Tamura M (2001) Interpretation of near-infrared spectroscopy signals: a study with a newly developed perfused rat brain model. *Journal of Applied Physiology*, *90*(5):1657–1662.

Hurks PPM (2012) Does instruction in semantic clustering and switching enhance verbal fluency in children? *The Clinical Neuropsychologist 26*(6):1019–1037.

Ikegashira A, Matsumoto Y, Morita Y (2009) English education in Japan – From kindergarten to university. In Reinelt, R. (Ed.) *Into the Next Decade with (2nd) FL Teaching* (pp. 16–40). Matsuyama, Japan: Rudolf Reinelt Research Laboratory EU. Retrieved October, 2016 from http://web.iec.ehime-u.ac.jp/reinelt/raineruto1/02RD2.pdf.

Ishihara Y, Fukuda T (2016) Immunohistochemical investigation of the internal structure of the mouse subiculum. *Neuroscience*, *337*, 242–266.

Islam MN, Takeshita Y, Yanai A, Imagawa A, Jahan MR, Wroblewski G, Nemoto J, Fujinaga R, Shinoda K. Immunohistochemical analysis of huntingtin-associated protein 1 in adult rat spinal cord and its regional relationship with androgen receptor. *Neuroscience, 340*:201–217

Islam MN, Fujinaga R, Yanai A, Jahan MR, Takeshita Y, Kokubu K, Shinoda K. (2012). Characterization of the "sporadically lurking HAP1-immunoreactive (SLH) cells" in the hippocampus, with special reference to the expression of steroid receptors, GABA, and progenitor cell markers. *Neuroscience*, *210*, 67–81.

Jahan, MR, Kokubu K, Islam MN, Matsuo C, Yanai A, Wroblewski G, ... Shinoda K (2015) Species differences in androgen receptor expression in the medial preoptic and anterior hypothalamic areas of adult male and female rodents. *Neuroscience*, *284*, 943–961.

Jones BF, Groenewegen HJ, Witter MP (2005) Intrinsic connections of the cingulate cortex in the rat suggest the existence of multiple functionally segregated networks. *Neuroscience*, *133*(1), 193–207.

Kakimoto Y, Nishimura Y, Hara N, Okada M, Tanii H, Okazaki Y (2009) Intrasubject reproducibility of prefrontal cortex activities during a verbal fluency task over two repeated sessions using multi-channel near-infrared spectroscopy *Psychiatry and Clinical Neurosciences 63*(4):491–499.

Kamei K, Matsuzaki Y, Hanada K, Nagano M, Nakahama K, Shinoda K (2001) Distribution of mRNA in the rat brain. Acta Anat Nippon 76(Suppl):A-91.

Kanzaki M (2010) Vocabulary size, TOEIC scores, and testwiseness. In A. M. Stoke (Ed.), *JALT2009 Conference Proceedings*. Tokyo: JALT. Retrieved October, 2016 from http://jalt-publications.org/archive/proceedings/2009/E106.pdf.

Katoh S, Simogaki H, Onodera A (1991) Development of the revised version of Hasegawa's Dementia Scale (HDS-R). *Rounen Seishin Igaku Zasshi 2*:1339–1347.

Kawagoe E (2004) A systematic study of actual conditions and the future: A survey of ESP education in medical schools and nursing schools. Kobe: Kobe City College of Nursing.

Keefe R (2004) The Brief Assessment of Cognition in Schizophrenia: reliability, sensitivity, and comparison with a standard neurocognitive battery. *Schizophrenia Research*, *68*(2-3): 283–297.

Kennan RP, Horovitz SG, Maki A, Yamashita Y, Koizumi H, Gore JC (2002) Simultaneous recording of event-related auditory oddball response using transcranial near infrared optical topography and surface EEG. *NeuroImage 16*(3, Part A):587–592.

Kenworthy J (1987) *Teaching English pronunciation*. London: Longman.

Koga, M., Fujinaga, R., & Shinoda, K. (2002). Interaction between HAP1 and polyglutaine protein in vitro: 57th Chugoku-Shikoku Conference of Japanese Association of Anatomists. *November*, *9*, 13.

Kobayashi Y, Amaral DG (2003) Macaque monkey retrosplenial cortex: II. Cortical afferents. *The Journal of Comparative Neurology*, *466*(1), 48–79.

Kobayashi Y, Amaral DG (2007) Macaque monkey retrosplenial cortex: III. Cortical efferents. *The Journal of Comparative Neurology*, *502*(5), 810–833.

Köhler C, Chan-Palay V, Steinbusch H (1981) The distribution and orientation of serotonin fibers in the entorhinal and other retrohippocampal areas. *Anatomy and Embryology*, *161*(3), 237–264.

Kojima M, Furukawa TA, Takahashi H, Kawai M, Nagaya T, Tokudome S (2002) Cross-cultural validation of the Beck Depression Inventory-II in Japan. *Psychiatry Research*, *110*(3):291–299.

Kroll JF, Bialystok E (2013) Understanding the consequences of bilingualism for language processing and cognition. *Journal of Cognitive Psychology 25*(5):497–514.

Kurume University Hospital (2014) Kurume University Hospital Homepage. Kurume, Japan. Retrieved June, 2014 from http://www.hosp.kurume-u.ac.jp (in Japanese)

Kuwabara H, Kasai K, Takizawa R, Kawakubo Y, Yamasue H, Rogers MA, ... Kato N (2006) Decreased prefrontal activation during letter fluency task in adults with pervasive developmental disorders: A near-infrared spectroscopy study. *Behavioural Brain Research 172*(2):272–277.

Ladefoged P (1982) *A course in phonetics*. New York: Harcourt Brace.

Li H, Wyman T, Yu Z, Li S, Li X (2003) Abnormal association of mutant huntingtin with synaptic vesicles inhibits glutamate release. *Human Molecular Genetics*, *12*(16), 2021–2030.

Lin, YF, Xu X, Cape A, Li S, Li XJ (2010) Huntingtin-associated Protein-1 Deficiency in Orexin-producing Neurons Impairs Neuronal Process Extension and Leads to Abnormal Behavior in Mice. *Journal of Biological Chemistry*, *285*(21), 15941–15949.

Li SH, Gutekunst CA, Hersch SM, Li XJ (1998) Association of HAP1 isoforms with a unique cytoplasmic structure. *Journal of Neurochemistry*, *71*(5), 2178–2185.

Li XJ, Sharp AH, Li SH, Dawson TM, Snyder SH, Ross CA (1996) Huntingtin-associated protein (HAP1): discrete neuronal localizations in the brain resemble those of neuronal nitric oxide synthase. *Proceedings of the National Academy of Sciences*, *93*(10), 4839–4844.

Luo L, Luk G, Bialystok E (2010) Effect of language proficiency and executive control on verbal fluency performance in bilinguals. *Cognition 114*(1):29–41.

Ma H, Hu J, Xi J, Shen W, Ge J, Geng F, ... Yao D (2014) Bilingual cognitive control in

language switching: An fMRI study of English-Chinese late bilinguals. *PLOS ONE*, *9*(9), e106468.

MacDonald ME, Ambrose CM, Duyao MP, Myers RH, Lin C, Srinidhi L., ... Harper PS (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell*, *72*(6), 971–983.

Martin A, Wiggs CL, Lalonde F, Mack C (1994) Word retrieval to letter and semantic cues: A double dissociation in normal subjects using interference tasks. *Neuropsychologia*, *32*(12):1487–1494..

Matsubara T, Matsuo K, Nakashima M, Nakano M, Harada K, Watanuki T, ... Watanabe Y (2014) Prefrontal activation in response to emotional words in patients with bipolar disorder and major depressive disorder *Neuroimage 85*:489-497.

Matsuo K, Kato T, Fukuda M, Kato N (2000) Alteration of hemoglobin oxygenation in the frontal region in elderly depressed patients as measured by near-infrared spectroscopy. *The Journal of Neuropsychiatry and Clinical Neurosciences 12*(4):465–471.

Matsuo K, Kouno T, Hatch JP, Seino K, Ohtani T, Kato N, Kato T (2007) A near-infrared spectroscopy study of prefrontal cortex activation during a verbal fluency task and carbon dioxide inhalation in individuals with bipolar disorder. *Bipolar Disorders 9*(8): 876–883.

McCroskey J, Fayer J, Richmond V (1985) Don't speak to me in English: Communication apprehension in Puerto Rico. Communication Quarterly, 33(3):185-192.

McDowd J, Hoffman L, Rozek E, Lyons KE, Pahwa R, Burns J, Kemper S. (2011) Understanding verbal fluency in healthy aging, Alzheimer's disease, and Parkinson's disease. *Neuropsychology 25*(2):210–225.

Metzger S, Rong J, Nguyen HP, Cape A, Tomiuk J, Soehn AS, ... Riess O (2008) Huntingtin-associated protein-1 is a modifier of the age-at-onset of Huntington's disease. *Human Molecular Genetics*, *17*(8), 1137–1146.

Miles, R. (2007). Oral presentations for English proficiency purposes. Reflections on English Language Teaching 8(2):103-110.

Ministry of Health, Labor, and Welfare (2010). Ishi, shikaishi, yakuzaishi chōsa no gaikyō (Overview of the survey of physicians, dentists, and pharmacists). Retrieved November, 2012 from http://www.mhlw.go.jp/toukei/saikin/hw/ishi/10/dl/kekka_1.pdf

Morton JB (2015) Still waiting for real answers *Cortex 73*:352–353.

Nakamura A (2012) The Determinants of Working Hours of Japanese Female Physicians: The Effects of Family Structures and Transitions into Part-time Status. Shakai Kagaku Kenkyu 64(1):45-68. (in Japanese)

Nakatani A (2006) The emergence of 'nurturing fathers': Discourses and practices of fatherhood in contemporary Japan. In: M. Rebick, A. Takenaka (eds). The Changing Japanese Family. New York: Routledge. pp. 94-108.

National Hospital Organization Kokura Medical Center (2014) Kokura Medical Center Homepage. Retrieved June, 2014 from http:// www.kokura-hp.jp/index.html (in Japanese)

National Hospital Organization Shikoku Cancer Center (2014) Shikoku Cancer Center Homepage. Retrieved June, 2014 from http://www.shikoku-cc.go.jp (in Japanese)

Nishida T (1988) Daigakusei no Komyunikeishon tuan. (Communication apprehension among Japanese college students) Kokusaikenkyu Nihon Daigaku 8:171-183.

North S (2009) Negotiating What's 'Natural': Persistent Domestic Gender Role Inequality in Japan. Social Science Japan Journal, 12: 1, available through Oxford Journals.

Obrig H, Villringer A (2003) Beyond the visible-imaging the human brain with light. *Journal of Cerebral Blood Flow & Metabolism 23*:1–18.

Ohata K (2004) Phonological differences between Japanese and English: Several potentially problematic areas of pronunciation for Japanese ESL/EFL Learners. *Language Learning 22*:29–41.

Ojima S, Nakata H, Kakigi R (2005) An ERP study of second language learning after childhood: Effects of proficiency. *Journal of Cognitive Neuroscience 17*(8): 1212–1228.

Okamura A (2006) How do Japanese researchers cope with language difficulties and succeed in scientific discourse in English?: Interviews with Japanese research article writers. The Economic Journal of Takasaki City University of Economics 48(3):61-78.

Oldfield RC (1971) The assessment and analysis of handedness: The Edinburgh inventory. *Neuropsychologia 9*(1):97-113.

O'Mara, SM, Commins S, Anderson M, Gigg J (2001) The subiculum: a review of form, physiology and function. *Progress in Neurobiology*, *64*(2), 129–155.

O'Mara SM, Sanchez-Vives MV, Brotons-Mas JR, O'Hare E (2009) Roles for the subiculum in spatial information processing, memory, motivation and the temporal control of

behaviour. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *33*(5), 782–790.

Otsubo T, Tanaka K, Koda R, Shinoda J, Sano N, Tanaka S, … Kamijima K (2005) Reliability and validity of Japanese version of the Mini-International Neuropsychiatric Interview. *Psychiatry and Clinical Neurosciences 59*(5):517–526.

Paap KR, Johnson HA, Sawi O (2016) Should the search for bilingual advantages in executive functioning continue? *Cortex 74*:305–314.

Patestas MA, Gartner LP (2016). *A textbook of neuroanatomy*. John Wiley & Sons: Hoboken, NJ.

Paulesu E, Goldacre B, Scifo P, Cappa S, Gilardi MC, Castiglioni I, … Fazio F (1997) Functional heterogeneity of left inferior frontal cortex as revealed by fMRI. *NeuroReport 8*(8):2011–2016.

Paxinos G, Watson C (2009) *The Rat Brain in Stereotaxic Coordinates:Compact Sixth Edition*. Academic Press.

Petrides M (2014) *Neuroanatomy of language regions of the human brain*. London: Academic Press.

Phillips GM (1991) Communication incompetencies: A theory of training oral performance behavior. Carbondale, IL: Southern Illinois University Press.

Pothuizen HHJ, Davies M, Albasser MM, Aggleton JP, Vann SD (2009) Granular and dysgranular retrosplenial cortices provide qualitatively different contributions to spatial working memory: evidence from immediate-early gene imaging in rats. *European Journal of Neuroscience*, *30*(5), 877–888.

Pribyl C, Keaten J, Sakamoto M (2001) The effectiveness of a skills-based program in reducing public speaking anxiety. Japanese Psychological Research, 43(3),148-155.

Pribyl C, Keaten J, Sakamoto M, Koshikawa F (1998) Assessing the cross-cultural content validity of the Personal Report of Communication Apprehension scale (PRCA-24). Japanese Psychological Researcher 40(1):47-53.

Ranck Jr., J (1984) Head direction cells in the deep layer of dorsal presubiculum in freely moving rats. *Soc Neurosci Abstr*.

Reiterer S, Hemmelmann C, Rappelsberger P, Berger ML (2005) Characteristic functional networks in high-versus low-proficiency second language speakers detected also during native language processing: An explorative EEG coherence study in 6 frequency bands. *Cognitive Brain Research 25*(2):566–578.

Reiterer S, Pereda E, Bhattacharya J (2009) Measuring second language proficiency with EEG synchronization: How functional cortical networks and hemispheric involvement differ as a function of proficiency level in second language speakers. *Second Language Research 25*(1):77–106.

Riecker A, Ackermann H, Wildgruber D, Meyer J, Dogil G, Haider H, Grodd W (2000) Articulatory/phonetic sequencing at the level of the anterior perisylvian cortex: A functional magnetic resonance imaging (fMRI) study. *Brain and Language 75*(2):259–276.

Rosen VM, Engle RW (1997). The role of working memory capacity in retrieval. *Journal of Experimental Psychology: General 126*(3):211–227.

Rodis O, Kariya N, Nishimura M, Matsumura S, Tamamura R (2011) Needs Analysis: Dental English for Japanese dental students. Asian EFL Journal 55:1-20.

Royal Society (2011). Knowledge, networks and nations: Global scientific collaboration in the 21st century. Retrieved November, 2012 from http://royalsociety.org/uploadedFiles/Royal_Society_Content/Influencing_Policy/Reports/2011-03-28-Knowledge-networks-nations.pdf

Rüschemeyer SA, Fiebach CJ, Kempe V, Friederici AD (2005) Processing lexical semantic and syntactic information in first and second language: fMRI evidence from German and Russian. *Human Brain Mapping 25*(2):266–286.

Salaj M, Druga R, Cerman J, Kubová H, Barinka F (2015) Calretinin and parvalbumin immunoreactive interneurons in the retrosplenial cortex of the rat brain: Qualitative and quantitative analyses. *Brain Research, 1627*, 201–215.

Sargolini F, Fyhn M, Hafting T, McNaughton BL, Witter MP, Moser MB, Moser EI (2006) Conjunctive Representation of Position, Direction, and Velocity in Entorhinal Cortex. *Science, 312*(5774), 758–762.

Savelli F, Yoganarasimha D, Knierim JJ (2008) Influence of boundary removal on the spatial representations of the medial entorhinal cortex. *Hippocampus, 18*(12), 1270–1282.

Schecklmann M, Ehlis AC, Plichta MM, Fallgatter AJ (2010) Influence of muscle activity on brain oxygenation during verbal fluency assessed with functional near-infrared spectroscopy. *Neuroscience 171*(2): 434–442.

ScienceWatch (2006) The 10 most-cited countries in clinical medicine, 1996-April 30, 2006. Retrieved November, 2012 from http://www.incites.com/countries/top10cli.html

Sheng G, Chang G, Lin JY, Yu ZX, Fang ZH, Rong J, ... Li XJ (2006) Hypothalamic huntingtin-associated protein 1 as a mediator of feeding behavior. *Nature Medicine*, *12*(5), 526–533.

Shi J, Sakatani K, Okamoto M, Yamaguchi Y, Zuo H (2014) Correlation between LIFG and autonomic activation during stressful tasks: A functional near-infrared spectroscopy (fNIRS) study. *Journal of Huazhong University of Science and Technology [Medical Sciences] 34*(5):663–671.

Shinoda K, Mori S, Ohtsuki T, Osawa Y (1992) An aromatase-associated cytoplasmic inclusion, the "stigmoid body", in the rat brain: I. Distribution in the forebrain. *Journal of Comparative Neurology*, *322*(3), 360–376.

Shinoda K, Nagano M, Osawa Y (1993) An aromatase-associated cytoplasmic inclusion, the "stigmoid body," in the rat brain: II. Ultrastructure (with a review of its history and nomenclature). *Journal of Comparative Neurology*, *329*(1), 1–19.

SoGoSurvey (2014) SoGoSurvey Homepage. Herndon, Virginia. Retrieved March, 2014 from http://www.sogosurvey.com

Solstad T, Boccara CN, Kropff E, Moser MB, Moser EI (2008) Representation of Geometric Borders in the Entorhinal Cortex. *Science*, *322*(5909), 1865–1868.

Spreen O, Benton AL (1969) *Neurosensory Center Comprehensive Examination for Aphasia: Manual of directions*. Victoria, B.C.: University of Victoria.

Stein M, Federspiel A, Koenig T, Wirth M, Lehmann C, Wiest R, ... Dierks, T (2009) Reduced frontal activation with increasing 2nd language proficiency. *Neuropsychologia 47*(13): 2712–2720.

Strangman G, Boas DA, Sutton JP (2002) Non-invasive neuroimaging using near-infrared light. *Biological Psychiatry 52*(7): 679–693.

Strangman G, Culver JP, Thompson JH, Boas DA (2002) A Quantitative Comparison of Simultaneous BOLD fMRI and NIRS Recordings during Functional Brain Activation. *NeuroImage 17*(2):719–731.

Strauss E, Sherman EMS, Spreen O (2006) *A Compendium of Neuropsychological Tests: Administration, Norms, and Commentary*. Oxford: Oxford University Press.

Suga M, Uetsuki M, Takizawa R, Araki T, Kasai K (2011) Phonological fluency is uniquely impaired in Japanese-speaking schizophrenia patients: Confirmation study. *Psychiatry and Clinical Neurosciences 65*(7):672–675.

Sugar J, Witter MP, van Strien NM, Cappaert NLM (2011) The Retrosplenial Cortex: Intrinsic Connectivity and Connections with the (Para)Hippocampal Region in the Rat. An Interactive Connectome. *Frontiers in Neuroinformatics*, *5*.

Sumiyoshi C, Ertugrul A, Yağcıoğlu AEA, Roy A, Jayathilake K, Milby A, ... Sumiyoshi, T (2014). Language-dependent performance on the letter fluency task in patients with schizophrenia. *Schizophrenia Research 152*(2–3):421–429.

Sumiyoshi C, Sumiyoshi T, Matsui M, Nohara S, Yamashita I, Kurachi M, Niwa S (2004) Effect of orthography on the verbal fluency performance in schizophrenia: examination using Japanese patients. *Schizophrenia Research 69*(1):15–22.

Swanson LW (2004) Brain maps III: structure of the rat brain: an atlas with printed and electronic templates for data, models, and schematics. Gulf Professional Publishing.

Tada M (2016) Recent reform to the English education system in Japan. *21st Century Education Forum 11*:21–29. Retrieved October, 2016 from http://repository.ul.hirosaki-u.ac.jp/dspace/bitstream/10129/5828/1/21SeikiForum_11_21.pdf.

Tahira, M (2012) Behind MEXT's new course of study guidelines. *The Language Teacher*, *36*(3):3–8.

Takeshita Y, Fujinaga R, Kokubu K, Islam MN, Jahan MR, YanaiA, ... ShinodaK (2011) Interaction of ataxin-3 with huntingtin-associated protein 1 through Josephin domain. Neuroreport, 22(5), 232-238.

Takeshita Y, Fujinaga R, Zhao C, Yanai A, Shinoda K (2006) Huntingtin-associated protein 1 (HAP1) interacts with androgen receptor (AR) and suppresses SBMA-mutant-AR-induced apoptosis. *Human Molecular Genetics*, *15*(15), 2298–2312.

Takizawa R, Kasai K, Kawakubo Y, Marumo K, Kawasaki S, Yamasue H, Fukuda M (2008) Reduced frontopolar activation during verbal fluency task in schizophrenia: A multi-channel near-infrared spectroscopy study. *Schizophrenia Research 99*(1–3): 250–262.

Takeshita, Y., Fujinaga, R., Kokubu, K., Islam, M. N., Jahan, M. R., Yanai, A., ... & Shinoda, K. (2011). Interaction of ataxin-3 with huntingtin-associated protein 1 through Josephin domain. *Neuroreport*, *22*(5), 232-238.

Tannenbaum RJ, Wylie EC (2004) *Mapping test scores onto the Common European Framework. ETS RR-05-18)*. Princeton, NJ: Educational Testing Service. Retrieved October, 2014 from https://www.ets.org/Media/Tests/TOEFL/pdf/CEFstudyreport.pdf.

Tannenbaum RJ, Wylie EC (2005) Mapping English language proficiency test scores onto the Common European Framework. Princeton, NJ: Educational Testing Service. Retrieved October, 2016 from https://doi.org/10.1002/j.2333-8504.2005.tb01995.x.

Tannenbaum RJ, Wylie EC (2013) *Mapping TOEIC and TOEIC Bridge Test Scores to the Common European Framework of Reference*. Princeton, NJ: Educational Testing Service. Retrieved October, 2016 from https://www.ets.org/Media/Research/pdf/TC2-06.pdf.

Taube JS (2007) The Head Direction Signal: Origins and Sensory-Motor Integration. *Annual Review of Neuroscience*, *30*(1), 181–207.

Taube JS, Muller RU, Ranck JB (1990) Head-direction cells recorded from the postsubiculum in freely moving rats. I. Description and quantitative analysis. *The Journal of Neuroscience*, *10*(2), 420–435.

Tazaki M, Nakane Y (1997) *WHO QOL 26 Japanese version*. Tokyo: Kaneko Shobo (in Japanese).

Toyomura A, Fujii T, Kuriki S (2011) Effect of external auditory pacing on the neural activity of stuttering speakers. *NeuroImage 57*(4):1507–1516.

Tupak SV, Badewien M, Dresler T, Hahn T, Ernst LH, Herrmann MJ, ... Ehlis AC (2012) Differential prefrontal and frontotemporal oxygenation patterns during phonemic and semantic verbal fluency. *Neuropsychologia 50*(7):1565–1569.

Tsuzuki D, Jurcak V, Singh AK, Okamoto M, Watanabe E, Dan I (2007) Virtual spatial registration of stand-alone fNIRS data to MNI space. *Neuroimage 34*(4):1506-1518.

Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, ... & Joliot M (2002) Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage 15*(1):273-289.

UNESCO Institute for Statistics (2007) Data Centre. Montreal, Canada. Retrieved November, 2012 from http://stats.uis.unesco.org/unesco/TableViewer/document.aspxReportId=143&IF_Language=eng

US Patent and Trademark Office (2010) Patent counts by country/ state and by year: utility patents January 1, 1963–December 31, 2009. US Patent and Trademark Office: Alexandria, VA, USA.

Vance T (1987) *An introduction to Japanese phonology*. Albany: State University of New York Press.

van Groen T, Wyss JM (1990b) The connections of presubiculum and parasubiculum in the rat. *Brain Research*, *518*(1–2), 227–243.

van Groen T, Wyss JM (1992) Connections of the retrosplenial dysgranular cortex in the rat. *Journal of Comparative Neurology*, *315*(2), 200–216.

van Groen T, Wyss JM (2003) Connections of the retrosplenial granular b cortex in the rat. *The Journal of Comparative Neurology*, *463*(3), 249–263.

Vann SD, Aggleton JP (2005) Selective Dysgranular Retrosplenial Cortex Lesions in Rats Disrupt Allocentric Performance of the Radial-Arm Maze Task. *Behavioral Neuroscience*, *119*(6), 1682–1686.

Vingerhoets G, Borsel JV, Tesink C, van den Noort M, Deblaere K, Seurinck R, ... Achten E (2003) Multilingualism: An fMRI study. *NeuroImage 20*(4): 2181–2196.

Wagner S, Sebastian A, Lieb K, Tüscher O, Tadić A (2014) A coordinate-based ALE functional MRI meta-analysis of brain activation during verbal fluency tasks in healthy control subjects. *BMC Neuroscience 15*(1):1.

Wallwork A (2010) English for presentations at international conferences. New York: Springer.

Wartenburger I, Heekeren HR, Abutalebi J, Cappa SF, Villringer A, Perani D, Fazio F (2003) Early setting of grammatical processing in the bilingual brain. *Neuron 37*(1):159–170.

Watanuki T, Matsuo K, Egashira K, Nakashima M, Harada K, Nakano M, ... Watanabe Y. (2016) Precentral and inferior prefrontal hypoactivation during facial emotion recognition in patients with schizophrenia: A functional near-infrared spectroscopy study. *Schizophrenia Research 170*(1):109-114.

Williams K, Andrade M. (2008) Foreign language learning anxiety in Japanese WFL university classes: Causes, coping, and locus of control. Electronic Journal of Foreign Language Teaching, 5(2):181-191. Retrieved January, 2013 from http://eflt.nus.edu.sg/v5n22008/williams.pdf

Witter MP, Amaral DG (2004) Hippocampal formation. In Paxinos, George (Ed.), *The Rat Nervous System. third ed.* (pp. 635–704). Elsevier Academic Press.

Witter MP, Groenewegen HJ (1990) Chapter 4 The subiculum: cytoarchitectonically a simple structure, but hodologically complex. In J. Z. and O. P. O. J. Storm-Mathisen (Ed.), *Progress in Brain Research* (Vol. 83, pp. 47–58). Elsevier.

Wroblewski G, Wroblewski J, Matsumoto T, Nozaki I, Kamura T, Kumashiro R, Shinoda K. (2014) Factors dissuading Japanese doctors from presenting more frequently at

international conferences: more than just the usual suspects? *Journal of Medical English Education 13*(3):55–64.

Wroblewski, G., Islam, M.N., Fujinaga, R., Jahan, M.R., Matsuo, C., Nemoto, J., et al. Characterization of the HAP1-immunoreactive cells in the subiculum and retrohippocampal formation in rat. Presentation at the 45th Annual Meeting of the Society for Neuroscience, Chicago, IL, October 17-21, 2015.

Wyss JM, van Groen T (1992) Connections between the retrosplenial cortex and the hippocampal formation in the rat: a review. *Hippocampus*, *2*(1), 1–11.

Yeung N, Nystrom LE, Aronson JA, Cohen JD (2006) Between-task competition and cognitive control in task switching. *The Journal of Neuroscience 26*(5):1429–1438.

APPENDIX

Publications from this thesis

A. Journals

Wroblewski G, Wroblewski J, Matsumoto T, Nozaki I, Kamura T, Kumashiro R, Shinoda K (2014) Factors dissuading Japanese doctors from presenting more frequently at international conferences: more than just the usual suspects? *Journal of Medical English Education 13*(3):55–64.

Wroblewski G, Matsuo K, Hirata K, Matsubara T, Harada K, Watanabe Y, Shinoda K (under revision) Effects of task language and second language proficiency on the neural correlates of phonemic fluency in native Japanese speakers: a functional near-infrared spectroscopy. *NeuroReport*.

B. Conference presentations

- **Greggory Wroblewski**, Md. Nabiul Islam, Chikahisa Matsuo, Akie Yanai, Ryutaro Fujinaga, Koh Shinoda. Immunohistochemical analysis of the retrosplenial–retrohippocampal area in rat. 16th Annual Meeting of the **Organization of Research-Integrated Groups in Neuroscience (ORIGIN), Shimonoseki, Yamaguchi, Japan.** 2015.08.28-29 **(Oral presentation).**

- **Greggory Wroblewski**, Md. Nabiul Islam, Ryutaro Fujinaga, Mir Rubayet Jahan, Chikahisa Matsuo, Jo Nemoto, Keisuke Tanaka, Kenyu Ishii, Akie Yanai, Koh Shinoda. Characterization of the HAP1-immunoreactive cells in the subiculum and retrohippocampal formation in rat. 45th Annual Meeting of the **Society for Neuroscience (SFN), Chicago, IL, USA**. 2015.10.17-21 **(Poster presentation).**

- **Greggory Wroblewski,** Md Nabiul Islam, Ryutaro Fujinaga, Chikahisa Matsuo, Mir Rubayet Jahan, Akie Yanai, Koh Shinoda. Characterization of

two novel HAP1-immunoreactive structures in the retrosplenial–retrohippocampal area in rat. 121st Annual Meeting of the **Japanese Association of Anatomists, Fukushima, JAPAN.** 2016.03.28-30 **(Poster presentation).**

- **Greggory Wroblewski,** Koji Matsuo, Keiko Hirata, Toshio Matsubara, Kenichiro Harada, Yoshifumi Watanabe, Koh Shinoda. Effects of task language and English proficiency on cortical activity during phonemic fluency tasks in Japanese–English bilinguals. 122nd Annual Meeting of the **Japanese Association of Anatomists, Nagasaki, JAPAN.** 2017.03.28-30 **(Poster presentation).**